20 May 2021
EMA/397273/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Klisyri

International non-proprietary name: tirbanibulin

Procedure No. EMEA/H/C/005183/0000

Note

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

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List of abbreviations

ADE	Acceptable Daily Exposure
AES	atomic emission spectroscopy
API	Active Pharmaceutical Ingredient
AR	assessment report
AS	Active Substance
ASM	Active Substance Manufacturer
ASMF	Active Substance Master File = Drug Master File
AUC	area under the curve
BDL	Below the limit of detection
CEP	Certificate of Suitability of the EP
CMS	Concerned Member State
CTD	common technical document
CoA	Certificate of Analysis
CQA(s)	critical quality attributes
CPP(s)	critical process parameters
CRS	Chemical reference substance
DL	Detection Limit
DMF	Dimethylformamide
DOM	Date of manufacture
DSC	Differential scanning Calorimetry
ECD	Electrochemical detection
EDMF	European Drug Master File
EDQM	European Directorate for the Quality of Medicines
ee	enantiomeric excess
EP	European Pharmacopoeia
FDA	US Food and Drug Administration
FID	Flame ionisation detection
FP	finished product
FPM	finished product manufacturer
FTIR	Fourier transmission infrared (spectroscopy)
GC	Gas chromatography
GMP	good manufacturing practice
HDPE	high density polyethylene
HPLC	High performance liquid chromatography
ICH	International conference on harmonization
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma-mass spectrometry
IPA	isopropyl alcohol
IPC	in-process control
IR	Infra-red
IVRT	*in vitro* release test
KF	Karl Fischer
LoA	Letter of Access
LDPE	low density polyethylene
LOD	Loss on Drying
LoD	Limit of detection
LoQ	Limit of Quantitation
MA	Marketing Authorisation
MAH	Marketing Authorisation holder
MCC	microcrystalline cellulose
MO	major objection
MS	Mass spectroscopy
NfG	Note for Guidance
NIR	Near infra-red
NLT	Not less than
NMR	Nuclear magnetic resonance
NMT	Not more than
PCTFE	Polychlorotrifluoroethene
PDA	Photo diode array
PDE	Permitted Daily Exposure
PET	polyethylene terephthalate

PG	propylene glycol
PVC	Polyvinyl chloride
PVdC	Polyvinyl dichloride
Ph.Eur.	European Pharmacopoeia
QL	Quantitation limit
QOS	Quality Overall Summary
QP	Qualified Person
(Q)SAR	Quantitative structure–activity relationship
QTPP	quality target product profile
RH	Relative Humidity
RMS	Reference member state
ROI	residue on ignition
RRt	Relative retention time
Rt	Retention time
RT	Room temperature
SAL	Sterility assurance level
SEM	Scanning electron microscopy
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TGA	Thermo-Gravimetric Analysis
TSE	Transmissible Spongiform Encephalopathy
UPLC	ultra performance liquid chromatography
UV	Ultra violet
XRD	X-Ray Diffraction
XRPD	X-Ray PowderDiffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Almirall, S.A. submitted on 6 February 2020 an application for marketing authorisation to the European Medicines Agency (EMA) for Klisyri, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 18 October 2018.

The applicant applied for the following indication:

Klisyri is indicated for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis of the face or scalp in adults.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

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

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0075/2019 on the granting of a (product-specific) waiver.

Information relating to orphan market exclusivity

Similarity

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

New active Substance status

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

Scientific advice

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
20 September 2018	EMA/CHMP/SAWP/420335/2018	*Dr Caroline Auriche, Dr Rune Kjeken*

28 March 2019	EMA/CHMP/SAWP/182021/2019	*Prof Livia Puljak, Ms Flora Musuamba Tshinanu*

The Scientific advice pertained to the following quality, non-clinical, and clinical aspects:

Quality:

- Starting material, drug substance polymorph, limit for potentially genotoxic/mutagenic impurities and the acceptability of the proposed control strategy.
- Drug substance specification and manufacturing processs.
- Application of complementary tests when harmonisation does not exist between United States Pharmacopeia and European Pharmacopeia.
- Drug product specification, primary packaging, extractables / leachables, elemental impurities, antimicrobial effectiveness testing, photostability forced degradation studies.
- Acceptability of the proposed comparability testing package for registration of the tubes for commercial supply.

Non-clinical:

- Acceptability of the overall non-clinical package for marketing authorisation application (MAA), including safety pharmacology package, toxicology and carcinogenicity studies.

Clinical:

- Design and adequacy of two identical phase 3 studies for a MAA, with regards to unblinding before initiation of part B and statistical methods in particular regarding assessment of the primary endpoint at week 8.
- Safety assessment and size of safety database.
- Acceptability of the overall development plan for MAA and that the MAA can be based upon 8-week efficacy and safety data from the Phase 3 studies, with follow-up data on recurrence provided post-approval.
- Proposed indication for MAA.
- Acceptability of the dermal safety testing package for MAA.
- Adequacy of the PK package including in vitro metabolism and protein binding data for MAA.
- Obviating the need for a dedicated QT/QTc study.
- Bioanalytical validation package for MAA.
- Plans and design for a post-approval study.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Peter Kiely Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	6 February 2020
The procedure started on	27 February 2020
The Rapporteur's first Assessment Report was circulated to all CHMP members on	18 May 2020
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	18 May 2020

The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	2 June 2020
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 June 2020
The applicant submitted the responses to the CHMP consolidated List of Questions on	11 December 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	1 February 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	11 February 2021
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	25 February 2021
The applicant submitted the responses to the CHMP List of Outstanding Issues on	22 March 2021
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	07 April 2021
The CHMP agreed on a 2nd list of outstanding issues to be sent to the applicant on	22 April 2021
The applicant submitted the responses to the CHMP List of Outstanding Issues on	27 April 2021
The Rapporteurs circulated the Joint Assessment Report on the responses to the 2nd List of Outstanding Issues to all CHMP members on	05 May 2021
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 Klisyri on	20 May 2021

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The applied indication is for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis of the face or scalp in adults.

Actinic keratosis (AK) is an ultra-violet (UV) light-induced pre-cancerous lesion of the skin that represents the initial intra-epidermal manifestation of abnormal keratinocyte proliferation (Röwert-Huber, 2007; Fernandez Figueras, 2017).

2.1.2. Epidemiology and risk factors, screening tools/prevention

Overall, the prevalence of AK ranges from 33% to 49% in adult males and 14% to 28% in females, depending on the country and the study population. AK is common in older, fair-skinned populations of European ancestry, and is more frequently observed in men (Flohil, 2013).

It is by far the most common lesion with malignant potential to arise on the skin. AK is mostly seen in fair-skinned persons on skin areas that have had long-term sun exposure (Salasche, 2000). Patients with AK tend to have Fitzpatrick type I or II skin (fair skin) which burns and does not tan. In the Fitzpatrick classification system skin-types range from very fair (Type I) to very dark (Type VI).

Epidemiological data show a high occurrence rate of AK. Regions with higher ultraviolet exposure have a higher prevalence of AK. In Europe, a prevalence of 15% in men and 6% in women has been documented. Over the age of 70 years, 34% of men and 18% of women were found to have AK (Memon et al., 2000).

AK represents a carcinoma in situ of the skin, and when left untreated, AK can progress to invasive SCC (Röwert-Huber, 2007; Werner, 2013; Fernandez Figueras, 2017). Cutaneous SCC represents 20% to 50% of skin cancers. Currently, no reliable estimates concerning the frequency of AK developing into invasive carcinoma can be given.

2.1.3. Biologic features, aetiology and pathogenesis

An actinic keratosis is caused by frequent or intense exposure to UV rays from the sun or tanning beds mostly in fair-skinned patients being susceptible to solar damage. The term actinic keratosis (AK) describes clinically ill-defined reddish to reddish-brown scaly lesions on erythematous base in areas damaged severely by sunlight. The term does not imply anything about the biology or histopathology (Roewert-Huber et al, 2007).

Actinic keratoses (AKs) have been recognised as precursor of cancer or of precancerous lesions in the past but today they are considered as an early in situ squamous cell carcinoma and are categorised in several classifications with subdivisions into three grades depending on the amount of atypical keratinocytes in the epidermis. The clinical classification following Olsen et al. assesses the severity degree of single AK lesions:

Grade 1: mild (slight palpability, with actinic keratoses felt better than seen)

Grade 2: moderate (moderately thick actinic keratoses that are easily seen and felt)

Grade 3: severe (very thick and/or obvious actinic keratoses)

2.1.4. Clinical presentation, diagnosis and stage/prognosis

AK presents as erythematous, scaly patches on the skin of the face, scalp, and extremities and can occur as a single lesion or multiple lesions or as an entire field ("field cancerisation"), such as in sun-exposed areas on the forehead or the back of the hand (Dodds, 2014; Figueras Nart, 2018). An AK may regress, persist unchanged, or progress to invasive squamous cell carcinoma. Furthermore, predicting which course each individual lesion will follow is impossible.

2.1.5. Management

AK treatment options consist in two broad categories: surgical destruction of the lesions (e.g. using cryosurgery or curettage with or without electrosurgery) and medical therapy (Hofbauer, 2014; Werner, 2015; de Berker, 2017; Leitlinienprogramm Onkologie, 2019).

The appropriate treatment is generally chosen based on the number of lesions present and therapy may be broadly categorised as either lesion-directed (e.g., cryosurgery) or field-directed (e.g., topical products).

Topical therapy is ideally suited to address multiple lesions and in particular in field cancerisation.

The following topical field treatments are currently approved in the EU:

- Imiquimod
- 5-fluorouracil [5-FU]
- 5-FU 0.5% in 10% salicylic acid
- Diclofenac sodium
- Aminolevulinic acid
- Methyl aminolevulinate

The above products vary in terms of tolerability/toxicity and treatment duration.

Cases of SCC shortly after treatment with Picato (ingenol mebutate) have been reported (Moreno Romero, 2015; Maglie, 2018), and a referral for the review of data on skin cancer with Picato was concluded by the European Medicines Agency (EMA) in April 2020 (EMA/248352/2020). The conclusion was that the medicine may increase the risk of skin cancer and that its risks outweigh its benefits. Picato is no longer authorised in the EU as the marketing authorisation was withdrawn on 11 February 2020 at the request of the MAH.

About the product

Tirbanibulin disrupts microtubules by direct binding to tubulin, which induces cell cycle arrest and apoptotic death of proliferating cells and is associated with disruption of Src tyrosine kinase signalling.

The initially claimed indication was for the topical field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis of the face or scalp in adults.

The finally approved indication was for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults.

Tirbanibulin ointment should be applied to the affected field on the face or scalp once daily for one treatment cycle of 5 consecutive days. A thin layer of ointment should be applied to cover the treatment field of up to 25cm^2 (see SmPC section 5.1).

Type of Application and aspects on development

The clinical development programme has been structured according to the regulatory advice received from Health Authorities.

Formal guidance was received from the EMA (see section 1.1 Scientific advice).

At the time of Scientific Advice, the development programme for the registration dossier consisted of 2 identical one-year pivotal studies. The CHMP suggested that due to the low but serious risk of conversion to squamous cell carcinoma, the applicant may need to gather additional long-term data (up to 3 years) and determine the related conversion rate. Given the slow disease progression rate, it could be accepted that longer-term than one-year recurrence-rate is provided post-approval. Plans for this longer-term recurrence study (sample size, duration of follow-up) should be part of the MA dossier. Since no active comparator arm is included in the pivotal phase 3 studies, the applicant could consider gathering not just safety but also efficacy data in an open, long-term comparative study, where tirbanibulin 1% ointment is compared to an established reference treatment.

The applicant is planning to conduct a Phase IV, long-term, randomised, active-controlled, investigator-blinded, safety and re-treatment study (M-14789-41) in adult patients with AK on the face or scalp.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as an ointment containing 10 mg/g of tirbanibulin as the active substance.

Other ingredients are: propylene glycol and glycerol monostearate 40-55.

The product is available in sachets with an inner layer of linear low-density polyethylene. Each sachet contains 250 mg of ointment.

2.2.2. Active Substance

General information

The chemical name of tirbanibulin is *N*-benzyl-2-(5-(4-(2-morpholinoethoxy)phenyl)pyridin-2-yl)acetamide. It corresponds to the molecular formula $C_{26}H_{29}N_3O_3$. Its relative molecular mass is 431.53 and it has the chemical structure shown in Figure 1.

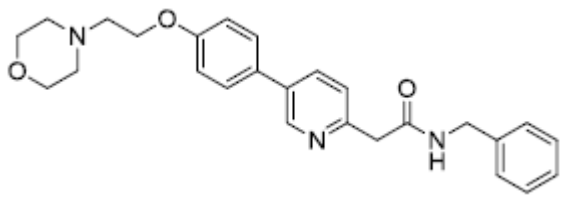


Figure 1. Chemical structure of tirbanibulin.

The structure of the active substance (AS) was adequately elucidated by a combination of elemental analysis, mass spectrometry (MS), IR spectroscopy and NMR spectroscopy, Physicochemical properties were investigated by thermogravimetric analysis, differential scanning calorimetry and x-ray powder diffraction (XRPD).

Tirbanibulin appears as white to off-white, non-hygroscopic, crystalline powder. It is freely soluble in dimethyl sulfoxide, slightly soluble in ethanol and ethyl acetate, and insoluble in water. The active substance pKas are 3.50, 6.16 (dibasic), and its partition coefficient LogP was found to be 2.78.

The active substance (AS) is achiral and has no stereocentres. Tirbanibulin exhibits polymorphism. Three polymorphic forms were identified. It has been shown that the manufacturing process consistently yields the thermodynamically most stable form. Polymorphism is controlled in the AS specification.

Manufacture, characterisation and process controls

A Major Objection (MO) was raised by CHMP concerning the QP declaration, which was incomplete, and was resolved with the provision of an updated QP declaration.

The manufacturing process consists of six steps. The choice of the starting materials has been justified in line with ICH Q11, they are controlled by suitable specifications, and are acceptable. Information on the manufacturers and suppliers of starting materials has been given. The manufacturing process has been described in sufficient detail. Satisfactory information on in-process controls (IPCs) tests and critical steps have been provided. Process intermediates were defined and are appropriately controlled The specifications and control methods for intermediate products and reagents have been presented and are acceptable.

The characterisation of the active substance and its impurities is in accordance with the EU guideline on chemistry of active substances. Potential and actual impurities were well discussed with regards to their origin and have been characterised. Carry-over of impurities has been discussed and results of purging studies have been presented. Genotoxic impurities have been adequately discussed and evaluated by two (Q)SAR prediction methodologies performed in line with M7. According to the outcome of this evaluation the impurities that need to be controlled in active substance have been identified and their limits have been justified. The overall control strategy is acceptable.

A summary of manufacturing process development activities has been provided. Changes introduced have been presented in sufficient detail and have been justified.

The AS container closure system complies with Ph. Eur. requirements for packaging materials. The material used in the manufacture of the primary packaging material meets the requirement of European Directive 10/2011 and all subsequent amendments including 2015/174 and Ph. Eur. 3.1.3.

Specification

The AS specification includes appropriate tests and limits for appearance (visual), identification (HPLC), water content (Ph. Eur.), residue on ignition (Ph. Eur.), palladium (Ph. Eur.), melting point (Ph. Eur.), assay, related substances, residual solvents , microbiological examination (Ph. Eur.) and crystal form.

Specifications for water content, residue on ignition, melting point, and assay are based on batch and stability results. The limits for the residual solvents and impurities were established in accordance with ICH Q3C and Q3A respectively. The limits for alkylsufonates are below the ICH M7 TTC based on a daily dose 20 µg/day. The proposed specifications are justified.

The presence of elemental impurities in the AS was evaluated in line with ICH Q3D. Results of batch analysis data from three batches obtained by a validated ICP-MS method were below the ICHQ3D PDE. Based on this, a test for elemental impurities is accepted not to be included in the specification.

The analytical methods used have been adequately described and validated in accordance with ICH guidelines. Satisfactory information regarding the reference standards used in the routine analysis of the AS has been presented.

Batch data was provided on representative batches. All the results comply with the proposed AS specification and demonstrate consistent manufacture and quality of the AS.

Stability

Stability data has been provided for three production scale batches packaged in the proposed container closure system. Stability data were provided for up to 36 months stored under long term conditions (30±2ºC / 75±5% RH, 2-8ºC and -20±5ºC) and for up to six months under accelerated conditions (40±2°C / 75±5% RH), according to the ICH guidelines.

Samples were tested for appearance, identification, water content, related substances, assay, residue on ignition, melting point, microbial limits and XRPD crystal form. Results met the specifications regardless of the storage condition. There was no change in the polymorphic form. No significant trends or variability were observed. The 30±2ºC / 75±5% RH condition is not precisely according to ICH Q1A (R2) where for long-term stability, recommended conditions are either 30±2°C/ 65±5% RH or 25±2°C/ 60±5% RH. However, because the studied storage conditions are harsher than the typical ones at long-term study this is not considered to raise any concerns.

Stress testing was performed at high humidity, high temperature, in the presence of acid, alkali, oxidant, under reducing conditions and exposure to light. Various degrees of degradation were observed. The impurities and assay methods were shown to be stability indicating.

Photostability testing was carried out on a commercial scale batch as per ICH Q1B. No degradation was found in the light-exposed and the control samples after the exposure of 50 days, indicating that AS is not sensitive to light (ICH conditions).

Based on the available stability data, the proposed re-test period and storage condition, is acceptable.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Klisyri 10 mg/g (1% w/w) ointment is a smooth, creamy white to off-white ointment free from foreign particulates. The ointment is composed of the active substance, tirbanibulin, formulated with compendial grade excipients, glycerol monostearate 40-55 Type I and propylene glycol, and packaged in single-use packets intended for topical use. Each sachet contains a nominal amount of 250 mg of ointment intended for only one single application, which translates into a maximum administration of 2.5 mg of tirbanibulin over a surface area up to 25 cm^2. The development of the finished product (FP) has been described in detail and was guided by the quality target product profile (QTPP).

The AS has sufficient solubility in propylene glycol, the solvent used in the formulation. Tirbanibulin can exist in three different polymorphic forms and the most thermodynamically stable form has been used in all clinical supplies and registration stability batches manufactured to date. Polymorphism and particle size are not considered critical quality attributes as tirbanibulin is dissolved in the formulation.

The excipients in tirbanibulin 1% ointment are compendial and commonly used in cutaneous formulations. Compatibility of the excipients with the active substance is inferred from the stability data on early prototype formulations and as demonstrated on Phase 3 and registration batches.

The production of the FP involves the dissolution of the AS before incorporation into the dosage form. A number of solvents were screened based on the solubility of tirbanibulin. Propylene glycol (PG) is used as solvent in the commercial formulation. The level of PG in the formulation ensures that the AS is completely dissolved in PG during formulation processing.

A series of thickening agents were evaluated for their compatibility in particular with regard to appearance of ointment and the miscibility between the solvents and thickening agents. Glycerol monostrearate 40-55 was chosen to be used as a thickening agent in the commercial formulation. The level of glycerol monostrearate 40-55 in the formulation ensures that the FP has the desired ointment consistency. The stability data demonstrated the AS remains solubilised in the FP under long-term and immediate storage conditions. To lower the risk of AS precipitation, the recommended storage condition for the FP is "do not freeze or refrigerate". Furthermore, various product batches manufactured with different AS batches all yielded smooth ointments with no AS precipitation under the recommended storage conditions for up to 24 months for Phase 3 batches and 12 months for registration batches.

Throughout product development, two formulations were used: formula 1 in early trials and formula 2, the proposed commercial formulation, in pharmacokinetic and phase 3 efficacy trials. The differences of composition are minor and have been described. In addition, changes to the container closure, manufacturer of AS, and manufacturing process parameters were introduced. These changes were evaluated via design of experiments (DoE) and the impact of changes to the formulation were compared by batch analysis and in vitro release test (IVRT) profile comparison. The batch results show the two formulations are comparable, with some difference in viscosity. The ratios of release rate for the 2 formulations (formula 1 and commercial) was determined using an in vitro release test method (IVRT). The development of the IVRT method, the validation, and the discriminating character over the drug load were evaluated and satisfactorily documented. The two formulations can be considered comparable. In addition, the commercial formulation was used in the pivotal phase 3 studies. The IVRT method used to compare formulations during development will be continued for stability testing of the registration lots of the FP and release of the process validation batches. Beyond the process validation batches, the IVRT test will only be employed to establish comparability according to the European guideline on quality and equivalence of topical products.

The evolution of the FP manufacturing process from initial prototypes to commercial formulation has been clearly described. The process was developed at small scale at a development site and was then transferred to a manufacturer which developed the commercial formulation (formula 2) and manufactured clinical supplies packaged into single use sachets. Subsequently, the manufacturing process used for Phase 3 clinical material was successfully transferred and optimised at the intended commercial manufacturer, which manufactured the process validation and stability batches. The changes to the manufacturing process are overall minor. The process parameters at the proposed commercial manufacturing site are the same as at the site that produced the clinical batches. The formulation evolution was discussed above and was supported by analytical and stability data.

The applicant has performed and justified a risk assessment on the impact of manufacturing steps on the critical quality attributes (CQAs) of the FP. Development studies and the results for several batches manufactured at commercial scale support the critical process parameters (CPPs) target set-points and ranges.

The CQAs of the FP that can potentially be impacted by the formulation and/or process variables were identified. All finished product CQAs are included in the FP specification.

A comparison of FP batches manufactured at the clinical batches manufacturing site and the proposed manufacturer was performed. The manufacturing transfer protocol addressed the minor changes in process between the two sites.

The bulk formulation and product in sachets were compared. Three batches from each site were tested and found comparable. There are no significant differences in any of the FP quality attributes between the batches from either site, including the IVRT assessment of one batch from each site. The results indicate that the in vitro release performance of FP batches manufactured at both sites are equivalent.

The container closure system consists from single-use sachets made from five-layer laminate material (web stock) that is heat sealed on four sides. The web stock is composed of linear LDPE (the product contact surface)/HPC/aluminum/LDPE/PET. A description including a schematic representation has been provided. Compliance with requirements of Ph. Eur. and EC Reg. 10/2011 as amended has been declared. A specification for identity of the material in contact with the finished product has been provided. Extractables and leachables were evaluated using ICH M7 (R1) as a guide. The results from these studies were provided. The Analytical Evaluation Threshold (AET) was estimated. Detected leachables were below the AET and overall below the ADI according to ICH M7. A registration lot will continue to be monitored for leachables at the long-term stability condition.

Manufacture of the product and process controls

A MO was raised concerning the manufacturing/importing authorisation (MIA) documentation of the proposed site of physical importation into the EU. Another MO was raised because the originally submitted GMP documentation for the non-sterility testing site and the physical-chemical testing site were older than 3 years; this was resolved by submission of updated compliance documentation.

The manufacturing process comprises the following main steps: slurry of the active substance in propylene glycol; mixing the slurry with propylene glycol and glycerol monostearate 40-55, heating under stirring until homogenisation in a single phase, cooling to form the bulk ointment; and filling into single use sachets. Critical steps of the process have been identified. The process parameters, the critical steps and IPCs are the same as determined in development of the FP. The methods and the limits of IPC parameters were described and are acceptable. The controls implemented assure that the manufacturing process is robust and can consistently produce FP meeting the proposed specifications.

There are no intermediates in the manufacturing process. The holding time of the product in bulk has been stated and was supported by relevant stability data and confirmed during process validation. All results including microbial purity comply with limits of specification. In addition, the start of shelf-life has been stated in line with relevant guideline CPMP/QWP/072/96.

A MO was raised with regard to the proposed maximum overfill and how the amount dispensed corresponds to the actual dose stated in the SmPC. This was addressed by provision of further clarification regarding the manufacturing process and materials, by additional user studies and by tightening the relevant IPC limits. Based on the additional provided data the MO has been resolved and the revised overfill range is justified. The FP is considered as a specialised pharmaceutical dosage form because of the low AS content (lower than 2% per unit). Therefore, an MO was raised requesting validation data from three full production scale batches as per the Guideline on Process Validation for Finished Products. The MO has been resolved since the requested data were provided in line with validation protocol initially presented in R.3.2. The critical process parameters for all critical equipment and non-critical equipment were documented and met expectations during bulk formulation and manufacture of the finished product. The whole manufacturing process is considered adequately validated.

Product specification

The finished product release and shelf life specifications include tests and limits for appearance (visual), identification (HPLC, UV), assay (HPLC), impurities (HPLC, UPLC), apparent viscosity (Ph. Eur.), and microbial limits (Ph. Eur.).

The product specification covers appropriate parameters for this dosage form as per the Ph. Eur. monograph for pharmaceutical form "Semi-solid preparations for cutaneous application". The acceptance criteria have been set based on requirements of relevant quality guidelines, Ph. Eur. and available results. Specification limits for viscosity were justified based on results from several batches at release and on stability. Overall, the risk of microbial contamination of tirbanibulin 1% ointment is determined as low. Nevertheless, microbiological quality of the finished product is controlled at release and at shelf life. The microbiological acceptance criteria were set based on Ph. Eur. 5.1.4 "Microbiological quality of non-sterile pharmaceutical and substances for pharmaceutical use" according to the standard acceptance criteria as specified for a cutaneous route of administration.

A satisfactory risk assessment summary on elemental impurities (EI) in accordance with the ICH Q3D guideline was provided. Since ICH Q3D does not provide a permitted daily exposure (PDE) for the cutaneous route, PDE limits for finished product (tirbanibulin 1% ointment) are based on the values set in ICH Q3D for parenteral routes of administration. The Acceptable Daily Exposure (ADE) limits are set to 30% of that value in accordance with ICH Q3D. Batch analysis data on 3 batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective ADE. Based on the risk assessment and the presented batch data 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.

An MO was raised concerning the potential risk of the presence of nitrosamine impurities in the finished product. In response to the MO, a risk evaluation was conducted and submitted 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). Taking into account the presented information and considering the topical use, duration of treatment and risk associated to the formula components (negligible or very low) the safety risk associated to the presence of nitrosamine impurities in the FP is negligible and thus there is no risk for the patients. Based on the information provided, no additional control measures are deemed necessary; the MO has been resolved.

A justification for not including a test for residual solvents in the specification was provided in line with ICH Q3C(R7) "Guideline for Residual Solvents" and Ph. Eur. 5.4 "Residual Solvents," taking into account the recommended maximum allowed limits. The maximum daily dose of tirbanibulin is well below 10 g (2.5 mg), and the AS and all excipients in the FP meet the limits given in Option 1 of Ph. Eur. 5.4 and ICH Q3C(R7). Based on this assessment, it is accepted that a test for residual solvents is not included in the FP specification.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. The analytical method for viscosity has changed in the course of development. The old method was used for release and stability of clinical batches. The new method is used for stability studies. An MO was raised requesting bridging data for the two methods in order to demonstrate that the proposed specifications for viscosity are representative of the clinical batches. Satisfactory bridging data were presented. In addition, the presented results are supportive of the proposed respective specifications. While differences are seen between the clinical and registration

batches, these are not considered significant and this is supported by IVRT results. The MO has been resolved.

Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

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

Batch analysis data have been provided on batches manufactured by the proposed manufacturer. Results from five smaller scale development batches used in various phases of the clinical trials and manufactured by the development sites were also presented. All the test results remain within the proposed specification limits demonstrating consistent product quality.

Stability of the product

Stability data from four commercial scale batches, stored for up to 24 months under long term conditions (25±2°C / 60±5% RH), for up to 18 months at 5°C/ ambient RH, and for up to 6 months under accelerated conditions (40±2ºC / 75±5% RH), according to the ICH guidelines, were provided. Data from one of the above batches stored for 12 months at 30±2°C / 75±5% RH was also presented. These primary stability batches were manufactured at the proposed manufacturing site and were packaged in the proposed commercial container closure system.

Stability samples were tested for appearance, assay, impurities, microscopy, IVRT, viscosity, seal integrity, and on a yearly basis, microbial limits. Appearance met the specification for all batches except at 5°C where the product was found to have a gritty consistency at the 6-month timepoint due to precipitation of the AS. There was no observed trend in viscosity, assay or impurities. All batches met all the other acceptance criteria across all stability storage conditions and timepoints.

Forced degradation studies showed that the FP is most susceptible to oxidation and heat, with modest degradation upon exposure to light (as confirmed in the photostability study described above). A known impurity was only detected under basic conditions. The stability-indicating character of the analytical methods has been demonstrated.

A thermal cycling study was conducted during which FP in the proposed commercial primary and secondary packaging was exposed to low and high temperature extremes. The study was designed to represent worst-case temperature stress by exposing product to both temperature extremes in each cycle. Results from the thermal cycling study indicate that temperature excursions encountered during storage or distribution are unlikely to have an impact on product quality.

Based on the overall submitted stability data, the proposed shelf-life of 2 years with the storage condition "do not refrigerate and freeze", as stated in SmPC sections 6.3 and 6.4, is acceptable.

Adventitious agents

No materials of human or animal origin are used in the manufacture of Klisyri. TSE statements for propylene glycol and glycerol monostearate 40-55 from the respective suppliers were provided.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and the finished has been presented in a satisfactory manner.

MOs raised regarding the QP declaration, the manufacturing authorisation and GMP certification of certain proposed sites in the supply chain have been addressed by provision of updated valid compliance documentation. The MO on the potential presence of nitrosamines in the finished product was resolved by performing a risk assessment in line with the requirements of the relevant published guidance documents. The MOs on the finished product manufacturing process were resolved by providing satisfactory process validation data and results from a sachet filling. Satisfactory bridging data was also provided to resolve the MO regarding the change of the viscosity method and the respective FP specification. The overall control strategy for the AS and the FP is adequately justified and is acceptable.

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

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.2.6. Recommendations for future quality development

None.

2.3. Non-clinical aspects

2.3.1. Introduction

In the non-clinical programme, data on the anti-proliferative activity and mechanism of action of tirbanibulin were submitted based on the applicant's own studies and on published studies. The applicant also submitted studies to identify possible secondary pharmacological effects and potential side effects. Pharmacokinetic data were submitted for single oral doses of tirbanibulin in mice and rats and single and multiple oral doses in rats and dogs, and for single intravenous doses in mice, rats, and dogs. Data for the dermal absorption of tirbanibulin were submitted for rats, rabbits, and minipigs. Further data submitted include the results of a single study of the dose-mass balance, metabolism, and tissue distribution of [^{3}H]tirbanibulin in rats, and results of *in vitro* metabolic studies in biomaterial from rat, dog, minipig, and human. The general toxicology programme for tirbanibulin consisted of dermal and oral studies in the mouse, rat, rabbit, minipig, and dog, including single and repeat dose, genetic, developmental and reproductive, and local tolerability toxicity studies. All pivotal Toxicology studies are claimed to be conducted in compliance with Good Laboratory Practice (GLP) regulations by the applicant. Two of the genotoxicity studies (Mouse lymphoma TK gene mutation assay [Study 7709-113]; Bone marrow micronucleus test in rat [Study 321-0044-GT]) and three of the reproductive toxicity studies (Study 321-0034-TX in rat and Studies 321-0047-TX and 321-0049-TX in rats and rabbits) were conducted at a site located in China, which is neither an OECD nor OECD-MAD member. As China does not adhere to the OECD MAD agreement, the Chinese GLP monitoring authority has not been assessed by its OECD peer. Thus, one condition of the GLP regulations is not fulfilled (see Discussion section). The aforementioned studies did not show any irregularities or signs of GLP non-compliance and based on inspections from an EU GLP monitoring authority there is evidence that this facility conducts studies in compliance with the OECD GLP regulations.

2.3.2. Pharmacology

In the non-clinical programme, primary pharmacodynamics data on the anti-proliferative activity and mechanism of action of tirbanibulin were obtained from the applicant's own studies and from published studies.

Primary pharmacodynamic studies

In vitro

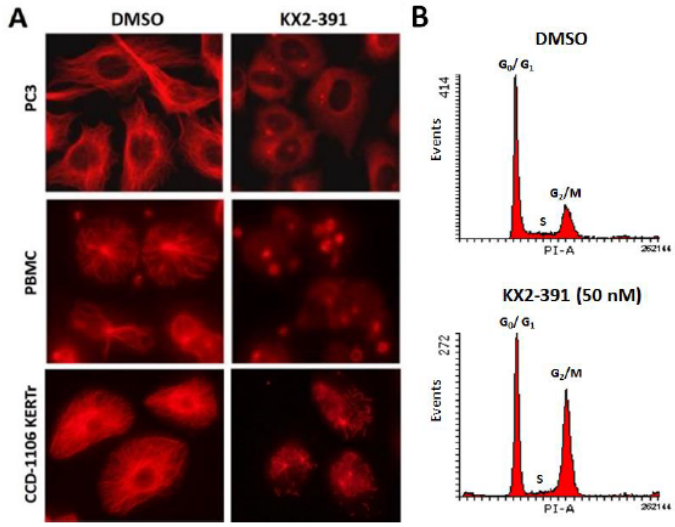
Study ATNXUS-KX01-001: Mechanism of Action and Anti-proliferative Activity *In Vitro*

This *in vitro* study investigated the mechanism of action of tirbanibulin (KX2-391) and its anti-proliferative activity in cell lines including keratinocytes.

Tubulin was identified as a specific drug target for tirbanibulin in various assays within this study using HT-29, PC-12, HUVEC, PC3-LN4, CCD-1106 KERTr and human peripheral blood mononuclear cells:

- α- and β-tubulins were identified as binders of tirbanibulin through LC-MS/MS; they were the most strongly labelled proteins when photoaffinity labelled proteins in colon cancer HT-29 cells were resolved by 2D gel analysis.
- Tubulin was observed to be specific binding target for tirbanibulin in photoaffinity labelling assays with purified tubulin and competitive binding assays with other tubulin binders.
- Dose-dependent inhibition of tubulin polymerisation *in vitro* was observed with tirbanibulin.
- In immunofluorescence assays tirbanibulin was observed to effectively disrupts the microtubule network *in vitro* in prostate cancer PC3 cells, human PBMCs, and immortalised keratinocyte CCD-1106 KERTr cells

Tirbanibulin resulted in a G2/M arrest in CCD-1106 KERTr cells, using flow cytometry (Figure 2). An increase in apoptotic cells, as indicated by annexin V and 7-amino-actinomycin D (7-AAD) staining, was observed in metastatic prostate PC3-LN4 cells and later in CCD-1106 KERTr keratinocytes. The collapse of the mitochondrial membrane potential, a characteristic event of early-stage apoptosis, was also observed in tirbanibulin-treated PC3-LN4 cells. Tirbanibulin treatment led to hyper-phosphorylation of Bcl-2, caspase 8 and 9 cleavage, activation of caspase 3, and subsequent poly (ADP-ribose) polymerase inhibitor (PARP) cleavage, as shown by immunoblot analysis.



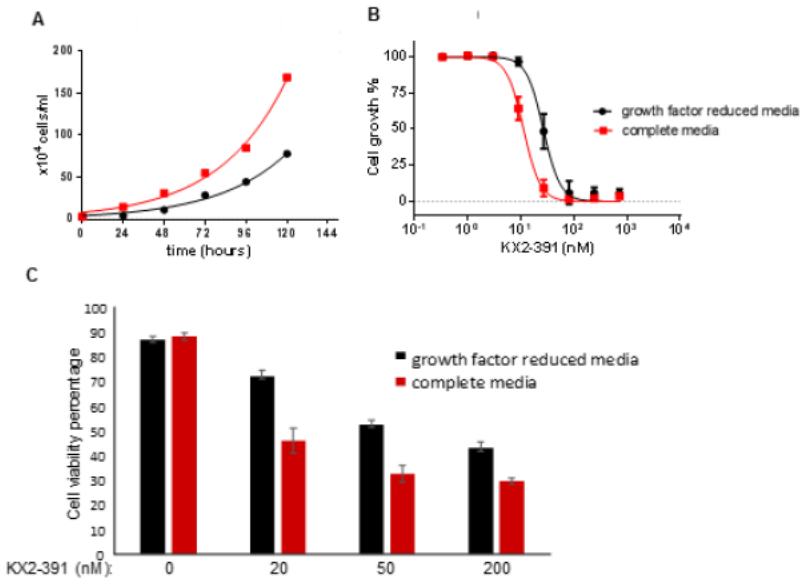
DMSO=dimethyl sulfoxide; G_0/G_1=growth phase 0/growth phase 1; G_2/M=growth phase 2/mitosis phase; KX2-391=tirbanibulin; PI=propidium iodide.

(A) PC3, PBMC, or CCD-1106 KERTr cells were treated with tirbanibulin (100, 125, and 200 nM, respectively) for 2 h. Cells were then fixed, permeabilised, and stained with an antibody to tubulin.

(B) CCD-1106 KERTr cells were incubated with DMSO or tirbanibulin (50 nM) for 40 h. Cells were permeabilised and stained with PI prior to analysis by flow cytometry.

Figure 2: Tirbanibulin disrupts microtubule architecture and arrests cells at the G2/M stage of the cell cycle (Study ATNXUS-KX01-001)

Given that tirbanibulin was thought to halt cell division at the phase of mitosis via inhibition of tubulin polymerisation, it was expected that fast-growing cells are more susceptible to tirbanibulin-induced growth inhibition than slowly growing cells. To test this hypothesis, slowly growing CCD-1106 KERTr keratinocytes were established by maintaining them in growth factor-reduced media. Following incubation with various concentrations of tirbanibulin, a significantly reduced growth rate was observed when cells were maintained in growth factor-reduced media for 72 h compared with cells in regular culture media, using analysis by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Figure 3). When cell growth inhibition and viability were compared between the 2 cell cultures with different growth rates after 72 h of tirbanibulin treatment, tirbanibulin was more effective in inhibiting cell growth and inducing cell death in the fast-growing cells compared to the slow-growing cells (GI50: 11 vs. 27 nM, $p<0.0001$, Student's t-test).



KX2-391=tirbanibulin; MTT=3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SD=standard deviation.

(A) CCD-1106 KERTr keratinocytes were cultured in regular media or growth factor reduced media (5% of regular media) and counted at various sampling times. CCD-1106 KERTr cells were treated with tirbanibulin at various concentrations and incubated in regular culture media or growth factor reduced media for 72 h, followed by MTT assay (B) or trypan blue staining (C); mean±SD cell viability percentage.

Figure 3: Tirbanibulin induces cell growth inhibition and cell death more potently in rapidly dividing cells (Study ATNXUS-KX01-001)

Study ATH001-01-p-00001: Bioactivity Comparison of Tirbanibulin and its Metabolites

Tirbanibulin has shown a potent anti-proliferative effect in the low nM range in a human keratinocyte cell line (CCD-1106 KERTr) and on a variety of tumour cell lines. None of the identified metabolites of tirbanibulin have significant *in vitro* anti-proliferative activity on CCD-1106 KERTr cells, including the major metabolite in human hepatocyte incubates, KX2-5036, which had no detectable activity at up to 20 µM.

Disruption of mitosis and Src signaling in breast cancer cell lines (Kim *et al*; 2007)

Nine breast cancer cell lines were treated with tirbanibulin *in vitro* and inhibitory effects were evaluated using an MTT assay. The luminal oestrogen receptor (ER)+ cell lines MCF7 and T47D, the HER2+ cell line SK-BR-3, and the triple negative breast cancer (TNBC) cell lines MDA-MB-231, MDA-MB-468, and BT-549 were sensitive to tirbanibulin with concentration of an inhibitor where the response (or binding) was reduced by 50% (IC_{50}) (synonymous with GI_{50} in the context of this publication) values <100 nmol/L, whereas TNBC cell lines Hs578T and HCC1937 could not be completely inhibited by tirbanibulin.

Table 1: Growth Inhibitory Effect of Tirbanibulin (Kim, 2017)

Cell line	Subtype	Mean±SD tirbanibulin IC_{50} [a] (nmol/L [b])
MCF7	Luminal (ER+)	41.8±1.0
T47D	Luminal (ER+/PR+)	43.5±42.3
BT-474	HER2+	128.6±7.6
SK-BR-3	HER2+	33.8±1.0
BT-549	Triple negative	46.7±1.9
MDA-MB-231	Triple negative	44.6±0.9
MDA-MB-468	Triple negative	61.3±1.7
HCC1937	Triple negative	>5000
Hs578T	Triple negative	>5000

ER=oestrogen receptor; GI_{50}=concentration of drug that inhibits cell proliferation by 50%; HER2=human epidermal growth factor receptor type 2; IC_{50}=concentration of an inhibitor where the response is reduced by half; PR=progesterone receptor; SD=standard deviation.

a) Synonymous with GI_{50} in the context of this publication.

b) Adapted from µmol.

Source: Kim, 2017.

To determine if tirbanibulin inhibited the activity of Src and focal adhesion kinase (FAK), Western blotting was performed to measure the concentrations of total and phosphorylated proteins after treatment. Phosphorylated Src (p-Src) in BT-549 cells significantly decreased following exposure to tirbanibulin. FAK and p130cas phosphorylation, which are known to be regulated by Src, also decreased. Furthermore, additional downstream signalling molecular-level changes associated with cell survival and proliferation (p-AKT, p-ERK, and p-STAT3) decreased in sensitive cell lines following 24 h of tirbanibulin treatment.

Src inhibitors are known to cause arrest of cell cycle phase G1 (Fabarius, 2008). Following tirbanibulin treatment, an arrest in G2/M cell cycle phase was observed. TNBC cells sensitive to tirbanibulin (BT-549, MDA-MB-231, and MDA-MB-468) displayed a 2- to 4-fold increase in the G2/M cell phase population, while G1 and S cell phases decreased. Conversely, the tirbanibulin-insensitive cell line (Hs578T) did not show increased G2/M cell phase population in response to up to 100 nM concentration of tirbanibulin. In addition, tirbanibulin treatment resulted in an increase in the number of multi-nucleated cells in MDA-MB-231 cells, which was presumably the result of microtubule polymerisation inhibition.

Disruption of Microtubule Network in Mucinous Ovarian Carcinoma Cells (Liu *et al*, 2013)

In vitro assays were performed to determine if tirbanibulin could directly affect the organisation of the microtubule network in RMUG-S and RMUG-L cells. RMUG-S and RMUG-L cells were treated with tirbanibulin for 24 or 48 h. These cells were then fixed and stained with fluorescein isothiocyanate (FITC)-conjugated anti-α/β tubulin antibodies and Hoechst 33342 or 4′,6-diamidino-2-phenylindole (DAPI) to observe the microtubule network and nuclei with fluorescence microscopy. Cells treated with tirbanibulin had a smaller microtubule network with a diffuse stain visible in the cytoplasm when compared with the vehicle control. Compared with RMUG-L cells, microtubules in RMUG-S cells were affected to a greater extent after treatment with tirbanibulin.

In vivo

In vivo data obtained from non-cutaneous mouse xenograft tumour models treated with oral tirbanibulin were provided (table below). In human breast, ovarian, and prostate tumour xenograft models treated with oral tirbanibulin for up to 30 days, a dose-dependent inhibition of tumour growth was observed, without significant adverse clinically relevant signs. In sections of these different tirbanibulin-treated xenograft tumours, alterations of the microtubule architecture were detected as a

diffuse tubulin staining pattern, as was an increased number of apoptotic tumour cells and reduced intracellular levels of phosphorylated Src and its downstream targets.

Table 2: ***In vivo*** **pharmacodynamics studies**

Study/ Reference	**Model**	**Treatment**	**Major Findings**
KX01src	Prostate Tumour Progression and Metastasis in an Orthotopic Nude Mouse Model using PC-3MM2 cells	3 days after xenografting mice (n=5) received 5 or 10 mg/kg/day of tirbanibulin or 15 mg/kg/day dasatinib for 28 days	After 42 days median tumour weight was significantly reduced in the 5 and 10 mg/kg tirbanibulin-treated groups compared to the vehicle control (1.16 and 0.35 vs. 2.27 g, respectively). The number of lymph node metastasis following dasatinib treatment (2/5) was comparable to 10 mg/kg tirbanibulin. The number of lymph node metastasis was decreased in the 5 and 10 mg/kg tirbanibulin-treated groups compared to control (4/5 and 2/5 vs. 5/5, respectively).
Kim *et al*., 2017	Subcutaneous MDA-MB-231 breast cancer cell xenograft model in mice	Tumours allowed to reach volume of 150 mm^3 and then treated with vehicle or 5 mg/kg bid (n=5) for 4 weeks	Tumour growth was significantly delayed in tirbanibulin-treated mice, Ki67 expression (indicative of proliferation) was decreased, TUNEL positive cells increased and phosphorylation of p-Src was decreased.
Liu *et al*., 2013	Mucinous ovarian cancer cells RMUG-S-ip2 or RMUG-L-ip2 cells were inoculated into the peritoneal cavity of mice	4 weeks after tumour initiation mice (n=10) were treated with vehicle, 5 mg/kg/day tirbanibulin, 5 mg/kg oxaliplatin twice weekly via ip injection or combination of tirbanibulin/oxaliplatin for 8 weeks	In the RUMG-S model combination therapy led to significantly greater tumour suppression effects than did either monotherapy (combination vs. tirbanibulin or oxaliplatin: 90% vs. 75% or 72%, $p<0.05$). In the RMUG-L model, although a significant reduction in tumour weight and number of nodules was observed in the combination group compared with the control group, the anti-tumour effects of the combination therapy did not differ from those of either monotherapy. With tirbanibulin and in combination with oxaliplatin tumour proliferation (Ki67) was reduced, angiogenesis (CD31) was reduced and apoptosis (cleaved caspase-3) was increased.

Anbalagan *et al*., 2012a	Oestrogen Receptor α-Positive Breast Cancer Model (MCF-7 cells) injected into both sides of the inguinal mammary fat pad of female athymic nude mice (BALB/c) mice	Oestradiol (E2) alone, E2+TAM, E2+tirbanibulin (1 mg/kg b.i.d.), and E2+tirbanibulin (1 mg/kg b.i.d.)+TAM for 30 days.	Tirbanibulin at the 1 mg/kg dose alone suppressed tumour growth significantly (36.6%) compared with the control group. Combination of TAM and tirbanibulin more potently inhibited tumour growth, yielding an inhibition rate of 57.4%. Src phosphorylation was inhibited by tirbanibulin and tirbanibulin+TAM. Ki67 expression was decreased by treatment with TAM and tirbanibulin+TAM. Angiogenesis was decreased by treatment with tirbanibulin+TAM.
Anbalagan et al., 2012b	MDA-MB-231 cells injected orthotopically and bilaterally into the mammary fat pads of female nude mice (2 tumours/mouse)	When tumours volume reached approx. 80 to 100 mm^3 treated with vehicle or 1 or 5 mg/kg administered b.i.d. by oral gavage for 28 days	Tirbanibulin at a dose of 1 and 5 mg/kg reduced MDA-MB-231 tumour volume (by 35.3% and 79.0%, respectively) compared with vehicle from Days 18 and 15, respectively. Tirbanibulin significantly inhibited Src and FAK phosphorylation, increased apoptosis, decreased expression of markers of proliferation and angiogenesis.

Secondary pharmacodynamic studies

To assess potential secondary pharmacodynamics of tirbanibulin a study evaluated the inhibitory effect of tirbanibulin on 63 molecular targets from a diverse range of therapeutic targets (Study 06-3061). These targets include receptors for neurotransmitters, steroids, second messengers, prostaglandins, growth factors, hormones, brain and GI peptides, as well as ion channels and enzymes. The inhibitory effect of tirbanibulin was tested at 1.0 µM. None of the targets was inhibited >50% by tirbanibulin, which is considered the threshold for follow-up evaluation. The risk of tirbanibulin having a clinically relevant interaction with any of the molecular targets tested in this study is low, given the maximum observed tirbanibulin plasma concentration of 1.09 ng/L (2.53 nM) observed in the maximal usage clinical trial (MUsT) (Study KX01-AK-007).

The kinase binding activity of tirbanibulin and the most abundant metabolite identified in human hepatocyte incubates, KX2-5036 was assessed using a KINOMEscan screening platform to quantitatively measure the interactions of tirbanibulin and KX2-5036 with more than 450 human kinases and disease-relevant mutant variants (Study ATH002-01-p-00001). In contrast to ponatinib, a multi-target kinase inhibitor used as a control, no competition activity >65% was observed for tirbanibulin at 5 µM, demonstrating that tirbanibulin did not inhibit the binding of the evaluated kinases to their substrates (bait ligands) under conditions of the study. Further, tirbanibulin did not bind to kinases in the Src kinase family.

Safety pharmacology programme

In vitro

hERG assay

In a non-GLP assay in HEK-293 cells stably expressing the hERG channel (Study 5152016) the IC_{50} for tirbanibulin for the hERG channel was 44 µM (18987 ng/mL). A subsequent GLP compliant study was completed in HEK-293 cells stably expressing the hERG channel (Study YY91QH). Tirbanibulin inhibited hERG tail current in a concentration-dependent manner. A concentration-response curve was determined using vehicle-corrected data plotted against the achieved concentrations. The IC_{50} value was 24.67 µM (10600 ng/mL). Terfenadine, a known inhibitor of the hERG channel, was used as a positive control at a submaximally effective concentration (50 nM).

