SCIENTIFIC DISCUSSION

1. Introduction

Soft tissue sarcoma (STS) constitutes a rare and heterogeneous group of tumours generally classified according to the normal tissue they mimic. Despite control of the primary site, about 50% of the patients ultimately succumb to metastases or local recurrence. In adults, there are only few active chemotherapeutic options available and the expected median survival after diagnosis of metastatic disease is about 1 year. Established first-line treatment options include doxorubicin and ifosfamide in mono-therapy or in combination regimens. DTIC is also regarded as an active drug. Despite numerous drugs being tested for phase II activity, there has been little progress with respect to new active compounds since the introduction of ifosfamide in the late eighties, the exception being imatinib for the treatment of gastrointestinal stromal tumours. From a regulatory perspective, doxorubicin, ifosfamide and DTIC are licensed in at least some EU countries for the treatment of STS.

Yondelis, formerly known as ET-743 PharmaMar, (ecteinascidin, trabectedin [INN]) is an anti-cancer medicinal product with claimed new mechanisms of action. It is a tetrahydro-isoquinoline and was originally extracted from the marine tunicate *Ecteinascidia turbinata* a colony forming tunicate that grows in the coastal platform of several temperate seas. Today a synthetic manufacturing process has been developed. Trabectedin is proposed to block the transcriptional activation of a subset of inducible genes without affecting their constitutive expression. Yondelis drug product is presented as a lyophilized powder for concentrate for solution for infusion, in vials of 0.25 mg and 1 mg trabectedin.

An application for a Marketing Authorisation was submitted through the Centralised Procedure in November 2001 for Yondelis in the treatment of patients with advanced STS, who had failed anthracyclines and ifosfamide, or had failed ifosfamide and were unsuitable to receive anthracyclines/ifosfamide. The demonstration of efficacy was based on three single-arm studies. Following the scientific assessment procedure the CHMP concluded that the benefit/risk profile of was not favourable. The negative opinion was mainly based on the fact that clinical efficacy had not been adequately demonstrated.

The current application includes data from a randomised phase II study in patients with liposarcoma or leiomyosarcoma. CHMP scientific advice has not been sought. At the time of submission of the marketing authorisation application, Yondelis had not been registered in any country. The claimed indications for trabectedin is as follows: Yondelis is indicated for the treatment of patients with advanced soft tissue sarcoma, after failure of anthracyclines and ifosfamide, or who are unsuited to receive these agents. Efficacy data are based mainly on liposarcoma and leiomyosarcoma patients. The proposed posology can be summarised as follows: The recommended dose is 1.5 mg/m^2 body surface area, administered as an intravenous infusion over 24 hours with a three-week interval between cycles. Administration through a central venous line is strongly recommended (se SPC, section 4.2).

2. Part II:Chemical, pharmaceutical and biological aspects

Introduction

Yondelis is a powder for concentrate for solution for infusion containing 0.25 mg and 1 mg of trabectedin per vial. Each vial is reconstituted with 5 ml and 20 ml of sterile water for injections, respectively. The reconstituted solution is a clear, colourless or slightly yellowish solution, essentially free of visible particles. The solution obtained has a concentration of 0.05 mg/ml and is for single-use only. Yondelis is supplied in either 10 ml or 25 ml Type I colourless glass vial with a grey bromobutyl rubber stopper with fluoropolymer coating. The stopper is sealed to the vial with an aluminium flip-off seal.

The excipients used in the preparation of Yondelis are sucrose, potassium dihydrogen phosphate, phosphoric acid and potassium hydroxide (for pH-adjustment), and water for injections.

Active Substance

The active substance trabectedin is a new chemical entity initially obtained by isolation from the marine tunicate *Ecteinascidia turbinata* by extraction and purification. A synthetic process was subsequently developed and the active substance is since then produced synthetically. The synthetic route starts from the secondary metabolite safracin-B, isolated from fermentation media of *Pseudomonas fluorescens*. The Active Substance Master File (ASMF) procedure was followed for the active substance.

Trabectedin is a white to off-white powder, soluble in polar organic solvents. Trabectedin is insoluble in hydrocarbons. In aqueous media, trabectedin is practically insoluble in water but solubility increases at acidic pH.

Trabectedin is a single enantiomer with a total of seven stereogenic centres. Most of these centres are linked together through a short bridge, which means that the stereochemistry of these centres is interrelated. No change has been observed in the crystalline form of trabectedin during storage, therefore, no evidence has been found of polymorphism.

- Manufacture

Trabectedin is manufactured utilising a three or five steps synthetic process., it was demonstrated that the trabectedin produced from the manufacturers is equivalent.

Confirmation of the chemical structure of the starting materials was provided by spectroscopic techniques and the impurity profile was characterised. The starting materials specification includes test for appearance, assay, identification, water content, impurities, heavy metals, and solvents.

A comparison between the synthetic and natural trabectedin has been performed by HPLC and TLC, IR and proton NMR, optical rotation and X-ray diffractometry. It was concluded that the active substance obtained by both processes is equivalent.

The structure of trabectedin was confirmed by elemental analysis, IR spectroscopy, high field NMR, UV spectroscopy, MS, X-ray crystallography and optical rotation.

- Specification

The active substance specification set by the manufacturers of the active substance are identical and the same as that of the Applicant. The analytical methods used for the release of the active substance are also equivalent in all sites.

