25 April 2025
EMA/161582/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Attrogy

International non-proprietary name: diflunisal

Procedure No. EMEA/H/C/006248/0000

Note

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

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List of abbreviations

ASMF	Active Substance Master File = Drug Master File
BCS	Biopharmaceutics Classification System
CFU	Colony Forming Units
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas chromatography mass spectrometry
GMP	Good Manufacturing Practice
HDPE	High Density Polyethylene
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LDPE	Low density polyethylene
NMT	Not more than
PDE	Permitted Daily Exposure
QTPP	Quality target product profile
RH	Relative Humidity
SmPC	Summary of Product Characteristics
TTC	Threshold of toxicological concern
USP	United States Pharmacopoeia
UV	Ultraviolet
XR(P)D	X-Ray (Powder) Diffraction

* General list of abbreviations. Not all of them will be used.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Purpose Pharma International AB submitted on 15 January 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Attrogy (diflunisal), through the centralised procedure falling within the Article 3(1) Indent 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 10 November 2022.

Diflunisal, was designated as an orphan medicinal product EU/3/22/2640 on 24 Jun 2022 in the following condition: treatment of ATTR amyloidosis.

Following the CHMP positive opinion on this marketing authorisation and at the time of the review of the orphan designation by the Committee for Orphan Medicinal Products (COMP), this product was withdrawn from the Community Register of designated orphan medicinal products on 10 June 2025 on request of the sponsor. The relevant orphan designation withdrawal assessment report can be found under the 'Assessment history' tab on the Agency's website:
https://www.ema.europa.eu/en/medicines/human/EPAR/attrogy

1.2. Legal basis, dossier content

The legal basis for this application refers to:

(Article 8(3) of Directive No 2001/83/EC) - complete and independent application

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0538/2023 on the granting of a (product-specific) waiver.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products Vyndaqel (tafamidis), Tegsedi (inotersen sodium), Onpattro (patisiran) and Amvuttra (vutrisiran). Assessment of these claims is appended.

1.4.2. Derogation(s) from market exclusivity

Not applicable.

1.4.3. Additional data exclusivity/marketing protection

Not applicable.

1.4.4. New active substance status

Not applicable.

1.5. Protocol assistance

The applicant did not seek Protocol assistance from the CHMP.

1.6. Steps taken for the assessment of the product

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

Rapporteur: Fátima Ventura Co-Rapporteur: Ewa Balkowiec Iskra

CHMP Peer reviewer(s): N/A

The application was received by the EMA on	15 January 2024
The procedure started on	01 February 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	26 April 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	06 May 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	30 May 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	02 December 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	15 January 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 January 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	26 January 2025
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	30 January 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	26 March 2025

The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the list of outstanding issues to all CHMP and PRAC members on	11 April 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the list of outstanding issues to all CHMP and PRAC members on	19 April 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Attrogy on	25 April 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The proposed indication of diflunisal is "for the treatment of transthyretin amyloid amyloidosis in adults with polyneuropathy".

For consistency purposes to the terminology and to the wording used recently with other products it is proposed to use the following wording:

Attrogy is indicated for the treatment of hereditary transthyretin-mediated amyloidosis in adult patients with stage 1 or stage 2 polyneuropathy.

This wording above is in accordance with the orphan designation of diflunisal ("Treatment of ATTR amyloidosis"),(https://ec.europa.eu/health/documents/community-register/2022/20220624156022/dec_156022_en.pdf).

Hereditary transthyretin amyloid amyloidosis (ATTRv amyloidosis, ATTR-FAP) is a rare lethal, autosomal dominant genetic disease caused by the aggregation of variant transthyretin (TTR); a thyroxine transport protein predominantly produced by the liver. More than 150 mutations have so far been recorded in the TTR gene on chromosome 18.

The most frequent form in EU is TTRmet30 mutation, which is endemic in EU countries (Portugal and Sweden).

The dissociation and subsequent aggregation of TTR may occur even in subjects without transthyretin gene mutations in certain conditions, such as aging, leading to an occurrence of wild-type transthyretin amyloidosis.

2.1.2. Epidemiology

The worldwide prevalence of hATTR-PN has been estimated at approximately 10,000 patients [Coelho, 2008]. In Europe, the incidence is estimated as 0.003 cases per 10,000 per year (or 0.3 new cases per year per 1 million inhabitants), with a prevalence estimate of 0.052 per 10,000 (or 5.2 cases per 1 million inhabitants) [Coelho et al., "A Guide to Transthyretin Amyloidosis", Amyloidosis Foundation, 2016 Edition]. In endemic regions the prevalence may exceed 90 per 10,000.

2.1.3. Biologic features, aetiology and pathogenesis

TTR is a 55 kD homotetrameric protein composed of 127-residue β-sheet-rich subunits. It is stable in its homotetramer form and functions as a transporter of thyroxin (T4) and retinol (vitamin A)-binding protein under physiological conditions. Most TTR mutations result in the production of TTR that is less stable than wild-type TTR, leading to aggressive and systemic amyloid deposition of variant TTR.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Early onset of symptoms between the third to fourth decades of life leads to rapid deterioration of the patients' health due to progression of autonomic and sensory-motor deficits, whereas the polyneuropathy slowly develops after late onset of the disease between the sixth and eighth decades of life. The average life expectancy is 3 to 15 years after diagnosis. Presence of significant cardiomyopathy is associated with poorer prognosis. Patients typically die from malnutrition and cachexia, renal failure and cardiac disease.

2.1.5. Management

Current treatment of ATTR-FAP may be considered under three headings: Liver transplant, and medical therapeutic agents, both approved drugs and off-label use.

Liver transplant

This approach removes the main source of mutated TTR but it does not prevent progression of cardiac disease because the wild-type TTR may continue to further expand existing amyloid deposits in the heart.

Currently approved drugs

Five drugs are currently approved in the EU in this indication:

- Tafamidis (Vyndaqel) binds with negative cooperativity to the two thyroxine binding sites on the native tetrameric form of transthyretin, preventing dissociation into monomers.
- Inotersen (Tegsedi) is a 2'-O-2-methoxyethyl phosphorothioate antisense oligonucleotide inhibitor of human transthyretin production. The selective binding of inotersen to the TTR messenger RNA causes the degradation of both mutant and wild type TTR mRNA. This prevents the synthesis of TTR protein in the liver, resulting in significant reductions in the levels of mutated and wild type TTR protein secreted by the liver into the circulation.
- Patisiran (Onpattro) is a double-stranded small interfering ribonucleic acid (siRNA) that specifically targets a genetically conserved sequence in the 3' untranslated region of all mutant and wild-type TTR mRNA. Patisiran is formulated as lipid nanoparticles to deliver the siRNA to hepatocytes, the primary source of TTR protein in the circulation. Through a natural process called RNA interference, patisiran causes the catalytic degradation of TTR mRNA in the liver, resulting in a reduction of serum TTR protein (Onpattro SmPC).
- Vutrisiran (Amvuttra) is an siRNA which targets variant and wild-type TTR mRNA. It is covalently linked to a ligand containing three N-acetylgalactosamine residues to enable delivery of the siRNA to hepatocytes. It causes the catalytic degradation of TTR mRNA in the liver, resulting in the reduction of variant and wild-type serum TTR protein levels.
- Eplontersen (Wainuza) is a N-acetylgalactosamine (GalNAc)-conjugated 2'-O-2-methoxyethyl-modified chimeric gapmer antisense oligonucleotide (ASO) with a mixed backbone of

phosphorothioate and phosphate diester internucleotide linkages. The GalNAc conjugate enables targeted delivery of the ASO to hepatocytes. The selective binding of eplontersen to the transthyretin (TTR) messenger RNA (mRNA) within the hepatocytes causes the degradation of both mutant and wild type (normal) TTR mRNA. This prevents the synthesis of TTR protein in the liver, resulting in significant reductions in the levels of mutated and wild type TTR protein secreted by the liver into the circulation.

Four of these drugs (inotersen, patisiran, vutrisiran, and eplontersen) require parenteral administration. Supplemental vitamin A is recommended for all four drugs. Additionally, patisiran requires premedication to reduce the risk of infusion-related reactions.

Off-label use - diflunisal
Although it has never been authorised for this indication, diflunisal is in current clinical practice widely used for the treatment of ATTR-FAP and is recommended in this role by some European countries' guidelines.

2.2. About the product

Diflunisal was previously authorised in several EU countries for traditional NSAID indications. For example, the Swedish authorisation for Donobid was granted in 1979. However, it has never been authorised for the treatment of ATTR amyloidosis (in Europe or elsewhere). All brands of diflunisal have now been withdrawn from all EU markets for commercial reasons (not because of safety concerns). In the US, the initiator (Dolobid) was also withdrawn, however, generic presentations are still available.

Diflunisal has been used for the treatment of ATTR amyloidosis for over 10 years in some EU and non-EU countries despite never having been authorised in this indication. This usage continues today despite the absence of an authorised product. In Sweden, diflunisal is available as an extemporaneous formulation and in the Netherlands it is available as an unlicenced medicine (Dolaced).

Published clinical trial evidence supporting such use began with Sekijima et al (2006) who confirmed the tetramer-stabilising effects in healthy volunteers.

2.3. Type of application and aspects on development

The repurposing of the previously marketed NSAID has led to an abridged clinical dossier that includes material from several sources:

- The original Merck, Sharp & Dohme dossier with regard to:
    - Clinical pharmacology
    - Original safety assessment of diflunisal
- A single pivotal trial of diflunisal in the treatment of ATTR conducted under the auspices of Prof John L. Berk of the Boston University School of Medicine with regard to efficacy of diflunisal in ATTR
- Post-marketing surveillance of diflunisal with reference to the safety of diflunisal in widespread clinical use.
- Published literature relating to the ATTR indication.

- A Patient Registry collecting experience gained with diflunisal in the ATTR indication treated at the Amyloidosis Centre, Umeå, Sweden.

The applicant received a national SA from MPA regarding regulatory aspects (Legal basis), overall content of the MAA dossier, size of the safety data base.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as film-coated tablets containing 250 mg of diflunisal as active substance.

Other ingredients are: microcrystalline cellulose (E460) (PH 101), pre-gelatinised starch (E1422), croscarmellose sodium (E468), silica, hydrophobic colloidal (E551), magnesium stearate, hydroxypropyl methylcellulose (E464) 2910 E5/hypromellose, macrogol 3350 (E1521), titanium dioxide (E171), sunset yellow aluminium lake (E110) and purified water.

The product is available in a HDPE bottle with polypropylene child-resistant tamper-evident screw cap with a liner as described in section 6.5 of the SmPC.

2.4.2. Active Substance

2.4.2.1. General information

The chemical name of diflunisal is [1,1'-biphenyl]-3-carboxylic acid, 2',4'-difluoro-4-hydroxy corresponding to the molecular formula $C_{13}H_8F_2O_3$. Diflunisal has a relative molecular mass of 250.20 and the following structure:

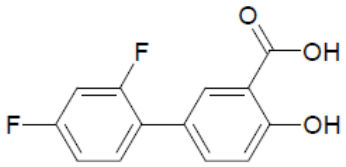


Figure 1: Active substance structure

The chemical structure of diflunisal was elucidated by a combination of elemental analysis, infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (^{1}H NMR) and mass spectroscopy. The solid-state properties of the active substance were studied by X-ray powder diffraction.

The active substance is a white to off-white powder which is practically insoluble in water and acidic aqueous conditions. Diflunisal is not hygroscopic and has a non-chiral molecular structure.

Polymorphism has been observed for diflunisal, and four polymorphs are reported in literature (Forms I, II, III and IV). Results from X-ray diffraction show that the polymorphic form obtained by the manufacturing process is consistently crystalline and shows peaks with 2θ value at 4.1, 13.4, 14.4, 14.8, 16.6, and 17.1 (± 0.3). This corresponds to a mixture of Form I (as the major form) and Form III. Polymorphism is controlled in the active substance specification as it was updated during the procedure.

2.4.2.2. Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

The active substance is manufactured at one manufacturing site, with further sites involved in the manufacture of intermediates. A major objection related to the QP declaration was resolved during the procedure. Compliance with EU GMP requirements at the active substance manufacturer was confirmed through an audit conducted in the last three years.

Diflunisal is synthesised in six main synthetic steps using well defined starting materials with acceptable specifications. During the procedure, a major objection was raised as the initially proposed starting material did not meet regulatory requirements and was therefore not acceptable. In response, the starting materials have been redefined to two starting materials to include an additional synthetic step. These starting materials are acceptable. The major objection is resolved.

The manufacturing process is described in sufficient detail and is acceptable.

The overall control strategy is adequate. Suitable in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities are well discussed with regards to their origin and characterised. A detailed discussion was on the potential presence of organic as well as inorganic impurities and residual solvents was provided. This includes also a detailed discussion on potential and actual impurities from the newly defined starting materials and their carry-over to the final active substance. The discussion is based on analysis of the synthetic route of the active substance as well as on batch data. A risk analysis of potentially mutagenic impurities in the active substance has been conducted in accordance with ICH M7 and the results are presented. Actual and potential impurities, including from the newly defined starting materials are classified based on Qualitative Structure-Activity Relationship assessment (QSAR). Three mutagenic impurities were identified. The limit for these impurities in the active substance was calculated using the TTC of 1.5 µg per person per day in line with ICH M7 and the maximum daily dose of Attrogy finished product. Batch data from three consecutive batches, obtained with a suitable analytical method is presented. The three mutagenic impurities were not detected. For one mutagenic impurity, purge factor calculations were provided in addition. Results from these calculations further confirm that this impurity is removed during the manufacturing process of the active substance. The information presented on mutagenic impurities and their control is satisfactory. It can be concluded that routine control in the active substance specification is not required.

The solvents used in the last step of the synthesis are adequately controlled in the active substance.

Inorganic impurities are routinely controlled in the active substance by testing for residue on ignition.

Routine control for one impurity with a suitable limit was included in the active substance specification during the procedure.

The active substance is packaged in low-density polyethylene (LDPE) bags which comply with Commission Regulation (EU) 10/2011, as amended.

2.4.2.3. Specification

The active substance specification includes tests for appearance, identity (IR, UV), assay (HPLC), residue on ignition (Ph. Eur.), impurities (HPLC), residual solvents (GC), loss on drying (Ph. Eur.), polymorph identification (X-ray diffraction), and particle size (sieve analysis).

The specification used by the active substance and finished product manufacturer respectively, are generally similar. Particle size is tested only by the finished prdouct manufacturer. The specification by the finished product manufacturer was established based on general compendial requirements, the USP monograph for diflunisal, information from the active substance manufacturer, ICH guidelines and in-house analysis. The specification and respective limits are well justified and cover all relevant parameters. A justification was provided for the absence of testing for microbial purity in the active substance, which was accepted. Limits for related substances are set in line with ICH Q3A(R2) and limits for residual solvents are in line with ICH Q3C(R8). A major objection was initially raised during the procedure on the lack of routine control of one impurity in the active substance. In response, control of this impurity at a suitable limit was added to the specification of the active substance. This resolved the major objection.

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

Batch analysis data from two production-scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Results are available from two formal stability studies.

From the first study, stability data from three production-scale batches of active substance from the proposed manufacturer stored in a container closure system representative of that intended for the market for up to 60 months under long term conditions (25ºC / 60% RH), for up to 12 months under intermediate conditions (30°C / 65% RH) and for up to 6 months under accelerated conditions (40ºC / 75% RH) according to the ICH guidelines were provided.

For the second subsequent study the stability protocol was revised, and the previous intermediate storage conditions were applied as long-term conditions. Stability data from three production-scale batches of active substance from the proposed manufacturer stored in a container closure system representative of that intended for the market for up to 60 months under long term conditions (30°C / 65% RH) and for up to 6 months under accelerated conditions (40ºC / 75% RH) according to the ICH guidelines were provided.

The analytical methods used were the same as used by the active substance manufacturer for release of the active substance and are stability indicating. The following parameters were tested: description, loss on drying, related substances, assay, polymorphism and in addition also chromatographic purity. All tested parameters were within the specifications.

Results from a stress test study were also conducted on one batch (stress conditions: temperature up to 105ºC, UV light, sunlight, aqueous alkaline, acidic and oxidative conditions). Degradation was only observed in aqueous acidic conditions at higher concentrations (1.0 HCl) and under oxidative conditions. Under the other stress conditions, the active substance remained stable. The test results confirmed the stability-indicting nature of the HPLC method used for assay and impurities testing.

Testing for the impurity diflunisal ester was only conducted at 60 months under the long-term conditions used in the second formal stability study. As the stability study under accelerated conditions did not include testing for diflunisal ester, the proposed temperature storage conditions as stated below are acceptable.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Attrogy finished product presents as light orange, capsule shaped, biconvex film-coated tablets. The tablets are engraved with "D250" on one side and are plain on the other side. The tablets are 6.35 mm wide and 14.29 mm long. The finished product is available in one strength (250 mg).

Attrogy was developed as an immediate-release film-coated tablet containing 250 mg of diflunisal as active substance.

The Quality Target Product Profile (QTPP) was defined and justified.

The pharmaceutical development has been sufficiently described. During pharmaceutical development, all attributes of the QTPP were monitored. In addition, Critical Quality Attributes (CQAs) were defined which were explicitly tracked in risk assessments (CQAs: assay, degradation products/impurities, content uniformity, and dissolution). The CQAs were those attributes that were considered to have the greatest potential to be altered by varying process parameters or the formulation during development studies.

The characteristics of the active substance are well understood. As further discussed above, several polymorphic forms of the active substance are known. The polymorphic form of the active substance is routinely controlled in the active substance specification and is a mixture of Form I (the major form) and Form III. It has been demonstrated that the manufacturing process of the finished product does not lead to changes in the polymorphic form.

Diflunisal is a BCS class II substance and is characterised by low solubility across the pH range of the gastrointestinal tract (pH 1.2–6.8) in aqueous buffer systems and by high permeability across cell membranes. The solubility is pH dependent and increases at higher pH.

The particle size of the active substance is routinely controlled in the active substance specification. These acceptance criteria for particle size are supported by results from manufacturing process development and dissolution development studies. The particle size distribution can have an impact on the manufacturing process (dry granulation) and content uniformity, but the risk is low as the distribution/bulk density is controlled during manufacture and the amount of active substance in the formulation is high (50%).

All excipients are well known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards, with the exception of the sunset yellow colourant used for film-coating which complies with in-house specifications. The colourants titanium dioxide and sunset yellow comply with Commission Regulation (EU) No 231/2012 for food additives. There are no novel excipients used in the finished product formulation. Sunset yellow is an excipient with a known physiological effect and is thus also listed in section 2 of the SmPC. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.4.1 of this report.

A rationale for the selection of each excipient was provided and the respective functionality was described. Compatibility of diflunisal active substance with the excipients was studied in binary mixtures and were found to be compatible. The compatibility is further supported by the results of finished product stability studies.

Formulation development has been described in detail. Formulation development focused on the evaluation of the high-risk formulation variables which were identified in an initial risk assessment. The development was conducted in several stages. The first development study was conducted to support the selection of excipients and identify the lead formulation. In the second development study, the composition variables were optimised. Based on the results of the second study, the optimised formulation was identified. The third formulation development study then focused on the identification of critical material attributes of the excipients. The acceptable range for the amount of each excipient as well as type and grade was studied regarding impact on product characteristics. The results are described in detail and led to the finalisation of the lead formulation for process optimisation studies. The final composition is justified and supported by the development studies.

The development of the dissolution method has been presented. During the procedure a major objection was initially raised related to the information provided on the dissolution method. The applicant was asked to further justify the selection of the dissolution method for routine testing and to further demonstrate the discriminatory power. Satisfactory responses on the points raised in the major objection were received and the major objection is resolved. The dissolution method used for routine release testing of the finished product is described. The selected dissolution conditions are appropriate, and the justification provided for the selection of the dissolution test medium and paddle rotation speed is satisfactory. The specification limit for routine testing is acceptable (not less than 80% (Q) dissolved in 30 minutes). During development, the impact of changes in the manufacturing process as well as the impact of changes in the formulation were tested and the dissolution test method used for these studies was described (900 mL medium pH 7.2, 75 rpm, apparatus 2/paddle). In particular, the impact of changes in particle size of the active substance and changes in tablet hardness on dissolution have been investigated. The discriminatory power of the dissolution method used for routine testing of the finished product has been demonstrated. Results of *in vitro* dissolution tests in three different buffers conducted with batches of finished product manufactured by the proposed commercial manufacturer are presented.

The development of the manufacturing process has been described. Risk analysis was used to identify the parameters and unit operations likely to have an impact on the quality of the finished product. The information presented on the development of the manufacturing process is satisfactory. Critical process parameters have been identified. Appropriate operating ranges and controls for critical steps were established based on results from development studies.

The start of shelf-life for the finished dosage form is defined in line with guideline CPMP/QWP/072/96.

A bulk holding time study was conducted to support the proposed bulk holding times (see below).

A risk assessment for microbial contamination of the finished product was provided. The microbial contamination throughout the manufacturing process is well controlled. Microbial contamination is also routinely controlled in the finished product.

The formulation used during clinical studies is the same as that intended for marketing.

The primary packaging is a HDPE bottle with polypropylene child-resistant tamper-evident screw cap with a liner. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured at one manufacturing site

The finished product is manufactured using a dry granulation process. The manufacturing process consists of five main steps: blending, compaction, compression, film coating and packaging. The process is considered to be a standard manufacturing process. The manufacturing process is described in sufficient detail and the batch formula is provided. Critical steps are clearly stated, and they are adequately controlled. The proposed commercial batch size is defined.

The maximum processing time has been defined. The holding time for the bulk tablets prior to bulk or final packaging has also been defined. The proposed hold times are acceptable.

A process validation scheme has been presented, and it is considered acceptable. The process will be validated on three consecutive batches of the minimum and maximum batch size before commercialisation. Based on batches manufactured so far, it has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (visual), average weight, identification (HPLC, UV), assay (HPLC), uniformity of dosage units by mass variation (Ph. Eur.), dissolution (in house), degradation products (HPLC), residual solvents (calculation) and microbial purity (Ph. Eur.).

The specification for the finished product is acceptable and includes all parameters necessary for this dosage form. Adequate justification for the proposed specification limits has been provided. The specification is in line with the requirements of relevant Ph. Eur. general monographs, ICH guidelines and was set based on available batch data.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities (option 2b). No elemental impurities were identified as having the potential to be present at a level of greater than 30% of the PDE limit for oral administration. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

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

Batch analysis results are provided for three batches manufactured at the minimum commercial batch size confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

2.4.3.4. Stability of the product

Stability data from three batches of finished product manufactured at the minimum commercial batch size stored for up to 24 months under long term conditions (25ºC / 60% RH) and for up to 6 months under accelerated conditions (40ºC / 75% RH) according to the ICH guidelines were provided. In addition, stability data was also provided for up to 6 months under intermediate conditions (30ºC / 65% RH). The batches of medicinal product are representative of those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, identity, assay, dissolution, degradation and microbial purity. The analytical procedures used are stability indicating. No significant changes have been observed, and all results were within specification.

A forced degradation study was conducted on one batch. The finished product in the solid state was exposed to thermal, heat/humidity and light stress and was found to be stable under the conditions tested.

In addition, one batch of finished product was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The appearance of tablets directly exposed to light showed a slight change in colour and therefore a storage condition is indicated in section 6.4 of the SmPC (see below).

A bulk holding time study was conducted on three batches of bulk finished product tablets packed in poly bags contained in HDPE pails stored up to 9 months at 25ºC / 60% RH. Samples were tested for appearance, identification, assay, dissolution, degradation products and microbial purity. The results support the proposed holding time for bulk tablets (24 months).

The finished product is packaged in multi-dose packs. The applicant committed to conduct an in-use stability study post-approval with finished product at the beginning and end of the shelf life (see below).

Based on available stability data, the proposed shelf-life of 2 years and storage condition "This medicinal product does not require any special storage conditions. Store in the original package in order to protect from light" as stated in the SmPC (sections 6.3 and 6.4) are acceptable.

2.4.3.5. Adventitious agents

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

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. Four major objections initially raised (related to the GMP compliance documentation of the active substance manufacturing site and the respective QP declaration, the designation of the active substance starting materials, the control of one impurity in the active substance specification and the dissolution method for the control of the finished product)

were resolved during the procedure. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

At the time of the CHMP opinion, there was a minor unresolved quality issue having no impact on the Benefit/Risk ratio of the product, which pertain to the in-use stability of the multi-dose packs. This point is put forward and agreed as a recommendation for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.4.6. Recommendation for future quality development

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

- To conduct an in-use stability study of the drug product at the beginning and end of the shelf life.

2.5. Non-clinical aspects

2.5.1. Introduction

Diflunisal is a difluorophenyl derivate of salicylic acid and is a non-steroidal anti-inflammatory drug (NSAID). The proposed therapeutic indication is for the treatment of transthyretin amyloid (ATTR) amyloidosis in adults with polyneuropathy, and the recommended dose is one 250 mg tablet taken with fluid twice daily. Diflunisal has been used for the treatment of ATTR amyloidosis for many (>10) years in some EU and non-EU countries despite never having been authorised in this indication.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Diflunisal's primary pharmacodynamic action results from its ability to stabilise transthyretin (TTR), distinct from traditional NSAIDs. TTR exists as a stable homotetramer in plasma, crucial for preventing amyloid formation. While thyroxine (T4) stabilises TTR tetramers, its therapeutic use is limited due to safety concerns. Diflunisal, mimicking T4's binding mode, exhibits appropriate stereochemistry to bind and stabilise TTR tetramers. Although a preclinical animal model was not available, human trials indicate that a dose of 250 mg twice daily achieves sufficient serum concentrations to stabilise TTR tetramers, effectively inhibiting amyloidogenesis.

2.5.2.2. Secondary pharmacodynamic studies

No new secondary PD studies have been provided by the applicant. Diflunisal's mechanism of action, akin to other NSAIDs like aspirin, involves blocking the cyclooxygenase enzyme in the arachidonic acid

cascade. Secondary pharmacodynamics studies, detailed in the MPA application dossier for Donobid in 1977, reveal diflunisal's superior anti-inflammatory, analgesic, and antipyretic properties compared to aspirin. Studies demonstrate diflunisal's efficacy in various inflammation models, including carrageenan-induced swelling, croton oil-induced inflammation, and adjuvant-induced arthritis, with favourable outcomes in rats and dogs. Additionally, diflunisal exhibits inhibitory effects on extracellular HMGB1, CBP and p300 lysine acetyltransferase activities, indicating potential therapeutic implications beyond its NSAID properties.

2.5.2.3. Safety pharmacology programme

The data provided on safety pharmacology studies are based on that presented for a marketing authorisation application in 1977. All the studies have been conducted prior to the introduction of the OECD's GLP principles.

The safety pharmacology studies were conducted on diflunisal as part of the MPA (Sweden) application dossier for Donobid in 1977. Diflunisal demonstrates a lower propensity for causing gastrointestinal ulcers compared to aspirin (ASA) in rats, requiring higher doses for adverse effects. It exhibits minimal impact on gastric secretion and intestinal propulsion in animal models. Diflunisal did not affect arterial pressure or heart rate in rats and dogs, and did not have any effects on the dog autonomic nervous system. While its potency in inhibiting platelet aggregation is comparable to ASA in guinea-pigs, it shows reduced efficacy in human platelets. Renal effects are observed at certain doses in rats, akin to those seen with ASA. Of note, diflunisal did not induce any behavioural central nervous system effects in mice or squirrel monkeys, nor affected respiration in anaesthetised dogs. Its biochemical activity includes moderate cyclic AMP antagonism and negligible impact on certain physiological processes. Comparative studies with ASA underscore diflunisal's distinct safety profile, particularly in terms of gastrointestinal effects and platelet aggregation inhibition.

The gastrointestinal and renal effects observed in nonclinical studies of diflunisal are representative of NSAIDs, and are clinically well-known for this NSAID. Appropriate warnings are summarised at Sections 4.4 and 4.8 of the SmPC for Attrogy Film-coated tablets 250 mg.

2.5.2.4. Pharmacodynamic drug interactions

The nonclinical pharmacodynamic drug-drug interaction studies on diflunisal, conducted in beagle dogs as part of the MPA (Sweden) application dossier for Donobid in 1977, suggest a favourable safety profile regarding interactions with bishydroxycoumarin (BHC) and tolbutamide. Diflunisal did not significantly affect prothrombin time or glucose tolerance when administered alone or in combination with these drugs in canine models. However, caution is advised based on clinical overview data, which highlight specific contraindications and warnings regarding concomitant use of diflunisal with certain medications, particularly other NSAIDs and ciclosporin, emphasizing the importance of careful clinical monitoring when administering diflunisal in combination therapy.

2.5.3. Pharmacokinetics

The ADME studies on diflunisal, detailed in the MPA (Sweden) *a*pplication dossier for Donobid in 1977, involved experiments in rats, dogs, monkeys and humans.

In some of the pharmacokinetics studies diflunisal ^{14}C-radiolabelled in the carboxylic acid group, was used. Radioactivity in biological samples was measured using liquid scintillation spectrometry. Various assays, including fluorescence and gas chromatography, were employed to analyse diflunisal and its metabolites in plasma and urine. Plasma protein binding was assessed by comparing diflunisal

concentrations before and after ultrafiltration. Additionally, HPLC and TLC were used to analyse diflunisal in cynomolgus monkeys, providing insights into absorption, distribution, metabolism, and excretion. While no validation of the analytical methods was provided, the ADME studies on diflunisal, conducted in rats, dogs, and humans, indicate comprehensive insights into its pharmacokinetics and metabolic pathways across species, contributing valuable data for understanding its behaviour in humans.

Absorption: In rats, diflunisal showed rapid and complete absorption, with peak plasma concentration (C_{max}) observed at 1-hour post-dosing for oral administration. Similarly, in dogs, diflunisal was rapidly absorbed, with C_{max} observed at 1-hour post-dosing for oral administration. Repeat dose toxicity studies in dogs showed dose-related plasma concentrations with no evidence of saturation or induction of metabolism. Cynomolgus monkey studies revealed rapid and well-absorbed diflunisal, with peak plasma radioactivity observed at 1-hour post-dosing. The parent drug accounted for the majority of plasma radioactivity. In humans, diflunisal absorption was also rapid and complete, with C_{max} observed at 2 hours post-dosing for both 50 mg and 500 mg doses. Plasma metabolites were minor, with the majority of plasma radioactivity attributed to the parent drug. The plasma half-life ranged from 5.6 to 9.8 hours. These findings demonstrate consistent pharmacokinetic behaviour of diflunisal across different species, with rapid absorption, plasma peak concentrations within hours of dosing. The results presented do not differentiate the data obtained for both sexes, which will not be a concern at this stage, given the extensive clinical experience with diflunisal.

Distribution: Male Sprague Dawley rats orally received 10 mg/kg ^{14}C-diflunisal. At 1-hour post-dosing, radioactivity was widely distributed, with stomach and blood showing the highest concentrations. After 24 hours, minimal tissue presence was observed, and at 48 hours, only low levels were found in plasma and erythrocytes. Limited placental transfer was observed in pregnant rats. Female rats orally received 10 mg/kg ^{14}C-diflunisal. After 1 hour, milk and plasma concentrations of diflunisal and metabolites were comparable, while higher levels were found in milk at 2- and 4-hours post-dosing. Pregnant rats orally received 10 mg/kg ^{14}C-diflunisal. Plasma concentrations were 38 µg/mL, with low concentrations observed in foetuses, placenta, and amniotic fluid. Pregnant cynomolgus monkeys orally received 20 or 60 mg/kg ^{14}C-diflunisal. At 4 hours post-dose, embryo concentrations ranged from 0.46 to 2.21 µg equivalents/g, representing 0.7-1.1% of maternal plasma concentrations. Diflunisal exhibited high PPB (~99%) in beagle dogs and humans. In vitro studies showed displacement effects for certain drugs. High PPB was also observed in rats, rabbits, and cynomolgus monkeys.

Metabolism: Male Sprague-Dawley rats were orally administered ^{14}C-diflunisal. In rat plasma, at least 70% of the radioactivity was intact diflunisal, while urine contained diflunisal and diflunisal ester glucuronide. In rat kidney microsomes, both acyl and phenolic glucuronides were formed. Biliary excretion of glucuronide conjugates and stable sulphate conjugation were observed. Covalent binding to tissues was noted after high-dose administration. Beagle dogs were orally administered ^{14}C-diflunisal, showing intact diflunisal as the primary component in plasma, and diflunisal glucuronide and a sugar phosphate derivative in urine. Human subjects orally received 50 or 500 mg ^{14}C-diflunisal. Plasma primarily contained intact diflunisal, while urine showed diflunisal and glucuronide conjugates as major metabolites, with a minor hydroxy metabolite also detected. Female cynomolgus monkeys orally received 60 mg/kg ^{14}C-diflunisal. Plasma primarily contained diflunisal, with glucuronide conjugates found in urine, resembling human metabolites.

Excretion: Following single oral or intravenous doses of 10 mg/kg ^{14}C-diflunisal in rats, approximately 50% of the urinary excretion products were conjugated. Diflunisal was rapidly absorbed orally, with biliary excretion contributing to elimination to a lesser extent compared to dogs. Dogs exhibited similar excretion patterns, with radioactivity equally distributed between urine and faeces after oral or intravenous administration of 10 mg/kg ^{14}C-diflunisal. Biliary excretion played a significant role in

elimination. After single oral doses of 50 mg or 500 mg ^{14}C-diflunisal in humans, the majority of excretion occurred via urine, with a small fraction in faeces. Urinary metabolites included ester glucuronide conjugates of diflunisal, representing 70-80% of urinary radioactivity.

Pharmacokinetic interactions: A study conducted on rats investigated the impact of diflunisal on drug metabolizing enzymes by measuring hexobarbital sleeping time. Male and female rats were orally administered 10, 30, or 90 mg/kg diflunisal at various intervals before hexobarbital administration. Results showed that diflunisal induced drug metabolizing enzymes in female rats treated for 4 days, leading to a reduction in hexobarbital sleeping time compared to control female rats. However, this effect was not observed in male rats. These data may be considered superseded by human clinical data generated over many years.

Overall, the pharmacokinetic data presented provide valuable insights into the behaviour of diflunisal across different species, aiding in our understanding of its pharmacological and toxicological dynamics.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

The data provided on single dose toxicity studies are based on that presented for a marketing authorisation application in 1977.

The summarised single dose toxicity studies have included studies with administration of diflunisal orally to mice, rats, rabbits and dogs and by intraperitoneal injection to mice. Studies with administration of diflunisal in combination with other drugs have also been conducted.

Determined LD50 after oral administration ranged from 185 mg/kg (infant rat) up to 826 mg/kg (young adult rat). The LD50 after intraperitoneal administration to mice was 180 mg/kg. Observed clinical signs of toxicity included ataxia, tremors, clonic convulsions, deep and/or slowed respiration, hyperventilation, ptyalism, lacrimation, emesis and diarrhoea.

Data from studies with administration of diflunisal in combination with other drugs - acetylsalicylic acid, indomethacin, bishydroxycoumarin, tolbutamide, hydrochlorothiazide, furosemide, chlorthalidone, ethanol, digitoxin and cyclobenzaprine – do not suggest an increased risk of acute toxicity due to administration of diflunisal in combination with the tested drugs.

2.5.4.2. Repeat dose toxicity

The data provided on repeated dose toxicity studies are based on that presented for a marketing authorisation application in 1977.

Repeated dose toxicity studies have been conducted in mice, rats and dogs with daily oral administration during up to 4, 59 and 58 weeks, respectively. Diflunisal was administered by oral gavage to mice and rats and by oral gavage or gelatine capsules to dogs.

The main targets of toxicity identified were the gastrointestinal tract and the kidneys, namely, with observation of perforations of the small intestine, ulcerative enteritis, gastric ulcers and erosions, and renal papillary oedema. These gastro-intestinal and renal findings were considered to be typical non-steroidal anti-inflammatory drugs (NSAIDs)-class related effects.

2.5.4.3. Genotoxicity

The data provided on genotoxicity are partly based on that presented for a marketing authorisation application in 1977. There are no remarkable findings and, therefore, no specific concern is identified for the proposed indication and posology.

The applicant also provided some information regarding *in vitro* and *in vivo* genotoxicity from the scientific literature as summarised below. Reference is made for this NSAID to the statement in the Dolobid (diflunisal) US Label*: "Diflunisal had no mutagenic activity after oral administration in the dominant lethal assay, in the Ames microbial mutagen test or in the V- 79 Chinese hamster lung cell assay."*

Assessment of this data is therefore not possible, and the only available information is contained in a document from FDA
*(*https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/018445s058lbl.pdf).

Regarding the genotoxicity tests with relevance for this analysis, the applicant quoted two more relevant studies. A full assessment of this data is not possible given that the information is contained in bibliographic references.

In vivo sister chromatid exchange (SCE) and chromosome aberrations studies were performed using six salicylic acid derivatives - including diflunisal - in bone marrow cells of mice. According to Giri *et al*, 1996, diflunisal was administered i.p. and orally by gavage. Diflunisal had increased SCEs and chromosome aberrations. Increased SCEs were observed at 50 and 100 mg/kg i.p., but not at 25 mg/kg i.p., and at 350 mg/kg after oral dosing. Increased chromosome aberrations were observed at 100 mg/kg i.p., but not at 25 or 50 mg/kg i.p., and at 350 mg/kg after oral dosing. Other NSAIDs including ASA have shown positive findings as well as negative results for clastogenicity in the published literature, as discussed by Giri *et al*, 1996.

The GADD45a-GFP (GreenScreen HC) reporter assay in the p53-competent human lymphoblastoid TK6 cell line included four compounds per 96 well microplate and nine dilutions per compound with the upper concentration limited by ICH S2B and in the absence of metabolic activation, as per Hastwell et al, 2009. For diflunisal, the highest concentration tested was 0.16 mM, which was the limit of solubility. Diflunisal was negative for genotoxicity in this assay supporting the overall conclusion of lack of genotoxic potential and carcinogenicity of this NSAID.

No non-clinical summary or tabulated summary was provided by the applicant. Data is scarce and mainly from old datasets. From the data provided, there were no positive findings in either in-vitro or in-vivo tests.

The totality of evidence available suggests that there are no specific genotoxic concerns arising from available data, clinical experience with diflunisal and taking into account the lack of carcinogenic potential observed for diflunisal.

2.5.4.4. Carcinogenicity

The summaries, study methods and results for the mouse and rat carcinogenicity studies are provided in the MPA (Sweden) application dossier for Donobid in 1977.