Table 3: Measured tirbanibulin concentrations in hERG assay

Nominal tirbanibulin concentration (µm)	Achieved tirbanibulin concentration (µM)	Duration of tirbanibulin exposure (min)	% Control mean±SEM
30	24.10	10	47.6±4.0
10	7.51	7	87.1±3.5
3	1.99	8	100.4±5.7

hERG=human ether-à-go go related gene; SEM=standard error of the mean.

KX2-5036, the major metabolite of tirbanibulin (KX2-391) in human hepatocyte incubates was also investigated in the hERG assay at a nominal concentration of 30 µM (the limit of solubility) and included the use of terfenadine as a positive control in a GLP compliant study (Study XV31HF). Analysis of the concentration of KX2-5036 attained in the perfusion apparatus showed that substance losses were between 0.0% and 0.3%. KX2-5036 had no significant effect (p=0.5328, unpaired Student's t-test) on hERG tail current when compared with vehicle-treated cells. Following time-matched vehicle correction, hERG tail current amplitude was 96.0±4.7% of baseline in the presence of KX2-5036 at a maximum achieved concentration of 30 µM.

In vivo

CNS

A non-GLP compliant study evaluated the potential of tirbanibulin to cause behavioural, neurological, or autonomic effects *in vivo*, using a Modified Irwin Screen in male Wistar rats at a single dose of 50 mg/kg via oral gavage (Study 509600-1024091). The animals were also observed for tirbanibulin- or vehicle-induced mortality during an 8-day observation period post-dose. Tirbanibulin scored zero (no effect) in all 32 observations in all 5 rats. A similar result was obtained for vehicle.

Cardiovascular and Respiratory

A GLP-compliant study evaluated potential pharmacologic effects that may have clinical relevance of intravenous (IV) doses of tirbanibulin on haemodynamic, respiratory, blood gas, and electrocardiographic parameters in conscious, telemetered Beagle dogs (Study QRV00015). All animals were treated via IV administration with 0 (vehicle control), 7.5, and 15 mg/kg of tirbanibulin in a cross-over design. The animals received vehicle on Day 1. Tirbanibulin was administered at 7.5 and 15 mg/kg on Days 4 and 11, respectively. The same 4 animals were dosed in each dose session. The time between sessions was extended after Day 4 to allow for an additional washout period between the test article doses.

IV administration of tirbanibulin to Beagle dogs at doses up to 15 mg/kg had no adverse effects on ECG waveforms, ECG intervals (PR, QRS, QT, or QTc), pH, pO_2, SO_2, or pCO_2. There were dose-dependent changes in blood pressure, heart rate, and respiratory rate. There was a moderate increase in blood pressure with a greater effect observed in diastolic pressure than systolic and mean pressures. These effects were observed after 7.5 and 15 mg/kg doses. Blood pressure increases were at a similar degree with these 2 doses but more prolonged at the higher dose. There was a moderate but transient increase in heart rate that began after the end of the infusion and returned to control levels by approximately 30 min after dosing in animals receiving 7.5 and 15 mg/kg of tirbanibulin. Heart rate was also elevated for approximately 8 to 12 or 20 h in animals receiving 7.5 and 15 mg/kg, respectively. In animals receiving 15 mg/kg tirbanibulin, a mild increase in respiratory rate beginning at the end of the infusion and lasting 120 min was observed, whereas a slight reduction was found 20 to 22 h after dosing.

Gastrointestinal system

A non-GLP compliant study evaluated the potential effects of both oral gavage and subcutaneous injection of tirbanibulin (KX01) on the propulsive motility in normal male and female Wistar rats (Study 21050). Groups receiving tirbanibulin orally were dosed at 2.5 or 10 mg/kg. Dasatinib was administered orally at 50 mg/kg. Animals treated subcutaneously were dosed with either 7.5, 15, or 30 mg/kg tirbanibulin or with 30 mg/kg dasatinib. Thirty minutes after single administration of tirbanibulin, vehicle, or dasatinib, 10 mL/kg of a 5% suspension of non-activated charcoal powder suspended in a 1% CMC solution in distilled water was administered to the rats via oral gavage. Tirbanibulin produced a small but statistically significant increase in mean (±standard error of the mean) intestinal transit time vs. the vehicle control following oral dosing at 5 and 10 mg/kg (82.9±1.4% and 83.1±1.7% vs. 76.0±1.8%) and subcutaneous dosing at 7.5, 15, and 30 mg/kg (75.0±1.9%, 76.9±1.2%, and 80.0±1.2% vs. 69.5±1.2%). Dasatinib at 50 mg/kg administered orally produced an increase in transit which was not significantly different from control due to the high variability of the response (81.7±5.9% vs. 76.0±1.8%).

A non-GLP compliant study evaluated the potential effects of tirbanibulin (KX01) on the propulsive motility (gastric emptying) in normal male and female Wistar rats (Study 21051). Groups receiving tirbanibulin orally were administered 2.5, 5, or 10 mg/kg. Dasatinib was administered orally at 50 mg/kg. Animals treated subcutaneously were dosed with either 7.5, 15, or 30 mg/kg of tirbanibulin or with 30 mg/kg dasatinib. Thirty minutes after administration of either drug or vehicle, 10 glass beads (size: 1 mm) were administered by oral gavage. After 60 min, the rats were euthanised and the stomach was removed. The number of beads remaining in the stomach was counted. Tirbanibulin did not produce any significant inhibition of gastric emptying of 1 mm glass beads when administered either orally or subcutaneously. There were no signs of gastric bloating or fluid in the stomach and intestines. There was no indication of gastric mucosal damage following administration of tirbanibulin. Dasatinib at 50 mg/kg orally produced a small but statistically significant inhibition of gastric emptying (93±3.0% vs. 99±1.0%), and on necropsy, the stomach and intestines appeared to be full of fluid.

Pharmacodynamic drug interactions

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

2.3.3. Pharmacokinetics

The absorption, distribution, metabolism, excretion and toxicity of tirbanibulin were initially investigated to support oral clinical studies in oncology indications. Therefore, the PK of tirbanibulin has been assessed in oral single (mice, rats, and dogs) and repeat (rats and dogs) dose studies and

following single dose IV administration (mice, rats, and dogs). The absorption of tirbanibulin following dermal application has been assessed in rats (repeat dose), rabbits (repeat dose), and minipigs (single and repeat dose). A single dose mass balance, metabolism, and tissue distribution study with [^{3}H]tirbanibulin was conducted in rats. *In vitro* metabolism studies in rat, dog, minipig (Göttingen and Bama), and human biomaterials have also been submitted.

2.3.3.1. Methods of analysis

In PK and toxicity studies, tirbanibulin concentrations in rat, rabbit, dog, and minipig plasma were measured using validated liquid chromatographic (LC) tandem mass spectrometric (MS) (LC-MS/MS) methods. Methods were also developed for the quantitation of the tirbanibulin metabolites KX2-5036 and KX2-5163 in rat and minipig plasma. The methods used for analysis were partially or fully validated across each calibration range.

Determination of the radioactivity in *in vivo* biological samples following administration of [^{3}H]tirbanibulin was carried out by HPLC-ARC™. Plasma samples with very low radioactivity were subjected to high performance LC separation, fraction collection, and detected by solid scintillation counting. Metabolite characterisation was accomplished by either LC-MS/MS, LC high resolution MS, or LC-MS, coupled with an appropriate radioactivity monitor, when applicable.

2.3.3.2. Absorption

Single dose studies

The PK parameters for tirbanibulin after a single dose administration are summarised in the table below. Study 7709-118 compared the PK of tirbanibulin (KX2-391) following single oral doses of 2 tirbanibulin salt forms (2HCl and mesylate), at the same free base equivalent to male (5 mg/kg) and female (2 mg/kg) Sprague-Dawley rats (4 males and 4 females per salt form). Study 7709-117 compared the PK of tirbanibulin (KX2-391) following single oral doses of 2 tirbanibulin salt forms (2HCl and mesylate), at the same free base equivalent (0.75 mg/kg) to male and female Beagle dogs (8 males and 8 females), in a cross-over design with a 7-day washout between doses, 4 males and 4 females received the 2HCl salt then mesylate salt while 4 other males and 4 other females first received the mesylate salt then 2HCl salt.

Table 4: PK parameters following single dose administration of tirbanibulin

Study ID	Species	N	Dose (mg/kg)	Route	Cmax (ng/mL)	Tmax (h)	AUC_{inf} (ng.h/mL)	F (%)
7709-109	CD-1 mice (M)	24	5 50	IV Oral	3220 5940	0.083 0.5	2021 13851	NA 59.6
7709-115	CD-1 mice (M)	21	5	Oral	524	0.5	801	49.3
PSA13080020	SD Rats (M)	5-10	6.3 12.4 bid	IV Dermal	3718 12	0.083 2	2303 -	NA 1.59
7709-107	SD Rats (M)	3	1 12.5 25 50	IV Oral Oral Oral	609 2310 3970 13400	0.083 0.5 0.5 0.5	326 2231 4383 16434	NA 55.5 54.1 102.1
7709-118	SD Rats	4	5 2HCl (m) 5 Mesylate (m) 2 2HCl (f)	Oral Oral Oral	499 528 470	0.25 0.25 0.25	536 409 590	NA 83.2 NA

			2 Mesylate (f)	Oral	757	0.375	1037	144
7709-108	Beagle dogs (M)	3	2.5	IV	1620	0.083	2303	NA
			12.5	Oral	3320	1	8653	89
			25	Oral	3590	1	10863	56.2
			50	Oral	5010	1	17124	42
7709-117	Beagle dogs	16	0.75 2HCl (m)	Oral	163	0.5	327	NA
			0.75 Mesyl. (m)	Oral	104	1	310	105
			0.75 2HCl (f)	Oral	78.1	0.75	239	NA
			0.75 Mesyl. (f)	Oral	81.9	0.5	294	125

2HCl=dihydrochloride; AUCinf =AUC from time 0 to infinite; Cmax=maximal observed concentration; F=female; F=percent bioavailability; M=male; tmax=time to maximal observed concentration

Repeat dose studies

Tirbanibulin plasma concentrations were determined by LC-MS/MS in repeat dose toxicology studies in Sprague-Dawley rats, NZW rabbits, Beagle dogs, and Bama minipigs. Additionally, plasma concentrations of the tirbanibulin metabolites KX2-5036 and KX2-5163 were determined by LC-MS/MS in Sprague-Dawley rats and Bama minipigs in repeat dose toxicology studies.

Rats

In 5 repeat dose dermal studies (392-0051-TX, 392-0053-TX, 321-0032-TX, 78539 and PSA13080020), Sprague-Dawley rats were dosed dermally with tirbanibulin ointment at doses ranging from 2 to 40 mg/kg/day tirbanibulin (percentage of tirbanibulin in the ointment ranged from 0.1% to 2%, respectively) for up to 28 days and, in the 3-month repeated dose study, with 4 cycles of 5 days of daily treatment with 23 days without treatment between cycles. Systemic exposure (C_{max} and AUC from time 0 to 24 h [AUC_{0-24h}]) to tirbanibulin increased with dose and was typically higher in females than males. In general, systemic exposure increased with repeat dosing up to 28 days but there was no accumulation after the 23-day treatment-free intervals of the 3-month repeat dose study. Plasma concentrations of 2 structurally confirmed metabolites of tirbanibulin were evaluated after up to 5 days of dermal treatment with tirbanibulin ointment (Study 392-0051-TX [KX2-5036] and Study 392-0053-TX [KX2-5036 and KX2-5136]). For KX2-5036, systemic exposure increased with repeat dosing. No consistent sex difference in systemic exposure was observed. Metabolite to parent (KX2-5036/tirbanibulin) ratios based on exposure (AUC_{0-24h}) ranged from 0.029 to 0.12. For KX2-5163, females typically had higher systemic exposure than males. The systemic exposure of KX2-5163 increased dose proportionally in both sexes on Day 1 but increased less than dose proportionally in both sexes on Day 5. Metabolite to parent (KX2-5163/tirbanibulin) ratios based on exposure (AUC_{0-24h}) ranged from 1.7 to 4.5.

In two repeat dose oral studies (7709-100 and 7709-102), Sprague-Dawley rats were dosed orally with a gavage solution of tirbanibulin at doses ranging from 0.625 mg/kg/dose (female) and 1.25 mg/kg/dose (male) to 5 mg/kg/dose 2 times a day for up to 28 days. For tirbanibulin, the systemic exposure (Cmax and AUC0-24h) generally increased with increasing oral doses, was roughly dose proportional, and was higher in females than males. No increase in systemic exposure (≥2-fold) was observed after repeat oral dosing of tirbanibulin.

NZ White Rabbits (PSA13100016)

Male NZW rabbits were dosed with tirbanibulin dermally b.i.d. (4 h/time, with 4 h interval) for 8 days using patches. Dermal doses ranged from 0.21 to 2.07 mg/kg/dose. The tmax for dermal dosing occurred 1 to 4 h post-dose. The systemic exposure (C_{max} and AUC) to tirbanibulin increased with increasing doses and with repeat dosing of dermal tirbanibulin.

Beagle Dogs

In 2 repeat dose oral studies in Beagle dogs (Study 7709-101 and Study 7709-103), animals were dosed orally with a gavage solution of tirbanibulin at doses ranging from 0.25 to 1 mg/kg/dose 2 times a day for up to 28 days. The tirbanibulin tmax generally occurred within the first 2 h post-dose. Systemic exposure (Cmax and AUC) to tirbanibulin generally increased with increasing doses (roughly dose proportional), and similar in males and females. No increase in systemic exposure was observed after repeat dosing.

Bama minipigs

In 4 repeat dose dermal studies (44103-13-835, 392-0052-TX, 392-0054-TX and 321-0033-TX), Bama minipigs were dosed dermally with tirbanibulin ointment at doses ranging from 2 to 40 mg/kg/day tirbanibulin for up to 28 days. For tirbanibulin, tmax was mainly observed between 2 and 8 h post-dose. Systemic exposure (C_{max} and $AUC_{0\text{-}24h}$) to tirbanibulin increased with dose. The exposure was higher in females than in males; In general, systemic exposure increased with repeat dosing but there was no accumulation after the 23-day recovery periods in the 3-month repeat dose study. Metabolite to parent ratios (KX2-5036/tirbanibulin and KX2-5163/tirbanibulin) based on plasma concentration were observed between 0.080 and 0.16 and between 0.31 and 0.59, respectively.

2.3.3.3. Distribution

In vitro

The extent of *in vitro* protein binding of tirbanibulin to plasma proteins was determined at concentrations ranging from 0.01 to 10 µg/mL in rat, rabbit, and dog plasma (pooled mixed sex) using equilibrium dialysis at 37°C for 4 h. Plasma protein binding of tirbanibulin over the range of 0.01 to 10 µg/mL was concentration independent. The average protein binding of tirbanibulin to rat, rabbit, and dog plasma was 92.3%, 94.0% and 81.9%, respectively (Study C18091).

The extent of *in vitro* protein binding of tirbanibulin to plasma proteins was also determined at concentrations ranging from 0.01 to 10 µg/mL in human plasma (pooled mixed sex) and 0.07% human α-1 acid glycoprotein in Dulbecco's phosphate buffered saline using equilibrium dialysis at 37°C for 4 h. The percent bound fraction of tirbanibulin ranged between 87.6% and 89.0% in human plasma and between 37.3% and 69.3% in human α-1 acid glycoprotein over the concentration range of 0.01 to 10 µg/mL. The plasma protein binding of tirbanibulin was concentration independent. The results showed that the binding of tirbanibulin to 0.07% human α-1 acid glycoprotein may reach saturation at concentrations >0.1 µg/mL (Study XBL17622).

The binding of KX2-5036, the pyridine acetamide metabolite of tirbanibulin, to the proteins in plasma from rats, rabbits, dogs, and humans and to human α-1 acid glycoproteins was assessed using equilibrium dialysis (Study C18092). Plasma binding of KX2-5036 over the range of 0.01 to 10 µg/mL was concentration independent. The average protein binding of KX2-5036 to rat, rabbit, dog, and human plasma and to human α-1 acid glycoproteins was 44.0%, 52.0%, 35.4%, 57.3%, and 3.9%, respectively.

The binding of tirbanibulin and KX2-5036, the pyridine acetamide metabolite of tirbanibulin, to plasma proteins in Bama minipig was assessed using equilibrium dialysis (Study 412036-2019052701-PPB). Binding of tirbanibulin and KX2-5036 to Bama minipig plasma was concentration independent over the range of 0.01 to 10 µg/mL (between 84.4% and 86.5% for tirbanibulin and between >34.3% to 48.1% for KX2-5036.

In vivo

A mass balance study which investigated tissue distribution was conducted with [^{3}H]tirbanibulin ([^{3}H]KX2-391) in male and female Sprague-Dawley rats, following a single oral administration. Fifteen male and 15 female rats received a single oral dose of [^{3}H]tirbanibulin (5 mg/kg free base; 200 µCi/kg) in purified water. Blood was collected at 15 and 30 min and 1, 2, 4, 8, 24, 48, and 72 h post-dose to measure blood and plasma concentrations of radioactivity and to determine PK parameters of total radioactivity. The peak concentration of the total radioactivity was found at 1 h post-dose for all organs, except for GI contents, for which the peak concentration was observed at 4 h post-dose. Within 72 h post-dose, the decrease of the radioactivity in all organs was in accordance with that in plasma. At all sampling times, liver, stomach, and intestines were the main tissues that contained higher radioactivity concentrations compared to plasma. Other organs containing higher radioactivity concentrations than plasma at some sampling times were adrenals, kidneys, and thyroids. Brain and bone had the lowest content of radioactivity. The radioactivity concentration in all tissues decreased significantly at 24 and 72 h. The radioactivity was detectable up to 72 h post-dose in the blood and plasma of intact male and female rats. The mean whole blood to plasma ratios ranged from 0.52 to 1.08, suggesting no significant binding to cellular components. No significant sex differences were observed for tissue distribution or PK parameters.

2.3.3.4. Metabolism

In vitro

The metabolism of tirbanibulin at a concentration of 1 µM was evaluated in the presence and absence of selective chemical inhibitors of 8 major CYP isozymes (CYP1A2, 2C8, 2C9, 2C19, 2D6, 3A4, 2B6, and 2E1). CYP isoforms responsible for tirbanibulin metabolism were confirmed by incubating tirbanibulin with individual recombinant human CYP enzymes (rCYP). Eight major CYP isozymes (rCYP1A2, 2C8, 2C9, 2C19, 2D6, 3A4, 2B6, and 2E1) were evaluated for their role in the metabolism of tirbanibulin. Tirbanibulin (1 µM) was incubated with individual rCYP isozymes (50 pmol/mL) for 30 min. The CYP3A4 inhibitor ketoconazole inhibited the metabolism of tirbanibulin 48.4% at a concentration of 5 µM. In addition, the recombinant human CYP3A4 system efficiently metabolised tirbanibulin 85.2% under the conditions of this study. The CYP2C8 inhibitor, quercetin, inhibited the metabolism of tirbanibulin 42.2% at a concentration of 20 µM. Recombinant human CYP2C8 did catalyse the metabolism of tirbanibulin but only 13.9% under the condition of this study. Metabolism by the CYP isoforms 1A2, 2B6, 2C9, 2C19, 2D6, and 2E1 was not observed in the chemical inhibitor or respective rCYP incubations (Study XBL08671).

Incubation with rat, dog, and human hepatocytes resulted in significant metabolism of tirbanibulin. Turnover was most extensive in rat hepatocytes with less than 10% of tirbanibulin remaining following incubation at 1 µM for 2 h. Turnover was also extensive, albeit significantly slower, in dog and human hepatocytes, with the extent of metabolism in the range of 50% following a 2 h incubation at 1 µM. Fourteen metabolites were characterised from the incubations in the hepatocytes of the 3 species. Human hepatocytes produced 5 metabolites, all of which were observed in either rat or dog hepatocytes and in either dog plasma or urine. No metabolites were found to be unique to human hepatocytes. Four of the metabolites were observed in rat plasma, all of which were also produced by rat hepatocytes, in addition to another 2 metabolites. Dog hepatocytes produced 6 metabolites, all of which and more were observed in dog plasma, dog urine, or both.

Table 5: Summary of estimated relative abundance of tirbanibulin and its metabolites in rat, dog, and human hepatocyte incubates, rat and dog plasma, and dog urine (Study 7709-114)

Compound	% of total MS peak area [a]					
	Hepatocytes			Plasma		Urine
	Rat	Dog	Human	Rat	Dog	Dog
M1	5.47	4.78	0.48	3.43	1.99	2.3
KX2-5163 (M2)	19.9	ND	ND	44.6	0.86	ND
M3	ND	ND	ND	ND	1.3	2.32
M4	ND	ND	ND	ND	0.34	1.06
M5	ND	ND	ND	ND	0.99	ND
M6	ND	ND	ND	ND	0.77	0.37
KX2-5183 (M7)	ND	1.00	ND	ND	1.13	0.63
M8	ND	0.03	1.73	ND	ND	0.38
KX2-5036 (M9)	34.8	52.6	56.2	9.21	21.2	27.3
Tirbanibulin	33.4	34.8	38.7	30.8	21.4	15.4
M10	ND	ND	ND	ND	2.93	1.77
KX2-5162 (M11)	2.08	5.22	2.5	ND	34.8	29.3
M12	ND	ND	ND	ND	2.46	1.77
KX2-5180 (M13)	3.33	1.42	ND	12.1	8.38	19.6
M14	1.05	ND	0.56	ND	1.49	ND

MS=mass spectrometry; ND=not detected.

a) Relative abundance is based on the assumption of equivalent ionisation of tirbanibulin and all metabolites. Therefore, the numbers presented in this table should be used with caution.

Tirbanibulin, at 1 and 10 µM, were extensively metabolised in minipig hepatocytes with 19.8% and 21.3% of tirbanibulin remaining after the 2 h incubation, respectively. In addition to unchanged tirbanibulin, 11 metabolites were tentatively identified by LC-HR-MS in minipig hepatocyte incubations (Study XBL18507).

In vivo

After a single oral dose of [^{3}H]tirbanibulin, unchanged drug and 18 metabolites, designated M1 to M18, were characterised in rat plasma, bile, urine, and faeces. M1 is an amide moiety hydrolysis product of M11. M2 and M3 identified in this study were confirmed as KX2-5036 and KX2-5163, respectively. M2 is a pyridine acetamide metabolite of tirbanibulin and M3 is a pyridine acetic acid metabolite of tirbanibulin or KX2-5036. Unchanged [^{3}H]tirbanibulin accounted for 4.19% and 7.49% of the total radioactivity in 0 to 24 h male and female rat plasma, respectively. Metabolites KX2-5036 and KX2-5163 (co-eluted in radioprofiles) were the major circulating metabolites, together accounting for 93.9% and 80.2% of the total radioactivity in male and female rat plasma, respectively. Three minor circulating metabolites, M16, M17, and M18, were also observed, accounting for 0.19%, 0.46%, and 0.45% for males, and 2.20%, 0.95%, and 6.61% for females, respectively (Study XBLC11643N).

Table 6: Percent distribution of [^{3}H]tirbanibulin and metabolites in rat plasma after a single oral administration of 5 mg/kg

Metabolite	AUC (ng.h-Eq./g)		% $AUC_{Total\ Radioactivity}$	
	Male	Female	Male	Female
[^{3}H]tirbanibulin	1049	1843	4.19	7.49
M2 (KX2-5036), M3 (KX2-5136) [a]	23503	19711	93.9	80.2
M16 (KX2-4764)	47.8	541	0.19	2.20
M17 (KX2-5180)	116	233	0.46	0.95
M18	113	1625	0.45	6.61
Total radioactivity	25024	24591	100	100

AUC=area under the concentration-time curve; ^{3}H=tritium.

a) M2 (KX2-5036) and M3 (KX2-5163) co-eluted in radioprofiles. M2 is a pyridine acetamide metabolite of tirbanibulin and M3 is a pyridine acetic acid metabolite of tirbanibulin or KX2-5036.

2.3.3.5. Excretion

Study XBLC11639N investigated mass balance, PK, metabolism, and tissue distribution of [^{3}H]tirbanibulin ([^{3}H]KX2-391) in male and female Sprague-Dawley rats, following a single oral administration. Three male and 3 female intact rats and 3 male and 3 female BDC rats were orally dosed with 5 mg/kg free base equivalent (200 µCi/kg) and housed individually in metabolism cages equipped for the separate collection of bile, urine, faeces, and cage rinse.

For intact animals, urine was collected at 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h post-dose. Faeces were collected at 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h post-dose. For BDC rats, bile (collected from bile duct-cannula) and urine were collected pre-dose and at 0 to 8, 8 to 24, 24 to 48, and 48 to 72 h post-dose. Faeces were collected at 0 to 24, 24 to 48, and 48 to 72 h post-dose.

In rats, faeces was the major route of excretion (61% to 71% of total radioactivity), mainly via biliary excretion (>80% of faecal radioactivity); 13% to 27% was excreted in urine. The majority of radioactivity was excreted within 24 h post-dose.

Table 7: Mean excretion (0 to 72 h) of dosed radioactivity from urine, cage rinse, and faeces after a single oral administration of [^{3}H]tirbanibulin at 5 mg/kg in Sprague-Dawley rats (Study XBLC11639N)

Group	Sex	% of dose excreted				Total dose recovered (%)
		Bile	Faeces	Urine	Cage wash/rinse	
Intact	Male	N/A	61.01	26.33	3.98	91.32
	Female	N/A	70.62	13.01	2.54	86.17
BDC	Male	61.83	8.29	22.79	0.92	93.82
	Female	64.05	10.38	15.25	1.14	90.81

BDC=bile duct-cannulated; ^{3}H=tritium; N/A=not applicable.

2.3.3.6. Pharmacokinetic drug interactions

CYP inhibition

Study C18087 evaluated the inhibition potential of tirbanibulin (KX2-391) and KX2-5036, the pyridine acetamide metabolite of tirbanibulin, for the enzymatic activities of human CYP isozymes CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 using pooled, mixed sex (n=150) HLM. Both direct and time-dependent inhibition (TDI) of tirbanibulin and KX2-5036 on CYP were evaluated. The direct inhibitory

potentials of tirbanibulin (0.09 to 90 µM) and KX2-5036 (0.1 to 100 µM) were tested separately *in vitro* in HLM (0.1 mg/mL), using CYP-specific marker substrate reactions.

For tirbanibulin, no/limited direct and TDI effects were observed on CYP1A2, 2B6, 2C8, and 2D6 under these experimental conditions. Tirbanibulin showed >50% direct inhibitory effects on 3 CYP-mediated reactions, CYP2C9, 2C19, and 3A4/5-mediated testosterone 6β-hydroxylation, with apparent direct IC_{50} values ranging from 35.2 to 73.5 µM. After a 30 min pre-incubation, the inhibitory effect of tirbanibulin increased on CYP2C9, 2C19, and 3A4/5 (both reactions) and IC_{50} shifted from 1.5- to 2-fold.

Table 8: Summary of direct and time-dependent inhibition of CYP isozymes by tirbanibulin (Study C18087)

CYP isoform	Marker substrate (concentration) [a]	Isoform-catalysed reaction	Direct IC_{50} [b] (µM)	Time-dependent IC_{50} [b] (µM)	IC_{50} shift fold [c]
1A2	Phenacetin (50 µM)	O-de-ethylation	>90	>90	N/A
2B6	Bupropion (50 µM)	Hydroxylation	>90	>90	N/A
2C8	Amodiaquine (2 µM)	N-de-ethylation	>90	>90	N/A
2C9	Diclofenac (5 µM)	4'-hydroxylation	73.5	47.3	1.6
2C19	S-mephenytoin (20 µM)	4'-hydroxylation	40.8	21.5	1.9
2D6	Bufuralol (5 µM)	1'-hydroxylation	>90	>90	N/A
3A4/5	Midazolam (2 µM)	1'-hydroxylation	>90 (~103)	67.8	1.5
3A4/5	Testosterone (50 µM)	6β-hydroxylation	35.2	17.6	2.0

CYP=cytochrome P450; IC_{50}=inhibitory concentration where the binding/activity is reduced by half; N/A=not applicable.

a) Incubation concentrations of marker substrates at or below their reported Michaelis Menten constant.

b) IC_{50} (inhibitor concentration that decreased the enzyme activity by 50%) was estimated by fitting the calculated % CYP activity data as a function of test article concentration to normalised sigmoidal inhibitory non-linear regression model using GraphPad Prism®.

c) IC_{50} shift folds=IC_{50}, direct/IC_{50}, time-dependent.

Note: >90=no inhibition greater than 50% within the concentration ranging 0.09 to 90 µM; data were calculated from triplicate measurements.

To establish if the TDI effect on CYP2C9, 2C19, and 3A4/5 that was seen in the initial experiments described above was irreversible, an additional set of experiments was conducted. A dilution method was used to determine the CYP2C9, 2C19, and 3A4/5 inhibition reversibility of tirbanibulin. Tirbanibulin over concentration ranges of 6.25 to 200 µM for CYP2C9, 3.44 to 110 µM for CYP2C19, 6.25 to 200 µM for CYP3A4/5-mediated midazolam 1'-hydroxylation, and 1.15 to 100 µM for CYP3A4/5-mediated testosterone 6β-hydroxylation, was incubated with HLM in the presence and absence of NADPH for 30 min. After the inactivation incubation, the HLM mixture was diluted 10-fold to buffer systems containing CYP-specific marker substrates at saturated concentrations, and the remaining CYP activities were monitored.

No/limited inhibitory effects of tirbanibulin on CYP2C9, 2C19, and 3A4/5 were observed after pre-incubating with HLM in the absence or presence of NADPH. However, tirbanibulin showed inhibitory effects on CYP3A4/5 after pre-incubating with HLM in the presence of NADPH after dilution. At the highest testing concentrations, the activity loss for CYP3A4/5-mediated midazolam 1'-hydroxylation and testosterone 6β-hydroxylation activities were 59.3% and 39.1%, respectively (table below).

Table 9: Summary of CYP2C9, 2C19, and 3A4/5 inhibition reversibility assay of tirbanibulin

CYP isoform	Marker substrate (concentration)	Isoform-catalysed reaction	Tirbanibulin concentration range (µM)	Apparent IC_{50} [a] (µM); pre-incubated		% activity loss [b] (at highest concentration)
				Without NADPH	With NADPH	
2C9	Diclofenac (40 µM)	4'-hydroxylation	6.25–200	>200	>200	5.9±2.2
2C19	S-mephenytoin (80 µM)	4'-hydroxylation	3.4375–110	>110	>110	7.2±4.0
3A4/5	Midazolam (20 µM)	1'-hydroxylation	6.25–200	>200	>200	59.3±6.2
3A4/5	Testosterone (400 µM)	6β-hydroxylation	1.1525–100	>100	>100	39.1±3.1

CYP=cytochrome p450; IC_{50}=inhibitory concentration where the binding/activity is reduced by half; NADPH=nicotinamide adenine dinucleotide phosphate.

a) Apparent IC_{50} values for tirbanibulin were determined by fitting normalised data to sigmoidal inhibitory non-linear regression model using GraphPad Prism.

b) % activity loss was determined as described in Section 4.2.2.6, Study C18087, Section 5.5.6.

Note: >100/110/200=no ≥50% inhibition was observed within the concentration ranges tested.

The MBI kinetics parameters of tirbanibulin on CYP3A4/5 for testosterone and midazolam were evaluated. Tirbanibulin at a concentration range of 6.25 to 200 µM was incubated with HLM in the presence of NADPH for 0, 2.5, 5, 10, 20, and 30 min. After the inactivation incubation, the HLM mixture was diluted 10-fold to buffer systems containing individual CYP3A4/5 marker substrates and the remaining CYP3A4/5 activities were monitored. The experimental MBI characterisation assay results are presented in the table below.

Table 10: Summary of mechanism-based inhibition characterisation assay results of tirbanibulin

CYP	Marker substrate (concentration)	K_I (µM) [a]	k_{inact} (min^{-1}) [a]	k_{inact}/K_I (mL/min/µmol)
3A4/5	Midazolam (20 µM)	78.91	0.04	0.51
3A4/5	Testosterone (400 µM)	87.38	0.04	0.46

CYP=cytochrome P450; IC_{50}=inhibitory concentration where the binding/activity is reduced by half; K_I=concentration of inactivator at half maximal rate of inactivation (K_{inact}); K_{inact}=maximal rate of inactivation.

a) Apparent K_I and k_{inact} values for tirbanibulin were determined by fitting inactivation rate constant data to non-linear regression model using GraphPad Prism.

For KX2-5036, no/limited direct and TDI effects were observed on CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 (both reactions) under experimental conditions. At 100 µM, remaining enzyme activity ranged from 91.6% to 107.5%.

CYP induction

Study XBL17621 evaluated the potential of tirbanibulin (KX2-391) to induce human CYP isozymes CYP1A2, 2B6, and 3A4 *in vitro* using cryopreserved plateable human hepatocytes from 3 individual donors. Under experimental conditions, >70% of hepatocytes were viable after 3-day treatment with tirbanibulin at 0.0003 to 1 µM. Tirbanibulin at 3 µM reduced the hepatocyte viability by approximately 45% relative to vehicle control. Therefore, the maximum concentration of tirbanibulin used in this study was 1 µM. CYP induction potential of tirbanibulin was evaluated by incubating the test article at 0.01, 0.1, and 1 µM with plated human hepatocytes from 3 individual donors. Tirbanibulin at concentrations of up to 1 µM showed no induction potential for the mRNA expression levels of CYP1A2, 2B6, and 3A4 in hepatocytes from 3 donors (table below). Respective positive controls under the same conditions showed significant induction relative to untreated hepatocytes. CYP 1A2, 2B6, and 3A4 expression levels were not affected by the treatment of the negative control, flumazenil.

Table 11: Summary of CYP mRNA induction by tirbanibulin in human hepatocytes

CYP isoform	Donor	mRNA induction-fold in 3 different donors				
		Tirbanibulin			Negative control	Positive control
		0.01 µM	0.10 µM	1.00 µM		
1A2	1	0.74	0.77	0.34	1.01	22.78
	2	0.90	1.04	0.64	0.97	23.45
	3	1.24	1.39	0.45	0.99	32.67
2B6	1	0.91	0.89	0.32	1.27	15.86
	2	0.78	0.89	0.27	1.00	10.05
	3	1.25	1.55	0.23	0.93	16.43
3A4	1	0.92	1.61	0.14	1.76	85.24
	2	0.56	1.05	0.23	1.04	47.25
	3	0.88	1.30	0.20	0.66	18.14

CYP=cytochrome p450; mRNA=messenger ribonucleic acid.

Note: Negative control: 25 µM flumazenil; positive control: 50 µM omeprazole for CYP1A2, 750 µM phenobarbital for CYP2B6 and 25 µM rifampin for CYP3A4.

Study C18089 evaluated the potential of KX2-5036, the pyridine acetamide metabolite of tirbanibulin, to induce human CYP isozymes CYP1A2, 2B6, and 3A4 *in vitro* using cryopreserved plateable human hepatocytes from 4 individual donors. CYP induction potential of KX2-5036 was evaluated by incubating the test article at 0.01, 0.03, 0.1, 0.3, 1, and 3 µM with plated human hepatocytes from 4 individual donors. Under experimental conditions, >95.6% of hepatocytes were viable after 3-day treatment with KX2-5036 at up to 3 µM. Therefore, the maximum concentration of KX2-5036 used in this study was 3 µM. KX2-5036 at concentrations of up to 3 µM showed no induction potential for the mRNA expression levels of CYP1A2, 2B6, and 3A4 in hepatocytes from 4 donors. Respective positive controls under the same conditions showed significant induction relative to untreated hepatocytes. CYP1A2, 2B6, and 3A4 expression levels were not affected by the treatment of the negative control, flumazenil.

Uptake and Efflux Transporter Inhibition and Substrate Assays

The ATP-dependent transport of tirbanibulin by MDR1, BCRP, BSEP, and MRP2 was evaluated in membrane vesicular assays. The transporter-specific ATP-dependent accumulation of tirbanibulin was <2-fold at all tested concentrations (0.1, 1, 10, and 100 µM), indicating that tirbanibulin is not a substrate for MDR1, BCRP, BSEP, or MRP2.

The inhibition potential of tirbanibulin on the transport activity of human MDR1, BCRP, BSEP, and MRP2 was tested in inside-out membrane vesicles. No significant inhibitions (<25%) with substrates by tirbanibulin at concentrations of up to 10 µM (4315 ng/mL), indicating that tirbanibulin is not an inhibitor for MDR1, BCRP, BSEP, or MRP2.

The inhibitory potential of tirbanibulin on the transport activities of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 was evaluated in cellular uptake inhibition assays. Under experimental conditions, <50% inhibition by tirbanibulin up to 100 µM was observed for OAT1 and OAT3. At 100 µM tirbanibulin, >50% inhibition was observed for MATE2-K, OATP1B1, and OATP1B3. At 10 and 100 µM tirbanibulin, >50% inhibition was observed for MATE1, OCT1, and OCT2.

The follow-up inhibition assays were performed in the presence of 7 concentrations of tirbanibulin to obtain IC_{50} values for MATE1, MATE2-K, OATP1B1, OATP1B3, OCT1, and OCT2. The estimated IC_{50} values ranged from 1.4 to 66.4 µM (604 to 28650 ng/mL), suggesting that tirbanibulin is an inhibitor for MATE1, MATE2-K, OATP1B1, OATP1B3, OCT1, and OCT2 (table below).

Table 12: Summary of IC_{50} values of tirbanibulin on human solute carrier transporters

Transporter	Probe substrate (concentration)	Mean tirbanibulin IC_{50} (μM) [a]
MATE1	Metformin (2 μM)	2.6
MATE2-K	Metformin (10 μM)	7.3
OATP1B1	Estradiol-17-β-glucuronide (1 μM)	66.4
OATP1B3	Cholecystokinin-8 (0.11 μM)	27.4
OCT1	Tetraethyl-ammonium (5 μM)	3.5
OCT2	Tetraethyl-ammonium (5 μM)	1.4

IC_{50}=inhibitory concentration where the binding/activity is reduced by half; MATE=multidrug and toxin extrusion protein; OATP=organic anion-transporting polypeptide; OCT=organic cation transporter.

a) IC_{50} are the mean of triplicate measurements.

Transporter-mediated uptake of tirbanibulin was tested in each of the MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2-expressing cell systems. Based on the results, tirbanibulin was not a substrate for MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 transporters.

Study C18090 evaluated The *in vitro* interaction between KX2-5036, the pyridine acetamide metabolite of tirbanibulin, and human ABC efflux transporters (MDR1, BCRP, BSEP, and MRP2) and SLC transporters (MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2) was evaluated.

The ATP-dependent transport of KX2-5036 by MDR1, BCRP, BSEP, and MRP2 was evaluated in membrane vesicular assays. The transporter-specific ATP-dependent accumulation of KX2-5036 was <2-fold at all tested concentrations in the MDR1 and MRP2 membrane vesicles. Greater than 2-fold accumulation of KX2-5036 at 0.1 and 1 μM was observed in BCRP. In a follow-up assay where transport was measured in the absence and presence of Ko143, a specific BCRP transport inhibitor showed that BCRP-mediated ATP-dependent accumulation of KX2-5036 did not change in the presence of 1 μM Ko143. Greater than 2-fold accumulation of KX2-5036 at 1 μM in BSEP-expressing membrane vesicles was observed. At 1 μM, the BSEP-mediated and ATP-dependent accumulation fold (3.5) was likely due the low ATP-dependent accumulation fold from the control vesicles (0.5), but not from the ATP-dependent accumulation fold from BSEP vesicles, because the ATP-dependent accumulation fold was <2 (1.6) from BSEP vesicles.

Inhibition potential of KX2-5036 on the transport activities of MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 was evaluated in cellular uptake inhibition assays. Under experimental conditions, <50% inhibition by KX2-5036 up to 100 μM was observed for MATE1, OAT1, OAT3, OATP1B1, OATP1B3, and OCT1. KX2-5036 at 100 μM inhibited MATE2-K and OCT2 by 67.6% and 77.7%, respectively. The follow-up inhibition assays were performed in the presence of 7 concentrations of KX2-5036 to obtain IC_{50} values for MATE2-K and OCT2. The calculated IC_{50} values are 63.1 and 9.1 μM for MATE2-K and OCT2, respectively.

Transporter-mediated uptake of KX2-5036 was tested in each of the MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2-expressing cell systems and the corresponding control systems. No accumulation >2-fold was observed for any transporter except OAT3. Follow-up substrate assays showed that the cellular accumulation observed was not inhibited by 100 μM probenecid, as specific inhibitor of OAT3.

2.3.4. Toxicology

Single dose toxicity

A GLP-compliant study evaluated the irritating dermal effects of tirbanibulin ointment and the plasma TK of tirbanibulin following a single dermal application of ointment to the intact skin of male and female (3 M/F) Bama minipigs (Study 44103-13-835). Ointment at a dose of 0.5 g ointment containing

either 0, 5 mg/g, or 10 mg/g of tirbanibulin was applied to the dosing area (2×3 cm) of each animal and covered with a gauze patch, which was held in place with non-irritating tape for approximately 4 h. After each 4-h exposure, residual test ointment was cleared using warm water. Animals were observed for up to 14 days post-dose. No mortality, moribundity, or any effect on clinical observations, body weight, or dermal observations was noted in the minipigs. All tirbanibulin plasma concentrations were below the lower limit of quantitation (LLOQ, <1 ng/mL). Therefore, no TK analysis could be performed.

Repeat dose toxicity

Table 13: Pivotal repeat dose toxicity studies with tirbanibulin

Study ID	Species/ Sex/ Number/ Group	Dose/Route	Duration	NOAEL
392-0053-TX	SD Rats; 4-8 M/F per group	Control, 0.1%, 0.5% or 1% ointment via dermal application to 10% BSA for 8 h	5-days with 21 days recovery	0.1%
321-0032-TX	SD Rats; 10 M/F per group	Control, 0.1%, 1% or 2% ointment via dermal application to 10% BSA for 20 h	28-days with 21 days recovery	0.1%
78539	SD Rats; 10 M/F per group	Control, 0.1%, 0.5% or 1% ointment via dermal application to 10% BSA for 8 h	4 cycles (5 days =1 cycle) with 23 days between cycles	1% for males; 0.1% for females
392-0054-TX	Bama minipigs/ 3-5 M/F per group	Control, 0.1%, 0.5% or 1% ointment via dermal application to 10% BSA with semi-occlusion for 8 h	5-days with 21 days recovery	1%
321-0033-TX	Bama minipigs/ 4-6M/F per group	Untreated, placebo, 0.1%, 1.0% or 2.0% ointment via dermal application to 10% BSA with semi-occlusion for 20-22 h	28-days with 14 day recovery	0.1%
78537	Gottingen minipigs/ 2-4 M/F per group	Untreated, placebo, 0.1%, 0.5% or 1% ointment via dermal application to 10% BSA with semi-occlusion for 20 h	4 cycles of 5-days dosing followed by 23 days treatment free	1%
7709-102	SD Rats 10 M/F per group	1.25, 2.5, or 5 mg/kg/dose b.i.d. (males) or 0.625, 1.25, or 2 mg/kg/dose b.i.d. (females)	4-weeks with 2-weeks recovery	1.25 mg/kg
7709-103	Beagle dogs; 3-5 M/F per group	0.25, 0.5, or 1/0.75 mg/kg/dose b.i.d. (males) or 0.625, 1.25, or 2 mg/kg/dose b.i.d. (females)	4-weeks with 2-weeks recovery	0.25 mg/kg (LOAEL)

F=female; F1=first filial; LOAEL=lowest observed adverse effect level; M=male; b.i.d.=twice daily; NOAEL=no observed adverse effect level

Dermal administration

Pivotal studies in rats and minipigs (Study 321-0032-TX and Study 321-0033-TX) assessed the possible toxic effects of 28 days of daily doses of tirbanibulin ointment applied to approximately 10% BSA (2 mL/kg) at body weight doses of 2 to 40 mg/kg/day (0.1% to 2% ointment concentration) for up to 20 h/day to male and female animals, with the application site being semi-occluded.

In rats (Study 321-0032-TX), toxicity, including death, occurred in the control group (due to animal wrapping) and in groups administered 20 and 40 mg tirbanibulin/kg/day for up to 28 days. Histology findings in thymus, spleen, lymph nodes, and bone marrow (femur and sternum) and associated haematologic changes occurred in males and females. In addition, adverse effects were seen histologically in the testes. Changes at the skin application sites included slight to severe eschar and/or erythema, and/or skin scaling, and/or ulceration. The NOAEL for systemic toxicity for tirbanibulin ointment was 2 mg/kg/day in both sexes. At this dose, mean tirbanibulin systemic exposure (AUC0-24h) was 205 (males)/216 (females) ng.h/mL. These exposure levels were comparable to systemic exposures not associated with toxicity following oral dosing in rats (Study 7709-102).

In minipigs (Study 321-0033-TX), treatment-related deaths occurred in groups administered 20 mg/kg and 40 mg/kg daily for up to 28 days. Histologic findings in these animals indicated that the deaths were related to sepsis and enteropathy. Treatment-related microscopic observations at the end of the dosing phase occurred in the digestive system (tongue, oesophagus, ileum, cecum, colon, rectum, and liver), lymphoid and haematopoietic tissues (thymus, mesenteric and axillary lymph nodes, and sternal bone marrow), and reproductive tissues (ovaries, vagina, testes, and epididymides). The NOAEL for tirbanibulin ointment was 2 mg/kg/day for 28 days in both sexes. At this dose, mean tirbanibulin systemic exposure (AUC0-24h) was 37.4 (males)/32.7 (females) ng.h/mL.

The toxicity of a less prolonged exposure to dermal tirbanibulin was assessed in 5-day studies in rats (Study 392 0053-TX) and minipigs (Study 392-0054-TX), in which tirbanibulin ointment was applied daily to approximately 10% BSA at doses of up to 20 mg/kg for 8 h/day for 5 consecutive days to male and female animals with no occlusion to the application site in the rat study and with semi-occlusion in the minipig study. Systemic toxicity was only observed in rats, was similar to that seen in the 28-day rat study and was related to tirbanibulin plasma exposure. Target organs include the intestine (epithelium), bone marrow, thymus, and testes. The NOAEL concentration for systemic toxicity of tirbanibulin ointment was 0.1% (2 mg/kg tirbanibulin) (exclusive of testicular effects) in male rats. The NOAEL for systemic toxicity was not defined in female rats because of the occurrence of mild bone marrow hypocellularity in 2/10 rats at 2 mg/kg/day. These bone marrow effects were not observed in any of the treatment groups at the end of the recovery phase, indicating these histopathological changes to be transient. Therefore, for female rats, 2 mg/kg/day was assigned the LOAEL for dermal tirbanibulin ointment. The NOAEL for systemic toxicity in minipigs was 20 mg/kg/day tirbanibulin, the highest tested dose. This corresponds to a systemic exposure that is ≥17 greater than maximal human exposure in the MUsT clinical study.

The 3-month repeated dose study in Sprague-Dawley rats tested dermal dosing of tirbanibulin ointment in 4 cycles of 5 consecutive days of treatment separated by 23 treatment-free days (Study 78539). For each treatment cycle, 3 doses of tirbanibulin were tested, with doses of 2, 10, 20 mg/kg in the first cycle and doses of 1, 5, 10 mg/kg in the subsequent 3 cycles (this reduction of dose in cycles 2-4 was due to mortality and adverse clinical signs in females in 10 mg/kg/day and 20 mg/kg/day groups). During tirbanibulin treatment, there were several cases of erythema and of oedema, of differing severity, at the dermal application site. No skin reactions were observed in the untreated control animals. The frequency and severity of erythema and oedema did not increase in a dose-dependent manner but was overall higher in females. At the end of the recovery period, there was full recovery, with no observed cases of erythema and oedema, except in the highest dose group, in which these reactions persisted in a few females until the end of the 4-week recovery period. There was no treatment-related effect on body weight or ophthalmoscopy or on parameters of clinical chemistry,

urinalysis or haematology and coagulation. In tirbanibulin treated animals, high incidences of ulcers, crust, mixed cell infiltrate and apoptosis in the basal layer of the epidermis were observed histopathologically in the skin. No differences were noted between males and females within a given treated group, or between the groups. Changes considered to be related to an immune suppressive effect were seen in the bone marrow, thymus and spleen of some females at 5 and 10 mg/kg/day. An increased incidence of extramedullary haematopoiesis was detected in the males of the groups treated with 1 and 5 mg/kg/day during cycles 2-4, which might be secondary to the inflammatory component of the treatment-related skin effects. These systemic effects were absent at the end of the recovery period. The TK data indicate tirbanibulin systemic exposure consistent with dermal dosing. Exposure was higher in females and C_{max} and AUC values increased dose-dependently. The maximum plasma concentration of tirbanibulin was reached at 1 to 2 h post dosing. Accumulation was not seen from one cycle to the next. The NOAEL values for males and female rats corresponds to a systemic exposure (based on AUC_{0-24h} values) that were 124- and 38-times, respectively, greater than maximal human exposure in the MUsT clinical study.