The active substance specifications include tests for description, identification (IR, HPLC and optical rotation), assay (HPLC), water content (coulometric determination), impurities (HPLC), residual solvents (GC), heavy metals, inorganic impurities and bacterial endotoxins.

The method for assay and related substances by HPLC were validated for specificity, linearity and range, limit of quantitation, precision, accuracy, stability of solutions and robustness. The GC method for residual solvents was validated for specificity, linearity and range, limit of quantitation, precision, accuracy and stability of solutions. The coulometric determination of water was validated for specificity, linearity, precision and accuracy.

Batch analysis data was performed on a total of 25 batches of trabectedin. All batches complied with the proposed set of specifications.

- Stability

Three commercial scale batches were stored at -20°C ± 5°C for 24 months (2 batches) and for 18 months (1 batch) in the proposed primary packaging. In addition, the effect of short term excursions outside the storage conditions was evaluated on one batch at 25°C/60% RH for 30 days and 5°C for 6 months. All the batches placed on stability studies complied with the requirements in the active substance specifications. The results from the short excursions study showed no significant changes at the end of the study periods for any parameter although there was a trend towards increased water content at higher temperature. Phostostability and stress testing using thermal, oxidative, acidic and alkaline conditions was also carried out. Stress conditions showed a slight increase in the impurities content and the photostability studies showed that the active substance is not affected by exposure to light.

The data provided is sufficient to confirm the proposed re-test period.

Drug Product

• Pharmaceutical Development

The aim of the pharmaceutical development was to obtain a formulation of trabectedin, which could be administered by intravenous infusion after appropriate dilution. The intended route of administration precluded the use of several excipients, such as stabilizers and non-aqueous solvents. Therefore, a simple aqueous solution was considered the most appropriate dosage form. Initially a formulation based in mannitol and phosphate buffer solution was designed. Further pharmaceutical development led to an improved sucrose-based formulation using synthetic trabectedin in phosphate buffer that was selected for scale up and commercialisation, that allows the storage of the product at 2-8°C.

- Adventitious Agents

Starting materials and excipients used for the manufacture of trabectedin powder for concentrate for solution for infusion do not contain materials that are of animal or human origin and are not in contact with materials of animal or human origin, during their manufacture.

- Manufacture of the Product

The manufacturing process for trabectedin powder for concentrate for solution for infusion is carried out under aseptic conditions in restricted areas and comprises: (1) sterilization and depyrogenation of primary packaging materials and equipment that comes into contact with the product (2) preparation of the bulk solution (3) sterilising filtrations (4) filling (5) lyophilization and stoppering (6) final packaging.

The validation of the manufacturing process was carried out at the proposed manufacturing sites The size of the validation batches corresponded to the proposed commercial manufacturing batch size. All the controls performed along the process validation complied with the pre-set acceptance criteria

- Product Specification

The specifications for both strengths of trabectedin powder for concentrate for solution for infusion at release and shelf-life are identical, exception being the related substances test, assay and the uniformity of content, which is only carried out at release. The specifications include tests for appearance before and after reconstitution (visual inspection), reconstitution time, pH, particulate matter, identification of trabectedin (UV and HPLC), assay (HPLC), related substances (HPLC), water content, uniformity of content (mass variation), bacterial endotoxins and sterility.

All analytical procedures used for testing the finished product were adequately described and validated. The majority of the methods were pharmacopoeial methods and therefore validation was deemed unnecessary. The endotoxin determination and sterility test were validated at both manufacturing sites. The HPLC method for the determination of assay and degradation products in the finished product was satisfactory validated for specificity, linearity, range, limits of quantitation and detection, precision (equipment and method repeatability) and accuracy. The intermediate precision, the stability of solutions and the robustness of the method were also evaluated.

- Stability of the Product

Stability data was provided on batches manufactured at both sites and stored at 5°C ± 3°C (long term conditions) for either 18 or 24 months, and under accelerated conditions (25°C ± 2°C / 60% RH ± 5% RH) for 6 months.

Stability studies included tests for appearance before and after reconstitution, reconstitution time, pH of reconstituted solution, particulate matter, identification, water content, assay, degradation products, sterility and bacterial endotoxins. The particulate matter sterility and endotoxins were tested at different checkpoints depending on the manufacturer and stability protocol.

Stability studies on the reconstituted and diluted solutions have also been performed for 30 hours at room temperature/ambient light and refrigerated conditions (5°C ± 3°C) in order to simulate the conditions typically encountered in a clinical setting. It was demonstrated that the drug product

reconstituted concentrated solutions are physically and chemically stable for 30 hours both at room temperature/ambient light and under refrigerated (5°C ± 3°C) conditions.

In addition, photostability studies were also performed to identify possible sensitivity to light of the drug product both in the solid state and after reconstitution with water. The results showed that trabectedin powder for concentrate for solution for infusion is not sensitive to light.

In conclusion, the real time stability data provided support the shelf life and storage conditions as stated in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

Trabectedin was initially obtained by isolation from *Ecteinascidia turbinata* but was subsequently produced synthetically, which resulted in a decrease in the level of impurities. The excipients used in the preparation of the product were chosen based on the physico-chemical properties of the active substance and the intended route of administration (intravenous), which precluded the use of several excipients. The results showed that both the active substance and the finished product can be manufactured reproducibly. This indicates that the product should have a satisfactory and uniform performance in the clinic. At the time of the CHMP opinion, there was a minor unresolved quality issue having no impact on the Benefit/Risk ratio of the product to be addressed as follow-up measure. The Applicant gave a Letter of Undertaking and committed to resolve the Follow Up Measures after the opinion, within an agreed timeframe.