An 82 week repeat dose oral dietary carcinogenicity study was performed in CD-1 mice (50/sex/treatment group) with dose levels of 10, 20 or 40 mg/kg/day diflunisal, and 2 control groups. There were no adverse clinical and physical signs including subcutaneous or intra- abdominal masses related to diflunisal treatment in any dose group. No deaths were attributed to treatment. Body weight and food consumption were not affected by diflunisal dosing. There were no treatment-related

ophthalmological findings. Histopathology of tissues was performed from all mice in the control groups and the high dose (40 mg/kg/day) group, and all suspected neoplastic and hyperplastic changes in tissues from mice at 10 and 20 mg/kg/day. In addition, sections of the gastrointestinal tract were routinely examined for degenerative or inflammatory changes. Semi-serial sections of both kidneys from all animals in the study were examined for papillary necrosis or oedema. There were no increased neoplastic or hyperplastic findings in diflunisal treatment groups, and no effects of treatment on incidence of renal papillary necrosis or oedema.

A 105 week repeat dose oral dietary carcinogenicity study was performed in Sprague Dawley (Charles River CD) rats (50/sex/treatment group), with dose levels of 10, 20 or 40 mg/kg/day diflunisal, and 2 control groups. There were no adverse clinical and physical signs including subcutaneous or intra-abdominal masses related to diflunisal treatment in any dose group. No deaths were attributed to treatment. Body weight and food consumption were not affected by diflunisal dosing. There were no treatment-related ophthalmological findings. Histopathology of tissues was performed from all rats in the control groups and the high dose (40 mg/kg/day) group, and all suspected neoplastic and hyperplastic changes in tissues from rats at 10 and 20 mg/kg/day. In addition, sections of the gastrointestinal tract were routinely examined for degenerative or inflammatory changes. There were no increased neoplastic or hyperplastic findings in diflunisal treatment groups. The incidence of focal inflammation and ulceration of the small intestine was slightly greater in rats dosed with diflunisal than the control rats, although there was not a dose-response effect.

There are no remarkable findings arising from the available data and despite the old dataset provided, the totality of evidence available – including the absence of genotoxicity potential along with the accumulated clinical experience using diflunisal – are suggestive of the absence of any carcinogenic concern. As stated in the Dolobid (diflunisal) US Label on the FDA website: *"Diflunisal did not affect the type or incidence of neoplasia in a 105-week study in the rat given doses up to 40 mg/kg/day (equivalent to approximately 1.3 times the maximum recommended human dose), or in long-term carcinogenic studies in mice given diflunisal at doses up to 80 mg/kg/day (equivalent to approximately 2.7 times the maximum recommended human dose). It was concluded that there was no carcinogenic potential for DOLOBID."*

2.5.4.5. Reproductive and developmental toxicity

Data on reproductive toxicity studies are based on that presented for a marketing authorisation application in 1977 and respective updates, and the publications by Rowland et al, 1987 and Clark et al 1984. Information on juvenile animal studies is also based on the Dolobid (diflunisal) US Label.

Developmental and reproductive toxicity studies have included studies on male and female fertility and early embryonic development (rat), embryo-foetal development (mouse, rat, rabbit, cynomolgus monkey), pre-post-natal development (rat), and juvenile animals (rat, dog).

Fertility and early embryonic development:

Fertility and early embryonic development studies comprised two separate studies in rats, one on male and the other on female fertility. In both studies, diflunisal was administered orally at a dose level up to 45 mg/kg/day. Males were treated during up to 70 days prior to mating with untreated females. Females were treated from 2 weeks prior to mating with untreated males up to gestation day 14 or parturition.

Effects observed were limited to an increase in the length of the gestation period in females treated with 45 mg/kg/day diflunisal, as observed with other NSAIDs. There were no effects on mating performance, male or female fertility, number of foetuses per litter, resorptions, implants,

teratogenicity or effects in the growth or survival of pups through the 21-day postpartum period of observation.

Embryo-foetal development:

Embryo-foetal development studies comprised studies in mice, rats, rabbits and cynomolgus monkeys, all with administration of diflunisal by the oral route. Pregnant mice, rats and cynomolgus monkeys received doses of diflunisal of up to 45, 45 and 80 mg/kg/day, respectively. In the various studies conducted in rabbits the animals received doses up to 45, or 60 mg/kg/day.

Abortions and teratogenicity observed in rabbits, most commonly axial skeletal defects, were attributed to severe maternal haemolytic anaemia induced following marked reductions in erythrocyte ATP levels in rabbits. The effects were considered to be unique to the rabbit.

No adverse effects on embryo-foetal development were observed in mice, rats or cynomolgus monkeys.

Pre-postnatal development:

A pre- postnatal development study was conducted in rats with oral administration of diflunisal from gestation day 15 up to postpartum day 21 at doses up 45 mg/kg/day.

At the maximum tested dose, the study revealed an increase in the length of the gestation period and, considered to be possibly related to this, an increase in the number of dead pups on the first postpartum day.

Juvenile animal studies:

Available data from the marketing application dossier for Donobid submitted to the Swedish Medical Products Agency (MPA) in 1977 and the Dolobid (diflunisal) US Label suggests that diflunisal is more toxic towards juvenile than adult animals. This is based on studies with single dose administration to rats and repeated dose administration to dogs.

2.5.4.6. Toxicokinetic data

The applicant provided an old non-clinical data package. Factual toxicokinetic data are limited to C_{max} and t_{max} values for the 14-week repeated dose toxicity study in dogs (Study #72-007-0). In this study, C_{max} values ranged from 61 up to 244 µg/mL, at Day 1, and from 65 up to 242 µg/mL, at Week 13.

For all repeated dose toxicity, an *in vivo* genotoxicity study, carcinogenicity and reproductive toxicity studies, the applicant has provided information on the tested doses, calculated the respective human equivalent doses, identified NOAEL and determined margins of exposure. For the repeated dose toxicity studies, the margins of exposure were calculated based on the animal dose; for the other studies, based on human equivalent doses. The applicant justified for the methodology followed to calculate the margins of exposure for the repeated dose toxicity studies based on the nature of the adverse effects observed in these studies, i.e., gastrointestinal toxicity.

2.5.4.7. Local Tolerance

Ocular and dermal irritation studies (TT #73-3632 and TT #71-3585) of diflunisal in rabbit have shown that the dry powder of diflunisal was severely irritating to the rabbit eye, and 5% and 10% suspensions of diflunisal showed very low irritancy and moderate irritancy, respectively, in the rabbit eye. The dry powder of diflunisal was non-irritating to intact and abraded skin sites of the rabbit.

The medicinal product is to be administered orally. Local tolerance for the oral route of administration has been investigated in different nonclinical studies with diflunisal. The main targets of toxicity identified in the repeated dose toxicity studies were the gastrointestinal tract and the kidneys, namely, with observation of perforations of the small intestine, ulcerative enteritis, gastric ulcers and erosions, and renal papillary oedema. These effects were considered to be typical NSAID class related effects. Local tolerance effects of diflunisal have been well-established in humans for 250 mg bid diflunisal as well as for higher doses of diflunisal, as summarised in the clinical overview and clinical module provided by the applicant and from the point of view of local tolerance, in terms of local irritancy at the site of administration/absorption, besides the expected reactions for NSAIDs, no specific concern arises from the available data.

2.5.4.8. Other toxicity studies

When considering the available data on other toxicity studies (including immunotoxicity, dependence and abuse potential, metabolites, phototoxicity, impurities and excipients, no specific toxicologic concern has been identified. In most cases, available clinical evidence supersedes non-clinical data. The SmPC adequately reflects the available evidence in this regard.

2.5.5. Ecotoxicity/environmental risk assessment

The Environmental Risk Assessment (ERA) provided by the applicant is in accordance with the *Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use* (EMEA/CHMP/SWP/4447/00, June 2006) and the Questions and Answers on *Guideline on the environmental risk assessment of medicinal products for human use'* document (EMA/CHMP/SWP/44609/2010 Rev. 1, 2016).

An ERA Phase I was conducted to consider the risk to the environment arising from the use of indicated for the treatment of adult patients with transthyretin amyloid amyloidosis with polyneuropathy.

Relevant endpoints, methods used, and results obtained were discussed and study results are summarised in table 1.

Table 1: Summary of main study results

Substance (INN/Invented Name): Diflunisal			
CAS-number (if available): 22494-42-4			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD123	**pH 5=2.12** **pH7 =0.57** **pH 9 =0.06**	Potential PBT (**N**
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	**No**	**not B**
	BCF		B/not B
Persistence	DT50 or ready biodegradability		P/not P
Toxicity	NOEC or CMR		T/not T
PBT-statement:	The compound is not considered as PBT nor vPvB		

Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$ refined	0.0029	μg/L	< 0.01 threshold **N**
Other concerns (e.g. chemical class)			**N**

2.5.6. Discussion on non-clinical aspects

As the presented non-clinical development of diflunisal was originally performed for the Swedish marketing authorisation of "Donobid" in the late 1970s, it is noticed that the non-clinical studies presented in Module 4 do not comply with current ICH, EMA and GLP regulations. However, the non-clinical dossier does not fulfil current CTD and eCTD standards of ICH M2 and M4 guidelines, although a complete module 2.4 nonclinical overview was provided addressing all pharmacology, PK and toxicology headings/endpoints. Instead, non-clinical investigations in Module 4 have been solely provided as "Non-eCTD electronic Submission" (NeeS).

The applicant did not conduct any new non-clinical studies. This MAA is based mainly on studies submitted to the Swedish Medical Products Agency (MPA) as a dossier for Donobid from 1977 and further documentation submitted to MPA after 1977. Moreover, relevant nonclinical information from published literature have been provided by the applicant.

Diflunisal's primary pharmacodynamic action results from its ability to stabilise transthyretin (TTR), distinct from traditional NSAIDs. Transthyretin exists as a stable homotetramer in plasma, crucial for preventing amyloid formation. While thyroxine (T4) stabilises TTR tetramers, its therapeutic use is limited due to safety concerns. Diflunisal, mimicking T4's binding mode, exhibits appropriate stereochemistry to bind and stabilise TTR tetramers. Although a preclinical animal model was not available, human trials indicate that a dose of 250 mg twice daily achieves sufficient serum concentrations to stabilise TTR tetramers, effectively inhibiting amyloidogenesis.

The active substance diflunisal, strength, pharmaceutical form and route of administration are the same for Attrogy Film-coated tablets 250 mg as for Donobid Film-coated tablets containing 250 mg diflunisal, although the indication and posology are different.

However, of note, diflunisal has well-documented clinical experience at daily doses higher than currently proposed for this new indication (up to 1500 mg per day for NSAID pain and anti-inflammatory indications). Therefore, from the NC perspective, reference to the available literature data and results of studies conducted to support other indications is considered acceptable taking into account the chosen legal basis for the dossier.

Moreover, no new safe concerns have been identified for the proposed new indication in clinical study in the treatment of transthyretin amyloid (ATTR) amyloidosis in adults with polyneuropathy.

Pharmacokinetics

In the *in vivo* conducted NC studies in rats, dogs and cynomolgus monkeys, diflunisal was completely and rapidly absorbed. After a single oral dose of 10 mg/kg diflunisal, the maximum plasma concentration (C_{max}) was observed at 1-hour post-dosing. From the studies conducted in rats it appears that diflunisal is widely distributed with the highest concentrations achieved in the stomach and blood. Diflunisal binds in ~99% to plasma proteins. Studies conducted in rats showed that in plasma, at least 70% of the radioactivity was intact diflunisal. In urine, both diflunisal and the diflunisal ester (acyl) glucuronide were identified. Metabolism of diflunisal was also characterised in the literature, including metabolism in humans. In cynomolgus monkeys, about a quarter was detected in

urine as diflunisal and three quarters present as diflunisal conjugates. In rat and dogs, diflunisal was excreted in urine and faeces. In humans, excretion was mainly via the urine. The available non-clinical data are limited. However, there are data regarding PK interactions in humans. Therefore, no additional PK NC studies are required.

Single dose toxicity

Based on the LD_{50} values, data on single dose toxicity studies with diflunisal do not indicate a risk of mortality at the intended therapeutic human dose (250 mg twice a day). Further information regarding toxic dose levels or doses without effect is not considered needed taking into account data from repeated dose toxicity studies and, most importantly, clinical experience with diflunisal.

Repeated dose toxicity

Repeated dose toxicity studies have been conducted in mice, rats and dogs during up to 4, 59 and 58 weeks, respectively.

In a 4-week repeat dose pilot study in mice, no signs of toxicity were observed at any of the dose levels tested (up to 40 mg/kg/day). In a 14-week repeat dose study in rats, white blood counts increase was observed after administration of 100 mg/kg/day doses. In a 13-week repeat dose oral gavage toxicity study in rats, no clinical signs, haematology, clinical chemistry or ophthalmological findings related to diflunisal were reported. In a 59-week repeat dose oral gavage toxicity study in rats dose levels of 10, 20 and 40 mg/kg/day diflunisal were evaluated. No clinical signs, body weight effects, haematology or ophthalmological findings related to diflunisal were observed. However, after 59 weeks, 2 rats at 40 mg/kg/day had gastro-intestinal ulcers. In a 58-week repeat dose oral capsule toxicity study in beagle dogs, the dose levels were 10, 20 or 40 mg/kg/day diflunisal. No drug-related food consumption, body weight, haematological, clinical chemistry, urinalysis or ophthalmological effects were reported. The NOAEL in the chronic toxicity studies in rats and dogs were set at 20 and 10 mg/kg/day, respectively.

Comparison of the doses without effect in animals *versus* the intended human therapeutic dose, in terms of weight per body weight and human equivalent doses, suggests that the gastro-intestinal effects observed in animals may represent a risk to the patients. The proposed SmPC includes in its sections 4.4, warnings regarding gastro-intestinal effects and risk of reduced kidney function, based on clinical experience.

Genotoxicity

The data provided on genotoxicity are partly based on that presented for a marketing authorisation application in 1977. Notwithstanding here are no remarkable findings and, therefore, no specific concern is identified for the proposed indication and posology.

The applicant provided scarce information regarding *in vitro* genotoxicity. The only reference made is the statement in the Dolobid (diflunisal) US Label*: "Diflunisal had no mutagenic activity after oral administration in the dominant lethal assay, in the Ames microbial mutagen test or in the V- 79 Chinese hamster lung cell assay."*

Assessment of this data is therefore not possible, and the only available information is contained in a document from FDA (https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/018445s058lbl.pdf).

Regarding the genotoxicity tests with relevance for this analysis, the applicant quoted two more relevant studies. A full assessment of this data is not possible given that the information is only contained in bibliographic references.

In vivo sister chromatid exchange (SCE) and chromosome aberrations studies were performed using six salicylic acid derivatives - including diflunisal - in bone marrow cells of mice. According to Giri *et al*, 1996, diflunisal was administered i.p. and orally by gavage. Diflunisal had increased SCEs and chromosome aberrations. Increased SCEs were observed at 50 and 100 mg/kg i.p., but not at 25 mg/kg i.p., and at 350 mg/kg after oral dosing. Increased chromosome aberrations were observed at 100 mg/kg i.p., but not at 25 or 50 mg/kg i.p., and at 350 mg/kg after oral dosing. Other NSAIDs including ASA have shown positive findings as well as negative results for clastogenicity in the published literature, as discussed by Giri *et al*, 1996.

The GADD45a-GFP (GreenScreen HC) reporter assay in the p53-competent human lymphoblastoid TK6 cell line included four compounds per 96 well microplate and nine dilutions per compound with the upper concentration limited by ICH S2B and in the absence of metabolic activation, as per Hastwell et al, 2009. For diflunisal, the highest concentration tested was 0.16 mM, which was the limit of solubility. Diflunisal was negative for genotoxicity in this assay supporting the overall conclusion of lack of genotoxic potential and carcinogenicity of this NSAID.

The totality of evidence available suggests that there are no specific genotoxic concerns arising from available data, clinical experience with diflunisal and with reference to the lack of carcinogenic potential of diflunisal.

Carcinogenicity

The summaries, study methods and results for the mouse and rat carcinogenicity studies are provided in the MPA (Sweden) Application dossier for Donobid in 1977.

An 82 week repeat dose oral dietary carcinogenicity study was performed in CD-1 mice (50/sex/treatment group) with dose levels of 10, 20 or 40 mg/kg/day diflunisal, and 2 control groups. There were no adverse clinical and physical signs including subcutaneous or intra- abdominal masses related to diflunisal treatment in any dose group. No deaths were attributed to treatment. Body weight and food consumption were not affected by diflunisal dosing. There were no treatment-related ophthalmological findings. Histopathology of tissues was performed from all mice in the control groups and the high dose (40 mg/kg/day) group, and all suspected neoplastic and hyperplastic changes in tissues from mice at 10 and 20 mg/kg/day. In addition, sections of the gastrointestinal tract were routinely examined for degenerative or inflammatory changes. Semi-serial sections of both kidneys from all animals in the study were examined for papillary necrosis or oedema. There were no increased neoplastic or hyperplastic findings in diflunisal treatment groups, and no effects of treatment on incidence of renal papillary necrosis or oedema.

A 105 week repeat dose oral dietary carcinogenicity study was performed in Sprague Dawley (Charles River CD) rats (50/sex/treatment group), with dose levels of 10, 20 or 40 mg/kg/day diflunisal, and 2 control groups. There were no adverse clinical and physical signs including subcutaneous or intra-abdominal masses related to diflunisal treatment in any dose group. No deaths were attributed to treatment. Body weight and food consumption were not affected by diflunisal dosing. There were no treatment-related ophthalmological findings. Histopathology of tissues was performed from all rats in the control groups and the high dose (40 mg/kg/day) group, and all suspected neoplastic and hyperplastic changes in tissues from rats at 10 and 20 mg/kg/day. In addition, sections of the gastrointestinal tract were routinely examined for degenerative or inflammatory changes. There were no increased neoplastic or hyperplastic findings in diflunisal treatment groups. The incidence of focal inflammation and ulceration of the small intestine was slightly greater in rats dosed with diflunisal than the control rats, although there was not a dose-response effect.

There are no remarkable findings arising from the available data and despite the old dataset provided, the totality of evidence available – including the absence of genotoxicity potential along with the

accumulated clinical experience using diflunisal – are suggestive of the absence of any carcinogenic concern. As stated in the Dolobid (diflunisal) US Label on the FDA website: *"Diflunisal did not affect the type or incidence of neoplasia in a 105-week study in the rat given doses up to 40 mg/kg/day (equivalent to approximately 1.3 times the maximum recommended human dose), or in long-term carcinogenic studies in mice given diflunisal at doses up to 80 mg/kg/day (equivalent to approximately 2.7 times the maximum recommended human dose). It was concluded that there was no carcinogenic potential for DOLOBID."*

Reproductive toxicity

Comparison of doses, in terms of weight per body weight and human equivalent doses, suggests that an increase in the length of the gestation period and in the number of dead pups observed in animals may represent a risk to the patients.

No adverse effects on embryo-foetal development were observed in mice, rats or cynomolgus monkeys. However, comparison of the dose levels tested in animals versus the intended therapeutic dose in humans – on the basis of weight per body weight and human equivalent doses - suggests low to null margins of exposures at the maximum tested dose in these animals.

Regarding information on fertility, embryo-foetal and pre-postnatal development in the proposed SmPC, no specific non-clinical data is included in section 4.6 of the SmPC. The proposed content of this section is based on the already available human experience with NSAIDs, which is considered acceptable.

Concerning results from juvenile animal studies, it is noted that, according to the proposed SmPC, the medicinal product is indicated for treatment in adults and its use "in the paediatric population is not recommended". Nevertheless, taking into account the limited data that suggests that diflunisal may be more toxic towards juvenile animals, information on juvenile animal studies has been adequately included in section 5.3 of the SmPC.

Other toxicity studies

When considering the available data on other toxicity studies (including immunotoxicity, dependence and abuse potential, metabolites, phototoxicity, impurities and excipients, no specific toxicologic concern has been identified. In most cases, available clinical evidence supersedes non-clinical data. The SmPC adequately reflects the available evidence in this regard.

Environmental Risk Assessment

In Phase I the PEC calculation is restricted to the aquatic compartment. PECSurfacewater was determined based on the refined Fpen (0.00001179) that resulted in a PECSurfacewater value of 0.0029 µg/L, far below the action limit of 0.01 µg/L. For refined Fpen, the highest prevalence (worst-case) value of transthyretin amyloidosis with polyneuropathy in the 20 EU countries was used, in line with the CHMP Q&A document of the Guideline EMA/CHMP/SWP/44609/2010 Rev. 1, 2016.

The OECD 123 Partition Coefficient (1-Octanol/Water): Slow-Stirring Method study was conducted according to Good Laboratory Practice (GLP) standards. The ion-corrected log Dow values for diflunisal at pH levels 5, 7, and 9 are recorded as 2.12, 0.57, and 0.06, respectively. Therefore, a screening for PBT of diflunisal will not be required.

It is concluded that the medicinal product is unlikely to represent a risk to the environment following its prescribed usage in patients.

The precautionary and safety measures taken, including the general statement on the SmPC and PL, to reduce any risk to the environment have been applied.

2.5.7. Conclusion on the non-clinical aspects

Given the existing clinical experience from long-term diflunisal therapy of human patients with higher doses of formerly authorised "Donobid" in Sweden or of the drug "Dolobid" in the USA, the general concerns regarding deficiencies in the non-clinical dossier are meanwhile outweighed, considering also that diflunisal is not genotoxic or carcinogenic and findings observed in the toxicology programme were apparently confined to those established for the pharmaceutical class of non-steroidal anti-inflammatory drugs (NSAIDs).

The applicant has not performed any new non-clinical studies with diflunisal, and no such studies are planned. It may be considered from a nonclinical perspective that Attrogy Film-coated tablets 250 mg has a positive benefit/risk balance for the treatment of transthyretin amyloid (ATTR) amyloidosis in adults with polyneuropathy.

The ERA report provided meets the ERA Guidelines and criteria, ensuring that diflunisal is unlikely to represent a risk to the environment following its prescribed usage in patients. The applicant provided the OECD 123 study report within the Day 180 responses for the PBT/vPvB screening assessment, confirming a lack of PBT/vPvB potential such that a definitive assessment was not required.

Considering the above data, Attrogy 250 mg film-coated tablets is not expected to pose a risk to the environment.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trial was performed in accordance with GCP as claimed by the applicant.

Tabular overview of clinical studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
H-23750	08 May 2006. Completed. 130/140. (8 centres in Italy, Japan, Sweden, UK, USA)	Double-blind. Placebo-controlled.	Diflunisal 250 mg orally twice daily. Matching placebo orally twice daily.	Biopsy-proven amyloid deposition. Signs of peripheral or autonomic neuropathy detectable by a neurologist.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Absorption

The oral absorption of diflunisal, when administered as capsules, seems to be fast with a tmax of around 1-4h. after the administration of 250 mg of diflunisal, a Cmax of around 30 mg/L was observed. Cmax is around 65 mg/L when the dose is 500 mg. Diflunisal may be considered a BCS class II drug.

Distribution

Diflunisal, as other NSAID, shows a very high (>99%) plasma protein binding that does not seem to saturate up to concentrations of 800 mg/L, much higher than the ones seen in vivo. The volume of distribution, although not calculated in the provided reports, based on the observed concentration values and some AUC values provided should be around 10 – 20L.

Elimination

The mass balance study after oral administration showed that most of the radioactivity was eliminated via the urine, with only a small proportion (<4%) excreted in the faeces. In urine, diflunisal Glucuronide conjugates accounted for ~95% of total urinary excretion and unchanged diflunisal accounted for ~5%.

Dose proportionality and time dependencies

The plasma disappearance rate appeared to be inversely related to dosage (the higher the plasma concentration, the slower the removal).

Special populations

The applicant only provided data for renal impaired subjects. Renal impairment increases the exposure and delays the elimination of diflunisal.

Pharmacokinetic interaction studies

The applicant presented several *in vivo* studies evaluating the DDI potential of diflunisal. These have shown that, at least indomethacin, hydrochlorothiazide and acenocoumarol exposures are modified by the co-administration with diflunisal.

Pharmacokinetics using human biomaterials

The applicant did not present any *in vitro* data on the DDI potential of diflunisal.

2.6.2.2. Pharmacodynamics

Mechanism of action

Diflunisal is a difluorophenyl derivate of salicylic acid. The primary pharmacodynamics of diflunisal relevant to the proposed indication is its ability to stabilise transthyretin (TTR) (separate and different from its traditional NSAID pharmacology).

Transthyretin exists in plasma as a noncovalent, homotetramer (a dimer of dimers) presenting two identical binding sites located in a channel formed by the dimer-dimer interface and crossing the

protein molecule; in this form the molecule is stable. The formation of amyloid is dependent on the dissociation of natively folded TTR tetramers into monomers.

Binding of thyroxine (T4) to TTR stabilises the tetramer but the use of the T4 hormone and its analogues in a therapeutic role is precluded by safety concerns. Attempts have therefore been made to identify other small molecules that exhibit the appropriate stereochemistry to bind to TTR.

Diflunisal exhibits the appropriate stereochemistry and studies have shown that diflunisal can bind to and stabilise TTR in its tetramer form (Sekijima et al, 2006; Tojo et al, 2006) hence preventing the dissociation to monomers. Like T4, diflunisal binds to what is referred to as the forward binding mode, where anionic substituents like carboxylate are positioned in the outer binding pocket engaging in electrostatic interaction with the Lys15 e-ammonium groups. A common pharmacophore among small molecule stabilisers of the T4 hormone binding pocket of TTR tetramer is a carboxylic acid connected through a rigid spacer to an aromatic moiety.

Primary and Secondary pharmacology

The applicant states that, although a preclinical animal model was not available, the primary pharmacodynamics of diflunisal in the treatment of ATTR amyloidosis has been demonstrated in human volunteers in the publication by Sekijima et al, 2006. In the 250 mg bid group, 12 hours after the 13th oral dose, the diflunisal serum concentration of 146 ± 39 μM was sufficient to afford a TTR binding stoichiometry exceeding 0.95 ± 0.13 (≈1.75 corrected). Diflunisal binding to TTR at this dose slowed urea-mediated dissociation and acid-mediated TTR aggregation, at least three-fold ($p<0.05$) in serum and *in vitro*, consistent with kinetic stabilisation of TTR. In summary, the authors conclude that diflunisal administered at a dose of 250 mg twice a day is sufficient to induce kinetic stabilisation on the tetrameric native state of TTR, preventing its dissociation required for amyloidogenesis in human serum.

In the study "A Comparison of the Effect of MK-647 and Aspirin on Fecal Blood Loss in Normal Volunteers", diflunisal was administered at the dose of 250 mg b.i.d. for two seven-day periods separated by a one week control period. Aspirin was administered at 750 mg q.i.d. and followed the same schedule.

MK-647 (250 mg b.i.d.) during two periods caused mean blood losses of 1.57 and 2.66 ml/day. Aspirin (750 mg q.i.d.) under comparable conditions caused blood losses of 34.33 and 14.7 ml/day.

No clinically significant drug related adverse reactions were reported for subjects receiving MK-647. One subject receiving aspirin developed gastric ulcer symptoms during the second treatment period. This reaction was considered probably drug related.

The applicant concluded that, at the doses employed in this study, MK-647 exhibited significantly less faecal blood loss than aspirin.

To further study the effect of diflunisal on platelets, the study entitled "A double-blind study to compare the effects of MK-647, indomethacin and placebo on platelet function following acute and chronic administration to normal male volunteers" was performed.

This was a randomised, double-blind, parallel clinical pharmacology study in which thirty subjects entered and completed the study (10 on each treatment).

All subjects received a single dose of medication (placebo, 250 mg MK-647, or 50 mg indomethacin) on day 1. During days 15 through 21, each subject received medication on a t.i.d. schedule as follows:

Group	Daily Doses		
	1st	2nd	3rd
Placebo	Placebo	Placebo	Placebo
MK-647	250 mg	Placebo	250 mg
Indomethacin	50 mg	50 mg	50 mg

After single or repeated doses, MK-647 caused no changes in the platelet function and blood coagulation parameters monitored. With the single or repeated doses studied, indomethacin appeared capable of altering bleeding time, and collagen induced platelet aggregation. No changes in the platelet function and blood coagulation parameters monitored were found in the placebo group. MK-647 had no effect on fasting blood glucose. No adverse experience was reported for the group receiving diflunisal.

The applicant concluded that MK-647 in a dosage regimen of 250 mg b.i.d. does not alter blood coagulation or platelet function. Indomethacin in single doses of 50 mg or in a dosage regimen of 50 mg t.i.d. prolongs bleeding time and alters collagen induced platelet aggregation.

An open study to investigate effects of diflunisal on platelet function and fasting blood glucose was performed, following a single 500 mg dose administration and after chronic administration of 500 mg b.i.d. in normal male volunteers. Five healthy male volunteers entered and completed the study. One additional subject entered and completed the multiple dose treatment part of the study. Following control observations, subjects were given a 500 mg oral dose of diflunisal and observed for seven days.

Multiple doses (500 mg b.i.d.) of diflunisal were then administered for seven days and the subjects observed for 12 days. Platelet function and other tests were carried out at designated times.

Single and multiple doses of diflunisal produced no clinically meaningful changes in blood platelet function. No drug related adverse reactions or abnormal laboratory values were reported.

The applicant concluded that, under the conditions of the study, diflunisal did not affect platelet function.

Given that there is no dedicated study on a potential dose-response relationship of diflunisal in the prolongation of QT interval and that ATTR can have a cardiomyopathy variant that can lead to electrophysiological changes the applicant has included in section 4.4 a reference regarding treatment of patients with prolonged QT interval along with the other proposed cardiac conditions.

On the non-clinical package provided, no information on the effect of diflunisal in the hERG channel was provided. In a 14-week repeat dose oral gavage toxicity study in beagle dogs (2/sex/dose and control group), the dose levels were 12.5-100 mg/kg/day for diflunisal and 25-200 mg/kg/day for the comparator, aspirin. It was reported that there were no adverse ECG findings for diflunisal treated animals.

A 58-week repeat dose oral capsule toxicity study (TT #73-005-0) in beagle dogs, diflunisal reported that there were no adverse ECG findings for diflunisal treated animals at dose levels of 10, 20 or 40 mg/kg/day.

On the safety report narrative presented by the applicant, several patients were reported to have arrhythmic events (ventricular tachycardias [1 subject], bundle branch block [1 subject], complete heart block [2 subjects], atrial flutter [1 subject].

2.6.3. Discussion on clinical pharmacology

Pharmacokinetics

Diflunisal was previously authorised in several EU countries for traditional NSAID indications. For example, the Swedish authorisation for Donobid was granted in 1979. However, it has never been authorised for the treatment of ATTR amyloidosis (in Europe or elsewhere). All brands of diflunisal have now been withdrawn from all EU markets for commercial reasons (not because of safety concerns). In the US, the initiator (Dolobid) was also withdrawn, however, generic presentations are still available.

In practice, diflunisal has been used for the treatment of ATTR amyloidosis for many (>10) years in a number of EU and non-EU countries despite never having been authorised in this indication. This usage continues today despite the absence of an authorised product. For example, in Sweden, diflunisal is available as an extemporaneous formulation and in the Netherlands, it is available as an unlicenced medicine (Dolaced).

Knowledge of the clinical pharmacology of diflunisal is derived from the work conducted by Merck Sharp & Dohme and included in the original marketing authorisation application dossier, to which the current applicant has access. The current applicant was, of course, not involved in this work and the details below are taken from the original documents.

Methods

It is clear that several analytical methods were available for use during the development of diflunisal. The exact method used is known for four of the Merck clinical pharmacology studies, but not for the remainder. All four methods included acidification and incubation steps which are likely to have ensured hydrolysis of any diflunisal sulphate. Information on the validation of the four known methods is limited and there is no reasonable possibility of further researching this. There is, however, evidence that linearity, reproducibility, sensitivity and risk of interference from other molecules were all taken into account. It is therefore considered highly likely that the resulting PK characterisation data are reliable.

Due to the date of the presented studies (all from the last century 70's) the PK data was evaluated based on non-compartmental analysis. This is, generally, acceptable. Only basic statistical analysis was provided.

Absorption

The oral absorption of diflunisal, when administered as capsules, seems to be fast with a t_{max} of around 1-4h. after the administration of 250 mg of diflunisal, a C_{max} of around 30 mg/L was observed. C_{max} is around 65 mg/L when the dose is 500 mg.

No IV administration study was provided and, thus, the absolute bioavailability was not determined. However, according to the mass balance study, after the oral administration of 50 mg or 500 mg as capsules, more than 90% of the dose was excreted in the urine, indicating a high permeability.

Diflunisal is an acid with a pKa of around 3, Practically insoluble at neutral or acidic pH. The highest dose solubility volume is > 250 mL. Therefore, diflunisal is considered a low solubility drug according to the Biopharmaceutics Classification System (BCS). As such, it may be considered a BCS class II drug.

The current application considers a new formulation of diflunisal and the applicant provided a bridge between its formulation and the one used in the pivotal clinical trial, supported by formulation

similarities, *in vitro* and *in vivo* data. As such, the extrapolation of the clinical pharmacological studies and, most relevant, the clinical safety and efficacy conclusion to the current formulation are accepted.

The oral administration of 250 mg of diflunisal in capsules with food does not seem to change significantly the PK of the drug. Although a slight delay in absorption and a small reduction in C_{max} was observed, the exposure does not seem to be significantly changed. In addition, the applicant provided published data from 1981 showing that food (in a high caloric meal) had little effect on the absorption of diflunisal.

Distribution

Diflunisal, as other NSAID, shows a very high (>99%) plasma protein binding that does not seem to saturate up to concentrations of 800 mg/L, much higher than the ones seen *in vivo*. The volume of distribution, although not calculated in the provided reports, based on the observed concentration values and some AUC values provided should be around 10 – 20L.

Elimination

The mass balance study after oral administration showed that most of the radioactivity was eliminated by the urine, with only a small proportion (<4%) excreted in the faeces. Of relevance, the elimination appears to be faster for lower doses, showing some non-linearity. Two metabolites were identified accounting for around 90% of the urine radioactivity, and diflunisal for the remaining 4-5%. The two metabolites were identified as glucuronide conjugates. An updated discussion on the elimination pathway of diflunisal was provided, referring that there are three diflunisal phase II conjugates metabolites, diflunisal acyl glucuronide, diflunisal phenolic glucuronide and diflunisal sulfate, that are excreted in the urine, along with smaller amounts of the parent, diflunisal. The elimination of diflunisal seems not to be capacity limited at the therapeutic dose of 250 mg BID. An acceptable updated version of part 5.2 Metabolism and elimination was provided.

The data provided in the mass balance study shows that the majority of the plasma radioactivity is related to diflunisal, although some latter papers were able to determine the 3 major metabolites in quantifiable concentrations in plasma. These works also confirmed that the majority of the circulating species is diflunisal itself. The applicant argues that two glucuronidation conjugation pathways and the sulfation conjugation pathway together provide an overall elimination pathway with capacity to compensate for inter-individual, including pharmacogenetic, enzymatic differences in patients treated at 250 mg twice daily. That seems indeed to be supported by a non-capacity limited elimination up to doses of 250 mg BID. Also, although there seems to exist sex-differences in the elimination of drugs undergoing glucuronide conjugation, small diflunisal PK differences were seen between male and females, were males have faster CL but similar elimination t1/2 of diflunisal.

The applicant did not discuss the intra- and inter-individual variability for diflunisal. However, according to the literature and based on published BE trials in healthy subjects, it should be low. Patel et al, 2012 (doi:10.1093/chromsci/bms181), reported an intra-individual variability of around 11% and 5% for C_{max} and AUCinf, respectively. The inter-individual variabilities for the same parameters were of around 15% and 21%.

Dose proportionality and time dependency

The provided ascending dose study showed that the elimination half-life of diflunisal increases with increasing doses, indicating the existence of a non-linearity in its PK. This is also confirmed by the more than linear increase in the AUC, based on the provided plots (again, even pages of the report were not provided and only partial information is available). Although the study only included 3 subjects, this finding was observed in other studies with different doses. This may be due to a capacity limited glucuronidation at higher doses where, typically, an increased elimination $t_{1/2}$ is observed.

There is also a non-linear plasma protein binding at higher doses but the effect of this in the elimination of the drug is not clear.

On multiple dose administrations, the increase in the elimination half-life with the increased doses was also observed, confirming the non-linearity observed in the single ascending dose study. Based on the stability of the $C_{through}$ concentrations over time after steady-state being observed, this non-linearity does not seem to be time-dependent.

Pharmacokinetics in the target population

The applicant did not perform any PK study in the intended population nor discussed if there is any expected difference to the healthy subjects and the previously considered patient populations. In this regard, the applicant discussed a recent publication from Tsai et al (2023) where plasma concentration data from participants in study H-23750 (the pivotal trial for this MAA) following long-term dosing at 250 mg twice daily were quantified. Reading that publication, is not clear at what time the blood sampling was done, thus is seams that it was at steady state but at any visiting time between two administrations. So, the values may vary from either close to C_{max} or C_{min} in a random way. In fact, the observed values presented a mean plasma concentration of 68.8 µg/ml, but grossly ranging from 19 µg/ml to 175 µg/ml. In the original Merck dossier, a PK study with 250 mg diflunisal twice daily for 7 days resulted in a C_{min} steady state concentration of 38 µg/ml. However, concentrations were as low as 26 µg/ml and as high as 77 µg/ml (first collection time was at 4h, when the C_{max} is expected to be at 1-2h). Based on the average profile, a $C_{average}$ of 47 µg/ml is observed with this data. Similarity in the two populations is further supported by some published results were a $C_{average}$ of 69 µg/ml was observed after diflunisal 250 mg BID to healthy volunteers (DOI: 10.1111/j.1365-2125.1990.tb03654.x).

Special populations

The applicant provided a study showing that renal impairment increases the exposure and delays the elimination of diflunisal. In the SmPC, this is said to be due to the fact that diflunisal is mainly eliminated by the kidneys. This is obviously incorrect as the amount of diflunisal eliminated unchanged in the urine is less than 5% of the dose. In any case, the studies initially presented have shown that, even for moderate insufficiency (defined as a Clcr of 10 to 50 mL/min), a significant delay in elimination was observed, with an elimination $t_{1/2}$ of 22h, compared to the usual value of 10h for healthy subjects. A published paper (DOI: 10.1002/phar.1983.3.2p2.9) related the elimination $t_{1/2}$ with CLcr with the following results showing a significant increase in the elimination half-life for CLcr < 30 mL/min. Based on the same, the proposal for contra-indication for the use of diflunisal in patients with CLcr < 30 ml/min is acceptable.