The 3-month repeated dose study in minipigs tested dermal dosing of tirbanibulin ointment containing tirbanibulin at 3 concentrations (1, 5, 10 mg/kg) in separate groups of animals (Study 78537). Each animal received a once daily dermal application on 5 consecutive days followed by 23 treatment-free days, after which the cycle of treatment was repeated 3 times. During tirbanibulin treatment, there were several cases of erythema, of differing severity, and a few cases of oedema, of mild severity, at the dermal application site. No skin reactions were observed in the control animals. The frequency and severity of erythema and oedema increased in a dose-dependent manner without any differences between sexes. At the end of the recovery period, there was full recovery, with no cases of erythema and oedema observed. Microscopically, treatment-related skin reactions were confined to the epidermis and were categorised as primary (outcome of direct pharmacology, ie increased mitotic figures and apoptosis in the basal cell layer) or secondary (sequelae to the primary changes). Following the 4-week treatment-free period, the basal cell layer in each treated animal was within normal limits. Body weight, food consumption, ECG parameters and ophthalmoscopy were unaffected by the treatment. No organ weight, macroscopic or microscopic changes occurred. No tirbanibulin ointment-related effects were observed on parameters of clinical chemistry, urinalysis or haematology and coagulation. The TK data indicate tirbanibulin systemic exposure consistent with dermal dosing. Exposure was higher in females and C_{max} and AUC values increased dose-dependently. The maximum plasma concentration of tirbanibulin was reached at 0.5 to 8 h post- dose. Accumulation was not seen from one cycle to the next. The NOAEL value for minipigs corresponds to a systemic exposure (based on AUC_{0-24h} vales) that is 17- and 23-times, in males and females, respectively, greater than maximal human exposure in the MUsT clinical study.

Local toxicity in both species following 5 and 28 days and 3 months of application was seen at all doses and included skin irritation and degeneration/necrosis in the epidermis/dermis. Toxicity was reversible or showed a tendency toward recovery following the 2 to 3-week recovery period in the respective studies.

Oral administration

Oral administration of tirbanibulin in rats twice daily (b.i.d.) for 28 days was generally well tolerated in males given up to 2.5 mg/kg/dose and females given up to 1.25 mg/kg/dose. The no observed adverse effect level (NOAEL) was defined as 1.25 mg/kg/dose in both sexes (Study 7709-102). At this dose, the mean tirbanibulin systemic exposure (Day 27 AUC0-24h) was 295 (males)/620 (females) ng.h/mL. It was not possible to assign a NOAEL value for tirbanibulin following b.i.d. oral administration for 28 days in dogs but the lowest dose of 0.25 mg/kg/dose b.i.d. was assigned the lowest adverse event level (LOAEL) for both sexes (Study 7709-103). Target organs following oral dosing included the thymus, testes, intestine, and bone marrow. GI toxicity and myelosuppression

were the most common systemic toxicities noted. Microscopic assessment of target tissue commonly showed villus atrophy and degeneration/loss of the intestinal epithelium and depletion of the bone marrow. These effects were expected based on the mode of action and were generally reversible or showed a tendency toward recovery following the 2- to 3-week recovery phases in the respective studies.

Genotoxicity

Bacterial reverse mutation test

A GLP-compliant study evaluated the ability of tirbanibulin to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes (Study 7709-112). The doses tested in the mutagenicity assay were selected based on the results of a DRF experiments. Tirbanibulin was checked for cytotoxicity for each of the 10 doses tested, where little or no cytotoxicity was observed in the DRF study. Therefore, the highest dose concentration of tirbanibulin used in the mutagenicity assay was the same dose as that tested in the DRF study (5000 µg). The doses tested were 33.3, 100, 333, 1000, 2500, and 5000 µg/plate. Formulation concentration verification analyses met acceptance criteria. The results from the positive controls (benzo[a]pyrene, 2-aminoanthracene, 2-nitrofluorene, sodium azide, ICR-191, 4-nitroquinoline-N-oxide) met the assay validity criteria. Precipitation was found in the bacterial background lawn at the 2 highest doses (2500 and 5000 µg/plate), and little to no cytotoxicity was observed for tirbanibulin.

Mouse Lymphoma TK Gene Mutation Assay

The ability of tirbanibulin (KX2-391) to induce forward mutations at the thymidine kinase locus was assessed in the mouse lymphoma cell line L5178Y TK±3.7.2C (L5178Y), in the presence or absence of an exogenous metabolic activation system (S9), as assayed by colony growth in the presence of trifluorothymidine (TFT resistance, TFTr) (Study 321-0045-GT). In the first definitive mutagenicity assay, precipitate was observed in treatment medium at $\geq$150 μ g/mL at the beginning and at the end of the treatment period. Since tirbanibulin showed a steep toxicity curve from the cell concentration determined on Day 2, plating for mutant frequency (MF), counting colony of MF and mutant colony sizing were not performed in the first definitive assay. A second definitive mutagenicity assay was performed based on the cytotoxicity data from the first definitive assay. In all 3 treatment conditions, the solvent controls met the study validity criteria for acceptable MF, cloning efficiency, and suspension growth. The results from the positive controls also met the study validity criteria. A minimum of 8 concentrations of tirbanibulin were evaluated in each treatment series.

Criteria for a positive or negative response in the assay, the Global Evaluation Factor (GEF) method was used. Briefly, for this multi-well version of the Mouse Lymphoma Assay, the GEF was 126×10^{-6}. The test article was considered to be clearly positive if, in any of the treatment conditions examined, the increase in MF above the concurrent background exceeds the GEF, i.e., the induced MF (IMF) was above the GEF, and the increase was concentration related. For the S9 activated 3-h exposure series, the IMF was 186.4 and 219.6 at 80 (with 13.8% RTG) and 85 µg/mL (with 14.2% RTG), respectively, and a weak dose-related increase was observed (linear regression analysis, R2=0.6804). In the non-activated 3-h exposure series, tirbanibulin did not induce a MF more than 126×10^{-6} over the concurrent solvent control at any dose concentration and a dose-related increase was not observed (linear regression analysis, R2=0.0381). In the non-activated 24-h exposure series, the IMF was 118.7, 184.8, and 240.1 at 0.018 (with 38.8% RTG), 0.019 (with 20.3% RTG), and 0.02 µg/mL (with 7.8% RTG), respectively, and a weak dose-related increase was observed (linear regression analysis, R2=0.6074).

Chromosomal Aberrations in Chinese Hamster Ovary Cells

A GLP-compliant study evaluated the ability of tirbanibulin to induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells with and without S9 (Study 7709-113). The vehicle and negative control cultures were in the historical control range for cells with chromosomal aberrations and the positive control cultures had significant increase in cells with chromosomal aberrations as compared with the vehicle control cultures. The high doses selected for analysis in the assay had approximately 50% toxicity as compared with the vehicle control cultures and/or had a precipitate at the end of the treatment period. In the confirmatory chromosomal aberrations assay, the treatment period with tirbanibulin was for approximately 20 h without metabolic activation and for 3 h with metabolic activation. The cell cultures were harvested approximately 20 h from the initiation of treatment. Tirbanibulin concentrations of 6.43, 12.9, 25.7, 41.3, 51.5, 77.2, 103, 147, 210, 255, 300, and 400 µg/mL were tested without metabolic activation and 25.7, 51.5, 103, 147, 210, 255, 300, and 400 µg/mL were tested with metabolic activation.

Cultures were treated with concentrations (% mitotic index reduction) of 25.7 (0), 41.3 (2), 51.5 (34), and 210 (47) µg/mL without metabolic activation and 51.5 (0), 103 (0), 147 (1), and 210 (0) µg/mL with metabolic. Precipitates were visible at concentrations ≥103 and ≥210 µg/mL in incubations without and with metabolic activation, respectively. Without metabolic activation, a significant increase in cells with chromosomal aberrations was observed in the cultures treated with 25.7, 51.5, and 210 µg/mL. No significant increase in polyploidy or endoreduplication was observed in the cultures analysed. With metabolic activation, a significant increase in cells with chromosomal aberrations was observed in the cultures treated with 147 and 210 µg/mL. No significant increase in polyploidy or endoreduplication was observed in the cultures analysed.

In vivo rat micronucleus test

A GLP-compliant study determined if tirbanibulin has clastogenic activity and/or has the ability to disrupt the mitotic apparatus via an induction of micronuclei in polychromatic erythrocyte cells (PCE) in the bone marrow of adult Sprague-Dawley rats (Study 321-0044-GT). Tirbanibulin was administered as free base, formulated in 3% (v/v) acetic acid in purified water. In the definitive micronucleus assay, 35 rats/sex were assigned to 7 groups. The dose concentrations were 7.5, 15, and 30 mg/kg in males and 3.75, 7.5, and 15 mg/kg in females, respectively. Vehicle was administered concurrently with the same treatment scheme as tirbanibulin. The positive control article (cyclophosphamide monohydrate) was given by single intraperitoneal (IP) injection at 20 mg/kg. The animals were necropsied at approximately at 24 or 48 h. One female rat in Group 1 (vehicle control) was found dead on Day 2. This animal showed abnormal respiratory sounds on Days 1 and 2. Given <5 analysable female rats were available in control Group 1, only the results obtained for females at the 48-h and males at the 24- and 48-h timepoints were evaluated for the effect of tirbanibulin on PCE micronucleus formation. Statistically significant increases in micronucleus formation ($p<0.05$, ANOVA) were observed following 30 mg/kg tirbanibulin in males and 15 mg/kg tirbanibulin in females, both at the 24- and 48-h sampling timepoints. Males dosed at 15 mg/kg also showed statistically significant increased micronucleus frequency ($p<0.05$, ANOVA) at the 24-h sampling timepoint when compared with the concurrent vehicle control.

A GLP-compliant study determined whether tirbanibulin has clastogenic activity and/or has the ability to disrupt the mitotic apparatus as evidenced by an induction of micronuclei in PCE in the bone marrow or by DNA damage in liver cells (Study 54715.00103) Tirbanibulin was administered by oral gavage to adult male and female Sprague-Dawley rats as the free base, after preparation in 5% DMSO in deionised water at concentrations of 0.075, 0.25, 0.75, and 2.5 mg/mL for administration to males and 0.0375, 0.125, 0.375, and 1.25 mg/mL for administration to females daily on 3 consecutive days. Approximately 3 h following the final dose, animals were sacrificed, and liver sections were collected for DNA damage assessment by the comet assay and blood samples were collected for micronucleus frequency assessment. The study met the validity criteria for the test. All animals survived until the

end of the study. In the micronucleus assay, there was a dose-dependent reduction in the frequency of PCE, which was evidence of bone marrow cytotoxicity, in both male and female animals exposed to tirbanibulin, which was statistically significant in males after a dose of 25 mg/kg/day and in females after a doses of 3.75 and 12.5 mg/kg/day. In 2 females, the concentrations of cytotoxicity after 12.5 mg/kg/day exceeded the maximum concentration recommended by the test guideline and these animals were excluded from the analysis. There was a dose-dependent increase in the frequency of micronucleated PCE (MN-PCE) in male rats, which was statistically significant after 25 mg/kg/day tirbanibulin, and in females, which was statistically significant after 3.75 and 12.5 mg/kg/day tirbanibulin.

In the comet assay of liver tissue, there was a dose-dependent increase in DNA damage that was statistically significant, compared to the negative control, in males exposed to 25 mg/kg/day tirbanibulin; however, all groups fell clearly within the laboratory's historical negative control range. There was also a statistically significantly positive result for induction of DNA damage in liver of female rats exposed to 1.25 mg/kg/day tirbanibulin. With no evidence of dose-dependency, response at only the second lowest dose, and all values within the historical control range, this result was considered a chance finding and not biologically relevant. Because the comet assay yielded some positive results in both male and female rats, the results were considered equivocal.

Table 14: Summary of genotoxicity studies

Type of test/study ID/GLP	Test system	Concentration range/ Metabolising system	Results
Gene mutations in bacteria/Study 7709-112/GLP	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537 *E. coli* WP2 *uvrA*	33.3-5000 μg/plate +/- S9	Negative
Gene mutations in mammalian cells/321-0045-GT/GLP	Mouse lymphoma L5178Y $TK^{+/-}$cells, TK-locus	-S9 for 3 h: 0.1-90 μg/ml +S9 for 3 h: 0.2-80 μg/ml -S9 for 24 h: 0.001 -0.022 μg/ml	Weakly positive
Gene mutations in mammalian cells/7709-113/GLP	Chromosomal Aberrations in CHO Cells	-S9: 6.43, 12.9, 25.7, 41.3, 51.5, 77.2, 103, 147, 210, 255, 300, and 400 μg/mL +S9: 25.7, 51.5, 103, 147, 210, 255, 300, and 400 μg/mL	-S9: Chrom. aberrations at 25.7, 51.5, and 210 μg/mL +S9: Chrom. Aberrations at 147 and 210 μg/mL
Chromosomal aberrations in vivo/ 321-0044-GT/GLP	Mouse, micronuclei in bone marrow	7.5, 15, and 30 mg/kg in males; 3.75, 7.5, and 15 mg/kg in females for 24 or 48 h	Positive in: males at 30 mg/kg at 24 & 48h and 15 mg/kg at 24 h; females at 15 mg/kg at 24 & 48h
Chromosomal aberrations in vivo/ 54715.00103/GLP	Mouse, micronuclei in bone marrow	0.75, 2.5, 7.5 and 25 mg/kg in males; 0.375, 1.25, 3.75 and 12.5 mg/kg in females daily for 3 days	Positive in micronucleus: males at 25 mg/kg; females at 3.75 and 12.5 mg/kg/

Carcinogenicity

No carcinogenicity studies were submitted.

Reproduction Toxicity

Fertility and early embryonic development

A GLP-compliant study of tirbanibulin on fertility and early embryonic development to implantation in adult Sprague-Dawley rats was performed with once daily oral administration of tirbanibulin in males and females prior to and during the mating period, as well as during early gestation in females (Study 321-0034-TX). The dose concentrations for this study were set to 1, 2, and 4 mg/kg/day for male rats and 0.25, 0.5, and 1 mg/kg/day for female rats via oral gavage. The control group was administered control formulation (3% (v/v) acetic acid in purified water, pH~2 to 3). The dose volume for females was 2.5 mL/kg. The dose volume for males was initially 10 mL/kg but was reduced to 5 mL/kg from Day 25 until termination due to mortality related to respiratory irritation in all groups, which resulted from the acetic acid in the vehicle. Males were treated for 9 weeks prior to mating and throughout mating to termination. Females were dosed for 2 weeks prior to mating and continued throughout mating and up to and including Gestation Day 7.

A total of 17 animals (2/24, 3/24, 5/24 and 4/24 male rats, and 2/24, 0/24, 1/24 and 0/24 female rats from control, low-dose, mid-dose and high-dose group respectively) were terminated early due to moribund condition or were found dead. Prior to termination, most animals were first noted with abnormal respiratory sounds, which was followed by distended abdomen, a slight to moderate thin appearance, laboured breathing, cold to touch, decreased activity, and/or unkempt. These mortalities may be related to larynx, trachea, or lung irritation resulting from the presence of acetic acid in the vehicle and were not considered to be treatment related. There were no treatment-related changes in mortality, clinical observations, body weight, gravid uterine weight, food consumption, oestrus cycle evaluation, cohabitation duration, fertility data (male mating index, male fertility index, female mating index, female fertility index, and fecundity index), macroscopic observations, epididymis and ovary weights, and litter data (number of corpora lutea, implantations, viable embryos, non-viable embryos, and pre- and post-implantation loss).

A decrease in testes weight (11.8% for absolute weight and 10.7% for relative weight) was noted in males at 4 mg/kg/day, which correlated with decreased sperm count (22.8%), decreased sperm motility (10.3%), increased incidences of abnormal sperm (47%), and increased incidence of degeneration of the seminiferous epithelium (characterised by variable degeneration/necrosis and loss of germinal cells, most notably affecting spermatocytes and spermatids) in testes.

The NOAEL of tirbanibulin for female fertility and early embryonic development to implantation in rats was considered to be 1 mg/kg/day, the highest dose tested in females in the study. The NOAEL of tirbanibulin for male fertility was 2 mg/kg/day. Plasma exposure to tirbanibulin at 2 mg/kg/day was projected to be 236 ng×h/mL (based on 1.25 mg/kg b.i.d. oral dosing in Study 7709-102, which was 58 times greater than human exposure in the maximal use PK human study (Study KX01-AK-007).

Embryo-foetal development

Rats

A pilot DRF study investigated the toxicity of tirbanibulin in adult pregnant female Sprague-Dawley rats, embryos, and foetuses following once daily oral gavage from Gestation Day 6 up to Gestation Day 17, with scheduled termination at Gestation Day 21 (Study 321-0046-TX). Tirbanibulin was administered at doses of 0, 5, 10, and 15 mg/kg/day formulated in 3% (v/v) acetic acid in purified water. Treatment-related mortalities (5/8 females at 15 mg/kg/day, 5/8 females at 10 mg/kg/day, and 1/8 female at 5 mg/kg/day) occurred in this study on Gestation Days 9 to 17. Two females receiving 10 mg/kg/day and 1 receiving 15 mg/kg/day were not pregnant at the time of death. Abnormal soft/watery stool, decreased activity, and cold to touch were observed at 10 and 15 mg/kg/day. Abnormal respiratory sounds, soiled coat, and thin and unkempt appearance were observed at 5, 10, and 15 mg/kg/day. Treatment-related decreases in body weight and food consumption occurred, ranging from 29% to 33% and 25% to 61%, respectively. A 100% post-implantation loss (embryonic

resorptions and/or early resorptions) was observed in all pregnant female rats receiving 5, 10, or 15 mg/kg/day.

The pivotal GLP compliant embryo-foetal development in Sprague-Dawley rats administered oral tirbanibulin once daily from Gestation Day 6 to 17 at doses of 0, 0.5, 1.25, and 2.5 mg/kg/day (Study 321-0047-TX). Animals received oral gavage doses of tirbanibulin formulated in 3% (v/v) acetic acid in purified water. Decreased body weight (up to 17.3%) were observed at 2.5 mg/kg/day during late-gestation (Gestation Day 15 to 21), and decreased gravid uterine weight (16.0% and 45.3%, respectively) were noted at 1.25 and 2.5 mg/kg/day, when compared to the control. No changes occurred in food consumption and absolute gestation weight gain. Therefore, these changes were considered to be induced by decreased foetal body weight and were not considered as an indication of maternal toxicity. An increased number of embryo deaths (embryonic resorptions) and/or decreased numbers of live foetuses were observed at 1.25 and 2.5 mg/kg/day when compared to control. The post-implantation loss at 1.25 and 2.5 mg/kg/day (11.0% and 71.1%, respectively) was higher than in the control group (post-implantation loss of 2.6%).

Decreased foetal body weight (9.2% and 23.3% respectively) and foetal crown-rump length (4.1% and 11.3%, respectively) were observed at 1.25 and 2.5 mg/kg/day when compared to control. In addition, increased incidence of foetuses (25.2% and 48.7%, respectively) and incidence of litters (63.6% and 100%, respectively) with skeletal variations were observed at 1.25 and 2.5 mg/kg/day when compared to control (incidence of foetuses and litters of 6.9% and 21.7%, respectively).

Foetal external, visceral, and skeletal malformations were observed at 1.25 and 2.5 mg/kg/day. External malformations included absent pinna, malpositioned pinna, small pinna, absent eye bulge, small lower jaw, cleft upper jaw, cleft palate, digit misshapen (forepaw), cranial meningocele, exencephaly, meningo-encephalocele, hyperextension hind limb, malrotated hind limb, localised and generalised subcutaneous oedema, bent, hooked, misshapen and thread-like tail, absent anus, and omphalocele. Visceral malformations included dilated lateral ventricle, eye absent, and absent lens. Skeletal malformations included split basisphenoid; absent, branched, misshapen and fused cervical arch; absent cervical vertebra; fused exoccipital and frontal, fused and misshapen lumbar arch; absent lumbar vertebra, supernumerary lumbar vertebra; absent rib, fused rib; misshapen scapula; absent sternebra, fused sternebra; and absent, misshapen, and fused thoracic arch.

There were no treatment-related changes in mortality, clinical observations, absolute gestation weight gain, food consumption, macroscopic observations, litter data (the number of corpora lutea, implantation sites, foetal death, pre-implantation loss), and sex ratio in the study. The NOAEL of tirbanibulin for maternal toxicity was considered to be 2.5 mg/kg/day. The mean C_{max} and AUC_{0-8h} on Gestation Day 17 following 2.5 mg/kg/day were 95.7 ng/mL and 417 ng×h/mL, respectively. The NOAEL of tirbanibulin for embryo-foetal developmental toxicity was considered to be 0.5 mg/kg/day. The mean C_{max} and AUC_{0-24h} on Gestation Day 17 following 0.5 mg/kg/day were 25.4 ng/mL and 90.2 ng×h/mL, respectively.

Rabbits

A non-GLP, pilot DRF study investigated the toxicity of tirbanibulin (KX2-391, administered as free base) on pregnant female New Zealand White (NZW) rabbits and embryo and foetuses following once daily oral administration of tirbanibulin at 0, 1, 3, and 6 mg/kg/day formulated in 3% (v/v) acetic acid in purified water from Gestation Day 6 up to Gestation Day 18 (321-0048-TX). Two of the 8 females in the control group, 1/8 female in the low-dose group, 1/8 female in the mid-dose group, and 2/8 females in the high-dose group mated but did not result in pregnancy. The other surviving females were observed to be pregnant. Three of the 8 high-dose animals were found dead or moribund euthanised (Gestation Day 17 to Gestation Day 21). Prior to death, decreased defaecation, inappetence, thin appearance, and/or white ears, body weight loss, and decreased food consumption

(up to 79%) were observed. Treatment-related and adverse external malformation (the first digit absent in forepaw) was observed at 3 mg/kg/day. Increased, but not statistically significant, post-implantation losses (30.6% and 28.6%, respectively) were observed at 1 and 3 mg/kg/day, when compared to the control (post-implantation loss is 2.6%). The NOAEL of tirbanibulin for maternal toxicity in rabbits was considered to be 3 mg/kg/day. The NOAEL of tirbanibulin for embryo-foetus developmental toxicity in rabbits was considered to be <1 mg/kg/day.

The definitive GLP-compliant embryo-foetal development study of tirbanibulin used doses of 0, 0.3, 1, and 3 mg/kg formulated in 3% (v/v) acetic acid in purified water in NZW rabbits when administered once daily orally from Gestation Day 6 to 18. In the Main Study animals, 4/25 females at control, 4/25 females at low-dose group, 3/25 females at mid-dose group, and 4/25 females at high-dose group were inseminated but this did not result in pregnancy.

There were no treatment-related changes on body weights, macroscopic observations, gravid uterine weight, the number of corpora lutea, implantation sites, viable foetuses, non-viable embryo-foetuses, placental examinations, pre- and post-implantation loss, and sex ratio following any dose concentrations in this study. One high-dose group animal at 3 mg/kg/day was found dead on Gestation Day 20. Before death, this animal was noted with moderate inappetence, decreased defecation, thin appearance, soft stool, and red materials in pan. No visible gross lesions were observed at necropsy. The reason for this death was undetermined but the mortality cannot be excluded as treatment related.

Inappetence and decreased defecation was observed in all groups. The incidence of inappetence and decreased defecation was much higher in the test article group than in control, and these clinical signs were considered treatment-related and non-adverse. Slight decreases (up to 25%, without statistical significance) in daily food consumption were noted at the high-dose group (3 mg/kg/day) during the dosing phase (Gestation Days 6 to 7, 9 to 10, 12 to 13, and 15 to 16). Slight and significant decreases in foetal weight (10.6%) and foetal crown-rump length (3.8%) were noted at 3 mg/kg/day, when compared to the control. Decreased foetal weight and crown-rump length correlated with external malformations of first digit absent in forepaw and increased skeletal variations or malformation.

Treatment-related and adverse malformations were noted following 3 mg/kg/day. External malformations (the first digit absent in forepaw) were observed in 23 of 161 foetuses in 8/19 litters. Visceral malformations (including right testis absent, lung lobe absent, gallbladder absent, right kidney and right ureter absent, dilated aorta, aortic arch, ductus arteriosus, and fused lung lobe) were observed in 6 of 161 foetuses in 4/19 litters. Treatment-related skeletal malformations and variations were noted following 3 mg/kg/day tirbanibulin. Skull skeletal malformations (fused frontal, nasal, premaxilla, or supernumerary parietal) were observed in 2 of 72 foetuses in 1/18 litters. Skull skeletal variations (hole in parietal) were observed in 2 of 72 foetuses in 2/18 litters. Unossified or incomplete ossified forepaw phalanx and unossified metacarpal (skeletal variations) was observed in 43 of 138 foetuses in 13/19 litters. Fused sternebra (skeletal malformation) were observed in 4 of 138 foetuses in 3/19 litters. Thoracic vertebra hemivertebra (skeletal malformation, at 10th thoracic vertebra) and branched ribs (skeletal malformation) were observed in 1 foetus, and fused ribs (skeletal malformation) were observed in 1 foetus at 3 mg/kg/day.

No treatment-related developmental abnormalities or effects occurred following administration of 0.3 or 1 mg/kg/day tirbanibulin. The NOAEL of tirbanibulin for maternal and embryo-foetus developmental toxicity in rabbits was considered to be 1 mg/kg/day. The mean C_{max} and $AUC_{0\text{-}24h}$ on Gestation Day 18 following 1 mg/kg/day were 144 ng/mL and 266 ng×h/mL, respectively.

Prenatal and postnatal development, including maternal function

A GLP-compliant study evaluated the effects of tirbanibulin on pregnant and lactating female Sprague-Dawley rats and on the development of the offspring and determined the TK of tirbanibulin, when administered once daily via oral gavage at 0, 0.5, 1.25, or 2.5 mg/kg (in 3% glacial acetic acid in reverse osmosis water) from implantation through weaning. Maternal toxicity (F_0) was assessed based on mortality, clinical observations, body weight, food consumption, and necropsy and caesarean section findings. Toxicity was assessed in the F_1 offspring based on clinical observations, body weight, food consumption, developmental landmark data, and neurobehavioral data. In adulthood, oestrous cyclicity was assessed in F_1 females. Additionally, F_1 females were mated with non-sibling F_1 males of the same dose group and were then sacrificed on Gestation Day 13 for assessment of reproductive indices, macroscopic observations, and caesarean section parameters.

Tirbanibulin-related early group sacrifice occurred on Lactation Day 8, 9, 10, 11, or 14 for dams administered 2.5 mg/kg/day tirbanibulin. Animals administered 2.5 mg/kg/day tirbanibulin were removed from the study due to high incidences of failure to produce a viable litter resulting in an insufficient group size for data evaluation; as such, these deaths were considered treatment related. Pups from these litters were sacrificed as they had not yet reached Postnatal Day 18 and would not have survived without the dam. No other tirbanibulin-related mortality occurred.

Adverse tirbanibulin-related reductions in body weight were noted during gestation for dams administered 2.5 mg/kg/day. Statistically significant reductions in body weight during gestation for animals of this group were likely due to the lack of viable foetuses noted at the Gestation Day 24 necropsy. Additionally, adverse tirbanibulin-related effects on natural delivery and litter indices were noted for animals administered 2.5 mg/kg/day. Slight treatment-related reductions (by 11%, compared with controls) in pup survival (live pups/litters with live pups) were observed from birth to Postnatal Day 4 for the group administered 2.5 mg/kg/day.

No tirbanibulin-related observations were noted in F0 animals administered 0.5 or 1.25 mg/kg/day during gestation or lactation. Audible respiration was noted for all groups during gestation, with the highest incidence in controls. Observations of audible and/or irregular respiration were also noted for all groups during lactation. Since these observations were noted at a high incidence in controls, they were attributed to the acetic acid content in the vehicle control article and were considered not treatment related. No remarkable effect on mean body weight, body weight gain, food consumption, delivery indices, or pup survival during gestation or lactation was noted in F0 animals administered 0.5 or 1.25 mg/kg/day tirbanibulin. No remarkable macroscopic observations were noted for any dose concentration at the scheduled necropsy.

No tirbanibulin-related clinical observations or mortality was observed for the F1 generation for groups administered 0.5 or 1.25 mg/kg/day, and no remarkable observations were noted at scheduled sacrifice. No tirbanibulin-related effects on spatial learning and memory were observed during neurobehavioral tests (locomotor activity, acoustic startle, or Morris Water Maze). During F1 maturation, the number of oestrous cycles for groups administered ≥0.5 mg/kg/day tirbanibulin was significantly reduced compared with controls and was considered treatment-related but not adverse as the number cycles in tirbanibulin-treated groups was within historical control data. Although corresponding reductions in mating and fertility indices and the number of pregnant females in these groups were noted, these indices did not reach statistical significance, compared with controls, and did not appear to impact mating, fecundity, or fertility indices or pregnancy outcome.

Toxicokinetic data

A summary of toxicokinetic parameters from the repeat dose toxicity studies is presented below.

Table 15: Steady-state exposure multiples at non-clinical systemic NOAEL relative to exposure at the proposed clinical dose for mean C_{max} (0.258 ng/mL) and mean AUC_{0-24h} (4.09 ng.h/mL) from Study KX01-AK-007 (MUsT)

Study ID	Daily Dose % or mg/kg	C_{max} (ng/mL)		AUC (ng.h/ml)		Animal:Human XXX Exposure Multiple			
						Cmax		AUC	
		♂	♀	♂	♀	♂	♀	♂	♀
5-day dermal GLP rat (392-0053-TX)	Control	0	0	0	0	-	-	-	-
	0.1%	48.1	130	306	657	186.4	503.9	74.8	160.6
	0.5%	348	583	1910	3260	1348.8	2259.7	467.0	797.1
	1%	398	486	2590	3310	1542.6	1883.7	633.3	809.3
28-day dermal GLP rat (321-0032-TX)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.1%	22.9	33.7	205	216	88.8	130.6	50.1	52.8
	1%	405	439	1660	1620	1569.8	1701.6	405.9	396.1
	2%	-	-	-	-	-	-	-	-
3-month dermal GLP rat (78539)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.1%	21.9	50.8	87.1	155	84.9	196.9	21.3	37.9
	0.5%	166	198	480	696	643.4	767.4	117.4	170.2
	1%	92	246	508	699	356.6	953.5	124.2	170.9
5-day dermal GLP minipig (392-0054-TX)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.1%	0.891	0.27	8.49	4.55	3.5	1.0	2.1	1.1
	0.5%	1.17	2.62	22.4	32.8	4.5	10.2	5.5	8.0
	1%	6.95	3.99	100	45.8	26.9	15.5	24.4	11.2
28-day dermal GLP minipig (321-0033-TX)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.1%	2.03	7.25	37.4	65.5	7.9	28.1	9.1	16.0
	1%	31.3	47.7	445	548	121.3	184.9	108.8	134.0
	2%	30.1	34.2	401	407	116.7	132.6	98.0	99.5
3-month dermal GLP minipig (78537)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.1%	0.239	0.325	4.25	6.45	0.9	1.3	1.0	1.6
	0.5%	1.75	1.84	34.2	35.3	6.8	7.1	8.4	8.6
	1%	3.60	5.79	68.7	95.1	14.0	22.4	16.8	23.3
28-day oral GLP rat (7709-102)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	1.25/0.625	41.7	59.3	295	374	161.6	229.8	72.1	91.4
	2.5/1.25	131	94.7	846	620	507.8	367.1	206.8	151.6
	5/2	170	173	1229	1206	658.9	670.5	300.5	294.9
28-day oral GLP dog (7709-103)		♂	♀	♂	♀	♂	♀	♂	♀
	Control	0	0	0	0	-	-	-	-
	0.25	33.8	31	298	266	131.0	120.2	72.9	65.0
	0.5	67.4	79.2	545	629	261.2	307.0	133.3	153.8
	5/2	151	141	1090	1263	585.3	546.5	266.5	308.8

Local Tolerance

Dermal Irritation Studies

Rats

The dermal irritancy potential of tirbanibulin ointment was evaluated on rats' skin following a single IV injection or 6 days of repeated dermal (topical) application in adult Sprague-Dawley rats in a non-GLP study (Study PSA13080020). Rats received b.i.d. applications of ointment base or tirbanibulin 1% ointment, respectively, for 5 days and a single application on Day 6. After each application, the dosing

area was covered with 1 layer of absorbent gauze secured by elastic wrap. Approximately 4 h after each dosing, the elastic wrap, tape, and absorbent gauze were removed, and residual test article or control ointment base was removed by serial washing with water/75% ethanol/propylene glycol. At the end of the study, a section of skin from the application area from animals was collected and prepared for histopathology evaluation. Histopathology examination determined minimal to mild multifocal hyperplasia in stratum spinosum of the epidermis in all animals in Groups 2 (ointment base) and 3 (1% tirbanibulin) and minimal focal round cell infiltration of the epidermis in Group 3.

Rabbits

A non-GLP study evaluated the dermal irritancy potential of tirbanibulin ointment (0.1, 0.25, 0.5 and 1%) and ointment base (propylene glycol) on intact rabbit skin after 8 days of repeated dermal application (Study PSA13100016). After each application, the dosing area (designated as 14×15 cm, about 10% BSA) was covered with 1 layer of absorbent gauze secured by non-irritating tape. The dermal irritation responses were determined and scored according to the Draize Score tables. In Draize scoring, most of the test animals showed slight to moderate erythema and oedema with a dose-dependent increase in severity. The general histopathological evaluation of the treatment sites showed that the test article showed some concentration of irritation as evidenced by epidermal thickening, cellular infiltration, and focal degeneration necrosis that also indicated a dose-response pattern of increasing severity with increase in dose. Based on these results, the test article and control article (tirbanibulin ointment and ointment base) administered to the NZW rabbits by repeated dermal application for 8 consecutive days caused slight to moderate irritation on the intact skin in vivo, with a dose-related trend in the severity of the irritation.

A non-GLP study evaluated the dermal irritancy potential of tirbanibulin ointment on intact rabbit skin and the TK following dermal application (Study PSA14010020). Tirbanibulin ointment was applied at levels of 0.005, 0.01, or 0.05 %. Each animal received repeated dermal application (b.i.d., 4 h/time, with a 4-h interval between treatments) for 14 consecutive days except Group 5 (no treatment control group) animals. For each application, the dosing area (designated as 14×15 cm, about 10% BSA) was covered with one layer of absorbent gauze and fixed by non-irritating tape. Most of the observed Draize scores were grade 0. The histopathological examination did not reveal any significant signs of inflammation, haemorrhage, or necrosis in test article treatment sites. Based on these results, tirbanibulin ointment, administered to the NZW rabbits by repeated dermal application for 14 consecutive days at different doses (up to 0.05% b.i.d.) did not cause dermal irritation on the intact skin in vivo. The CIIs calculated were 0, 0, 0.07, and 0.1 following tirbanibulin doses of 0.005, 0.01, or 0.05 % ointment b.i.d., respectively. The CII of the no treatment control group was 0.

Ocular Irritation

A GLP-compliant study evaluated the acute ocular toxicity of tirbanibulin ointment following a single ocular administration to male NZW rabbits (Study 4103-13-830). Tirbanibulin 1% ointment was administrated to the left eye of each animal once at a dose volume of 0.1 g/eye. The untreated right eye of each rabbit served as a control. After 24 h of treatment, the left eye of each rabbit was rinsed with saline. During ocular lesion evaluation in the treated eye, slight conjunctiva redness was noted in 3/3 rabbits from 1 h up to 3 days post-dose and slight to mild conjunctiva chemosis was observed in 3/3 rabbits at 1 h post-dose. No abnormalities were noted in the cornea or iris. The results indicated that tirbanibulin 1% ointment was an irritant in the rabbit ocular irritation assay. The irritation cleared within 3 days post-dose.

A GLP study evaluated the ocular irritation potential of tirbanibulin 1% ointment in an ECVAM-validated EpiOcular tissue model (OECD Test Guideline 492) (Study 031-19). This study tested tirbanibulin in the to be marketed tirbanibulin ointment base formulation (90% [w/w] Propylene Glycol USP and 10% [w/w] mono- and diglycerides NF). Fifty µL of each test (ointment base) and control article (water as

negative control, methyl acetate as positive control) were delivered to the surface of the tissue, followed by an incubation for 30 min, a 12-min soak to remove any excess test or control article, and a further 2-h incubation period without test or control article. The tissue was further incubated for 3 h with a dye (MTT reagent). Viability >60% of the negative control is non-irritating while viability ≤60% of the negative control is considered irritating. The positive control demonstrated irritating potential, whereas tirbanibulin 1% ointment did not display any irritation potential, as measured by cell viability compared to the cell viability after application of the negative control.

Other toxicity studies

Antigenicity

A GLP-compliant study evaluated the contact allergenic potential of tirbanibulin ointment using the mouse local lymph node assay (LLNA) (Study 8301030). Tirbanibulin ointment at concentrations of 0.01, 0.1, and 1.0%, or tirbanibulin ointment base (vehicle) at a concentration of 0% were administered to 5 mice/dose group. Tirbanibulin ointment was applied to the dorsal aspect of the ears of the mice to evaluate contact allergenic potential based on the induction of lymphocyte proliferation from the auricular lymph nodes. Mice were dosed once daily for 3 consecutive days. Following the 3-day dosing period and a subsequent 2 days without treatment, a single IV injection of [^{3}H]thymidine was then administered to each mouse. Approximately 5 h following the [^{3}H]thymidine injection, mice were sacrificed, and the auricular lymph nodes were collected intact and pooled for each individual animal. all animals in the 25% (v/v) hexylcinnamaldehyde positive control group exhibited slight erythema (corresponding to a score of 1) on both ears between Days 2 and 5. There were no observations noted for the mice in the other treatment groups. The mean SI value for the hexylcinnamaldehyde positive control was 6.4. Exposure of animals to tirbanibulin ointment at 0.01, 0.1, and 1% (w/w) resulted in mean SI values of 1.2, 0.8, and 3.1, respectively. The differences in mean dpm values in mice treated with high dose, tirbanibulin ointment 1% were statistically significant when compared with the corresponding tirbanibulin ointment vehicle group (2910 vs. 939 mean dpm, $p \leq 0.05$). Acceptance criteria for identification of a test article as a contact sensitiser in the LLNA included an SI of 3.0 or greater at any dose concentration, statistically significant differences from control values, and evidence of a dose response. Tirbanibulin 1% ointment met 2 of those criteria.

A GLP-compliant study following the principles of the Buehler test evaluated the skin sensitisation potential of tirbanibulin ointment following epidermal application to Guinea pigs (Study 44103-13-837). Tirbanibulin ointment was administered as a topical dose to the flanks of Guinea pigs to evaluate skin sensitisation potential. For induction, neomycin sulphate (Group 1), ointment base (vehicle) (Group 2) or tirbanibulin 1% and 4% ointment (Groups 3 and 4, respectively) were administered via test patch to the left flank dosing areas (3×3 cm) for approximately 6 h/dose on Days 1, 7, and 14. For the challenge on Day 27, neomycin sulphate (Group 1), tirbanibulin ointment base (vehicle) (Group 2), or tirbanibulin 1% and 4% ointment (Groups 3 and 4, respectively) were administered via test patch to the right flank dosing areas (3×3 cm) for approximately 6 h. Following the induction doses, slight irritation, oedema, and/or erythema were noted in all Guinea pigs in the positive control and tirbanibulin 1% and 4% ointment dose groups, following the second and third induction doses. Following the challenge dose, slight oedema and/or erythema was noted in 2 positive control (neomycin sulphate) animals, 4 tirbanibulin 1% ointment animals, and 5 tirbanibulin 4% ointment animals 48 h post-dose.

Metabolites

No dedicated metabolic toxicity studies were performed.

Impurities

The specification for the drug product only contains a single impurity, Impurity C, above the ICH Q3B qualification threshold. The drug lots used for dermal studies in rats and minipigs of 5 and 28-days duration were spiked with levels of Impurity C ≥ 4.1%. In all instances based on the NOAEL for the study the data suggested that the margin of exposure was at 16-fold that at which patients will be exposed to. In particular, considering the similarities of the skin of minipigs to humans, the calculated margins of exposure were 1004 for the 5-day study and 89-fold for the 28-day study. Therefore, the proposed drug product specification of 2% for Impurity C is considered qualified from a toxicological perspective.

Phototoxicity

A GLP-compliant study evaluated the phototoxicity of tirbanibulin (KX01) following a single dermal application and UVA (365 nm) irradiation in NZW rabbits (Study 44103-13-831). Tirbanibulin 1% ointment, vehicle (ointment base), or 0.1% Methoxsalen solution (8-Mop, positive control; 0.1 mL/site) were applied to each of 2 dorsal dose application sites/animal. Following 15 min of drying post-application, 1 of each of the 2 application sites was exposed to UVA irradiation (365 nm) at 10 J/cm^2 for a duration of 40 to 50 min.

No mortalities were noted in this study. There were no treatment-related effects on clinical observations, body weight, or body weight changes. Sites treated with the positive control, 0.1% Methoxsalen solution, with UVA exposure demonstrated moderate to severe erythema and mild to severe oedema beginning 24 h post-UVA exposure, which had not resolved 72 h post-UVA exposure in all 6 rabbits. In contrast, there were no abnormalities noted in any other application sites following application and UVA exposure.

2.3.5. Ecotoxicity/environmental risk assessment

Summary of main study results

Substance (INN/Invented Name): Tirbanibulin			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 (LogD assay)	<3.1	Potential PBT (N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0028	μg/L	> 0.01 threshold (N)

Tirbanibulin PEC $_{surfacewater}$ value is below the action limit of 0.01 μg/L. and is not a PBT substance as log K_{ow} does not exceed 4.5. Therefore, tirbanibulin is not expected to pose a risk to the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

The molecular mechanism of how tirbanibulin binds to tubulin resulting in the induction of cell cycle arrest have been sufficiently elucidated. The provided *in vitro* studies have shown that tirbanibulin binds to tubulin, inhibiting its polymerisation and resulting in the disruption of the microtubule network, which leads to G2/M cell cycle arrest. Additionally, tirbanibulin induces apoptosis through the activation of both intrinsic and extrinsic pathways and has potent anti-proliferative activity against a variety of cancer cell lines, including melanoma, SCC, and multi-drug resistant cancer cell lines.

Tirbanibulin induces cell growth inhibition and cell death more potently in rapidly dividing cells. The effects on Src kinase signalling are ambiguous, with the weight of evidence suggesting that any effects on this signalling pathway are indirect and a result of microtubule disruption. Of particular relevance, considering the indication for the treatment of actinic keratosis of the face and scalp, is the data from the immortalised keratinocyte cell line, CCD-1106 KERTr. The more rapidly dividing keratinocytes were more susceptible to the cell growth inhibitory effects of tirbanibulin. This could potentially suggest that the keratinocytes in the lesions of patients may be more susceptible to the effects of tirbanibulin than any surrounding keratinocytes.

Although the *in vivo* studies are limited to the investigation of tirbanibulin in xenograft tumour models, there is sufficient rationale and non-clinical evidence to support its potential for efficacy in AK patients.

No safety pharmacology studies were performed using topical administration. Oral gavage dosing was used for the CNS studies and for the investigations on the GI system while IV administration was used for the cardiovascular and respiratory study. Statistically significant shortening of the PR, QRS and QTc intervals were seen. The changes seen were deemed to be within an acceptable physiological range. Considering the differences in the route of administration and the fact that no ECG changes were seen following dermal application in minipigs in the repeat toxicity studies, these observations are not considered clinically relevant. In the GI safety pharmacology studies no effect was seen on gastric emptying, however, motility was statistically increased in the lower GI tract with both oral and subcutaneous administration. The differences measured were small and considering the topical nature of application unlikely to be of any clinical significance for the current application.

Considering the topical nature of the treatment the absence of pharmacodynamic drug interaction studies is considered appropriate.

Pharmacokinetics

All methods and limits of quantification were adequate regarding specificity and sensitivity to support the kinetic analyses of tirbanibulin.

The PK profile has been examined in the nonclinical species used for the toxicity studies. The absorption following dermal application was higher in rat compared to the minipig, however, the minipig is typically seen as a better model and more reflective of the absorption in humans. Considering the clinical route of administration, the most relevant study in rats using dermal application with a 1% ointment suggested low bioavailability of 1.59% after 24 h. The metabolic profile of the nonclinical species is reflective of that seen in humans and there are no metabolites in humans that are not present at similar or higher levels in the nonclinical species.

The mass balance study in rats revealed that the main route of excretion of tirbanibulin was via biliary excretion into the faeces with urinary excretion as a minor route and no differences between the sexes.

The potential for drug-drug interactions was investigated using the standard battery of *in vitro* studies looking at CYP enzyme inhibition and induction as well as the potential for tirbanibulin to be an inhibitor or substrate for the various transporters. A time dependent inhibition was seen for CYP2C9, CYP2C19 and CYP3A4/5 by tirbanibulin. The inhibition of CYP2C9 and CYP2C19 was reversible whilst the inhibition of CYP3A4/5 was irreversible. Considering the maximum clinically measured levels of ~ 2.5 nM for tirbanibulin, these findings do not lead to any concern for inhibition of CYP2C9, CYP 2C19 or CYP3A4/5 in the clinical setting. Tirbanibulin was identified as an inhibitor of MATE1, MATE2-K, OATP1B1, OATP1B3, OCT1, and OCT2. The lowest calculated IC_{50} was 1.4 μM for OCT2 which, considering the previously mentioned maximum clinically measured levels of ~ 2.5 nM for tirbanibulin, the clinical relevance can be considered low.

Given the route of administration (topical), the dual route of CYP metabolism, the low daily dose of tirbanibulin (up to 2.5 mg), the short duration of dosing (5 days), and the low systemic exposure (subnanomolar mean Cmax), it is considered unlikely that co-administration of strong CYP3A4/5 or CYP2C8 inhibitors will adversely affect systemic tirbanibulin exposure.

The potential for drug-drug interactions has been adequately addressed in vitro and any potential interactions are unlikely considering the measured inhibition constants and the low levels of systemic exposure seen after clinical use (see SmPC section 4.5).

Toxicology

Several of the toxicology studies were conducted at a site located in a country which is neither an OECD nor OECD-MAD member. This includes two of the genotoxicity studies (Mouse lymphoma TK gene mutation assay [Study 7709-113]; Bone marrow micronucleus test in rat [Study 321-0044-GT]) and three of the reproductive toxicity studies (Study 321-0034-TX in rat and Studies 321-0047-TX and 321-0049-TX in rats and rabbits). Therefore, the claimed GLP status of these studies is not accepted. However, it should be noted that three additional genotoxicity studies were conducted in an OECD country (Ames study [Study 7709-112]; Chromosomal aberrations in CHO cells [Study 7709-113]; Micronucleus and Comet assay in rats [Study 54715.00103]) as well as a reproductive toxicology study (Study 8379929). In addition, the most relevant repeat dose toxicity study in minipigs with 5 days dermal administration followed by 23 days off treatment for a total of 4 cycles was performed in an OECD country. The aforementioned studies did not show any irregularities or signs of GLP non-compliance and based on inspections from an EU GLP monitoring authority there is evidence that this facility conducts studies in compliance with the OECD GLP regulations. Therefore, although none of the non-GLP studies are considered pivotal to the assessment, they can be accepted as supportive for the risk assessment.

Concerning the single dose toxicity studies, the proposed studies did not allow to determinate the lethal or sub-lethal dose or the maximum tolerated dose (MTD) for tirbanibulin. However, as outlined in ICH M3(R1) such studies are not required. The acute toxicity can be inferred from the observations in the repeat dose studies in rats after oral and dermal administration. In these studies, mortality was typically seen after between 6 to 8 days dosing and the systemic exposure measured was always > 1,000 ng.h/mL. Tirbanibulin is to be marketed in sachets containing at least 250 mg of tirbanibulin 10 mg/g ointment, i.e. 2.5 mg tirbanibulin, intended for topical administration. Even when applying the complete sachet topically, systemic exposure is estimated to be 140-fold lower (7 ng.h/mL) than the doses associated with mortality. Even if a patient would inadvertently ingest a whole sachet, systemic exposure is estimated to be 50-fold lower than the doses associated with mortality.

Significant toxicities have been seen, including mortalities, at high levels of systemic exposure. The toxicities were consistent across species and in line with the pharmacology of the compound as a microtubule disruptor. The tissues where toxicity was observed contained rapidly dividing cell populations. In the pivotal dermal toxicity studies in minipigs following a similar posology to that of the proposed indication there was no systemic toxicity evident with only local site reactions which is similar to that seen with clinical use. The systemic exposure at the NOAEL in this study was at least 9-fold of that measured clinically.