3. Part III: Toxico-pharmacological aspects

Introduction

Trabectedin binds to the minor groove of DNA, bending the helix to the major groove. This binding to DNA triggers a cascade of events affecting several transcription factors, DNA binding proteins, and DNA repair pathways, resulting in perturbation of the cell cycle. Trabectedin has been shown to exert antiproliferative *in vitro* and *in vivo* activity against a range of human tumour cell lines and experimental tumours, including malignancies such as sarcoma, breast, non-small cell lung, ovarian and melanoma.

Pharmacology

- Primary pharmacodynamics

The antitumour effect of trabectedin has been investigated in a panel of human tumour cell lines of mesenchymal origin or directly established from tissue specimens obtained from untreated sarcoma patients. In 23/29 human tumour cell lines of mesenchymal origin exposed to trabectedin in vitro for 72-96 hours, the IC_{50} value was below the expected C_{max} in patients (13 nM). Those with higher IC_{50} values included 2/4 chondrosarcomas, 1/2 fibrosarcomas, 1/4 osteosarcomas, 1/1 malignant peripheral nerve sheath tumour and 1/1 nephroblastoma. In the most sensitive cell lines (HT-1080, M8805, HS-90, M9110, HS-16, HS-18, HS-30, HS-42), trabectedin was more potent (IC_{50} = 0.0002-0.3 nM) than methotrexate (IC_{50} = 15-230 nM), doxorubicin (IC_{50} = 10-160 nM), etoposide (IC_{50} = 16-90 nM) and paclitaxel (IC_{50} = 0.2-1.2). There was no obvious correlation between the sensitivity seen for trabectedin and the comparators. Furthermore, trabectedin was highly effective in cisplatin-resistant sublines of osteosarcoma U-2OS and Ewing's sarcoma Saos-2 cells and in methotrexate-resistant osteosarcoma cells. When tested in SK-LMS-1 leiomyosarcoma, OSA-FH osteosarcoma or CHSA chondrosarcoma cells, there was no difference between the antiproliferative effects of natural and synthetic trabectedin.

Trabectedin was tested for antitumour activity in a number of sarcoma xenografts of murine and human origin (Table 1).

Table 1. Antitumour effect in sarcoma xenografts of murine and human origin

Tumour origin	Cell line	Route and schedule	Dose (mg/m²/day)	Tumour growth inhibition (%)	Reference
Mouse fibrosarcoma	UV2237	IV qdx1	0.6	63	Meco et al. (2003)
Mouse ovarian reticulosarcoma	M5076	IV q7dx2	0.45	64	Donald et al. (2003)
Human chondrosarcoma	CHSA	IV qdx2	0.3	76	Morioka et al. (2003)
Human osteosarcoma	OSA-FH	IV qdx5	0.12	67	Faircloth et al. (2001)
Human rhabdomyosarcoma	TE-671	IV qdx1	0.6	33	Donald et al. (2003)

In vivo, trabectedin had significant antitumour effects in five sarcoma xenograft models of murine or human origin, causing 33-76% tumour growth inhibition at dose levels between 0.12-0.6 mg/m^2/treatment.

There is a substantial body of published data addressing the mode of action of trabectedin. In summary, these findings suggest that trabectedin binds to DNA by alkylating guanine at the exocyclic N2 position in the minor groove and bends the DNA towards the major groove, triggering a cascade of events affecting several transcription factors and DNA repair pathways. These events result in delayed entry into the S phase and arrest in the G2/M interphase, leading to apoptosis through a p53-independent mechanism. Other mechanistic investigations have shown that trabectedin may induce topoisomerase I cleavage complexes and cause disorganisation in the microtubule network around the nuclear membrane. These effects, however, were only observed in cells exposed to trabectedin concentrations that were higher than those required to induce cytotoxicity and above the therapeutically active levels in cancer patients. They are therefore unlikely to be clinically relevant.

- Secondary pharmacodynamics

Secondary pharmacodynamics comprised studies of the myelotoxic effects of trabectedin in normal haematopoietic cells in vitro. Dog CFU-GM progenitors were markedly more sensitive to the myelotoxic effects of trabectedin than those of murine or human origin. In human and murine cells, the IC_{50} values were within or not much above expected plasma levels in patients (13 nM); CFU-Meg progenitors were slightly more sensitive than CFU-GM progenitors.

- Safety pharmacology programme

Safety pharmacology studies comprised a modified Irwin's test in rats, a HERG assay and a test for cardio-respiratory effects in anaesthetised Cynomolgus monkeys. In the modified Irwin's test, groups of 5 male rats were injected IV with a single dose of 0, 12.5, 25 or 50 µg/kg of trabectedin (about 0, 75, 150 and 300 µg/m^2). There were no signs of adverse behavioral effects, neurological impairment or adverse autonomic responses at any dose level. In the HERG assay, trabectedin had no effect over a concentration range of 10-1000 nM. At 10,000 nM (7600 ng/mL), a 10% reduction relative to solvent was observed. In groups of 4 male Cynomolgus monkeys anaesthetised with pentobarbitone and infused with 0 or 90 µg/kg (1 mg/m^2) of trabectedin IV over 60 minutes, there was a transient fall of about 20 mm Hg in diastolic arterial blood pressure after 180 minutes, but no effect on PR, QT, QTcF, QTcV intervals and QRS duration), ECG waveform and rhythm, left ventricular variables, cardiac output, stroke volume, arterial blood variables or respiratory variables. The C_{max} level attained in this study was 10-11 ng/mL, which is similar to that observed in patients.