There is scarce information about the relevance of hepatic impairment of the PK of diflunisal. Cirrhosis does not seem to influence the plasma clearance of total (bound + unbound) diflunisal although changing significantly the unbound fraction of diflunisal and significantly impairing the plasma clearance of unbound diflunisal. In this regard, the contraindication of use of diflunisal in Severe hepatic impairment seems appropriate. No specific studies focusing on the effect of weight, gender and ethnicity on diflunisal PK were performed during the Merck development period. Only a later study focusing on the gender effect is available in the literature. As discussed before, although there seems to exist sex-differences in the elimination of drugs undergoing glucuronide conjugation, only small diflunisal PK differences were seen between male and females, where males have faster CL but similar elimination $t_{1/2}$ of diflunisal. So, this does not support any relevant dose change due to gender. Regarding weight and ethnicity, no data are available. The applicant argues, however, that in the phase III trial, patients with a range of weights from 39 to 119 kg were included and no dose adjustment was considered needed. Regarding ethnicity, although most of the patients were Caucasians, no major differences were seen in the other ethnicities included. Also, no important ethnic

PK implication have emerged during the long period in which diflunisal was widely used in its NSAID indications.

Overall, it is agreed that the balance of the available evidence suggests that weight, gender and ethnicity do not have PK implications sufficient to warrant specific SmPC advice and/or dose adjustment.

No specific studies focusing on the effect of age on diflunisal PK were performed during the Merck development period. Again, only a later published study focusing on the investigation of the PK of a single 500 mg dose of diflunisal in young adults and healthy elderly subjects was provided. In this study, with a small number of subjects (8 vs 7), no statistically significant differences were observed in the overall PK parameters that were compared (except marginally in Vd, with a p=0.05). Based on this, it can be accepted that age, by itself, is not a relevant factor in diflunisal PK.

Diflunisal is not proposed to be used in the paediatric population. A waiver was accepted by the Paediatric Committee on the grounds that the specific medicinal product does not represent a significant therapeutic benefit over existing treatments.

Pharmacokinetic interactions studies

The applicant did not present any data nor discussion on the *in vitro* potential for DDI both at the metabolic or drug transport level, either as perpetrator or victim level. This is a major limitation on the interpretation of the several known interactions observed *in vivo* for diflunisal. For example, it was reported that the plasma concentration of indomethacin was increased with concomitant oral dosages of diflunisal in humans and it is known that both indomethacin and diflunisal are glucuronidated in humans. It was shown in the literature that, indeed, diflunisal inhibited the indomethacin glucuronidation in HLM with IC_{50} values ranging from 100 to 231 microM. In HIM, inhibition of the indomethacin glucuronidation by diflunisal was even more potent with IC50 values of 15.2-48.7 microM. Also, diflunisal was shown to inhibit the Organic anion transporter 1, for example, being this a possible explanation for the known effect of diflunisal on the reduction of hydrochlorothiazide elimination.

The applicant presented several *in vivo* studies evaluating the DDI potential of diflunisal. These have shown that, at least indomethacin, hydrochlorothiazide and acenocoumarol exposures are modified by the co-administration with diflunisal. Due to the lack of *in vitro* studies, the mechanisms behind these interactions are yet to be explained.

Pharmacodynamics

The applicant has provided bibliographic data on the mechanism of action of diflunisal. The primary pharmacodynamics of diflunisal relevant to the proposed indication is its ability to stabilise transthyretin (TTR), preventing the dissociation of TTR tetramers into monomers and consequent deposition. The mechanism of action description is currently reflected in section 5.1 of the SmPC.

Diflunisal exhibits the appropriate stereochemistry and studies have shown that diflunisal can bind to and stabilise TTR in its tetramer form (Sekijima et al, 2006; Tojo et al, 2006) hence preventing the dissociation to monomers. Like T4, diflunisal binds to what is referred to as the forward binding mode, where anionic substituents like carboxylate are positioned in the outer binding pocket engaging in electrostatic interaction with the Lys15 e-ammonium groups. A common pharmacophore among small molecule stabilisers of the T4 hormone binding pocket of TTR tetramer is a carboxylic acid connected through a rigid spacer to an aromatic moiety.

The applicant has provided very limited data on the primary pharmacodynamics of diflunisal in ATTR.

The applicant states that, although a preclinical animal model was not available, the primary pharmacodynamics of diflunisal in the treatment of ATTR amyloidosis has been demonstrated in human volunteers in the publication by Sekijima et al, 2006. In the 250 mg bid group, 12 hours after the 13th oral dose, the diflunisal serum concentration of 146 ± 39 μM was sufficient to afford a TTR binding stoichiometry exceeding 0.95 ± 0.13 (≈1.75 corrected). Diflunisal binding to TTR at this dose slowed urea-mediated dissociation and acid-mediated TTR aggregation, at least three-fold ($p<0.05$) in serum and *in vitro*, consistent with kinetic stabilisation of TTR. In summary, the authors conclude that diflunisal administered at a dose of 250 mg twice a day is sufficient to induce kinetic stabilisation on the tetrameric native state of TTR, preventing its dissociation required for amyloidogenesis in human serum.

No information on the correlation of TTR stabilisation and clinical outcome or efficacy endpoints was provided by the sponsor. Nevertheless, diflunisal is not the first-in-class of TTR stabilisers for ATTR and indirect correlation of the mechanism of action to clinical outcome can be performed.

Considering the secondary pharmacodynamics of diflunisal in the context of ATTR treatment, main safety concerns would be related to its anti-inflammatory effects and associated adverse events. With the data provided on laboratory measurements, some of these events could be analysed. Although there was some variation in the baseline values, with a proportion of patients presenting with abnormal values, the median of diflunisal and placebo were within the accepted normal range and were comparable.

Haematology parameters are particularly relevant in the case of gastric adverse events, correlated with the COX-1 inhibitory activity of diflunisal, along with the possibility of anti-platelet effect. Although median values for platelet count was also found to be similar in baseline and subsequent measurements, a proportion of patients was reported to have significant changes during this period. Given the relevance of platelet count and thrombocytopenia in possible GI bleeding severity due to diflunisal mediated mucous membrane injury, a focused analysis of these cases was justified and presented by the applicant. As a result, text regarding these effects was included in section 4.4.

TTR is a known carrier for vitamin A and medicines that decrease TTR level are known to reduce vitamin A levels. In the data provided by the applicant, including literature and *in vitro* data, there is no data supporting the effect on diflunisal binding to TTR and alter its ability to function as vitamin A carrier. The applicant also has not found vision impairment effects related to diflunisal.

There is no dedicated study on a potential dose-response relationship of diflunisal in the prolongation of QT interval. Taking into consideration that ATTR can have a cardiomyopathy variant that can lead to electrophysiological changes, a proper characterisation of the effect of diflunisal is relevant. Given the absence of data on cardiac amyloidosis patients the applicant has included a reference regarding administration in patients with prolonged QT interval in section 4.4 along with the other proposed cardiac conditions.

The study for evaluation of interaction with oral anticoagulants was performed with diflunisal and acenocumarol, however with several study limitations. The study involved only a total of 6 participants and all of them were healthy individuals instead of patients. Furthermore, although from the same pharmacological class, acenocumarol is rarely used in clinical practice nowadays, limiting the application of these results given that clinical correlation, for example in magnitude of effect, to the most commonly used warfarin was not established. For example, acenocoumarol has a 1.8h half-life, while warfarin has a half-life of 24–33h. Given the prevalence of valvular disease in patients with ATTR associated cardiomyopathy, the enlightenment of the safety profile of coadministration with warfarin is very relevant. This has been reflected in section 4.4 of the SmPC.

Haematology parameters are particularly relevant in the case of gastric adverse events, correlated with the COX-1 inhibitory activity of diflunisal, along with the possibility of anti-platelet effect. Although median values for platelet count was also found to be similar in baseline and subsequent measurements, a proportion of patients was reported to have significant changes during this period. Given the relevance of platelet count and thrombocytopenia in possible GI bleeding severity due to diflunisal mediated mucous membrane injury, a focused analysis of these cases was presented by the applicant.

The applicant has presented information regarding the effect of diflunisal in patients with the V30M and non-V30M mutation. In a publication by Tojo et al (2006), serum samples from 37 FAP patients with 10 different mutations were assessed for TTR stability following treatment with diflunisal. The applicant has provided in the main pivotal efficacy study, that 56.3% of patients randomised to diflunisal (n=36) had the V30M mutation and 43.8% had a non-V30M mutation (n=28).

Information on the genotype distribution of the 28 patients with non-V30M mutations included in the pivotal study and randomised to diflunisal was included in section 5.1.

The applicant has provided very limited data on the PK/PD relationship of diflunisal in ATTR. The information provided by the applicant to support the PK/PD profile of diflunisal on ATTR is derived from the publication of Sekijima and colleagues (2013), entitled "Orally administered diflunisal stabilises transthyretin against dissociation required for amyloidogenesis". In this study, the authors concluded that diflunisal, when dosed orally at a level of 250 mg bid, results in a serum concentration of 230 mM, 4 h after the 13th dose, leading to a corrected TTR binding stoichiometry of 1.9, above the 1:1 stoichiometry needed for complete kinetic stabilisation of TTR. Therefore, it is concluded that diflunisal administered at a dose of 250 mg twice a day is sufficient to impose kinetic stabilisation on the tetrameric native state of TTR, preventing its dissociation required for amyloidogenesis in human serum.

The sponsor has based the dose decision on a previous Phase 1 trial performed to determine the safety, tolerability, and TTR stabilising activity of administration of diflunisal. Based on the results from this study, the dose of 250 mg was considered by the applicant as achieving high drug: TTR binding stoichiometry in plasma, inhibiting amyloid fibril formation without inducing short-term renal injury and therefore chosen as the dose used in further clinical trials.

2.6.4. Conclusions on clinical pharmacology

Pharmacokinetics

The overall clinical pharmacokinetics of diflunisal are well characterised.

Pharmacodynamics

The applicant has addressed all the Clinical Pharmacodynamics issues raised in the assessment process.

2.6.5. Clinical efficacy

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
H-23750	08 May 2006. Completed. 130/140. (8 centres in Italy, Japan, Sweden, UK, USA)	Double-blind. Placebo-controlled.	Diflunisal 250 mg orally twice daily. Matching placebo orally twice daily.	Biopsy-proven amyloid deposition. Signs of peripheral or autonomic neuropathy detectable by a neurologist.

2.6.5.1. Dose response studies

The applicant did not present dose-response studies of diflunisal for the treatment of amyloidosis.

However, the Pharmacokinetics and pharmacodynamics of diflunisal for this indication has been adequately addressed. Off label use as discussed below also supports the efficacy of the 250 mg bid dose.

2.6.5.2. Main studies

Study #1 H23750: The Effect of Diflunisal on Familial Amyloidosis - A Randomised, Double-Blind, Placebo-Controlled, International Multi-Centre Trial of Diflunisal on Neurologic Disease Progression in 200 Familial Amyloid Subjects

Methods

Study Participants

Patients with (Inclusion criteria):

1. Biopsy proven amyloid deposition

2. Genotyping of variant TTR

3. Signs of mild to moderate peripheral or autonomic neuropathy -- detectable by a neurologist (performance status <= 3)

4. Age >= 18 and <= 75 years

5. Negative βHCG testing and contraception for sexually active women of child-bearing potential

Exclusion criteria:

1. Use of non-study NSAIDs within 30 days of enrolment

2. Other causes of sensorimotor polyneuropathy

a. Vitamin B12 deficiency

b. HIV patients on anti-retroviral drugs

c. *Diabetes mellitus* (Hgb A1C > 6.2%)

d. Chronic alcoholism (> 6 ounces hard liquor daily for 10 or greater years)

3. Co-morbidities with anticipated survival <2 years or liver transplantation in <1 yr

4. Liver transplantation

5. End-stage neuropathic disease (performance status > 3, parenteral nutrition, bedsores)

6. NYHA class IV (cardiac symptoms at rest & with minimal exertion)

7. Pregnancy or unwillingness to use contraception by women of childbearing age

8. Renal insufficiency (creatinine clearance < 30 ml/min)

9. Active or recent non-haemorrhoidal GI bleeding (within past 18 months)

10. Current anti-coagulation therapy, non-study NSAID or aspirin use

11. AST, ALT or Total Bilirubin > twice *or three (4th and 5th amendment)* times the upper limit of normal lab value

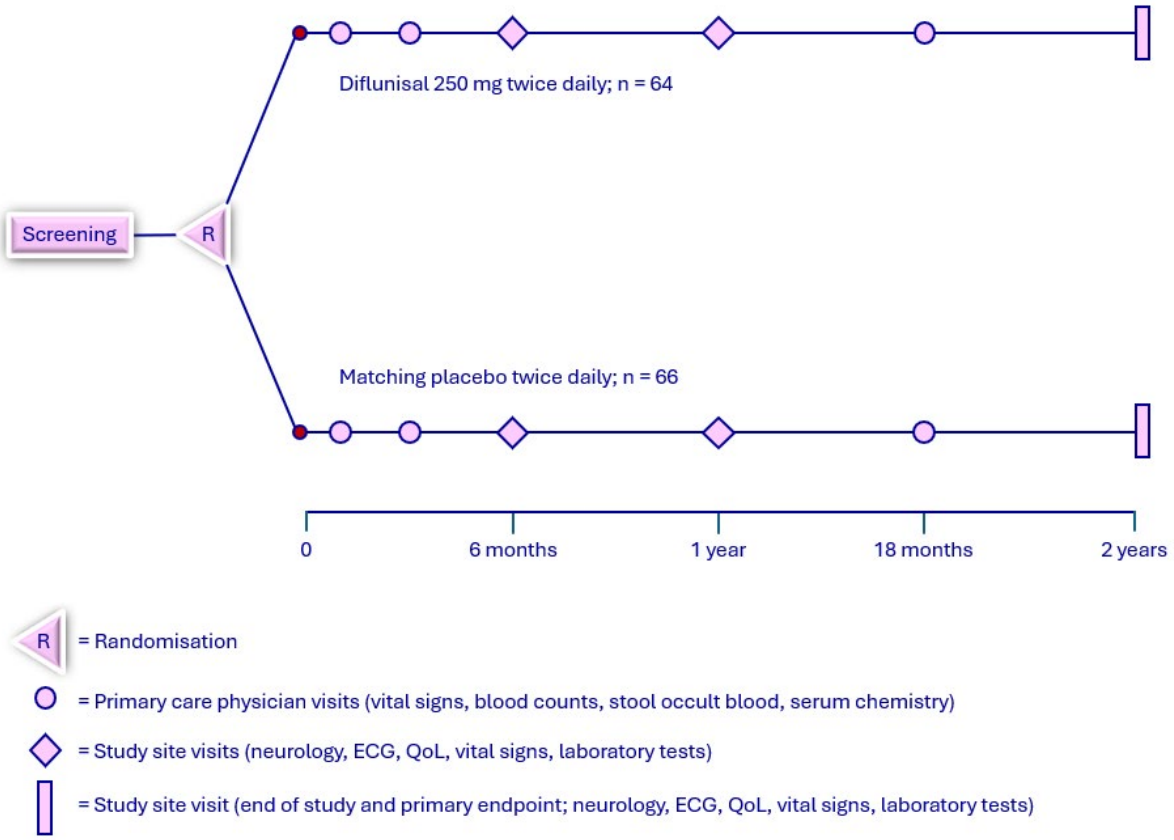
12. Non-steroidal or aspirin allergy/hypersensitivity

13. Thrombocytopenia (< 100,000 platelets/mm3)

14. Previous participation in this study

15. Inability or unwillingness of subject or legal guardian/representative to give written informed consent

Figure 2: Study schematic for Study H23750



Treatments

Eligible subjects were randomised to the treatment arms (diflunisal or placebo) in a 1:1 ratio.

The study drug was packaged according to the randomisation schemes and labelled only with a number. A study pharmacist at each clinical site dispensed the next available study drug kit as directed by the randomisation scheme, and the study identification number on the drug kit was assigned to the study subject. Duration of treatment: 24 months.

Test product, dose and mode of administration, batch number:

- Diflunisal (over-encapsulated tablets; OET) 250 mg orally twice daily.
- Diflunisal batch number DL00003348, OET batch number 33131B0.

Reference product, dose and mode of administration, batch number:

- Matching placebo, orally twice daily, batch number 33131A0

Concomitant treatment

Use of any medications other that the study treatment from enrolment until study discontinuation was to be recorded on the appropriate CRF. Each time a concomitant medication was used, the medication name, dose, route, frequency, and start/stop dates were to be recorded.

Prohibited Therapy

- Concomitant use of non-study non-steroidal anti-inflammatory agents (other than aspirin ≤160 mg daily) and/or anticoagulants was prohibited while enrolled in the study.

- Subjects could not take HIV anti-retroviral medications during study participation.
- Previous liver transplantation was an exclusion criterion for the study. Subjects undergoing liver transplantation were to be terminated from the trial.

Lifestyle considerations

- Subjects could not ingest more than 6 ounces of hard liquor (equals 6 units of alcohol) per day.

There was no rescue treatment planned.

Objectives

Primary objective

- To determine whether diflunisal inhibits (peripheral and autonomic neuropathic) disease progression in subjects with FAP.

Secondary objectives

- To examine TTR stability and amyloidogenicity in FAP subjects randomly assigned to the placebo and diflunisal treatment groups, correlating disease progression with TTR stability.
- To compare the detection of FAP neurologic disease progression by a highly quantitative composite testing instrument (Neuropathy Impairment Score [NIS] + 7) versus a clinical neurologic scoring system (Kumamoto Scale).
- To define the natural history of ATTR cardiomyopathy.

The secondary objectives of the study were considered exploratory in nature.

Given the extended duration of the inclusion period, as knowledge on the disease grew, another secondary objective to determine whether diflunisal would also inhibit the progression of ATTR cardiomyopathy was added. Additionally, change in QoL was added as a secondary objective for the same reason.

The primary hypotheses being tested are as follows.

H0: Effect in Diflunisal group = Effect in Placebo group

H1: Effect in Diflunisal group ≠ Effect in Placebo group

Outcomes/endpoints

Primary endpoint

The primary outcome measure in this study was the NIS+7 nerve tests ("NIS+7") composite assessment. This score combines the NIS, with a neurologist's clinical assessment of muscle weakness, sensory loss, and decreased muscle stretch reflexes, with 5 nerve conduction study (NCS) attributes derived from 3 lower extremity nerves, vibration detection threshold, and heart rate response to deep breathing (HRdb; Dyck, 2019).

A difference of 2 points in NIS+7 score was defined by the international Peripheral Nerve Society to represent the minimal clinically detectable change in polyneuropathy progression that is detectable by neuromuscular experts (Peripheral Nerve Society 1995). A 2-point change in NIS+7 could, for example, reflect a 25% decline in muscle strength and a 50% decrease in one of the other NIS assessments.

Secondary endpoints

The secondary outcome measures specified in the SAP were as follows:

Kumamoto clinical neurologic scale

The Kumamoto clinical neurologic scale score is a 14-item clinical neurological scale of motor, sensory and autonomic nerve function combined with heart and kidney end organ measures (Tashima, 1997). This score ranges from 0 to 96 points, with higher scores indicating greater neurological deficits.

Clinical neurology assessment measures

- NIS – a component of the primary outcome.
- Lower limb function (NIS-LL).
- Neuropathy Symptoms and Change (NSC).

Modified body mass index

The modified body mass index (mBMI) is the product of the subject's BMI ([weight in kilograms] divided by [height in meters]^2) and the serum albumin concentration (in g/L). Lower scores indicate worse nutritional status and have been shown to correlate with survival in ATTR-FAP.

Echocardiographic readings

- M-mode measurements (left atrial, ventricle, inferior vena cava).
- 2-D measurements (LV ejection fraction, Doppler transmitral & pulmonary venous flows).

Short Form General Health Survey 36

The 36-item Short Form General Health Survey (SF-36) is a questionnaire that measures QoL. It consists of 8 scales measuring physical and mental health. The score ranges from 0 to 100 points, with lower scores indicating diminished status.

Amyloid content in aspirated fat tissue

Amyloid content in aspirated fat tissue was to be evaluated as positive or negative by two independent, blinded investigators at Boston University, scoring all slides in a randomised order by semi-quantitative measures. Quantitative assessments of variant TTR in fat tissue were planned to be performed in a subset of subjects using a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (ELISA).

Furthermore, progression of aTTR cardiomyopathy and QoL were added as secondary objectives. SF-36 was part of the initial protocol and maintained through the study, but no specific tool was added to quantify progression of cardiomyopathy.

Sample size

It was planned to enrol 70 evaluable subjects per treatment arm (anticipating a 30% dropout rate, this translated to 100 subjects per arm) yielding a power slightly in excess of 0.80 to detect an effect size of 0.5 (a 1.8-point difference in NIS+7 scoring), with a 2-sided statistic at alpha level 0.05.

A difference of 2 points in NIS+7 score was defined by the international Peripheral Nerve Society to represent the minimal change in polyneuropathy progression that is detectable by neuromuscular experts (Peripheral Nerve Society 1995). A 2-point change in NIS+7 could, for example, reflect a 25% decline in muscle strength and a 50% decrease in one of the other NIS assessments.

Randomisation and blinding (masking)

Randomisation was performed in permuted blocks of 2 or 4 stratified for ATTR genotype (non-V30M vs V30M) and study site. Investigators, other study staff and study subjects were blinded to treatment assignments.

A DSMB monitored the trial and had access to unblinded trial data. The study biostatistician, who was the only person at the Sponsor side with access to the unblinded randomisation scheme, prepared data for review at the DSMB meetings. The blind was to be preserved throughout the study unless, in the opinion of the investigator, this placed the subject at an undue risk. If unblinding occurred, it was required that the Sponsor be notified immediately. No unblinding occurred during the study.

There are concerns on the maintained blinding of the study, as it was stated that 10 pts in the placebo but not in the diflunisal group may have started to take commercially available diflunisal during the study.

Statistical methods

Population	Patients with symptomatic FAP and identified TTR mutation who would not encounter exclusion criteria prior to study start under any treatment assignment.
Treatment condition<s>	The applicant did not explicitly provide the estimand policy with the argument that at the time the study was conducted the estimand approach had not been published yet. Longitudinal analysis Assignment to diflunisal in the hypothetical scenario of no discontinuation compared to assignment to placebo in the hypothetical scenario of no discontinuation.
Endpoint (variable)	NIS+7 at Month 24
Population-level summary	Difference in LS means between groups
Intercurrent events and strategy to handle them	
Loss of efficacy – Liver transplantation?	Hypothetical
Loss of efficacy – Switch to active treatment (diflunisal? Tafamidis??)	Hypothetical
Adverse event leading to discontinuation	Hypothetical

Death	Hypothetical
Loss to follow-up	Hypothetical

Planned analyses

The originally-planned analyses as set out in the Statistical Analysis Plan adopted on 13 December 2012 (i.e., before the blind was broken). These are referred to in the results sections below as the "originally-planned analyses". They include:

- A primary longitudinal analysis; this does not consider the potential impact of missing data.
- The originally-planned sensitivity analysis; this includes a mixture of fixed value and multiple imputation under a missing at random (MAR) assumption and hence can be said to be more appropriate.
- A responder analysis as an additional way to evaluate the primary outcome.
- Subgroup analyses to confirm findings and study possible effect modification.

Primary endpoint:

The primary endpoint was the change in Neuropathy Impairment Score + 7 nerve tests (NIS+7) composite score from baseline to the Month 24-assessment.

Measured sorting to change from baseline (LS Mean)

Secondary endpoints:

Changes from baseline to Month 24 were evaluated in the following secondary outcome measures:

• Kumamoto Neurologic Scale score

• Neuropathy Impairment Score (NIS) – a component of the primary outcome

• Lower limb function (NIS-LL)

• Neuropathy Symptoms and Change (NSC) score

• Modified body mass index (mBMI)

• Echocardiographic readings

• 36-item Short Form General Health Survey (SF-36)

• Amyloid content in aspirated fat tissue

All analyses of efficacy endpoints were performed on the intent-to-treat (ITT) population, and these are considered as the main analyses. Analyses in a per protocol (PP) population would be subject to considerable bias given high dropout rate in the study and were thus not performed.

The main original analysis for both primary and secondary efficacy endpoints (the "primary analysis") was an analysis of covariance (ANCOVA), where missing data were assumed missing at random (MAR). Baseline, Month 12 and Month 24 values were included in a single model as repeated measures within subject.

In total, 10 subjects randomised to placebo stopped taking study drug and instead acquired diflunisal outside of the study or were prescribed open-label diflunisal as rescue treatment. Seven of these

subjects continued in the study. It cannot be ruled out that this affected the results of the ITT population.

Planned subgroup analyses

Subgroup analyses were planned by gender, geographical region, mutation type, and disease severity at entry (based on NIS+7 of <45 points [stage I] or ≥45 points [stage II-IV]). None of these variables was seen to significantly influence the results.

There were also subgroup analyses to confirm findings and study possible effect modification, namely NIS, NIS-LL, Kumamoto scale, SF-36 Physical and Mental.

Post-hoc analyses

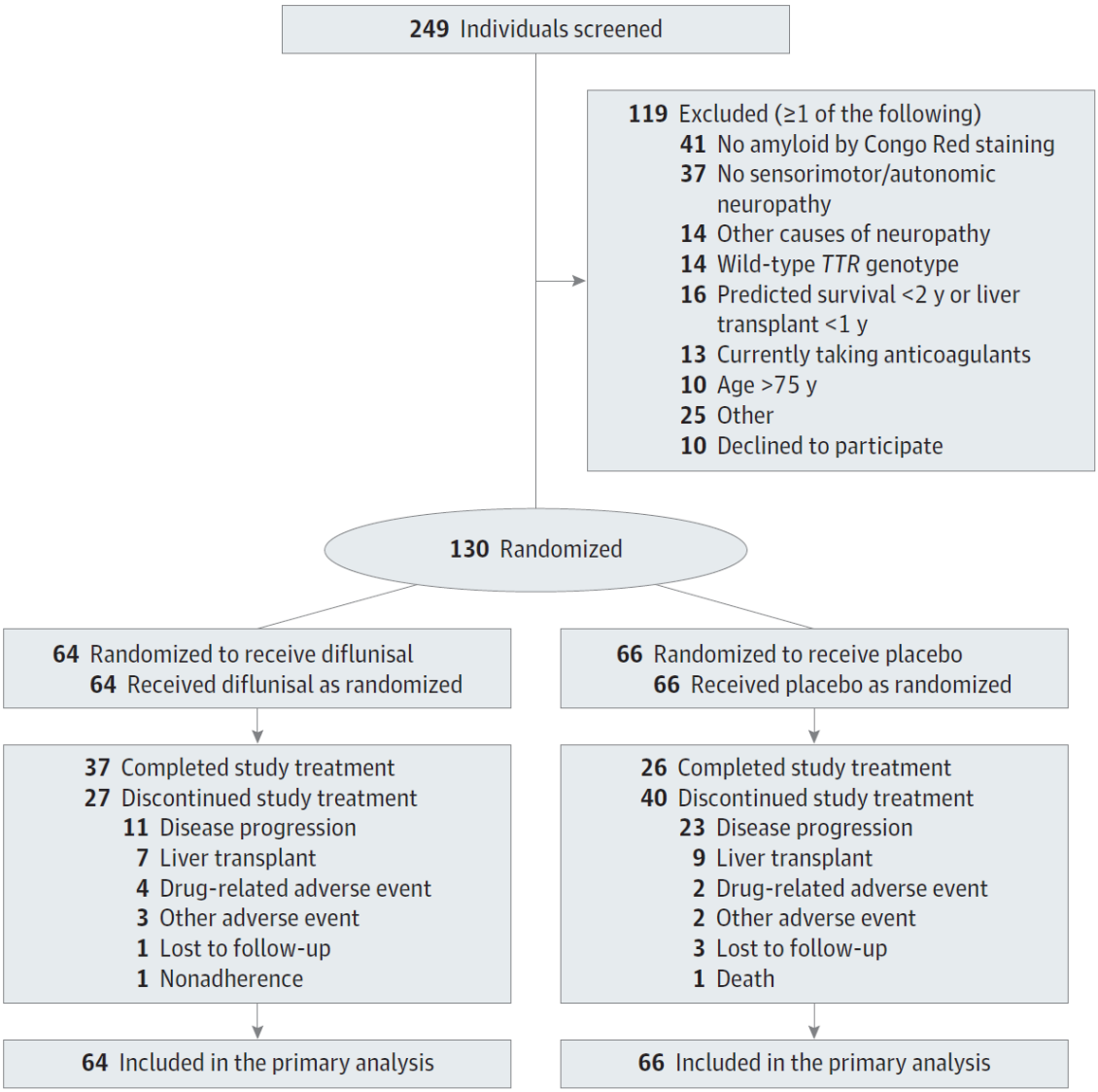
Additional, post hoc sensitivity analyses were captured in an Addendum, dated 13 September 2023, to the original Statistical Analysis Plan. These analyses are referred to in the results sections below as "post hoc sensitivity analyses". They include:

- A multiple imputation analysis "(i)" whereby intermittent missing data were multiply imputed to generate a monotone missing data pattern and then the remaining missing data were imputed assuming MAR; note, and in contrast to the originally defined MI analysis, no fixed value imputation was included.
- A "jump to placebo" analysis "(ii)" whereby missing observations in both the experimental and placebo groups are imputed as missing not at random (MNAR) using only data observed in the placebo group (i.e., instantaneous loss of efficacy and conversion to placebo is assumed upon cessation of diflunisal therapy).
- A tipping point analysis "(iii)" which assesses the robustness of results, again under a MNAR assumption, successively imputing missing data in the experimental arm under an increasingly punitive penalty in order to identify the degree of penalty that needs to be applied to subjects with missing data in the experimental arm in order to force the loss of statistical significance seen in the primary analysis. If the degree of missing data penalty to rendering the primary analysis non-significant is implausibly large, then this suggests that the primary endpoint analysis is robust.

Inversely, the initial per protocol analysis was not performed.

Results

Participant flow



Among the 63 participants who completed study treatment, analysable primary outcome data were obtained for 60 (placebo, n=23; diflunisal, n=37); 3 in the placebo group had inadmissible data for the Neuropathy Impairment Score plus 7 nerve tests (NIS+7). Among the 67 in whom study drug was discontinued prior to 2 years (placebo, n=40; diflunisal, n=27), 2-year primary outcome data (NIS+7) were obtained for 8 participants (placebo, n=5; diflunisal, n=3).

Recruitment

There is no information on the period of recruitment. Still, study dates are provided to allow indirect information on this. Study start is stated in clinicaltrials.gov as of Feb2006, and the first patient enrolled in May 2006. Since the last patient out occurred in Dec2012, one would expect that recruitment has occurred between Feb 2006 and Dec 2010, almost 5 years.

Conduct of the study

Major amendments have been implemented along the long study duration. The protocol amendments have been detailed in the D80 clinical report. Pt enrolment was only started after the second amendment was produced, and while there is no information on which protocol version was in place when patients were enrolled at each study centre, the applicant has stated that the study started with the second version.

Baseline data

Numbers analysed

Table 1: Disposition of patients in Study H-23750

	Diflunisal	Placebo	Total
No. planned:	70	70	140
No. randomised and treated:	64	66	130
Males, n (%)	43 (67.2%)	44 (66.7%)	87 (66.9%)
Females, n (%):	21 (32.8%)	22 (33.3%)	43 (33.1%)
Mean age (range), years:	60.3 (24-76)	59.2 (27-75)	59.7 (24-76)
TTR Mutation, n (%): V30M	36 (56.3%)	35 (53.0%)	71 (54.6%)
TTR Mutation, n (%): Other	28 (43.8%)	31 (47.0%)	59 (45.4%)
Polyneuropathy disability (PND) stage, n (%): 0-I	28 (43.8%)	21 (31.8%)	49 (37.7%)
Polyneuropathy disability (PND) stage, n (%): II	18 (28.1%)	23 (34.8%)	41 (31.5%)
Polyneuropathy disability (PND) stage, n (%): IIIA	11 (17.2%)	8 (12.1%)	19 (14.6%)
Polyneuropathy disability (PND) stage, n (%): IIIB	3 (4.7%)	10 (15.2%)	13 (10%)
Polyneuropathy disability (PND) stage, n (%): IV	4 (6.3%)	4 (6.1%)	8 (6.2%)

No. analysed for efficacy: Intent-to-treat (ITT) analysis set:	64 (100%)	66 (100%)	130 (100%)
No. analysed for safety:	64 (100%)	66 (100%)	130 (100%)
No. completed treatment:	37 (57.8%)	26 (39.8%)	63 (48.5%)

Category	**Diflunisal n (%)**	**Placebo n (%)**	**Total (N=249) n (%)**
Randomised, treated and included in ITT analysis	64 (100.0)	66 (100.0)	130 (100.0)
Had primary endpoint data at Month 12	50 (78.1)	39 (59.1)	89 (68.5)
Had primary endpoint data at Month 24	40 (62.5)	30 (45.5)	70 (53.8)

Sixty-seven subjects (51.5%) discontinued study treatment before completing the 2-year protocol, including 27 (42.2%) from the diflunisal group and 40 (60.6%) from the placebo group. Disease progression (11 subjects [17.2%] in the diflunisal group and 23 subjects [34.8%] in the placebo group) and orthotopic liver transplantation (7 [10.9%] diflunisal and 9 [13.6%] placebo) were the leading reasons for dropout.

Outcomes and estimation

Originally planned analyses

The results of these analyses are summarised below

Table 2: Originally-planned analyses of change from baseline in NIS+7

Analysis	**Timepoint**	**Diflunisal CFB (N=64) LS Mean (95% CI)**	**Placebo CFB (N=66) LS Mean (95% CI)**	**Difference (Placebo - Diflunisal)**	**p-value**
Longitudinal	Month 12	6.2 (2.8, 9.6)	12.5 (8.6, 16.4)	6.4 (1.2, 11.6)	0.0170
	Month 24	8.2 (2.9, 13.6)	26.3 (20.2, 32.4)	18.0 (9.9, 26.2)	<.0001
Multiple imputation	Month 12	6.4 (3.1, 9.6)	12.5 (8.6, 16.4)	6.1 (1.1, 11.1)	0.0169
	Month 24	8.7 (3.3, 14.1)	25.0 (18.4, 31.6)	16.3 (8.1, 24.5)	0.0001

NIS+7 = Neuropathy Impairment Score plus 7 nerve tests; ITT = Intent-to-treat; N = Number of subjects that were randomly assigned to treatment sequence; LS = Least-squares; CI = Confidence interval; CFB = Change from baseline.

Table 14.2.2.1 Longitudinal Analyses of Primary and Secondary Outcomes - Primary Analysis
(Intent to Treat Population)

Endpoint	Timepoint	Diflunisal Change from Baseline LS Mean (95% C.I.)	Placebo Change from Baseline LS Mean (95% C.I.)	Difference (Placebo - Diflunisal)	P-Value
NIS+7 Composite Score	Month 12	6.2 (2.8, 9.6)	12.5 (8.6, 16.4)	6.4 (1.2, 11.6)	0.0170
	Month 24	8.2 (2.9, 13.6)	26.3 (20.2, 32.4)	18.0 (9.9, 26.2)	<.0001
NIS Score	Month 12	4.1 (1.2, 6.9)	10.1 (6.9, 13.3)	6.0 (1.7, 10.3)	0.0065
	Month 24	6.4 (1.6, 11.2)	23.2 (17.8, 28.5)	16.8 (9.6, 24.0)	<.0001
NIS-Lower Limb Score	Month 12	3.2 (1.3, 5.2)	6.0 (3.9, 8.2)	2.8 (-0.1, 5.7)	0.0564
	Month 24	3.8 (0.9, 6.6)	12.1 (8.9, 15.3)	8.3 (4.1, 12.6)	0.0002
Kumamoto Composite Score	Month 12	1.9 (0.1, 3.7)	4.1 (2.1, 6.2)	2.3 (-0.5, 5.0)	0.1025
	Month 24	3.1 (1.1, 5.1)	8.0 (5.8, 10.3)	5.0 (1.9, 8.0)	0.0015
Modified-BMI	Month 12	-18.7 (-51.6, 14.1)	-38.5 (-74.9, -2.1)	-19.8 (-68.8, 29.2)	0.4261
	Month 24	-33.7 (-69.3, 1.8)	-67.9 (-108.1, -27.7)	-34.1 (-87.8, 19.5)	0.2105
SF-36 Physical Component Score	Month 12	0.7 (-1.1, 2.5)	-1.9 (-3.9, 0.2)	-2.6 (-5.3, 0.1)	0.0589
	Month 24	1.2 (-1.2, 3.7)	-4.9 (-7.6, -2.1)	-6.1 (-9.8, -2.5)	0.0013
SF-36 Mental Component Score	Month 12	2.5 (-0.0, 5.1)	0.8 (-2.0, 3.6)	-1.7 (-5.5, 2.1)	0.3674
	Month 24	3.5 (0.4, 6.7)	-0.9 (-4.4, 2.5)	-4.5 (-9.2, 0.2)	0.0620

Analyses presented are based on a mixed model repeated measures (MMRM) in which missing data are assumed to be missing at random. The model includes terms for treatment group, month and treatment group by month interaction with month as a repeated measure.

LS: Least-squares; C.I.: Confidence interval; BMI: body mass index; NIS: Neuropathy Impairment Score; NIS+7: NIS plus 7 nerve tests; NIS-LL: Neuropathy Impairment Score of the Lower Limbs; SF-36: 36-Item Short-Form Health Survey.

The longitudinal analysis showed that subjects randomised to diflunisal had significantly less progression of polyneuropathy than those assigned to placebo.

The primary analysis does not consider missing data, which are prominent in this trial. Therefore, the originally-planned sensitivity analysis, which includes multiple imputation (MI) under missing at random (MAR) assumption, can be said to be a more appropriate analysis. This analysis corroborated the results from the longitudinal analysis.

The inhibitory effect of diflunisal on neuropathy progression was also detectable at Month 12 in both analyses.