Epithelial cell hyperplasia was seen at the dermal application site which is likely indicative of cell regeneration. The hyperplasia was observed in tissues which undergo a high turnover rate and is likely secondary to degeneration of epithelial tissues following apoptotic and necrotic cell death induced by the pharmacological action of tirbanibulin. In the dermal study in minipigs the dermal hyperplasia observed was attributable to the action of tirbanibulin inducing apoptosis of the basal cell layer of the epidermis and the subsequent reparative hyperplasia to replace the layer of epidermis. The findings almost completely reversed after recovery and it is hypothesised that if this recovery time was

extended total recovery would be likely. The findings in relation to the dermal hyperplasia occurred in animals with healthy skin as a direct result of the pharmacological activity of tirbanibulin inducing cell death of healthy keratinocytes. In the context of treatment of actinic keratosis, the same pharmacological activity results in cell death of keratinocytes undergoing uncontrolled proliferation.

There was a slightly higher incidence of treatment-emergent skin cancers with tirbanibulin (see clinical section) and the potential of local tumours arising from the topical use of tirbanibulin, however, the weight of evidence provided suggest that risk is low. Nevertheless, local skin tumours in the treatment area will be monitored as an important potential risk, including an imposed PASS, which is considered appropriate to address any concerns for potential effects in the treatment area where exposure levels will be highest.

The results of the Bacterial Reverse Mutation Assay with a confirmatory assay indicated that under the conditions of this study, tirbanibulin is not mutagenic.

No carcinogenicity studies were submitted. Considering that duration of treatment is limited to five days carcinogenicity studies would not be expected and therefore, the absence of carcinogenicity studies is considered acceptable.

In embryo-foetal development studies in rats and rabbits, embryonic and foetal toxicity, including foetal malformations, occurred at multiples of 22 times and 65 times greater than human exposure in the maximal use pharmacokinetic human study. In a pre- and postnatal development study in rats, reductions in fertility and increased embryo-foetal lethality were seen in the offspring of treated females.

In a fertility and early embryonic development study in rats, decrease in testes weight which correlated with decreased sperm count, decreased sperm motility, increased incidences of abnormal sperm, and increased incidence of degeneration of the seminiferous epithelium, considered indicative of male fertility toxicity, occurred at multiples of 58 times greater than human exposure in the maximal use pharmacokinetic human study. However, there were no changes in male mating or fertility indices.

Tirbanibulin was a threshold based genotoxin, with a margin of exposure of at least 22-fold based on AUC and 174-fold based on C_{max} levels from the dose levels in which no genotoxic events were seen and which is based on a GLP compliant study performed in an OECD country. Moreover, it is likely the observed genotoxicity was a result of the induction of apoptosis within the same cells. The effects of reproductive toxicity are reflected in the SmPC. Tirbanibulin has been demonstrated to be both teratogenic and genotoxic and there is therefore potential for genetic damage at the level of the germ cells and/or conceptus. However, as already discussed there is a threshold for the genotoxic effects seen. Comparing C_{max} margins of exposure, which is most appropriate for this type of effects for an indirect acting clastogenic compound, a margin of 174-fold exists relative to the levels measured clinically following dermal application. Therefore, based on the totality of the data (short duration of exposure with systemic exposure in patients at a margin of exposure 174-fold lower than the NOAEL (based upon Cmax) for genotoxicity in vivo) it can be considered acceptable to not include the contraceptive measures in the SmPC of the recently published "Response from SWP to CMDh questions regarding Genotoxicity and Contraception" (EMA/CHMP/SWP/74077/2020).

Tirbanibulin ointment is not recommended during pregnancy and in women of childbearing potential not using contraception. A risk to the newborns/infants cannot be excluded. Consideration should be given on whether to discontinue breast-feeding or to discontinue/abstain from tirbanibulin ointment therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman.

Concerning immunotoxicity effects, a primary pharmacology study on tirbanibulin treatment showed a significant induction of IL-1α, and a moderate induction of IL-8 in cultures of the human keratinocyte

cell line CCD-1106 KERTr. However, as previously discussed these are related to the pharmacological actions of tirbanibulin inducing cell death. Whilst some evidence of immunosuppression, including hypocellularity of the bone marrow, were seen in the repeat dose toxicity studies at high exposure levels, no such effects have been seen clinically and are likely related to the very low systemic exposure seen with topical use.

Local tolerance studies showed that tirbanibulin ointment is a moderate contact sensitiser to the skin (Buehler assay in guinea pigs and in murine LLNA test). Dermal irritation tests in rats and in rabbits were positive and the severity of dermal findings increased with increases in dose, but this was not confirmed in humans (see clinical safety).

No dedicated metabolic toxicity studies were performed which is acceptable. Systemic exposure to the metabolites, KX2-5036 and KX2-5163, were measured in the repeat dose toxicity study in rats and minipigs after dermal application. The results of these suggest that exposures well in excess of that seen clinically have been achieved and therefore, these can be considered qualified from a nonclinical perspective.

Based on the findings of Study 44103-13-831, tirbanibulin 1% ointment demonstrated no phototoxicity.

2.3.7. Conclusion on the non-clinical aspects

Tirbanibulin disrupts microtubules by direct binding to tubulin, which induces cell cycle arrest and apoptotic death of proliferating cells and is associated with disruption of Src tyrosine kinase signalling.

Non-clinical data revealed no special hazard for humans based on conventional studies of safety pharmacology and repeated dose toxicity.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

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

- Tabular overview of clinical studies

Study ID Number of sites Location(s) CSR status	Study type	Study objective(s)	Study design	Key inclusion criteria	Sample size Gender Age	Treatment
KX01-AK-01-US 1 site US Complete Section 5.3.3.2, Synopsis and Final CSR	PK	Safety, PK, efficacy	Phase I, non-randomised, uncontrolled, open-label study	Patients with AK on the dorsal forearms 4 to 8 AK lesions in an area of 25 cm^2 or 8 to 16 AK lesions in an area of 100 cm^2	30 total 19 M, 11 F 47 to 79 yrs	Tirbanibulin 1% ointment, 50 mg over 25 cm^2 area or 200 mg over 100 cm^2 area, once daily for 3 or 5 days
KX01-AK-007 2 sites US Complete Section 5.3.3.2, Synopsis and Final CSR	PK	PK under maximal use conditions	Phase I, non-randomised, uncontrolled, open-label study	Patients with AK on the face or scalp, 4 to 8 AK lesions in an area of 25 cm^2	18 total 15 M, 3 F 43 to 83 yrs	Tirbanibulin 1% ointment, over 25 cm^2 area once daily for 5 days
KX01-AK-003 31 sites US Complete Section 5.3.5.1, Synopsis and Final CSR	Efficacy, Safety	Efficacy, safety	Phase III, randomised, double blind, vehicle-controlled, parallel-group, multi-centre study	Patients with AK on the face or scalp, 4 to 8 AK lesions in an area of 25 cm^2	351 total 301 M, 50 F 45 to 96 yrs	Tirbanibulin 1% ointment or vehicle over 25 cm^2 area once daily for 5 days
KX01-AK-004 31 sites US Complete Section 5.3.5.1, Synopsis and Final CSR	Efficacy, Safety	Efficacy, safety	Phase III, randomised, double blind, vehicle-controlled, parallel-group, multi-centre study	Patients with AK on the face or scalp, 4 to 8 AK lesions in an area of 25 cm^2	351 total 308 M, 43 F 46 to 92 yrs	Tirbanibulin 1% ointment or vehicle over 25 cm^2 area once daily for 5 days
KX01-AK-002 16 sites US Complete Section 5.3.5.2, Synopsis and Final CSR	Efficacy, Safety, PK	Efficacy, safety, PK	Phase IIa, non-randomised, uncontrolled, open-label study	Patients with AK on the face or scalp, 4 to 8 AK lesions in an area of 25 cm^2	168 total 148 M, 20 F 45 to 90 yrs	Tirbanibulin 1% ointment, 50 mg over 25 cm^2 area once daily for 3 days or 5 days
KX01-AK-006 1 site US Complete Section 5.3.5.4, Synopsis and Final CSR	Dermal Safety	Sensitizing potential	Phase I, randomised, controlled, evaluator-blinded, within-subject comparison study	Healthy subjects	261 total 57 M, 204 F 18 to 75 yrs	Tirbanibulin 1% ointment or placebo under open patch conditions 3 times a week for 3 weeks for induction followed by 1 challenge
KX01-AK-008 1 site US Complete Section 5.3.5.4, Synopsis and Final CSR	Dermal Safety	Phototoxic potential	Phase I, randomised, double-blind, controlled, within-subject comparison study	Healthy subjects	31 total 7 M, 24 F 21 to 70 yrs	Tirbanibulin 1% ointment or placebo, single dose plus/minus ultraviolet irradiation
KX01-AK-009 1 site US Complete Section 5.3.5.4, Synopsis and Final CSR	Dermal Safety	Photoallergic potential	Phase I, randomised, double-blind, controlled, within-subject comparison study	Healthy subjects	64 total 7 M, 57 F 26 to 75 yrs	Tirbanibulin 1% ointment or placebo under open patch conditions 2 times a week with irradiation for induction; plus 1 challenge with irradiation
KX01-AK-010 1 site US Complete Section 5.3.5.4, Synopsis and Final CSR	Dermal Safety	Local tolerability after repeated applications under occlusive, semi-occlusive, and open patch conditions	Phase I, randomised, controlled, evaluator-blinded, within-subject comparison study	Healthy subjects	36 total 9 M, 27 F 25 to 73 yrs	Tirbanibulin 1% ointment or placebo under occlusive, semi-occlusive, and open patch conditions, 3 times a week for 3 weeks

AK=actinic keratosis; F=female; ID=identifier; M=male; PK=pharmacokinetic; US=United States; yrs=years.

2.4.2. Pharmacokinetics

All clinical PK data were derived from 3 clinical pharmacology studies conducted in patients with actinic keratosis (AK) to determine tirbanibulin PK parameters following topical application of tirbanibulin 1% ointment (KX01-AK-01-US, KX01-AK-002 and KX01-AK-007).

Two formulations of tirbanibulin ointment were used during non-clinical and clinical development: Formulation I and the to-be-marketed formulation (TBM) formulation of tirbanibulin 1% ointment. Formulation I was used in non-clinical studies and early Phase I and Phase II clinical studies. The TBM formulation was used in the pivotal non-clinical 5-day toxicology studies and the clinical MUsT PK, Phase I dermal safety, and pivotal Phase III studies.

Bioanalytical Methods

Method Validation

Methods for the determination of tirbanibulin (KX2-391), KX2-5036 and KX2-5163 were validated.

Method 07047 (version M03 and M04) was utilised during clinical studies KX01-AK-01-US and KX01-AK-002. This method utilises a liquid-liquid extraction procedure to isolate KX2-391 from 0.1 mL of human plasma, and a reverse-phase HPLC column to separate KX2-391 and the internal standard (IS) from the matrix. The LC-MS/MS instrument was in positive electrospray ionisation-multiple reaction monitoring (ESI-MRM) mode to quantitate KX2-391 and the associated (IS). Initially, method 07047 was developed using a structural analogue of KX2-391 as the IS, but this was later changed to an isotopically labelled form of KX2-391, KX2-391-d4 (KX2-3472), during subsequent amendments.

Due to the limited sensitivity of method to detect KX2-391 in clinical samples (0.1 ng/mL), the new method was developed to provide a better characterisation of the PK profile of KX2-391 (0.01 ng/mL) during study KX01-AK-007. The method utilises a Supported-Liquid Extraction (SLE) procedure to extract the analyte from K_2EDTA human plasma, a reverse-phase HPLC column to elute KX2-391 and the IS, and an LC-MS/MS instrument with positive ESI-MRM mode for quantification.

An additional method was developed to assess metabolites KX2-5036 and KX2-5163 during study KX01-AK-007. Similar to methods for assessment of KX2-391, a reversed-phase HPLC column was used to elute KX2-5036 and KX2-5163 from plasma, and a LC-MS/MS instrument for quantification.

Bioanalysis of Samples

During the analysis of participant samples, spiked CS and QC standards were extracted to permit the determination of the concentration of KX2-391, KX2-5036 and KX2-5163, in addition to the assessment of accuracy and precision. All samples were analysed within the established long-term stability range. Incurred sample reanalysis (ISR) was performed for KX2-391 during KX01-AK-01-US and KX01-AK-007, but not KX01-AK-002 due to the number of samples at LLOQ or below the limit of quantification (BLQ). Moreover, ISR was not performed for KX2-5036 and KX2-5163 during KX01-AK-007 given the majority of samples near LLOQ or BLQ.

Absorption

The PK data from studies using Formulation I (KX01-AK-01-US and KX01-AK-002) were uninterpretable or incomplete. Therefore, an assessment of bioequivalence based on clinical PK data comparing Formulation I and the TBM formulation was not possible. Comparison of formulations and manufacturing methods for tirbanibulin 1% ointment was done through an assessment of *in vitro* release rates (*in vitro* release testing [IVRT]) and *in vitro* permeability testing (IVPT).

IVRT demonstrated that the clinical formulations used during development and intended for marketing have comparable release characteristics. IVRT also demonstrated the comparability of drug products manufactured at different facilities and formulated with drug substance from different manufacturers (detailed in the Quality section).

The *in vitro* skin penetration study TER-158-17.00 was conducted to determine the penetration profiles of tirbanibulin into and through *ex vivo* human cadaver skin using vertical diffusion cells. The average cumulative amount of drug in the media after 24 hours of application was 14,451 ng and was comparable across all 4 different formulations tested. The data indicated that drug permeating through the skin into the media, in general, starts at around 2 hours after application and appears to plateau after 10 hours for most formulations. The maximum average flux of tirbanibulin occurs between 8 and 10 hours after application. The drug concentrations achieved in the epidermis and dermis showed that tirbanibulin penetrates through the different layers of the skin.

Distribution

The protein binding of tirbanibulin and KX2-5036 in human plasma, as determined by equilibrium dialysis, was approximately 88% (Study XBL17622) and 57% (Study C18092), respectively.

Elimination

In vitro, tirbanibulin was mainly metabolised by CYP3A4/5 and, to a lesser extent, by CYP2C8 (Study XBL08671).

In vitro studies, together with an *in vivo* radiolabelled study in rats, identified 6 primary metabolites. Two metabolites were identified as possible drug-related metabolites in humans. One metabolite (KX2-5036), a pyridine acetamide metabolite of tirbanibulin, was identified as a potentially abundant human metabolite because it was the most abundant metabolite in human hepatocytes. Another metabolite (KX2-5163), a pyridine acetic acid metabolite of tirbanibulin, was also identified because it was the most abundant metabolite in both rat and dog plasma.

A metabolite scan of pooled plasma from Study KX01-AK-007 was performed to identify metabolites by high resolution mass spectrometry (Study C19017). The low peak intensities in the scan prevented structural confirmation of metabolites and suggested the metabolites identified were present at very low concentrations in human plasma. All metabolites were the result of dealkylation, hydrolysis, oxidation, and/or hydroxylation and reflected the metabolic pathways observed in the non-clinical studies. In addition to KX2-5036, 2 other metabolites (KX2-5180 and M477/1) were identified by the scan. The low peak intensity in the scan suggested that these 2 metabolites were present at very low concentrations in human plasma.

The structurally confirmed human metabolites (KX2-5136, KX2-5036, and KX2-5180) were shown to have no or little anti-proliferative activity in a keratinocytes cell growth assay with metabolite half-maximal growth inhibition concentrations ≥300-fold that of tirbanibulin (Study ATNXUS-KX01-001).

Pharmacokinetics of metabolites

In the maximal use study KX01-AK-007, plasma concentrations for the main metabolites KX2-5036 and KX2-5163 were below the LLOQ (0.05 ng/mL for both metabolites) for all collected samples in 14 and 13 of the 18 included subjects, respectively. The metabolite concentrations, which were only within 2-fold of the LLOQ, were inadequate for estimating PK parameters except Cmax.

In 3 of 4 patients who had complete Day 5 profiles with at least 1 of the 2 metabolites, the metabolite concentrations were only a fraction of the parent tirbanibulin concentrations, and in the fourth patient,

both metabolites were slightly lower than the parent concentration. The highest observed individual plasma concentration of tirbanibulin, KX2-5036 and KX2-5163 were 1.09 ng/mL (0.598 nM), 0.0925 ng/mL (0.27 nM), and 0.121 ng/mL (0.35 nM), respectively.

Pharmacokinetics in the target population

Study KX01-AK-01-US

This was a Phase I, single-centre, uncontrolled, open-label study to evaluate the safety, tolerability, and PK of tirbanibulin 1% ointment following topical application to the dorsal forearm in adult patients with AK.

The study enrolled 30 patients and was divided into 4 cohorts:

- Tirbanibulin 1% ointment, topical, 2 mg/cm^2 (50 mg ointment; 0.5 mg of tirbanibulin) on a 25 cm^2 area with 4 to 8 clinically typical AK lesions on the dorsal forearm, daily for 3 consecutive days in Cohort 1 (n=4) and daily for 5 consecutive days in Cohort 3 (n=8).
- Tirbanibulin 1% ointment, topical, 2 mg/cm^2 (200 mg ointment; 2.0 mg of tirbanibulin) on a 100 cm^2 area with 8 to 16 clinically typical AK lesions on the dorsal forearm, daily for 3 consecutive days in Cohort 2 (n=10) and daily for 5 consecutive days in Cohort 4 (n=8).

Of the 30 patients enrolled, 6 (20%) had no samples with measurable tirbanibulin concentrations, and 24 (80%) had at least 1 post-dose sample with measurable tirbanibulin concentrations. Several patients had intermittently elevated tirbanibulin plasma concentrations followed by low or BLQ values. These aberrant values may be the result of collecting PK samples from the same arm where the treatment was applied. Since the study protocol did not specify that samples were to be drawn from the opposite arm, the study site drew samples based on ease of venous access and patient preference. Based on these findings, the PK results were not interpretable and PK parameters were not estimated.

Study KX01-AK-002

This was a Phase IIa, open-label, non-randomised, uncontrolled study to evaluate the activity, safety, and PK of tirbanibulin 1% ointment in adult patients with AK on the face or scalp.

Study treatment consisted of topical application of tirbanibulin 1% ointment at a dose of 2 mg/cm^2 (50 mg ointment; 0.5 mg of tirbanibulin) on a 25 cm^2 area with 4 to 8 clinically typical AK lesions on the face or scalp, once daily for 5 days (Cohort 1, n=84) or 3 days (Cohort 2, n=84).

In Cohort 1, only 15 of 336 (4%) samples analysed had tirbanibulin plasma concentrations above the LLOQ on Day 1 following the initial dose. Of the pre-dose samples on Day 5, 8 of 84 (10%) patients had quantifiable tirbanibulin plasma concentrations. Of the 4-hour post-dose samples on Day 5, measurable plasma concentrations were detected in 41 of 83 (49%) patients (range: 0.108 to 0.576 ng/mL).

In Cohort 2, only 33 of 332 (10%) samples analysed had tirbanibulin plasma concentrations above the LLOQ on Day 1 following the initial dose. Of the pre-dose samples on Day 3, 14 of 84 (17%) patients had quantifiable tirbanibulin plasma concentrations. At 4 hours post-dose on Day 3 measurable plasma concentrations were detected in 45 of 83 (54%) patients (range: 0.101 to 1.420 ng/mL).

All measurable plasma concentrations were below 1.5 ng/mL, except for 3 aberrant samples. The aberrant samples were re-analysed, and cross-contamination was suspected. The maximum individual plasma concentration (excluding the 3 aberrant values) across both cohorts and all days of PK

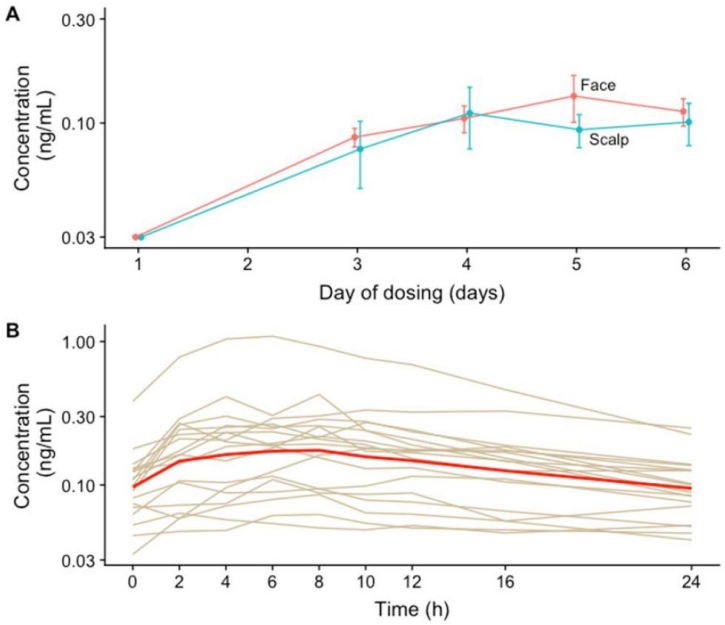
sampling was 1.42 ng/mL at 4 hours post-dose on Day 3. Based on the low number of patients with tirbanibulin concentrations above the LLOQ and amenable to PK analysis, and the fact that samples were only obtained up to 4 hours post-dose, PK parameters were not determined.

Study KX01-AK-007 (MUsT)

This was a Phase I, open-label, uncontrolled, non-randomised, parallel group, maximal usage trial (MUsT) to evaluate the safety and PK of tirbanibulin 1% ointment in adults with actinic keratosis (AK) lesions on the face or scalp.

Tirbanibulin 1% ointment was applied under maximal use conditions, defined as the application of a small amount (total dose up to 250 mg from a single-dose unit) of ointment over 25 cm^2 of the face (n=9) or balding scalp (n=9) that contains at least 6 AK lesions (severe conditions), once daily for 5 consecutive days. Study treatment was self-administered once daily, using the single-dose sachet to mimic clinical usage. The sachets were weighed after use to determine the dose administered.

All subjects completed 5 days of treatment. The average tirbanibulin dose applied was 137mg (45 mg), ~55% of the full dose possible (250 mg) and the average dose applied was similar in the face and scalp groups. All patients had measurable but low tirbanibulin concentrations at daily trough (pre-dose) sampling times and post-dose sampling times on Day 5. The pre-dose concentration (Ctrough) data demonstrated that steady state was achieved following the third dose (72 hours) of 5 daily doses (see figure below).



Daily tirbanibulin trough concentrations and Day 5 PK parameters of maximum observed concentration (Cmax) and area under the concentration-time curve (AUC) from time 0 to 24 hours (AUC0-24h) generated by non-compartmental analysis are summarised in the table below. All patients had peak concentrations lower than 0.428 ng/mL following the Day 5 dose, except for 1 patient, who had a peak concentration of 1.09 ng/mL.

Treatment Group	Day:	1	3	4	5	6	5		
	Descriptive Statistics	KX2-391 Trough Concentration (ng/mL)					C_{max} (ng/mL)	t_{max} (h)	AUC_{24h} (h*ng/mL)
Face (N = 9)	Mean	0.00	0.0861	0.105	0.133	0.111	0.340	-	5.0
	SD		0.0250	0.0444	0.0969	0.0476	0.297	-	3.9
	CV%	-	29.0	42.4	72.9	43.1	87.4	-	78.7
	Median	0.00	0.0771	0.0918	0.108	0.0879	0.270	6.0	4.04
	Min	0.00	0.0588	0.0571	0.0625	0.0636	0.109	2.0	1.78
	Max	0.00	0.124	0.190	0.383	0.219	1.09	9.8	15.0
Scalp (N = 9)	Mean	0.00	0.0760	0.111	0.0932	0.099	0.176	-	3.18
	SD		0.0778	0.105	0.0487	0.0677	0.102	-	1.92
	CV%	-	102.3	94.7	52.2	68.1	57.8	-	60.4
	Median	0.00	0.0391	0.0760	0.0812	0.102	0.179	7.8	3.3
	Min	0.00	0.0232	0.0273	0.0329	0.0413	0.0623	2.0	1.2
	Max	0.00	0.271	0.368	0.179	0.254	0.333	10.0	6.7
Combined (N = 18)	Mean	0.00	0.0811	0.1078	0.1131	0.105	0.258	-	4.09
	SD	-	0.0563	0.0782	0.0772	0.0571	0.231	-	3.15
	CV%	-	69	73	68	54	90	-	77
	Median	0.00	0.0718	0.0806	0.1033	0.0949	0.234	6.91	3.86
	Min	0.00	0.0232	0.0273	0.0329	0.0413	0.0623	2.0	1.2
	Max	0.00	0.271	0.368	0.383	0.254	1.09	10.0	15.0

The terminal elimination half-life, apparent clearance, and apparent volume of distribution of tirbanibulin and its metabolites could not be accurately estimated or were not applicable, due to the low systemic exposure.

There was a trend towards a lower Cmax and AUC0-24h on Day 5 for patients with scalp treatment (~49% in Cmax and ~32% in AUC) (table below). This trend remained after excluding 1 outlier patient.

Table 9: ANCOVA Statistical Comparison of Face and Scalp KX2-391 PK Exposure Parameters

Test (T)	Reference (R)	Parameter	Test (T) GeoLSM	Ref (R) GeoLSM	GMR% (T/R)	CI90% Lower, Upper
Face	Scalp	AUC_{0-24}	3.82	2.90	131.75	86.32, 201.08
(n=9)	(n=9)	C_{max}	0.24	0.16	149.47	96.99, 230.32

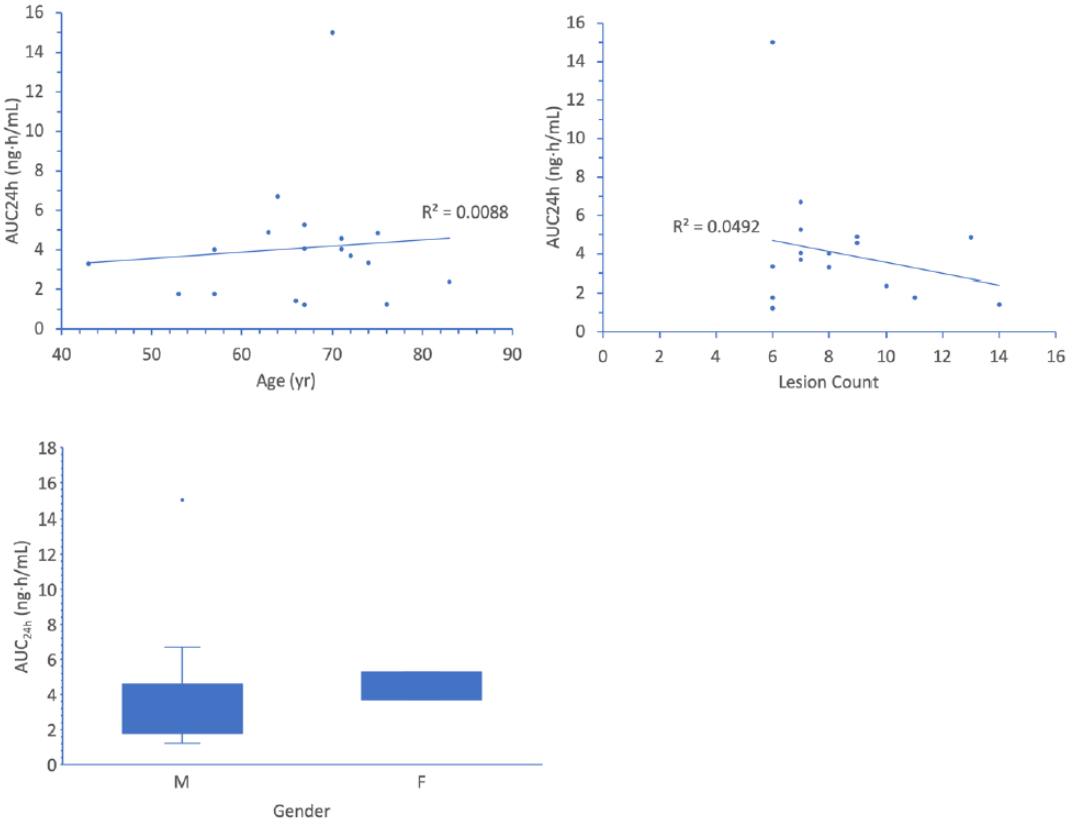
Dose proportionality and time dependencies

No data were provided.

Special populations

Dedicated clinical studies in patients with renal or hepatic impairment were not conducted.

In the maximal use study (MUsT) KX01-AK-007, Intrinsic factors (gender, age and lesion counts) did not show an apparent effect on exposure (below figure). The effect of ethnicity was not evaluated because all patients were White and predominantly not Hispanic or Latino.



Pharmacokinetic interaction studies

In the MUsT clinical study KX01-AK-007, the tirbanibulin Cmax was 0.258 ng/mL (0.598 nM) and the highest individual plasma concentration was 1.09 ng/mL (2.53 nM). Based on the observed plasma protein binding of tirbanibulin in human plasma of approximately 88%, the maximum free plasma concentration after topical application was approximately 0.13 ng/mL.

Considering the low estimated maximum free plasma concentration of 0.13 ng/mL tirbanibulin after topical application in the MUsT clinical Study KX01-AK-007:

- The potential for tirbanibulin to affect concomitant medications through direct inhibition of CYP3A4/5 enzymes in clinical use is low, as the lowest observed IC50 value for CYP inhibition was 35.2 µM.
- There is no potential for tirbanibulin to affect concomitant medications through the induction of CYP enzymes, as tirbanibulin showed no induction potential for CYP1A2, 2B6, or 3A4 at a concentration of up to 1 µM (431.5 ng/mL).

In the MUsT clinical study (Study KX01-AK-007), the highest observed plasma concentration for metabolite KX2-5036 was 0.09257 ng/mL (0.2785 nM) and the free fraction of KX2-5036 in human plasma at 0.01 µM was 0.45 (Study C18092). Based on the observed plasma protein binding of KX2-5036 in human plasma of approximately 57%, the maximum free plasma concentration of KX2-5036 following dermal application was <0.05 ng/mL. Altogether, these data indicate that KX2-5036 has no potential to affect concomitant medication at the level of the CYP enzymes or membrane transporters at systemically achieved exposures of tirbanibulin at the proposed clinical dose regimen.

Pharmacokinetics using human biomaterials

In vitro, tirbanibulin was mainly metabolised by CYP3A4 and, to a lesser extent, by CYP2C8.

In terms of metabolites, the DDI potential for KX2-5036 was studied *in vitro*. Results showed that KX2-5036 did not inhibit CYP enzymes directly or through TDI at 100 µM, did not induce CYP1A2, 2B6, and 3A4 in human hepatocytes at 3 µM, and KX2-5036 was not a substrate for MDR1, BCRP, MRP2, BSEP, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2. KX2-5036 showed <50% inhibition on MDR1, BCRP, MRP2, BSEP, MATE1, OAT1, OAT3, OATP1B1, OATP1B3 and OCT1 at concentrations of up to 100 µM.

Metabolite KX2-5163 was not tested for CYP inhibition, CYP induction, and CYP transporter potential because this metabolite was not found in human hepatocytes. The maximum concentration observed in the MUsT PK study was 0.121 ng/mL (0.35 nM) and were lower than tirbanibulin.

2.4.3. Pharmacodynamics

Mechanism of action

The mechanism of action of tirbanibulin involves induction of p53, and a subsequent G2/M cell cycle arrest, leading to inhibition of cell proliferation. In vitro, tirbanibulin has been tested in human keratinocytes and several melanoma cell lines. In these cells, a GI50 value of < 50 nM has been established, suggesting potent inhibition of cell proliferation and promotion of apoptosis are likely to occur in the epidermal and dermal skin layers following topical application of tirbanibulin.

Primary and Secondary pharmacology

No clinical pharmacodynamic endpoints were included in the clinical studies, and no concentration-effect was established in non-clinical and clinical studies.

2.4.4. Discussion on clinical pharmacology

Bioanalytical Methods

Certificates of analysis for reference standards were provided. A statement on GLP compliance, in addition to a quality assurance regarding adherence to the study protocol and SOPs were also provided.

In general, all validation reports provide sufficient data pertaining to the appropriateness of each method. Originally, method 07047 was established using an analogue of KX2-391 as the internal standard, but this was later changed to an isotopically labelled form of KX2-391 (KX2-391-d4). Ideally, a cross-validation study should have been performed directly comparing the performance of the method using each internal standard to analysis a common set of QC samples. Ultimately, 07047 was replaced by 18141-M01 due to insufficient sensitivity.

The bioanalytical assessment reports exhibit acceptable calibration curve and QC sample performance. Each analytical run included QC, blank and zero samples, respectively. All samples were analysed within the established long-term storage period.

ISR was performed for KX2-391 during KX01-AK-01-US and KX01-AK-007, but not KX01-AK-002 due to the number of samples at or below the limit of quantification (BLQ). Similarly, ISR was not performed for KX2-5036 and KX2-5163. Due to the limited sensitivity of method 07047 and 19052-

M01 to determine plasma concentrations of KX2-391, KX2-5036 and KX2-5163 in clinical samples, any ISR data provides no meaningful information on method reproducibility for the associated clinical studies.

ISR for KX01-AK-007 was performed outside of established long-term stability for method 18141-M01 (i.e. 252 vs 194 days), however, results were still within acceptable limits.

Pharmacokinetics

All clinical PK data were derived from 3 clinical pharmacology studies (KX01-AK-01-US, KX01-AK-002 and KX01-AK-007) conducted in patients with actinic keratosis (AK) to determine tirbanibulin PK parameters following topical application of tirbanibulin 1% ointment.

Two formulations of tirbanibulin ointment were used during non-clinical and clinical development: Formulation I and the to-be-marketed formulation (TBM) formulation of tirbanibulin 1% ointment. Comparison of formulations for tirbanibulin 1% ointment was done through an assessment of *in vitro* release rates (*in vitro* release testing [IVRT], detailed in Quality section) and *in vitro* permeability testing (IVPT). The *in vitro* skin permeability study (TER-158-17.00) showed tirbanibulin, in a 1% ointment formulation, permeates into human (cadaver) skin layers, while a fraction permeates through the skin.

The PK data from KX01-AK-01-US and Studies KX01-AK-002 were limited by an assay that was not sensitive (LLOQ: 0.1 ng/mL), and a large percentage of samples (51% to 81% after the final dose) with tirbanibulin plasma concentrations below the LLOQ. Both studies used an early drug product (Formulation I) and provided incomplete PK information. Furthermore, Study KX01-AK-01-US, enrolled patients with AK on the dorsal forearm, which is not representative of the intended clinical population with AK on the face and scalp. Despite this, plasma exposure data indicated that tirbanibulin is minimally absorbed following 3 or 5 days of treatment with tirbanibulin 1% ointment. In Study KX01-AK-002, the maximum observed individual plasma concentration of tirbanibulin across cohorts and all days of PK sampling was 1.420 ng/mL.

The maximal usage Study KX01-AK-007 was the only study that determined PK parameters for tirbanibulin 1% ointment following topical application once daily for 5 consecutive days. This study utilised a more sensitive assay (LLOQ: 0.01 ng/mL) and all patients' tirbanibulin plasma concentrations could be measured. Steady-state tirbanibulin plasma concentrations were reached after the third dose. AUC0-24h and Cmax were somewhat lower for scalp application compared with face, which could be due to potential difference in dermal structure of the two sites. Key PK values confirm low systemic exposure with subnanomolar plasma concentrations for both parent drug and its metabolites. The overall mean Cmax for tirbanibulin was 0.258 ng/mL (0.598 nM), and AUC0-24h of 4.09 ng·h/mL and the highest observed individual plasma concentration was 1.09 ng/mL (2.53 nM) following the final Day 5 treatment to the face. Large inter-subject variability was observed, which was considered to be mainly due to one subject with the highest concentration.

Study KX01-AK-007 confirmed the very low plasma concentrations for metabolites KX2-5036 and KX2-5163 after topical application of tirbanibulin 1% ointment under maximal use conditions. Concentrations of the two metabolites were below the limit of quantitation in the majority of patients, and when measurable, were only a fraction of the parent tirbanibulin concentration in human plasma. Considering that after 5 elimination half-lives the amount of tirbanibulin in the body is negligible, it is expected that 5 days after last treatment no tirbanibulin will be available in plasma.

In the maximal use Study KX01-AK-007, the intrinsic factors age, sex, and AK lesion count had no apparent effect on the PK of tirbanibulin in the small sample size studied. It should be noted that participants included in this study were of an age that is comparable with the patient population to be treated (range 43-83 years) and had comorbidities typical of the target population. No formal studies

of tirbanibulin ointment in patients with hepatic or renal impairment have been conducted. Due to the low systemic exposure to tirbanibulin after topical application of tirbanibulin ointment once daily for 5 days, changes in hepatic or renal function are unlikely to have any effect on the elimination of tirbanibulin. Therefore, no dose adjustments are considered needed.

Pharmacodynamics

No clinical pharmacodynamic endpoints were included in the clinical studies, and no concentration-effect was established in non-clinical and clinical studies. This is acceptable since tirbanibulin is applied topically and exerts its effects locally.

The lack of a dedicated QT/QTc study was endorsed in previous Scientific Advice, since no signal arose either from hERG and telemetered dog non-clinical studies, nor from the completed clinical studies, including oral administration with tirbanibulin exposures ~100-fold higher than those following 5-days of topical administration.

In the safety pharmacology evaluation of tirbanibulin, the hERG IC_{50} was found to be 44 uM (approximately 20 µg/mL), which is over 500-fold higher than the highest human plasma concentration observed following topical administration. The majority of the plasma concentrations from the clinical trials (KX01-AK-01-US, KX01-AK-002 and KX01-US-007) were below 2 ng/mL or below the LLOQ. No electrocardiographic effects occurred in a nonclinical cardiovascular safety study of tirbanibulin up to 15 mg/kg IV in telemetered dogs. Therefore, tirbanibulin appears to have a low risk of QT prolongation.

In the maximal use Study KX01-AK-007, there was no apparent association between steady-state Ctrough and time-matched QTcF or ΔQTcF in 18 subjects. Since the highest Ctrough was greater than mean Cmax, the Ctrough-QT has some value even though the ECG was not measured at Tmax.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology is considered sufficiently characterised.

2.5. Clinical efficacy

2.5.1. Dose response studies

Study KX01-AK-01-US: A phase 1, single-center, safety, tolerability, and pharmacokinetic, study of KX2-391 ointment 1.0% in subjects with actinic keratosis

Study KX01-AK-01-US was a single-centre, open-label, safety, tolerability, and PK study of KX2 391 ointment 1.0% administered topically to the dorsal forearm of subjects with AK. The study was conducted in 3 periods: a screening/Baseline Period, a treatment period, and a follow-up period. A total of 32 subjects were planned to be enrolled; 30 subjects were enrolled and treated.

Study population

Eligible subjects were adults with clinically typical AK lesions on the dorsal forearm: **4 to 8 lesions** in a contiguous area of **25 cm^2** for Cohorts 1 and 3 and **8 to 16 lesions** in a contiguous area of **100 cm^2** for Cohorts 2 and 4. Subjects should not have used prohibited medications including AK treatment or systemic immunomodulatory agents and cytotoxic agents. Women of child-bearing potential and men who were sexually active with female partners of childbearing potential must be using a highly effective method of contraception.

Treatments

KX2-391 Ointment 1%, 50 mg (25 cm^2 area) or 200 mg (100 cm^2 area), once daily for 3 or 5 days, topically applied.

Duration for Cohort 1 and Cohort 2:

- Planned subject participation period up to 73 days (up to 28 days screening period, 3 consecutive treatment days, 42 days follow-up period)

For Cohort 3 and Cohort 4:

- Planned subject participation period up to 73 days (up to 28 days screening period, 5 consecutive treatment days, 40 days follow-up period)

Primary Objectives:

- To assess the **safety** and **tolerability** of KX2-391 ointment 1.0% in subjects with actinic keratosis (AK)
- To assess the **pharmacokinetics** (PK) of KX2-391 ointment 1.0% in subjects with AK

Secondary Objective:

To evaluate the **activity** of KX2-391 ointment 1.0% administered topically to the dorsal forearm in subjects with AK

Study design

This study was conducted in 4 sequential cohorts:

- Cohort 1: N=4, KX2-391 ointment 1%, topical, 2 mg/cm^2 (50 mg of study medication; 0.5 mg of KX2-391) on a **25 cm^2 area** with 4-8 clinically typical AK lesions on the dorsal forearm, daily for **3 consecutive days**
- Cohort 2: N=10, KX2-391 ointment 1%, topical, 2 mg/cm^2 (200 mg of study medication; 2.0 mg of KX2-391) on a **100 cm^2 area** with 8-16 clinically typical AK lesions on the dorsal forearm, daily for **3 consecutive days**
- Cohort 3: N=8, KX2-391 ointment 1%, topical, 2 mg/cm^2 (50 mg of study medication; 0.5 mg of KX2-391) on a **25 cm^2 area** with 4-8 clinically typical AK lesions on the dorsal forearm, daily for **5 consecutive days**
- Cohort 4: N=8, KX2-391 ointment 1%, topical, 2 mg/cm^2 (200 mg of study medication; 2.0 mg of KX2-391) on a **100 cm^2 area** with 8-16 clinically typical AK lesions on the dorsal forearm, daily for **5 consecutive days**

All subjects were to be followed for 45 days (42 days post-dosing for the 3-day regimen and 40 days post-dosing for the 5-day regimen).

The study database was locked on 9th August 2017. The sample size chosen for this study was based on practical considerations for a pilot study and was not based on power calculations. It was considered that 32 subjects should allow a good estimate of dermal absorption. During the study 2 additional cohorts were added. Duration of dosing was increased from 3 to 5 days to see if further activity could be achieved, as in the original 3-day treatment cohorts, beneficial clinical activity had been noted. Of the 30 subjects enrolled and treated, 29 (97%) completed the study. Any protocol deviations were not considered to have affected the overall evaluation of activity or safety.

Results

Baseline characteristics

A total of 30 male and female adult subjects with AK were enrolled. Most subjects had a history of AK for >5 years. Subjects ranged in age from 47 to 79 years (median overall was 62.5 years); all but one were classified as white, with 19 males (63%) and 11 females (37%). Weight ranged from 100 to 305 pounds [median 197.5 pounds, (89.58 kg)].

Baseline AK lesions in Cohorts 1 and 3 (25 cm^2 treatment area) ranged from 4-6 lesions (median 5 and 6 lesions, respectively) and for Cohorts 2 and 4 (100 cm^2 treatment area) ranged from 8-16 (median 11.5 and 11.0 lesions, respectively).

One subject in cohort 2 had a concomitant medicine for AK in the study treatment area (Liquid nitrogen).

Eleven (37%) subjects had a history of basal cell carcinoma (BCC), 5 subjects (17%) previously had squamous cell carcinoma (SCC), and 1 subject had a history of melanoma. Thirteen subjects (43%) had some type of surgical procedure or Mohs surgery for the treatment of BCC, SCC, and/or melanoma.

Outcomes

Summary of median (min, max) AK lesion counts by cohort and visit

Activity AK Lesion Counts	Cohort 1 N=4	Cohort 2 N=10	Cohort 3 N=8	Cohort 4 N=8
Day 1 (Baseline)	5.0 (4, 5)	11.5 (8, 16)	6.0 (5, 6)	11.0 (10, 16)
Day 4	5.0 (4, 5)	9.0 (6, 16)	Not done[a]	Not done[a]
Day 10	5.0 (4, 5)	7.0 (6, 11)	5.0 (5, 6)	10.0 (10, 10)
Day 17	4.5 (3, 5)	7.0 (5, 15)	5.0 (4, 6)	10.0 (10, 11)
Day 31	3.5 (1, 5)	7.0 (1, 15)	1.0 (0. 6)	6.0 (0, 11)
Day 45	2.5 (0, 4)	4.0 (1, 10)	0.5 (0, 4)	4.5 (0, 12)

AK = actinic keratosis; Max = maximum, Min = minimum.
a: Lesion counts were not performed in these cohorts on this day, per protocol.
Cohort 1 = 25 cm^2 treatment area/3 consecutive treatment days/50 mg ointment administered.
Cohort 2 = 100 cm^2 treatment area/3 consecutive treatment days/200 mg ointment administered.
Cohort 3 = 25 cm^2 treatment area/5 consecutive treatment days/50 mg ointment administered.
Cohort 4 = 100 cm^2 treatment area/5 consecutive treatment days/200 mg ointment administered.

Secondary outcomes: AK lesion clearance by Day 45

Cohort	Subjects with 100% Clearance	Subjects with ≥75% - <100% Clearance	Total Number of Subjects n (%)
Cohort 1 (N=4)	01-08	01-02	2 (50)
Cohort 2 (N=10)[a]	0	01-19, 01-21, 01-34	3 (30)
Cohort 3 (N=8)	01-39, 01-43, 01-49, 01-64	01-51	5 (63)
Cohort 4 (N=8)	01-70	01-73, 01-74, 01-75	4 (50)

a: Subject 01-23 withdrew consent on Day 2 of treatment.
Cohort 1 = 25 cm^2 treatment area/3 consecutive treatment days/50 mg ointment administered.
Cohort 2 = 100 cm^2 treatment area/3 consecutive treatment days/200 mg ointment administered.
Cohort 3 = 25 cm^2 treatment area/5 consecutive treatment days/50 mg ointment administered.
Cohort 4 = 100 cm^2 treatment area/5 consecutive treatment days/200 mg ointment administered.

Activity was seen in all cohorts, with the most complete clearance (0 lesions) seen in Cohort 3 with 4 of 8 subjects (50%); other cohorts had at most, 1 subject who demonstrated complete clearance.

Study KX01-AK-002: A phase 2a, open-label, multicentre, activity and safety study of KX2-391 ointment 1% in subjects with actinic keratosis on the face or scalp

This Phase 2 study was a multicentre, non-randomised, open-label, activity, safety, tolerability, and PK study of KX2-391 ointment 1% administered topically to the face or scalp of subjects with AK in 2 sequential cohorts: a 5-day dosing regimen (Cohort 1) and a 3-day dosing regimen (Cohort 2).

The study consisted of screening, treatment (5 or 3 consecutive days), follow-up (up to Day 57), and recurrence follow-up periods (3, 6, 9 and 12 months post-Day 57).

Study population

The study population consisted of adults with stable, clinically typical AK. Subjects must have had a treatable AK area on the face or scalp that measured 25 cm^2 and contained between 4 and 8 AK lesions.

Treatments

A dose of 50 mg of KX2-391 ointment 1% was applied topically once daily for 5 consecutive days (Cohort 1) or for 3 consecutive days (Cohort 2) to the 25 cm^2 treatment area (2 mg/cm^2; 0.5 mg KX2-391 total). No comparator treatment was used in this study. No study treatments were administered in the recurrence follow-up period.

Key objectives

The primary objective of this study was:

- To evaluate the **activity** of KX2-391 Ointment 1% administered topically to the face or scalp in subjects with AK by determining complete response rate, defined as 100% clearance at Day 57

One of the secondary objectives of this study was:

- To assess **dose regimens** by contrasting 5-day treatment with 3-day treatment in terms of the activity and safety of KX2-391 Ointment 1% in subjects with AK on the face or scalp

Results

Baseline characteristics

A total of 168 subjects (84 subjects per cohort) were enrolled into the study. Subjects were primarily male: 76 (90%) in Cohort 1 and 72 (86%) in Cohort 2. The age range was 45 to 90 years and 51 to 89 years, respectively, with median ages of 70 and 67 years; 62 subjects (74%) and 53 subjects (63%) were aged ≥65 years. Race in all but 1 subject was classified as White.

Per protocol, all subjects entered with a range of 4 to 8 AK lesions; the median number of lesions was 6.0 in Cohort 1 and 5.0 in Cohort 2, with means of 5.8 and 5.4, respectively. Treatment location was on the face in 44 subjects (52%) in Cohort 1 and in 66 subjects (79%) in Cohort 2, and on the scalp in 40 subjects (48%) in Cohort 1 and 18 subjects (21%) in Cohort 2. Fitzpatrick skin type was balanced for Cohorts 1 and 2. 98% of subjects had Classic AK.

Prior medications were generally typical for an elderly population. There were no concomitant medications administered that were judged to have interfered with treatment activity.

Because study treatment was administered in a clinic setting, treatment compliance was 100% overall for subjects in each cohort and 100% at each study visit for each cohort.