- Pharmacodynamic drug interactions

Pharmacodynamic drug interactions studies have focused on potential synergism associated with concomitant or sequential use of trabectedin and other antineoplastic agents such as doxorubicin and cisplatin, which was investigated in vivo using xenografts of different origin.

Pharmacokinetics

PK studies were primarily conducted in the rat and the Cynomolgus monkey using a validated LC-MS/MS method with a limit of detection of 0.05 ng/mL for rat plasma and 0.02 ng/mL for monkey plasma. Distribution and metabolism studies used trabectedin labeled with ^{14}C in a metabolically stable position.

In both species, trabectedin plasma levels dropped rapidly at the end of the infusion, followed by a much more gradual decrease, suggesting multi-compartmental kinetics. Although the number of animals investigated was limited (1-2 per dose level), there were no obvious differences in exposure

between sexes or between the first and the last treatment cycle and exposures increased fairly proportionally with dose. In the monkey, estimates of terminal half-lives ranged from 2 to 6 days. The apparent volume of distribution (94.4-287 L/kg) was larger than total body water indicating extensive distribution in the tissues, and systemic clearance was moderate to high (1.43-3.68 L/h.kg). Similar findings were obtained in a population analysis in patients treated with trabectedin 1500 $\mu g/m^2$ every 3 weeks, indicating that trabectedin plasma kinetics is similar in humans and the Cynomolgus monkey.

Tissue distribution studies were conducted in non-pregnant animals. Studies in the rat using ^{14}C-labelled drug substance showed trabectedin to be rapidly and extensively distributed to peripheral tissues including bone marrow, lymphoid organs, gastro-intestinal tissue, liver, kidney and muscle. After peak time, total radioactivity levels declined very slowly and appreciable amounts remained in the carcass at 1 week post-dose, indicating significant retention of trabectedin and/or its metabolites, particularly in females. In the monkey, trabectedin levels were determined in target organs of toxicity. The derived tissue to plasma concentration ratios were estimated at 7.85-73.4 in liver, 11.5-27.1 in kidney and 5.11-68.6 in bone marrow, indicating that tissue concentrations were greater than corresponding plasma concentration by a 5 to 70-fold factor.

Trabectedin was a substrate for P-glycoprotein (P-gp) in vitro and experiments with P-gp knock-out mice showed elevated levels of total radioactivity in the brain (13-fold increase) and testis (2-fold increase) relative to the wild-type controls. Less pronounced increases were observed in other P-gp expressing organs such as the small intestine and heart whereas parent drug levels were similar in the plasma and liver of both strains.

Plasma protein binding of trabectedin was studied in vitro in plasma from mice, rats, rabbits, dogs, Cynomolgus monkeys and humans, and blood distribution was examined in the same species except the monkey. Plasma protein binding was high (90-99%) in all species studied, including man, and the blood to plasma ratio ranged from 0.69 to 1.51 across species, reflecting the observed differences in the free fraction.

Metabolism studies carried out in vitro showed that the hepatic metabolism of trabectedin is fast and extensive. The hepatic metabolism of trabectedin displayed substantial species differences; however, human and monkey liver fractions had similar metabolic profiles and all metabolites produced by human material were also detected in Cynomolgus monkey fractions. The major in vitro metabolic routes identified in man and monkey include *N*-demethylation (ET-729 formation), *O*-demethylation, carboxylic acid formation with and without additional oxidation, mono-oxidation and di-oxidation, aliphatic ring opening and demethylation in combination with mono-oxidation or with di-oxidation. Whereas ET-729 was detected after incubation with hepatic subcellular fractions of all species in vitro, only trace amounts were found in monkeys in vivo and it was not detected in plasma, urine or bile of trabectedin-treated patients. Several major CYP isoforms including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2D6, 2E1, 3A4 and 3A4 were able to metabolise trabectedin in vitro, however, CYP3A4 was shown to be the main isoform at low, therapeutically achievable concentrations.

In rats, mice and humans, the excretion of trabectedin-related radioactivity was slow and occurred principally via the faeces (90-97% of the recovered radioactivity). Biliary excretion of trabectedin and its metabolites was confirmed in bile duct cannulated rats, but did not appear to involve P-gp transport as the excretion pattern was similar between wild-type and P-gp knock-out mice. In the rat, male animals exhibited greater faecal excretion and less body retention of trabectedin-related material than females, but no such sex-differences have been found in humans. There were no excretion studies in the Cynomolgus monkey and no studies of the excretion of trabectedin in milk in any species.

PK interactions caused by plasma protein binding were not investigated as therapeutic plasma levels are too low (typically less than 10 nM) to cause concern. Although trabectedin is metabolised in the liver by CYP3A4, there was no clear evidence of cytochrome P450 inhibition or induction in in-vitro studies using human liver microsomes, human hepatocytes or ex-vitro liver microsomes from rats and Cynomolgus monkeys treated with 3-weekly infusions of up to 300 and 1200 $\mu g/m^2$ of trabectedin for 3-4 cycles.