Table 14.2.2.3.1 Multiple Imputation Analysis of Primary and Secondary Outcomes
(Intent to Treat Population)

Endpoint	Timepoint	Diflunisal Change from Baseline LS Means (95% C.I.)	Placebo Change from Baseline LS Means (95% C.I.)	Difference (Placebo - Diflunisal)	P-Value
NIS+7 Composite Score	Month 12	6.4 (3.1, 9.6)	12.5 (8.6, 16.4)	6.1 (1.1, 11.1)	0.0169
	Month 24	8.7 (3.3, 14.1)	25.0 (18.4, 31.6)	16.3 (8.1, 24.5)	0.0001
NIS Score	Month 12	4.2 (1.5, 7.0)	10.1 (6.9, 13.3)	5.9 (1.8, 10.0)	0.0052
	Month 24	6.7 (1.9, 11.4)	22.8 (17.2, 28.4)	16.1 (9.0, 23.2)	<.0001
NIS-Lower Limb Score	Month 12	3.3 (1.4, 5.1)	6.0 (3.9, 8.2)	2.8 (-0.0, 5.6)	0.0513
	Month 24	3.8 (1.0, 6.7)	12.1 (8.7, 15.5)	8.2 (4.0, 12.5)	0.0002
Kumamoto Composite Score	Month 12	1.9 (0.0, 3.7)	4.1 (1.9, 6.4)	2.3 (-0.6, 5.2)	0.1214
	Month 24	3.2 (1.1, 5.3)	8.1 (5.7, 10.6)	4.9 (1.7, 8.1)	0.0025
Modified-BMI	Month 12	-19.7 (-54.1, 14.7)	-40.3 (-75.4, -5.2)	-20.6 (-69.0, 27.9)	0.4055
	Month 24	-35.2 (-73.6, 3.3)	-65.1 (-107.4, -22.7)	-29.9 (-85.7, 25.9)	0.2928
SF-36 Physical Component Score	Month 12	0.8 (-0.9, 2.5)	-1.9 (-3.8, -0.1)	-2.8 (-5.2, -0.3)	0.0302
	Month 24	1.5 (-0.8, 3.7)	-4.9 (-7.6, -2.2)	-6.4 (-9.8, -2.9)	0.0003
SF-36 Mental Component Score	Month 12	2.3 (0.1, 4.5)	0.6 (-1.7, 3.0)	-1.7 (-4.9, 1.5)	0.2996
	Month 24	3.7 (1.0, 6.4)	-1.1 (-4.3, 2.0)	-4.9 (-9.0, -0.7)	0.0216

Responder analysis

By responder analysis (assigning treatment failure to all study dropouts and subjects with a ≥2-point increase in NIS+7 score), 19 (29.7%) of subjects in the diflunisal group were found successful at Month 24 compared to 6 (9.4%) of subjects in the placebo group (p=0.007).

Responder analysis of Month 12 data did not meet statistical significance.

Table 14.2.2.2 Analysis of NIS+7 Composite Score Responder Status
(Intent to Treat Population)

Category	Result	Diflunisal (N=64)	Placebo (N=66)	Total (N=130)	Risk Ratio (95% C.I.)	P-Value
Success at 12 months	Yes	17 (26.6)	9 (14.1)	26 (20.3)	1.89 (0.91-3.92)	0.123
	No	47 (73.4)	55 (85.9)	102 (79.7)		
Success at 24 months	Yes	19 (29.7)	6 (9.4)	25 (19.5)	3.17 (1.35-7.41)	0.007
	No	45 (70.3)	58 (90.6)	103 (80.5)		

Secondary endpoints

Kumamoto clinical neurologic scale

The originally-planned analyses of this endpoint are summarised below.

Table 3: Originally-planned analyses of change from baseline in Kumamoto clinical neurologic scale

Analysis	Timepoint	Diflunisal CFB (N=64) LS Mean (95% CI)	Placebo CFB (N=66) LS Mean (95% CI)	Difference (Placebo - Diflunisal)	p-value
Longitudinal	Month 12	1.9 (0.1, 3.7)	4.1 (2.1, 6.2)	2.3 (-0.5, 5.0)	0.1025
	Month 24	3.1 (1.1, 5.1)	8.0 (5.8, 10.3)	5.0 (1.9, 8.0)	0.0015
Multiple imputation	Month 12	1.9 (0.0, 3.7)	4.1 (1.9, 6.4)	2.3 (-0.6, 5.2)	0.1214
	Month 24	3.2 (1.1, 5.3)	8.1 (5.7, 10.6)	4.9 (1.7, 8.1)	0.0025

Clinical neurology assessment measures: NIS

The originally-planned analyses of this endpoint are summarised below.

Table 4: Originally-planned analyses of change from baseline in NIS

Analysis	Timepoint	Diflunisal CFB (N=64) LS Mean (95% C.I.)	Placebo CFB (N=66) LS Mean (95% C.I.)	Difference (Placebo - Diflunisal)	p-value
Longitudinal	Month 12	4.1 (1.2, 6.9)	10.1 (6.9, 13.3)	6.0 (1.7, 10.3)	0.0065
	Month 24	6.4 (1.6, 11.2)	23.2 (17.8, 28.5)	16.8 (9.6, 24.0)	<.0001
Multiple imputation	Month 12	4.2 (1.5, 7.0)	10.1 (6.9, 13.3)	5.9 (1.8, 10.0)	0.0052
	Month 24	6.7 (1.9, 11.4)	22.8 (17.2, 28.4)	16.1 (9.0, 23.2)	<.0001

Clinical neurology assessment measures: lower limb function (NIS-LL)

The originally-planned analyses of this endpoint are summarised below.

Table 5: Originally-planned analyses of change from baseline in NIS-LL

Analysis	Timepoint	Diflunisal CFB (N=64) LS Mean (95% C.I.)	Placebo CFB (N=66) LS Mean (95% C.I.)	Difference (Placebo - Diflunisal)	p-value
Longitudinal	Month 12	3.2 (1.3, 5.2)	6.0 (3.9, 8.2)	2.8 (-0.1, 5.7)	0.0564
	Month 24	3.8 (0.9, 6.6)	12.1 (8.9, 15.3)	8.3 (4.1, 12.6)	0.0002
Multiple imputation	Month 12	3.3 (1.4, 5.1)	6.0 (3.9, 8.2)	2.8 (-0.0, 5.6)	0.0513
	Month 24	3.8 (1.0, 6.7)	12.1 (8.7, 15.5)	8.2 (4.0, 12.5)	0.0002

Clinical neurology assessment measures: Neuropathy Symptoms and Change (NSC)

Data on this secondary endpoint was provided during the review procedure. The data show consistent benefits for diflunisal in terms of an increased probability of response relative to placebo at both 12 and 24 months for all domains based on the ITT population, with broadly similar findings based on the PP analysis.

Modified body mass index

The primary analysis showed that although subjects in the diflunisal group had a numerically smaller reduction in mBMI LS mean than the placebo group both at Month 12 and Month 24, the differences between groups were not statistically significant.

Echocardiographic readings

All measurements were similar between the treatment groups at baseline as well as at Month 12 and Month 24. No obvious changes could be seen in any of the parameters between study timepoints. As expected, there were no statistically significant differences between the treatment groups in LS mean change from baseline for any parameter in either of the original statistical analyses.

Short Form General Health Survey 36

The originally-planned analyses of this endpoint are summarised below.

Table 6: Originally-planned analyses of change from baseline in SF-36

Analysis	Month	Comp-onent	Diflunisal CFB (N=64) LS Mean (95% C.I.)	Placebo CFB (N=66) LS Mean (95% C.I.)	Difference (Placebo - Diflunisal)	p-value
Longitudinal	12	Physical	0.7 (-1.1, 2.5)	-1.9 (-3.9, 0.2)	-2.6 (-5.3, 0.1)	0.0589
		Mental	2.5 (-0.0, 5.1)	0.8 (-2.0, 3.6)	-1.7 (-5.5, 2.1)	0.3674
	24	Physical	1.2 (-1.2, 3.7)	-4.9 (-7.6, -2.1)	-6.1 (-9.8, -2.5)	0.0013
		Mental	3.5 (0.4, 6.7)	-0.9 (-4.4, 2.5)	-4.5 (-9.2, 0.2)	0.0620
Multiple imputation	12	Physical	0.8 (-0.9, 2.5)	-1.9 (-3.8, -0.1)	-2.8 (-5.2, -0.3)	0.0302
		Mental	2.3 (0.1, 4.5)	0.6 (-1.7, 3.0)	-1.7 (-4.9, 1.5)	0.2996
	24	Physical	1.5 (-0.8, 3.7)	-4.9 (-7.6, -2.2)	-6.4 (-9.8, -2.9)	0.0003
		Mental	3.7 (1.0, 6.4)	-1.1 (-4.3, 2.0)	-4.9 (-9.0, -0.7)	0.0216

Amyloid content in aspirated fat tissue

Overall, at screening, about two thirds of the subjects were positive for amyloid deposition in fat tissue by this method of analysis. At Month 12 and Month 24, respectively, the distribution of amyloid positive and amyloid negative subjects among those with available data appeared to shift towards equal proportions in the diflunisal group (18 subjects [28.1%] were positive and 17 [26.6%] were negative at Month 24). This was not seen in the placebo group (17 subjects [25.8%] were positive and

6 [9.1%] were negative at Month 24); however, the large amount of missing data confounded the interpretation.

Post-hoc analyses

Primary endpoint

In the contemporary MI analysis under MAR assumption, the significance for the Month 24 result was retained (difference in LS mean change from baseline between groups 16.3 points [p=0.0002]). In the Jump to Placebo analysis (under MNAR), significance was not retained. In the Tipping Point analysis (under MNAR), a Tipping Point Delta of 15.7 (96.2% of the effect seen in the primary analysis) at Month 24 was observed.

Secondary endpoints

Kumamoto clinical neurologic scale

The contemporary MI analysis supported the significant difference at Month 24 (p=0.0260) but the Jump to Placebo analysis did not (p=0.0889).

The Tipping Point Delta at Month 24 was 1.5, indicating that to lose the statistical significance of the original sensitivity analysis would require the missing data of diflunisal subjects to have a 1.5 unit reduction in treatment effect; such a reduction is not unlikely.

Clinical neurology assessment measures: NIS

The contemporary MI analysis supported the significant differences at Months 12 and 24 (p=0.0250 and 0.0002 respectively) but the Jump to Placebo analysis did not (p=0.0534 and 0.0859 respectively).

The Tipping Point Delta of 15.5 points at Month 24 indicates that to lose the statistical significance of the original sensitivity analysis would require the missing data of diflunisal subjects to have a 15.5 unit reduction in treatment effect, a near complete inversion. Such an inversion of the benefit seen for subjects treated with diflunisal is considered extreme and unlikely.

Clinical neurology assessment measures: lower limb function (NIS-LL)

The contemporary MI analysis supported the significant differences at Month 24 (p=0.0017) but the Jump to Placebo analysis did not (p=0.1826).

The Tipping Point Delta at Month 24 was 4.3, indicating that to lose the statistical significance of the original sensitivity analysis would require the missing data of diflunisal subjects to have a 4.3 unit reduction in treatment effect. Given the extent of missing data in the placebo group, which limits the measured neurologic decline in that group, such a reduction is considered unlikely.

Clinical neurology assessment measures: Neuropathy Symptoms and Change (NSC)

This secondary endpoint was not analysed due to data not being accessible at the time of clinical study report compilation. However, data was made accessible during the evaluation. The applicant has analysed them in the same manner as reported by Dyck et al (2020). The analysis examined the proportion of patients with stabilised/improved symptoms over time. For the overall total score and the score within each subdomain, if the median change in symptom score was ≥0, the subject was considered as stabilised/improved and thus classified as a responder. The analysis was performed at 12 and 24 months based on:

- Patients with non-missing data (labelled as the PP population).
- All randomised patients including those with missing data that were classified as non-responders (labelled as the ITT population).

Exact odds ratios (ORs), Exact 95% CIs and Fisher's Exact test p-values were used to compare the proportion of responders between treatment groups. The results of these analyses are shown below.

For the ITT analysis, response rates and ORs consistently favoured diflunisal-treated patients across all domains and overall. OR estimates ranged from 1.5 to 2.0, indicating a 50% to 100% increase in the odds of response with diflunisal as compared to placebo across all subdomains.

For the PP analysis at 12 months, response rates were the same for diflunisal and placebo for the Total Score and Head & Chest Weakness subdomain scores, and were similar for Upper Limb Weakness (83% vs 87%, diflunisal vs placebo) and Sensation Symptoms (79% vs 82%, diflunisal vs placebo) subdomains. For all other subdomains, ORs favoured diflunisal-treated patients.

For the PP analysis at 24 months, and similar to the 12 months analysis, response rates were the same for diflunisal and placebo for the Total Score and for the Head and Chest Weakness subdomain scores. They were also similar for the Upper Limb Weakness subdomain (83% vs 86%, diflunisal vs placebo). For all other subdomains, ORs favoured diflunisal-treated patients. OR estimates ranged from 1.16 to 1.33, indicating a 16% to 33% increase in the odds of response for diflunisal-treated patients as compared to placebo treated patients.

It should be noted that the study was neither designed nor powered for the NSC and so statistically significant outcomes were not anticipated. Nevertheless, the data show consistent benefits for diflunisal in terms of an increased probability of response relative to placebo at both 12 and 24 months for all domains based on the ITT population, with broadly similar findings based on the PP analysis.

Table 7: Analysis of Neuropathy Symptoms and Change (NSC)

Response = Percentage of Patients with Stabilized/Improved Symptoms Over Time

Population	Time (months)	Parameter	N Diflunisal	% [N] Diflunisal	Miss Difn	N Placebo	% [N] Placebo	Miss Pcbo	Exact Odds Ratio	95% CI	Exact P-value
ITT	Month 12	NSC (1-36)	66	72.4% [47/65]	26.6% [17/64]	66	59.1% [39/66]	40.9% [27/66]	1.906	(0.859 , 4.214)	0.1223
		Head & Chest Weakness (1-9)	66	72.4% [47/65]	26.6% [17/64]	66	59.1% [39/66]	40.9% [27/66]	1.906	(0.859 , 4.214)	0.1223
		UL Weakness (10-15)	66	60.9% [39/65]	26.6% [17/64]	66	51.5% [34/66]	40.9% [27/66]	1.464	(0.691 , 3.127)	0.2652
		LL Weakness (16-19)	66	57.8% [37/65]	26.6% [17/64]	66	43.9% [29/66]	40.9% [27/66]	1.761	(0.825 , 3.714)	0.1594
		Sensation Symptoms - All Sites (20-29)	66	57.8% [37/65]	26.6% [17/64]	66	48.5% [32/66]	40.9% [27/66]	1.452	(0.689 , 3.084)	0.3737
		Sensation Loss (20-22)	66	56.7% [35/65]	26.6% [17/64]	66	40.9% [27/66]	40.9% [27/66]	1.736	(0.822 , 3.705)	0.1622
		Sensation Physiologic (23-29)	66	65.6% [42/64]	26.6% [17/64]	66	50.0% [33/66]	40.9% [27/66]	1.899	(0.899 , 4.117)	0.1027
		Sensation Pain (25-29)	66	68.8% [44/64]	26.6% [17/64]	66	51.5% [34/66]	40.9% [27/66]	2.059	(0.955 , 4.521)	0.0673
		Autonomic (30-36)	66	72.4% [47/65]	26.6% [17/64]	66	59.1% [39/66]	40.9% [27/66]	1.906	(0.859 , 4.214)	0.1223
ITT	Month 24	NSC (1-36)	66	56.3% [36/64]	43.8% [28/64]	66	42.4% [28/66]	57.6% [38/66]	1.737	(0.823 , 3.706)	0.1610
		Head & Chest Weakness (1-9)	66	56.3% [36/64]	43.8% [28/64]	66	42.4% [28/66]	57.6% [38/66]	1.737	(0.823 , 3.706)	0.1610
		UL Weakness (10-15)	66	46.9% [30/64]	43.8% [28/64]	66	36.4% [24/66]	57.6% [38/66]	1.539	(0.722 , 3.309)	0.2993
		LL Weakness (16-19)	66	62.5% [28/64]	43.8% [28/64]	66	27.3% [18/66]	57.6% [38/66]	2.062	(0.938 , 4.627)	0.0745
		Sensation Symptoms - All Sites (20-29)	66	62.5% [28/64]	43.8% [28/64]	66	31.8% [21/66]	57.6% [38/66]	1.660	(0.768 , 3.634)	0.2214
		Sensation Loss (20-22)	66	37.5% [24/64]	43.8% [28/64]	66	25.8% [17/66]	57.6% [38/66]	1.722	(0.767 , 3.932)	0.2105
		Sensation Physiologic (23-29)	66	50.0% [32/64]	43.8% [28/64]	66	36.4% [24/66]	57.6% [38/66]	1.762	(0.820 , 3.748)	0.1635
		Sensation Pain (25-29)	66	53.1% [34/64]	43.8% [28/64]	66	39.4% [26/66]	57.6% [38/66]	1.736	(0.821 , 3.712)	0.1631
		Autonomic (30-36)	66	56.3% [36/64]	43.8% [28/64]	66	40.9% [27/66]	57.6% [38/66]	1.868	(0.875 , 3.953)	0.1151
PP	Month 12	NSC (1-36)	67	100 % [67/67]	0.0 % [0 /67]	39	100 % [39/39]	0.0 % [0 /39]	--	(-- , --)	--
		Head & Chest Weakness (1-9)	67	100 % [67/67]	0.0 % [0 /67]	39	100 % [39/39]	0.0 % [0 /39]	--	(-- , --)	--
		UL Weakness (10-15)	67	83.0% [39/47]	0.0 % [0 /67]	39	87.2% [34/39]	0.0 % [0 /39]	0.720	(0.168 , 2.776)	0.9170
		LL Weakness (16-19)	67	78.7% [37/47]	0.0 % [0 /67]	39	74.4% [29/39]	0.0 % [0 /39]	1.272	(0.413 , 3.928)	0.9226
		Sensation Symptoms - All Sites (20-29)	67	78.7% [37/47]	0.0 % [0 /67]	39	82.1% [32/39]	0.0 % [0 /39]	0.811	(0.233 , 2.684)	0.9138
		Sensation Loss (20-22)	67	74.5% [35/47]	0.0 % [0 /67]	39	69.2% [27/39]	0.0 % [0 /39]	1.292	(0.452 , 3.709)	0.7641
		Sensation Physiologic (23-29)	67	89.4% [42/47]	0.0 % [0 /67]	39	84.6% [33/39]	0.0 % [0 /39]	1.520	(0.352 , 6.882)	0.7363
		Sensation Pain (25-29)	67	93.6% [44/47]	0.0 % [0 /67]	39	87.2% [34/39]	0.0 % [0 /39]	2.130	(0.385 , 14.732)	0.5144
		Autonomic (30-36)	67	100 % [67/47]	0.0 % [0 /67]	39	100 % [39/39]	0.0 % [0 /39]	--	(-- , --)	--
PP	Month 24	NSC (1-36)	36	100 % [36/36]	0.0 % [0 /36]	28	100 % [28/28]	0.0 % [0 /28]	--	(-- , --)	--
		Head & Chest Weakness (1-9)	36	100 % [36/36]	0.0 % [0 /36]	28	100 % [28/28]	0.0 % [0 /28]	--	(-- , --)	--
		UL Weakness (10-15)	36	83.3% [30/36]	0.0 % [0 /36]	28	85.7% [24/28]	0.0 % [0 /28]	0.826	(0.155 , 2.998)	1.0000
		LL Weakness (16-19)	36	77.8% [28/36]	0.0 % [0 /36]	28	64.3% [18/28]	0.0 % [0 /28]	1.926	(0.562 , 6.816)	0.3623
		Sensation Symptoms - All Sites (20-29)	36	77.8% [28/36]	0.0 % [0 /36]	28	75.0% [21/28]	0.0 % [0 /28]	1.164	(0.306 , 4.350)	1.0000
		Sensation Loss (20-22)	36	66.7% [24/36]	0.0 % [0 /36]	28	60.7% [17/28]	0.0 % [0 /28]	1.299	(0.408 , 4.077)	0.8163
		Sensation Physiologic (23-29)	36	88.9% [32/36]	0.0 % [0 /36]	28	85.7% [24/28]	0.0 % [0 /28]	1.327	(0.233 , 7.907)	0.9900
		Sensation Pain (25-29)	36	96.4% [26/36]	0.0 % [0 /36]	28	92.9% [26/28]	0.0 % [0 /28]	1.302	(0.089 , 19.079)	1.0000
		Autonomic (30-36)	36	100 % [36/36]	0.0 % [0 /36]	28	96.4% [27/28]	0.0 % [0 /28]	1.296	(0.068 , <999.99)	0.4375

Figure 3: Percentage of patients with stabilised/improved symptoms over time (ITT at enrolment)

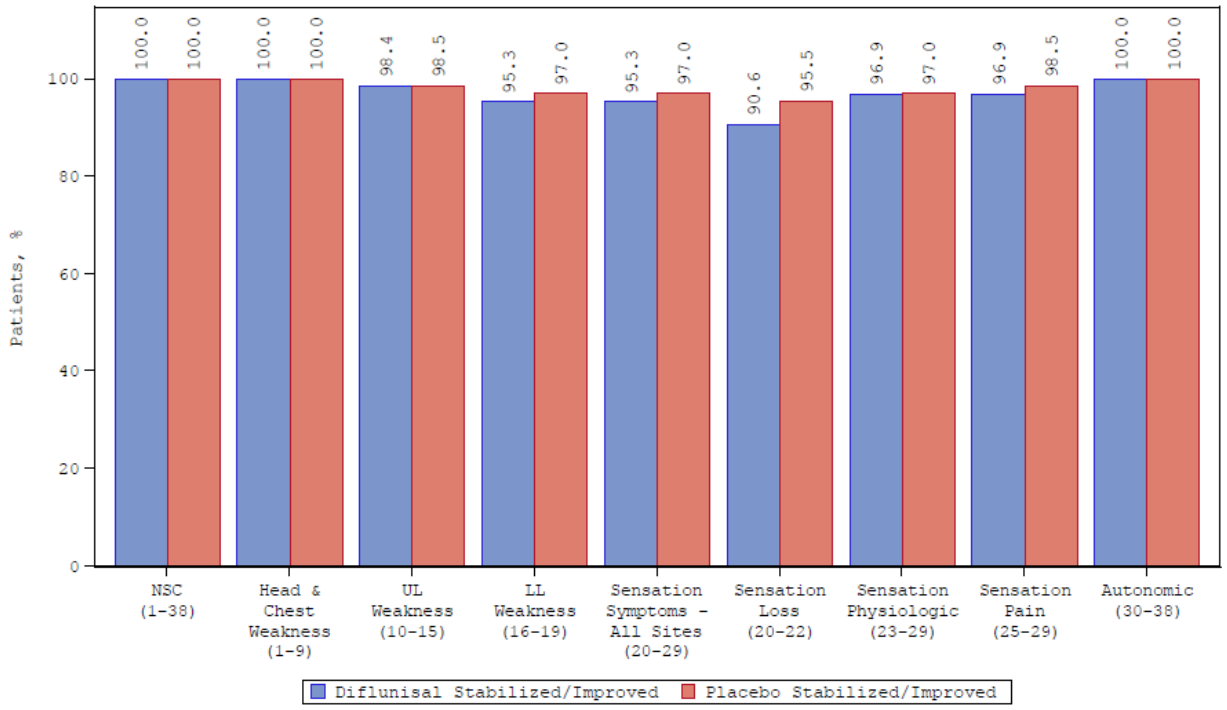


Figure 4: Percentage of patients with stabilised/improved symptoms over time (ITT at 12 months)

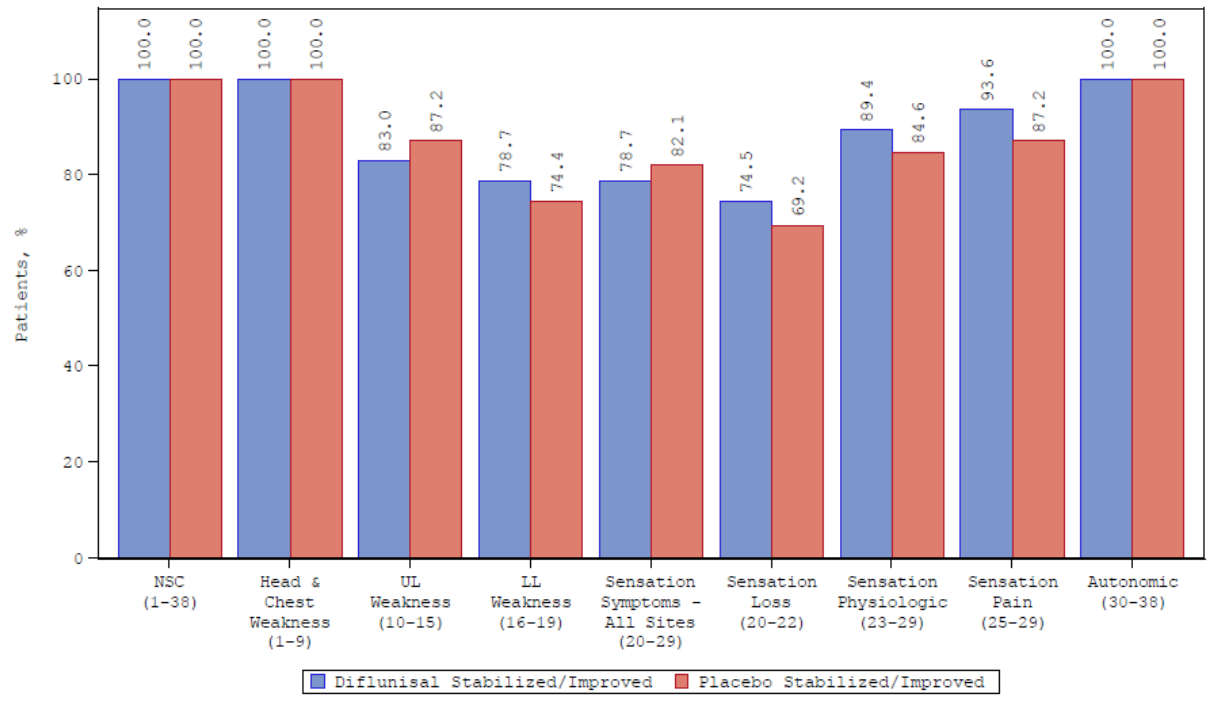
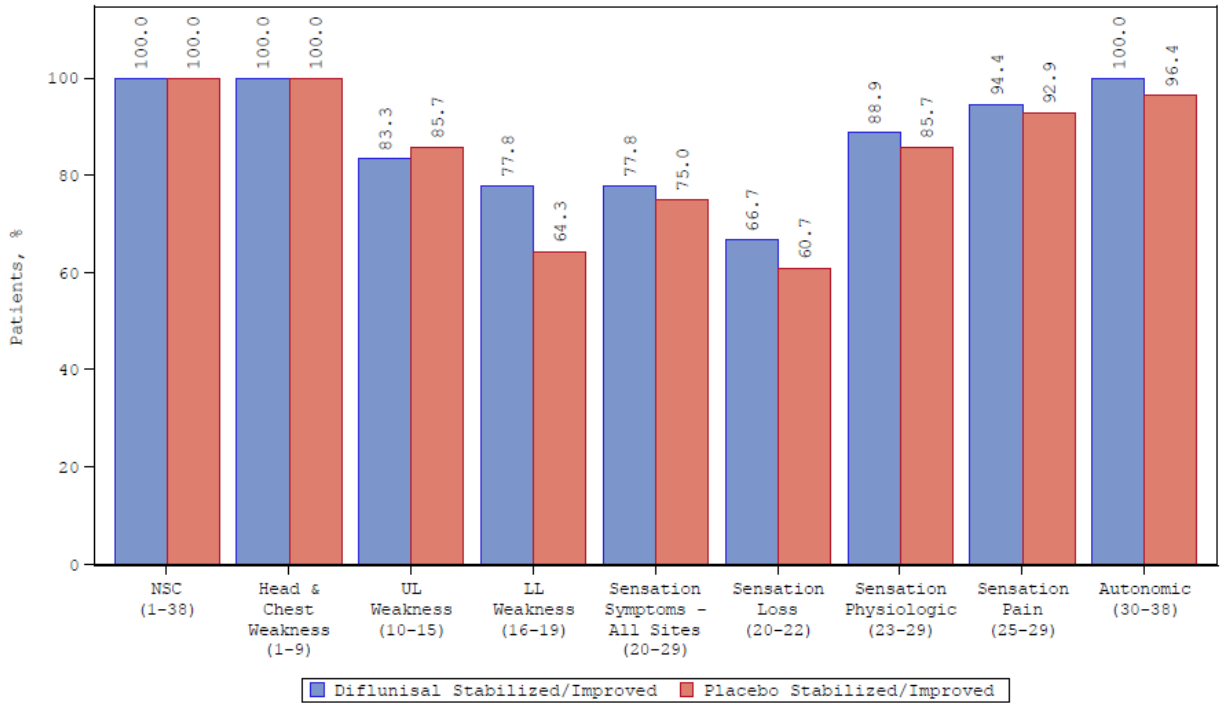


Figure 5: Percentage of patients with stabilised/improved symptoms over time (ITT at 24 months)



The data supports primary endpoint results.

Modified body mass index

Data not discussed.

Echocardiographic readings

Data not discussed.

Short Form General Health Survey 36

The contemporary MI analysis and Jump to Placebo analysis both supported the significant differences in the physical component at Month 24 (p=0.0074 and 0.0486 respectively). Neither post hoc analysis detected significant differences in the mental component at either time point.

The Tipping Point analysis was not performed for the SF-36 metal component score as the difference between groups was not significant in the contemporary MI sensitivity analysis.

Ancillary analyses

Subgroup analysis

The primary endpoint results for the ITT population were analysed for effect modification by the variables gender, geographical region, mutation type, and disease severity at entry (based on NIS+7 of <45 points [stage I] or ≥45 points [stage II-IV]). None of these variables was seen to significantly influence the results.

Summary of main efficacy results

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

Table 8: Summary of efficacy for trial

Title: The Effect of Diflunisal on Familial Amyloidosis. A Randomised, Double-Blind, Placebo-Controlled, International Multi-Centre Trial of Diflunisal on Neurologic Disease Progression in 200 Familial Amyloid Subjects.			
Study identifier	Protocol: H-23750 ClinicalTrials.gov: NCT00294671 EudraCT: 2006-001066-16		
Design	Multi-centre, multi-national, randomised, double-blind, parallel group, placebo-controlled trial.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		24 months Not applicable Not applicable
Hypothesis	Superiority		
Treatments groups	Diflunisal		250 mg diflunisal twice daily for 2 years. N=64 randomised; N=37 completed.
	Placebo		Placebo twice daily for 2 years. N=66 randomised; N=26 completed.
Endpoints and definitions	Primary endpoint	NIS+7	Change in NIS+7 score from baseline to Month 24.
	Secondary endpoints	Kumamoto scale	Change in Kumamoto score from baseline to Month 24.
		NIS	Change in NIS from baseline to Month 24.
		NIS-LL	Change in NIS-LL from baseline to Month 24.
		SF-36 Physical	Change in SF-36 Physical from baseline to Month 24.
		SF-36 Mental	Change in SF-36 Mental from baseline to Month 24.
Database lock	1 May 2013		

Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat at 2-year time point.		
Descriptive statistics and estimate of variability	Treatment group	Diflunisal	Placebo
	Number of subjects	64	66
	NIS+7 (longitudinal) LS mean (95% CI)	+8.2 (2.9, 13.6)	+26.3 (20.2, 32.4)
	NIS+7 (multiple imputation) LS mean (95% CI)	+8.7 (3.3, 14.1)	+25.0 (18.4, 31.6)
	Kumamoto scale (longitudinal) LS mean (95% CI)	3.1 (1.1, 5.1)	8.0 (5.8, 10.3)
	Kumamoto scale (multiple imputation) LS mean (95% CI)	3.2 (1.1, 5.3)	8.1 (5.7, 10.6)
	NIS (longitudinal) LS mean (95% CI)	6.4 (1.6, 11.2)	23.2 (17.8, 28.5)
	NIS (multiple imputation) LS mean (95% CI)	6.7 (1.9, 11.4)	22.8 (17.2, 28.4)
	NIS-LL (longitudinal) LS mean (95% CI)	3.8 (0.9, 6.6)	12.1 (8.9, 15.3)
	NIS-LL (multiple imputation) LS mean (95% CI)	3.8 (1.0, 6.7)	12.1 (8.7, 15.5)
	SF-36 Physical (longitudinal) LS mean (95% CI)	1.2 (-1.2, 3.7)	-4.9 (-7.6, -2.1)
	SF-36 Physical (multiple imputation) LS mean (95% CI)	1.5 (-0.8, 3.7)	-4.9 (-7.6, -2.2)
	SF-36-Mental (longitudinal) LS mean (95% CI)	3.5 (0.4, 6.7)	-0.9 (-4.4, 2.5)

	SF-36-Mental (multiple imputation) LS mean (95% CI)		3.7 (1.0, 6.4)	-1.1 (-4.3, 2.0)
Effect estimate per comparison	Primary endpoint NIS+7 (longitudinal)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	18.0	
		95% CI	(9.9, 26.2)	
		P-value	p<0.0001	
	Primary endpoint NIS+7 (multiple imputation)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	16.3	
		95% CI	(8.1, 24.5)	
		P-value	p<0.0001	
	Secondary endpoint Kumamoto scale (longitudinal)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	5.0	
		95% CI	(1.9, 8.0)	
		P-value	p<0.0015	
	Secondary endpoint Kumamoto scale (multiple imputation)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	4.9	
		95% CI	(1.7, 8.1)	
		P-value	p<0.0025	
	Secondary endpoint NIS (longitudinal)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	16.8	
		95% CI	(9.6, 24.0)	
		P-value	p<0.0001	
	Secondary endpoint NIS (multiple imputation)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	16.1	
		95% CI	(9.0, 23.2)	
		P-value	p<0.0001	
	Secondary endpoint NIS-LL (longitudinal)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	8.3	
		95% CI	(4.1, 12.6)	
		P-value	p=0.0002	
	Secondary endpoint NIS-LL (multiple imputation)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	8.2	
		95% CI	(4.0, 12.5)	
		P-value	p=0.0002	
	Secondary endpoint SF-36 Physical (longitudinal)	Comparison groups	Diflunisal vs placebo	
		Difference between groups	-6.1	
		95% CI	(-9.8, -2.5)	
		P-value	p=0.0013	

	Secondary endpoint SF-36 Physical (multiple imputation)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-6.4
		95% CI	(-9.8, -2.9)
		P-value	p=0.0003

Effect estimate per comparison	Secondary endpoint SF-36 Mental (longitudinal)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-4.5
		95% CI	(-9.2, 0.2)
		P-value	p=0.062
	Secondary endpoint SF-36 Mental (multiple imputation)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-4.9
		95% CI	(-9.0, -0.7)
		P-value	p=0.0216
Notes	A degree of missing longitudinal data was inevitable given the long duration of the study and the severity of the condition. The longitudinal analyses above do not consider the impact of missing data. The multiple imputation (MI) analyses were originally-planned sensitivity analyses. These took missing data into account by including a mixture of fixed value and multiple imputation under a missing at random (MAR) assumption. Additional post-hoc sensitivity analyses were also performed: • An MI analysis whereby intermittent missing data were multiply imputed to generate a monotone missing data pattern and then the remaining missing data were imputed assuming MAR (no fixed value imputation was included). • A "jump to placebo" analysis whereby missing observations in both the experimental and placebo groups were imputed as missing not at random (MNAR) using only data observed in the placebo group (i.e. instantaneous loss of efficacy and conversion to placebo was assumed upon cessation of diflunisal therapy). • A tipping point analysis which assessed the robustness of results under an MNAR assumption, successively imputing missing data in the experimental arm under an increasingly punitive penalty to identify the degree of penalty that needed to be applied to subjects with missing data in the experimental arm in order to force the loss of statistical significance seen in the primary analysis. These analyses are referred to as "MI/MAR", "Jump to Placebo" and "Tipping Point" in the secondary analyses reported in the remainder of this table.		
Analysis description	**Secondary (post-hoc sensitivity) analyses**		
Analysis population and time point description	Intent to treat at 2-year time point.		
Descriptive statistics and estimate of variability	Treatment group	Diflunisal	Placebo
	Number of subjects	64	66
	NIS+7 (MI/MAR) LS mean (95% CI)	+6.2 (1.4, 11.1)	+22.6 (15.6, 29.5)

	NIS+7 (Jump to Placebo) LS mean (95% CI)	13.1 (6.7, 19.54)	22.0 (12.3, 31.7)
	Kumamoto scale (MI/MAR) LS mean (95% CI)	2.6 (0.5, 4.6)	6.9 (3.8, 9.9)

	Kumamoto scale (Jump to Placebo) LS mean (95% CI)	4.2 (1.7, 6.7)	7.4 (4.3, 10.5)
	Kumamoto scale (Tipping Point) Tipping Point Delta % Loss of Treatment to Render p>0.05	$\Delta = 1.5$ 30.5%	
	NIS (MI/MAR) LS mean (95% CI)	4.8 (0.6, 9.0)	19.7 (26.3, 13.1)
	NIS (Jump to Placebo) LS mean (95% CI)	0.9 (0.5, 16.9)	19.2 (10.0, 28.4)
	NIS (Tipping Point) Tipping Point Delta % Loss of Treatment to Render p>0.05	Δ =15.5 96.1%	
Descriptive statistics and estimate of variability	NIS-LL (MI/NAR) LS mean (95% CI)	2.5 (-0.2, 5.2)	10 (6.1, 13.9)
	NIS-LL (Jump to Placebo) LS mean (95% CI)	5.8 (2.2, 9.4)	9.7 (4.0, 15.5)
	NIS-LL (Tipping Point) Tipping Point Delta % Loss of Treatment to Render p>0.05	Δ =4.3 52.2%	
	SF-36 Physical (MI/MAR) LS mean (95% CI)	2.0 (-0.5, 4.5)	-3.8 (-7.4, -0.2)
	SF-36 Physical (Jump to Placebo) LS mean (95% CI)	0.1 (-2.7, 2.9)	-3.8 (-7.6, 0.0)
	SF-36 Physical (Tipping Point) Tipping Point Delta % Loss of Treatment to Render p>0.05	Δ =4.6 -72.3%	

SF-36-Mental (MI/MAR) LS mean (95% CI)	3.2 (0.3, 6.2)	-0.7 (-4.2, 2.9)
SF-36-Mental (Jump to Placebo) LS mean (95% CI)	2.3 (-1.2, 5.9)	-0.7 (-4.9, 3.4)
SF-36-Mental (Tipping Point) Tipping Point Delta % Loss of Treatment to Render p>0.05	NA	

Effect estimate per comparison	Primary endpoint NIS+7 (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	16.3
		95% CI	(8.1, 24.6)
		P-value	p=0.0002
	Primary endpoint NIS+7 (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	8.9
		95% CI	(-1.0, 18.8)
		P-value	p=0.0774
	Primary endpoint NIS+7 (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	10.4
		95% CI	(0.0, 20.9)
		P-value	p<0.0496
		Tipping Point delta	15.7
	Secondary endpoint Kumamoto scale (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	4.3
		95% CI	(0.5, 8.0)
		P-value	p=0.026
	Secondary endpoint Kumamoto scale (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	3.2
		95% CI	(-0.5, 6.8)
		P-value	p=0.0889
	Secondary endpoint Kumamoto scale (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	3.9
		95% CI	(0.0, 7.7)
		P-value	p=0.0480
		Tipping Point delta	1.5
	Secondary endpoint NIS (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	14.9
		95% CI	(7.3, 22.5)
		P-value	p=0.0002

	Secondary endpoint NIS (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	8.2
		95% CI	(-1.2, 17.7)
		P-value	p=0.0859
	Secondary endpoint NIS (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	9.1
		95% CI	(0.0, 18.2)
		P-value	p=0.0492
		Tipping Point delta	15.5

Effect estimate per comparison	Secondary endpoint NIS-LL (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	7.5
		95% CI	(2.9, 12.1)
		P-value	p=0.0017
	Secondary endpoint NIS-LL (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	3.9
		95% CI	(-1.9, 9.7)
		P-value	p=0.1826
	Secondary endpoint NIS-LL (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	5.8
		95% CI	(0.0, 11.5)
		P-value	p=0.0487
		Tipping Point delta	4.3
	Secondary endpoint SF-36 Physical (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-5.9
		95% CI	(-10.1, -1.6)
		P-value	p=0.0074
	Secondary endpoint SF-36 Physical (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-3.9
		95% CI	(-7.7, 0.0)
		P-value	p=0.0486
	Secondary endpoint SF-36 Physical (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	4.0
		95% CI	(0.0, 7.9)
		P-value	p=0.0485
		Tipping Point delta	4.6
	Secondary endpoint SF-36 Mental (MI/MAR)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-3.9
		95% CI	(-8.4, 0.6)

		P-value	p=0.0854
	Secondary endpoint SF-36 Mental (Jump to Placebo)	Comparison groups	Diflunisal vs placebo
		Difference between groups	-3.1
		95% CI	(-8.0, 1.8)
		P-value	p=0.2175
	Secondary endpoint SF-36 Mental (Tipping Point)	Comparison groups	Diflunisal vs placebo
		Difference between groups	Not calculated
		95% CI	
		P-value	
		Tipping Point delta	

Clinical studies in special populations

The applicant did not specifically address this issue, and did not provide the data to populate the table below.