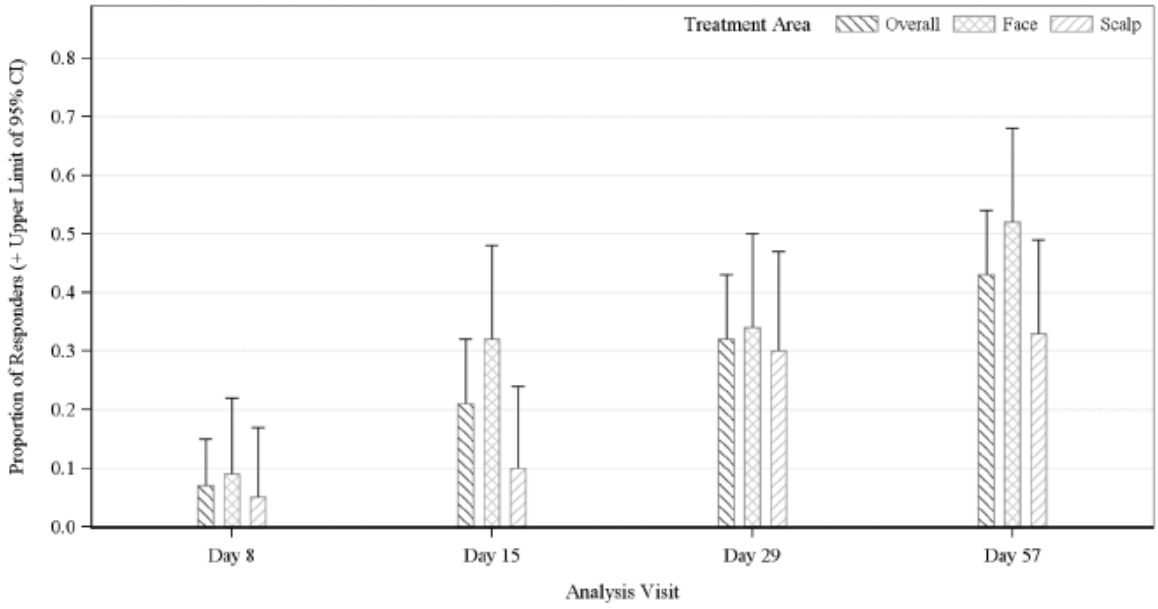
Outcomes

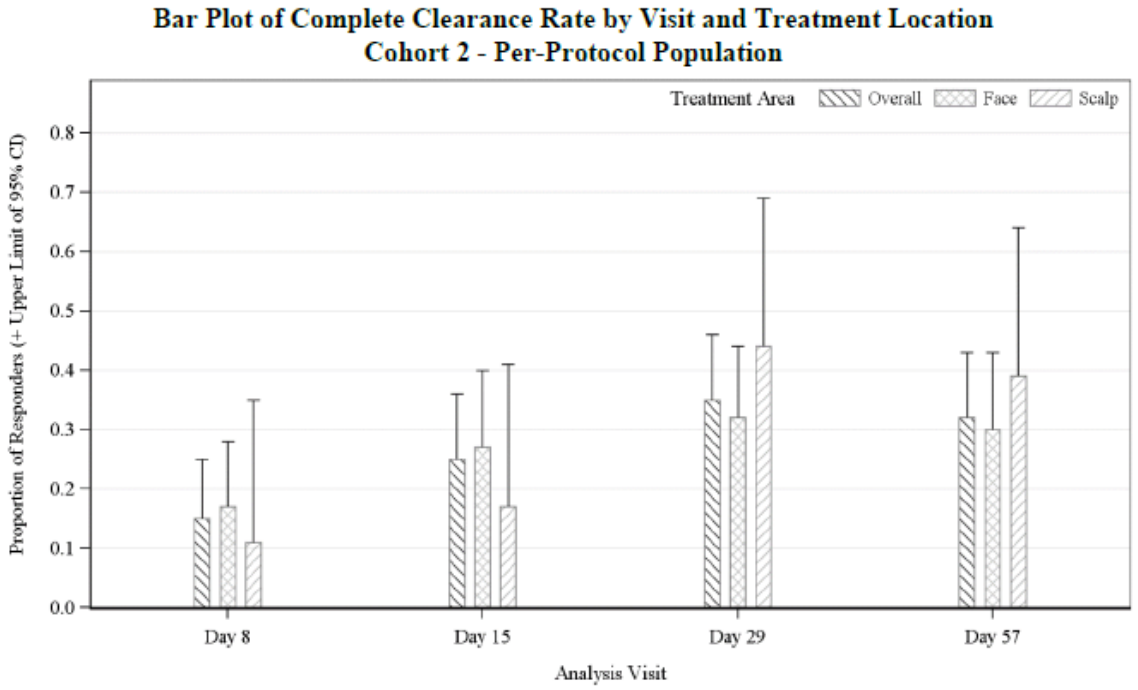
AK Clearance rate at Day 57 by treatment locations and duration (5 days versus 3 days)

	Cohort 1			Cohort 2		
Clearance Rate	**Total N=84**	**Face N=44**	**Scalp N=40**	**Total N=84**	**Face N=66**	**Scalp N=18**
	Proportion (95% Confidence Interval)					
100%	0.43 (0.32, 0.54)	0.52 (0.37, 0.68)	0.33 (0.19, 0.49)	0.32 (0.22, 0.43)	0.30 (0.20, 0.43)	0.39 (0.17, 0.64)
≥75%	0.56 (0.45, 0.67)	0.66 (0.50, 0.80)	0.45 (0.29, 0.62)	0.52 (0.41, 0.63)	0.53 (0.40, 0.65)	0.50 (0.26, 0.74)

Note: Evaluable Set = group of protocol-eligible subjects who received 5 days (Cohort 1) or 3 days (Cohort 2) of study treatment and completed Day 1 and Day 57 AK lesion evaluations.
Cohort 1 = 25 cm^2 treatment area; 5 consecutive treatment days.
Cohort 2 = 25 cm^2 treatment area; 3 consecutive treatment days.

Bar Plot of Complete Clearance Rate by Visit and Treatment Location
Cohort 1 - Per-Protocol Population





Actinic Keratosis Lesion Counts by Visit and Treatment Location prior to the Recurrence Follow-up Period: 5-day treatment – PPS

Treatment Location	Screening	Baseline	Day 8	Day 15	Day 29	Day 57
Face						
n	44	44	40	43	44	44
Mean (SD)	5.5 (1.35)	5.4 (1.31)	4.5 (2.51)	2.3 (2.04)	1.8 (1.71)	1.3 (1.94)
Median	5.0	5.0	4.5	3.0	2.0	0
Min, max	4, 8	4, 8	0, 10	0, 7	0, 6	0, 9
Scalp						
n	40	40	37	37	40	40
Mean (SD)	6.2 (1.48)	6.2 (1.39)	5.1 (2.75)	4.5 (2.96)	3.1 (2.63)	2.6 (2.49)
Median	6.0	6.0	5.0	5.0	3.5	2.0
Min, max	4, 8	4, 8	0, 9	0, 9	0, 8	0, 8
Overall						
n	84	84	77	80	84	84
Mean (SD)	5.8 (1.45)	5.8 (1.41)	4.8 (2.63)	3.3 (2.72)	2.4 (2.27)	1.9 (2.29)
Median	6.0	6.0	5.0	3.5	2.0	1.0
Min, max	4, 8	4, 8	0, 10	0, 9	0, 8	0, 9

Note: Values given are actual values.
Cohort 1 = 25 cm^2 treatment area; 5 consecutive treatment days.

Actinic Keratosis Lesion Counts by Visit and Treatment Location prior to the Recurrence Follow-up Period: 3-day treatment – PPS

Treatment Location	Screening	Baseline	Day 8	Day 15	Day 29	Day 57
Face						
n	66	66	55	64	66	66
Mean (SD)	5.4 (1.19)	5.4 (1.20)	3.7 (2.55)	2.8 (2.53)	2.3 (2.37)	2.0 (2.06)
Median	5.0	5.0	4.0	2.0	2.0	1.0
Min, max	4, 8	4, 8	0, 9	0, 9	0, 9	0, 7
Scalp						
n	18	18	17	18	18	18
Mean (SD)	5.3 (1.19)	5.3 (1.18)	4.0 (2.35)	3.1 (2.36)	2.1 (2.35)	1.6 (1.75)
Median	5.0	5.0	4.0	2.5	1.5	1.5
Min, max	4, 7	4, 7	0, 7	0, 7	0, 7	0, 6
Overall						
n	84	84	72	82	84	84
Mean (SD)	5.4 (1.18)	5.4 (1.19)	3.8 (2.49)	2.9 (2.48)	2.3 (2.35)	1.9 (1.99)
Median	5.0	5.0	4.0	2.0	2.0	1.0
Min, max	4, 8	4, 8	0, 9	0, 9	0, 9	0, 7

Note: Values given are actual values.

Cohort 2 = 25 cm^2 treatment area; 3 consecutive treatment days.

Recurrence Rates During the Recurrence Follow-up Period by Cohort, Treatment Location, and Visit – Recurrence Follow-up Set						
	Cohort 1			Cohort 2		
Visit	Total N=36	Face N=23	Scalp N=13	Total N=27	Face N=20	Scalp N=7
	Proportion (95% Confidence Interval)					
3 months	0.39 (0.25, 0.57)	0.30 (0.16, 0.53)	0.54 (0.30, 0.81)	0.41 (0.25, 0.61)	0.40 (0.22, 0.64)	0.43 (0.16, 0.83)
6 months	0.48 (0.33, 0.65)	0.40 (0.23, 0.63)	0.62 (0.37, 0.86)	0.45 (0.28, 0.65)	0.45 (0.27, 0.69)	0.43 (0.16, 0.83)
9 months	0.51 (0.35, 0.68)	0.40 (0.23, 0.63)	0.69 (0.45, 0.91)	0.50 (0.32, 0.70)	0.52 (0.32, 0.76)	0.43 (0.16, 0.83)
12 months	0.57 (0.41, 0.73)	0.40 (0.23, 0.63)	0.85 (0.61, 0.98)	0.70 (0.51, 0.87)	0.73 (0.50. 0.91)	0.62 (0.28, 0.94)
Recurrence Follow-up Set = group of subjects who achieved complete clearance at Day 57. Recurrence rates were estimated with a Kaplan-Meier method.						

KX2-391 was below the lower limit of quantification (LLOQ) of 0.1 ng/mL for the majority of plasma samples collected. At 4 hours after the last dose, about half of the subjects in both cohorts had quantifiable plasma concentrations. The maximum individual plasma concentration across both cohorts and all days of PK sampling did not surpass 2 ng/mL. Based on these findings and the limited number of plasma concentrations above the LLOQ, PK parameters were not determined.

2.5.2. Main studies

Studies KX01-AK-003 and KX01-AK-004

Both Phase III studies (Studies KX01-AK-003 and KX01-AK-004) were double-blind, randomised, vehicle-controlled, multi-centre Phase III studies and that evaluated the efficacy and safety of tirbanibulin 1% ointment when applied daily to a treatment area of 25 cm^2 for 5 consecutive days in

adults with AK on the face or scalp. These 2 studies were identical in terms of study design, patient entry criteria, and assessments, and were independently conducted at non-overlapping study sites.

Enrolment was controlled so that approximately two thirds of subjects enrolled will be treated on the face and approximately one third of subjects enrolled will be treated on the scalp. A sufficient number of subjects will be screened to randomize approximately 300 subjects. At each site, a minimum of 10 subjects and a maximum of 20 subjects were projected to be enrolled.

An overview of the study design is presented in the figure below.

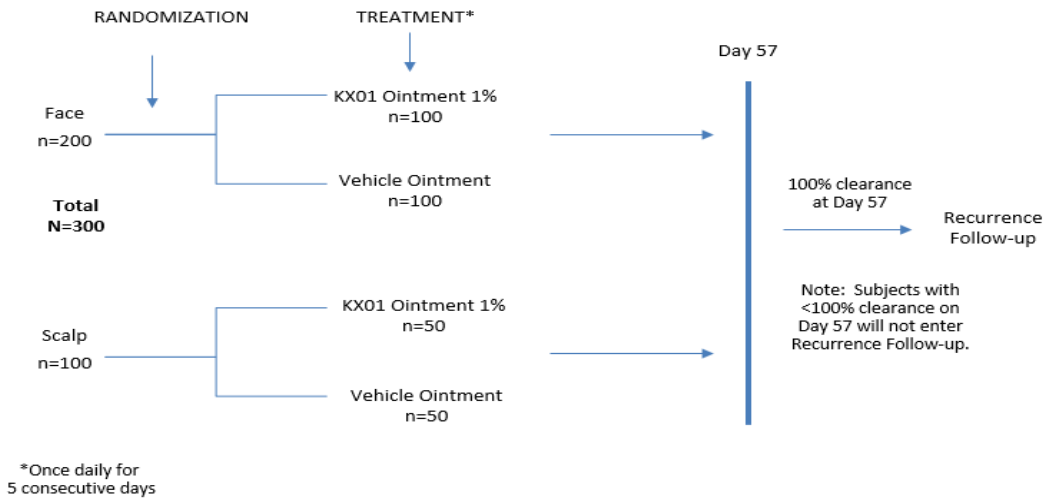


Figure 4: Study design for studies KX01-AK-003 and KX01-AK-004

The study consisted of Screening, Treatment, Response Assessment, and Recurrence Follow-up Periods.

After a Screening Period of up to 28 days, subjects returned to the site for confirmation of eligibility. Eligible subjects were randomised on Day 1 to treatment in a 1:1 (KX2-391 Ointment 1% or Vehicle) ratio in each treatment area subgroup. If subjects had eligible lesions in both locations, the decision of which location to treat was made by the Investigator.

The treatment area was marked with an indelible marker at Baseline (Day 1 pre-dose) at the investigational site. Subjects were given verbal and written instructions on self-administration of study drug/vehicle and a study kit containing 5 daily single-dose packets (one for each day of treatment). The first dose was applied by the subject under the supervision of study site personnel. Subjects then took home the study kit containing the remaining single-dose packets of study drug for daily self-administration on the next 4 consecutive days.

Subjects returned to the clinical sites for assessments at Response Assessment visits on Days 5, 8, 15, 29, and 57.

All subjects who had unresolved LSRs, hypo- or hyperpigmentation, and/or scarring in the treatment area, or treatment-related AEs at Day 57 returned for additional follow-up, which included

standardised photography of the treatment area, every 7 to 28 days until resolved, returned to baseline, or considered stabilised by the Investigators.

Subjects who achieved complete (100%) clearance of AK lesions in the treatment area at Day 57 continued in the Recurrence Follow-up Period to determine recurrence rate and safety for up to 12 months following the Day 57 Visit. Visits during the Recurrence Follow-up Period occurred every 3 months. Subjects who developed AK lesions in the treatment area during the Recurrence Follow-up Period were discontinued at the time of recurrence.

Methods

Study Participants

Inclusion Criteria

Subjects were eligible for participation in the study if they met all of the following inclusion criteria:

1. Males and females ≥18 years old
2. A treatment area on the face or scalp that
    - was a contiguous area measuring 25 cm^2
    - contained 4 to 8 clinically typical, visible, and discrete AK lesions.
3. Subjects who, in the judgment of the Investigator, were in good general health based on medical history, physical examination (PE) findings, vital signs, ECGs, clinical chemistry, haematology, and urinalysis results
4. Females had to be postmenopausal (>45 years of age with at least 12 months of amenorrhea), surgically sterile (by hysterectomy, bilateral oophorectomy, or tubal ligation); or, if of childbearing potential, had to be using highly effective contraception for at least 30 days or 1 menstrual cycle, whichever was longer, prior to study treatment and had to agree to continue to use highly effective contraception for at least 30 days following their last dose of study treatment. Highly effective contraception included oral hormonal contraceptives, hormonal contraceptive implant, injection or patch, intrauterine device or complete abstinence from sexual intercourse.
5. Sexually active males who had not had a vasectomy, and whose partner was reproductively capable, had to agree to use barrier contraception from Screening through 90 days after their last dose of study treatment.
6. All subjects had to agree not to donate sperm or eggs or to attempt conception from Screening through 90 days following their last dose of study treatment.
7. Females of childbearing potential had to have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day 1 prior to randomisation.
8. Willing to avoid excessive sunlight or ultraviolet (UV) light exposure, including the use of tanning beds, to the face or scalp.
9. Able to comprehend and were willing to sign the informed consent form (ICF).

Exclusion Criteria

Subjects were not eligible for participation in the study if they met any of the following:

1. Clinically atypical and/or rapidly changing AK lesions on the treatment area, e.g., hypertrophic, hyperkeratotic, recalcitrant disease (had cryosurgery on 2 previous occasions) and/or cutaneous horn.
2. Location of the treatment area was
    - on any location other than the face or scalp
    - within 5 cm of an incompletely healed wound
    - within 5 cm of a suspected basal cell carcinoma (BCC) or SCC.
3. Had been previously treated with KX2-391 Ointment.
4. Anticipated need for in-patient hospitalisation or in-patient surgery from Day 1 to Day 57.
5. Treatment with 5-fluorouracil (5-FU), imiquimod, ingenol mebutate, diclofenac, photodynamic therapy, or other treatments for AK within the treatment area or within 2 cm of the treatment area within 8 weeks prior to the Screening Visit.
6. Use of the following therapies and/or medications within 2 weeks prior to the Screening Visit:
    - Cosmetic or therapeutic procedures (eg, use of liquid nitrogen, surgical excision, curettage, dermabrasion, medium or greater depth chemical peel, laser resurfacing) within the treatment area or within 2 cm of the selected treatment area
    - Acid-containing therapeutic products (eg, salicylic acid or fruit acids, such as alpha and beta-hydroxyl acids and glycolic acids), topical retinoids, or light chemical peels within the treatment area or within 2 cm of the selected treatment area
    - Topical salves (nonmedicated/nonirritant lotion and cream were acceptable) or topical steroids within the treatment area or within 2 cm of the selected treatment area;

    artificial tanners within the treatment area or within 5 cm of the selected treatment area.
7. Use of the following therapies and/or medications within 4 weeks prior to the Screening Visit:
    - Treatment with immunomodulators (eg, azathioprine), cytotoxic drugs (e.g., cyclophosphamide, vinblastine, chlorambucil, methotrexate) or interferons/interferon inducers.
    - Treatment with systemic medications that suppress the immune system (e.g., cyclosporine, prednisone, methotrexate, alefacept, infliximab).
8. Use of systemic retinoids (e.g., isotretinoin, acitretin, bexarotene) within 6 months prior to the Screening Visit.
9. History of sensitivity and/or allergy to any of the ingredients in the study medication.
10. Skin disease (e.g., atopic dermatitis, psoriasis, eczema) or condition (e.g., scarring, open wounds) that, in the opinion of the Investigator, might have interfered with the study conduct or evaluations, or which exposed the subject to unacceptable risk by study participation.
11. Other significant uncontrolled or unstable medical diseases or conditions that, in the opinion of the Investigator, would have exposed the subject to unacceptable risk by study participation.
12. Females who were pregnant or nursing.

13. Participated in an investigational drug trial during which an investigational study medication was administered within 30 days or 5 half-lives of the investigational product, whichever was longer, before dosing.

Treatments

Treatments Administered in Study KX01-AK-003 and KX01-AK-004

Treatment	Strength	Size of Treatment Area	Number Applications and Frequency	Study Days Administered
KX2-391 Ointment 1%	1%	25 cm^2	1 application once daily for 5 consecutive days	Days 1–5
Vehicle Ointment	NA	25 cm^2	1 application once daily for 5 consecutive days	Days 1–5

KX2-391 Ointment 1% was supplied in single-use packets each of which contained 250 mg of the ointment, equivalent to 2.5 mg of KX2-391 free base. Vehicle ointment was supplied in the same single-use packets each of which contained 250 mg of the ointment without the active drug. Each packet was for use as a single-dose application.

Subjects were instructed to apply the study drug preferably early in the day and at approximately the same time every day for a total of 5 days. The first dose was self-administered in the clinic under the supervision of study site personnel on Day 1; the remaining doses were self-administered on the next 4 consecutive days at home.

Concomitant or Prohibited Medications / Therapy:

During the Recurrence Follow up Period sunblock and non-medications topical products could be used in the treatment area. Other concomitant medication used for the treatment of AEs in the treatment area and those that could affect the assessment of AK lesion recurrence in the treatment area were entered in the CRF. Any drug products or treatment that might influence or make the effect of study treatment up to Day 57 and during the Recurrence Follow-up Period were prohibited. Direct sun or UV exposure to the treatment area was to be avoided throughout the study, or if not possible from Day 15 was acceptable with the use of a sunblock. Any subject who started systemic or topical therapy for the treatment of AK was withdrawn from the study.

Objectives

Primary Objective

The primary objective of both studies was to evaluate the efficacy of topical KX2-391 Ointment 1% once daily for 5 consecutive days compared with vehicle control in terms of 100% clearance at Day 57 in the treatment of adults with AK, when applied to a contiguous area of 25 cm^2 on the face or scalp.

The secondary objectives of the study were:

- To evaluate the safety of topical KX2-391 ointment 1% once daily for 5 consecutive days in terms of LSRs and other safety evaluations, such as AEs and laboratory assessments.
- To compare the rates of partial response defined as ≥75% clearance of AK lesions in the treatment area on the face or scalp at Day 57 between the KX2-391 Ointment 1%-treated group and vehicle-treated group.

- To determine the recurrence rate of AK in the treatment area up to 12 months post-Day 57 in subjects who had complete (100%) clearance at Day 57 after 5 consecutive days of treatment with KX2-391 Ointment 1%.
- To evaluate the safety of topical KX2-391 Ointment 1% within the treatment area during the Recurrence Follow-up Period.

Outcomes/endpoints

The primary endpoint:

The primary endpoint was the complete (100%) clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with no clinically visible AK lesions in the treatment area.

The secondary endpoints:

Key secondary endpoint:

- Partial clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with a ≥75% reduction in the number of AK lesions identified at Baseline (Day 1 predose) in the treatment area

Additional secondary endpoint:

- Recurrence rate of AK lesions in subjects who achieved complete clearance at Day 57. Recurrence was defined as appearance of any AK lesions in the treatment area, including those recurred (i.e., reappearance of previously cleared lesions) or newly identified.

Safety:

- Evaluation of LSRs, pigmentation and scarring in the treatment area, AEs, SAEs, events of special interest, clinical laboratory data, and other safety assessments (vital signs, physical examinations [PEs], electrocardiograms [ECGs])
- AEs within the treatment area after Day 57 up to 12 months post-Day 57.

Sample size

The sample size was estimated based on the primary efficacy endpoint, complete (100%) clearance, at the Day 57 visit, for the comparison of KX2-391 Ointment 1% and Vehicle control. By using a Pearson Chi-square method, a sample size of 100 scalp-treated subjects and 200 face-treated subjects, both of which have 1:1 treatment allocation ratio, provided a >90% power to detect a 20% difference (30% for active treatment and 10% for vehicle control) with a 2-tailed significant level of 0.05.

The 30% response rate for complete (100%) clearance at the Day 57 visit for active treatment was assumed based on unpublished results from the 5-day treatment cohort in the Phase 2 study KX01-AK-002.

The 10% response rate of complete (100%) clearance at Day 57 for Vehicle control was conservatively assumed per literature reports.

Randomisation

Enrollment was controlled so that approximately two-thirds of subjects enrolled were treated on the face and approximately one-third of subjects enrolled were treated on on the scalp. Eligible subjects were randomised in a double-blinded manner to treatment in a 1:1 (KX2-391 Ointment 1% or Vehicle)

ratio in each treatment area subgroup. Enrollment numbers and study kits were distributed sequentially from two lists, one for face and one for scalp.

Blinding (masking)

This was a double-blind study.

Statistical methods

Study hypothesis:

Treatment with KX2-391 Ointment 1% administered topically once daily for 5 consecutive days will demonstrate a greater complete clearance (defined as 100% clearance of clinically typical and visible actinic keratosis [AK] lesions at Day 57) than vehicle ointment administered topically once daily for 5 consecutive days in adults with AK on the face or scalp.

The analysis sets were defined as follows:

- **Intent-To-Treat (ITT) Population:** all randomised subjects. This was the primary efficacy population.
- **Per-Protocol (PP)/Evaluable Population** (hereafter referred to as the PP population)**:** all randomised subjects who received at least 4 of the 5 doses, conformed to the entry criteria in the protocol, did not receive concomitant medications that could affect efficacy, and returned for the Final Visit on Day 57.
- **Safety Population**: all randomised subjects who received at least one dose of study treatment

Recurrence follow-up period

- **Recurrence Follow-up Population:** all subjects in the ITT Population who achieved complete clearance at the Day 57 visit.

Treatment/Response Assessment Periods: Complete clearance

The Day 57 complete (100%) clearance rate was analysed using a Cochran-Mantel-Haenszel (CMH) model controlling for treatment location and treatment group (primary analysis). Before applying the CMH method, a Breslow-Day test with a significance level of 10% was used to explore heterogeneity of the odds ratios across treatment location subgroups.

Further, the Pearson Chi Square (used to power the study) was applied to demonstrate basic agreement with the CMH.

The primary efficacy analysis was performed with the ITT population and was repeated with the PP/Evaluable population to support the primary efficacy analysis results.

Low enrolment sites were pooled to create analysis sites with approximately 20 subjects in each pool.

Another CMH test adjusting for treatment group and analysis site (secondary analysis) was performed to ensure concordance with the primary efficacy endpoint analyses described above (data not shown). To explore heterogeneity of the odds ratios across analysis sites, the Breslow-Day test at a significance level of 10% was applied. A finding of statistical significance in this test was followed by exploratory analyses to identify outlier study sites.

The outlier sites were discussed and an exploratory analysis excluding the outlier sites could be carried out to estimate the impact of site-by-treatment interactions.

Complete (100%) clearance and its associated 95% confidence interval (CI) at each analysis site or at each study site were also provided.

Treatment/Response Assessment Periods: Partial clearance

To control for multiplicity, the partial (≥75%) clearance rate was examined using a step-down gatekeeping testing strategy for the overall type I error rate, i.e., the primary endpoint served as a gatekeeper for the key secondary endpoint. The complete (100%) clearance rate was tested initially; if, and only if it was statistically significant at the 0.05 significance level, then the partial (≥75%) clearance rate was statistically tested at the same significance level.

Recurrence Follow-up Period

Recurrence was defined as appearance of any AK lesions in the treatment area, including those recurred (i.e., reappearance of previously cleared lesions) or newly identified. Recurrence rates were estimated based on a Kaplan-Meier method at each post-Day 57 analysis visit. Subjects with missing AK assessments in the Recurrence Follow-up Period were considered as censored at their last AK assessment.

A Kaplan-Meier plot was created for the recurrence rate in the KX2-391 Ointment 1% group.

The number of subjects at risk, the number of subjects with recurrence, and the number of censored subjects were presented at each analysis visit by treatment location in the KX2-391 Ointment 1% group.

Listing of subject status for the Kaplan-Meier analysis in the Recurrence Follow-up Period was provided.

Missing data were not imputed. Subjects with missing AK lesion assessments due to early termination were considered as censored on the analysis visit at which they discontinued.

Treatment/Response Assessment Periods: **AK Lesion Count**

The number of AK lesions and the change from baseline in lesion count at each visit were summarised using descriptive statistics (i.e., mean, standard deviation [SD], median, minimum and maximum) by treatment location (face or scalp) and treatment group for the ITT population and the PP population. T-test-based statistical comparisons of change from baseline between the 2 treatment groups at each visit were performed.

In addition, line plots were provided to present mean (± standard error) of AK lesion count by treatment group and treatment location at each visit. T-test-based statistical comparisons of AK lesion counts between the 2 treatment groups at each visit were performed.

Subgroup Analysis

To indicate concordance with the overall results, the primary and the key secondary efficacy endpoints were tabulated and displayed graphically in subgroups such as treatment location (face or scalp), gender, age (<65 or ≥65 years), baseline AK lesion count (4, 5, 6 or 7, 8), skin type (Fitzpatrick I/II or III/IV/V/VI).

A Clopper-Pearson Exact 95% CI was provided for each estimation, but no statistical test was performed between subgroups.

Missing Data

For the primary and the key secondary efficacy endpoint analyses, the subjects who discontinued prior to Day 57 visit will be considered as non-responders. Due to extremely low number of subjects who discontinued prior to Day 57 visit, no sensitivity analyses for the primary and the key secondary efficacy endpoints will be performed.

Changes to the Planned Analyses Before Treatment Unblinding:

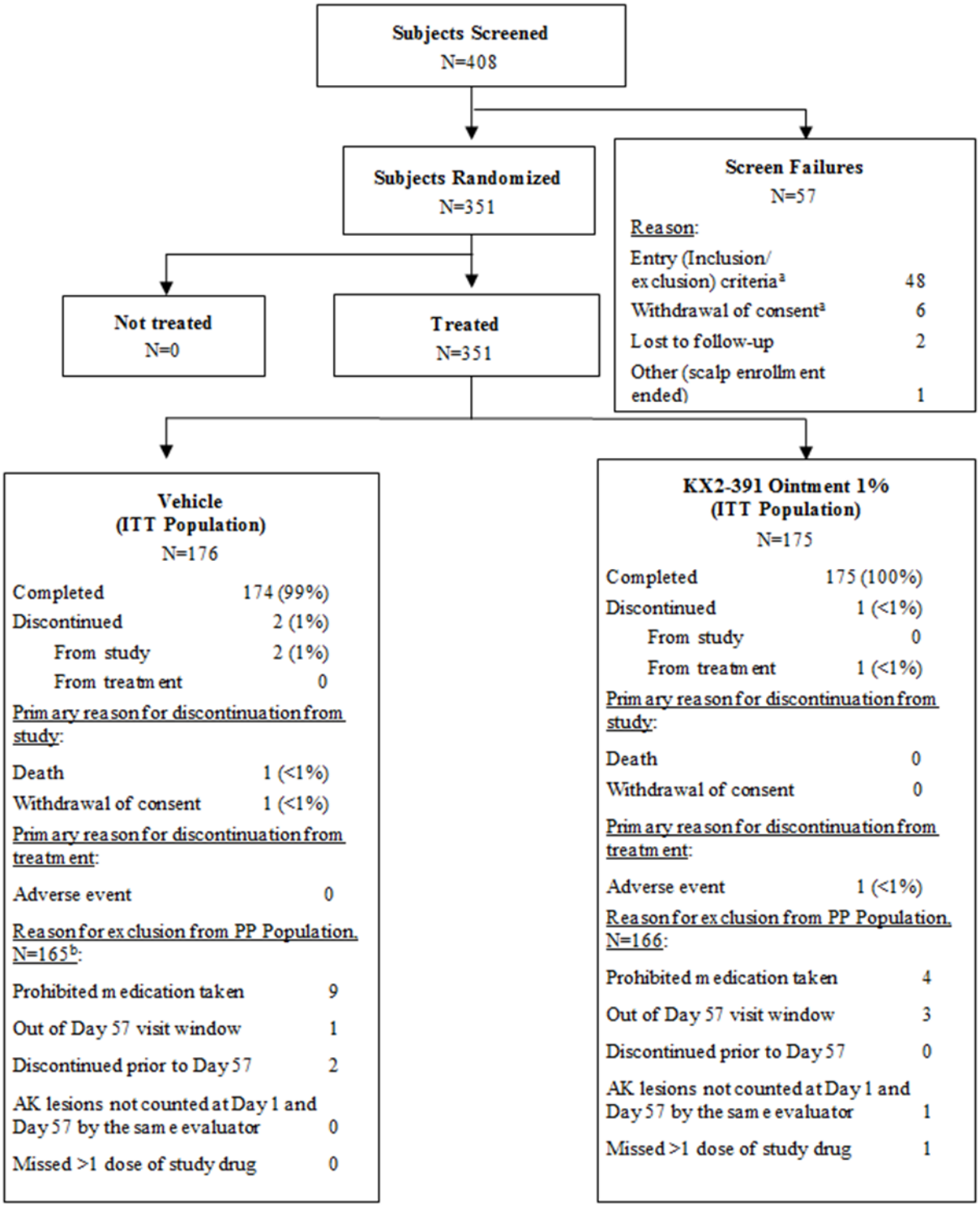
In the SAP v2.0 that was signed off after the Blind Data Review Meeting but before database lock, the Sponsor made the following changes to the planned analyses as only 2 of the 351 subjects discontinued from the study. No other analyses were changed.

Analysis Specified in SAP v1.0	Changes in SAP v2.0
Primary and Key Secondary Analysis: Impute missing data at Day 57 visit with the last observation carried forward method	Primary and Key Secondary Analysis: Consider subjects with missing AK lesion count at Day 57 visit as nonresponders

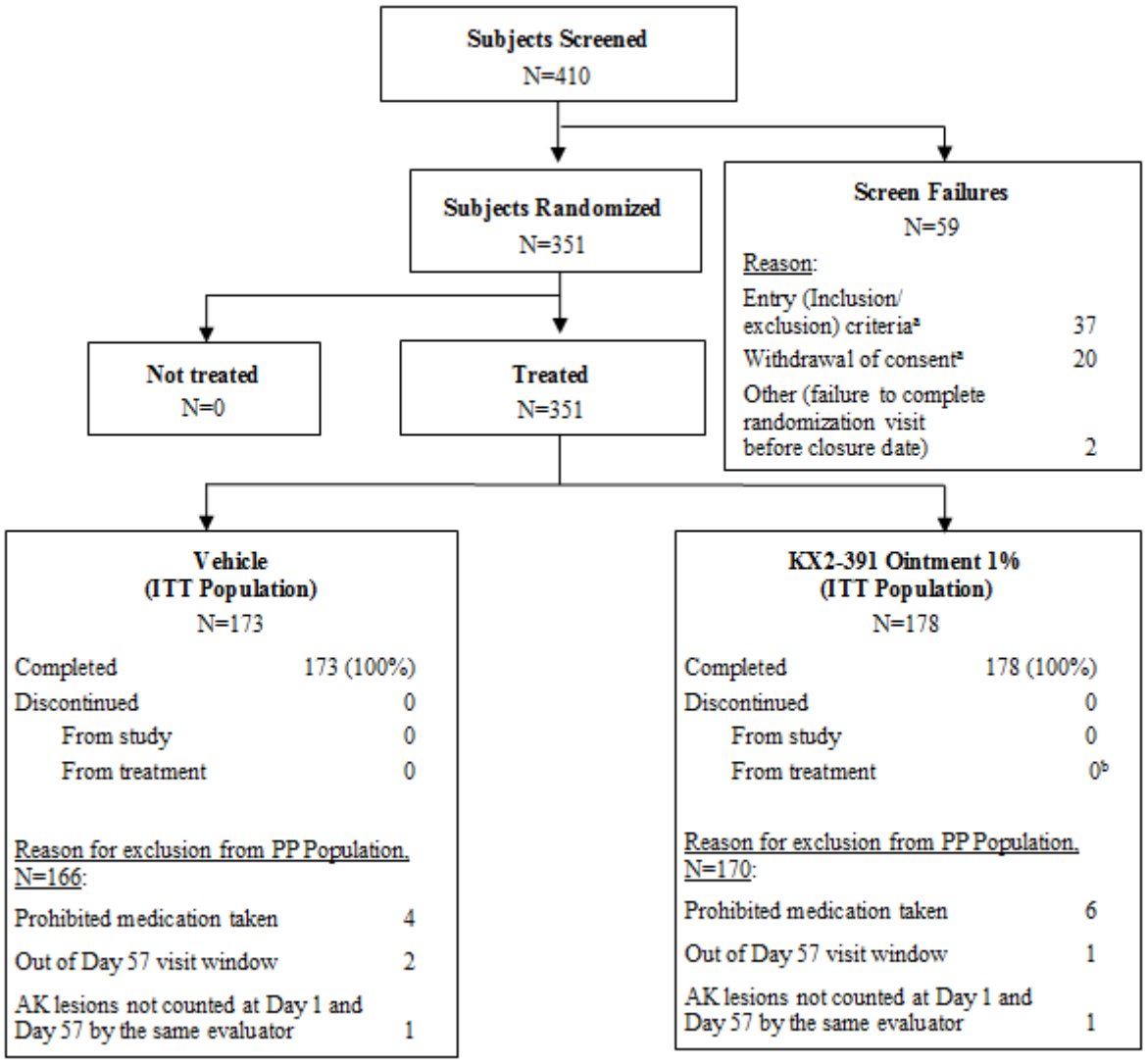
Results

Participant flow

Subject Disposition up to the Day 57 Visit in KX01-AK-003



Subject Disposition up to the Day 57 Visit in KX01-AK-004


Subjects Screened
N=410
Subjects Randomized
N=351
Screen Failures
N=59
Reason:
Entry (Inclusion/ exclusion) criteria[a] 37
Withdrawal of consent[a] 20
Other (failure to complete randomization visit before closure date) 2
Not treated
N=0
Treated
N=351
Vehicle
(ITT Population)
N=173
Completed 173 (100%)
Discontinued 0
From study 0
From treatment 0
Reason for exclusion from PP Population, N=166:
Prohibited medication taken 4
Out of Day 57 visit window 2
AK lesions not counted at Day 1 and Day 57 by the same evaluator 1
KX2-391 Ointment 1%
(ITT Population)
N=178
Completed 178 (100%)
Discontinued 0
From study 0
From treatment 0[b]
Reason for exclusion from PP Population, N=170:
Prohibited medication taken 6
Out of Day 57 visit window 1
AK lesions not counted at Day 1 and Day 57 by the same evaluator 1

Subject Disposition During the Treatment/ Response Assessment Period (up to Day 57) – ITT/Safety Population KX01-AK-003

	Vehicle n (%)	KX2-391 Ointment 1% n (%)	Overall n (%)
Number of subjects dosed	176	175	351
Number of subjects who completed Day 57 visit	174 (99)	175 (100)	349 (>99)
Number of subjects who achieved 100% AK clearance at Day 57 visit	8 (5)	77 (44)	85 (24)
Number of subjects who entered the Recurrence Follow-up Period	7 (4)	77 (44)	84 (24)
Number of subjects who did not enter the Recurrence Follow-up Period	1 (<1)	0	1 (<1)
Number of subjects who discontinued study prior to Day 57 visit	2 (1)	0	2 (<1)
Withdrew consent [a]	1 (<1)	0	1 (<1)
Death	1 (<1)	0	1 (<1)

Subject Disposition During the Treatment/ Response Assessment Period (up to Day 57) – ITT/Safety Population KX01-AK-004

	Vehicle n (%)	KX2-391 Ointment 1% n (%)	Overall n (%)
Number of subjects screened			410
Number of subjects who failed screening			59
Number of subjects in the ITT/Safety Population	173	178	351
Number of subjects who completed Day 57 visit	173 (100)	178 (100)	351 (100)
Number of subjects who achieved 100% AK clearance at Day 57 visit	22 (13)	97 (54)	119 (34)
Number of subjects who entered the Recurrence Follow-up Period	22 (13)	96 (54)	118 (34)
Number of subjects who did not enter the Recurrence Follow-up Period	0	1 (<1)	1 (<1)
Number of subjects who discontinued study prior to Day 57 visit	0	0	0

Recruitment

In KX01-AK-003 the study period was between 18 Sep 2017 (first subject first visit) and 24 Apr 2019 (last subject last visit).

In KX01-AK-004 the study period was between 15 Sep 2017 (first subject first visit) and 24 Apr 2019 (last subject last visit).

Conduct of the study

Protocol Amendments for Both studies KX01-AK-003 and KX01-AK-004

The original protocol was approved on 29 Jun 2017. There was 1 protocol amendment on 18 Feb 2018. The amendment was submitted to the IRB for approval in accordance with local requirements.

The changes covered a revision how lesions would be counted during the Recurrence Follow-up Period to include any lesion within the treatment area and not lesions completely in the treatment area, in order to address concern regarding possible underestimation of recurrence, and some clarifications regarding the statistical analysis. A summary of important changes instituted is provided below:

Change	Rationale
Revised lesion count wording during the Recurrence Follow-up Period to indicate that lesions 'within the treatment area' should be counted. Previously it was stated that lesions counted must be completely in the treatment area.	To address concern regarding possible underestimation of recurrence.
Deleted reference to analysis of treatment/location interaction to show concordance.	To provide clarification; the face and scalp are analyzed as a whole; the face and scalp will also be analyzed separately to show consistency as to treatment location.
Provided language about pooling sites and additional analysis for site-to-site variability.	To address potential variability among sites.
Provided additional language about how recurrence rates were to be analyzed.	Clarification of analysis for recurrence rate.
Added language for partial response rate analysis.	To address multiplicity issues for the secondary efficacy endpoint.
Revised language regarding missing data handling/sensitivity and subgroup analyses.	Clarification for these efficacy analyses.
Deleted reduction in AK lesion count during Days 1–57 as a secondary objective/endpoint.	To limit the number of secondary objectives/endpoints being measured in the study.

Protocol deviations

Study KX01-AK-003

Most deviations were related to visits/study assessments that occurred outside the allowed time window or visits that were missed or improperly performed.

Three subjects had used prohibited medication/therapies within 4 weeks of Screening (Exclusion Criterion 7) and were randomised and then dosed by study sites in error and were recorded as protocol deviations.

These 3 subjects were not displayed in the Listing of Screening Failure Subjects.

They were included in the ITT and the Safety populations for analysis but were excluded from the PP population.

Twenty subjects (including the 3 subjects mentioned above) were excluded from the PP analysis for various protocol deviations.

Study KX01-AK-004

Most deviations were related to visits/study assessments that occurred outside the allowed time window, visits/study procedures that were missed or improperly performed, subjects who received a prohibited medication/therapy, or noncompliance with study drug or application instructions.

Fifteen subjects were excluded from the PP analysis for various protocol deviations.

Baseline data

Demographic and Other Baseline Characteristics: KX01-AK-003 and KX01-AK-004

KX01-AK-003

	Vehicle	KX2-391 Ointment 1%	Overall
Number of subjects in the ITT/Safety Population	176	175	351
Age (years)			
n	176	175	351
Mean (SD)	70.2 (9.41)	69.5 (8.55)	69.9 (8.99)
Median	69.5	70.0	70.0
Min, max	45, 96	48, 86	45, 96
Age group (years), n (%)			
<65	42 (24)	51 (29)	93 (26)
≥65	134 (76)	124 (71)	258 (74)
Sex, n (%)			
Female	22 (13)	28 (16)	50 (14)
Male	154 (88)	147 (84)	301 (86)
Race, n (%)			
American Indian or Alaska native	1 (<1)	0	1 (<1)
White	175 (>99)	175 (100)	350 (>99)
Ethnicity, n (%)			
Hispanic or Latino	5 (3)	2 (1)	7 (2)
Not Hispanic or Latino	171 (97)	173 (99)	344 (98)
Location of treatment area, n (%)			
Face	121 (69)	119 (68)	240 (68)
Scalp	55 (31)	56 (32)	111 (32)
Number of Baseline AK Lesions, n (%)			
4	31 (18)	26 (15)	57 (16)
5	50 (28)	58 (33)	108 (31)
6	40 (23)	40 (23)	80 (23)
7	24 (14)	25 (14)	49 (14)
8	31 (18)	26 (15)	57 (16)

KX01-AK-004

Vehicle	KX2-391 Ointment 1%	Overall
173	178	351
173	178	351
70.2 (8.86)	69.1 (8.69)	69.7 (8.78)
70.0	69.0	70.0
46, 92	46, 90	46, 92
39 (23)	56 (31)	95 (27)
134 (77)	122 (69)	256 (73)
23 (13)	20 (11)	43 (12)
150 (87)	158 (89)	308 (88)
0	1 (<1)	1 (<1)
173 (100)	177 (>99)	350 (>99)
8 (5)	11 (6)	19 (5)
165 (95)	167 (94)	332 (95)
118 (68)	119 (67)	237 (68)
55 (32)	59 (33)	114 (32)
21 (12)	21 (12)	42 (12)
57 (33)	50 (28)	107 (30)
42 (24)	48 (27)	90 (26)
34 (20)	28 (16)	62 (18)
19 (11)	31 (17)	50 (14)

	Vehicle	KX2-391 Ointment 1%	Overall	Vehicle	KX2-391 Ointment 1%	Overall
Number of AK lesions at baseline						
n	176	175	351	173	178	351
Mean (SD)	5.9 (1.35)	5.8 (1.28)	5.8 (1.31)	5.8 (1.20)	6.0 (1.27)	5.9 (1.24)
Median	6.0	6.0	6.0	6.0	6.0	6.0
Min, max	4, 8	4, 8	4, 8	4, 8	4, 8	4, 8
Baseline weight (kg)						
n	176	175	351	172	178	350
Mean (SD)	87.6 (16.2)	86.9 (15.1)	87.3 (15.7)	84.7 (15.39)	89.1 (18.07)	86.9 (16.93)
Median	84.5	86.4	85.5	83.05	86.55	84.80
Min, max	50.8, 138.2	51.8, 139.5	50.8, 139.5	52.3, 140.4	45.0, 167.0	45.0, 167.0
Fitzpatrick skin type, n (%)						
Type I	21 (12)	19 (11)	40 (11)	17 (10)	30 (17)	47 (13)
Type II	121 (69)	104 (59)	225 (64)	103 (60)	96 (54)	199 (57)
Type III	31 (18)	43 (25)	74 (21)	48 (28)	45 (25)	93 (26)
Type IV	3 (2)	9 (5)	12 (3)	4 (2)	6 (3)	10 (3)
Type V	0	0	0	1 (<1)	0	1 (<1)
Type VI	0	0	0	0	1 (<1)	1 (<1)

For study **KX01-AK-003** the number of lesions was similar between the 2 treatment groups, with most subjects having 5 (108 subjects [31%]) or 6 (80 subjects [23%]) lesions in the treatment area.

In study **KX01-AK-004** the number of lesions was similar between the 2 treatment groups, with most subjects having 5 (107 subjects [30%]) or 6 (90 subjects [26%]) lesions in the treatment area.

Similar proportion of subjects in each treatment group had previous AK treatment **KX01-AK-003** (87% and 83% for Vehicle and KX2-391 Ointment 1%, respectively); **KX01-AK-004:** 72% and 74% for Vehicle and KX2-391 Ointment 1%, respectively).

34% and 36% (overall) of patients had prior field treatment **KX01-AK-003** and **KX01-AK-004** respectively.

Previous AK treatments in **KX01-AK-003** were predominantly localised, e.g., cryotherapy; 75% and 74% for Vehicle and KX2-391 Ointment 1%, respectively. For **KX01-AK-0034** they were recorded as 67% and 63% for Vehicle and KX2-391 Ointment 1%, respectively.

Many enrolled subjects ([47%] **KX01-AK-003 and** [42%] **KX01-AK-004)** had a history of skin cancer (**KX01-AK-003 ;** 105 had SCCs, 124 had BCCs, and 24 had melanomas: **KX01-AK-004** ; 79 had SCCs, 110 had BCCs, and 15 had melanomas).

They were similarly distributed between the 2 treatment groups: **KX01-AK-003 :** 89 (51%) in Vehicle and 77 (44%) in KX2-391 Ointment 1%.), **KX01-AK-004**: 72 (42%) in Vehicle and 75 (42%) in KX2-391 Ointment 1%.

Both studies had reported similar medical conditions among subjects, 96% and 98% **KX01-AK-003** and 99% and 97% **KX01-AK-004** of subjects in the Vehicle and KX2-391 Ointment 1% groups, respectively. These conditions are common in an elderly population and occurred in similar proportions of subjects in the 2 groups, e.g., surgical and medical procedures, neoplasms metabolic and nutritional, vascular, musculoskeletal and connective tissue, gastrointestinal, and immune system disorders.

Approximately 89% (**KX01-AK-003)** to 92% **(KX01-AK-004)** of subjects in each treatment group received at least 1 concomitant medication. The most prevalent medications given to ≥15% of subjects in each group were acetylsalicylic acid, simvastatin, multivitamin, atorvastatin, Lisinopril metformin,

and omeprazole. These concomitant medications were given for medical conditions typical for this age group.

Compliance to the 5-day self-application treatment regimen was >99%. All but 1 subject in both studies received the planned 5 daily doses. Subject 313-501 (KX2-391 Ointment 1%) stopped treatment after Day 3 because of a traumatic wound in the treatment area (scalp) which was unrelated to study treatment. Subject 412-502 (KX2-391 Ointment 1%) missed the Day 5 dose but completed the study through Day 57.