Toxicology

- Single dose toxicity

Single-dose toxicity studies were carried out in mice, rats, dogs and Cynomolgus monkeys administered trabectedin by IV bolus injection or short-term infusion. The maximum non-lethal dose was 450 $\mu g/m^2$ in mice, 300 $\mu g/m^2$ in female and 450 $\mu g/m^2$ in male rats, 700 $\mu g/m^2$ in dogs and 1050 $\mu g/m^2$ in the Cynomolgus monkey. Common clinical signs included diarrhoea, emesis, inappetence, lethargy, and loss of body weight. Predominant toxicities included myelosuppression characterised by neutropoenia, thrombocytopoenia, leukopoenia associated to hypocellularity in the bone marrow, and atrophy of the lymphoid tissues, and hepatotoxicity, characterised by increased serum transaminases, and focal hepatocyte necrosis in all species. In rodents and dogs, acute cholangitis, hepatobiliary fibrosis and proliferation were observed as well. This toxicity was irreversible in rats, and more pronounced in female animals. In rodents, microscopic evaluation further revealed epithelial necrosis and ulceration in the gastro-intestinal tract and pancreatic acinar single cell necrosis and inflammation at the injection site.

- Repeated dose toxicity

Repeat-dose toxicity studies were conducted in mice, rats, dogs and Cynomolgus monkeys and are summarised in Table 2. In all studies, trabectedin was administered IV either as a bolus injection or a 3- or 24-hour infusion.

Table 2. Summary of repeat-dose toxicity studies

Study ID	GLP	Species/sex/number per group	Duration and dose ($\mu g/m^2$)	Type of IV administration	NTEL ($\mu g/m^2$)	Principal findings
4311-M001-94	Yes	Mouse/M+F/10	5 days 120, 180, 240	Bolus injection	< 120	Mortality, bone marrow depletion
1589/2	Yes	Mouse/M/20	5 days 60, 120	Bolus injection	< 60	Mortality, reduced body weight, increased liver weight and enzymes, bone marrow and spleen depletion and reversible blood cell reductions
1589/5	Yes	Mouse/M/10-15	q 3 w x 1-3 60, 300	Bolus injection	< 60	Mortality, reduced body weight, injection site lesions, gall bladder oedema and reversible blood cell reductions
1589/3	Yes	Rat/M/10	5 days 12, 105	Bolus injection	< 12	Reduced body weight and WBC, increased liver enzymes and atrophy or necrosis in liver, bile ducts, thymus, spleen and GI tract
M049-95	Yes	Rat/F/15	5 days 30, 60, 90	Bolus injection	< 30	Mortality, reduced body weight, RBC parameters and WBC, increased liver enzymes, injection site lesions and atrophy or necrosis in liver, bile ducts, thymus, spleen and GI tract
M050-95	No	Rat/F/5	1-3 5-day treatments each followed by a 30-day break 15, 30, 60	Bolus injection	< 15	As above
5536	Yes	Rat/M+F/10	q 3 w x 3 15, 60, 150, 300, 450	3-hour infusion	< 15	As above, but lesions more severe in females than males
M008-95	Yes	Dog/M+F/1	5 days 140, 280	Bolus injection	< 140	Diarrhoea, prostration, reduced body weight, blood cell reduction, increased liver enzymes, microscopic lesions (atrophy, necrosis or inflammation) in multiple organs
196	Yes	Dog/M+F/2	5 days 100, 160, 220	Bolus injection	< 100	As above in HD and MD animals, but limited to reversible liver and bone marrow pathology in LD dogs

Study ID	GLP	Species/sex/number per group	Duration and dose (µg/m²)	Type of IV administration	NTEL (µg/m²)	Principal findings
5537	Yes	Cynomolgus monkey/M+F/2	q 3 w x 3-4 300-1440 (range-finding)	3- or 24-hour infusion	< 300	Mortality, reduced body weight, RBC parameters and WBC, increased liver weight and enzymes, increased kidney weight, injection site lesions and hypoplasia of bone marrow, spleen and thymus
TOX5702	Yes	Cynomolgus monkey/M+F/2	q 1-3 w x 3-4 120-840 (range-finding)	3-hour infusion	< 120	Mortality, reduced RBC parameters and WBC, increased liver enzymes, intestinal lesions, injection site lesions and hypoplasia of bone marrow, spleen and thymus
TOX6809	Yes	Cynomolgus monkey/M+F/2	q 3 w x 4 300, 600, 840	24-hour infusion	< 300	Study terminated prematurely because of severe injection site lesions
TOX7106	Yes	Cynomolgus monkey/M+F/2	q 3 w x 4-8 300, 600, 780	3-hour infusion through central v. cava catheter	< 300	Mortality due to neutropoenia and intestinal infections, dropsy, reduced RBC parameters and WBC, increased liver enzymes, severe injection site lesions

In mice, the target organs of toxicity comprised the bone marrow, liver and injection site.

In rats, trabectedin induced dose-dependent hepatotoxicity characterised by increased transaminases, ALP and bilirubin, focal hepatocyte necrosis, cholangitis, bile duct inflammation, proliferation, and fibrosis. Most of these changes failed to recover three weeks after the last injection and were far more pronounced in females. Myelosuppression was characterized by reduced bone marrow cellularity and diffuse atrophy of the lymphoid tissues. These changes recovered partially or completely after each treatment cycle. In addition, inflammation and necrosis at the injection site and single cell necrosis, ulceration and epithelial atrophy in the gastro-intestinal tract were common.