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials			
Non Controlled trials			

2.6.5.3. In vitro biomarker test for patient selection for efficacy

Not applicable.

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

Not applicable.

2.6.5.5. Supportive study(ies)

Below is described the only document referring to the registry database. The database name and properties are not specifically provided.

Provided title: Diflunisal Registry Analysis: A Brief Abbreviated Statistical Analysis Report

Background and objectives:

Dr Jonas Wixner is as specialist physician in the Department of Medicine, Unit of Gastroenterology and Hepatology at the Umeå University Hospital, Sweden. Dr Wixner run the Amyloidosis Centre at Umeå and, in so doing, has compiled a registry of 123 subjects with FAP who have been treated with diflunisal in clinical practice.

This purpose and objectives of this abbreviated statistical report are to:

(i) Examine these registry data and estimate, in so far as is possible, the rates of change in key clinical outcomes over time following treatment with diflunisal;

(ii) And, where possible, seek to combine these rate of change estimates with data collected in the Berk et al 2013 randomised controlled trial (EudraCT number 2006-001066-16) via augmented Bayes analysis.

Registry Data

Given the nature of registries, data are not collected in a systematic fashion at regular intervals post initiation of diflunisal treatment, rather data are collected idiosyncratically from patient to patient according to the nature and severity of their underlying disease. Nevertheless, sufficient data were captured to allow the analysis of Kumamoto score, PND score, FAP score, mBMI, NYHA classification and Karnofsky performance score. These variables are defined as follows:

- Kumamoto score is a 14-item clinical neurological scale of motor, sensory and autonomic nerve function combined with heart and kidney end organ measures [Tashima 1997]. This score ranges from 0 to 96 points, with higher scores indicating greater neurological deficits.
- Polyneuropathy disability (PND) score and familial amyloidotic polyneuropathy (FAP) stage are related as defined as,

Polyneuropathy Disability (PND)	
Score	Description
0	No impairment
I	Sensory disturbances, preserved walking capability
II	Impaired walking capability but ability to walk without a stick or crutches
IIa	Walking only with the help of 1 stick or crutch
IIIb	Walking with the help of 2 sticks or crutches
IV	Confined to a wheelchair or bedridden

Familial Amyloidotic Polyneuropathy (FAP)	
Stage	Description
0	No symptoms
1	Unimpaired ambulation; mostly mild sensory and motor neuropathy in the lower limbs
1	Assistance with ambulation needed; mostly moderate impairment progression to the lower limbs, upper limbs and trunk
2	Assistance with ambulation needed; mostly moderate impairment progression to the lower limbs, upper limbs and trunk
2	Assistance with ambulation needed; mostly moderate impairment progression to the lower limbs, upper limbs and trunk
3	Wheelchair-bound or bedridden; severe sensory and motor neuropathy of all limbs

- mBMI is defined as the product of the subject's BMI ([weight in kilograms] divided by [height in meters]2) and the serum albumin concentration (in g/L).
- NYHA Classification
- Karnofsky Performance Score

A total of 118/123 registry subjects were included in analyses. Five subjects were missing a visit date such that the timing of assessments vis-à-vis the start of diflunisal treatment could not be determined and, therefore, these subjects could not be included in the analysis. While all N=118 remaining subjects were included, not all had data recorded for each of the parameters of interest. Thus, in

practice, each analysis involved fewer that N=118. The exact numbers included for any given analysis are displayed within SAS Tables in Appendices 2 and 3 relating which are also presented in abbreviated summary tables displayed within the text of this short report.

Statistical Methods

General

Given the lack of systematic data collection at predefined time intervals post initiation of diflunisal, the usual and simple summaries of data by clinic visit are not possible. Further, visit-windowing of data is also not possible given the high degree of inter-subject variability in the timing of assessment and data collection. With such data in hand, the most appropriate methodology to assess rate of change over time a random coefficients mixed effects modelling. This directly accommodates variability between subjects in both the timing of data collection and the number of data collection timepoints and, in so doing, provides unbiased estimates of rate of change over time with a standard error that reflects the extent of inter-subject variability.

Random Coefficients Mixed Effects Modelling

The random coefficients mixed effects model is of the form:

$yit = (\beta 0 + \xi i\ ) + (\beta 1 + \eta i\ ) \cdot tij + eij$

Where,

yit is the parameter value for subject $i = 1 \ldots N$ at timepoint $j = 1, \ldots k$

$\beta 0$ is the fixed intercept effect.

$\beta 1$ is the fixed effect of slope over time

tij is time of the jth parameter value in subject i

$\xi i$is the random effect of subject i intercept

$\eta i$is the random effect of subject i on slope over time

eij is the random error for subject i at timepoint j

The random effects, ξi and ηi are assumed independent and identically distributed normally distributed with variance components $\sigma\xi$ 2 and $\sigma\eta$ 2, and, independently, the random error eij is normally distributed with variance $\sigma 2$. The change in parameter value from time zero to time T is thus given by $\beta 1T$.

Given the estimated values of $\beta 0$ and $\beta 1$, ie, $\beta\ \hat{}\ _0$ and $\beta\ \hat{}\ _1$, along with their standard errors, $SE\ \hat{}(\beta\ \hat{}\ _0)$ and $SE\ \hat{}(\beta\ \hat{}\ _1)$, estimated covariance, $Cov(\beta\ \hat{}\ _0,\beta\ \hat{}\ _1)$ and estimated random error variance $\sigma\hat{}2$, the overall expected value of yit, $E[yit]$, is given by

$E[yit] = \beta\ \hat{}\ _0 + \beta\ \hat{}\ _1 \cdot t$

with variance V

$V[yit] = V[\beta\ \hat{}\ _0 + \beta\ \hat{}\ _1 \cdot t] = [SE\ \hat{}(\beta\ \hat{}\ _0)]^2 + [SE\ \hat{}(\beta\ \hat{}\ _1)]^2 \cdot t2 + 2Cov(\beta\ \hat{}\ 0,\beta\ \hat{}\ 1)\ t$

$V[yit]$ then provides the basis for construction of a confidence bank around the expected value $E[yit]$ and, similarly, confidence band on the expected individual value $E[yit]$ can be constructed using $V[yit] = V[yit] + \sigma\hat{}^2$.

Bayesian Augmentation

Bayesian augmentation involves the analysis of randomised controlled trial (RCT) data whereby one or more of the randomised treatment arms are strengthened by combination, or 'augmentation', with relevant external trial data that serve as an informative prior. Randomised arms without relevant, external data are augmented with a non-informative prior. The goal of Bayesian augmentation is to provide more precise inferences and higher power than the analysis the RCT data can provide in and of themselves.

Based upon data presented in the Berk et al 2013 RCT, Bayesian augmentation was possible for Kumamoto score and mBMI.

Results

Population Description

Of the n=118 subjects included in the analyses, 88/118 (75%) were male, 81/118 (69%) had neuropathy at onset, 110/118 (93%) had a TTR mutation of which the major genotype was VM30 in 102/118 (86%) of subjects.

The estimated mean (SD) time in receipt of diflunisal therapy was 3.4 (2.0) years, with a range of (0.1 to 10.2) years. The mean (SD) age at FAP diagnosis was 68.2 (9.6) years and mean (SD) duration of FAP since diagnosis was 7.6 (4.4) years.

In the Berk et al RCT, subjects treated with diflunisal were similar in terms of gender with 67% male but were younger than registry subjects with mean (SD) age of 61 (11.7) years. The fraction of subjects with a VM30 mutation was 56% in the Berk et al RCT, and so was rather lower than the corresponding fraction of registry subjects. As captured below in Section 4.2, mean Kumamoto score for registry subjects at the start of diflunisal therapy was 12.2, similar to that seen in the Berk et al RCT with a mean score of 15.3. PND mean class at the start of diflunisal therapy was 3 (where PND class 0=1, PND class I =2, PND class II = 3, PND class IIIA=4, PND class IIIB=5 and PND class IV = 6), which is similar to the mean baseline PND score of 2.8 based on data reported by Berk et al. And mBMI was also similar being 936 kg/m2×g/L at the start of diflunisal therapy and 1024 kg/m2×g/L in the Berk et al RCT. Other variables at the time of start of diflunisal therapy were Karnofsky score of 80, NYHA mean class of 1.5 (where class I=1, class II=2, class III=3, class IIIA=3, class IIIB=4 and class IV=5) and FAP mean class of 1.4 (where FAP class 0/PND class 0 =1, FAP class 1/PND class I =2, FAP class 1/PND class II = 3, FAP class 2/PND class IIIA = 4, FAP class 2/PND class IIIB = 5 and FAP class 3/PND class IV = 6).

Random Coefficients Mixed Effects Modelling Results

The results of random coefficients analyses of Kumamoto score, Karnofsky performance score, PND score, mBMI, NYHA classification and FAP score are summarised in text tables T10 and T11 below.

Apart from Kumamoto Score and NYHA class, all variables examined were associated with statistically significant slope estimates over time:

• For Karnofsky performance score, the mean value at start of diflunisal therapy (i.e. the intercept estimate) was 80 with slope $\hat{\beta}1 = -0.1311$, $p = 0.0038$, which, translates to an annual change of -1.57 units with 95% CI (-1.94, -1.21).

• For PND score, the mean value at start of diflunisal therapy was 3.1 with slope $\hat{\beta}1 = 0.016$, $p = 0.0001$, which, translates to an annual change of 0.19 with 95% CI (0.13 , 0.25).

• For mBMI, the mean value at start of diflunisal therapy was 936 with slope $\hat{\beta}_1 = -1.1568$, $p = 0.0004$, which, translates to an annual change of -13.9 units with 95% CI (-20.9, -6.84).

• For FAP class, the mean value at start of diflunisal therapy was 1.35 with slope $\hat{\beta}_1 = 1.348$, $p = 0.0001$, which, translates to an annual change of 0.07 with 95% CI (0.05, 0.10).

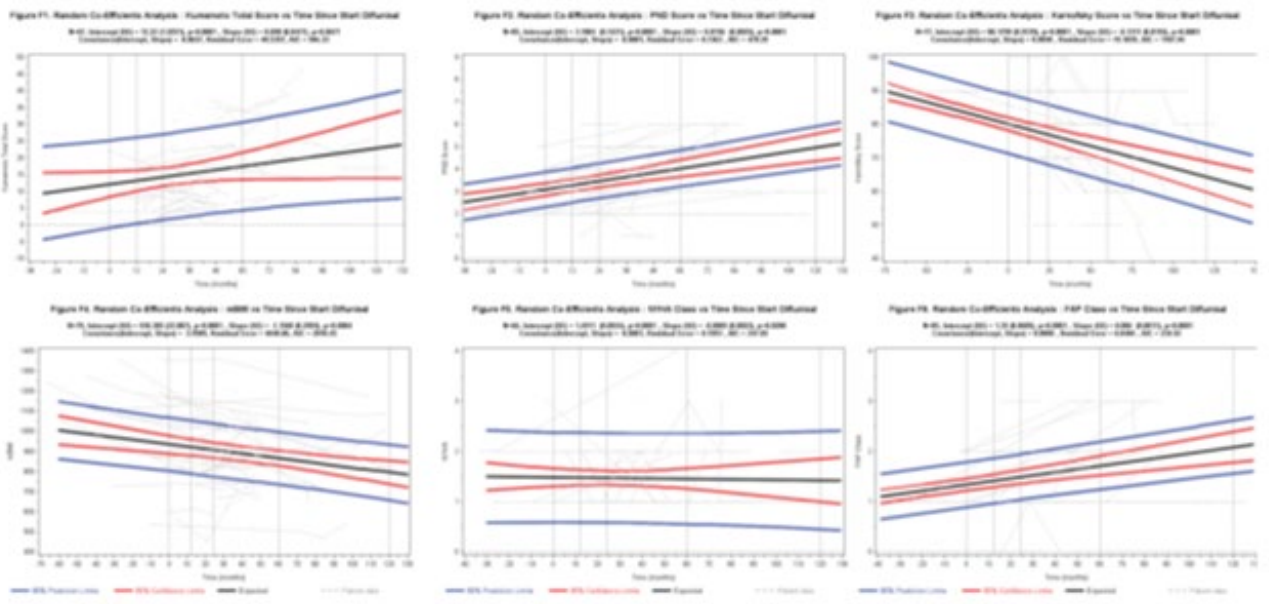
Table 9: Random coefficients Model Parameter Estimates

Variable	N	Intercept [SE] $\hat{\beta}_0\ [\widehat{SE}(\hat{\beta}_0)]$	Slope [SE] $\hat{\beta}_1\ [\widehat{SE}(\hat{\beta}_1)]$	$Cov(\hat{\beta}_0,\hat{\beta}_1)$	Residual Error, $\hat{\sigma}^2$
Kumamoto Score	67	12.22 (1.9251), p=0.0001	0.090 (0.0477), p=0.0677	-0.0637	40.5357
Karnofsky Score	77	80.189 (0.973), p=0.0001	-0.1311 (0.0155), p=0.0001	0.0038	19.1839
PND Score	85	3.100 (0.1431), p=0.0001	0.016 (0.0025), p=0.0001	-0.0001	0.1363
mBMI	79	936.31 (22.81), p=0.0001	-1.1568 (0.2993), p=0.0004	-3.9589	4040.86
NYHA Class	66	1.491 (0.0924), p=0.0001	-0.0005 (0.0022), p=0.8280	-0.0001	0.1993
FAP Class	85	1.348 (0.0605), p=0.0001	0.006 (0.0011), p=0.0001	0	0.0484

Table 10: Estimated Mean [SE] Change over time and 95% CI

Time Since Start Diflunisal (months)	Kumamoto Score Mean Change [SE], 95%CI	Karnofsky Score Mean Change [SE], 95%CI	PND Score Mean Change [SE], 95%CI
12	1.08 [0.572] (-0.04, 2.20)	-1.57 [0.186] (-1.94, -1.21)	0.19 [0.030] (0.13 , 0.25)
24	2.15 [1.144] (-0.09, 4.40)	-3.15 [0.372] (-3.87, -2.42)	0.37 [0.060] (0.26 , 0.49)
36	3.23 [1.717] (-0.13, 6.60)	-4.72 [0.558] (-5.81, -3.63)	0.56 [0.091] (0.38 , 0.74)
48	4.31 [2.289] (-0.18, 8.79)	-6.29 [0.744] (-7.75, -4.83)	0.75 [0.121] (0.51 , 0.99)
60	5.39 [2.861] (-0.22, 10.99)	-7.86 [0.929] (-9.69, -6.04)	0.94 [0.151] (0.64 , 1.23)
72	6.46 [3.433] (-0.27, 13.19)	-9.44 [1.115] (-11.6, -7.25)	1.12 [0.181] (0.77 , 1.48)
84	7.54 [4.005] (-0.31, 15.39)	-11.0 [1.301] (-13.6, -8.46)	1.31 [0.211] (0.90 , 1.72)
96	8.62 [4.577] (-0.36, 17.59)	-12.6 [1.487] (-15.5, -9.67)	1.50 [0.242] (1.02 , 1.97)
108	9.69 [5.150] (-0.40, 19.79)	-14.2 [1.673] (-17.4, -10.9)	1.68 [0.272] (1.15 , 2.22)
120	10.77 [5.722] (-0.44, 21.98)	-15.7 [1.859] (-19.4, -12.1)	1.87 [0.302] (1.28 , 2.46)
12	-13.9 [3.591] (-20.9, -6.84)	-0.006 [0.0261] (-0.057 , 0.045)	0.07 [0.013] (0.05 , 0.10)
24	-27.8 [7.182] (-41.8, -13.7)	-0.011 [0.0521] (-0.114 , 0.091)	0.15 [0.027] (0.10 , 0.20)
36	-41.6 [10.773] (-62.8, -20.5)	-0.017 [0.0782] (-0.170 , 0.136)	0.22 [0.040] (0.15 , 0.30)
48	-55.5 [14.365] (-83.7, -27.4)	-0.023 [0.1043] (-0.227 , 0.182)	0.30 [0.053] (0.20 , 0.40)
60	-69.4 [17.956] (-105 , -34.2)	-0.029 [0.1303] (-0.284 , 0.227)	0.37 [0.066] (0.24 , 0.50)
72	-83.3 [21.547] (-126 , -41.1)	-0.034 [0.1564] (-0.341 , 0.272)	0.45 [0.080] (0.29 , 0.61)
84	-97.2 [25.138] (-146 , -47.9)	-0.040 [0.1825] (-0.398 , 0.318)	0.52 [0.093] (0.34 , 0.71)
96	-111 [28.729] (-167 , -54.7)	-0.046 [0.2086] (-0.454 , 0.363)	0.60 [0.106] (0.39 , 0.81)
108	-125 [32.320] (-188 , -61.6)	-0.051 [0.2346] (-0.511 , 0.409)	0.67 [0.120] (0.44 , 0.91)
120	-139 [35.911] (-209 , -68.4)	-0.057 [0.2607] (-0.568 , 0.454)	0.75 [0.133] (0.49 , 1.01)

Figure 6: Random coefficients analysis results



Bayesian Augmentation Results

The results of the Bayesian Augmentation analysis are provided in text Table 12. For ease of reference, the Test Table 13 replicates the results for Kumamoto score and mBMI as presented in the Clinical Study Report (CSR) prepared for the Berk et al. study.

As can be seen, when the Berk et al CSR results are augmented with the diflunisal registry data, treatment effect estimates for both Kumamoto Score and mBMI at 12 and 24 months remain similar to those based on the Berk et al RCT data alone, however the Bayesian CIs narrows due to increased precision and the probability of superiority reinforces the CSR based result, surpassing the strength of evidence offered by the usual p-value.

Table 11: Bayesian augmentation results

Variable	Time Point (months)	Placebo CFB [SE] 95% CI	Diflunisal CFB [SE] 95% CI	Placebo vs Diflunisal CFB [SE] 95% CI	1-sided P-value	Pr(Diflunisal Superior)
Kumamoto Score	12	4.2 [1.15] (2.0 , 6.5)	1.4 [0.65] (0.1 , 2.7)	2.8 [1.32] (0.2 , 5.4)	0.0176	98.2%
	24	6.9 [1.54] (3.9 , 9.9)	2.4 [0.74] (0.9 , 3.8)	4.5 [1.70] (1.2 , 7.8)	0.0045	99.6%
mBMI	12	-45.0 [19.99] (-84.6, -5.8)	-18.7 [11.26] (-40.7, 3.5)	-26.3 [22.97] (-71.4, 18.7)	0.1257	87.5%
	24	-71.2 [24.12] (-118 , -23.8)	-29.2 [11.27] (-51.3, -6.9)	-42.1 [26.62] (-94.1, 10.4)	0.0572	97.2%

Registry data provide a diflunisal GammaNormal prior and are combined in a Bayesian fashion with the diflunisal data in Berk et al CSR.
A non-informative GammaNormal prior is used for placebo data in Berk et al CSR.

Table 12: Replication of Berk et al CSR Tables 20 and 21

Variable	Time Point (months)	Placebo CFB 95% CI	Diflunisal CFB 95% CI	Placebo vs Diflunisal CFB 95% CI	1-sided P-value	2-sided P-value
Kumamoto Score	12	4.2 (2.0, 6.5)	1.8 (0.0, 3.6)	2.4 (-0.4,5.3)	0.0476	0.0951
	24	6.9 (3.8, 9.9)	2.6 (0.5, 4.6)	4.3 (0.5, 8.0)	0.0130	0.0260
mBMI	12	-45.0 (-84.5, 5.5)	-19.7 (-53.1, 13.7)	-25.3 (-76.2, 25.6)	0.1640	0.3280
	24	-71.3 (-119, 23.3)	-20.8 (-54.2, 12.5)	-50.4 (-106, 5.1)	0.0374	0.0748

Summary

The characteristics of the n=118 registry subjects included in the analysis in this report were broadly similar to the diflunisal treated subjects in Berk et al; Kumamoto score at the start of diflunisal therapy

was similar as was PND class and mBMI. However, registry subjects were older (mean age 68 years vs 61 years in Berk et al) and the fraction of subjects with a VM30 mutation was higher (86% vs 56% in the Berk et al). Over the period of diflunisal treatment, there was an upward trend in Kumamoto score, PND class and FAP class score, a downward trend in Karnofsky score and mBMI while NYHA class score remained flat. These data suggest a progression of disease over time as would be expected, albeit only gradual. This can be seen by examining mean change in parameter values after 24 months of diflunisal therapy; the mean change in Kumamoto score was 2.15 95% CI (-0.09, 4.40), Karnofsky score was -3.15 95% CI (-3.87, -2.42), PND score was 0.37 95% CI (0.26, 0.49), mBMI was -27.8 95% CI (-41.8, -13.7), NYHA class score was -0.011 95% CI (-0.114, 0.091) and FAP class score was 0.15 95% CI (0.10, 0.20).

Finally, Bayesian augmentation of the Berk et al data with the diflunisal registry data served to enhance treatment effect estimates versus placebo; the probability diflunisal therapy is superior to placebo after 24 months was 99.6% and 97.2% for Kumamoto score and mBMI respectively.

The applicant has provided further external data upon request:

SUPPORT FROM EXTERNAL DATA

External support for the efficacy of diflunisal in the treatment of hereditary transthyretin amyloid polyneuropathy (ATTRv-PN) (previously known as familial amyloid polyneuropathy, FAP) may be derived from four published studies which were not sponsored by the applicant. These are summarised in date order below.

Takahashi et al, 2014

Design

- *Structure:* Prospective open-label single centre study.
- *Population:* Consecutive patients with FAP and a Val30Met mutation from an endemic area in Japan.
- *Sample size:* 6.
- *Diflunisal dose and treatment duration:* 250 mg twice daily for 4.4 ± 0.9 years.
- *Endpoints (none defined as primary):*
    - Clinical manifestations.
    - Stage of FAP.
    - Kumamoto FAP score.
    - Modified body mass index (mBMI).
    - Grip power.
    - Medical Research Council (MRC) sum score.
    - Nerve conduction studies (NCS).
    - Electrocardiogram (ECG), ECG Holter monitor test and echocardiography.
    - ^{123}iodinemetaiodobenzylguanidine (^{123}I-MIBG) imaging.

Results

Clinical manifestations

One patient discontinued diflunisal therapy after 6 months due to a finding of haematuria on urinalysis. His renal function remained normal, and haematuria cleared after discontinuing diflunisal.

Among the five patients who continued diflunisal treatment, four had symptoms of autonomic neuropathy at baseline, including orthostatic hypotension, alternating diarrhoea and constipation. The autonomic symptoms (orthostatic hypotension and gastrointestinal symptoms) resolved in two patients within one month after starting diflunisal treatment. Motor and sensory symptoms gradually progressed in four patients, but not in the fifth.

FAP stage

FAP stage progressed from 1 to 2 in one patient and remained stable in the other four.

Kumamoto FAP score

In the five assessable patients, Kumamoto score rose from 16.0 ± 4.1 at baseline to 23.0 ± 8.0 after 3 years treatment (p=0.07).

Modified body mass index

In the five assessable patients, mBMI fell from 857.5 ± 67.4 at baseline to 818.7 ± 88.8 after 3 years treatment (p=0.31).

Grip power

In the five assessable patients, grip power fell from 34.0 ± 2.3 kg at baseline to 28.8 ± 8.2 kg after 3 years treatment (p=0.34).

MRC sum score

Motor sum scores deteriorated in three patients.

Nerve conduction studies

At baseline, sensory nerve action potentials (SNAPs) were not evoked in most patients, and compound muscle action potentials (CMAPs) were below the normal ranges in four out of six patients. NCS remained stable during diflunisal treatment.

ECG, Holter and echocardiography

In addition to ECG abnormalities at baseline, two patients developed first-degree atrioventricular block during the observational period.

On echocardiography, ejection fraction (EF) was maintained over 50% during the whole observation period. Mean left ventricular (LV) wall thickness increased slightly, and exceeded the normal range.

Impaired LV relaxation patterns as shown by decreased ratio of mitral peak velocity of early filling to mitral peak velocity of late filling (E/A ratio) and prolonged deceleration time appeared in five patients.

Ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e') (E/e') values were elevated in three patients at baseline suggesting elevated LV filling pressure. During the observation period, the E/e' value of two patients tended to improve or remained stable.

^{123}I-MIBG imaging

At baseline, ^{123}I-MIBG imaging showed the reduction of early and delayed H/M ratios and an increase of washout rate in all but one patient. After treatment, the delayed H/M ratio showed statistically significant improvement three years after the treatment in all the five available patients.

Authors' conclusions

"Our results indicated that diflunisal would be effective for autonomic symptoms of late-onset FAP with a TTR Val30Met mutation. This treatment would be helpful for late-onset FAP patients who are not considered indications for LTs [i.e. liver transplant]. Further study with large sample size in late-onset FAP is required to ensure the efficacy and safety of diflunisal."

Sekijima et al, 2015

Design

- *Structure:* Prospective open-label single centre study.
- *Population:* Japanese patients with biopsy proven amyloid deposition and mutant TTR genopositivity who exhibited signs of peripheral or autonomic neuropathy.
- *Sample size:* 40.
- *Diflunisal dose and treatment duration:* 250 mg twice daily for 2 to 116 months (mean ± SD: 38.0 ± 31.2 months).
- *Endpoints (none defined as primary):*
    - Clinical FAP score.
    - Modified BMI.
    - Intra-ventricular septum + left ventricular posterior wall thickness.
    - Ejection fraction.
    - Ulnar nerve CMAP.
    - Tibial nerve CMAP.
    - Plasma BNP.
    - Plasma hANP.

Results

The results are summarised in Table 14 which is derived from Tables 2 and 3 of the paper (*Sekijima et al, 2015*).

Table 13: Results from Sekijima et al, 2015

	Baseline to 12 months (n=28)	12 to 24 months (n=21)	24 to 36 months (n=16)	36 to 48 months (n=14)	After 48 months (n=11)	Throughout the course (n=28)
Change in outcome per year						
Clinical FAP score	0.74 ± 1.46	1.48 ± 2.91	0.63 ± 3.00	1.57 ± 3.74	0.87 ± 1.34	0.98 ± 1.39
Modified BMI	-33.5 ± 56.0	-8.4 ± 63.8	-44.8 ± 95.2	-10.7 ± 96.6	-51.6 ± 56.8	-28.1 ± 25.6
IVS+LVPW (mm)	0.64 ± 2.75	0.17 ± 3.10	0.64 ± 3.6	-0.49 ± 3.23	-0.18 ± 1.38	0.25 ± 1.74
Ejection fraction (%)	0.04 ± 6.99	-0.94 ± 5.95	-1.77 ± 6.04	-0.52 ± 6.62	3.73 ± 6.97	-0.21 ± 3.76
Percent changes in outcome per year						
Ulnar nerve CMAP	-15.4 ± 33.0	1.8 ± 39.3	16.3 ± 48.1	-22.9 ± 38.7	-2.7 ± 19.6	-6.7 ± 18.1
Tibial nerve CMAP	-23.9 ± 59.2	-26.6 ± 35.1	3.6 ± 71.2	-43.9 ± 36.3	-4.2 ± 25.3	-29.2 ± 38.6
Plasma BNP	30.9 ± 69.6	11.2 ± 49.6	24.2 ± 62.1	30.0 ± 81.4	-6.4 ± 27.9	11.7 ± 42.5
Plasma hANP	41.2 ± 87.5	17.0 ± 74.0	14.1 ± 66.0	19.5 ± 42.8	11.8 ± 29.2	11.7 ± 32.7

FAP, familial amyloid polyneuropathy; mBMI, modified body mass index; IVS, intra-ventricular septum; LVPW, left ventricular posterior wall; CMAP, compound muscle action potential; BNP, brain natriuretic peptide; hANP, human atrial natriuretic peptide.

Authors' conclusions

"The deterioration rate of clinical FAP score in our diflunisal treated hereditary ATTR amyloidosis patients (1.0/year) was nearly identical to that in the previous clinical trial of this drug (1.4/year) [*Berk et al, 2013; the single pivotal trial of the application*] and much better than the natural history of untreated hereditary ATTR amyloidosis (3.3–7/year). In addition, deterioration rates of clinical FAP score were lower after 2 years of treatment, suggesting sustaining effects of diflunisal.

Longitudinal analyses of ulnar and tibial nerve CMAP amplitude, plasma BNP and hANP, cardiac wall thickness, and EF also demonstrated sustaining effects of diflunisal on both neurological and cardiac functions. Notably, ulnar CMAP amplitude, cardiac wall thickness, and EF did not show deterioration at all after 24 months of treatment. The only exception was mBMI, which deteriorated consistently throughout the course…"

Wixner et al, 2019

Design

- *Structure:* Open-label observational study.
- *Population:* Swedish patients with hereditary transthyretin (ATTRm) amyloidosis.
- *Sample size:* 54, of whom 17 completed the study.

- *Diflunisal dose and treatment duration:* 250 mg twice daily for 23 months.
- *Primary endpoint:* Change in the Kumamoto scale.
- *Secondary endpoints:*
    - Change in mBMI.
    - Change in plasma albumin x BMI.
    - Change in cardiac function (septal thickness, plasma proBNP).

Results

Change in the Kumamoto scale

For patients who completed the study protocol, total Kumamoto scores remained stable across the 3 timepoints of baseline, 12 months and 24 months (median score 13 vs 16 vs 17.5, p=0.21), as did the sub-scores for sensory neuropathy, autonomic neuropathy and organ dysfunction. However, motor neuropathy scores had increased slightly over time (0 vs 2.5 vs 4.5, p=0.02).

Change in modified body mass index

No significant changes were found for mBMI (1028 vs 918 vs 982; p=0.06).

Change in plasma albumin x BMI

The paper does not report the results for this secondary endpoint.

Change in septal thickness

Cardiac septum thickness increased over time (16.5 vs 16.5 vs 18 mm; p=0.01). No significant changes were found for those who had completed 12 months.

Change in plasma proBNP

No significant changes were found for plasma proBNP (532 vs 412 vs 457 ng/l; p=0.19).

Authors' conclusions

"Although limited by high dropout rates, mainly due to liver transplantation and study closure [*Note; the study closed because the supply of diflunisal ran out*], the DFNS01 trial supports the safety and efficacy of diflunisal for ATTRm amyloidosis, and the results are in line with the previous placebo-controlled trial [*Berk et al, 2013; the single pivotal trial of the application*]. Total Kumamoto scores and nutritional status remained stable, however, motor neuropathy scores and cardiac septum thickness increased significantly during the study, which suggests that complete disease stabilisation is not achieved on group level. No obvious difference in outcome was noted with regard to amyloid fibril type, but the number of patients was low. Further studies are needed to evaluate the long-term effect of diflunisal and whether all sub-groups of patients have the same beneficial treatment effect."

Chao et al, 2024

Design

- *Structure:* Single-centre prospective study of patients receiving diflunisal or tafamidis compared to a historical control group of patients receiving no treatment.
- *Population:* Taiwanese patients with ATTRv-PN as evidenced by transthyretin pathogenic mutation, clinical evidence of sensorimotor or autonomic neuropathic symptoms and evidence of axonal polyneuropathy on nerve conduction studies or skin biopsy.

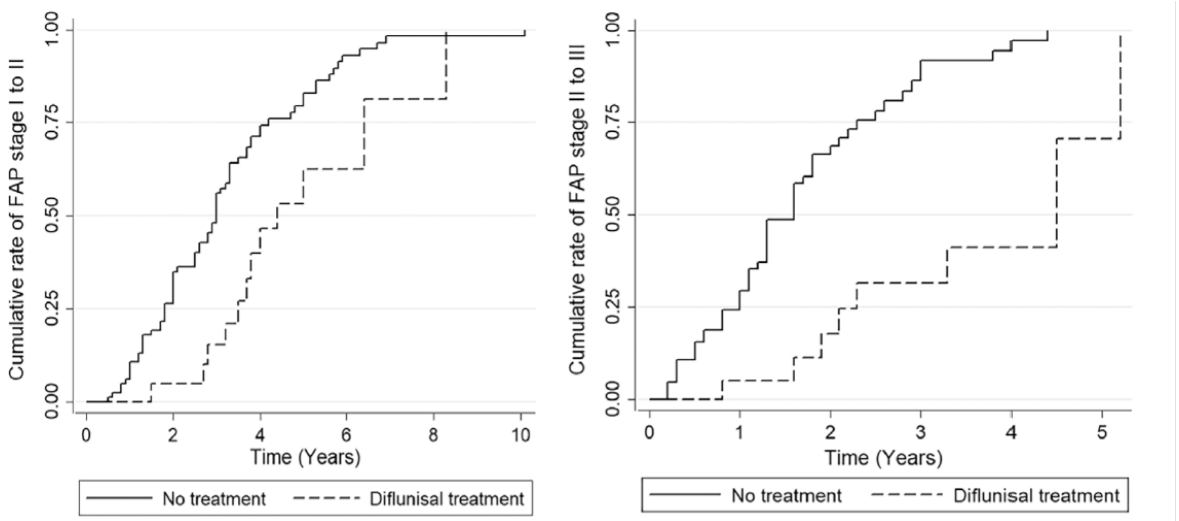
- *Sample size:* Diflunisal 35; tafamidis 22; historical no-treatment controls 85.
- *Dose and treatment duration:*
    - Diflunisal: 500 mg/day (n=23); 375 mg/day (n=4); 250 mg/day (n=8); 31.6 ± 15.3 months.
    - Tafamidis: 61 mg once daily; 35.3 ± 11.5 months.
- *Endpoints (none defined as primary):*
    - Transition times of FAP stage 1 to 2 and 2 to 3.
    - Nerve conduction studies.

Results

Transition times of FAP stage 1 to 2 and 2 to 3

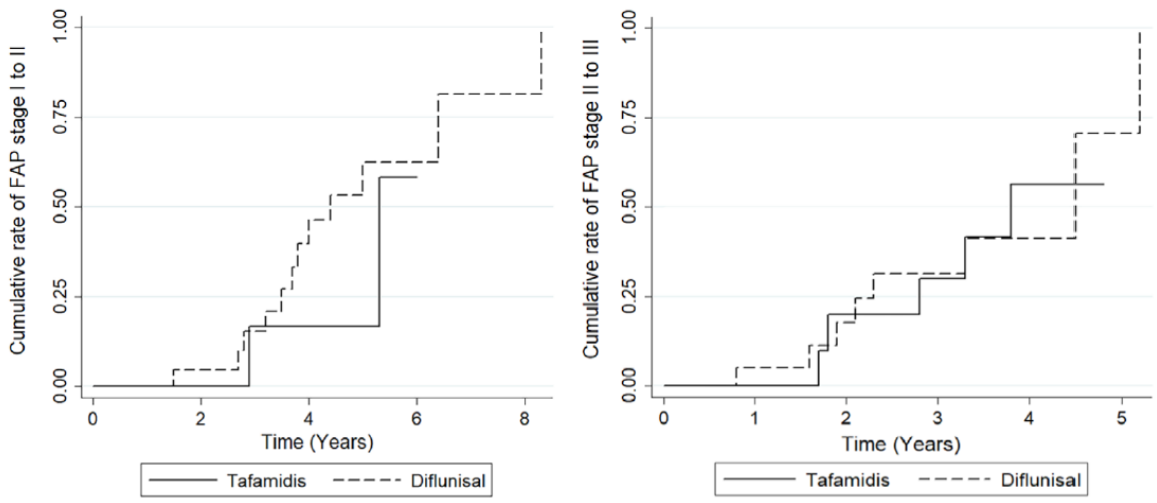
The paper presents separate comparisons of diflunisal vs no treatment (Figure 7) and diflunisal vs tafamidis (Figure 8).

Figure 7: Change in FAP stage: diflunisal vs no treatment



Diflunisal treatment significantly delayed the transition of FAP Stage 1 to 2 (HR=0.43; 95% CI 0.23-0.79; p=0.007) and of FAP Stage 2 to 3 (HR=0.18; 95% CI 0.08-0.43; p<0.001).