Numbers analysed

Study KX01-AK-003

Summary of Numbers of Subjects in Each Analysis Set by Treatment Group Up to Day 57 Visit

	Vehicle	KX2-391 Ointment 1%	Overall
ITT/Safety Population, n	176	175	351
Per-Protocol/Evaluable Population, n (%)	165 (94)	166 (95)	331 (94)
Days in Study (ITT/Safety Population)			
n	176	175	351
Mean (SD)	57.0 (4.09)	57.5 (3.15)	57.2 (3.66)
Median	57.0	57.0	57.0
Min, Max	29, 78	47, 77	29, 78
Days in Study (Per-Protocol/Evaluable Population)			
n	165	166	331
Mean (SD)	57.2 (2.41)	57.5 (2.63)	57.3 (2.53)
Median	57.0	57.0	57.0
Min, Max	50, 69	51, 70	50, 70

Summary of Subjects Who Are Excluded from the Per-Protocol/Evaluable Population by Treatment Group

	Vehicle n (%)	KX2-391 Ointment 1% n (%)	Overall n (%)
Number of subjects in the ITT/Safety Population	176	175	351
Number of subjects in the Per-Protocol/Evaluable Population	165 (94)	166 (95)	331 (94)
Number of subjects excluded from the Per-Protocol/Evaluable Population	11 (6)	9 (5)	20 (6)
Prohibited Medication Taken	9 (5)	4 (2)	13 (4)
Out of Day 57 Visit Window (Day 50 - day 71, inclusive)	1 (<1)	3 (2)	4 (1)
Discontinued Prior to Day 57	2 (1)	0	2 (<1)
AK Lesions Not Counted at Day 1 and Day 57 by the Same Evaluator	0	1 (<1)	1 (<1)
Missed >1 Dose of Study Drug	0	1 (<1)	1 (<1)

Study KX01-AK-004

Summary of Numbers of Subjects in Each Analysis Set by Treatment Group Up to Day 57 Visit

	Vehicle	KX2-391 Ointment 1%	Overall
ITT/Safety Population, n	173	178	351
Per-Protocol/Evaluable Population, n (%)	166 (96)	170 (96)	336 (96)
Days in Study (ITT/Safety Population)			
n	173	178	351
Mean (SD)	57.0 (3.07)	56.9 (2.45)	56.9 (2.77)
Median	57.0	57.0	57.0
Min, Max	50, 81	45, 65	45, 81
Days in Study (Per-Protocol/Evaluable Population)			
n	166	170	336
Mean (SD)	56.8 (2.16)	56.9 (2.30)	56.9 (2.23)
Median	57.0	57.0	57.0
Min, Max	50, 65	50, 65	50, 65

Summary of Subjects Who Are Excluded from the Per-Protocol/Evaluable Population by Treatment Group

	Vehicle n (%)	KX2-391 Ointment 1% n (%)	Overall n (%)
Number of subjects in the ITT/Safety Population	173	178	351
Number of subjects in the Per-Protocol/Evaluable Population	166 (96)	170 (96)	336 (96)
Number of subjects excluded from the Per-Protocol/Evaluable Population	7 (4)	8 (4)	15 (4)
Prohibited Medication Taken	4 (2)	6 (3)	10 (3)
Out of Day 57 Visit Window (Day 50 - day 71, inclusive)	2 (1)	1 (<1)	3 (<1)
AK Lesions Not Counted at Day 1 and Day 57 by the Same Evaluator	1 (<1)	1 (<1)	2 (<1)

Outcomes and estimation

Primary and key secondary endpoint

Study KX01-AK-003

The KX2-391 Ointment 1% group had significantly higher (p<0.0001) complete (100%) clearance than the Vehicle group (overall, 44% versus 5%, respectively). In addition, significantly higher rates of complete (100%) clearance for KX2-391 Ointment 1% were observed when the face (p<0.0001 versus Vehicle) and scalp (p<0.0001 versus Vehicle) subgroups were analysed separately.

Clearance Rates at Day 57 by Treatment Location and Treatment Group – ITT Population (primary analysis)

Clearance Rate	Vehicle	KX2-391 Ointment 1%	p-Value
100% [a]			
Overall	8/176 (5%)	77/175 (44%)	<0.0001 [b]
Face	7/121 (6%)	60/119 (50%)	<0.0001 [c]
Scalp	1/55 (2%)	17/56 (30%)	<0.0001 [c]
≥75% [d]			
Overall	29/176 (16%)	119/175 (68%)	<0.0001 [b]
Face	23/121 (19%)	90/119 (76%)	<0.0001 [c]
Scalp	6/55 (11%)	29/56 (52%)	<0.0001 [c]

AK = actinic keratosis; ITT = Intent-to-Treat.

a Complete (100%) clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with no clinically visible AK lesions in the treatment area.

b Based a Cochran-Mantel-Haenszel test stratified by treatment location.

c Based on a Pearson Chi-square test within each subgroup.

d Partial (≥75%) clearance rate is defined as the proportion of subjects at Day 57 with a ≥75% reduction in the number of AK lesions identified at Baseline (Day 1 predose) in the treatment area.

Clearance Rates at Day 57 by Treatment Location and Treatment Group – PP Population

Clearance Rate	Vehicle	KX2-391 Ointment 1%	p-Value
100% [a]			
Overall	8/165 (5%)	76/166 (46%)	<0.0001 [b]
Face	7/112 (6%)	60/114 (53%)	<0.0001 [c]
Scalp	1/53 (2%)	16/52 (31%)	<0.0001 [c]
≥75% [d]			
Overall	29/165 (18%)	114/166 (69%)	<0.0001 [b]
Face	23/112 (21%)	87/114 (76%)	<0.0001 [c]
Scalp	6/53 (11%)	27/52 (52%)	<0.0001 [c]

AK = actinic keratosis; PP = Per Protocol.

a Complete (100%) clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with no clinically visible AK lesions in the treatment area.

b Based on a Cochran-Mantel-Haenszel test stratified by treatment location.

c Based on a Pearson Chi-square test within each subgroup.

d Partial (≥75%) clearance rate is defined as the proportion of subjects at Day 57 with a ≥75% reduction in the number of AK lesions identified at Baseline (Day 1 predose) in the treatment area.

Study KX01-AK-004

The KX2-391 Ointment 1% group had significantly higher (p<0.0001) complete (100%) clearance than the Vehicle group (overall, 54% versus 13%, respectively). In addition, significantly higher rates of complete (100%) clearance for KX2-391 Ointment 1% were observed when the face (p<0.0001 versus Vehicle) and scalp (p=0.0003 versus Vehicle) subgroups were analysed separately.

Clearance Rates at Day 57 by Treatment Location and Treatment Group – ITT Population (primary analysis)

Clearance Rate	Vehicle	KX2-391 Ointment 1%	p-Value
100%[a]			
Overall	22/173 (13%)	97/178 (54%)	<0.0001[b]
Face	16/118 (14%)	73/119 (61%)	<0.0001[c]
Scalp	6/55 (11%)	24/59 (41%)	0.0003[c]
≥75%[d]			
Overall	34/173 (20%)	136/178 (76%)	<0.0001[b]
Face	26/118 (22%)	95/119 (80%)	<0.0001[c]
Scalp	8/55 (15%)	41/59 (69%)	<0.0001[c]

AK = actinic keratosis; ITT = Intent-to-Treat.

a Complete (100%) clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with no clinically visible AK lesions in the treatment area.

b Based on a Cochran-Mantel-Haenszel test stratified by treatment location.

c Based on a Pearson Chi-square test within each subgroup.

d Partial (≥75%) clearance rate is defined as the proportion of subjects at Day 57 with a ≥75% reduction in the number of AK lesions identified at Baseline (Day 1 predose) in the treatment area.

Clearance Rates at Day 57 by Treatment Location and Treatment Group – PP Population

Clearance Rate	Vehicle	KX2-391 Ointment 1%	p-Value
100%[a]			
Overall	21/166 (13%)	94/170 (55%)	<0.0001[b]
Face	16/114 (14%)	70/113 (62%)	<0.0001[c]
Scalp	5/52 (10%)	24/57 (42%)	0.0001[c]
≥75%[d]			
Overall	33/166 (20%)	130/170 (76%)	<0.0001[b]
Face	26/114 (23%)	91/113 (81%)	<0.0001[c]
Scalp	7/52 (13%)	39/57 (68%)	<0.0001[c]

AK = actinic keratosis; PP = Per Protocol.

a Complete (100%) clearance rate of AK lesions, defined as the proportion of subjects at Day 57 with no clinically visible AK lesions in the treatment area.

b Based on a Cochran-Mantel-Haenszel test stratified by treatment location.

c Based on a Pearson Chi-square test within each subgroup.

d Partial (≥75%) clearance rate is defined as the proportion of subjects at Day 57 with a ≥75% reduction in the number of AK lesions identified at Baseline (Day 1 predose) in the treatment area.

For the primary endpoint (complete [100%] clearance at the Day 57 Visit), the p-values of the Breslow-Day test were not significant (p>0.1) in either the ITT population or the PP population for the CMH analysis adjusting for analysis sites.

For the secondary endpoint (partial [75%] clearance at the Day 57 visit), the p-values of the Breslow-Day test were significant (p<0.1) in both the ITT population and the PP population for the CMH

analysis adjusting for analysis sites. To evaluate the heterogeneity indicated across analysis sites, the difference of partial (≥75%) clearance rate at Day 57 between the 2 treatment groups was calculated and then the analysis sites were sorted by this difference. For both the ITT and PP populations, KX2-391 ointment 1% was favoured over vehicle at all analysis sites with a ≥40% difference for 13 of the 17 analysis sites. Consistency was demonstrated across all analysis sites.

Additional secondary endpoint

Recurrence rate of AK lesions - FOLLOW-UP PERIOD

Subjects who did not return for follow-up were censored. This occurred for 4 subjects in **Study KX01-AK-003** who had KX2-391 Ointment 1% treatment on the face (1 was lost to follow-up and 3 withdrew consent) and 1 subject in the Vehicle group (withdrew consent). 1 patient in **Study KX01-AK-004** withdrew consent.

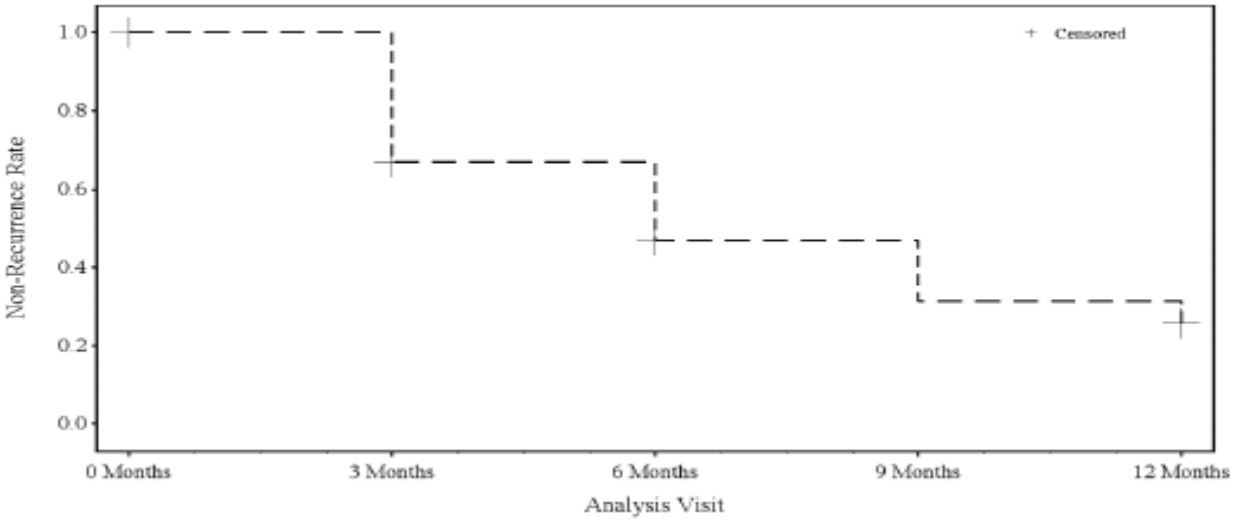
In study **KX01-AK-003** at 12 months post-Day 57, 18 KX2-391 Ointment 1%-treated subjects (23%) had not developed recurrent AK lesions in the treatment area – 16/60 (26%) who received treatment on the face and 2/17 (12%) who received treatment on the scalp.

In study **KX01-AK-004** 27 KX2-391 Ointment 1%-treated subjects (28%) had not developed recurrent AK lesions in the treatment area – 23/73 (31%) who received treatment on the face and 4/24 (17%) who received treatment on the scalp

Summary of Recurrence Status and Rates by Treatment Location and Analysis Visit, Recurrence Follow-up Population, KX2-391 Ointment 1% Group study KX01-AK-003

Treatment Location Analysis Visit	Number of Subjects			KM Estimate
	At Risk n	With Recurrence n (%)	Censored n (%)	
Overall				
3 months post-Day 57	76[a]	25 (33)	1 (1)	0.33
6 months post-Day 57	50	15 (30)	2 (4)	0.53
9 months post-Day 57	33	11 (33)	0	0.69
12 months post-Day 57	22	4 (18)	18 (82)	0.74
Face				
3 months post-Day 57	59	20 (34)	1 (2)	0.34
6 months post-Day 57	38	10 (26)	2 (5)	0.51
9 months post-Day 57	26	7 (27)	0	0.64
12 months post-Day 57	19	3 (16)	16 (84)	0.70
Scalp				
3 months post-Day 57	17	5 (29)	0	0.29
6 months post-Day 57	12	5 (42)	0	0.59
9 months post-Day 57	7	4 (57)	0	0.82
12 months post-Day 57	3	1 (33)	2 (67)	0.88

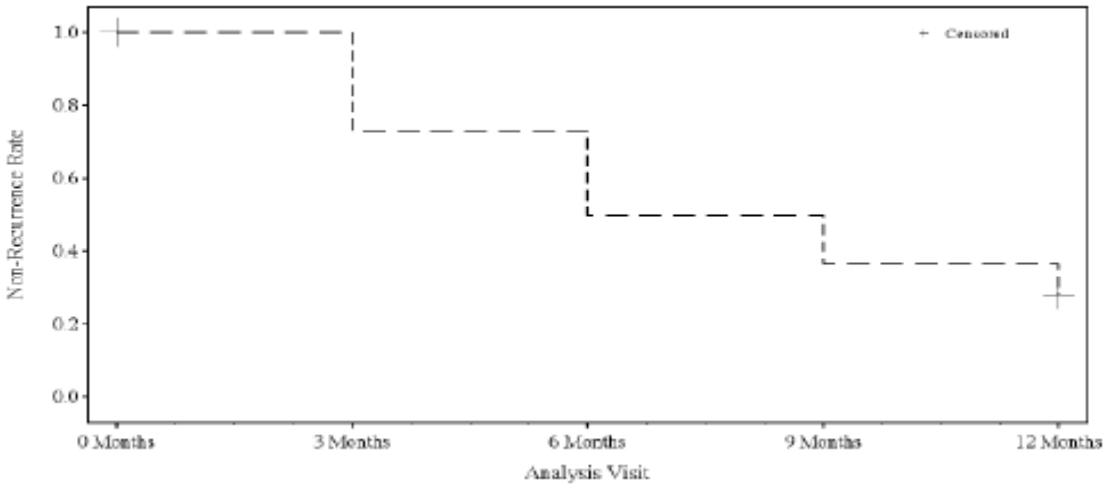
Kaplan-Meier Plot for Recurrence Analysis, Recurrence Follow-up Population, KX2-391 Ointment 1% Group study KX01-AK-003



Summary of Recurrence Status and Rates by Treatment Location and Analysis Visit, Recurrence Follow-up Population, KX2-391 Ointment 1% Group study KX01-AK-004

	Number of Subjects			
Treatment Location Analysis Visit	At Risk n	With Recurrence n (%)	Censored n (%)	KM Estimate
Overall				
3 months post-Day 57	96[a]	26 (27)	0	0.27
6 months post-Day 57	70	22 (31)	0	0.50
9 months post-Day 57	48	13 (27)	0	0.64
12 months post-Day 57	35	8 (23)	27 (77)	0.72
Face				
3 months post-Day 57	72	20 (28)	0	0.28
6 months post-Day 57	52	15 (29)	0	0.49
9 months post-Day 57	37	7 (19)	0	0.58
12 months post-Day 57	30	7 (23)	23 (77)	0.68
Scalp				
3 months post-Day 57	24	6 (25)	0	0.25
6 months post-Day 57	18	7 (39)	0	0.54
9 months post-Day 57	11	6 (55)	0	0.79
12 months post-Day 57	5	1 (20)	4 (80)	0.83

Kaplan-Meier Plot for Recurrence Analysis, Recurrence Follow-up Population, KX2-391 Ointment 1% Group study KX01-AK-004



Other endpoint: Reduction in AK lesion counts

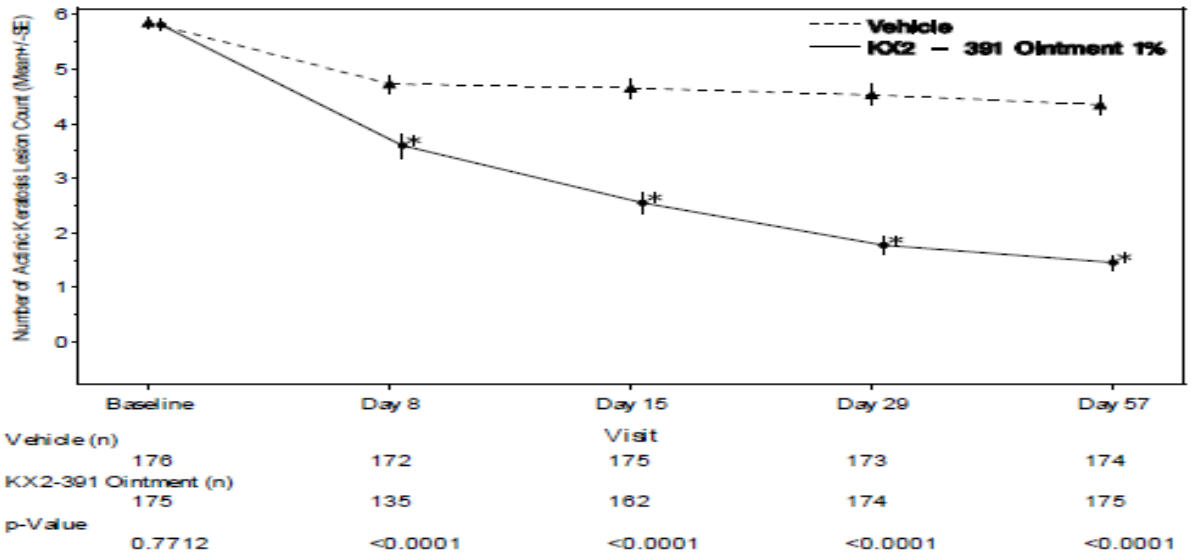
Study KX01-AK-003

Overall, there was a median reduction in AK lesion count of 83% for KX2-391 Ointment 1% compared with 20% for Vehicle at Day 57.

Summary of Actinic Keratosis Lesion Counts by Visit and Treatment Location up to Day 57 Visit – ITT Population – Change from Baseline – Study KX01-AK-003

Treatment Location	Day 8		Day 15		Day 29		Day 57	
	Vehicle	KX2-391	Vehicle	KX2-391	Vehicle	KX2-391	Vehicle	KX2-391
Overall								
n	172	135	175	162	173	174	174	175
Mean (SD)	-1.1 (1.76)	-2.2 (2.65)	-1.2 (1.75)	-3.2 (2.36)	-1.3 (1.91)	-4.0 (2.05)	-1.5 (1.90)	-4.4 (1.96)
Median (%)	0.0 (0)	-1.0 (-25%)	0.0 (0)	-4.0 (-61%)	-1.0 (-13%)	-4.0 (-80%)	-1.0 (-20%)	-5.0 (-83%)
Min, max	-7, 3	-8, 4	-7, 2	-8, 4	-7, 4	-8, 1	-7, 5	-8, 0
p-Value	<0.0001		<0.0001		<0.0001		<0.0001	
Face								
n	118	87	121	111	120	118	119	119
Mean (SD)	-1.2 (1.77)	-2.4 (2.67)	-1.3 (1.79)	-3.7 (2.18)	-1.5 (1.97)	-4.4 (1.87)	-1.5 (1.90)	-4.6 (1.80)
Median (%)	0.0 (0)	-2.0 (-25%)	0.0 (0)	-4.0 (-80%)	-1.0 (-13%)	-5.0 (-86%)	-1.0 (-25%)	-5.0 (-100%)
Min, max	-7, 1	-8, 2	-7, 1	-8, 1	-7, 4	-8, 0	-7, 5	-8, 0
p-Value	0.0004		<0.0001		<0.0001		<0.0001	
Scalp								
n	54	48	54	51	53	56	55	56
Mean (SD)	-1.0 (1.75)	-1.8 (2.60)	-1.0 (1.65)	-2.3 (2.49)	-1.1 (1.76)	-3.4 (2.24)	-1.5 (1.93)	-3.8 (2.20)
Median (%)	0.0 (0)	-1.0 (-20%)	0.0 (0)	-2.0 (-33%)	0.0 (0)	-4.0 (-65%)	-1.0 (-20%)	-4.0 (-75%)
Min, max	-5, 3	-8, 4	-5, 2	-8, 4	-5, 2	-7, 1	-5, 3	-8, 0
p-Value	0.0587		0.0033		<0.0001		<0.0001	

Line Plot for Actinic Keratosis Lesion Count by Visit and Treatment Group (Mean ± SE) – ITT Population - Study KX01-AK-003

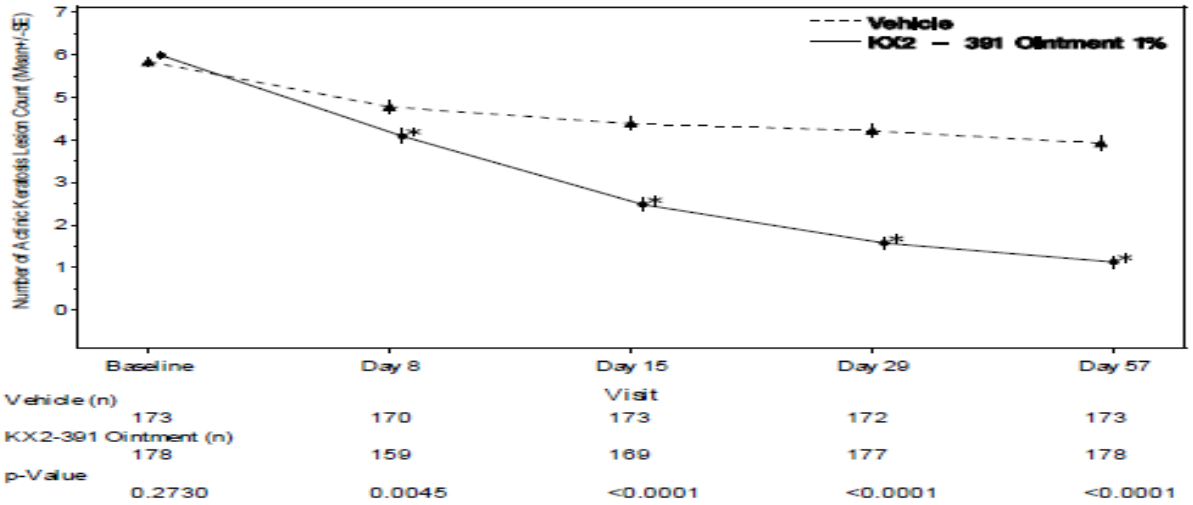


Study KX01-AK-004

Summary of Actinic Keratosis Lesion Counts by Visit and Treatment Location up to Day 57 Visit – ITT Population – Change from Baseline - Study KX01-AK-004

Treatment Location	Day 8		Day 15		Day 29		Day 57	
	Vehicle	KX2-391	Vehicle	KX2-391	Vehicle	KX2-391	Vehicle	KX2-391
Overall								
n	170	159	173	169	172	177	173	178
Mean (SD)	-1.1 (1.72)	-1.9 (2.25)	-1.5 (1.83)	-3.5 (2.29)	-1.6 (1.98)	-4.4 (2.12)	-1.9 (2.09)	-4.9 (2.02)
Median (%)	0.0 (0)	-1.0 (-25%)	-1.0 (-14.3%)	-4.0 (-60.0%)	-1.0 (-14.3%)	-5.0 (-83.3%)	-1.0 (-25.0%)	-5.0 (-100.0%)
Min, max	-8, 1	-7, 3	-7, 1	-8, 3	-7, 2	-8, 1	-8, 2	-8, 1
p-Value	0.0001		<0.0001		<0.0001		<0.0001	
Face								
n	115	104	118	114	117	118	118	119
Mean (SD)	-1.1 (1.85)	-2.1 (2.35)	-1.5 (1.87)	-3.7 (2.26)	-1.8 (2.07)	-4.4 (2.02)	-2.0 (2.14)	-4.9 (1.98)
Median (%)	0 (0.0%)	-2.0 (-25.0%)	-1.0 (-16.7%)	-4.0 (-66.7%)	-1.0 (-20.0%)	-5.0 (-83.3%)	-1.5 (-25.0%)	-5.0 (-100.0%)
Min, max	-8, 1	-7, 3	-7, 1	-8, 3	-7, 0	-8, 1	-8, 2	-8, 1
p-Value	0.0005		<0.0001		<0.0001		<0.0001	
Scalp								
n	55	55	55	55	55	59	55	59
Mean (SD)	-0.9 (1.43)	-1.5 (2.02)	-1.4 (1.76)	-3.3 (2.34)	-1.2 (1.73)	-4.4 (2.33)	-1.7 (1.98)	-4.8 (2.12)
Median (%)	0 (0.0%)	-1.0 (-16.7%)	0 (0.0%)	-4.0 (-57.1%)	0 (0.0%)	-5.0 (-80.0%)	-1.0 (-16.7%)	-5.0 (-87.5%)
Min, max	-5, 0	-7, 2	-6, 0	-8, 2	-7, 2	-8, 0	-7, 0	-8, 0
p-Value	0.0943		<0.0001		<0.0001		<0.0001	

Line Plot for Actinic Keratosis Lesion Count by Visit and Treatment Group (Mean ± SE) – ITT Population - Study KX01-AK-004



	Baseline	Day 8	Day 15	Day 29	Day 57
Vehicle (n)	173	170	173	172	173
KX2-391 Ointment (n)	178	159	169	177	178
p-Value	0.2730	0.0045	<0.0001	<0.0001	<0.0001

Ancillary analyses

Subgroup analyses of complete and partial clearance rates

Complete clearance rates

Subgroup Analysis of Complete (100%) Clearance at Day 57 – ITT Population Study KX01-AK-003

	Vehicle N = 176	KX2-391 Ointment 1% N = 175	p-Value [a]
Sex			
Female	3/22 (14%)	17/28 (61%)	0.0007
Male	5/154 (3%)	60/147 (41%)	<0.0001
Age			
<65 years old	1/42 (2%)	23/51 (45%)	<0.0001
≥65 years old	7/134 (5%)	54/124 (44%)	<0.0001
Baseline Lesion Count			
4 to 6 AK lesions	7/121 (6%)	61/124 (49%)	<0.0001
7 to 8 AK lesions	1/55 (2%)	16/51 (31%)	<0.0001
Fitzpatrick Skin Type			
I or II	7/142 (5%)	55/123 (45%)	<0.0001
III/IV/V/VI	1/34 (3%)	22/52 (42%)	<0.0001

Subgroup Analysis of Complete (100%) Clearance at Day 57 – ITT Population Study KX01-AK-004

	Vehicle N = 173	KX2-391 Ointment 1% N = 178	p-Value[a]
Sex			
Female	3/23 (13%)	17/20 (85%)	<0.0001
Male	19/150 (13%)	80/158 (51%)	<0.0001
Age			
<65 years old	4/39 (10%)	35/56 (63%)	<0.0001
≥65 years old	18/134 (13%)	62/122 (51%)	<0.0001
Baseline Lesion Count			
4 to 6 AK lesions	16/120 (13%)	72/119 (61%)	<0.0001
7 to 8 AK lesions	6/53 (11%)	25/59 (42%)	0.0002
Fitzpatrick Skin Type			
I or II	15/120 (13%)	68/126 (54%)	<0.0001
III/IV/V/VI	7/53 (13%)	29/52 (56%)	<0.0001

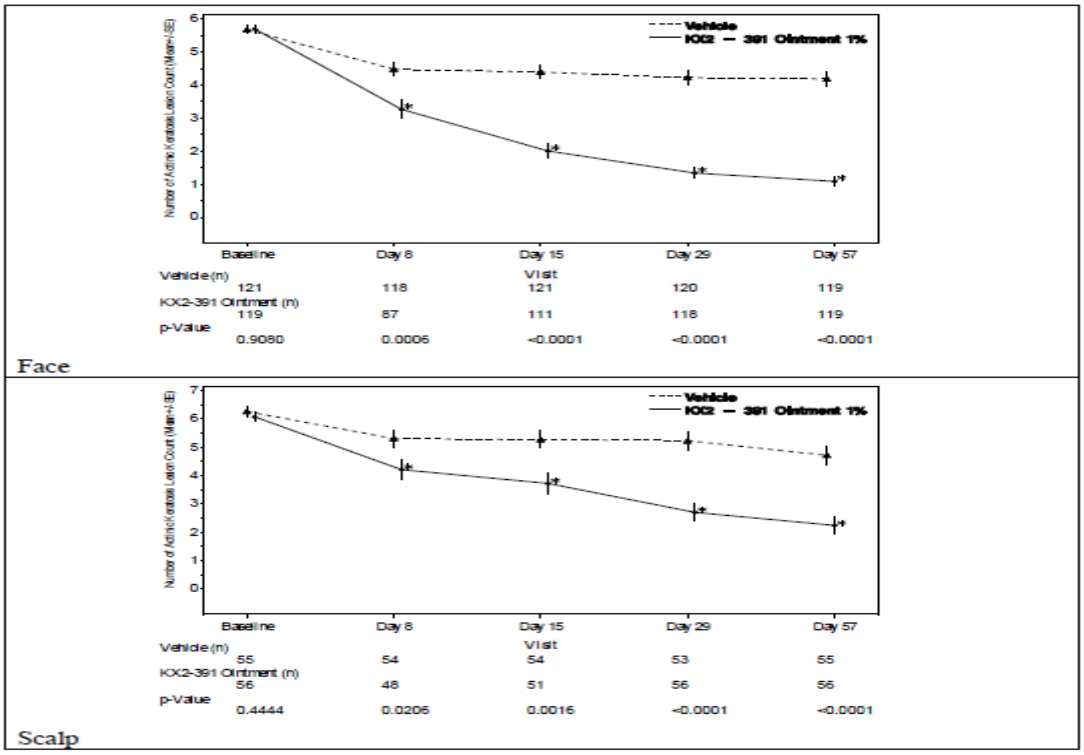
Partial (≥75%) Clearance

Similar results were observed for the demographic/baseline subgroup analysis of partial (≥75%) clearance at Day 57 (i.e., higher clearance rates for KX2-391 Ointment 1% group than for the Vehicle group): $p<0.0001$ in all subgroups in the ITT population and the PP population.

AK lesion counts per visit

For mean AK lesion counts, a statistically significant ($p<0.0001$) difference between treatments in favour of KX2-391 Ointment 1% was seen at Days 15, 29, and 57 in the face subgroup and at Days 29 and 57 in the scalp subgroup.

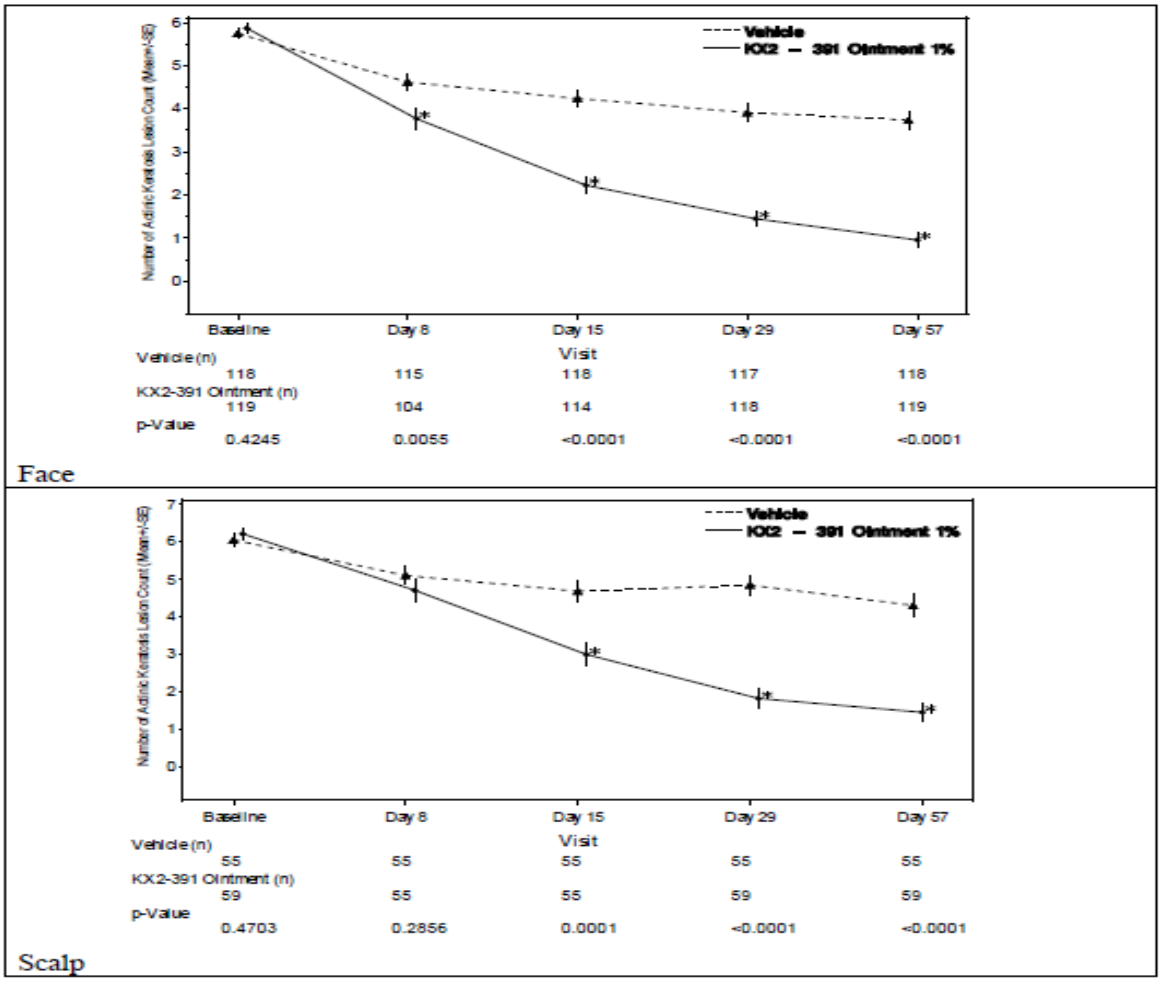
Line Plot for Actinic Keratosis Lesion Count by Visit and Treatment Group (Mean ± SE) for Face and Scalp Treatment Locations – ITT Population Study KX01-AK-003



Note: Asterisk indicates significant difference of the mean of AK lesion counts ($p \leq 0.05$, based on a t-test) at a specified visit between 2 treatment groups.

AK = actinic keratosis; ITT = Intent-to-Treat; n = number of subjects at a specified visit; SE = standard error.

Line Plot for Actinic Keratosis Lesion Count by Visit and Treatment Group (Mean ± SE) for Face and Scalp Treatment Locations – ITT Population Study KX01-AK-004



Face	Baseline	Day 8	Day 15	Day 29	Day 57
Vehicle (n)	118	115	118	117	118
KX2-391 Ointment (n)	119	104	114	118	119
p-Value	0.4245	0.0055	<0.0001	<0.0001	<0.0001

Scalp	Baseline	Day 8	Day 15	Day 29	Day 57
Vehicle (n)	55	55	55	55	55
KX2-391 Ointment (n)	59	55	55	59	59
p-Value	0.4703	0.2856	0.0001	<0.0001	<0.0001

Note: Asterisk indicates significant difference of the mean of AK lesion counts ($p \leq 0.05$, based on a t-test) at a specified visit between 2 treatment groups.

Summary of main studies

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

Table 16. Summary of efficacy for trial KX01-AK-003

Title: A Phase 3, Double-Blind, Vehicle-Controlled, Randomized, Parallel Group, Multicenter, Efficacy and Safety Study of KX2-391 Ointment 1% in Adult Subjects with Actinic Keratosis on the Face or Scalp			
Study identifier	KX01-AK-003		
Design	This was a multicentre, randomised, double-blind, vehicle-controlled, parallel-group study to evaluate the efficacy and safety of KX2-391 Ointment 1% administered topically to the face or scalp of adult subjects with actinic keratosis. Enrolment was controlled so that approximately two-thirds of subjects enrolled were treated on the face and approximately one-third of subjects enrolled were treated on the scalp. The study consisted of Screening, Treatment, Response Assessment (up to Day 57), and Recurrence Follow-up Periods. After Screening, subjects returned to the site for confirmation of eligibility. Eligible subjects were randomised to treatment on Day 1 in a 1:1 (KX2-391 Ointment 1% or vehicle) ratio in each treatment area subgroup. Subjects who achieved 100% clearance of AK lesions in the treatment area at Day 57 continued in the recurrence Follow up period to determine recurrence rate and safety at visits every 3 months for up to 12 months.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		57 days 28 days 12 months post-Day 57, only for subjects with complete clearance at Day 57 (Recurrence follow-up phase).
Hypothesis	Superiority		
Treatments groups	Test treatment		Tirbanibulin 1% ointment, Main phase: 57 days, N=175 (119 face, 56 scalp) Recurrence follow-up phase: up to 12 months post-Day 57, N=77
	Reference treatment		Vehicle, Main phase: 57 days, N=176 (121 face, 55 scalp) Recurrence follow-up phase: up to 12 months post-Day 57, N=8
Endpoints and definitions	Primary endpoint	Complete Clearance rate at Day 57	Proportion of subjects with no AK lesions (100% clearance) in the treatment area at Day 57.
	Key Secondary endpoint	Partial Clearance rate at Day 57	Proportion of subjects with a reduction from baseline ≥75% in the number of AK lesions in the treatment area at Day 57
	Secondary endpoint	Number of AK lesions at each visit	Number of AK lesions in the treatment area at each visit
	Secondary endpoint	Sustained clearance rate[(a)] after 12 months post-Day 57	Proportion of subjects with no AK lesions occurring in the treatment area during 12 months, after being Complete Clearance at Day 57
Database lock	10th June 2019		

Results and Analysis			
Analysis description	**Primary Analysis: Complete clearance and Partial clearance at Day 57 analysed by means of Cochran-Mantel Haenszel (CMH) adjusting for treatment location**		
Analysis population and time point description	Intent to treat population defined as all randomised subjects Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	175	176
	Complete Clearance (proportion)	44%	5%
	95% CI	37%, 52%	2%, 9%
	Partial Clearance (proportion)	68%	16%
	95% CI	61%, 75%	11%, 23%
	Change from baseline in lesion count at Day 57 (mean)	-4.4	-1.5
	SD	1.96	1.90
Effect estimate per comparison	Primary endpoint Complete clearance rate at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	39%
		95% CI	31%, 48%
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance rate at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	52%
		95% CI	42%, 60%
		P-value	<0.0001*
	Secondary endpoint Change from baseline in lesion count at Day 57	Comparison groups	Comparison between Tirbanibulin 1% ointment and vehicle
		t-test	N.A.
		P-value	<0.0001*
Analysis population and time point description	Per protocol population defined as all randomised subjects who received at least 4 of the 5 doses, conformed to the entry criteria in the protocol, did not receive concomitant medications that could affect efficacy, and returned for the Final Visit on Day 57 Complete clearance and Partial Clearance at Day 57		

Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	166	165
	Complete Clearance (proportion)	46%	5%
	95% CI	38%, 54%	2%, 9%
	Partial Clearance (proportion)	69%	18%
	95% CI	61%, 76%	12%, 24%
	Change from baseline in lesion count at Day 57 (mean)	-4.4	-1.6
	SD	1.95	1.84

Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	41%
		95% CI	32%, 49%
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	51%
		95% CI	41%, 60%
		P-value	<0.0001*
	Secondary endpoint Change from baseline in lesion count at Day 57	Comparison groups	Comparison between Tirbanibulin 1% ointment and vehicle
		t-test	N.A.
		P-value	<0.0001*
Analysis population and time point description	Recurrence follow-up population defined as all subjects in the ITT Population who achieved complete clearance at the Day 57 visit. Sustained clearance rate at 12 months post-Day 57		

Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects entering in the recurrence follow-up	77	8
	Sustained clearance rate (KM estimate)	26%	N.D.
	95% CI	16%, 36%	N.D.
Analysis description	**Secondary analysis: Complete clearance and Partial clearance at Day 57 analysed by means of CMH adjusting for Analysis site**		

Analysis population and time point description	Intent to treat population defined as all randomised subjects Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	175	176
	Complete Clearance (proportion)	44%	5%
	95% CI	37%, 52%	2%, 9%
	Partial Clearance (proportion)	68%	16%
	95% CI	61%, 75%	11%, 23%

Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	N.D.
		95% CI	N.D.
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	N.D.
		95% CI	N.D.
		P-value	<0.0001*

Analysis population and time point description	Per protocol population defined as all randomised subjects who received at least 4 of the 5 doses, conformed to the entry criteria in the protocol, did not receive concomitant medications that could affect efficacy, and returned for the Final Visit on Day 57 Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	166	165
	Complete Clearance (proportion)	46%	5%
	95% CI	38%, 54%	2%, 9%
	Partial Clearance (proportion)	69%	18%
	95% CI	61%, 76%	12%, 24%

Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by analysis site	N.D
		95% CI	N.D.
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by analysis site	N.D.
		95% CI	N.D.
		P-value	<0.0001*
Source: From CSR, CC Primary Analysis (ITT, PP) Table 14.2.1.1, CC Secondary Analysis (ITT, PP) Table 14.2.1.2, CC Effect (ITT) Figure 14.2.1.3.3, CC Effect (PP) Figure 14.2.1.3.4, PC Primary Analysis (ITT, PP) Table 14.2.2.1, PC Secondary Analysis (ITT, PP) Table 14.2.2.2, PC Effect (ITT) Figure 14.2.2.3.3, PC Effect (PP) Figure 14.2.2.3.4, Lesion count (ITT) Table 14.2.3.1, Lesion count (PP) Table 14.2.3.4, Non-Recurrence by Kaplan-Meier Table 14.2.4.2. N.D. Not Determined N.A. Not Available * statistically significant (p=<0.05) (a) In the Protocol and SAP, only Recurrence rate was defined. Results of Non-Recurrence by Kaplan-Meier estimate (sustained clearance) presented in table 14.2.4.2 of the CSR			

Table 17. Summary of efficacy for trial KX01-AK-004

Title: A Phase 3, Double-Blind, Vehicle-Controlled, Randomized, Parallel Group, Multicenter, Efficacy and Safety Study of KX2-391 Ointment 1% in Adult Subjects with Actinic Keratosis on the Face or Scalp		
Study identifier	KX01-AK-004	
Design	This was a multicentre, randomised, double-blind, vehicle-controlled, parallel-group study to evaluate the efficacy and safety of KX2-391 Ointment 1% administered topically to the face or scalp of adult subjects with actinic keratosis. Enrollment was controlled so that approximately two-thirds of subjects enrolled were treated on the face and approximately one-third of subjects enrolled were treated on the scalp. The study consisted of Screening, Treatment, Response Assessment (up to Day 57), and Recurrence Follow-up Periods. After Screening, subjects returned to the site for confirmation of eligibility. Eligible subjects were randomised to treatment on Day 1 in a 1:1 (KX2-391 Ointment 1% or vehicle) ratio in each treatment area subgroup. Subjects who achieved 100% clearance of AK lesions in the treatment area at Day 57 continued in the recurrence Follow up period to determine recurrence rate and safety at visits every 3 months for up to 12 months.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	57 days 28 days 12 months post-Day 57, only for subjects with complete clearance at Day 57 (Recurrence follow-up phase).
Hypothesis	Superiority	

Treatments groups	Test treatment		Tirbanibulin 1% ointment, Main phase: 57 days, N=178 (119 face, 59 scalp) Recurrence follow-up phase: up to 12 months post-Day 57, N=97
	Reference treatment		Vehicle, Main phase: 57 days, N=173 (118 face, 55 scalp) Recurrence follow-up phase: up to 12 months post-Day 57, N=22
Endpoints and definitions	Primary endpoint	Complete Clearance rate at Day 57	Proportion of subjects with no AK lesions (100% clearance) in the treatment area at Day 57.
	Key Secondary endpoint	Partial Clearance rate at Day 57	Proportion of subjects with a reduction from baseline ≥75% in the number of AK lesions of in the treatment area at Day 57
	Secondary endpoint	Number of AK lesions at each visit	Number of AK lesions in the treatment area at each visit
	Secondary endpoint	Sustained clearance rate[(a)] after 12 months post-Day 57	Proportion of subjects with no AK lesions occurring in the treatment area during 12 months, after being Complete Clearance at Day 57
Database lock	10th June 2019		

Results and Analysis

Analysis description	**Primary Analysis: Complete clearance and Partial clearance at Day 57 analysed by means of Cochran-Mantel Haenszel (CMH) adjusting for treatment location**		
Analysis population and time point description	Intent to treat population defined as all randomised subjects Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	178	173
	Complete Clearance (proportion)	54%	13%
	95% CI	47%, 62%	8%, 19%
	Partial Clearance (proportion)	76%	20%
	95% CI	69%, 82%	14%, 26%
	Change from baseline in lesion count at Day 57 (mean)	-4.9	-1.9
	SD	2.02	2.09
Effect estimate per comparison	Primary endpoint Complete clearance rate at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle

		Cochran-Mantel-Haenszel test stratified by treatment location	42%
		95% CI	32%, 51%
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance rate at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	57%
		95% CI	47%, 65%
		P-value	<0.0001*
	Secondary endpoint Change from baseline in lesion count at Day 57	Comparison groups	Comparison between Tirbanibulin 1% ointment and vehicle
		t-test	N.A.
		P-value	<0.0001*
Analysis population and time point description	Per protocol population defined as all randomised subjects who received at least 4 of the 5 doses, conformed to the entry criteria in the protocol, did not receive concomitant medications that could affect efficacy, and returned for the Final Visit on Day 57 Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	170	166
	Complete Clearance (proportion)	55%	13%
	95% CI	47%, 63%	8%, 19%
	Partial Clearance (proportion)	76%	20%
	95% CI	69%, 83%	14%, 27%
	Change from baseline in lesion count at Day 57 (mean)	-4.9	-1.9
	SD	2.01	2.11
Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	43%
		95% CI	33%, 51%
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	57%

		95% CI	47%, 65%
		P-value	<0.0001*
	Secondary endpoint Change from baseline in lesion count at Day 57	Comparison groups	Comparison between Tirbanibulin 1% ointment and vehicle
		t-test	N.A.
		P-value	<0.0001*
Analysis population and time point description	Recurrence follow-up population defined as all subjects in the ITT Population who achieved complete clearance at the Day 57 visit. Sustained clearance rate at 12 months post-Day57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects entering in recurrence follow-up	97	22
	Sustained clearance rate (KM estimate)	28%	N.D.
	95% CI	20%, 37%	N.D.
Analysis description	**Secondary analysis: Complete clearance and Partial clearance at Day 57 analysed by means of CMH adjusting for Analysis site**		
Analysis population and time point description	Intent to treat population defined as all randomised subjects Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	178	173
	Complete Clearance (proportion)	54%	13%
	95% CI	47%, 62%	8%, 19%
	Partial Clearance (proportion)	76%	20%
	95% CI	69%, 82%	14%, 26%
Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	N.D.
		95% CI	N.D.
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by treatment location	N.D.
		95% CI	N.D.

		P-value	<0.0001*
Analysis population and time point description	Per protocol population defined as all randomised subjects who received at least 4 of the 5 doses, conformed to the entry criteria in the protocol, did not receive concomitant medications that could affect efficacy, and returned for the Final Visit on Day 57 Complete clearance and Partial Clearance at Day 57		
Descriptive statistics and estimate variability	Treatment group	Tirbanibulin 1% ointment	Vehicle
	Number of subjects	170	166
	Complete Clearance (proportion)	55%	13%
	95% CI	47%, 63%	8%, 19%
	Partial Clearance (proportion)	76%	20%
	95% CI	69%, 83%	14%, 27%
Effect estimate per comparison	Primary endpoint Complete clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by analysis site	N.D
		95% CI	N.D.
		P-value	<0.0001*
	Key Secondary endpoint Partial clearance at Day 57	Comparison groups	Difference between Tirbanibulin 1% ointment and vehicle
		Cochran-Mantel-Haenszel test stratified by analysis site	N.D.
		95% CI	N.D.
		P-value	<0.0001*
Source: From CSR, CC Primary Analysis (ITT, PP) Table 14.2.1.1, CC Secondary Analysis (ITT, PP) Table 14.2.1.2, CC Effect (ITT) Figure 14.2.1.3.3, CC Effect (PP) Figure 14.2.1.3.4, PC Primary Analysis (ITT, PP) Table 14.2.2.1, PC Secondary Analysis (ITT, PP) Table 14.2.2.2, PC Effect (ITT) Figure 14.2.2.3.3, PC Effect (PP) Figure 14.2.2.3.4, Lesion count (ITT) Table 14.2.3.1, Lesion count (PP) Table 14.2.3.4, , Non-Recurrence by Kaplan-Meier Table 14.2.4.2 N.D. Not Determined N.A. Not Available * statistically significant (p=<0.05) (a) In the Protocol and SAP, only Recurrence rate was defined. Results of Non-Recurrence by Kaplan-Meier estimate (sustained clearance) presented in table 14.2.4.2 of the CSR			

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

The results of a post-hoc pooled efficacy analysis were submitted. This analysis was done with the pooled ITT Populations of the identical Phase III pivotal Studies KX01-AK-003 and KX01-AK-004.

The purpose of this pooling was to provide a more precise estimate of the overall treatment effect. The same statistical methodology as in the individual trials was used for the pooling, adding 'trial factor' in the statistical models to account for possible variability across trials. Therefore, the primary and key

secondary variables, proportion of patients with complete clearance at Day 57 and proportion of patients with Partial Clearance at Day 57 respectively, were analysed for the comparison between active and vehicle arm by means of CMH test, stratified by treatment location (face or scalp) and by study (KX01-AK-003 and KX01-AK-004). The Breslow-Day test was used to assess the heterogeneity across trials.