In dogs, the target organs of toxicity comprised the bone marrow, liver and gut.

In monkeys, repeated i.v. dosing every three weeks resulted in mortality at doses of 420 µg/m² and above. Myelosuppression was characterized by anaemia, neutropoenia, thrombocytopoenia, leucopoenia associated with depletion in the lymphoid organs and hypocellularity in the bone marrow. Most of these changes recovered at least partially between treatment cycles, with exception of anaemia, which was slightly cumulative. Dose-dependent local intolerance at the injection site resulted in severe thrombophlebitis extending to adjacent tissues. Hepatotoxicity in monkeys was less pronounced than in rats, not systematically observed and was found to be reversible, non-cumulative in nature and characterised by transient increase in transaminase levels, rare increase in ALP, hepatocellular hypertrophy, focal hepatocyte degeneration and necrosis, and mixed inflammatory cells in the sinusoids and portal tracts, without biliary damage. Kidney tubular dilatation, with flattened epithelium and slight epithelial degeneration/necrosis were seen in some animals at high doses. Finally, focal necrotic ulcerations of the colon/rectum were reported, sometimes with bacteria-mediated intestinal inflammatory, hemorrhagic and necrotic lesions, in the animals sacrificed or found dead after the first cycles of treatment. A NTEL was not established in any species.

- Genotoxicity

Trabectedin was evaluated for its potential to induce mutations in bacteria, chromosome aberrations in mammalian cells in vitro and micronucleated polychromatic erythrocytes in mice in vivo. Trabectedin proved genotoxic in all three tests (data not shown).

- Carcinogenicity

No carcinogenicity studies have been conducted.

- Reproduction Toxicity

Trabectedin was tested in conventional developmental toxicity studies in the rat and rabbit (data not shown).

- Toxicokinetic data

Toxicokinetic evaluations were conducted in rats and monkeys. Based on AUC levels, animal to human exposure ratios did not exceed 0.3 in any study. Since a NTEL could not be established, animal to human safety ratios cannot be calculated. It is nevertheless obvious that trabectedin, like other cytotoxic agents, is a highly toxic drug requiring careful monitoring of the target organs of toxicity such as the liver, blood forming organs and gastro-intestinal tract.

- Local tolerance

Local tolerance was examined in male rabbits after single and repeated IV and perivenous dose administration. Single intravenous injection induced a dose-related subacute inflammation, oedema with focal fibrinoid necrosis and endothelial necrosis. Repeated IV administrations (4 times) increased the local irritation. After single perivenous doses of 12 and 120 $\mu g/m^2$ at concentrations of 2.5 and 25 µg/mL in a total volume of 0.3 mL, marked irritation (swelling of the injection site, mild to severe erythema, haematoma and vessel dilation) was seen. These changes corresponded histologically to ulceration, focal fibrinoid necrosis, cutaneous epithelial necrosis, subacute inflammation, and oedema. Similar injection site lesions were a consistent finding in all toxicity studies in which trabectedin was administered by IV injection or infusion, irrespective of species.

Ecotoxicity/environmental risk assessment

No environmental risk assessment has been submitted.

Discussion on the non-clinical aspects

Pharmacodynamics

Trabectedin binds to the minor groove of the DNA, bending the helix to the major groove. This binding to DNA triggers a cascade of events affecting several transcription factors, DNA binding proteins, and DNA repair pathways, resulting in perturbation of the cell cycle.

Preclinical data indicate that trabectedin has limited effect on the cardiovascular, respiratory and central nervous system at exposures below the therapeutic clinical range, in terms of AUC.

The effects of trabectedin on cardiovascular and respiratory function have been investigated *in vivo* (anesthetized Cynomolgus monkeys). A 1-hr infusion schedule was selected to attain maximum plasma levels (C_{max} values) in the range of those observed in the clinic. The plasma trabectedin levels attained were 10.6 ± 5.4 (C_{max}), higher than those reached in patients after infusion of 1500 $\mu g/m^2$ for 24 (C_{max} of 1.8 ± 1.1 ng/ml) and similar to those reached after administration of the same dose by 3-hr infusion (C_{max} of 10.8 ± 3.7 ng/ml).

Pharmacokinetics

No studies have been submitted on the potential impact of other CYP substrates on the metabolism of trabectedin. However, Brandon et al. (2005) reported that the cytotoxic activity of trabectedin could be increased in combination with the CYP inhibitors metyrapone (3A4), phenanthrene (substrate for 2E1, 3A4), piperonyl butoxide (3A), proadifen (2C9, 2E1, 3A4), ritonavir (3A4), and warfarin (2C9, 2C19) in the Hep G2 cell line in vitro, suggesting that combination therapy of trabectedin with CYP inhibitors, e.g. other anticancer drugs, could lead to changes in hepatotoxicity and therefore might be of clinical importance. The same applies to co-administration of trabectedin and other Pg-p substrates.