Figure 8: Change in FAP stage: diflunisal vs tafamidis

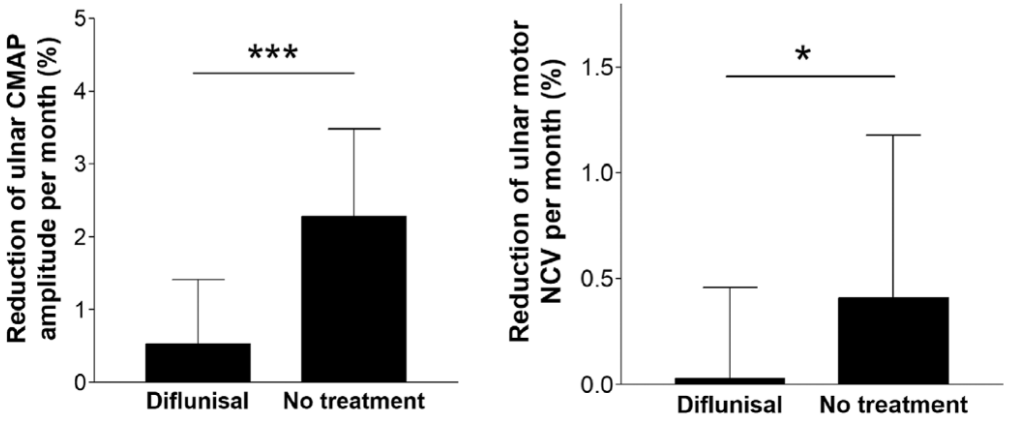


ATTRv-PN patients treated with diflunisal and those treated with tafamidis did not differ between groups in progression from the onset of FAP Stage 1 to 2 (p=0.332) or from the onset of Stage 2 to Stage 3 (p=0.993).

Nerve conduction studies

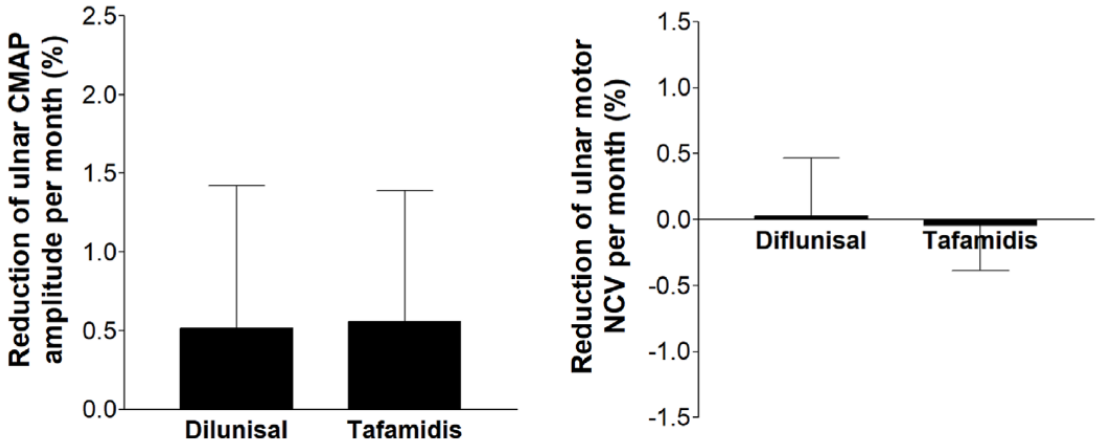
Results are again presented separately for the comparisons of diflunisal vs no treatment (Figure 9) and diflunisal vs tafamidis (Figure 10).

Figure 9: Nerve conduction studies: diflunisal vs no treatment



The reduction in the amplitude of the ulnar CMAP per month (%) from baseline was significantly lower in patients treated with diflunisal than in treatment-naïve patients (p<0.001), as was the decrease in ulnar motor conduction velocity (%) per month from baseline (p=0.027).

Figure 10: Nerve conduction studies: diflunisal vs tafamidis



The reduction in amplitude of CMAP per month (%) from baseline (p=0.889) and the decrease in ulnar motor conduction velocity per month from baseline (p=0.623) were similar in patients treated with diflunisal and tafamidis.

Authors' conclusions

"Both diflunisal and tafamidis can inhibit acid-mediated A97S-TTR aggregation by effectively stabilizing tetrameric A97S-TTR. Diflunisal has been proven to delay the progression of both polyneuropathy and cardiomyopathy without discernible differences in the progression of biomarkers compared to tafamidis in late-onset ATTRv-PN patients. Thus, diflunisal may become an obtainable, cost-effective and practical alternative treatment for ATTRv amyloidosis in selected patients."

Applicant's Comments

Taken individually, each of these studies has weaknesses:

- None of the studies were blinded.
- Three had no control group while Chao et al (2024) had non-randomised tafamidis patients and historical treatment-naïve patients as controls.
- The sample size in Takahashi et al (2014) was very small.
- The only study with a European population (Wixner et al, 2019) lacks detail in its published report.

Nevertheless, taken as a body they provide a credible independent supportive background to the results of the single pivotal trial:

- In all cases the overall results are concordant with the finding from the pivotal study that diflunisal has efficacy in the treatment of ATTRv amyloidosis; there are no published studies that contradict that finding.
- All four teams of independent authors conclude that diflunisal is effective in this indication.

Chao et al (2024) is particularly supportive in that, in spite of its design limitations, it shows diflunisal to be significantly more effective than no treatment and to have similar efficacy to tafamidis. This is despite the fact that, in this study, tafamidis was given at the 61 mg dose. According to Section 5.2 of the tafamidis 61 mg SmPC "the relative bioavailability of tafamidis 61 mg is similar to tafamidis meglumine 80 mg at steady-state. Tafamidis and tafamidis meglumine are not interchangeable on a

per mg basis." Therefore, the dose of tafamidis used in this study corresponds to 4-fold the dose authorised for use in the ATTRv-PN indication in Europe.

In addition to the publications discussed above, it is relevant to keep in mind the patient registry data presented in Module 2.5 of the initial MAA. These data concerned n=118 ATTRv-PN patients treated for a mean of 3.4 years (maximum 10.2 years). The registry data were compared vs the pivotal trial data and used to augment the trial data in a Bayesian analysis. The full report of this work is available in Module 5.3.5.3. Briefly, participants in the trial and in the registry were comparable with the exception of age and the proportion of patients with a Val30Met mutation:

Table 14: Baseline characteristics of pivotal trial and registry subjects

Characteristic	Pivotal trial	Registry
Age (years; ±SD)	61 (±11.7)	75.8 (estimated) †
Gender	67% m, 33% f	75% m, 25% f
Val30Met TTR mutation	56%	86%
Kumamoto score (mean)	15.3	12.2
PND score (mean)	2.8	3
mBMI (mean; kg/m^2·g/L)	1024	936

† The estimate is derived from the mean age at diagnosis (68.2 years) plus the mean time elapsed since diagnosis (7.6 years).

Over 24 months, changes in clinical status of registry patients derived from random coefficients mixed effects modelling were consistent with the expectation of gradual disease progression over time:

- Kumamoto score: +2.15 (95% CI: -0.09, 4.40).
- PND score: +0.37 (95% CI: 0.26, 0.49).
- FAP class score: +0.15 (95% CI: 0.10, 0.20)
- Karnofsky score: -3.15 (95% CI: -3.87, -2.42).
- mBMI: -27.8 kg/m^2·g/L (95% CI: -41.8, -13.7).
- NYHA class score: -0.011 (95% CI: -0.114, 0.091).

For Kumamoto score and mBMI, Bayesian augmentation of the pivotal trial data with the registry data yielded very high probabilities that diflunisal is superior to placebo for the augmented dataset at both 12 and 24 months:

Table 15: Bayesian augmentation results

Variable	Timepoint	Probability that diflunisal is superior to placebo for the augmented dataset
Kumamoto score	12 months	98.2%
	24 months	99.6%
mBMI (kg/m^2·g/L)	12 months	87.5%

	24 months	97.2%

As noted in Module 2.5, the registry data are highly consistent with the results of the pivotal clinical trial and provide valuable reassurance regarding their reliability.

Finally, based on the available evidence, diflunisal has been recommended as an effective therapy for the treatment of ATTRv in published national and international treatment guidelines. Particularly noteworthy among those is the International Society of Amyloidosis guidelines which rate the level of evidence supporting use of diflunisal on a par with evidence supporting use of the other currently available pharmacological therapies (Ando et al, 2022).

Mechanistic argument

The mechanistic rationale for the use of diflunisal to treat ATTRv amyloidosis was presented in sections 2.5.1.1 and 2.5.1.3 of Module 2.5. The relevant text is reproduced below for convenience:

TTR is a 55 kD homotetrameric protein composed of 127-residue β-sheet-rich subunits. It is stable in its homotetramer form and functions as a transporter of thyroxin (T4) and retinol (vitamin A)-binding protein under physiological conditions. It is widely accepted that the dissociation of natively folded TTR tetramers into monomers is a crucial step in the disease process, particularly in the aggregation of amyloid fibrils in ATTRv amyloidosis. Most TTR mutations result in the production of TTR that is less stable than wild-type TTR, leading to aggressive and systemic amyloid deposition of variant TTR (Koike & Katsuno, 2019).

The dissociation and subsequent aggregation of TTR may occur even in subjects without transthyretin gene mutations in certain conditions, such as aging, leading to an occurrence of wild-type transthyretin amyloidosis (ATTRwt); (Koike & Katsuno, 2019).

The pharmacology of diflunisal relevant to the proposed indication is its ability to stabilise transthyretin (this is separate and different from its traditional NSAID pharmacology).

As discussed above, TTR exists in plasma as a noncovalent, homotetramer (a dimer of dimers) presenting two identical binding sites located in a channel formed by the dimer-dimer interface and crossing the protein molecule (Corazza et al, 2019); in this form the molecule is stable.

The formation of amyloid is dependent on the dissociation of natively folded TTR tetramers into monomers (Koike & Katsuno, 2019). Binding of T4 to TTR stabilises the tetramer but the use of the hormone and its analogues in a therapeutic role is precluded by safety concerns. Attempts have therefore been made to identify other small molecules that exhibit the appropriate stereochemistry to bind to TTR. Diflunisal exhibits the appropriate stereochemistry and several studies have shown that diflunisal can bind to and stabilise TTR in its tetramer form (Sekijima et al 2006, Tojo K et al, 2006) hence preventing the dissociation to monomers.

This mechanism of action was independently investigated by Chao et al (2024) who studied interactions of A97S-TTR with tafamidis and diflunisal by measuring the reduction in the nuclear magnetic resonance (NMR) peak intensity using two-dimensional [^{15}N, ^{1}H]-NMR spectroscopy. In both cases, the resonance peaks that significantly shifted as a result of drug binding were mainly located at the dimer-dimer interface, previously reported as the thyroxine-binding site. They also evaluated whether tafamidis and diflunisal inhibited the amyloidogenicity of A97S *in vitro*, revealing that both compounds equally and almost sufficiently reduced fibril formation. In light of these experiments, the authors conclude:

"In summary, these structural and biochemical assessments indicated that both diflunisal and tafamidis effectively stabilise A97S-TTR and provided a foundation for treating ATTRv patients with either diflunisal or tafamidis."

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The applicant has provided data from study H-23750 to determine whether diflunisal inhibits (peripheral and autonomic neuropathic) disease progression in subjects with familial amyloid polyneuropathy. Diflunisal is a NSAID which has been on the market since the late 70s.

The study was conducted between May 2006 and Dec 2012, but results have been retrieved and analysed in 2023. The analyses foreseen in the original SAP were carried out. Further analyses were also carried out, as specified in a SAP amendment.

Although there is a statement in the CSR that the study was in full compliance with GCP, there is another extract in section 1.9 where it is stated: "While the study complied in principle with GCP, its administrative focus was on generating a peer-reviewed publication (Berk et al, 2013) and the resources did not exist within the university environment to produce an immediate ICH-style clinical study report." The Neuropathy Symptoms and Change (NSC) data was not available for assessment due to data not being accessible at the time of clinical study report compilation. However, data was made accessible during the evaluation.

There has not been a thorough dose-finding strategy, but the selected dose was considered by the applicant to be an adequate compromise between estimated efficacy and safety. The justification for the dose selection was presented during the assessment procedure.

The applicant did not seek advice neither for the development of study H 23750 nor for the retrieval of the data in 2023.

The study design (randomised placebo-controlled, double blind, two year duration) and the study population enrolled is considered adequate and to represent the most treatable population, but deviates from the proposed broad population of the intended indication. The applicant proposed that the indication may include aTTR pts with any type of neuropathy plus with wild-type TTR and not only aTTRv pts; and the broad population also includes very advanced FAP patients, but very few stage 3 were included. Since the initially claimed indication was too broad considering the study population a question was raised to the applicant in order to limit the indication. The choice of placebo was adequate since there were no disease modifying treatments available in 2006. Given the long study duration though, tafamidis became available in late 2011. Based on data provided during the review it was concluded that the later drop-outs may not have been related to the meanwhile availability of an approved agent for the treatment of FAP.

The original SAP and the 2023 revised SAP did not express the estimand policies for the study, and dealing with missing data was not initially sought for. The applicant argues that when the study was conducted ICH E9 (R1) the estimand approach had not been published and that the best approach was to provide a "treatment policy" as per Intent to treat philosophy. The results presented were the full ITT.

The random distribution between study arms seemed to have favoured the diflunisal arm as compared to the placebo arm, and this may have impacted on the worsening of placebo patients and on the drop-out rate. Furthermore, there was a significant patient drop-out in both study arms, but more prominent in the placebo arm (60.2% in placebo vs 42.2% in diflunisal did not complete treatment).

The applicant provided a description of the individual drop-outs with timelines, and there was no reason to suspect that underlying factors might justify the discrepancies.

Baseline data is not available for disease duration at study entry, which is a valuable patient characteristic for B/R assessment.

Efficacy data and additional analyses

Efficacy results are as follows:

Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat at 2-year time point.		
Descriptive statistics and estimate of variability	Treatment group	Diflunisal	Placebo
	Number of subjects	64	66
	NIS+7 (longitudinal) LS mean	+8.2 (2.9, 13.6)	+26.3 (20.2, 32.4)
	NIS+7 (multiple imputation) LS mean (95% CI)	+8.7 (3.3, 14.1)	+25.0 (18.4, 31.6)
	NIS-LL (longitudinal) LS mean (95% CI)	3.8 (0.9, 6.6)	12.1 (8.9, 15.3)
	NIS-LL (multiple imputation) LS mean (95% CI)	3.8 (1.0, 6.7)	12.1 (8.7, 15.5)

Effect estimate per comparison	Primary endpoint NIS+7 (longitudinal)	Comparison groups	Diflunisal vs placebo
		Difference between groups	18.0
		95% CI	(9.9, 26.2)
		P-value	p<0.0001
	Primary endpoint NIS+7 (multiple imputation)	Comparison groups	Diflunisal vs placebo
		Difference between groups	16.3
		95% CI	(8.1, 24.5)
		P-value	p<0.0001
	Secondary endpoint NIS-LL	Comparison groups	Diflunisal vs placebo
		Difference between groups	8.3

	(longitudinal)	95% CI	(4.1, 12.6)
		P-value	p=0.0002
	Secondary endpoint NIS-LL (multiple imputation)	Comparison groups	Diflunisal vs placebo
		Difference between groups	8.2
		95% CI	(4.0, 12.5)
		P-value	p=0.0002

The primary endpoint, NIS+7 is a composite endpoint. It has been validated to assess disease progression, but NIS-LL has been more linked to ambulation and disease disability, and has been favoured for the assessment of disease modifying FAP drugs. The secondary endpoints for the study evolved during the 6 years of study conduct. In the originally planned analyses, potential impact of missing data was not included. But post hoc analyses cannot constitute a primary analysis from a regulatory perspective.

The results presented are in line with those observed with tafamidis regarding the magnitude of effect. The secondary endpoints are also consistent and point towards a beneficial effect of diflunisal as compared to placebo.

The reference to the 1.8 difference was made by the applicant when describing the sample size determination, and this value, and not the 2-point value, was considered by the applicant for the selection of the sample size. It is nice to know that the applicant considered the 2-point difference as the minimal detectable difference by the trained neurologist. Still, the minimal detectable difference is different from the minimal clinically important difference. Along the 2-year duration of the study, the responder threshold should be based on published data discussing the NIS+7 / mNIS+7 in FAP like Aaron Yarlas, 2021 (J Neurol. 2021 Jun 14;269(1):323–335. doi: 10.1007/s00415-021-10635-1).

The applicant discussed the correlation in the study between NIS relates scores and global / QoL tools and justified the responder threshold.

The correlation between the NIS and the SF-36 Physical and SF-36 Mental component scores was examined using random coefficients analysis. The dependent variable was the within subject change from baseline to 12 and 24 months for (i) NIS+7 and (ii) NIS-LL. The independent variable was the SF-36 item score change from baseline to 12 and 24 months.

The resulting relationships between the NIS and SF-36 endpoints and the corresponding intercept and slope estimates are provided in Figure 11 through Figure 14 below.

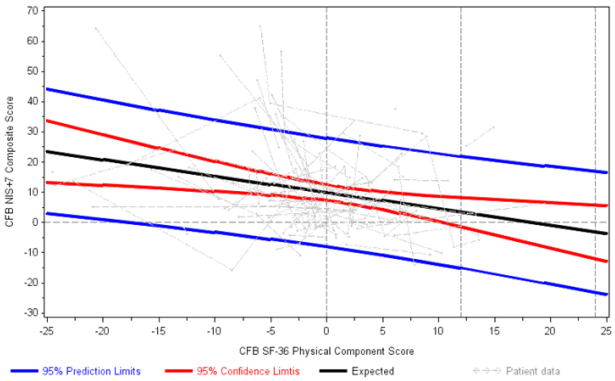
As shown in Figures below, relevant associations were observed for both the NIS+7 (p=0.0064) and the NIS-LL (p=0.0046) vs the SF-36 Physical Component score, with smaller (i.e. positive) changes in the NIS scores being associated within improvement in physical score. For the NIS+7, no statistically significant association was observed vs the SF-36 Mental Component score (Figure 13; p=0.87). However, as shown in Figure 14, a trend towards an association was observed for the NIS-LL (p=0.0563) with smaller (i.e. positive) changes in the NIS being associated within improvement in physical score.

Figure 11 and Figure 12: Relationship between change in NIS+7 and NIS-LL and change in SF-36 Physical Component Score

NIS+7

N=90, Intercept (SE) = 9.983 (1.3357), p=0.0001,
Slope (SE) = -.541 (0.1918), p=0.0064

Covariance (Intercept, Slope) = -0.0469, Residual Error = 83.0713, AIC = 1254.62



NIS-LL

N=92, Intercept (SE) = 4.881 (0.7505), p=0.0001,
Slope (SE) = -.260 (0.0887), p=0.0046

Covariance (Intercept, Slope) = 0.0032, Residual Error = 24.7903, AIC = 1093.90

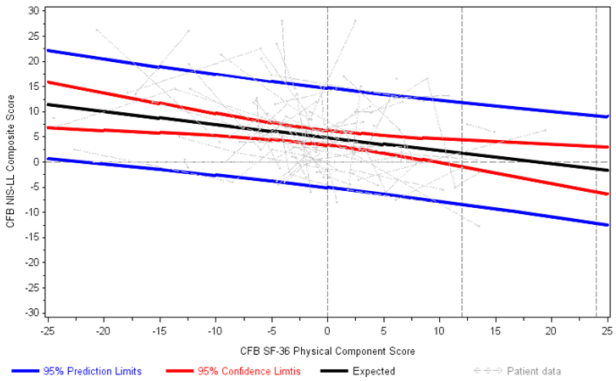
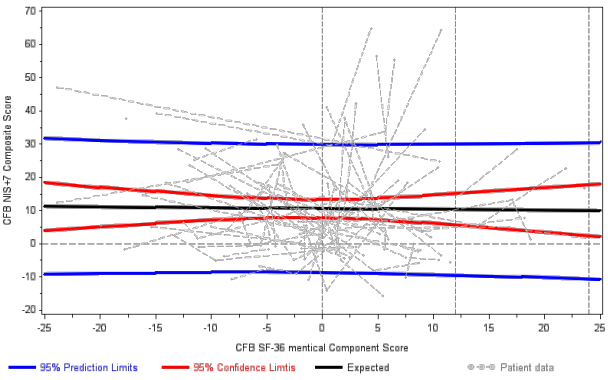


Figure 13 and Figure 14: Relationship between change in NIS+7 and NIS-LL and change in SF-36 Mental Component Score

NIS+7

N=90, Intercept (SE) = 10.68 (1.4220), p=0.0001,
Slope (SE) = -.024 (0.1435), p=0.8652

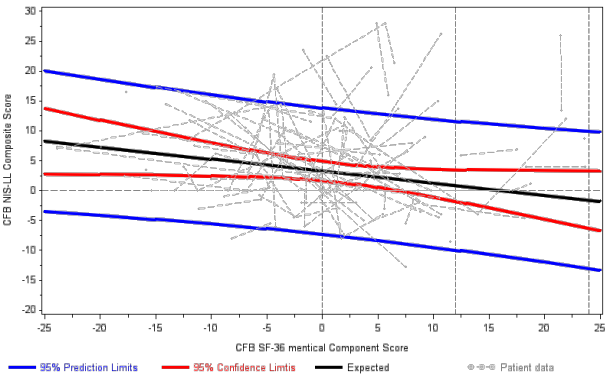
Covariance (Intercept, Slope) = -0.0237, Residual Error = 95.0723, AIC = 1272.17



NIS-LL

N=92, Intercept (SE) = 3.289 (0.8227), p=0.0002,
Slope (SE) = -.199 (0.1013), p=0.0563

Covariance (Intercept, Slope) = -0.0147, Residual Error = 28.3549, AIC = 561.84



Seventy subjects per arm were expected to be included in the Study in line with the sample size calculation. However, only 64 and 66 subjects, respectively were included in diflunisal and placebo arms.

The overall number of subjects who had primary endpoint data at Month 24 was relatively low (40 (62.5%) and 30 (45.5%) in the diflunisal and the placebo groups.

Registry driven data was presented regarding the off-label use of diflunisal in Swedish FAP patients. These patients are older than the Study H-23750 patients, and constitute a more diverse phenotype of

FAP than the patients with the common ATTR-PN Val Met30 mutation. The registry data has also been proposed to assist the study H 23750 with a Bayesian augmentation. Given the differences in study population, this was not considered adequate. The applicant did not discuss the fact that the Swedish registry included only the SE FAP population, which have a distinct behaviour from the remainder EU and ROW population, both in age at onset (usually SE patients have a later start of symptoms) and slower progression. The population overlapped in stages 0-1 but not on stage 2, which is understandable for a clinical trial vs. registry (stage 2 patients are possibly the population where the higher benefit can be identified). It is unfortunate that the registry lacks data on important aspects such as drop-out or treatment adherence. With all these caveats (population, type of data acquired, no search for adherence and reasons for drop out) the study is of little help on the confirmation of the results from the main study.

The registry data can be considered a supportive study with very little support for the main study.

The applicant has provided additional external data consisting of 4 small sample, open label or externally controlled studies, to support efficacy and mechanistic data on PD. The applicant has also repeated the data presented at the initial submission, which had been previously discussed and is therefore not repeated now.

External data

Takahashi et al, 2014

This was an ATTR PN FAP stage 1 study involving 6 late onset patients. An open label design, with 250 mg bid diflunisal administered for about 4.4 years. No primary endpoint was established, but there were clinical and neurophysiological endpoints collected. One patient withdrew due to haematuria, the other 5 remained in the study for >4 years.

All patients progressed, worsening either motor or global endpoints, but the clinical significance of the worsening or the loss of function is not compared to any natural history data. Reference is made to dysautonomic symptoms in a way that it is understood that they have stabilised, but no real evidence on this was provided. The conclusions on the value of diflunisal for the treatment of late onset FAP patients is thus not supported on the basis of this study.

Sekijima et al, 2015

This was an ATTR PN FAP stage 1 study involving 40 patients. A prospective, single centre open label study. No primary endpoint was established, some clinical and electrophysiological neurological and surrogate cardiac endpoints. There was either worsening (mBMI) or worsening followed by stabilisation with a magnitude of 1/3 the observed in natural history studies. The conclusions only apply to the studied population, but are noteworthy.

Wixner et al, 2019

This was a study on Swedish patients, similar to the Swedish population presented in a supportive study in the original submission, with follow up to 24 months. It is not clear whether this reflects the same patients or not. 54 patients, of whom 1/3 completed the study. An open label study. Primary endpoint was Kumamoto scale.

For the study completers, patients were clinically stable as per the Kumamoto scale, and mBMI did not change significantly. However, motor neuropathy scores increased significantly during the study, as well as cardiac thickness. The dropout rate may have been mostly related to hepatic transplantation.

Chao et al, 2024

This was a single centre prospective study of patients receiving either diflunisal or tafamidis compared to an historical control without directed treatment. Conducted in Taiwan patients with ATTRv-PN. Sample size diflunisal 35 tafamidis 22, historical control 85 patients.

No endpoint defined as primary. Time to transition FAP stages from 2 to 3 seemed to overlap between tafamidis and diflunisal, but not from 1 to 2, where between years 3 and 5 patients on diflunisal progressed quicker to stage 2.

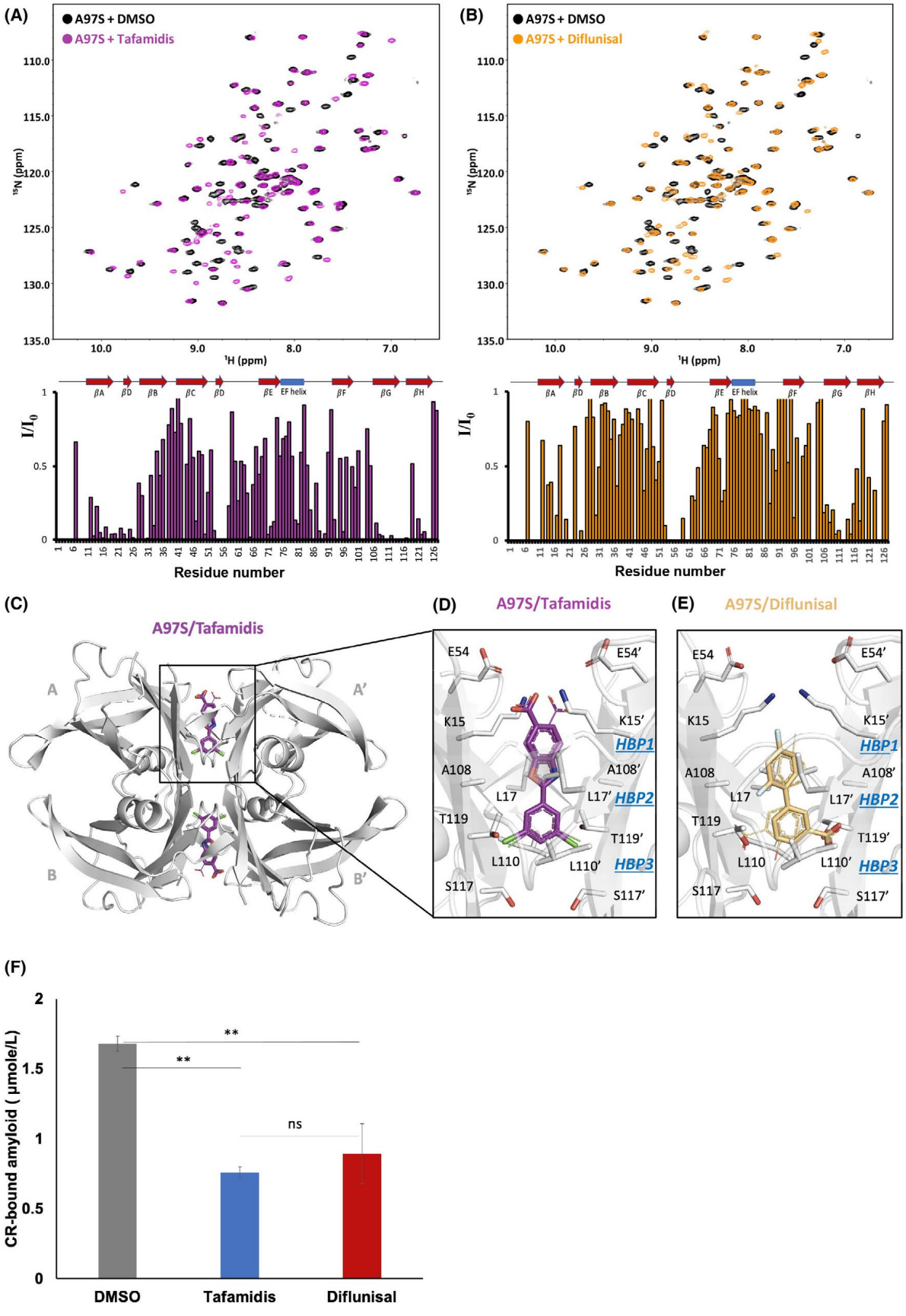
Mechanistic discussion

The mechanism of action of diflunisal on ATTR PN was independently investigated by Chao et al (2024). Interactions of A97S-TTR with tafamidis and diflunisal were studied by measuring the reduction in the nuclear magnetic resonance (NMR) peak intensity using two-dimensional [^{15}N, ^{1}H]-NMR spectroscopy. In both cases, the resonance peaks that significantly shifted as a result of drug binding were mainly located at the dimer-dimer interface, previously reported as the thyroxine-binding site. They also evaluated whether tafamidis and diflunisal inhibited the amyloidogenicity of A97S *in vitro*, revealing that both compounds equally and almost sufficiently reduced fibril formation. In light of these experiments, the authors conclude:

"In summary, these structural and biochemical assessments indicated that both diflunisal and tafamidis effectively stabilise A97S-TTR and provided a foundation for treating ATTRv patients with either diflunisal or tafamidis."

Figure from Chao et al, 2024 doi: 10.1002/acn3.52158. Structural analyses of stabiliser binding sites in A97S-TTR. (A) NMR studies on tafamidis and diflunisal binding to A97S-TTR.

Comparison of 2D TROSY-HSQC spectra: 15N-labeled A97S-TTR with (purple) and without (black) tafamidis. Intensity fluctuations observed in individual residues of A97S-TTR upon tafamidis binding. (B) 2D TROSY HSQC spectra of 15N-labeled A97S-TTR with (orange) and without (black) the presence of diflunisal. Changes in the intensity of each residue of A97S-TTR due to diflunisal binding. The ratio of NMR signal intensities, denoted as I/I0, refers to the signal intensity of stabiliser-bound A97S-TTR. (C) Crystal structures of the A97S-TTR ligand complexes. Global view of TTR-A97S bound to tafamidis (PDB ID: 8YQD). The overall structure is represented as a white cartoon, and tafamidis is shown as purple sticks. (D) Close-up view of one of the tafamidis binding sites in the TTR-A97S/tafamidis complex structure. Two different binding modes of tafamidis are shown as purple sticks and lines. (E) Two different binding modes of diflunisal are shown as orange sticks and lines. The side chains of TTR-interacting residues are labelled and represented by white sticks. (F) Both tafamidis and diflunisal effectively inhibited the acid-mediated aggregation of A97S-TTR. The A97S-TTR samples were incubated in the absence or presence of tafamidis or diflunisal at a molar ratio of 1:2 (A97S:drug) at pH 4.0 for 6 days, and the acid-induced fibrils of A97S-TTR were quantified using Congo red (CR) dye.


(A)
A97S + DMSO
A97S + Tafamidis
^{15}N (ppm)
^{1}H (ppm)
I/I$_0$
Residue number
(B)
A97S + DMSO
A97S + Diflunisal
^{15}N (ppm)
^{1}H (ppm)
I/I$_0$
Residue number
(C)
A97S/Tafamidis
A
A'
B
B'
(D)
A97S/Tafamidis
E54
E54'
K15
K15'
HBP1
A108
A108'
L17
L17'
HBP2
T119
T119'
L110
L110'
HBP3
S117
S117'
(E)
A97S/Diflunisal
E54
E54'
K15
K15'
HBP1
A108
A108'
L17
L17'
HBP2
T119
T119'
L110
L110'
HBP3
S117
S117'
(F)
CR-bound amyloid (µmole/L)
**
**
ns
DMSO
Tafamidis
Diflunisal

The mechanistic effect can be considered sufficiently robust in particular for patients where the risk is lower, for whom a higher B/R can be foreseen.

Although this is a disease which may affect late adolescence, the study enrolled adult patients between 24 and 76 years of age. No analyses were provided regarding the response according to age or age at the start of symptoms.

2.6.7. Conclusions on the clinical efficacy

The efficacy of diflunisal in ATTR-PN has been studied in a single-phase III trial. There was a difference between diflunisal and placebo of 18 points as per the primary endpoint NIS+7, which, also considering the totality of evidence from published off label use has been considered externally valid.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

Pivotal safety data are derived from the study H-23750 (randomised, placebo-controlled, parallel-group, double-blind, multi-centre, corresponding to Phase 3), in which 130 subjects were randomised to diflunisal 250 mg twice daily (n=64) of placebo (n=66) for a planned treatment period of 2 years. This study was conducted between April 2006 and May 2013.

The safety population included all randomised subjects who took at least one dose of study medication (N=130), i.e., it was identical to the ITT Population (N=130). Demographics and baseline clinical characteristics of the study H-23750 are presented below.

Table 16: Summary of demographic and baseline characteristics (safety population)

	Diflunisal	Placebo	Total
No. randomised and treated:	64	66	130
Males, n (%)	43 (67.2%)	44 (66.7%)	87 (66.9%)
Females, n (%):	21 (32.8%)	22 (33.3%)	43 (33.1%)
Mean age (range), years:	60.3 (24-76)	59.2 (27-75)	59.7 (24-76)
TTR Mutation, n (%):			
V30M	36 (56.3%)	35 (53.0%)	71 (54.6%)
Other	28 (43.8%)	31 (47.0%)	59 (45.4%)
Polyneuropathy disability (PND) stage, n (%):			
0-I	28 (43.8%)	21 (31.8%)	49 (37.7%)
II	18 (28.1%)	23 (34.8%)	41 (31.5%)
IIIA	11 (17.2%)	8 (12.1%)	19 (14.6%)
IIIB	3 (4.7%)	10 (15.2%)	13 (10%)
IV	4 (6.3%)	4 (6.1%)	8 (6.2%)

No. analysed for safety:	64 (100%)	66 (100%)	130 (100%)
No. completed treatment:	37 (57.8%)	26 (39.8%)	63 (48.5%)

Sixty-three subjects (48.5%) completed the study, including 37 (57.8%) in the diflunisal group and 26 (39.4%) in the placebo group. Sixty-seven subjects (51.5%) discontinued study treatment before completing the 2-year protocol, including 27 (42.2%) from the diflunisal group and 40 (60.6%) from the placebo group. Disease progression (11 subjects [17.2%] in the diflunisal group and 23 subjects [34.8%] in the placebo group) and orthotopic liver transplantation (7 [10.9%] diflunisal and 9 [13.6%] placebo) were the leading reasons for dropout.

In total, 10 subjects randomised to placebo stopped taking study drug and instead acquired diflunisal outside of the study or were prescribed open-label diflunisal as rescue treatment. Seven of these subjects continued in the study. It cannot be ruled out that this affected the results of the ITT population. As requested, the applicant discussed how the higher drop-out rate and the occurrence of existent open-label diflunisal treatment in the placebo group might have affected the interpretation of safety data results. For that, the applicant presented a comprehensive list of adverse events reported by patients who (might had) switched to open-label diflunisal, along with the causality assessments and dates of onset for those events. All in all, none of the reported AEs from these patients was considered to be a serious adverse event and none was rated as severe, thus it can be agreed that the overall qualitative assessment of the safety of diflunisal does not change for the requested indication.

The mean treatment compliance was above 90% in both treatment groups during the first 6 months of the study. Between Months 6 and 12, mean (SD) compliance dropped to 87.3 (13.8)% in the diflunisal group and 90.7 (7.8)% in the placebo group. Adherence dropped to 76% (N=49) in the diflunisal group and 86% (N=37) in the placebo group. During the second year of the study, mean (SD) compliance was 86.2 (15.2)% in the diflunisal group and 84.8 (12.8)% in the placebo group. Adherence was 82% (N=39) in the diflunisal group and 82% (N=27) in the placebo group.

Table 17: Compliance and adherence to randomised treatment (safety population)

Period Parameter		Diflunisal (N=64)	Placebo (N=66)	Total (N=130)
Baseline to Month 6	n	56	48	104
Compliance (%)	Mean	91.0	92.5	91.7
	SD	8.80	7.91	8.39
Adherence (n [%])	Yes	50 (78.1)	46 (69.7)	96 (73.8)
	No	6 (9.4)	2 (3.0)	8 (6.2)
Month 6 to Month 12	n	49	37	86
Compliance (%)	Mean	87.3	90.7	88.8
	SD	13.78	7.75	11.64
Adherence (n [%])	Yes	37 (57.8)	32 (48.5)	69 (53.1)
	No	12 (18.8)	5 (7.6)	17 (13.1)
Month 12 to Month 24	n	39	27	66
Compliance (%)	Mean	86.2	84.8	85.6
	SD	15.24	12.79	14.20
Adherence (n [%])	Yes	32 (50.0)	22 (33.3)	54 (41.5)
	No	7 (10.9)	5 (7.6)	12 (9.2)

Source: Table 14.1.8
Compliance (%) between two visits is calculated as:
(actual number of capsules taken / expected number of capsules taken) * 100.
Adherence is defined as a compliance of ≥80% for the period.
Note: if the date of first dose is unavailable, the date of enrolment is used in calculation of compliance.
For adherence, percentages in the table are based on the total number of subjects within each cohort

Exposure (in days) to the randomised study treatment was calculated as: (date of discontinuation of study drug - date of first dose) + 1. If the date of first dose was unavailable, the date of enrolment was used instead (the protocol stipulated that study interventions should begin within one day of randomisation). The mean (SD) duration of study treatment exposure was 562.2 (240.32) days in the diflunisal group and 456.4 (266.90) days in the placebo group. Exposure to study drug is summarised in the Table 19 below.