Sensitivity analyses done in each individual trial were also replicated with the pooled data adding 'trial factor' in all statistical models. Furthermore, subgroup analyses for the pooled data (treatment location, gender, age, Fitzpatrick skin type, and number of AK baseline lesions) were done using CMH. The Breslow-Day method was used to test heterogeneity across trials. Missing values were treated exactly the same way as in each individual trial (considered NR). Changes from baseline in AK lesions count were analysed by means of descriptive statistics for the pooled data, similar to the approach used in each individual trial. For the 1-year Recurrence Follow-up Period, the pooled recurrence rate and sustained clearance was estimated using KM estimates on the pooled data. A Cox model was performed to test the statistical significance of the trial factor.

In addition, a sensitivity estimand approach up to Day 57 similar to the one done for the individual Phase III pivotal studies was done on the pooling of the Phase III studies. Missing data were imputed as NR and the results were entered to NR for all patients who took prohibited medication before Day 57, from the time they took their first prohibited medication onwards. Due to the low number of missing observations and given that most of the patients taking prohibited medication before Day 57 were already NR, the difference between the main pooled analysis and the post-hoc estimand analysis was minimal.

Results are presented in the tables below.

Table 18: Analysis of Complete Clearance Rate at Day 57 Visit Adjusting for Treatment Location and Trial Factor (Pooled ITT Population) - Post-Hoc Statistical Analyses (KX01-AK-003 and KX01-AK-004)

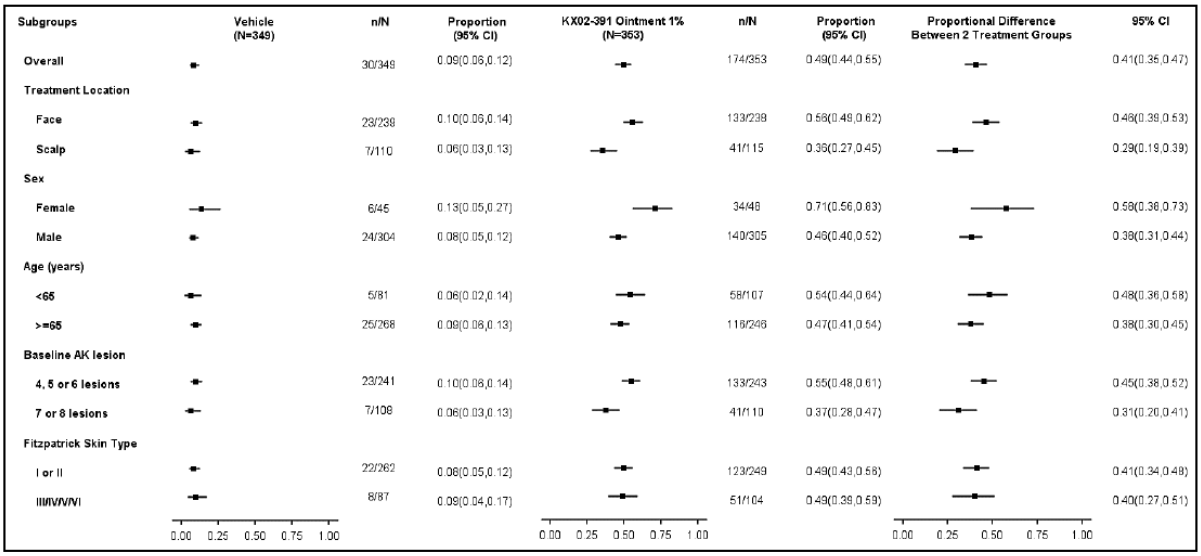
		Vehicle N=349 n(%)	Ointment 1% N=353 n(%)	Cochran-Mantel-Haenszel p-Value (a)
Complete Clearance Rate at Day 57	Total	349 (100.0%)	353 (100.0%)	
	No	319 (91.4%)	179 (50.7%)	
	Yes	30 (8.6%)	174 (49.3%)	
	Binomial Proportion	0.0860	0.4929	<.0001
	95% Clopper-Pearson confidence interval	(0.06 , 0.12)	(0.44 , 0.55)	
	Breslow-Day test p-value (b)		0.3380	

(a) p-value is from Cochran-Mantel-Haenszel test stratified treatment location and trial factor
(b) p-value is from Breslow-Day method to test heterogeneity across treatment location and trial factor

Table 19: Treatment Location Subgroup Analysis of Complete Clearance Rate at Day 57 Visit by Treatment Group Adjusting for Trial Factor (Pooled ITT Population)

			Vehicle N=349 n(%)	Ointment 1% N=353 n(%)	Cochran-Mantel-Haenszel p-Value (a)
FACE	Complete Clearance Rate at Day 57	Total	239 (100.0%)	238 (100.0%)	
		No	216 (90.4%)	105 (44.1%)	
		Yes	23 (9.6%)	133 (55.9%)	
		Binomial Proportion	0.0962	0.5588	<.0001
		95% Clopper-Pearson confidence interval	(0.06 , 0.14)	(0.49 , 0.62)	
		Breslow-Day test p-value (b)		0.3593	
SCALP	Complete Clearance Rate at Day 57	Total	110 (100.0%)	115 (100.0%)	
		No	103 (93.6%)	74 (64.3%)	
		Yes	7 (6.4%)	41 (35.7%)	
		Binomial Proportion	0.0636	0.3565	<.0001
		95% Clopper-Pearson confidence interval	(0.03 , 0.13)	(0.27 , 0.45)	
		Breslow-Day test p-value (b)		0.1925	

(a) p-value is from Cochran-Mantel-Haenszel test stratified by trial factor
(b) p-value is from Breslow-Day method to test heterogeneity across trial factor



Note 1: Subjects who discontinued prior to the Day 57 visit are considered as non-responders.

2: Confidence interval is from a binomial Clopper-Pearson method.

Age is derived as the integer part of ((informed consent date - birth date + 1) /365.25). If only birth year is collected, the subject is assumed to be born on July 1.

Figure 5: Forest plot of subgroup analysis of complete clearance rate at day 57 visit by treatment group (pooled ITT population)

Table 20: Analysis of partial (≥75%) clearance rate at day 57 visit by treatment location and trial factor (pooled ITT population)

		Vehicle N=349 n(%)	Ointment 1% N=353 n(%)	Cochran-Mantel-Haenszel p-Value (a)
Partial (≥75%) Clearance Rate at Day 57	Total	349 (100.0%)	353 (100.0%)	
	No	286 (81.9%)	98 (27.8%)	
	Yes	63 (18.1%)	255 (72.2%)	
	Binomial Proportion	0.1805	0.7224	<.0001
	95% Clopper-Pearson confidence interval	(0.14 , 0.22)	(0.67 , 0.77)	
	Breslow -Day test p-value (b)		0.8834	

(a) p-value is from Cochran-Mantel-Haenszel test stratified by treatment location, trial factor
(b) p-value is from Breslow-Day method to test heterogeneity across treatment location, trial factor

Table 21: Treatment location subgroup analysis of partial (≥75%) clearance rate at day 57 visit by treatment group adjusting for trial factor (pooled ITT population)

			Vehicle N=349 n(%)	Ointment 1% N=353 n(%)	Cochran-Mantel-Haenszel p-Value (a)
FACE	Partial (≥75%) Clearance Rate at Day 57	Total	239 (100.0%)	238 (100.0%)	
		No	190 (79.5%)	53 (22.3%)	
		Yes	49 (20.5%)	185 (77.7%)	
		Binomial Proportion	0.2050	0.7773	<.0001
		95% Clopper-Pearson confidence interval	(0.16 , 0.26)	(0.72 , 0.83)	
		Breslow-Day test p-value (b)		0.8978	
SCALP	Partial (≥75%) Clearance Rate at Day 57	Total	110 (100.0%)	115 (100.0%)	
		No	96 (87.3%)	45 (39.1%)	
		Yes	14 (12.7%)	70 (60.9%)	
		Binomial Proportion	0.1273	0.6087	<.0001
		95% Clopper-Pearson confidence interval	(0.07 , 0.20)	(0.51 , 0.70)	
		Breslow-Day test p-value (b)		0.5435	

(a) p-value is from Cochran-Mantel-Haenszel test stratified by trial factor
(b) p-value is from Breslow-Day method to test heterogeneity across trial factor

Table 22: Post-day 57 recurrence rates across studies and pooling (ITT population)

	Tirbanibulin 1% ointment			
	KX01-AK-003 (N = 77)	KX01-AK-004 (N = 97)	Pooling KX01-AK-003 and KX01-AK-004 (N=172)	KX01-AK-002 (N = 36)
3 months	33%	27%	30%	39%
6 months	53%	50%	51%	48%
9 months	69%	64%	66%	51%
12 months	74%	72%	73%	57%

ITT = Intent-to-Treat.

Source: Section 5.3.5.2, CSR KX01-AK-002, Table 14.2.3; Section 5.3.5.1, CSR KX01-AK-003, Table 14.2.4.1; Section 5.3.5.1, CSR KX01-AK-004, Table 14.2.4.1, and Section 5.3.5.3, Post-Hoc Statistical Analysis Report, Table 25.

Clinical studies in special populations

	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	298/702	185/702	31/702
Non Controlled Trials	88/198	34/198	4/198

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The outcomes from Study KX01-AK-01-US indicated that the most promising dose was that applied to Cohort 3 subjects (25 cm^2 treatment area/5 consecutive treatment days). The data also imply that treatment of a smaller body surface area (e.g. 25cm^2) which contains fewer AK lesions might be more efficacious than treating a larger area. The results of this Phase 1 study inform on the rationale for design of the subsequent Phase 2 and 3 studies. The 25 cm^2 treatment area was chosen for use in the Phase IIa study to further determine the better treatment duration and select the dose to be used in the pivotal Phase III studies. In both cohorts of Study KX01-AK-01-US, a decrease from baseline in AK lesion counts was noted by Day 15. Even with small numbers, consistency among subgroups was noted. By Day 29, lesion counts for each cohort showed meaningful reductions from baseline that continued up to Day 57.

Study KX01-AK-002 was a non-randomised, open label study of tirbanibulin administered topically to the face or scalp of subjects for either 5 or 3 consecutive days. The 5-day treatment regimen had a higher percentage of subjects with 100% clearance at Day 57 compared with the 3-day regimen (43% vs 32%, respectively). Most but not all of the recurrence happened within the first 6 months of post-Day 57 follow-up. More subjects with AK lesions on the face in the 5 day cohort (60%) had maintained 100% clearance than those in the 3 day cohort (27%), while subjects with AK lesions on the scalp had high recurrence rates in both cohorts (85% and 62%, respectively). Most subgroups had too few subjects to provide meaningful interpretation of results regarding the effect of subgroup on complete clearance rate. This small Phase II study supports the trial of tirbanibulin for 5 consecutive days in the Phase III studies.

The 2 pivotal efficacy Studies KX01-AK-003 and KX01-AK-004 were identical double-blind, randomised, vehicle-controlled, multi-centre Phase III studies that evaluated the efficacy and safety of tirbanibulin 1% ointment when applied once daily to a treatment area of 25 cm^2 for 5 consecutive days in adults with AK on the face or scalp. These 2 studies were identical in terms of study design, patient entry criteria, and assessments, and were independently conducted at non-overlapping study sites.

These studies were conducted only in the US however it is agreed that the condition should not be different in the EU. Furthermore, the studies were placebo (Vehicle) controlled, therefore influence of different treatments is not an issue.

The applicant did not include an active-control study design. They reported that this was because they had already started the clinical trials before seeking CHMP advice and there was no gold standard for the evaluation of efficacy in AK. The applicant argued that there was no standard of care for AK treatment in the EU. However, there were several treatments licensed in the EU including similar topical treatments which would have been beneficial to contextualise the efficacy of this proposed product.

Both studies were sufficiently powered to assess complete clearance, combining face and scalp lesions.

Patients enrolled had AK lesions on the face or scalp over contiguous area of 25 cm^2 containing 4 to 8 stable AK lesions. The lesions were not histologically confirmed, diagnosis was clinical and there was no efficacy assessment in other anatomical areas. The distribution was in accordance with planned subject enrolment.

Eligible patients were not allowed to have clinically atypical and/or rapidly changing AK lesions in the treatment area, and they were not allowed to use prohibited medicines, including AK treatment or systemic immunomodulatory agents and cytotoxic drugs. Approximately two-thirds of patients were enrolled in the face treatment subgroup and one-third in the scalp treatment subgroup. Overall the enrolled population is accepted and appears to have been well balanced between the arms. Patients enrolled were exclusively white, 84% to 90% of patients were male and 90% to 98% of patients had Fitzpatrick skin phototype I, II, or III. the efficacy was studied in non-hyperkeratotic lesions (Olsen grade 1) and there is no data with use in patients in hyperkeratotic lesions (Olsen 2 or 3). The indication in 4.1 of the SmPC was revised to reflect this.

Patients received topical treatment for 5 days followed by efficacy and safety assessments up to Day 57 and a 1-year Recurrence Follow-up Period. There was no retreatment of lesions for patients who had an incomplete response or those which recurred following initial clearance. There are no available data on retreatment with tirbanibulin in patients who had a recurrence of their AK lesions, or on those who developed new lesions. Therefore, it is unclear whether recurrent lesions would respond similarly to what was observed in the pivotal studies. Since there are no data on retreatment, it was reflected in section 4.2 of the SmPC that if recurrence occurs, or new lesions develop within the treatment area, other treatment options should be considered. Administration is restricted to single use. Efficacy on re-treatment will be investigated in the imposed PASS study which will also review AK histological progression to in situ or invasive SCC following treatment over a 3-year period.

A dermatologist (investigator or Sub investigator) performed a count of clinically visible AK lesions (lesion count) for all subjects at Response Assessment visits on Days 8, 15, 29, and 57. The same investigator or Sub investigator conducted the lesion count at all visits for an individual subject.

The investigator or sub investigator could use the transparency and/or photograph from baseline to locate the treatment area. Actinic keratosis lesion counts from previous visits were not to be used to assist in the assessment of AK lesion count at the current visit. Only AK lesions completely within the 25 cm^2 treatment area were to be counted.

The primary endpoint of complete clearance (100% reduction from Baseline of AK lesions in the treatment area) at Day 57, is considered stringent and clinically meaningful in the treatment of AK.

A key secondary endpoint was partial clearance (≥75% reduction from Baseline of AK lesions in the treatment area) at Day 57, which is less clinically meaningful but still shows a degree of benefit. Total lesion count was also measured. Overall, the chosen endpoints are supported.

In line with CHMP scientific advice a sensitivity estimand approach up to Day 57 was used, comprising the same analysis as described in the Statistical Analysis Plan, but taking into account the inter-current events.

Pooled analyses for primary and key secondary efficacy endpoints (complete and partial clearance rates at Day 57), reduction in AK lesion count up to Day 57, and sustained clearance rates were conducted with the pooled ITT Populations of the identical Phase III studies.

Additionally, subgroup analyses for the pooled primary efficacy data to assess treatment location (face and scalp), sex, age (<65 and >65 years), Fitzpatrick skin phototype (I/II and III/IV/V/VI), and

baseline AK lesion count (4 to 6 and 7 to 8 lesions) were done using CMH. The Breslow-Day method was used to test heterogeneity across studies.

There was one protocol amendment in both pivotal studies which involved a revised lesion count in the recurrence follow up period to indicate that lesions 'within the treatment area' are to be counted, previously the lesions 'must be completely within the treatment area'. This led to a more conservative estimation of the recurrence rate, including also those actinic keratosis lesions partially inside the treatment area.

Efficacy data and additional analyses

Overall, the key findings from the proof-of-concept Phase I Study (KX01-AK-01-US) and Phase IIa (Study KX01-AK-002) helped to determine and support the use of a 5 day once-daily regimen in the Phase III trials.

Both pivotal studies (KX01-AK-003 and KX01-AK-004) met the primary endpoint for complete clearance with statistically significant results for treatment versus placebo for both face and scalp and for each location separately.

Pivotal study KX01-AK-003 showed a difference in complete clearance of 39%, 44% and 28% in favour of tirbanibulin in the overall treatment field (face + scalp), the face and the scalp respectively. In study KX01-AK-004 the corresponding values were an improvement in complete clearance of 41%, 47% and 30% in the overall treatment field (face + scalp), the face and the scalp respectively.

Pooled analysis with the pooled ITT populations of the two studies showed a 40% improvement in favour of tirbanibulin for complete clearance and 54% improvement for partial clearance, both statistically significant ($p<0.0001$). A consistent effect was seen between both studies and between ITT and PP analysis. A lower effect was observed with scalp lesions versus facial ones (29% versus 46%, ITT analysis).

The overall mean reduction in AK lesion count and the mean reduction in AK lesion count to day 57 on the face or scalp with tirbanibulin 1% ointment treatment were statistically significantly greater compared with vehicle treatment at all-time points assessed.

In the efficacy pooling, the median percent reduction from baseline (median baseline lesion count: 6) at Day 57 was 87.5% in the tirbanibulin 1% ointment group and 20.0% in the vehicle group.

Both studies had a recurrence follow up period. There was a steady recurrence of lesions in the follow up period over time, which was similar between both studies, although the rate of recurrence was higher on the scalp compared to the face. In study **KX01-AK-003** at 12 months post-Day 57, 16 patients among 60 (26%) who received treatment on the face and 2 patients among 17 (12%) who received treatment on the scalp had a recurrence. In study **KX01-AK-004,** 23 of 73 (31%) patients who received treatment on the face and 4 of 24 (17%) patients who received treatment on the scalp recurred. In the pooled analyses of the 2 Phase III studies: 70% of patients up to 3 months, 49% up to 6 months, 34% up to 9 months, and 27% up to 12 months showed sustained clearance, showing a gradual loss of effect over time.

The applicant's clinical development programme was generally in line with CHMP advice. CHMP had recommended the applicant to gather additional long-term safety data (up to 3 years) and to determine the related conversion rate of AK lesions to SCC or BCC. However, it could be accepted that longer-term data are provided post-approval.

The applicant will conduct a Phase IV, long-term, randomised, active-controlled, investigator-blinded, safety and re-treatment study (M-14789-41) in adult patients with AK on the face or scalp (see RMP,

PASS category 1 study). The draft protocol was submitted and is broadly acceptable. The study objectives are to determine the incidence of AK progression to SCC after treatment with tirbanibulin 1% ointment, and to evaluate the safety and efficacy of re-treatment. This long-term study is in the planning phase and will be conducted in EU countries and Russia. In contrast to the completed Phase IIa and Phase III studies, patients will be re-treated with tirbanibulin 1% ointment (up to 4 treatment cycles per year), if necessary. The protocol will be submitted for approval.

2.5.4. Conclusions on the clinical efficacy

Overall the observed improvement in complete and partial clearance rates with Klisyri is considered clinically relevant for single period of 5-day use on the scalp and head. The use of this product is restricted to single use treatment 5 days to a maximal area of 25 cm^2.

2.6. Clinical safety

The clinical development programme for topical tirbanibulin 1% ointment for the treatment of AK included a total of 9 clinical studies. Safety results from 2 Phase III Studies, KX01-AK-003 and KX01-AK-004, and the Phase IIa Study KX01-AK-002 form the basis for this MAA. Key results from the Phase I studies in patients with AK (Studies KX01-AK-01-US and KX01-AK-007) and in healthy subjects (Studies KX01-AK-006, KX01-AK-008, KX01-AK-009, and KX01-AK-010) are also presented at the end of the section.

Patient exposure

Pivotal Phase III Studies KX01-AK-003 and KX01-AK-004

Pooling

The 2 pivotal Phase III Studies KX01-AK-003 and KX01-AK-004 were identical in terms of objectives, study design, patient population, safety assessments, and safety endpoints. Both studies evaluated the safety of the proposed treatment regimen, tirbanibulin 1% ointment once daily for 5 consecutive days, in adult patients with AK on the face or scalp, with a 1-year safety follow-up.

Therefore, an integrated analysis of safety was performed based on pooled data from all evaluable patients in these 2 studies to maximise exposure information and to assess overall safety of the proposed dose regimen of tirbanibulin in patients with AK.

The pooled data for tirbanibulin 1% ointment was compared with pooled data for the vehicle control. For reference, the safety data for each individual Phase III study are presented alongside the pooled safety data as well as the individual safety data of the Phase IIa Study KX01-AK-002.

Due to differences in treatment duration, treatment location, treatment area, and study population, the safety results in the Phase IIa and Phase I studies in patients (KX01-AK-002, KX01-AK-01-US, and KX01-AK-007) and the dermal safety studies in healthy subjects (KX01-AK-006, KX01-AK-008, KX01-AK-009, and KX01-AK-010) were not pooled, but are presented separately.

Exposure

Summary of exposure to tirbanibulin 1% ointment across safety studies

	Patients enrolled	Patients exposed	Patients exposed to the proposed dose range	Patients with long term* safety data
Placebo-controlled	1094	741	353	173
Active-controlled	0	0	0	0
Open studies	198	198	0	63
Post marketing	0	0	0	0
Compassionate use	0	0	0	0
Total	1292	939	353	236

* The Recurrence Follow-up Population In Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002 with a 12-month Safety Follow-up, was defined as all patients in the ITT Population who achieved complete clearance at the Day 57 visit.

A total of 1310 patients/subjects have been enrolled and treated in 9 clinical studies for the evaluation of tirbanibulin 1% ointment in the treatment of AK: 2 Phase III studies, 1 Phase IIa study, 2 Phase I studies in patients with AK, and 4 Phase I studies in healthy subjects. Of the 1310 patients/subjects, 961 (569 patients with AK and 392 healthy subjects) received the active treatment tirbanibulin and 349 received vehicle ointment. When types of studies were considered, 1094 patients/subjects were enrolled in double-blind or evaluator-blind studies and 216 were enrolled in open-label studies. Of the 569 patients, 455 patients received the proposed treatment regimen, tirbanibulin 1% ointment applied to the face or scalp once daily for 5 consecutive days and 84 patients for 3 days; and the remaining 30 patients received tirbanibulin 1% ointment on the dorsal forearm, up to an area of 100 cm^2 and up to once daily for 5 consecutive days.

Adverse events

Common AEs

Safety assessment up to Day 57:

The most common TEAEs (PTs) across Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002 were the following:

- Application site pain: 4% to 13% of patients across the tirbanibulin 1% ointment groups, and 3% of patients in each of the vehicle groups.
- Application site pruritus: 7% to 11% of patients across the tirbanibulin 1% ointment groups, and 5% to 8% of patients in the vehicle groups.
- Upper respiratory tract infection: 3% to 4% of patients across the tirbanibulin 1% ointment groups, and 4% to 6% of patients in the vehicle groups.

No other specific TEAE was reported by more than 5% of patients across Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002.

The incidence of application site AEs (pruritus and pain) were higher in the pooled tirbanibulin 1% ointment group (9% and 10% patients, respectively) than in the pooled vehicle group (6% and 3% patients, respectively). These application site AEs were all considered to be related to study treatment.

The incidence of upper respiratory tract infection was similar between both the pooled tirbanibulin (4% patients) and pooled vehicle groups (5% patients), and the incidence of viral respiratory tract infection was similar for the 2 pooled treatment groups (3% patients each).

Summary of Treatment-emergent Adverse Events up to Day 57 Occurring in ≥2% of Patients in any Treatment Group - Phase III Patient Studies: KX01-AK-003, KX01-AK-004 (Pooled and by Study) and Phase II Patient Study: KX01-AK-002 (Safety Population)

	Tirbanibulin 1% ointment, 5 day, once daily				Vehicle, 5 day, once daily		
Preferred Term n (%)	KX01-AK-003 (N=175)	KX01-AK-004 (N=178)	Pooled analysis (N=353)	KX01-AK-002 (N=84)	KX01-AK-003 (N=176)	KX01-AK-004 (N =173)	Pooled analysis (N=349)
Application site pain[a]	11 (6)	24 (13)	35 (10)	3 (4)	6 (3)	5 (3)	11 (3)
Application site pruritus	13 (7)	19 (11)	32 (9)	6 (7)	8 (5)	13 (8)	21 (6)
Benign prostatic hyperplasia	0	0	0	2 (2)	1 (<1)	0	1 (<1)
Bronchitis	3 (2)	1 (<1)	4 (1)	0	4 (2)	0	4 (1)
Cough	1 (<1)	1 (<1)	2 (<1)	0	0	3 (2)	3 (<1)
Diarrhoea	0	1 (<1)	1 (<1)	2 (2)	1 (<1)	1 (<1)	2 (<1)
Dizziness	1 (<1)	0	1 (<1)	3 (4)	0	0	0
Headache	3 (2)	1 (<1)	4 (1)	2 (2)	2 (1)	2 (1)	4 (1)
Hypertension	1 (<1)	1 (<1)	2 (<1)	2 (2)	1 (<1)	0	1 (<1)
Muscle strain	0	0	0	2 (2)	0	0	0
Nasopharyngitis	0	0	0	2 (2)	0	0	0
Sinusitis	1 (<1)	0	1 (<1)	0	2 (1)	3 (2)	5 (1)
Skin abrasion	4 (2)	3 (2)	7 (2)	0	5 (3)	3 (2)	8 (2)
Skin injury	0	0	0	3 (4)	0	0	0
Squamous cell carcinoma	4 (2)	2 (1)	6 (2)	1 (1)	1 (<1)	1 (<1)	2 (<1)
Upper respiratory tract infection	6 (3)	7 (4)	13 (4)	0	7 (4)	10 (6)	17 (5)
Urinary tract infection	1 (<1)	0	1 (<1)	1 (1)	0	3 (2)	3 (<1)
Viral upper respiratory tract infection	3 (2)	8 (4)	11 (3)	0	5 (3)	4 (2)	9 (3)

CSR=clinical study report; MedDRA=Medical Dictionary for Regulatory Activities.

a) Includes pain, tenderness, stinging, and burning sensation at the application site.

Note: Results are presented for the Safety Population, which in each study was defined as all patients who received at least 1 dose of study treatment.

Adverse events were coded with MedDRA v18.1 in Study KX01-AK-002 and with MedDRA v20.0 in Studies KX01-AK-003 and KX01-AK-004.

Recurrence Follow-up Period:

During the Recurrence Follow-up Period, a few TEAEs were reported in no more than 1 patient across the studies in the tirbanibulin 1% ointment group. No TEAEs were reported across the studies in the vehicle group. Two patients in Study KX01-AK-004 had 1 TEAE each (BCC [outside the treatment area] and colon cancer, both unrelated to study treatment and not serious). In Study KX01-AK-002, 1 patient had a severe serious TEAE of cerebrovascular accident which resolved with sequelae and was not considered related to treatment.

Treatment-Related AEs

Safety assessment up to Day 57:

Table 2.7.4-13 Summary of Treatment-related Treatment-emergent Adverse Events up to Day 57 Occurring in ≥2% of Patients in any Treatment Group – Phase III Patient Studies: KX01-AK-003, KX01-AK-004 (Pooled and by Study) and Phase II Patient Study: KX01-AK-002 (Safety Population)

	Tirbanibulin 1% ointment, 5 day, once daily				Vehicle, 5 day, once daily		
Preferred Term n (%)	KX01-AK-003 (N=175)	KX01-AK-004 (N=178)	Pooled analysis (N=353)	KX01-AK-002 (N=84)	KX01-AK-003 (N=176)	KX01-AK-004 (N =173)	Pooled analysis (N=349)
Application site pain[a]	11 (6)	24 (13)	35 (10)	3 (4)	6 (3)	5 (3)	11 (3)
Application site pruritus	13 (7)	19 (11)	32 (9)	5 (6)	8 (5)	13 (8)	21 (6)
Dizziness	0	0	0	2 (2)	0	0	0

CSR=clinical study report; MedDRA=Medical Dictionary for Regulatory Activities.

a) Includes pain, tenderness, stinging, and burning sensation at the application site

Note: Results are presented for the Safety Population, which in each study was defined as all patients who received at least 1 dose of study treatment.

Adverse events were coded with MedDRA v18.1 in Study KX01-AK-002 and with MedDRA v20.0 in Studies KX01-AK-003 and KX01-AK-004.

The most common treatment-related TEAEs (PTs) across Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002 were the following:

- Application site pain: 4% to 13% of patients across the tirbanibulin 1% ointment groups, and 3% of patients in each of the vehicle groups.
- Application site pruritus: 6% to 11% of patients across the tirbanibulin 1% ointment groups, and 5% to 8% of patients in the vehicle groups.

No other specific treatment-related TEAE was reported by more than 2 patients across Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002.

The incidence of treatment-related TEAEs of application site pruritus and pain were higher in the pooled tirbanibulin 1% ointment group (9% and 10% patients, respectively) than in the pooled vehicle group (6% and 3% patients, respectively).

Recurrence Follow-up Period:

None of the TEAEs reported during the Recurrence Follow-up Period were treatment-related across the studies in the tirbanibulin 5-day dosing groups.

Severity

Safety assessment up to Day 57:

The majority of TEAEs across Studies KX01-AK-003, KX01-AK-004, and KX01-AK-002 were of mild or moderate severity and the frequency of severe TEAEs was low.

Severe TEAEs across Studies KX01-AK-003, KX01-AK-004 (pooled analysis) were reported in 3 patients in the tirbanibulin 1% ointment group and 7 patients in the vehicle group, and in KX01-AK-002 from 1 patient. Of these severe TEAEs, only application site pruritus (tirbanibulin 1% ointment group in Study KX01-AK-003) was considered by the investigator to be related to study treatment, and the event was not serious and resolved.

Among the patients with treatment-related TEAEs in tirbanibulin 1% ointment group, 1 (<1%) patient had severe treatment-related AEs (application site pruritus), 12 (3%) patients had moderate treatment-related AEs (the events of moderate severity included application site pruritus, application site pain, headache, application site nodule, application site scab, skin odour abnormal, and viral upper respiratory tract infection), and the remaining 43 (12%) patients had treatment-related AEs of mild severity. With the exception of 1 AE of moderate severity (application site pain), all treatment-related AEs in the vehicle group were of mild in severity. All treatment-related AEs resolved. No patient discontinued treatment due to treatment-related AEs.

Recurrence Follow-up Period:

During the Recurrence Follow-up Period, severe TEAEs were reported in 3 patients across the studies in the tirbanibulin 1% ointment group:

- In Study KX01-AK-003, 1 patient had 3 severe and serious TEAEs of sepsis, hairy cell leukaemia, haemoglobin decreased; none were treatment-related and all resolved except the AE of hairy cell leukaemia.
- In Study KX01-AK-004, 1 patient had colon cancer, which was not serious, not treatment-related, and which resolved.
- In Study KX01-AK-002, 1 patient had a severe serious TEAE of cerebrovascular accident, which was not treatment-related, and which resolved with sequelae on Day 350. The patient was discontinued from the study on Day 415 because of the clinically significant AE.

Table 26 Summary of Overall Adverse Events Up to Day 57 – Studies KX01-AK-002, KX01-AK-003, and KX01-AK-004 - Safety Population

	Tirbanibulin Ointment 1%				Vehicle	
	KX01-AK-002 3 day n (%)	KX01-AK-002 5 day n (%)	KX01-AK-003 5 day n (%)	KX01-AK-004 5 day n (%)	KX01-AK-003 5 day n (%)	KX01-AK-004 5 day n (%)
Number of subjects in the Safety Population	84	84	175	178	176	173
Number of subjects with any adverse events	21 (25)	35 (42)	66 (38)	71 (40)	62 (35)	73 (42)
Number of subjects with any treatment-emergent adverse events (a)	18 (21)	34 (40)	57 (33)	67 (38)	57 (32)	67 (39)
Number of subjects with any adverse events that are at least possibly related to study drug (b)	3 (4)	9 (11)	20 (11)	36 (20)	16 (9)	19 (11)
Number of subjects with any serious adverse events	2 (2)	2 (2)	0	1 (<1)	2 (1)	4 (2)
Number of subjects with any severe adverse events	2 (2)	1 (1)	2 (1)	1 (<1)	2 (1)	4 (2)
Number of subjects with any adverse events leading to treatment discontinuation	0	0	1 (<1)	0	0	0
Number of subjects with any adverse events leading to study discontinuation	0	0	0	0	0	0
Number of subjects who died	0	0	0	0	1 (<1)[c]	0

a Treatment-emergent adverse events are those start on or after the first dose or those worsen after the first dose. Adverse events, which cannot be determined due to missing value, are considered as treatment-emergent.

b Treatment-related adverse events are adverse events with relationship to study treatment as 'Definitely Related', 'Possibly Related', 'Probably Related' or unknown.

c Death was unrelated to study treatment (suicide).

Adverse Events of Special Interest

Skin Cancer

Medical History of Skin Cancer

In the Phase III Studies KX01-AK-003 and KX01-AK-004, a large proportion of patients had a history of skin cancer (47% [166 of 351 patients] and 42% [147 of 351 patients], respectively). Patients with a history of skin cancer were similarly distributed between the tirbanibulin 1% ointment and vehicle groups in each of the Phase III studies. The most common types of skin cancer in the medical history of patients in the Phase III studies included BCC, SCC, and melanoma.

A tabulated patient listing of treatment-emergent skin cancers (i.e., skin cancers with start date after the start of study treatment) reported in Studies KX01-AK-003, KX01-AK-004 is provided in the table below.

No skin cancers were reported in Study KX01-AK-007 or the Phase I dermal safety studies in healthy subjects. Except for 1 skin cancer, all of the 23 treatment-emergent skin cancers in 19 patients occurred outside the treatment area. None of the treatment-emergent skin cancers were related to study treatment. The occurrence of treatment-emergent skin cancers was similar between the tirbanibulin 1% ointment and vehicle groups for the 2 Phase III studies.

Table 42 Tabulated Listing of Treatment Emergent Skin Cancers by Study and Treatment – Studies KX01-AK-003 and KX01-AK-004 – Safety Population

Study	Treatment Group	Subject Number	Treatment Location	Skin Cancer, verbatim term	Start Date	End Date	Inside Treatment Area
KX01-AK-003	Vehicle	[redacted]	Face	SCC, right temple	Day 43	Day 43	No
			Face	SCC, right ear	Day 11	Ongoing	No
			Face	BCC, nodular type, right lateral scalp	Day 5	Day 73	No
			Scalp	BCC, right postauricular	Day 29	Day 84	No
	tirbanibulin ointment 1%		Face	Melanoma in situ, chest	Day 55	Day 78	No
				SCC in situ, left cheek/jawline	Day 55	Day 78	No
			Face	Possible SCC, left cheek	Day 55	Day 78	No
				Possible SCC, right helix	Day 55	Day 78	No
				Possible SCC, left forearm	Day 55	Day 78	No
			Face	BCC, right nose	Day 7	Day 70	No
				SCC, right ear	Day 7	Day 70	No
			Scalp	SCC	Day 74	Day 98	Yes
			Scalp	SCC, left dorsal hand	Day 15	Day 31	No
KX01-AK-004	Vehicle		Face	BCC, right superior nasal side wall	Day 15	Ongoing	No
			Face	SCC, posterior neck	Day 17	Day 30	No
			Face	SCC, left anterior shoulder	Day 43	Day 57	No
	tirbanibulin ointment 1%		Face	BCC, left side of neck	Day 8	Day 56	No
			Face	BCC, left temple	Day 99	Day 118	No
			Scalp	Melanoma in situ, right upper back	Day 43	Day 64	No
			Face	SCC, left forearm	Day 7	Day 33	No
			Face	SCC, right medial forehead	Day 57	Ongoing	No

BCC = basal cell carcinoma; SCC = Squamous cell carcinoma.
Source: CSR KX01-AK-003 Listings 16.2.1.2, 16.2.7.2, 16.2.7.2.1; CSR KX01-AK-004 Listings 16.2.1.2, 16.2.5, 16.2.7.2, 16.2.7.2.2.

The table below provides a tabulation of subjects with treatment-emergent skin cancers reported in Studies KX01-AK-002 and KX01-AK-01-US. In Study KX01-AK-002, 1 subject in Cohort 1 (5-day treatment) and 2 subjects in Cohort 2 (3-day treatment) developed skin cancer during the study. In Study KX01-AK-01-US, 1 subject in Cohort 4 (5-day 100cm2 treatment area) developed BCC. In all subjects, the treatment-emergent skin cancer occurred outside the treatment area. None of the skin cancers were considered to be related to study drug.

Table 43 Tabulated Listing of Treatment Emergent Skin Cancers by Study and Treatment – Studies KX01-AK-002 and KX01-AK-01-US – Safety Population

Study	Treatment Group	Subject Number	Treatment Location	Skin Cancer, verbatim term	Start Date	End Date	Inside Treatment Area
KX01-AK-002	tirbanibulin ointment 1% daily for 5 days (Cohort 1)	[redacted]	Scalp	SCC	Day 3	Day 53	No
	tirbanibulin ointment 1% daily for 3 days (Cohort 2)		Scalp	SCC, left pre-auricular area	Day 36	Day 36	No
			Face	SCC, scalp	Day 43	Day 72	No
KX01-AK-01-US	tirbanibulin ointment 1% 200 mg daily for 5 days over 100 cm^2 (Cohort 4)		Dorsal forearm	BCC	Day 3	Day 25	No

BCC = basal cell carcinoma; SCC = Squamous cell carcinoma.
a A safety narrative was not written for Subject 01-71; however, a hyperlink is provided to the source for this information in CSR KX01-AK-01-US Listing 16.2.7.

Ocular Exposure

No cases of ocular exposure to tirbanibulin 1% ointment or vehicle were reported across all studies in patients with AK or healthy subjects. None of the 6 ocular TEAEs reported during the Phase III and IIa studies were related to study treatment, and all resolved.

Overdose

Overdose happened in 1 study of the clinical programme for safety.

One patient in the 5-day treatment group of Study KX01-AK-002 received 10 times the protocol-specified dose (500 mg tirbanibulin 1% ointment) for each of the 5 applications received but did not experience TEAEs or LSRs of higher incidence or severity than patients who received the protocol-specified dose of 50 mg per application. The patient completed the Treatment Period and had his last dose of study treatment on Day 5 after 5 days of dosing. The patient had complete clearance at Day 57 and entered the Recurrence Follow-up Period. The patient discontinued from the study on Day 240 due to AK recurrence. Another 2 patients also had an overdose in the 3-day dosing group in Study KX01-AK-002.

Pregnancy

One pregnancy occurred during the clinical development programme (Study KX01-AK-006) and no pregnancies reported in the phase 2 or phase 3 trials. There are no data on excretion of tirbanibulin/metabolites in human or animal milk. The effects of tirbanibulin on the breastfed infant, or its effects on milk production, are unknown.

Local Skin Reactions

Local tolerability was assessed by evaluating the following signs: erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation, and erosion/ulceration, which were referred to as LSRs. For the Phase III Studies and the Phase II study, evaluations of LSRs, pigmentation, and scarring were conducted during the Treatment and Follow-up Periods only, not during Recurrence Follow-up.

Summary of Local Skin Reactions (up to Day 57) – Pooled Phase III Patient Studies KX01-AK-003 and KX01-AK-004 (Safety Population)

	Absent (0)		Mild (1)		Moderate (2)		Severe (3)	
LSR sign Analysis visit	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)
Erythema, n (%)								
Baseline [a]	263 (75)	257 (74)	82 (23)	84 (24)	8 (2)	8 (2)	0	0
Day 5 [b]	32 (9)	194 (56)	131 (38)	138 (40)	173 (50)	13 (4)	10 (3)	0
Day 8 [c]	22 (6)	224 (65)	116 (33)	117 (34)	199 (57)	5 (1)	14 (4)	0
Day15 [d]	69 (20)	243 (70)	226 (64)	95 (27)	57 (16)	10 (3)	0	0
Day 29 [e]	239 (68)	271 (79)	106 (30)	72 (21)	7 (2)	2 (<1)	0	0
Day 57 [f]	292 (83)	291 (84)	58 (16)	54 (16)	3 (<1)	2 (<1)	0	0
Maximal Post-Baseline Grade [g]	9 (3)	160 (46)	95 (27)	165 (47)	227 (64)	24 (7)	22 (6)	0
Flaking/Scaling, n (%)								
Baseline [a]	253 (72)	241 (69)	96 (27)	100 (29)	4 (1)	8 (2)	0	0
Day 5 [b]	115 (33)	214 (62)	190 (55)	120 (35)	39 (11)	11 (3)	2 (<1)	0
Day 8 [c]	49 (14)	226 (65)	122 (35)	104 (30)	152 (43)	16 (5)	27 (8)	0
Day15 [d]	124 (35)	229 (66)	171 (49)	107 (31)	52 (15)	11 (3)	5 (1)	1 (<1)
Day 29 [e]	280 (80)	253 (73)	71 (20)	89 (26)	1 (<1)	3 (<1)	0	0
Day 57 [f]	299 (85)	273 (79)	53 (15)	71 (20)	1 (<1)	3 (<1)	0	0
Maximal Post-Baseline Grade [g]	24 (7)	154 (44)	130 (37)	159 (46)	168 (48)	35 (10)	31 (9)	1 (<1)
Crusting, n (%)								
Baseline [a]	326 (92)	316 (91)	24 (7)	29 (8)	3 (<1)	4 (1)	0	0
Day 5 [b]	263 (76)	316 (92)	71 (21)	26 (8)	12 (3)	3 (<1)	0	0
Day 8 [c]	202 (58)	316 (91)	98 (28)	29 (8)	43 (12)	1 (<1)	7 (2)	0
Day15 [d]	285 (81)	321 (92)	59 (17)	26 (7)	8 (2)	1 (<1)	0	0
Day 29 [e]	343 (97)	327 (95)	9 (3)	16 (5)	0	2 (<1)	0	0
Day 57 [f]	345 (98)	324 (93)	7 (2)	20 (6)	1 (<1)	3 (<1)	0	0
Maximal Post-Baseline Grade [g]	176 (50)	288 (83)	120 (34)	52 (15)	50 (14)	9 (3)	7 (2)	0

LSR sign Analysis visit	Absent (0)		Mild (1)		Moderate (2)		Severe (3)	
	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)	Tirbanibulin (N=353)	Vehicle (N=349)
Swelling, n (%)								
Baseline [a]	352 (>99)	347 (>99)	1 (<1)	2 (<1)	0	0	0	0
Day 5 [b]	251 (73)	337 (98)	76 (22)	7 (2)	18 (5)	1 (<1)	1 (<1)	0
Day 8 [c]	246 (70)	341 (99)	81 (23)0	5 (1)	22 (6)	0	1 (<1)	0
Day15 [d]	315 (89)	341 (98)	34 (10)	7 (2)	3 (<1)	0	0	0
Day 29 [e]	347 (99)	345 (100)	5 (1)	0	0	0	0	0
Day 57 [f]	351 (>99)	346 (>99)	1 (<1)	1 (<1)	01 (<1)	0	0	0
Maximal Post-Baseline Grade [g]	217 (61)	332 (95)	102 (29)	16 (5)	32 (9)	1 (<1)	2 (<1)	0
Vesiculation/Pustulation, n (%)								
Baseline [a]	352 (>99)	349 (100)	1 (<1)	0	0	0	0	0
Day 5 [b]	332 (96)	345 (100)	13 (4)	0	1 (<1)	0	0	0
Day 8 [c]	334 (95)	345 (>99)	16 (5)	1 (<1)	0	0	1 (<1)	0
Day15 [d]	349 (>99)	346 (>99)	2 (<1)	2 (<1)	1 (<1)	0	0	0
Day 29 [e]	349 (>99)	344 (>99)	2 (<1)	1 (<1)	0	0	1 (<1)	0
Day 57 [f]	353 (100)	347 (100)	0	0	0	0	0	0
Maximal Post-Baseline Grade [g]	324 (92)	346 (>99)	25 (7)	3 (<1)	2 (<1)	0	2 (<1)	0
Erosion/Ulceration, n (%)								
Baseline [a]	349 (99)	347 (>99)	4 (1)	2 (<1)	0	0	0	0
Day 5 [b]	333 (96)	342 (>99)	10 (3)	3 (<1)	3 (<1)	0	0	0
Day 8 [c]	327 (93)	340 (98)	19 (5)	6 (2)	5 (1)	0	0	0
Day15 [d]	338 (96)	345 (>99)	11 (3)	3 (<1)	3 (<1)	0	0	0
Day 29 [e]	352 (100)	343 (>99)	0	2 (<1)	0	0	0	0
Day 57 [f]	353 (100)	346 (>99)	0	1 (<1)	0	0	0	0
Maximal Post-Baseline Grade [g]	311 (88)	339 (97)	33 (9)	10 (3)	9 (3)	0	0	0

LSR=local skin reaction.

a) Total number of patients assessed at baseline for each LSR sign: 353 patients for the tirbanibulin group, 349 patients for the vehicle group.

b) Total number of patients assessed at Day 5 for each LSR sign: 346 patients for the tirbanibulin group, 345 patients for the vehicle group.

c) Total number of patients assessed at Day 8 for each LSR sign: 351 patients for the tirbanibulin group, 346 patients for the vehicle group.

d) Total number of patients assessed at Day 15 for each LSR sign: 352 patients for the tirbanibulin group, 348 patients for the vehicle group.

e) Total number of patients assessed at Day 29 for each LSR sign: 352 patients for the tirbanibulin group, 345 patients for the vehicle group.

f) Total number of patients assessed at Day 57 for each LSR sign: 353 patients for the tirbanibulin group, 347 patients for the vehicle group.

g) Total number of patients assessed at for the maximal post-baseline grade for each LSR sign: 353 patients for the tirbanibulin group, 349 patients for the vehicle group.

Local tolerability signs were graded using a 4-point scale: absent (0), mild (1), moderate (2), and severe (3) and referred to as LSRs in Studies KX01-AK-003 and KX01-AK-004.

At baseline, both treatment groups were well matched for the presence of LSRs, primarily consisting of mild erythema (23% to 24% patients) or flaking/scaling (27% to 29% patients) at the treatment site.

Following treatment, LSRs were transient in both treatment groups but were more frequent in the tirbanibulin 1% ointment group compared with vehicle. The difference in incidence of LSRs were more pronounced for the moderate to severe category across the 2 treatment groups, though severe LSRs were overall infrequent. LSRs peaked by Day 8 and, by Day 29, all signs were at or close to their baselines in both incidence and severity.

Erythema and flaking/scaling were frequently observed and were mostly mild to moderate in severity. LSRs of crusting, swelling, vesiculation/pustulation, and erosion/ulceration occurred less frequently, and were generally mild. Severe LSRs were infrequent among patients treated with tirbanibulin 1% ointment. The highest incidence of severe LSRs was flaking/scaling at Day 8 (27 patients, 8%), erythema at Day 8 (14 patients, 4%) and at Day 5 (10 patients, 3%); all other signs occurred in <1% to 2% of patients. The severe LSR of flaking was reported in 1 patient in the vehicle group. Generally, LSRs were self-limited and did not require treatment. All patients with residual LSRs were followed until resolution or stabilisation.

Overall, severe LSRs occurred in 46 (13%) patients in the tirbanibulin 1% ointment group and in 1 (0.3%) patient in the vehicle group for the pooled safety analysis (Studies KX01-AK-003 and KX01-AK-004). One patient discontinued treatment due to a TEAE during the tirbanibulin 1% ointment

development programme up to day 57 which was unrelated to study treatment. In the individual studies, severe LSRs occurred in 14 (8%) patients in the tirbanibulin 1% ointment group and there were none in the vehicle group (Study KX01-AK-003), and in 32 (18%) patients in the tirbanibulin 1% ointment group and in 1 (0.6%) patient in the vehicle group (Study KX01-AK-004).

The analysis of maximal post-baseline LSRs greater than baseline provides the worst case observed for incidence, and the highest severity, irrespective of study visit after baseline. Incidence and severity of LSRs were higher in the tirbanibulin 1% ointment group than the vehicle group. Within the tirbanibulin 1% ointment group, the most commonly occurring LSRs were moderate erythema (63% patients) and flaking/scaling (47% patients); less frequently reported were mild crusting and mild swelling (30% and 29% patients, respectively); least frequently reported were mild vesiculation/pustulation and mild erosion/ulceration (7% and 9% patients, respectively). The incidence of patients with severe LSR was highest for flaking/scaling (9% patients), followed by erythema (6% patients), and then all other LSR categories (≤2% patients). No patient experienced severe erosion/ulceration in the tirbanibulin 1% ointment group.

In Study KX01-AK-002, LSRs were assessed using a 5-point scale ranging from Grades 0 (not present), 1 (minimal), 2 (mild), 3 (moderate), and 4 (worst). At baseline, 15 (18%), 18 (21%), and 5 (6%) patients in the tirbanibulin 1% ointment 5-day dosing group had Grade 1 erythema, flaking/scaling, or crusting, respectively; other LSRs (swelling, vesiculation/pustulation, erosion/ulceration) were not present. In the tirbanibulin 1% ointment 5-day dosing group, the majority of LSRs were Grade 1 and Grade 2, and consisted of erythema and flaking/scaling.