Toxicology

Myelosupression and hepatoxicity were identified as the primary toxicity for trabectedin. Findings observed included hematopoietic toxicity (severe leucopenia, anemia, and lymphoid and bone marrow depletion) as well as increases in liver function tests, hepatocellular degeneration, intestinal epithelial necrosis, and severe local reactions at the injection site. Renal toxicological findings, mainly histopathological changes, were detected in multi-cycle toxicity studies conducted in monkeys. Since the interpretation of these toxicological results might have been hampered due to the occurrence of

secondary histological kidney lesions, uncertainly attributable to the severe local reactions at the injection site, caution must be guaranteed in the interpretation of these renal findings, and treatment-related renal toxicity cannot be excluded.

Trabectedin is genotoxic both *in vitro* and *in vivo*. Long-term carcinogenicity studies have not been performed.

Fertility studies with trabectedin were not performed but limited histopathological changes were observed in the gonads in the repeat dose toxicity studies. Considering the nature of the compound (cytotoxic and mutagenic), it is likely to affect the reproductive capacity.

Trabectedin, like other cytotoxic agents, is a highly toxic drug requiring careful monitoring of the target organs of toxicity such as the liver, blood forming organs and gastro-intestinal tract.

Trabectedin was a strong irritant causing severe injection site inflammation, necrosis and fibrosis in all IV toxicity studies, irrespective of species, as well as in a conventional rabbit test for local tolerance using single and repeated IV and perivenous dose administration. It is therefore appropriate that the SPC recommends administration by slow intravenous infusion through a central venous line.

The proposed impurity specifications comprise six substances at levels above the qualification threshold. These have been qualified in single- and repeat-dose toxicity studies and/or clinical studies using batches of drug substance or drug product containing impurity levels in excess of the proposed specifications. None of the impurities has been tested for genotoxicity, but since trabectedin is itself a genotoxic substance intended for the treatment of cancer patients with a poor prognosis, this is not a cause for concern.

No environmental risk assessment has been submitted. The small number of patients suffering from the proposed indication would preclude any substantial environmental exposure to the drug.

4. Part IV: Clinical aspects

Introduction

The clinical development program supporting the efficacy of trabectedin in the treatment of STS includes one ongoing pivotal, randomised, unblinded, dose-finding, Phase-II study (ET743-STS-201) and 3 completed Phase II, non-controlled studies, ET-B-005-98, ET-B-008-98, and ET-B-017-99. The original Phase II studies included a variety of different histological types of STS, whereas the randomised, pivotal Phase II study was restricted to patients with L-sarcoma (liposarcoma and leiomyosarcoma).

Yondelis must be administered under the supervision of a physician experienced in the use of chemotherapy. Its use should be confined to qualified oncologists or other health professionals specialised in the administration of cytotoxic agents. The recommended dose is 1.5 mg/m^2 body surface area, administered as an intravenous infusion over 24-hr with a three-week interval between cycles. Administration through a central venous line is strongly recommended (see SPC section 6.6).

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.

Pharmacokinetics

- Absorption

Trabectedin is administered as a constant rate, intravenous infusion. Therefore, the clinical pharmacology program did not include conventional studies that assess bioavailability, bioequivalence, food, and effect.

- Distribution

Trabectedin is extensively bound to plasma proteins. The mean free (unbound) fraction, evaluated by equilibrium dialysis, was 2.23% and 2.72% at 10 ng/mL and 100 ng/mL, respectively. Trabectedin was more extensively bound to alpha-1 acid glycoprotein (free fraction, 9.95% at 0.05 g/dL of protein and 3.34% at 0.1 g/dL of protein) as compared to albumin (free fraction, 13.98% at 4.3 g/dL of protein) in purified solutions of each protein at physiological concentrations. Binding to plasma proteins was also assessed by ultracentrifugation. The free fraction was 5.8% at a total trabectedin concentration of approximately 3 ng/ml.

The ratio of trabectedin concentration in blood versus plasma averaged 0.89 during *in vitro* studies. Thus, trabectedin distributes to the cellular components of whole blood to some degree.

The population estimate of distribution volume in the central compartment is 16.1 L and 13.9 L in male and female patients, respectively. The distribution volume at steady state is 6070 L and 5240 L, respectively.

- Elimination

Trabectedin is metabolized by various routes that produce a wide range of metabolites upon incubation with human hepatic subcellular fractions and hepatocytes. Those identified include *N*-demethylation (formation of N-desmethyl trabectedin or ET-729), *O*-demethylation, carboxylic acid formation with and without additional oxidation, mono-oxidation and di-oxidation, aliphatic ring opening, and demethylation in combination with mono-oxidation or with di-oxidation. No appreciable glucuronidation of trabectedin was demonstrated. In addition, no metabolites were formed after incubation with trabectedin with human whole blood. Although most major CYP enzymes metabolize trabectedin *in vitro*, CYP3A4 is considered to be the main CYP enzyme responsible for the oxidative metabolism of trabectedin at clinically relevant concentrations.

Experiments were conducted using membrane preparations of *E. coli* expressing individual human CYPs together with human reductase. Disappearance of trabectedin (5 µM or 3.81 µg/mL) and concomitant formation of radiolabelled metabolites after incubation with CYP1A2, 2A6, 2C8, 2C9, 2C18, 2D6, 2E1, and 3A4 was observed. Virtually no metabolite formation was seen with CYP1B1, 2C19, and 4A11. *In vitro* experiments with cDNA expressed human enzymes indicated CYP2C9, 2D6, 2E1, and 3A4 metabolize trabectedin (50 µM or 38.1 µg/mL), whereas no notable metabolism by CYP1A1, 1A2, 2A6, 2B6, 2C8, and 2C19 was observed.