Table 18: Exposure to diflunisal in pivotal study (days)

	Diflunisal (N=64)	**Placebo (N=66)**	**Total (N=130)**
Mean	562.3	456.4	508.6
SD	240.32	266.90	258.70
Median	721.0	513.5	639.5
Min	5	1	1
Max	829	787	829

Out of the 64 patients in the diflunisal arm, 51 patients had a duration of exposure ≥12 months (34 079 patient-days), 4 patients had a duration of exposure 6 to<12 months (989 patient-days), 5 patients had a duration of exposure 3 to<6 months (792 patient-days), 3 patients had a duration of exposure 1 to<3 months (124 patient-days) and 1 patient had a duration of exposure <1 month (5 patient-days). Taking into consideration that ATTR amyloidosis is an orphan disease and the safety profile of diflunisal has been defined during more than 40 years of marketed use (usually in higher doses: 250 mg twice daily for ATTR-FAP vs 500 mg twice daily for NSAID indications), this safety database could be acceptable for an orphan drug. Diflunisal was first authorised in Europe in Sweden in the late 1970s with the last MA withdrawal being from the UK in 2015. This historic use encompassed chronic use (e.g., for osteoarthritis and rheumatoid arthritis) and was mainly at higher doses than is proposed for ATTR amyloidosis.

In several documents of the dossier the applicant mentioned the Umea registry (where patients have been treated for a mean of 3.4 years and a maximum of 10.2 years) as a source of evidence that reassures the safety collective experience data for this indication. However, the applicant recognised the presence of several methodological flaws in what concerns to this data source and the data retrieved: patients could be seen once or twice a year (meaning no fixing data collection points), data collection was the responsibility of the treating physician in a non-structured way, patients were free to opt out at any time; all in all one cannot exclude several sources of bias when interpretating the data retrieved. As supportive data, the applicant presented also publications considering three open-label trials of diflunisal in ATTR (Takahashi et al 2014, Sekijima et al 2015 and Wixner et al, 2019). However, the sample sizes of the three studies are very small, the study designs are all open-label and uncontrolled, and the methods to collect safety data are poorly described. Furthermore, the information relevant to safety is presented only in summarised form in all the three publications provided by the applicant. Thus, the informative values of published studies are considered low.

2.6.8.2. Adverse events

Overall, similar proportions of subjects experienced AEs in the two treatment groups. The dropout rate in the study was lower in the diflunisal group than in the placebo group, which can affect the interpretation of differences between number of subjects reporting AEs in each group. In addition, 7 subjects in the placebo group discontinued study treatment and started on open-label diflunisal, yet continued study evaluations. In the safety population, those subjects are included as randomised, i.e., in the placebo group.

An overview of all AEs and SAEs is presented for the safety population in the table 20 below.

Table 19: Overview of adverse events (safety population)

Category	Diflunisal (N=64) n (%) [E]	Placebo (N=66) n (%) [E]	Total (N=130) n (%) [E]
Adverse Event (AE)	56 (87.5) [353]	54 (81.8) [303]	110 (84.6) [656]
Mild	47 (73.4) [190]	43 (65.2) [146]	90 (69.2) [336]
Moderate	43 (67.2) [126]	40 (60.6) [118]	83 (63.8) [244]
Severe	14 (21.9) [26]	14 (21.2) [28]	28 (21.5) [54]
Life-Threatening	5 (7.8) [7]	7 (10.6) [8]	12 (9.2) [15]
Fatal	4 (6.3) [4]	3 (4.5) [3]	7 (5.4) [7]

Category	Diflunisal (N=64) n (%) [E]	Placebo (N=66) n (%) [E]	Total (N=130) n (%) [E]
Treatment emergent AE (TEAE)	56 (87.5) [349]	54 (81.8) [303]	110 (84.6) [652]
Drug related TEAE	29 (45.3) [59]	25 (37.9) [61]	54 (41.5) [120]
TEAE leading to discontinuation	14 (21.9) [32]	9 (13.6) [14]	23 (17.7) [46]
Drug related TEAE leading to discontinuation	8 (12.5) [11]	6 (9.1) [9]	14 (10.8) [20]
Serious TEAE	21 (32.8) [65]	24 (36.4) [59]	45 (34.6) [124]
TEAE with outcome of death	4 (6.3) [4]	3 (4.5) [3]	7 (5.4) [7]

AE = Adverse event; TEAE = Treatment-emergent adverse event; N = Number of subjects that were randomly assigned to treatment sequence; n = Number of subjects with adverse events; E = number of adverse events. Percentages are based on the total number of subjects within each cohort.

A total of 656 AEs were reported for 110/130 subjects (84.6%) during the study; 56/64 subjects (87.5%) in the diflunisal group and 54/66 subjects (81.8%) in the placebo group. Of these, 652 AEs were collected after study drug initiation and were therefore considered treatment-emergent (i.e., TEAEs).

Most AEs were mild or moderate in severity. Fifty-four events in 28 subjects (21.5%) were graded as severe; these occurred in 14 subjects (21.9%) in the diflunisal group and 14 subjects (21.2%) in the placebo group. A total of 15 AEs were considered life-threatening; these occurred in 5 subjects (7.8%) in the diflunisal group and 7 subjects (10.6%) in the placebo group.

Analysis of adverse events by organ system

In the diflunisal group (N=64), most TEAEs were reported within the SOCs Infections and infestations (39.1% of subjects), Gastrointestinal disorders (35.9%), Nervous system disorders (35.9%), General disorders and administration site conditions (29.7%), and Musculoskeletal and connective tissue disorders (29.7%). In the placebo group (N=66), most TEAEs were reported within the SOCs Infections and infestations (40.9%), Gastrointestinal disorders (37.9%), Nervous system disorders (30.3%), Renal and urinary disorders (18.2%), Respiratory, thoracic and mediastinal disorders (16.7%), and Investigations (16.7%).

For most SOCs, TEAEs were reported for a similar proportion of subjects in the diflunisal and placebo groups. However, TEAEs were reported for a greater proportion of subjects in the diflunisal group for the SOCs Musculoskeletal and connective tissue disorders (19 subjects [29.7%] in the diflunisal group vs 8 subjects [12.1%] in the placebo group) and General disorders and administration site conditions (19 subjects [29.7%] in the diflunisal group vs 7 subjects [10.6%] in the placebo group).

The table below summarises the TEAEs reported by 5% or more of patients in either treatment group.

Table 20: TEAEs by system organ class and preferred term (>5% of subjects)

System Organ Class Preferred Term	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Any TEAE	**56 (87.5)**	**54 (81.8)**	**110 (84.6)**
Infections and infestations	**25 (39.1)**	**27 (40.9)**	**52 (40.0)**

System Organ Class Preferred Term	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Urinary tract infection	8 (12.5)	14 (21.2)	22 (16.9)
Influenza	5 (7.8)	2 (3.0)	7 (5.4)
Gastroenteritis viral	2 (3.1)	4 (6.1)	6 (4.6)
Sinusitis	4 (6.3)	2 (3.0)	6 (4.6)
Gastrointestinal disorders	**23 (35.9)**	**25 (37.9)**	**48 (36.9)**
Nausea	10 (15.6)	13 (19.7)	23 (17.7)
Vomiting	9 (14.1)	9 (13.6)	18 (13.8)
Diarrhoea	5 (7.8)	7 (10.6)	12 (9.2)
Abdominal pain upper	2 (3.1)	6 (9.1)	8 (6.2)
Dyspepsia	7 (10.9)	1 (1.5)	8 (6.2)
Nervous system disorders	**23 (35.9)**	**20 (30.3)**	**43 (33.1)**
Headache	6 (9.4)	6 (9.1)	12 (9.2)
Syncope	6 (9.4)	4 (6.1)	10 (7.7)
Carpal tunnel syndrome	4 (6.3)	3 (4.5)	7 (5.4)
Musculoskeletal and connective tissue disorders (No PTs reaching the 5% frequency threshold)	**19 (29.7)**	**8 (12.1)**	**27 (20.8)**
General disorders and administration site conditions	**19 (29.7)**	**7 (10.6)**	**26 (20.0)**
Oedema peripheral	5 (7.8)	3 (4.5)	8 (6.2)
Chest pain	4 (6.3)	0 (0)	4 (3.1)
Fatigue	4 (6.3)	0 (0)	4 (3.1)
Cardiac disorders	**15 (23.4)**	**9 (13.6)**	**24 (18.5)**
Cardiac failure	4 (6.3)	0 (0)	4 (3.1)
Respiratory, thoracic and mediastinal disorders	**12 (18.8)**	**11 (16.7)**	**23 (17.7)**
Cough	6 (9.4)	6 (9.1)	12 (9.2)
Injury, poisoning and procedural complications	**13 (20.3)**	**9 (13.6)**	**22 (16.9)**
Fall	4 (6.3)	5 (7.6)	9 (6.9)
Investigations	**11 (17.2)**	**11 (16.7)**	**22 (16.9)**
Occult blood positive	6 (9.4)	5 (7.6)	11 (8.5)
Renal and urinary disorders	**10 (15.6)**	**12 (18.2)**	**22 (16.9)**
Renal failure	4 (6.3)	2 (3.0)	6 (4.6)

System Organ Class Preferred Term	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Vascular disorders	**11 (17.2)**	**7 (10.6)**	**18 (13.8)**
Hypertension	7 (10.9)	2 (3.0)	9 (6.9)
Eye disorders (No PTs reaching the 5% frequency threshold)	**7 (10.9)**	**9 (13.6)**	**16 (12.3)**
Metabolism and nutrition disorders (No PTs reaching the 5% frequency threshold)	**4 (6.3)**	**9 (13.6)**	**13 (10.0)**
Psychiatric disorders	**5 (7.8)**	**7 (10.6)**	**12 (9.2)**
Depression	3 (4.7)	5 (7.6)	8 (6.2)
Skin and subcutaneous tissue disorders (No PTs reaching the 5% frequency threshold)	**6 (9.4)**	**6 (9.1)**	**12 (9.2)**
Surgical and medical procedures (No PTs reaching the 5% frequency threshold)	**5 (7.8)**	**3 (4.5)**	**8 (6.2)**

TEAE = Treatment-emergent adverse event; N = Number of subjects that were randomly assigned to treatment sequence; n = Number of subjects with adverse events. Percentages, presented in brackets, are based on the number of subjects within each cohort.

Treatment-emergent AEs assessed as possibly, probably, or likely related to study drug were categorised as "drug related". A total of 119 TEAEs assessed as "drug related", were reported for 54/130 subjects (41.5%); 29/64 (45.3%) in the diflunisal group and 25/66 (37.9%) in the placebo group. The most common drug related TEAEs (PT; reported for >5% of subjects in any group) were nausea (6 subjects (9.4%) in the diflunisal group and 8 subjects (12.1%) in the placebo group), dyspepsia (7 subjects (10.9%) in the diflunisal group and 1 subject (1.5%) in the placebo group), occult blood positive (5 subjects (7.8%) in the diflunisal group and 4 subjects (6.1%) in the placebo group), oedema peripheral (5 subjects (7.8%) in the diflunisal group and 2 subjects (3.0%) in the placebo group), abdominal pain upper (2 subjects (3.1%) in the diflunisal group and 5 subjects (7.6%) in the placebo group), renal failure (4 subjects (6.3%) in the diflunisal group and 2 subjects (3.0%) in the placebo group) and vomiting (2 subjects (3.1%) in the diflunisal group and 4 subjects (6.1%) in the placebo group).

2.6.8.3. Serious adverse event/deaths/other significant events

Serious Treatment-Emergent Adverse Events

Overall, TEAEs assessed as serious were reported for 45/130 subjects (34.6%): 21 subjects (32.8%) in the diflunisal group and 24 subjects (36.4%) in the placebo group. In the diflunisal group (N=64), most serious TEAEs were reported within the SOCs cardiac disorders (12.5% of subjects), nervous system disorders (12.5%), infections and infestations (9.4%), and gastrointestinal disorders (7.8%). In the placebo group (N=66), most serious TEAEs were reported within the SOCs infections and

infestations (12.1% of subjects), cardiac disorders (10.6%), nervous system disorders (9.1%), and gastrointestinal disorders (7.6%).

Of the SAEs, 14 events were assessed as related to study treatment (6 events in the diflunisal group and 8 events in the placebo group). A total of 7 subjects (5.4%) had TEAEs with outcome of death; 4 subjects (6.3%) were in the diflunisal group and 3 subjects (4.5%) in the placebo group. None of the deaths was considered drug related.

Most of the serious TEAEs by PT were reported for one or two subjects. The serious TEAEs (PTs) reported for ≥3 subjects are listed in the table below.

Table 21: Serious adverse events reported for 3 or more subjects

Event	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Vomiting	5 (7.8)	4 (6.1)	9 (6.9)
Nausea	3 (4.7)	4 (6.1)	7 (5.4)
Syncope	3 (4.7)	2 (3.0)	5 (3.8)
Cardiac failure congestive	3 (4.7)	1 (1.5)	4 (3.1)

Deaths

A total of 7 deaths were reported, 4 in the diflunisal group and 3 in the placebo group. Five of the events leading to death were judged to be unrelated to the study drug and the remaining 2 events were judged as unlikely to be related to the study drug. Six of the reported deaths occurred after the subject had discontinued study drug.

Additionally, one subject (07-132) originally in the placebo group died 2 months after withdrawing from the study to have a liver transplantation. This was discovered when follow-up information to previous SAEs was sought. The subject was no longer being evaluated or followed for new adverse events, hence the cause of death (post-operative complications) was not reported as an SAE within the study.

Table 22: Summary of treatment-emergent adverse events leading to death by system organ class and preferred term (safety population)

System Organ Class Preferred Term	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Any TEAE	4 (6.3)	3 (4.5)	7 (5.4)
Cardiac disorders	3 (4.7)	2 (3.0)	5 (3.8)
Cardio-respiratory arrest	2 (3.1)	2 (3.0)	4 (3.1)
Cardiac failure	1 (1.6)	0	1 (0.8)
General disorders and administration site conditions	1 (1.6)	1 (1.5)	2 (1.5)
Death	1 (1.6)	0	1 (0.8)
Sudden death	0	1 (1.5)	1 (0.8)

Source: Table 14.3.1.7

TEAE = Treatment-emergent adverse event; N = Number of subjects that were randomly assigned to treatment sequence; n = Number of subjects with adverse events

Percentages, presented in brackets, are based on the number of subjects within each cohort

ADRs of special interest, serious ADRs and deaths causally related to the medicinal product.

Most of the serious TEAEs were assessed as not related or unlikely related to study treatment. However, 14 events were assessed as possibly or probably related to study treatment. These were 4 events of cardiac failure congestive (2 in the diflunisal group and 2 in the placebo group), 3 events of vomiting (1 in the diflunisal group and 2 in the placebo group), 2 events of nausea (1 in the diflunisal group and 1 in the placebo group), and one event each of urinary tract infection (placebo) haematuria (placebo), upper gastrointestinal haemorrhage (diflunisal), renal failure (placebo), and post procedural haemorrhage (diflunisal).

A total of 7 subjects had TEAEs with outcome of death. None of the deaths was considered drug related by the applicant. Nevertheless, regarding the fatal SAEs 'heart failure (cardiac failure)' and 'cardiorespiratory arrest' in the diflunisal arm, although co-existing risk factors might not be in favour of the causal role of diflunisal, more information or at least a thorough discussion on causal assessment is needed before drawing a firm conclusion. The applicant provided the discussion on the causal assessment for both fatal SAEs in Participant 01-742 and in Participant 01-806, based on the information available. The death of Participant 01-742 occurred more than 15 years ago and the death of Participant 01-806 more than 13 years ago. The applicant acknowledged that in both cases, it was not possible to gather more information beyond the content of the SAE narrative provided. This is acknowledged. In both participants, causes of death were assessed by the investigator as being unrelated to diflunisal and were seen as features of ATTR amyloidosis.

2.6.8.4. Laboratory findings

Clinical chemistry

Descriptive summaries of clinical chemistry measurements (serum albumin, serum creatinine, BUN, AST, ALT, total bilirubin) were provided. Overall, the applicant stated there were no major differences in the median values or ranges in any of the clinical chemistry measurements (serum albumin, serum creatinine, blood urea nitrogen [BUN], aspartate aminotransferase [AST], alanine aminotransferase [ALT], bilirubin) at the different timepoints or between the treatment groups. Median values were all within generally accepted normal ranges defined in the medical literature.

- Serum albumin: By local laboratory reference ranges, 6 subjects of 59 subjects with available data (10.2%) in the diflunisal group and 11 subjects out of 63 with available data (17.5%) in the placebo group presented with abnormal values for serum albumin at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (1-4 subjects [2.6% - 13.3%] at each timepoint). The same was seen in the placebo group (3-4 subjects [10.0% - 13.0%]). Of subjects in the diflunisal group with abnormal baseline values, roughly half had abnormal values also at later timepoints. In the placebo group, a similar picture was seen, except at Month 24 when 4 out of 5 subjects with abnormal values at baseline (and with available data at Month 24) again had abnormal values of serum albumin. There were no AE reports relating to serum albumin.

- Serum creatinine: By local laboratory reference ranges, 14 subjects out of 59 subjects with available data (23.7%) in the diflunisal group and 19 subjects out of 63 with available data (30.2%) in the placebo group presented with abnormal values for serum creatinine at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (2-4 subjects [5.4% - 13.8%] at each timepoint). The same was seen in the placebo group (1-3 subjects [2.9% - 7.9%]). Of subjects in the diflunisal group with abnormal baseline values (i.e., 14 subjects), and who had data available at the respective timepoint, 60-80% had abnormal values also at later timepoints. In the placebo

group, a similar picture was seen. Adverse events of PT Blood creatinine increased were reported for 2 subjects (3.0%) in the placebo group.

- Blood urea nitrogen: By local laboratory reference ranges, 17 subjects out of 56 subjects with available data (30.4%) in the diflunisal group and 19 subjects out of 60 subjects with available data (31.7%) in the placebo group presented with abnormal values for BUN at baseline. Of those with data from subsequent measurements, 2-6 subjects (6.1% - 18.8%) in the diflunisal group, and 0-3 subjects (0.0% - 15.8%) in the placebo group, with normal baseline values shifted to have abnormal values. Of the subjects in the diflunisal group with abnormal baseline values (i.e., 17 subjects), and who had data available at the respective timepoint, most (80-90%) tended to have abnormal values also at later timepoints. In the placebo group, a similar picture was seen. There were no AE reports relating to BUN.

- Aspartate aminotransferase: By local laboratory reference ranges, 8 subjects out of 59 subjects with available data (13.6%) in the diflunisal group and 6 subjects out of 63 subjects with available data (9.5%) in the placebo group presented with abnormal values for AST at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (1-3 subjects [2.4% - 9.1%] at each timepoint). The same was seen in the placebo group (0-1 subjects [0.0% - 3.1%]). Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, roughly half had abnormal values also at later timepoints. In the placebo group, a similar picture was seen. An AE of PT Transaminases increased was reported for 1 subject (1.6%) in the diflunisal group.

- Alanine aminotransferase: By local laboratory reference ranges, 6 subjects out of 59 subjects with available data (10.2%) in the diflunisal group and 5 subjects out of 63 subjects with available data (7.9%) in the placebo group presented with abnormal values for ALT at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (0-4 subjects [0.0% - 9.3%] at each timepoint). The same was seen in the placebo group (0-2 subjects [0.0% - 4.9%]). Of subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, roughly half had abnormal values also at later timepoints. In the placebo group, a similar picture was seen. An AE of PT Transaminases increased was reported for 1 subject (1.6%) in the diflunisal group.

- Total bilirubin: By local laboratory reference ranges, 7 subjects out of 59 subjects with available data (11.9%) in the diflunisal group and 8 subjects out of 63 subjects with available data (12.7%) in the placebo group presented with abnormal values for total bilirubin at baseline. Data from further measurements during the study were inaccessible at the time of CSR compilation. There were no AE reports relating to total bilirubin.

Haematology

Descriptive summaries of haematology measurements (haemoglobin, haematocrit, white blood cell count, platelet count) were provided. Overall, the applicant stated there were no major differences in the median values or ranges in any of the haematology measurements at the different timepoints or between the treatment groups. Median values were all within generally accepted normal ranges defined in the medical literature.

- Haemoglobin: By local laboratory reference ranges, 17 subjects out of 59 subjects with available data (28.8%) in the diflunisal group and 19 subjects out of 63 subjects with available data

(30.2%) in the placebo group presented with abnormal values for haemoglobin at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (0-3 subjects [0.0% - 8.8%] at each timepoint). Slightly more subjects shifted from normal to abnormal in the placebo group (2-6 subjects [8.0% - 27.3%]). Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, 50-86% had abnormal values also at later timepoints. A similar picture was seen in the placebo group. Adverse events of PT anaemia were reported for 2 subjects (3.0%) in the placebo group.

- Haematocrit: By local laboratory reference ranges, 22 subjects out of 56 subjects with available data (39.3%) in the diflunisal group and 22 subjects out of 59 subjects with available data (37.3%) in the placebo group presented with abnormal values for haematocrit at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (1-4 subjects [0.0% - 14.3%] at each timepoint). The same was seen in the placebo group (1-5 subjects [3.6% - 25.0%]). Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, approximately 40-60% had abnormal values also at later timepoints. In the placebo group, the corresponding proportions were 70-80%. Adverse events of PT Haematocrit decreased were reported for 3 subjects (4.7%) in the diflunisal group and 2 subjects (3%) in the placebo group.

- White blood cell count: By local laboratory reference ranges, 4 subjects out of 59 subjects with available data (6.8%) in the diflunisal group and 3 subjects out of 63 subjects with available data (4.8%) in the placebo group presented with abnormal values for WBC count at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (0-4 subjects [2.2% - 8.3%] at each timepoint). The same was seen in the placebo group (2-4 subjects [4.8% - 11.1%]). Of the subjects in the diflunisal group with abnormal baseline WBC values, and who had data available at the respective timepoint, there was only data for 1 subject at each of the later timepoints. Values were in the abnormal range for 2 out of 3 subsequent visits but there was no data from later visits than Month 6. Of subjects with abnormal baseline values in the placebo group, there was 1 subject with abnormal values at each of the subsequent timepoints, except at Month 18, where both subjects with available data had normal WBC counts. There were no AEs reported relating to white blood cell count.

- Platelet count: By local laboratory reference ranges, 4 subjects out of 55 subjects with available data (7.3%) in the diflunisal group and 4 subjects out of 61 subjects with available data (6.6%) in the placebo group presented with abnormal values for platelet count at baseline. Of those with data from subsequent measurements, few of the subjects in the diflunisal group with normal baseline values shifted to have abnormal values (1-6 subjects [3.2% - 14.6%]). The same was seen in the placebo group (1-4 subjects [2.5% - 15.4%]). Of the few subjects in the diflunisal group with abnormal baseline values, normal values at later timepoints were only seen in single subjects. In the placebo group, 2-3 of the subjects with measurements at Months 1-6 were normal, and from Month 12, the only available measurement at each timepoint was normal. Adverse events of PT Thrombocytopenia were reported for 2 subjects: 1 subject (1.6%) in the diflunisal group and 1 subject (1.5%) in the placebo group. One subject in the diflunisal group (subject 05-142), discontinued the study drug due to thrombocytopenia (90 000/µl) at the Month 1 visit. As per the protocol, study drug would be withheld at a platelet count <100 000/µl. The event was assessed to be unlikely related to study drug.

Miscellaneous tests

Evaluation of HbA1C, vitamin B12, and β-HCG were done at baseline only, in order to verify subjects' eligibility for the study. Samples for evaluation of brain natriuretic peptide (BNP) and N-terminal prohormone brain natriuretic peptide (NT-proBNP), markers for congestive heart failure (CHF), were collected at baseline and additionally at Months 6, 12, and 24. All subjects (48/48) in the diflunisal group and 47/50 subjects in the placebo group had normal values of HbA1C by local laboratory reference ranges, as available. For vitamin B12, 40/47 in the diflunisal group and 47/50 subjects in the placebo group had normal values by local laboratory reference ranges, as available. No enrolled subjects were found to be pregnant as per β-HCG analysis.

- Brain natriuretic peptide and N-terminal prohormone brain natriuretic peptide

  The median values of BNP were largely similar between the treatment groups at any timepoint and remained in the same range over time. All median values of BNP were within generally accepted normal range as defined in the medical literature, except for the diflunisal group at Month 6, where the median value was just above the ULN. By local laboratory reference ranges, 28 subjects out of 44 subjects with available data (63.6%) in the diflunisal group and 28 subjects out of 42 subjects with available data (66.7%) in the placebo group presented with abnormal values for BNP at baseline. Of those with data from subsequent measurements, 2-4 subjects (14.3% - 25.0%) in the diflunisal group with normal baseline values shifted to have abnormal values. In the placebo group, the corresponding shift was seen in 0-2 subjects (0.0% - 22.2%) at each timepoint. Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, all had abnormal values also at later timepoints. In the placebo group, a similar picture was seen. There were no AEs reported relating to BNP.

  Only a minor proportion of the subjects had measurements for NT-proBNP; 14/64 subjects in the diflunisal group and 17/66 subjects in the placebo group at baseline. At Month 24, measurements were only available for 9 subjects in the diflunisal group and 5 subjects in the placebo group. Due to local laboratory ranges not being completely provided, not all subjects could be categorised as normal/abnormal. The baseline median values for NT-proBNP in both groups at were around the level commonly proposed for suspicion of CHF (>400 pg/mL). Over time, median values in the diflunisal group remained stable. In the placebo group, however, NT-proBNP median values doubled at Months 6 and 12 compared to baseline, and at Month 24, the median value in this group had increased several-fold. From the increase, the median values in the placebo group were 3- to 9-fold above the upper limit of normal as defined in the medical literature (<300 pg/mL; Mosby's Diagnostic & Laboratory Test Reference 14th Ed, 2019). By local laboratory reference ranges, 9 subjects out of 9 subjects with available data (100%) in the diflunisal group and 10 subjects out of 14 subjects with available data (71.4%) in the placebo group presented with abnormal values for NT-proBNP at baseline. Of those with data from subsequent measurements, for subjects in the placebo group with normal baseline values, only abnormal values were reported (1-2 subjects at each timepoint). No subjects in the diflunisal group with normal baseline values had measurements at later timepoints. Of the subjects with abnormal baseline values in both treatment groups, all had abnormal values also at later timepoints. There were no AEs reported relating to NT-proBNP.

Urinalysis

Urinalysis, including urine creatinine, urine protein and creatinine clearance, from 24-hour urine collection were evaluated at baseline and at Months 6, 12, and 24.

- Urine creatinine: The median urine creatinine amounts in the 24-hour urine specimens were largely similar between the treatment groups and remained in the same range over time. Median values were within most accepted normal ranges. By local laboratory reference ranges, 31 subjects out of 53 subjects with available data (58.5%) in the diflunisal group and 33 subjects out of 58 subjects with available data (56.9%) in the placebo group presented with abnormal values for urine creatinine at baseline. Of those with data from subsequent measurements, 1-5 subjects (5.6% - 31.3%) in the diflunisal group with normal baseline values shifted to have abnormal values. In the placebo group, the corresponding shift was seen in 2-5 subjects (13.3% - 55.6%). Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, 60-90% had abnormal values also at later timepoints. In the placebo group with abnormal baseline values, approximately two thirds of the subjects at each timepoint had abnormal values. There were no AEs reported that related to urine creatinine.

- Urine protein: Median values of urine protein over 24 hours were largely similar between the treatment groups and remained in the same range over time. All median values were within generally accepted normal range defined in the scientific literature. By local laboratory reference ranges, 11 subjects out of 52 subjects with available data (21.1%) in the diflunisal group and 12 subjects out of 56 subjects with available data (21.4%) in the placebo group presented with abnormal values for urine protein at baseline. Of those with data from subsequent measurements, 4-7 subjects (15.2% - 20.6%) in the diflunisal group with normal baseline values shifted to have abnormal values. In the placebo group, the corresponding shift was seen in 3-4 subjects (10.7% - 25.0%). Of the subjects in the diflunisal group with abnormal baseline values, and who had data available at the respective timepoint, 77-100% had abnormal values also at later timepoints. In the placebo group with abnormal baseline values, 55-80% of the subjects at each timepoint had abnormal values. An adverse event of PT Proteinuria was reported for 1 subject (1.6%) in the diflunisal group.
- Creatinine clearance: While being required for eligibility assessment from the start of the study, estimated creatinine clearance was only added to the Screening labs CRF from when version 4 of the protocol was implemented (March 2009). Hence, subjects enrolled earlier did not have this parameter reported to the study database. Median values of creatinine clearance at baseline were around the lower limit of most accepted normal ranges, and similar between the two treatment groups. Data from further measurements during the study were inaccessible at the time of CSR compilation. Due to only 2 sites providing local reference ranges for creatinine clearance, a normal/abnormal categorisation for this parameter at the individual subject level was not done. One subject (08-170) had a reported estimated creatinine clearance of 3.5 mL/min at screening. Since creatinine clearance values of <30 mL/min constituted an exclusion criterion, and since the subject had serum- and 24-hour urine creatinine levels within the normal ranges of the local laboratory, the creatinine clearance value was recalculated. The resulting estimated creatinine clearance was 77.6 mL/min. The cause of the reported erroneous value is unknown.

Stool Guaiac

To assess gastrointestinal status, stool samples were collected to test for the presence of faecal occult blood. In the diflunisal group, 0-6 subjects (less than 10%) had positive stool guaiac tests at any of the assessments. In the placebo group, 1-3 subjects (4.5% or less) had a positive test at any of the assessments. Adverse events of the PT Occult blood positive were reported for 6 subjects (9.4%).

2.6.8.5. Safety in special populations

Table 23: Summary of adverse events according to age category

	Diflunisal			Placebo		
MedDRA Terms	Age <65 (N=35) n (%)	Age 65-74 (N=27) n (%)	Age 75-84 (N=2) n (%)	Age <65 (N=40) n (%)	Age 65-74 (N=25) n (%)	Age 75-84 (N=1) n (%)
Total AEs	30 (85.7%)	24 (88.9%)	2 (100%)	30 (75.0%)	23 (92.0%)	1 (100%)
Serious AEs – Total	10 (28.6%)	11 (40.7%)	0 (0%)	13 (32.5%)	11 (44.0%)	0 (0%)
- Fatal	3 (8.6%)	1 (3.7%)	0 (0%)	1 (2.5%)	2 (8.0%)	0 (0%)
- Life-threatening	2 (5.7%)	3 (11.1%)	0 (0%)	3 (7.5%)	4 (16.0%)	0 (0%)
AE leading to drop-out	6 (17.1%)	7 (25.9%)	1 (50%)	5 (12.5%)	4 (16.0%)	0 (0%)
Psychiatric disorders	0 (0%)	5 (18.5%)	0 (0%)	3 (7.5%)	4 (16.0%)	0 (0%)
Nervous system disorders	12 (34.3%)	10 (37.0%)	1 (50%)	14 (35.0%)	6 (24.0%)	0 (0%)
Injury, poisoning and procedural complications	8 (22.9%)	4 (14.8%)	1 (50%)	3 (7.5%)	6 (24.0%)	0 (0%)
Cardiac disorders	6 (17.1%)	9 (33.3%)	0 (0%)	5 (12.5%)	4 (16.0%)	0 (0%)
Vascular disorders	5 (14.3%)	5 (18.5%)	1 (50%)	5 (12.5%)	2 (8.0%)	0 (0%)
Cerebrovascular disorders	0 (0%)	0 (0%)	1 (50%)	1 (2.5%)	0 (0%)	0 (0%)
Infections and infestations	14 (40.0%)	11 (40.7%)	0 (0%)	12 (30.0%)	15 (60.0%)	0 (0%)
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	7 (20.0%)	8 (29.6%)	0 (0%)	5 (12.5%)	6 (24.0%)	0 (0%)
Skin and subcutaneous tissue disorders	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)

Table 24: Safety profile according to PND stage 0-I vs stage II-IV

	Diflunisal		Placebo	
MedDRA Terms	PND 0-I (N=28) n (%)	PND II-IV (N=36) n (%)	PND 0-I (N=21) n (%)	PND II-IV (N=45) n (%)
Total AEs	24 (85.7%)	32 (88.9%)	19 (90.5%)	35 (77.8%)
Serious AEs – Total	9 (32.1%)	12 (33.3%)	6 (28.6%)	18 (40.0%)
- Fatal	1 (3.6%)	3 (8.3%)	0 (0%)	3 (6.7%)
- Life-threatening	2 (7.1%)	3 (8.3%)	1 (4.8%)	6 (13.3%)
AE leading to drop-out	3 (10.7%)	11 (30.6%)	2 (9.5%)	7 (15.6%)
Psychiatric disorders	0 (0%)	5 (13.9%)	2 (9.5%)	5 (11.1%)
Nervous system disorders	10 (35.7%)	13 (36.1%)	10 (47.6%)	10 (22.2%)
Injury, poisoning and procedural complications	4 (14.3%)	9 (25.0%)	1 (4.8%)	8 (17.8%)
Cardiac disorders	5 (17.9%)	10 (27.8%)	3 (14.3%)	6 (13.3%)
Vascular disorders	6 (21.4%)	5 (13.9%)	2 (9.5%)	5 (11.1%)
Cerebrovascular disorders	1 (3.6%)	0 (0%)	1 (4.8%)	0 (0%)
Infections and infestations	11 (39.3%)	14 (38.9%)	8 (38.1%)	19 (42.2%)
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	5 (17.9%)	10 (27.8%)	3 (14.3%)	8 (17.8%)

Table 25: Safety profile according to genotype (V30M vs non-V30M)

	Diflunisal		Placebo	
MedDRA Terms	V30M (N=36) n (%)	Non-V30M (N=28) n (%)	V30M (N=35) n (%)	Non-V30M (N=31) n (%)
Total AEs	29 (80.6%)	27 (96.4%)	28 (80.0%)	26 (83.9%)
Serious AEs – Total	10 (27.8%)	11 (39.3%)	9 (25.7%)	15 (48.4%_
- Fatal	1 (2.8%)	3 (10.7%)	1 (2.9%)	2 (6.5%)
- Life-threatening	2 (5.6%)	3 (10.7%)	2 (5.7%)	5 (16.1%)
AE leading to drop-out	7 (19.4%)	7 (25.0%)	4 (11.4%)	5 (16.1%)
Psychiatric disorders	2 (5.6%)	3 (10.7%)	7 (20.0%)	0 (0%)
Nervous system disorders	10 (27.8%)	13 (46.4%)	10 (28.6%)	10 (32.3%)
Injury, poisoning and procedural complications	5 (13.9%)	8 (28.6%)	3 (8.6%)	6 (19.4%)
Cardiac disorders	8 (22.2%)	7 (25.0%)	3 (8.6%)	6 (19.4%)
Vascular disorders	7 (19.4%)	4 (14.3%)	2 (5.7%)	5 (16.1%)
Cerebrovascular disorders	1 (2.8%)	0 (0%)	0 (0%)	1 (3.2%)
Infections and infestations	12 (33.3%)	13 (46.4%)	12 (34.3%)	15 (48.4%)
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	7 (19.4%)	8 (28.6%)	4 (11.4%)	7 (22.6%)

2.6.8.6. Safety related to drug-drug interactions and other interactions

No data on drug interactions were collected in the pivotal H-23750 study.

Drug-drug interactions studies were conducted in healthy volunteers and diabetic patients with the conclusions shown in the table below.

Table 26: Drug-drug interactions with diflunisal

Drug and study reference	Effect of drug on diflunisal	Effect of diflunisal on drug
Acetylsalicylic acid (Merck study #16, Perrier)	2400 mg daily (given as 4 x 600 mg in divided doses) lowered diflunisal levels; smaller doses had no effect	None observed
Indomethacin (Merck study #20, Schepper)	Temporary increase in indomethacin levels resolved after 7 days	None recorded
Naproxen (Merck study #21, Dresse)	None observed	None recorded
Tolbutamide (Merck study #19, Gilbert)	None observed	None recorded
Hydrochlorothiazide (Merck study #22, Kappas)	Increased plasma levels, decreased urinary excretion, antagonised hyper uricaemic effect	None recorded
Phenprocoumon (Merck study #17, Vermijlen)	None observed	None observed
Acenocoumarol (Merck study #18, Morselli)	Prolongation of prothrombin time	None recorded

Among these interactions, the only one considered at risk of being clinically relevant was that with acenocoumarol.

In the study "A Comparison of the Effect of MK-647 and Aspirin on Fecal Blood Loss in Normal Volunteers", MK-647 was administered at the dose of 250 mg b.i.d. for two seven-day periods separated by a one week control period. Aspirin was administered at 750 mg q.i.d. and followed the same schedule.

MK-647 (250 mg b.i.d.) during two periods caused mean blood losses of 1.57 and 2.66 ml/day. Aspirin (750 mg q.i.d.) under comparable conditions caused blood losses of 34.33 and 14.7 ml/day.

No clinically significant drug related adverse reactions were reported for subjects receiving MK-647. One subject receiving aspirin developed gastric ulcer symptoms during the second treatment period. This reaction was considered probably drug related.

The applicant concluded that, at the doses employed in this study, MK-647 exhibited significantly less faecal blood loss than aspirin.

In the study "A single blind study to determine the possible interaction of MK-647 with sintrom in patients on long term sintrom therapy", possible interactions of diflunisal with acenocumarol were investigated.

This was a single-blind study in normal volunteers who had been receiving the oral anticoagulant SINTROM (acenocoumarol) for two months. When the study began, the subjects received placebo on days -14 to -1, MK-647 (375 mg b.i.d.) on days 1-7, and placebo (b.i.d.) on days 8 and 9. The subjects also received SINTROM throughout the study, and they were hospitalised beginning day 1. The objectives of the study were to (1) study the effects of multiple doses of MK-647 on prothrombin time, plasma levels of SINTROM and protein binding of SINTROM; (2) correlate any changes observed with MK-647 plasma levels; (3) acquire additional short-term safety and tolerance data on MK-647.

There were two protocol deviations: (1) normal volunteers were studied instead of patients, because of Italian IND regulations, and (2) the protein binding of SINTROM was not measured, because of a lack of the necessary technology in the investigator's laboratory.

Three (50%) of the six subjects exhibited a lengthening of prothrombin time and a lowering of clotting factor VII while receiving MK-647 plus SINTROM. Plasma SINTROM levels were lowered in all patients receiving MK-647 plus SINTROM.

2.6.8.7. Discontinuation due to adverse events

In total, 23 subjects (17.7%) reported 46 TEAEs leading to discontinuation of study drug; 14 subjects (21.9%) with 32 events in the diflunisal group and 9 subjects (13.6%) with 14 events in the placebo group. Drug-related TEAEs leading to discontinuation were registered for 14 subjects (10.8%); 8 subjects (12.5%) with 11 events in the diflunisal group and 6 subjects (9.1%) with 9 events in the placebo group.