LSRs began by Day 2 and peaked by the end of treatment; by Day 15, there were more patients with ≥Grade 2 erythema or flaking/scaling; by Day 29, all LSRs were close to baselines; and by Day 57, almost all patients had all LSRs as not present or Grade 1. The tirbanibulin treatment was well tolerated and the LSRs were self-limited and did not require treatment.

In Study KX01-AK-002, composite LSR score was defined as the sum (possible range, 0 to 24) of the individual LSR grades for each sign at each visit. The composite LSR scores for the tirbanibulin 1% ointment 5-day dosing group were consistent with the trends observed for individual LSR grades in that by Day 29; signs were at or below the baseline scores.

Maximal post-baseline Grade 2 or Grade 3 were reported mainly for erythema, flaking/scaling, and crusting LSRs; 1 patient had Grade 4 erythema and flaking/scaling in the tirbanibulin 1% ointment 5-day dosing group. Swelling, vesiculation/pustulation, and erosion/ulceration were generally not present or were presented at post-baseline Grade 1.

Pigmentation and Scarring

The incidences of hypopigmentation, hyperpigmentation, and scarring were similar between the tirbanibulin 1% ointment and vehicle groups. The number of patients in the vehicle and tirbanibulin 1% ointment groups with pigmentation or scarring remained relatively consistent over time and was generally lower at Day 57 than at baseline.

The number of patients with pigmentation/scarring peaked by the end of treatment, were at or below baseline by Day 15, and were at baseline for hyperpigmentation, and below baseline for scarring and hypopigmentation by Day 57.

Serious adverse event/deaths/other significant events

In total, 8 patients had a serious TEAE during the Treatment/Follow-Up Period in the pooled dataset: 3 in the tirbanibulin 5-day dosing groups and 5 in the vehicle groups. Of these, 3 patients in the

tirbanibulin 5-day dosing group had a serious TEAE during the Recurrence Follow-up Period. None of the serious TEAEs were considered to be related to study treatment. Two patients did not recover from the serious TEAE (1 patient in the tirbanibulin 1% ointment group continued to have hairy cell leukaemia and 1 patient in the vehicle group died due to completed suicide).

Safety Assessment up to Day 57:

Three patients reported 5 serious TEAEs across the studies in the tirbanibulin 1% ointment groups: benign prostatic hyperplasia (2 events), intestinal obstruction (severe), pulmonary embolism (severe), and chest pain; and 5 patients reported 7 serious TEAEs (all severe) across the vehicle groups: myocardial infarction, aortic valve stenosis, completed suicide, arthralgia, intervertebral disc degeneration, complete atrioventricular block, and bacteraemia. None of the serious TEAEs were treatment-related.

Recurrence Follow-up Period:

During the Recurrence Follow-up Period, 3 patients reported 5 serious TEAEs following daily application for 5 days in the tirbanibulin 1% ointment groups: 1 patient (3 serious TEAEs of sepsis, hairy cell leukaemia, haemoglobin decreased) in Study KX01-AK-003, 2 patients (serious TEAEs of cerebrovascular accident and VIIth nerve paralysis) in Study KX01-AK-002, and no serious TEAE in Study KX01-AK-004. None of the serious TEAEs were considered related to study treatment.

Laboratory findings

Clinical Laboratory Evaluations

No clinically meaningful changes from baseline were observed for haematology, blood chemistry, or urinalysis parameters across the studies in patients with AK. There were no clinically significant abnormalities that were considered related to tirbanibulin.

Vital Signs and Physical Examinations

No clinically meaningful changes from baseline were observed in vital signs across the studies in patients with AK. No clinically meaningful changes in physical examination findings over time were observed across the 5 studies in patients with AK.

Minimal mean body weight changes were noted from baseline to Day 57 in all treatment groups in the Phase III studies and the Phase IIa study.

Electrocardiograms

When tirbanibulin was used as intended in clinical practice (daily application of tirbanibulin 1% ointment for 5 consecutive days), information on the effects of tirbanibulin on cardiac repolarisation, using both direct measures of QT in human studies and in a series of non-clinical studies, indicate that tirbanibulin does not affect cardiac repolarisation.

Phase III Patient Studies: KX01-AK-003 and KX01-AK-004

Few clinically significant treatment-emergent ECG abnormalities were observed in Studies KX01-AK-003 and KX01-AK-004. These were mostly asymptomatic arrhythmias (including sinus bradycardia, ventricular premature beats, atrial premature beats, first-degree heart block, T-wave inversion, or flat/low negative T waves) that did not require intervention and were not considered related to study treatment.

ERT Cardiac Safety Report

ECG data from Days 5 and 15 in the 2 Phase III studies were combined as part of a pooled analysis exploring the potential effect of tirbanibulin 1% ointment on ECG parameters. For this analysis, the 12-lead ECGs were centrally read and interpreted by technical experts. There were no treatment effects on QTcF and other ECG intervals/parameters (HR, RR, PR, QRS, and T wave morphology) of clinical or regulatory concern.

Phase II Patient Study: KX01-AK-002

In Study KX01-AK-002, 2 abnormal, clinically significant ECG findings in 1 patient were reported as TEAEs: ECG QT prolonged and defect conduction interventricular, both TEAEs were reported on Day 57 with moderate severity. The patient remained asymptomatic and stable, and had a significant medical history of hypertension and hypothyroidism. Both TEAEs were considered not related to study treatment and resolved by Day 68 without treatment.

The few other clinically significant ECG abnormalities that were observed included first-degree heart block in 2 patients (1 patient each on Days 8 and 57) and prolonged QT at Day 57 in 2 additional patients. None of these patients were symptomatic.

No other changes of clinical importance in ECG readings over time were reported.

Safety in special populations

The table below summarises the pooled incidence of treatment-related TEAEs by the subgroups age, gender, treatment location, and baseline AK lesion group for patients in the tirbanibulin 1% ointment and vehicle groups in from the Phase III Studies KX01-AK-003 and KX01-AK-004, up to Day 57.

Table 2.7.4-23 Summary of Treatment-related TEAEs by Subgroups in ≥2% of Patients in Any Subgroup Category (up to Day 57) – Pooled Phase III Patient Studies KX01-AK-003 and KX01-AK-004 (Safety Population)

Subgroup category Preferred term	Number (%) of patients			
	Tirbanibulin 1% ointment 5-days, once daily		Vehicle 5-days, once daily	
Age	**<65 years (N=107)**	**≥65 years (N=246)**	**<65 years (N=81)**	**≥65 years (N=268)**
Any treatment-related TEAE [a]	18 (17)	38 (15)	8 (10)	27 (10)
Application site pain	12 (11)	23 (9)	3 (4)	8 (3)
Application site pruritus	10 (9)	22 (9)	4 (5)	17 (6)
Gender	**Male (N=305)**	**Female (N=48)**	**Male (N=304)**	**Female (N=45)**
Any treatment-related TEAE [a]	48 (16)	8 (17)	34 (11)	1 (2)
Application site pain	30 (10)	5 (10)	11(4)	0
Application site pruritus	26 (9)	6 (13)	20 (7)	1 (2)
Treatment location	**Face (N=238)**	**Scalp (N=115)**	**Face (N=239)**	**Scalp (N=110)**
Any treatment-related TEAE [a]	41 (17)	15 (13)	27 (11)	8 (7)
Application site pain	26 (11)	9 (8)	9 (4)	2 (2)
Application site pruritus	23 (10)	9 (8)	18 (8)	3 (3)
Baseline AK lesion group	**4, 5, or 6 Lesions (N=243)**	**7 or 8 Lesions (N=110)**	**4, 5, or 6 Lesions (N=241)**	**7 or 8 Lesions (N=108)**
Any treatment-related TEAE [a]	34 (14)	22 (20)	28 (12)	7 (6)
Application site pain	22 (9)	13 (12)	9 (4)	2 (2)
Application site pruritus	21 (9)	11 (10)	16 (7)	5 (5)

AE=adverse event; AK=actinic keratosis; N=number of patients in the Safety Population under each subgroup category; TEAE=treatment-emergent adverse event.

a) TEAEs are AEs with relationship to study treatment as 'Definitely Related', 'Possibly Related', 'Probably Related', or unknown.

Note: Percentages are based on number of patients in the Safety Population under each subgroup (N).

Age

No notable difference was observed in safety between the 2 age subgroups treated with tirbanibulin 1% ointment.

The number of patients <65 years of age was lower than those ≥65 years of age in both treatment groups for the pooled studies. The overall incidences of treatment-related TEAEs was similar between patients who are <65 years of age and those ≥65 years of age in the tirbanibulin 1% ointment (17% versus 15% patients, respectively) and same in vehicle groups (10% patients for both subgroups). Treatment-related TEAEs were predominantly application site pain and pruritus, and were similarly distributed between the 2 age subgroups. Other treatment-related TEAEs were too few for meaningful comparison between the 2 subgroups.

Gender

Overall there were no meaningful difference in safety between the gender subgroups.

There were a greater number of male patients than female patients in the both the tirbanibulin 1% ointment (305 versus 48 patients, respectively) and vehicle (304 versus 45 patients, respectively) treatment groups in the pooled studies. There was a relatively greater difference in the incidence of treatment-related TEAEs between the tirbanibulin 1% ointment and vehicle groups in case of female patients (17% versus 2% patients, respectively) than for male patients (16% versus 11% patients, respectively). However, due to the relatively lower number of female patients, these data need to be interpreted with caution.

Treatment-related TEAEs in the tirbanibulin 1% ointment group were predominantly application site pain and pruritus (10 and 9% patients, respectively). Although there was a smaller number of female patients compared to males, the incidence of application site pain and application site pruritus appeared to be similar between the gender subgroups in the tirbanibulin 1% ointment group: 10% and 9% in male patients, and 10% and 13% in female patients.

Treatment Location:

There was a greater number of patients with treatment location face than patients with treatment location scalp in the both the tirbanibulin 1% ointment (238 versus 115 patients, respectively) and vehicle (239 versus 110 patients, respectively) treatment groups in the pooled studies. The overall incidence of treatment-related TEAEs was slightly higher for face subgroup than the scalp subgroup for both the tirbanibulin 1% ointment (17% versus 13% patients, respectively) and vehicle (11% versus 7% patients, respectively) groups.

Treatment-related TEAEs were predominantly application site pain and pruritus and were similarly reported in the face subgroups (11% and 10% patients, respectively) and scalp subgroups (8% and 8% patients, respectively). Other treatment-related TEAEs were too few for meaningful comparison between the subgroups.

Baseline AK Lesion Group:

There was a greater number of patients with 4 to 6 baseline AK lesions than 7 to 8 baseline AK lesions in the both the tirbanibulin 1% ointment (243 versus 110 patients, respectively) and vehicle (241 versus 108 patients, respectively) treatment groups in the pooled studies.

The overall incidence of treatment-related TEAEs is slightly lower in subgroup with 4 to 6 baseline AK lesions (14% patients) than that with 7 to 8 baseline AK lesions (20% patients) in the tirbanibulin 1% ointment group.

Treatment-related TEAEs were predominantly application site pain and pruritus and were similarly reported in both subgroups with 9% and 9% patients, respectively, in the subgroup 4 to 6 baseline AK lesions and 12% and 10% patients, respectively, in the subgroup with 7 to 8 baseline AK lesions. Other treatment-related TEAEs were too few for meaningful comparison between the 2 subgroups.

Renal/hepatic impairment

No formal studies of tirbanibulin 1% ointment in patients with hepatic or renal impairment were submitted (see discussion on clinical safety).

Safety related to drug-drug interactions and other interactions

Low systemic exposure with a sub-nanomolar (0.598 nM) mean Cmax was observed in PK Study KX01-AK-007. Therefore, no clinical drug-drug interaction studies were submitted (see discussion on clinical safety). *In vitro* drug-drug interaction studies showed that tirbanibulin and its metabolite KX2-5036

have no potential to affect or be affected by concomitant medication at the level of the CYP enzymes or membrane transporters at the maximum clinical exposure observed.

Given the route of administration (topical), the short duration of dosing (5 days), the low systemic exposure (sub-nanomolar mean Cmax), and the *in vitro* data, there is no potential for tirbanibulin to be affected by concomitant medications that are inhibitors or inducers of CYP3A4 and CYP2C8.

Discontinuation due to adverse events

Safety assessment up to Day 57

One patient discontinued treatment due to a TEAE during the tirbanibulin 1% ointment development programme. The patient in the tirbanibulin group of Study KX01-AK-003 discontinued treatment on Day 3 because of a traumatic wound in the treatment area (scalp) which was unrelated to study treatment. The wound was healed by Day 15 and the patient did not discontinue from the study

Recurrence Follow-up Period

No patients experienced TEAEs leading to discontinuation from the Recurrence Follow-up Period in the Studies KX01-AK-003 and KX01-AK-004. In Study KX01-AK-002, 1 patient was discontinued from the Recurrence Follow-up period due to cerebrovascular accident, though the event had resolved.

Post marketing experience

No post-marketing data were provided.

Safety in phase I studies

Phase I studies in patients with AK (Studies KX01-AK-01-US and KX01-AK-007)

- Study KX01-AK-01-US: a Phase I, uncontrolled, open-label study to evaluate the safety, tolerability, and pharmacokinetics (PK) of tirbanibulin 1% ointment in adults with AK on the dorsal forearm.
- Study KX01-AK-007: a Phase I, open-label, non-randomised, uncontrolled, parallel-group study to evaluate the safety and PK of tirbanibulin 1% ointment in adults with AK on the face or scalp.

Monitoring Periods for Safety Assessments:

- Day 45 for the Phase I Study KX01-AK-01-US
- Day 29 for the Phase I Study KX01-AK-007

In Study KX01-AK-01-US, no patients had a severe TEAE or a treatment-related TEAE. No patients died, or had a serious TEAE, or discontinued due to a TEAE. In the ITT Population (n=30) in Study KX01-AK-01-US, 4 (13%) patients had a TEAE: 1 (10%) patient who received 200 mg over 100 cm^2 for 3 days, 1 (13%) patient who received 50 mg over 25 cm^2 for 5 days, and 2 (25%) patients who received 200 mg over 100 cm^2 for 5 days.

In Study KX01-AK-007, 4 (22%) patients had 5 TEAEs. One (11.1%) patient in the face group and 3 (33.3%) patients in the scalp group experienced TEAEs. One (11.1%) patient in the scalp group

experienced a treatment-related TEAE; all other TEAEs were classified as not related to the treatment. There were no serious, severe or fatal, or TEAEs leading to study treatment discontinuation.

KX01-AK-01-US: Local skin reactions were generally mild and transient, resolving quickly after completing study treatment and most were resolved by the end of observation at Day 45.

KX01-AK-007: Local skin reaction signs on the treatment area were mostly transient, all were mild to moderate in erythema and flaking/scaling. Local skin reactions generally appeared after treatment, peaked around Day 8, and resolved or returned to baseline by Day 29.

In Studies KX01-AK-01-US and KX01-AK-007 in patients, pigmentation and scarring were not assessed.

Phase I studies in healthy subjects - Dermal safety studies (Studies KX01-AK-006, KX01-AK-008, KX01-AK-009, and KX01-AK-010)

KX01-AK-010

Study Design: Study KX01-AK-0010 was a randomised, single-centre, controlled, evaluator-blinded, within-subject comparison study of tirbanibulin 1% ointment and vehicle ointment under occlusive, semi-occlusive, and open patch conditions in healthy subjects. Additionally, 0.9% saline was used as negative control and patched under occlusive conditions only. The primary objective was to determine the tolerability of tirbanibulin 1% ointment after repeated topical application to the healthy skin of humans under occlusive, semi-occlusive, and open patch study conditions to determine which patch conditions would be used for a Repeat Insult Patch Test (RIPT) study (Study KX01-AK-006) to be conducted after completion of this study.

All subjects had fields designated for the study treatment patches and negative control patch at 7 randomly assigned, 2×2 cm per patch area at adjacent sites in the infrascapular region, for the purpose of determining irritation potential.

- Occlusive patch condition: 1 sachet (~250 mg) of study treatment on pad and adhered to designated area with adhesive that covered the pad and edges of pad.
- Semi-occlusive patch condition: 1 sachet (~250 mg) of study treatment on pad and adhered to designated area with adhesive at the edges of pad.
- Open patch condition: 1 sachet (~250 mg) of study treatment on designated area, covered with polyester gauze and taped down at 4 corners of gauze.

Applications by study personnel and evaluation took place at the study site 3 times a week for 3 consecutive weeks and a total of 9 applications approximately 48 to 72 (±4) hours apart.

Evaluation of dermal reactions at the application sites was assessed clinically using a visual scale rating the degree of erythema, oedema, and other signs of cutaneous irritation. In addition, safety was assessed by evaluation of any AEs reported during the study.

Subject Disposition: In Study KX01-AK-010, 36 subjects were randomised, and 30 subjects completed the study. Six subjects discontinued: 1 subject discontinued for a TEAE that was not related to study treatment and 5 subjects withdrew consent

Safety Results: A total of 36 subjects were randomised, included in the Safety Population, and analysed for safety. A summary of the key safety findings is provided below.

Evaluation of Skin Irritation

- Under occlusive and semi-occlusive patch conditions, significant irritation was observed at the tirbanibulin site: 32 (88.9%) subjects had a cumulative irritation score of 3 or greater. The vehicle site exhibited lower irritation under occlusive patch conditions, where only 2 (5.6%) subjects had a score of 3 or greater.
- Under open patch conditions, 8 (22.2%) subjects had a cumulative irritation score of 3 or greater at the tirbanibulin site. No subjects exhibited irritation at the vehicle site.
- Discontinuation of patch application was similar following tirbanibulin application under occlusive and semi-occlusive conditions, with 32 subjects discontinued by Day 11. Tirbanibulin application under open conditions had a lower discontinuation rate and discontinuation occurred later than tirbanibulin application under occlusive or semi-occlusive conditions, with 8 subjects who discontinued application by Day 22.

Systemic Safety

- One TEAE was reported in 1 (2.8%) subject during the study. The TEAE (contact dermatitis - tape reaction around all sites) was mild in severity, not considered related to study treatment, and led to the subject's discontinuation from the study
- There were no severe, serious, or fatal TEAEs reported.
- There were no ESIs reported.

KX01-AK-006

Study Design: Study KX01-AK-006 was a randomised, single-centre, controlled, evaluator-blinded, within-subject comparison study of tirbanibulin 1% ointment, vehicle ointment, and negative control under open patch conditions in healthy subjects. The primary objective was to determine the potential of tirbanibulin 1% ointment to induce sensitisation by repeated topical application to the healthy skin of humans under controlled conditions. In addition, safety was assessed by evaluation of any AEs reported during the study.

During the Induction Phase, tirbanibulin 1% ointment, vehicle (supplied in single-use sachets that contained at least 250 mg of the investigational product), and negative control were applied by study personnel to randomly assigned, adjacent sites of 2×2 cm on the infrascapular area of the back 3 times a week for 3 consecutive weeks (9 applications). Evaluation of dermal reactions at the application sites were assessed clinically approximately 48 to 72 hours post-dose using a visual scale that rated the degree of erythema, oedema, and other signs of cutaneous irritation.

Following the Induction Phase, subjects entered a 10- to 14-day Rest Period, after which they entered the Challenge Phase, which consisted of one 48-hour application to a naïve site on the opposite side of the back. Observations based on the grading at the naïve site during Challenge Phase and the patterns of reactivity during the Induction Phase provided a basis for an interpretation of contact sensitisation. Notations on the appearance of the skin were made using a protocol-defined scoring system.

A re-challenge was to be performed if a cutaneous response observed during the Challenge Phase indicated possible sensitisation (Grade 3 or higher) and/or at the discretion of the investigator. A narrative description of reactions in the Challenge and Re-challenge Phases were reported together with the opinion of the investigator as to whether such reactions were felt to be indicative of contact sensitisation.

A total of 10 applications (9 applications in the Induction Phase and 1 during the Challenge Phase) were made over a period of approximately 6 to 8 weeks.

Subject Disposition: In Study KX01-AK-006, 261 subjects were randomised, 232 subjects completed the Induction Phase, and 229 subjects completed both the Induction and Challenge phase of the study. Thirty-two subjects discontinued: 2 subjects discontinued due to AEs that were unrelated to the study treatment, 14 subjects withdrew consent, and 16 subjects were lost to follow-up.

Safety Results: The Safety Population included 261 subjects, the Cumulative Irritancy Population in the Induction Phase included 232 subjects, and the Sensitisation Population in the Challenge Phase included 229 subjects. A summary of the key safety findings is provided below.

Sensitisation

In the opinion of the investigator, no subjects required a Re-challenge in the study. There were no signs observed at any of the evaluations following the removal of the challenge patch for all study treatments suggestive of contact sensitisation. The response scores of 1 and 2 observed at the tirbanibulin 1% ointment and vehicle sites were consistent with the contact irritation seen during Induction, rather than sensitisation.

- The mean and total cumulative irritation scores during the Induction Phase and the response scores during the Challenge Phase were consistent with contact irritation at the tirbanibulin 1% ointment site. There was greater contact irritation associated with tirbanibulin 1% ointment compared to vehicle ointment or 0.9% saline.

Systemic Safety

1. Twenty-two TEAEs were reported in 21 (8.0%) subjects. The most frequently reported TEAEs were headache (7 subjects, 2.7%), nasopharyngitis (6 subjects, 2.3%), and rhinorrhoea (3 subjects, 1.1%).
2. None of the TEAEs were treatment related.
3. One severe TEAE was reported in 1 (0.4%) subject. The subject had a headache that was not related to study treatment and the event resolved.
4. One (0.4%) subject experienced a serious TEAE of mild dyspnoea that was considered not related to study treatment and the event resolved. The study treatment was discontinued, and the subject was discontinued from the study.
5. One (0.4%) subject experienced a TEAE of nausea that led to treatment discontinuation. The event was of moderate intensity, considered not related to study treatment, and the event resolved.
6. There were no fatal TEAEs reported.
7. There was 1 ESI: 1 subject had a positive urine pregnancy test at the end of study assessment. There were no pregnancy-associated AEs or SAEs reported.
8. There were no other ESIs reported.

KX01-AK-008

Study Design: Study KX01-AK-008 was a single-centre, randomised, double-blind, controlled, within-subject comparison study of tirbanibulin 1% ointment and vehicle ointment in healthy subjects. The primary objective was to determine the phototoxic potential of tirbanibulin 1% ointment when topical application to healthy skin was followed by light exposure. In addition, safety was assessed by evaluation of any AEs reported during the study.

A total of 4 application sites (2×2 cm) were marked in the infrascapular region: 1 single-use sachet (~250 mg) per site of tirbanibulin 1% ointment was applied to 2 sites and vehicle ointment was applied to 2 sites by study personnel. Each study treatment was applied according to the

randomisation scheme under semi-occlusive patch conditions once during the study. After approximately 24 (±2) hours, all patches were removed and 1 application site for each treatment was irradiated with full-spectrum UV light (UVA/UVB), and 1 site for each treatment remained non-irradiated. The irradiation dose of each subject was calculated using the minimal erythema dose (MED) that was determined on Day 1. The irradiated and non-irradiated sites were compared with each other and with a pre-designated, untreated irradiated site. All sites were examined approximately 24 (±2) hours after patch application (upon patch removal) and at approximately 24 and 48 hours post-irradiation. Cutaneous reactions at the application sites were evaluated using a visual scale that rated the degree of erythema, oedema, and other signs of cutaneous irritation. The total study duration was 4 days for each subject.

Safety Results: A total of 31 subjects were randomised, included in the Safety Population and the Phototoxicity Analysis Population, and completed the study. A summary of the key safety findings is provided below.

Evaluation of phototoxicity

- No subject had oedema even though some had an erythema score of 1 or 2; therefore, no one met the criterion of having phototoxicity. Thus, the dermal response scores for all subjects were attributed to irritation by tirbanibulin 1% ointment or vehicle ointment rather than phototoxicity.
- The tirbanibulin 1% ointment irradiated site had a higher mean dermal response score (average of the sum of erythema and oedema scores for Day 3 and Day 4) than the vehicle ointment irradiated site and the untreated irradiated control site; 0.94, 0.77, and 0.23, respectively. The tirbanibulin 1% ointment non-irradiated site had a higher mean dermal response score than the vehicle ointment non-irradiated site; 0.89 and 0.69, respectively.
- There was no evidence of a difference in mean dermal response scores of tirbanibulin 1% ointment irradiated and non-irradiated (p=0.5977). Similarly, there was no difference in mean dermal response scores of vehicle ointment irradiated and non-irradiated (p=0.3796). This supports the lack of phototoxic activity for both tirbanibulin 1% ointment and the vehicle ointment.
- There were no statistically significant differences observed between tirbanibulin 1% ointment and vehicle ointment at the irradiated sites. The mean dermal response score of tirbanibulin 1% ointment at the non-irradiated site was significantly higher than that of the vehicle ointment at the non-irradiated sites (p=0.0364).
- There was no indication of phototoxicity present among the subjects in this study.

Systemic Safety

- One TEAE was reported by 1 (3.2%) subject during the study. The TEAE of headache was of mild severity and considered to be related to treatment by the investigator.
- There were no severe, serious, or fatal TEAEs reported. No subject discontinued study treatment due to TEAEs.
- There were no ESIs reported during the study.

KX01-AK-009

Study Design: Study KX01-AK-009 was a randomised, double-blind, single-centre, controlled, within-subject comparison study of tirbanibulin 1% ointment, vehicle ointment, and an untreated, irradiated, open (non-occluded) control site in healthy subjects. The primary objective was to determine the

photo-allergic potential of tirbanibulin 1% ointment when topical application to healthy skin was followed by light exposure. In addition, safety was assessed by evaluation of any AEs reported during the study.

A total of 8 application sites (2×2 cm each) were marked on the subject's back and distributed so that 4 sites were on each side of the back: 1 side was used for induction and 1 side for challenge. One set of application sites on the back was designated for irradiation after approximately 24 (±4) hours of study treatment application and the other set remained non-irradiated. An additional site was marked on the back that did not receive treatment but was irradiated at Challenge to serve as an untreated irradiated control.

During the 3-week Induction Phase, study treatment (a small amount of ointment from single-use sachets containing approximately 250 mg of tirbanibulin 1% ointment or vehicle ointment) was applied by study personnel to 2 sites twice each week for approximately 24 (±4) hours under open patch conditions (6 applications). After 24 (±4) hours, all application sites were evaluated, and 1 application site of each treatment was irradiated with 2 times the subject's MED (determined on Day 1). The sites were evaluated by a trained evaluator. All sites were re-evaluated post-irradiation, at approximately 48 to 72 hours after irradiation. These procedures were performed each week for 3 weeks. Dermal reactions at the application sites were evaluated using a visual scale that rated the degree of erythema, oedema, and other signs of cutaneous irritation.

At the end of the Induction Phase, subjects entered a 10- to 17- day Rest Period, and then a Challenge Phase. At Challenge, 1 single-use sachet of tirbanibulin 1% ointment or vehicle ointment was applied to each of 2 naïve sites once for approximately 24 (±4) hours under open patch conditions, after which all sites were evaluated, and 1 site of each study treatment and the additional untreated site were irradiated. The sites were examined for dermal reactions at approximately 24 (±4), 48 (±4), and 72 (±4) hours post-irradiation. A re-challenge was to be performed if a cutaneous response observed during the Challenge Phase indicated possible photosensitisation or at the discretion of the investigator.

The safety endpoints for this study were irritation responses during the Induction Phase, positive responses at Challenge (i.e., reactions indicative of a sensitisation response) and AEs.

Subject Disposition: In Study KX01-AK-009, 64 subjects were randomised, 61 subjects completed the Induction Phase, and 59 subjects completed both the Induction and Challenge Phase of the study. Five subjects discontinued: 1 subject discontinued due to an AE not related to study treatment, 1 subject discontinued for a protocol violation, and 3 subjects withdrew consent.

Safety Results: A total of 64 subjects were included in the analysis of AEs. A total of 61 (95.3%) subjects completed the Induction Phase and were included in the analysis of local tolerability (photo-irritation), and 59 (92.2%) subjects completed the Challenge Phase and were included in the analysis of photosensitisation. A summary of the key safety findings is provided below.

Evaluation of Photosensitisation

- Local tolerability (photo-irritation) was analysed in the Induction Phase and is a component of photo-allergy but does not necessarily indicate photo-allergy. Although there were statistically significant differences between mean irritation scores of the study treatment sites, these differences were not clinically significant because of the low level of the dermal response scores. The non-irradiated sites showed less irritation than the irradiated sites. There was no difference in irritation between the tirbanibulin 1% ointment and vehicle irradiated sites. The tirbanibulin 1% ointment non-irradiated site showed statistically significant more irritation than the vehicle non-irradiated site.

- Based on the investigator's assessment, reviewing the results of the study treatments irradiated versus non-irradiated sites, as well as tirbanibulin 1% ointment versus vehicle and control, no subject showed evidence of photosensitisation during the Challenge Phase of the study.

Safety

- Six TEAEs were reported by 5 (7.8%) subjects during the study. One TEAE (nasopharyngitis) was reported in 2 (3.1%) subjects, and the other 4 events were reported in 1 subject each. None of the TEAEs were considered related to study treatment.
- There were no severe, serious, or fatal TEAEs reported.
- One subject discontinued study treatment and study due to a TEAE of upper respiratory infection that was moderate in severity and not related to study. There were no ESIs reported during the study.

2.6.1. Discussion on clinical safety

Phase II and phase III patient studies

The safety database was mainly based on studies KX01-AK-002, KX01-AK-003 and KX01-AK-004. The phase III studies KX01-AK-003 and KX01-AK-004 have the same study design, population and treatment regimens and were thus pooled together for their safety analysis.

With the exception of AEs connected to the application site (application site pain and pruritus), the number of patients that experienced AEs was generally similar and low across all treatment arms. Most of these AEs were mild to moderate.

The most common AEs up to day 57 were application site pain (10 vs 3%), application site pruritus (9 vs 6%) and upper respiratory tract infection (4 vs 5%) for the pooled tirbanibulin 1% and vehicle treatment arms respectively. Only 1 AE, application site pruritus, was severe and considered related to tirbanibulin.

In the follow up period a few TEAEs were reported in no more than 1 patient across the studies in the tirbanibulin 1% ointment group and none in the vehicle group. While safety was followed for one year this was only for patients with a complete response at day 57 resulting in only a moderate proportion of tirbanibulin patients and a small proportion of vehicle treated patients being followed up for safety beyond day 57. However due to the low systemic absorption and mechanism of action of tirbanibulin, AEs related to treatment are generally not expected beyond the initial day 57 period (with the possible exception of skin cancer, discussed further below).

Application site pain, application site pruritus and dizziness were considered related to tirbanibulin treatment and did not occur in the follow-up period. Application site pain and application site pruritus have been included in section 4.8 of the SmPC.

Pregnancy, overdose and ocular exposure were closely monitored during the studies. There were no pregnancies reported in the phase II and phase III clinical trials. The effects of tirbanibulin on pregnancy or breast feeding are unknown (see SmPC section 4.6). However, due to the low systemic absorption of the ointment effects on pregnancy or breast feeding are considered unlikely. There were 3 cases of overdose during the clinical development programme of tirbanibulin including one case where 10 times the amount of product was applied for 5 days, however these overdoses did not cause any safety concerns. None of the ocular adverse events observed during the clinical development programme were considered related to study treatment. The SmPC and PIL reflects that contact with

the eyes should be avoided and tirbanibulin ointment may cause eye irritation. In the event of accidental contact with the eyes, the eyes should be rinsed immediately with large amounts of water, and the patient should seek medical care as soon as possible.

Furthermore, tirbanibulin ointment must not be ingested. If accidental ingestion occurs, the patient should drink plenty of water and seek medical care. Tirbanibulin ointment should not be used on the inside of the nostrils, on the inside of the ears, or on the lips.

Application of tirbanibulin ointment is not recommended until the skin is healed from treatment with any previous medicinal product, procedure or surgical treatment and should not be applied to open wounds or broken skin where the skin barrier is compromised (see section 4.2).

Skin cancer was also an adverse event of special interest. There was a slightly higher incidence of treatment-emergent skin cancers with tirbanibulin 10 of 353 (2.8%) compared to the vehicle 7 of 349 (2.0%) subjects. It is noted that some of the tirbanibulin patients had developed more than 1 skin cancer. Many cases were confounded with a history of skin cancers. Furthermore, the short duration of safety follow-up suggests many of these skin cancers were pre-existing and only 1 of these skin cancers occurred in the treatment field (SCC, tirbanibulin arm). The SCC in the treatment field developed on day 74 of the study and was resolved by day 98. This patient had a history of BCC, and the SCC was considered as not related to tirbanibulin by the investigator. However, it is noted that some of the other skin cancers also developed in head and neck areas and close to the treatment field. Overall there is limited information on the potential risk of developing skin cancer. A relevant warning was added in the SmPC to highlight that changes in the appearance of actinic keratosis could suggest progression to invasive squamous cell carcinoma, and that clinically atypical lesions for actinic keratosis or suspicious for malignancy should be appropriately managed (see SmPC section 4.4).

While an association between tirbanibulin use and skin cancer development is not established, tirbanibulin is through disruption of the cellular microtubule network and the safety database did not include a sufficient number of patients to rule out a higher incidence of skin cancer over time.

Skin tumours in treatment area is included in the RMP as an important potential risk, which is considered appropriate to identify this potential effect in the treatment area where exposure levels will be highest.

In order to further evaluate this risk the applicant will also conduct and submit the results of study M-14789-41, a phase 4, multicentre, randomised, investigator-blinded, active-controlled, parallel-group study (Post-authorisation safety study (PASS)). The proposed study will be conducted to evaluate the incidence of invasive SCC and to assess the long-term safety and efficacy of tirbanibulin 10 mg/g ointment. Since the potential risk of developing skin cancer is considered critical, the PASS study M-14789-41 is considered key to the benefit/risk and included as a category 1 PASS. The protocol will be submitted for approval.

Local skin reactions in the treated area, including erythema, flaking/scaling, crusting, swelling, erosion/ulceration, and vesiculation/pustulation, may occur after topical application of tirbanibulin ointment as commonly reported in patients (>1% to <10%) treated with tirbanibulin. Treatment effect may not be adequately assessed until resolution of local skin reactions (see SmPC section 4.4). Most LSRs were mild to tolerate and reached a peak around day 8 after treatment before dropping back down to baseline levels by day 57. However, it is noted that there were no formal assessments between days 5, 8 and 15 when potentially the peak severity of LSRs might have occurred. The levels of pigmentation and scarring in patients was similar between tirbanibulin and vehicle control groups.

Numerically there were a lower number of SAEs in vehicle groups than tirbanibulin groups. Overall the level of SAEs was low and no SAE was considered related to treatment. There was one death in the clinical development programme but this was in a vehicle treatment arm.

No clinically meaningful changes from baseline were observed for haematology, blood chemistry, urinalysis, vital signs or physical examinations across the pivotal studies in patients treated with tirabanibulin. A thorough QT study is not considered needed due to results of non-clinical studies and PK data demonstrating that systemic exposure is negligible. In addition, the clinical data from the phase III studies showed no excess of cardiac-related TEAEs or clinical importance in ECG readings with tirbanibulin as compared with vehicle treatments. The risk of any cardiac adverse events is considered low.

There were no meaningful differences in the numbers of AEs for patients treated with tirbanibulin with respect to age (<65 v >65 years), sex, treatment locations (face vs scalp) and the number of baseline lesions (4-6 vs 7-8). An adequate number of elderly patients have been recruited to the studies. This is in line with the demographic distribution of AK lesions. Paediatric patients were not recruited to the clinical development programme as AK is not reported in children.

No effects on renal or hepatic impairment are expected due to the negligible systemic absorption of the ointment.

No systemic drug-drug interactions are expected given the low systemic absorption of this product and the short duration of dosing.

Only 1 patient discontinued tirbanibulin treatment due to an AE (traumatic wound) that was not considered related to the treatment.

Phase I patient studies

There were no serious or severe AEs, and no AEs that led to discontinuation of treatment in either of the phase 1 patient studies. There was a similar trend for LSRs in healthy subjects as there was for patients.

Dermal safety studies

The pilot study KX01-AK-010 confirmed that occluded and semi-occluded patch testing were not tolerable in healthy volunteers. Open patch conditions were thus selected for the next study KX01-AK-006.

In the potential sensitizing study KX01-AK-006 tirbanibulin 1% ointment was observed to cause irritancy in the majority of subjects. The conclusion of the application is that tirbanibulin 1% ointment does not cause potential sensitisation. The level of AEs was low throughout the study.

The results of phototoxicity study KX01-AK-008 were presented. It is noted that this study was performed under semi-occlusive conditions and is therefore not directly comparable to the open patch conditions used in studies KX01-AK-006 and KX01-AK-009. However, there were no significant differences in mean dermal scores for irradiated and non-irradiated tirbanibulin treatments and therefore no evidence of potential phototoxicity.

In the photosensitising study KX01-AK-009, there were no meaningful differences in mean dermal scores for challenge irradiated and non-irradiated tirbanibulin and therefore no evidence of potential photosensitisation.

Overall, tirbanibulin was well tolerated and apart from LSRs, application site pain and application site pruritus, there were similar levels of AEs in the tirbanibulin treatment group compared to the vehicle

control group. The lack of comparator data is considered acceptable given the safety profile of the product and considering comparative data will be available from the phase 4 trial M-14789-41.

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

2.6.2. Conclusions on the clinical safety

Overall tirbanibulin 1% ointment has a tolerable safety profile, most AEs were mild to moderate in severity, and reversible.

Although a causal association with malignancy cannot be currently established, the potential to develop skin cancers is considered a safety concern due the lack of long-term safety data.

The results of a 3-year post authorisation safety study (PASS, study M-14789-41) against an active comparator expected to enrol 270 subjects in the tirbanibulin arm will be provided (see RMP, category 1 study) to address the potential risk of developing skin tumours. This study will provide both longer term safety and efficacy data.

The CHMP considers the following measures necessary to address issues related to safety:

Annex II PASS: In order to further investigate the risk of progression of actinic keratosis (AK) to squamous cell carcinoma (SCC) in adult patients with non-hyperkeratotic, non-hypertrophic actinic keratosis (AK) treated with tirbanibulin, the MAH should conduct and submit the results of the phase 4, multicentre, randomised, investigator-blinded, active-controlled, parallel-group study M-14789-41 conducted according to an agreed protocol (due date Q2 2026).

2.7. Risk Management Plan

Safety concerns

Table 23: Summary of Safety Concerns

Important identified risks	None
Important potential risks	Skin tumours in treatment area
Missing information	None

Pharmacovigilance plan

Table: On-going and Planned Additional Pharmacovigilance Activities

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Tirbanibulin Post-Authorisation Safety Study (PASS). (Study M-14789-41)	To evaluate the incidence of invasive SCC and to assess the long-term safety and efficacy of tirbanibulin 10 mg/g ointment.	Skin tumours in treatment area	Study Protocol submission to Competent Authorities Interim Results Final Clinical Study Report	Q4 of 2021 Q1 of 2024 Q2 of 2026
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
None				

Risk minimisation measures

Table 24: Summary table of risk minimisation activities by safety concern

Safety concern	Risk minimisation measures
Skin tumours in treatment area	Routine risk minimisation measures: • SmPC section 4. 2, section 4.4 and section 5.1. • PL. Section 2 and Section 3

Safety concern	Risk minimisation measures
	Additional risk minimisation measures: • None

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.7 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

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

Periodic Safety Update Reports submission requirements

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 applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 14.12.2020. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant compared the structure of tirbanibulin with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers tirbanibulin to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

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, Klisyri (tirbanibulin) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU and it has a PASS imposed at the time of authorisation.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Klisyri is intended for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults.

3.1.2. Available therapies and unmet medical need

The appropriate treatment is generally chosen based on the number of lesions present and therapy may be broadly categorised as either lesion-directed (e.g., cryosurgery) or field-directed (e.g., topical products).

While there are a number of licensed therapies available in the EU that are effective in AK treatment adherence with topical AK treatments is reported to be poor, with approximately 90% of patients being non-adherent or non-persistent with therapy (Shergill, 2013). Therefore, there is a continued need for additional AK treatment options.

3.1.1. Main clinical studies

The main evidence of efficacy is from two replicate phase III studies, KX01-AK-003 and KX01-AK-004. They were double-blind, randomised, vehicle-controlled, multi-centre Phase III studies that evaluated the efficacy and safety of tirbanibulin 1% ointment when applied daily to a treatment area of 25 cm^2 for 5 consecutive days in adults with AK on the face or scalp.

3.2. Favourable effects

A statistically significant improvement was observed for complete clearance at day 57 in both studies ($p<0.0001$) in the tirbanibulin 1% ointment group compared with the vehicle group (KX01-AK-003 overall: 44% versus 5%). A similar effect was observed in study KX01-AK-004 with an overall complete clearance rate of 54% in the tirbanibulin 1% ointment arm versus 13% in the placebo arm, respectively ($p<0.0001$).

There were very high completion rates therefore both ITT and PP analyses were similar and there were no issues regarding missing data.

Complete clearance rates for the facial lesions were consistent between both studies KX01-AK-003 and KX01-AK-004 with a difference from placebo of 47% and 46% respectively ($p<0.0001$).

Complete clearance rates on the scalp were lower than the rates on the face and reported difference from placebo of 28% (p<0.0001) and 30% (p 0.0003) in studies KX01-AK-003 and KX01-AK-004 respectively.

3.3. Uncertainties and limitations about favourable effects

There are no available data on retreatment with tirbanibulin in patients who had a recurrence of their AK lesions, or on those who developed new lesions. Therefore, it is unclear whether recurrent lesions would respond similarly to what was observed in the pivotal studies. Therefore, administration is restricted to single use. Efficacy on re-treatment will be investigated in the imposed PASS study which will also review AK histological progression to in situ or invasive SCC following treatment over a 3-year period.

3.4. Unfavourable effects

Most AEs reported in the clinical trials with tirbanibulin 1% ointment were mild to moderate in severity, and reversible. The overall rates for AEs and SAEs were low across all treatment arms with higher incidences of local skin reactions (LSRs), including erythema (91%), flaking/scaling (82%), crusting (46%), swelling (39%), erosion/ulceration (12%), and vesiculation/pustulation (8%) at the application site. Furthermore, application site pruritus (9.1%) and pain (9.9%) were reported.

There was a slightly higher incidence of treatment-emergent skin cancers reported with tirbanibulin 10 of 353 (2.8%) compared to the vehicle 7 of 349 (2.0%) subjects. Some of the tirbanibulin patients had developed more than 1 skin cancer and many case reports were confounded with a history of skin cancers. The short duration of safety follow-up suggested many of these skin cancers were pre-existing and only 1 of these skin cancers occurred in the treatment field (SCC, tirbanibulin arm).

3.5. Uncertainties and limitations about unfavourable effects

There is no comparative safety data and no long-term safety data available to address concerns about the potential risk of developing skin cancer.

While an association between tirbanibulin use and skin cancer development is not established, the mode of action of tirbanibulin is stated to be through disruption of the cellular microtubule network and the safety database did not include a sufficient number of patients to rule out a higher incidence of skin cancer over time.

While the risk of skin cancer is considered to be low from a preclinical and PK perspective, it cannot be completely excluded that an increase in skin cancer development can occur over time. Skin tumours in treatment area is included as an important potential risk in the RMP and will be closely monitored. Furthermore, the incidence of progression to skin cancer over 3 years will be followed in the phase IV PASS study M-14789-41 to be conducted against an active comparator.

3.6. Effects Table

Table 25: Effects Table for tirbanibulin for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults (09 September 2019 cutoff)

Effect	Short Description	Unit	Tirbanibulin	Vehicle	Uncertainties/ Strength of evidence	References
Favourable Effects						
Complete clearance rate (primary endpoint)	Number of subjects with no AK lesions in the treatment area at day 57	N (%)	174/353 (49%)	30/349 (9%)	p<0.0001	KX01-AK-003 & KX01-AK-004
Partial clearance rate	Number of subjects with ⩾ 75% reduction in the number of AK lesions at day 57	N (%)	255/353 (72%)	63/349 (18%)	p<0.0001	KX01-AK-003 & KX01-AK-004
Sustained clearance rate	Proportion of subjects with no AK lesions occurring during 12 months, after being at complete clearance	%	27%	-		KX01-AK-003 & KX01-AK-004
Unfavourable Effects						
AEs	Up to Day 57	No. of events (%)	Tirbanibulin 137/353 (39%)	Placebo 135/349 (39%)		KX01-AK-003 & KX01-AK-004
SAEs	Up to Day 57	No. of events (%)	Tirbanibulin 1/353 (0.3)	Placebo 6/349 (1.7%)		KX01-AK-003 & KX01-AK-004
Treatment emergent skin cancer	Follow-up	No. of subjects affected (%)	Tirbanibulin 10/353 (2.8%)	Placebo 7/349 (2%)	No long-term safety data	KX01-AK-003 & KX01-AK-004

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

A significant improvement in complete clearance of AK lesions at day 57 following treatment with tirbanibulin was observed over vehicle. A consistent effect was seen in both replicate studies. The effects were seen in both facial and scalp lesions, however a greater effect was observed in facial lesions compared to the scalp.

Patients were followed up for 1 year and there was a recurrence rate of 74% and 72%, one year post day 57 in study KX01-AK-003 and KX01-AK-004 respectively. There was a higher recurrence rate for scalp lesions versus facial lesions.

There are limitations with the clinical programme. There is only evidence for a single treatment confined to the head and face and confined to non-hyperkeratotic, non-hypertrophic AK lesions. There was a significant recurrence for previously treated and cleared lesions 12 months after the primary endpoint at day 57. This occurred at a higher extent on the scalp compared to the face. Given the natural history of the disease, it is likely that patients could develop additional AK lesions in the future. However, intermittent use of tirbanibulin has not been studied. Therefore, the product information recommends a single treatment duration of 5 days only and up to a maximum surface area of 25 cm^2. Initiation and oversight should be performed by a physician (see SmPC).

It is important to further examine longer term safety in terms of skin cancer risk with this medicinal product and whether retreatment of recurrent lesions is effective and safe. In line with the previous CHMP advice on this issue, the applicant will conduct a Phase IV PASS which is a randomised, active-controlled, investigator-blinded, safety and re-treatment study (M-14789-41) in adult patients with AK on the face or scalp over 3 years with tirbanibulin 1% ointment.

3.7.2. Balance of benefits and risks

Efficacy has only been studied in non-hyperkeratotic lesions (Olsen scale 1). Since there is no data with use in patients in hyperkeratotic lesions Olsen 2 or 3 or in use in patients with basal cell carcinoma (BCC) the indication was restricted to patients with Olsen grade 1 lesions.

The efficacy of Klisyri as a single treatment duration of 5 days is considered established for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults. The safety profile is considered acceptable in general, however long-term safety data are not yet available and the potential risk for skin cancer is unknown and will be evaluated as part of the Study M-14789-41 which will provide longer term safety and efficacy data.

3.8. Conclusions

The overall benefit/risk of Klisyri for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults is positive.

Divergent positions are appended to this report.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the benefit-risk balance of Klisyri is favourable in the following indication:

Klisyri is indicated for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (Olsen grade 1) of the face or scalp in adults.

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.

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 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.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
PASS: In order to further investigate the risk of progression of actinic keratosis (AK) to squamous cell carcinoma (SCC) in adult patients with non-hyperkeratotic, non-hypertrophic actinic keratosis (AK) treated with tirbanibulin, the MAH should conduct and submit the results of the phase 4, multi-centre, randomised, investigator-blinded, active-controlled, parallel-group study M-14789-41 conducted according to an agreed protocol.	30/06/2026

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

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that tirbanibulin is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

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

Appendix

1. Divergent positions to the majority recommendation

Appendix: DIVERGENT POSITION DATED 20 May 2021
Klisyri EMEA/H/C/005183/0000

The undersigned CHMP members did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Klisyri (tirbanibulin 1% ointment) indicated for the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis of the face or scalp in adults.
The reasons for divergent opinion are based on the insufficient demonstration of the safety profile of Klisyri. If most AEs were mild to moderate in severity, the main concern remains the lack of long-term safety data with regards to the potential to develop skin cancers.
Overall, we consider that the current data do not allow a proper assessment of the safety profile of Klisyri for the following reasons:

- Too few patients have been enrolled in phase III studies (only 353 patients received the proposed treatment regimen and only 107 patients followed-up to approximately 1 year after the first day of application of tirbanibulin (11 months of total length of follow-up) and 83 at the time point of 14 months after first application.);

- The skin retention time is a key factor to explore the potential carcinogenic effect of a topical treatment for the development of skin cancer, especially Squamous Cell Carcinoma, but no such data have been provided.

- The mechanism of action of tirbanibulin is worrying: it is similar to the vinca-alkaloids class. This class is known to be associated to many serious adverse effects, especially risk of cancer.

As a conclusion, we are of the opinion the benefit/risk ratio cannot be determined for Klisyri in the field treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis of the face or scalp in adults.

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