As supra-therapeutic concentrations were used, another set of experiments was conducted aimed at identifying the major CYP enzymes involved at clinically relevant concentrations of trabectedin (7.62 nM or 10 ng/mL). Trabectedin was incubated together with pooled human liver microsomes (male and female donors) and the disappearance of unchanged drug from the incubation mixture was quantified. Selective chemical inhibitors and inhibitory antibodies directed against specific CYPs were used as diagnostic tools.

Trabectedin metabolism was markedly diminished by chemical inhibitors of CYP3A4 (ketoconazole and troleandomycin) and selective inhibitory antibodies directed towards this enzyme. Chemical inhibitors and/or antibodies of other CYP enzymes had no effect. The latter experiments strongly suggest CYP3A4 is the predominant CYP enzyme responsible for the hepatic metabolism of trabectedin.

Study ET-A-013-01 was mass balance study of ET-743 administered as a 3- or 24-hour intravenous infusion to patients with advanced cancer conducted in order to characterize the metabolic profile and routes of excretion of trabectedin in patients with solid tumors (mass balance and metabolism study). The study included 8 patients and was an open-label, non-randomized study including full PK sampling during Cycle 1. Urine and fecal samples were collected for metabolite profiling and identification. A majority of the radio labelled material excreted was recovered in the faeces (57.6% of the dose) and smaller amounts recovered in the urine (5.8% of the dose) over a collection interval of up to 24 days and 10 days, respectively (ET-A-013-01). Negligible quantities of unchanged drug were recovered in urine (<1% of the dose) and faeces, confirming the extensive metabolism of trabectedin *in vivo*. The insignificant urinary excretion of unchanged trabectedin was confirmed during 3 other Phase I studies.

The active metabolite ET-729, which was extracted from faecal samples only under acidic conditions, accounted for a minor fraction of the total radioactivity recovered in faeces and has not been recovered in urine.

The population pharmacokinetics model used a single clearance parameter to describe elimination of trabectedin by all routes (Population Pharmacokinetic Analysis of Trabectedin (ET-743) in Patients With Cancer). The typical value of trabectedin plasma clearance and the corresponding intersubject variability were 31.5 L/h and 51%, respectively.

Most trabectedin-derived metabolites have not been identified due to, in part, the low concentrations of metabolites, low faecal extraction recoveries, and complex metabolite profile of trabectedin. N-desmethyl-trabectedin (ET-729) is a pharmacologically active metabolite of trabectedin. Its concentrations in plasma were below the limit of quantification of 0.1 ng/mL in samples collected from 14 patients administered trabectedin as a 3-hour or 24-hour infusion during 6 Phase I and II studies. The glucuronide conjugates of trabectedin could not be measured in plasma samples of trabectedin-treated patients. The metabolism of trabectedin was investigated using urine and faeces collected from patients with cancer administered a single-dose of 14C-labelled trabectedin as a 3- or a 24-h intravenous infusion (ET-A-013-01). Radio-chromatograms of faeces showed that trabectedin was extensively metabolized to several radio labelled metabolites including ET-745 (carbonyl metabolite), ET-731, and ETM-217. Metabolites ET-729, ET-759A, and ETM-259 were recovered in faeces only under acidic conditions. Metabolites recovered in urine included ET-745, ET-759A, ETM-259, and ETM-204. There was no evidence that unchanged trabectedin undergoes direct glucuronidation. However, at least one oxidative metabolite appears to be glucuronidated prior to urinary excretion.

These results are supported data indicating that urine and bile samples of trabectedin-treated patients did not contain measurable concentrations of ET-729 or glucuronide conjugates of trabectedin.

Both ET-745 and ET-759A are known degradants of trabectedin. In addition, the "ETM compounds" may have been produced via fragmentation of trabectedin and/or its metabolites in the mass spectrometer during sample analysis. Therefore, it is not clear if these compounds are genuine metabolites or artefacts.

- Dose proportionality and time dependencies

Dose-proportional pharmacokinetics were demonstrated for the 3-hour intravenous infusion as well as for the 24 hour infusion.

- Special populations

A formal clinical study to evaluate the impact of renal impairment on the pharmacokinetics of trabectedin has not not performed since only a minor fraction of the total dose is excreted in the urine as unchanged drug or derived metabolites.

A population analysis showed no relationship between the concentration of liver enzymes in serum (ranges: AST, 0.15 to 3.49 x ULN; ALT, 0.03 to 4.76 x ULN; alkaline phosphatase, 0.17 to 6.82 x ULN; LDH, 0.25 to 20.38 x ULN; total bilirubin, 0.08 to 4.00) and the plasma clearance of trabectedin.

Clinically significant association between gender and plasma clearance was not observed during the population pharmacokinetic analysis.

A study to evaluate the impact of race/ethnicity on the clinical pharmacokinetics of trabectedin has not been performed.

In light of the available information on the distribution and elimination of trabectedin, clinically significant effects of race/ethnicity on the pharmacokinetics of trabectedin are not expected and dose adjustments are not recommended.

The population pharmacokinetic analysis indicated that the plasma clearance and distribution volume of trabectedin is not influenced by patient age (range, 19 to 83 years).