The most frequently reported TEAEs leading to discontinuation or interruption of study drug were occult blood positive, reported for 3 subjects (4.7%) in the diflunisal group and 2 subjects (3.0%) in the placebo group, and nausea, reported for 3 subjects (4.7%) in the diflunisal group and 1 subject (1.5%) in the placebo group.

Table 27: TEAEs leading to discontinuation by system organ class and preferred term

System Organ Class Preferred Term	Diflunisal (N=64) n (%)	Placebo (N=66) n (%)	Total (N=130) n (%)
Any TEAE	8 (12.5)	6 (9.1)	14 (10.8)
Investigations	3 (4.7)	3 (4.5)	6 (4.6)
Occult blood positive	3 (4.7)	2 (3.0)	5 (3.8)
Haematocrit decreased	1 (1.6)	1 (1.5)	2 (1.5)
Blood creatinine increased	0	1 (1.5)	1 (0.8)
Gastrointestinal disorders	2 (3.1)	1 (1.5)	3 (2.3)
Nausea	2 (3.1)	1 (1.5)	3 (2.3)
Upper gastrointestinal haemorrhage	1 (1.6)	0	1 (0.8)
Vomiting	1 (1.6)	0	1 (0.8)
Nervous system disorders	1 (1.6)	1 (1.5)	2 (1.5)
Headache	1 (1.6)	1 (1.5)	2 (1.5)
Renal and urinary disorders	0	2 (3.0)	2 (1.5)
Renal failure	0	2 (3.0)	2 (1.5)
Cardiac disorders	1 (1.6)	0	1 (0.8)
Cardiac failure	1 (1.6)	0	1 (0.8)
Eye disorders	1 (1.6)	0	1 (0.8)
Ocular hypertension	1 (1.6)	0	1 (0.8)
General disorders and administration site conditions	0	1 (1.5)	1 (0.8)
Malaise	0	1 (1.5)	1 (0.8)

2.6.8.8. Post-marketing experience

There is no post-marketing experience with diflunisal in the ATTR-FAP indication. Post-marketing experience with diflunisal in its traditional NSAID indications is extensive. Diflunisal was previously authorised in Sweden (between 1979 and 2007) and several other European countries where different generic versions were available. All brands of diflunisal have now been withdrawn from all EU markets for commercial reasons (not because of safety concerns). In the US, the initiator was also withdrawn, however, generic presentations are still available. The post-authorisation experience of diflunisal

products refers to safety data for other indications than ATTR-FAP and higher doses than those applicable for Diflunisal AO Pharma. It should be noted that diflunisal marketing authorisation was withdrawn from for commercial reasons, not because of any concerns regarding safety.

Although it has never been authorised for this indication, diflunisal is in current clinical practice widely used for the treatment of ATTR-FAP and is recommended in this role by a number of European guidelines (Adams 2016; Ando 2022; Conduluci 2021; Swedish Council for New Therapies 2023). In practice, diflunisal has been used for the treatment of ATTR amyloidosis for many (>10) years in a number of EU and non-EU countries despite never having been authorised in this indication. This usage continues today despite the absence of an authorised product. For example, in Sweden, diflunisal is available as an extemporaneous formulation and in the Netherlands, it is available as an unlicenced medicine (Dolaced).

2.6.9. Discussion on clinical safety

Pivotal safety data are derived from the study H-23750 (randomised, placebo-controlled, parallel-group, double-blind, multi-centre, corresponding to Phase 3), in which 130 subjects were randomised to diflunisal 250 mg twice daily (n=64) of placebo (n=66) for a planned treatment period of 2 years. This study was conducted between April 2006 and May 2013 and the report presented by the applicant was compiled using the summaries and listings generated retrieved from the available datasets and documentation.

The median duration of study treatment exposure was 562.2 (240.32) days in the diflunisal group and 456.4 (266.90) days in the placebo group. Out of the 64 patients in the diflunisal arm, 51 patients had a duration of exposure ≥12 months (34 079 patient-days). Taking into consideration that ATTR amyloidosis is an orphan disease and the safety profile of diflunisal has been defined during more than 40 years of marketed use, the size of the safety database could be acceptable for an orphan drug. It is noteworthy that the dose to be used in the ATTR-FAP indication is lower than that routinely used historically for NSAID indications (250 mg twice daily for ATTR-FAP vs 500 mg twice daily for NSAID indications) and although it has never been authorised for ATTR-FAP indication, diflunisal is in current clinical practice for the treatment of ATTR-FAP, being recommended by some of European clinical guidelines.

The applicant mentioned the Umea registry, where patients have been treated for a mean of 3.4 years and a maximum of 10.2 years, as a source of evidence that reassures the safety collective experience data for this indication. However, the applicant recognised the presence of several methodological flaws: patients could be seen once or twice a year (meaning no fixing data collection points), data collection was the responsibility of the treating physician in a non-structured way, patients were free to opt out at any time; all in all, one cannot exclude several sources of bias when interpretating the data retrieved. Though, the applicant mention that there were no safety signals relating to bleeding or renal failure, according to the information provided by the registry coordinator.

In the pivotal trial, adverse events were reported for a total of 110 subjects (84.6%) and for a similar number of subjects in each of the treatment groups. Nevertheless, it should be noted the higher drop-out rate and the occurrence of open-label diflunisal treatment in the placebo group. The fact that the medicinal product under investigation was commercially available in several of the study countries where the trial was conducted made the risk for dropouts high, as subjects experiencing functional deterioration could choose an open-label drug regimen. As a consequence, the higher drop-out rate and the occurrence of open-label diflunisal treatment in the placebo group might had impacted the interpretation of the reported adverse events. In total, 10 subjects randomised to placebo stopped

taking the study drug and instead acquired diflunisal outside of the study or were prescribed open-label diflunisal as rescue treatment. Seven of these subjects continued in the study. Therefore, it cannot be ruled out that this affected the results of the ITT population. As requested, the applicant discussed how the higher drop-out rate and the occurrence of existent open-label diflunisal treatment in the placebo group might have affected the interpretation of safety data results. For that, the applicant presented a comprehensive list of adverse events reported by patients who (might have) switched to open-label diflunisal, along with the causality assessments and dates of onset for those events. All in all, none of the reported AEs from these patients was considered to be a serious adverse event and none was rated as severe, thus one can concur with the applicant that the overall qualitative assessment of the safety of diflunisal does not change for the requested indication.

Most AEs were mild or moderate in severity. Fifty-four events in 28 subjects (21.5%) were graded as severe; these occurred in 14 subjects (21.9%) in the diflunisal group and 14 subjects (21.2%) in the placebo group. A total of 15 AEs were considered life-threatening; these occurred in 5 subjects (7.8%) in the diflunisal group and 7 subjects (10.6%) in the placebo group. The most frequently reported TEAEs occurred in the SOCs Infections and infestations (39.1% of subjects in the diflunisal group vs 40.9% of subjects in the placebo group), Gastrointestinal disorders (35.9% vs 37.9%), and Nervous system disorders (35.9% vs 30.3%). Adverse events were reported more frequently in the diflunisal group for the SOCs Musculoskeletal and connective tissue disorders (29.7% of subjects in the diflunisal group vs 12.1% of subjects in the placebo group) and General disorders and administration site conditions (29.7% vs 10.6%). An imbalance of overall cardiac disorders events, including serious and drug related events which were more frequently reported in the diflunisal arm were observed. Moreover, the applicant discussed the cardiovascular and renal safety of diflunisal for the proposed indication, analysing the data from the pivotal trial and taking into consideration the existent information from this product – not only from the Swedish SmPC for diflunisal but also the former UK SmPC. As result, the applicant proposed changes in the section 4.4 of the SmPC on renal and cardiovascular safety, adding and strengthening the existing text with additional information taken from the former (but more recent) UK SmPC.

Overall, serious TEAEs were reported for a total of 45 subjects (34.6%); 21 subjects (32.8%) in the diflunisal group and 24 subjects (36.4%) in the placebo group. In the diflunisal group (N=64), most serious TEAEs were reported within the SOCs cardiac disorders (12.5% of subjects), nervous system disorders (12.5%), infections and infestations (9.4%), and gastrointestinal disorders (7.8%). Of the reported SAEs, 14 events were assessed as related to study treatment (6 events in the diflunisal group and 8 events in the placebo group). Among the 6 SAEs assessed as drug related in the diflunisal group, 2 were cardiac failure congestive, 1 vomiting, 1 nausea, 1 upper gastrointestinal haemorrhage and 1 post procedural haemorrhage. A total of 7 subjects (5.4%) had TEAEs with outcome of death; 4 of these subjects (6.3%) were in the diflunisal group and 3 subjects (4.5%) in the placebo group. None of the deaths was considered drug related by the applicant. A total of 15 AEs considered life-threatening were reported in 5 subjects (7.8%) in the diflunisal group and 7 subjects (10.6%) in the placebo group.

Treatment-emergent AEs leading to discontinuation (including drug interruption) were reported for a total of 23 subjects (17.7%); slightly more frequently in the diflunisal group (21.9%) than in the placebo group (13.6%). The most frequently reported TEAEs leading to discontinuation or interruption of study drug were occult blood positive and nausea, both are included in the section 4.8 of the SmPC. In what concerns to the proposed product information, the applicant presented substantial changes to the initial SmPC proposed, taking into account not only the diflunisal SmPC from Sweden (dated 2004) but also the UK SmPC from 2015. Further, the applicant stated that the revised SmPC submitted incorporates current NSAID class labelling (European SmPCs for ibuprofen and naproxen were used by the applicant to inform these revisions). Regarding the revised section 4.8, a proper description of the

used methodology was provided. The revised version proposed by the applicant took into account: a) the current standard MedDRA SOCs, b) current standard frequency categories and frequency definitions, c) action taken in case of disagreement on frequency between SmPC (the higher frequency was used) and d) action taken in case of a different frequency being observed in Study H-23750 than that cited in the legacy SmPCs (the higher frequency was used). Overall, the applicant provided satisfactory responses/amendments to the SmPC.

Considering the secondary pharmacodynamics of diflunisal in the context of ATTR treatment, main safety concerns would be related to its anti-inflammatory effects and associated adverse events. With the data provided on laboratory measurements, some of these events could be analysed. Although there was some variation in the baseline values, with a proportion of patients presenting with abnormal values, the median of diflunisal and placebo were within the accepted normal range and were comparable. Renal effects were not observed in the data provided, with no significant change in median serum creatinine nor BUN until month 24 of administration, as well as parameters measured by urinalysis. However, it is reported by the applicant that some patients shifted from normal to abnormal values throughout the treatment period. Although mild elevations of hepatic transaminases and jaundice are reported to happen with diflunisal administration (as per diflunisal label as anti-inflammatory) the study data provided by the applicant do not show changes in transaminases. The applicant has provided baseline bilirubin values, but no follow-up values, nevertheless, the study data indicates that no related adverse events were reported. Haematology parameters are particularly relevant in the case of gastric adverse events, correlated with the COX-1 inhibitory activity of diflunisal, along with the possibility of anti-platelet effect. No significant change in haematocrit or haemoglobin levels were observed in the data provided by the applicant. Alterations reported were mostly in patients with already abnormal baseline values. Although median values for platelet count were also found to be similar in baseline and subsequent measurements, a proportion of patients was reported to have significant changes during this period. Given the relevance of platelet count and thrombocytopenia in possible GI bleeding severity due to diflunisal mediated mucous membrane injury, a focused analysis of these cases is justified. Regarding other measurements, one to be noted is the BNP and nt-proBNP in which variations were observable in both groups. These variations were more notorious in the placebo group. This could be correlated to the cardiomyopathy aspects of ATTR and natural evolution of the disease in some patients. The evaluation of presence of occult blood by stool guaiac test showed that some individuals presented with positive occult blood. Although placebo group has also reported of positive occult blood tests, the mean is higher for diflunisal group.

In line with this and although median values of safety laboratory parameters (6, 12 and 24 months) were not found to be significantly different for diflunisal population, a variable proportion of patients was reported to have significant changes during this period. The applicant presented an analysis of the percentage of patients with changes from baseline without indication of the absolute values and clinical correlation, only disperse information in individual patients' narratives was provided. Hence, the applicant was asked to present an analysis of this data, particularly for renal and haematological effects, and discuss clinical correlations. In 3 patients that presented with haematocrit drop, it was mild and not clinically significant, with reductions accompanied by haemoglobin levels not correlated to a diagnosis of anaemia. In one patient, haematocrit and haemoglobin reduction led to levels compatible with the diagnosis of anaemia. Blood was detected in faeces and blood loss from GI bleeding was assumed from the investigator. This patient had lower than normal levels of haematocrit and haemoglobin at baseline and therefore already had some anaemia risk. This risk is identified and included in the SmPC as potential adverse effects, as well as in the warnings. The event in the patient with elevated transaminases was categorised by the investigator as non-serious, expected, mild and unlikely to be related to study drug. The elevation was mild, temporary and reversible and it did not lead to suspension of treatment. The patient had already higher than normal ALT levels at baseline.

Transthyretin is a known carrier for vitamin A and medicines that decrease TTR level are known to reduce vitamin A levels. Although diflunisal does not reduce TTR levels it alters its structure by binding to it. The applicant has not provided data supporting the absence of effect of diflunisal binding to TTR on its ability to function as vitamin A carrier and blindness was reported as an AE in diflunisal group. Thus, the applicant was asked to discuss the effect of diflunisal binding to TTR in the vitamin A carrier function. In the responses, the applicant has stated that the blindness case reported in the diflunisal group was clearly unrelated to vitamin A transport, since the cause of blindness was identified to be from embolic nature. The applicant has additionally stated is not aware of any data on the relationship between conformational change in TTR as a result of diflunisal binding and its ability to carry RBP. The applicant then made some biochemical considerations based on literature to support this observation. In summary, the applicant concluded that, while diflunisal binding to TTR might affect its ability to carry RBP, there is no evidence that this translates into clinically relevant adverse effects.

No post marketing data have been provided by the applicant. However, since diflunisal was approved for the treatment of pain, the applicant was asked to provide all the available safety data. The applicant obtained under freedom of information rules, access to a post-authorisation safety update report issued by Merck and covering the period 1 June 1991 to 31 March 1996. It is estimated that in this time period, the exposure covered 1-2 million patient-years. A total of 177 spontaneous reports fulfilled the criteria for inclusion in this safety update. Of these, 55 reports were sent to regulatory authorities on an expedited basis. During this period there were 7 reports of a drug overdose with diflunisal and 7 spontaneous reports of possible drug interaction with diflunisal. During this reporting period there were 8 spontaneous reports of fatal outcome.

2.6.10. Conclusions on the clinical safety

Overall, diflunisal was well tolerated and there were no major safety findings in the pivotal study that altered the established safety profile of diflunisal based on existent accumulated experience.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns

Table 28: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.7.3. Risk minimisation measures

None.

2.7.4. Conclusion

The CHMP and PRAC considered that the updated risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

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

2.8.2. Periodic Safety Update Reports submission requirements

Based on the scientific reason described below, the PRAC is of the opinion that a separate entry in the EURD list for Attrogy is needed, as it cannot follow the already existing entry for diflunisal with PSUR intervals of nine years. The target population of Attrogy differs in some aspects from the broad original target population of diflunisal. Patients with transthyretin amyloidosis with polyneuropathy may have different degrees of progressing heart and renal impairment, depending on the type of mutation and duration of disease, as amyloid may be deposited in these organs as well as in peripheral nerves. A PSUR interval of nine years is considered too long. As Attrogy has an orphan indication, a short interval in not meaningful either as case reports and literature publications likely will need some time to reach a cumulative level necessary to allow for any sound conclusion. It is therefore considered that the PSUR cycle for Attrogy should be 3 years.

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion.

The data lock point (DLP) of Attrogy will be aligned with diflunisal to November 3. However, the PSUR cycle duration should initially be 3 years (next DLP 03/11/2028, submission date 01/02/2029) for the reasons mentioned above. Eventually, the cycle duration might be prolonged and synchronised with diflunisal.

2.9. Non-Conformity of paediatric studies

Not applicable

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Attrogy (diflunisal) is not included in the additional monitoring list.

3. Benefit-Risk Balance

3.1. Therapeutic context

3.1.1. Disease or condition

The target indication applied for by the applicant is for transthyretin amyloid amyloidosis in adults with polyneuropathy. The studied population pertained to aTTRv FAP patients only.

The aim of the treatment is to delay disease progression and disability.

3.1.2. Available therapies and unmet medical need

Current treatment of ATTR-FAP includes Liver transplant and medical therapeutic agents. The EU medicinal approved drugs are tafamidis, inotersen, patisiran, vutrisiran and eplontersen.

Diflunisal - previously marketed as a NSAID - has been used as an off-label agent for the treatment of FAP in some countries where it is available. The mode of action of diflunisal is supposed to be similar to tafamidis.

Despite progress in its current treatment and management, FAP is still incurable. Survival from symptom start is 11 years on average.

3.1.3. Main clinical studies

The main evidence of efficacy submitted is a single phase III multicentre, randomised, placebo controlled double blinded study, comparing diflunisal (n=64) vs placebo (n=66) in symptomatic FAP adult patients.

It should be signalled that during most of the study period (08 May 2006 - 09 Dec 2012) no other disease specific therapeutic agent was available. Exception is Vyndaqel which was approved by the EC on November 16, 2011.

3.2. Favourable effects

The change from baseline in mean placebo subtracted difference of diflunisal at month 24:

- in NIS+7 was 18.0 points (95% CI 9.9, 26.2; p<0.001).
- in NIS-LL was 8.3 points (95% CI 4.1, 12.6; p<0.01)
- in NIS score was 16.8 points (95% CI 9.6, 24.0; p<0.001)
- in SF-36 Physical was -6.1 points (95% CI -9.8, -2.5; p<0.001).

The applicant has also used a local Umeå registry as supplemental data, both in support of efficacy data and for a Bayesian augmentation of the clinical trial results. Given the nature of registries, data are not collected in a systematic fashion at regular intervals post initiation of diflunisal treatment, rather data are collected idiosyncratically from patient to patient according to the nature and severity of their underlying disease. Nevertheless, sufficient data were captured to allow the analysis of Kumamoto score, PND score, FAP score, mBMI, NYHA classification and Karnofsky performance score. The characteristics of the n=118 registry subjects included in the analysis in this report were broadly similar to the diflunisal treated subjects in Berk et al; Kumamoto score at the start of diflunisal therapy was similar as was PND class and mBMI. However, registry subjects were older (mean age 68 years vs 61 years in Berk et al) and the fraction of subjects with a VM30 mutation was higher (86% vs 56% in the Berk et al). Over the period of diflunisal treatment, there was an upward trend in Kumamoto score, PND class and FAP class score, a downward trend in Karnofsky score and mBMI while NYHA class score remained flat. These data suggest a progression of disease over time as would be expected, albeit only gradual. This can be seen by examining mean change in parameter values after 24 months of diflunisal therapy; the mean change in Kumamoto score was 2.15 95% CI (-0.09, 4.40), Karnofsky score was -3.15 95% CI (-3.87, -2.42), PND score was 0.37 95% CI (0.26, 0.49), mBMI was -27.8 95% CI (-41.8, -13.7), NYHA class score was -0.011 95% CI (-0.114, 0.091) and FAP class score was 0.15 95% CI (0.10, 0.20). Finally, Bayesian augmentation of the Berk *et al* data with the diflunisal registry data served to enhance treatment effect estimates versus placebo; the probability diflunisal therapy is superior to placebo after 24 months was 99.6% and 97.2% for Kumamoto score and mBMI respectively.

As supportive data the applicant also presented 4 additional publications with safety and effects data of diflunisal in patients with ATTR. Data from three different open-label studies is summarised below.

In the study conducted by Takahashi et al (2014), an open-label intervention study without a control group carried out in an endemic district in Japan where most FAP patients were late-onset, the authors concluded that diflunisal might be effective especially for autonomic dysfunction in late-onset FAP with a TTR Val30Met mutation. In the study conducted by Sekijima et al (2015), the authors concluded that longitudinal analyses examining data collected at baseline, 24 months, and after 24 months confirmed the sustaining effects of diflunisal on both neurological and cardiac functions. Overall, it was concluded that clinical effects are sustained after 2 years of treatment, however clinical symptoms deteriorated slowly in most patients, indicating that diflunisal cannot stop disease progression of hereditary ATTR amyloidosis completely. In the study conducted by Wixner (2019), a 24-month open-label observational study designed to monitor the effect of diflunisal 500 mg daily (250 mg twice daily) in hereditary amyloidosis, the authors concluded overall that although limited by high dropout rates, mainly due to liver transplantation and study closure, the results were in line with the placebo-controlled trial. All in all, in what regards to safety data results retrieved, these three small open-label studies did not raise any new concerns regarding the safety profile of diflunisal.

3.3. Uncertainties and limitations about favourable effects

To support the MAA the applicant submitted results of a single pivotal study, which was conducted by an academic group. The target dose was selected based on the Sekijma et al study (2006), which evaluated 3 dose strengths of 125mg, 250 mg and 500 mg, administered bid. The choice of the dose was based on the diflunisal-TTR binding stochiometric approach which showed comparable stabilisation of TTR in 250 mg bid doses and 500 mg bid doses. The applicant clarified that classical phase II b study aimed to select and adequate dose would not be feasible in this population of patients. Unlike what the applicant states, a phase 2b study could have been performed in these patients if carried on

before 2009-2010; afterwards the window of opportunity would be lost, given the publicly available results of tafamidis, rendering the complete phase 2 and even the phase 3 unfeasible (in fact, even the phase 3 study was affected near its end). The applicant provided results of Tsai et al, 2023 study, which showed concentration-dependent TTR subunit exchange rate. Doubt remains on whether this may be the best dose for the individual patient and results of this study should be treated with caution due to huge interpatient differences in diflunisal concentrations. Nevertheless, it is acknowledged that conducting a dose-selection study would not be feasible and it can be agreed that a totality of the available data support the proposed posology. All things considered, it is understood why those responsible for the study at that time have decided to go for the 250 mg dosing.

The applicant states that some FAP type 3 patients have been included (4 in diflunisal arm, 4 in placebo arm). However, according to the exclusion criteria, the end-stage neurologic disease (parenteral nutrition, bedsores, debilitating orthostasis, performance status >3 [confined to bed >75% per day]), limited survivorship (<2 years), liver transplantation in <1 year, and genotype positive/disease-free kindred were to be excluded. Therefore it is prudent to exclude stage 3 and 4 patients from the indication, for both efficacy and safety reasons: it is unlikely that with the expected MoA of diflunisal there may be a response in late stage disease, as is well known for tafamidis; on the other hand, these advanced patients almost invariably already have significant renal involvement, and the use of a NSAID such as diflunisal is not welcomed. The applicant agreed to restrict the indication to FAP stage 1 and 2 patients in line with the CHMP recommendation.

The PEP of the Study - NIS+7 was met from a formal perspective, the LS mean change in NIS+7 score from baseline to Month 24 was 8.7 points (95% CI, 3.3 to 14.14 points) in the diflunisal group and 25.0 points (95% CI, 18.4 to 31.6 points) in the placebo group. The difference in LS means between groups of 16.3 points (95% CI, 8.1 to 24.5 points; $p < 0.001$).

In a post-hoc MI analysis an estimated difference in LS mean NIS+7 score change from baseline between the diflunisal and placebo groups was comparable to the primary analysis: 16.3 points (95% CI, 8.1 to 24.6; p=0.0002) at Month 24, and 5.8 points (95% CI, -0.1 to 11.7; p=0.0552) at Month 12. However, in the Jump to Placebo sensitivity analysis the differences were not statistically significant (p=0.1061 and p=0.0774, respectively).

In a responder analysis 29.7% of subjects in the diflunisal group were found successful at Month 24 compared to 9.4% of subjects in the placebo group (p=0.007).

Risk ratio analysis indicated a 3-fold higher probability of response in the diflunisal vs the placebo group (RR 3.17; 95% CI, 1.35 to 7.41).

Secondary endpoint, the mean change in NIS from baseline to Month 24 was met, achieving 6.4 points (95% CI, 1.6 to 11.2 points) in the diflunisal group and 23.2 points (95% CI, 17.8 to 28.5 points) in the placebo group. The difference in LS means between groups was 16.8 points (95% CI, 9.6 to 24.0), p<0.0001. No statistically significant differences were reported in the 36-item Short Form Health Survey (SF-36).

However, in an originally planned analyses, potential impact of missing data was not included. It should be noted that very high number of subjects discontinued the study - 42.2% from the diflunisal group and 60.6% from the placebo group. The overall number of subjects who had primary endpoint data at Month 24 was relatively low (40 (62.5%) and 30 (45.5%) in the diflunisal and the placebo groups. The presented data do not explain the differences between the timing of dropping out of the study arms: while in diflunisal, the highest percentage of drop-out occurred between month 12 and month 24, in the placebo arm the highest percentage loss occurred between baseline and month 6. This is not readily explained by the intercurrent events, since intercurrent events were well balanced between arms apart from disease progression, and the magnitude of this event was not so high as to

explain the current difference in timing. The applicant provided information on the patient trajectory of both arms and there was no evidence that an underlying occult factor might be responsible for the difference observed.

In summary, the main uncertainty on the quality of the data presented have been mitigated. The clinical trial was reported to be academic, and not for registration purposes. Data was gathered between 2006 and 2012 and the study report was performed in 2023. There was a significant lack of data for many of the endpoints, and a higher-than-expected attrition rate.

Furthermore, the applicant reported that 10 patients from the placebo arm (and none from the diflunisal arm) may have started to use commercially available diflunisal. The applicant has provided data on the 10 patients allocated to placebo that decided to start on diflunisal. Of these, 7 continued follow-up and 3 stopped the study. The reasons for change to diflunisal were identified in 4 cases, and were related to the sense of worsening and for the sensed improving with 2 brothers; all other switches lacking justification. While it may seem odd that all switching to diflunisal occurred in the placebo arm (as statistically unlikely if expected at random, less so but still unlikely assuming the not random effect of disease progression) the fact is that no evidence exists that patients or others may have unblinded the study treatment. The conclusion from the applicant that switching placebo to diflunisal does not affect assessment of the effect of diflunisal as compared to placebo, as it would underestimate diflunisal may not be completely followed, since it is the B/R balance that is at stake, and not just the blinded efficacy value. Still, given the duration of follow-up under placebo treatment of these patients, it can be assumed that these 10 patients may have not significantly impacted the B/R analysis.

The applicant argues that when the study was conducted ICH E9 (R1) estimand approach had not been published and that the best approach was to provide a "treatment policy" as per Intent to treat philosophy.

Also, there was no prespecified methodology for Type I Error control. Regarding this aspect, the applicant states that, for the 6 pre-specified secondary endpoints available at 24 months, there was a good behaviour of 4 endpoints (NIS, NIS-LL, Kumamoto score and SF-36 physical score), which reached statistical significance even with Bonferroni adjustment. For the first 3 endpoints this was no surprise, since they are part of the primary endpoint or assess similar aspects as assessed by the primary endpoint. This leaves the SF-36 as a relevant secondary endpoint. The SF-36 mental score was not significant, but the result might be nonetheless relevant since it accommodates aspects not assessed with NIS scores, and the modified BMI which was also non-significant, and it is regretful, since weight loss is a very important marker of loss of control in the disease. It may be solely due to the small sample size and missing data.

In spite of the NIS-LL better correlate with deambulation and patient self-sufficiency, and has been more favoured in the assessment of FAP products, the NIS+7 score has been validated and used for the assessment of FAP. Given the requirement for both clinical and neurophysiological assessment for NIS+7 but not to NIS-LL (clinical assessment), this may have increased the missing data reported.

The random distribution between study arms may have favoured the diflunisal arm as compared to the placebo arm, and this may have impacted on the worsening of placebo patients and on the drop-out rate. There is a high patient drop-out in both study arms, more prominent in the placebo arm (60.2% in placebo vs 42.2% in diflunisal did not complete treatment).

The applicant has also used a local Umeå registry as supplemental data, both in support of efficacy data and for a Bayesian augmentation of the clinical trial results. The FAP registry population differs from the population in the study, in terms of disease severity and age. Efficacy data from the registry

has been intermittently registered. Since NIS+7 (nor NIS) was not available for assessment, augmentation has been performed for two secondary endpoints, the Kumamoto score and mBMI.

3.4. Unfavourable effects

In the pivotal trial, adverse events were reported for a total of 110 subjects (84.6%) and for a similar number of subjects in each of the treatment groups. Nevertheless, it should be noted the higher drop-out rate and the occurrence of open-label diflunisal treatment in the placebo group.

In total, 10 subjects randomised to placebo stopped taking the study drug and instead acquired diflunisal outside of the study or were prescribed open-label diflunisal as rescue treatment. Seven of these subjects continued in the study.

Most AEs were mild or moderate in severity. Fifty-four events in 28 subjects (21.5%) were graded as severe; these occurred in 14 subjects (21.9%) in the diflunisal group and 14 subjects (21.2%) in the placebo group. A total of 15 AEs were considered life-threatening; these occurred in 5 subjects (7.8%) in the diflunisal group and 7 subjects (10.6%) in the placebo group. The most frequently reported TEAEs occurred in the SOCs Infections and infestations (39.1% of subjects in the diflunisal group vs 40.9% of subjects in the placebo group), Gastrointestinal disorders (35.9% vs 37.9%), and Nervous system disorders (35.9% vs 30.3%). Adverse events were reported more frequently in the diflunisal group for the SOCs Musculoskeletal and connective tissue disorders (29.7% of subjects in the diflunisal group vs 12.1% of subjects in the placebo group) and General disorders and administration site conditions (29.7% vs 10.6%). There was also an imbalance of cardiac disorders, which were more frequently reported in the diflunisal arm.

Treatment-emergent AEs assessed as related to study drug were reported for a total of 54 subjects (41.5%); 29 subjects (45.3%) in the diflunisal group and 25 subjects (37.9%) in the placebo group.

Overall, serious TEAEs were reported for a total of 45 subjects (34.6%); 21 subjects (32.8%) in the diflunisal group and 24 subjects (36.4%) in the placebo group. In the diflunisal group (N=64), most serious TEAEs were reported within the SOCs cardiac disorders (12.5% of subjects), nervous system disorders (12.5%), infections and infestations (9.4%), and gastrointestinal disorders (7.8%). Of the reported SAEs, 14 events were assessed as related to study treatment (6 events in the diflunisal group and 8 events in the placebo group). Among the 6 SAEs assessed as drug related in the diflunisal group, 2 were cardiac failure congestive, 1 vomiting, 1 nausea, 1 upper gastrointestinal haemorrhage and 1 post procedural haemorrhage. A total of 7 subjects (5.4%) had TEAEs with outcome of death; 4 of these subjects (6.3%) were in the diflunisal group and 3 subjects (4.5%) in the placebo group. None of the deaths was considered drug related by the applicant.

Treatment-emergent AEs leading to discontinuation (including drug interruption) were reported for a total of 23 subjects (17.7%); slightly more frequently in the diflunisal group (21.9%) than in the placebo group (13.6%). The most frequently reported TEAEs leading to discontinuation or interruption of study drug were occult blood positive and nausea.

Regarding the safety related to drug-drug interactions, no data were collected in the pivotal H-23750 study.

Although median values of safety laboratory parameters (6, 12 and 24 months) were not found to be significantly different for diflunisal population, a variable proportion of patients was reported to have significant changes during this period.

As supportive data the applicant also presented 3 additional publications with safety and effects data of diflunisal in patients with ATTR retrieved from three different open-label studies.

In the study conducted by Takahashi et al (2014), an open-label intervention study without a control group carried out in an endemic district in Japan where most FAP patients were late-onset, the authors concluded that diflunisal might be effective especially for autonomic dysfunction in late-onset FAP with a TTR Val30Met mutation. In the study conducted by Sekijima et al (2015), the authors concluded that longitudinal analyses examining data collected at baseline, 24 months, and after 24 months confirmed the sustaining effects of diflunisal on both neurological and cardiac functions. Overall, it was concluded that clinical effects are sustained after 2 years of treatment, however clinical symptoms deteriorated slowly in most patients, indicating that diflunisal cannot stop disease progression of hereditary ATTR amyloidosis completely. In the study conducted by Wixner (2019), a 24-month open-label observational study designed to monitor the effect of diflunisal 500 mg daily (250 mg twice daily) in hereditary amyloidosis, the authors concluded overall that although limited by high dropout rates, mainly due to liver transplantation and study closure, the results were in line with the placebo-controlled trial. All in all, in what regards to safety data results retrieved, these three small open-label studies did not raise any new concerns regarding the safety profile of diflunisal.

3.5. Uncertainties and limitations about unfavourable effects

Please see discussion on the quality of data, both from the clinical trial and the registry as discussed in section 3.3., and impact safety as well as efficacy.

Adverse events reported more frequently in the diflunisal arm occurred in the Musculoskeletal and connective tissue disorders, general disorders and administration site conditions, and also cardiac disorders, which were more frequently reported in the diflunisal arm. The higher cardiac frequency of events is of concern, given the cardiac involvement of amyloid disorders patients.

There were 4 deaths in the diflunisal and 3 deaths in the placebo group.

Drug-drug interactions have been described, namely including other NSAID and VKA, but no data is available regarding the new NOACs. A clear reference to DOACs (direct oral anticoagulants) should be added to PI. It is acknowledged that there is limited literature on diflunisal administration in DOAC-treated patients, however there is undoubtedly enough pharmacological rational to extrapolate the bleeding risk to this class as well. It is stated in the SmPC of currently approved DOACs that there is potential for interaction between NSAIDs and DOACs leading to increased risk of bleeding and therefore it is requested that this information is also provided clearly in the SmPC of diflunisal.

For the Umeå registry, which is presented as a source of evidence that reassures the safety collective experience data for this indication, the applicant did not provide safety data results (methods of collection, listing of adverse events). These patients are older than the Study H-23750 patients, and constitute a more diverse phenotype of FAP than the patients with the common ATTR-PN Val Met30 mutation. The registry data has also been proposed to assist the study H 23750 with a Bayesian augmentation. Given the differences in study population, this was not considered adequate. It is noted that the overall registry population included mostly elderly patients. Therefore, the overall value of the Registry is considered very limited.

Safety data from other sources was also not adequately discussed.

3.6. Effects table

Table 29: Effects table for diflunisal

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
NIS+7	Change from baseline in NIS+7 after 24 months treatment	Points (95% CI)	8.2 (2.9, 13.6)	26.3 (20.2, 32.4)	Data quality / relevant difference	study H-23750
NIS	Change from baseline in NIS after 24 months treatment	Points (95% CI)	6.4 (1.6, 11.2)	23.2 (17.8, 28.5)	Data quality / relevant difference	study H-23750
NIS-LL	Change from baseline in NIS-LL after 24 months treatment	Points (95% CI)	3.8 (0.9, 6.6)	12.1 (8.9, 15.3)	Data quality / relevant difference	study H-23750
SF-36 Physical	Change from baseline in SF-36 physical component after 24 months treatment	Score (95% CI)	1.2 (-1.2, 3.7)	-4.9 (-7.6, -2.1)	Data quality / relevant difference	study H-23750
Unfavourable Effects						
Dyspepsia	Gastrointestinal dyspepsia events	Number (%)	7 (10.9)	1 (1.5)	Data quality	study H-23750
Musculoskeletal / connective tissue	Musculoskeletal and connective tissue disorders events (not discriminated)	Number (%)	19 (29.7)	8 (12.1)	Data quality, not discussed	study H-23750
Chest pain	Chest pain vents	Number (%)	4 (6.3)	0 (0)	Data quality, not discussed	study H-23750
Cardiac failure	Cardiac failure events	Number (%)	4 (6.3)	0 (0)	Data quality	study H-23750
Hypertension	Hypertension as adverse event	Number (%)	7 (10.9)	2 (3.0)	Data quality	study H-23750

Abbreviations:
Notes:

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The initial statistical analysis plan was complemented with the post-hoc analyses which have been sufficiently detailed. The applicant argues that when the study was conducted ICH E9 (R1) estimand approach had not been published and that the best approach was to provide a "treatment policy" as per Intent to treat philosophy.

The very high number of study discontinuations and its implications have been sufficiently mitigated with the presented analyses and new data from the previously missing key secondary endpoint.

There are uncertainties regarding representativeness of the study population. However, the applicant agreed to restrict the indication to FAP stage 1 and 2 patients in line with the CHMP recommendation.

Generally, the PEP of the Study was met – the LS mean change in NIS+7 score from baseline to Month 24 was 8.7 points (95% CI, 3.3 to 14.14 points) in the diflunisal group and 25.0 points (95% CI, 18.4 to 31.6 points) in the placebo group. The difference in LS means between groups of 16.3 points (95% CI, 8.1 to 24.5 points; $p < 0.001$).

At Month 12, the LS mean change in NIS+7 score from baseline to Month 12 reached statistical significance (a difference of 6.1 points (95% CI, 1.1 to 11.1 points; p=0.0169).

The magnitude of effect and the consistency of improvement of both NIS related endpoints (including Kumamoto scale which has similar to NIS items) and SF-36 are considered clinically relevant in the stage 1 / 2 enrolled population. Although there is an approved agent with a similar MoA, its access has been limited and diflunisal is used off-label in countries where it is available. The other 3 approved agents are for parenteral administration and not so convenient.

The difference in response according to intrinsic characteristics of the population is not known. There was also not a response regarding mBMI which is a prognosis surrogate expected to parallel NIS+7 response.

A safety database from a study trial with follow-up for two years is considered sufficient in this orphan disease.

No safety data was provided from the supportive registry, which was claimed to be a testimony of maintenance of efficacy and safety. Notwithstanding, the external data from publications on the off label use of diflunisal mitigate this lack of medium to long-term data from the initial submission.

3.7.2. Balance of benefits and risks

The concerns regarding the pivotal study results reliability related to the statistical analysis, dose selection and study population as well as the balance of efficacy and safety data have been sufficiently detailed with the data and information presented by the applicant during the assessment of the MA application. A sufficient bridge between the Merck formulation and the proposed formulation was provided.

From a clinical efficacy/safety perspective approval of MAA is acceptable.

3.8. Conclusions

The overall benefit/risk balance of Attrogy is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Attrogy (diflunisal) is not similar to Vyndaqel (tafamidis), Tegsedi (inotersen sodium), Onpattro (patisiran) and Amvuttra (vutrisiran) within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Attrogy is favourable in the following indication(s):

> Treatment of *hereditary transthyretin-mediated amyloidosis (ATTRv) in adult patients with stage 1 or stage 2 polyneuropathy.*

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

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

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

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

Not applicable.