SCIENTIFIC DISCUSSION

1 Introduction

Kidney cancer represents approximately 2% of all cancers worldwide and is responsible for 2% of all cancer-related deaths. Renal cell carcinomas (RCC), which comprise the majority of kidney tumours, are epithelial tumours in nature. Approximately 30% of RCC patients have metastases at the time of initial diagnosis. For the last 20 years, immunotherapy based on treatment with cytokines has been the standard of care for patients with advanced RCC. The two different cytokines used for the treatment of advanced RCC are interferon-alpha (IFN-alpha) and interleukin-2 (IL-2).

RCC, which originates in the renal cortex, accounts for up to 85% of malignant kidney tumours. Based on histological classification, approximately 75% of RCC are of the clear cell type, 15% are papillary, 5% are chromophobic and the remaining comprises a variety of tumour types. The poor prognosis of clear cell RCC correlates with the appearance of higher nuclear grade and the presence of a sarcomatoid (spindle) cell morphology. Papillary and chromophobic RCC are associated with a more favourable prognosis than clear cell RCC.

Treatment and prognosis are dependent on the disease stage at initial diagnosis. In Stage I-III surgical procedures are part of standard care. It has been shown that 50% of patients diagnosed with Stage I can be cured by nephrectomy, whereas almost all of the 30% patients presenting with Stage IV are incurable. Without intervention, patients with advanced RCC have a median overall survival (OS) of approximately one year and a 5-year survival rate of ≤ 10%. Surgical resection may be appropriate for selected patients, including those with isolated metastases. However, the disease often recurs, even when the primary and metastatic sites are aggressively resected. Radiation therapy can provide significant palliation to painful metastases. Use of conventional chemotherapy has generally been very limited because of low rate of responders to therapy leading to a poor rate of success.

IFN-alpha therapy is approved in several EU member states on the basis of data from randomised trials showing a survival benefit in previously untreated RCC patients. A meta-analysis of studies evaluating IFN-alpha-containing regimens with non-IFN-alpha-containing regimens showed objective response rates (ORR) of 14% with IFN-alpha (4-33%) and 8% without IFN-alpha (3-27%) (Hernberg M et al., *J. Immunother.* 1999,:22:145-154).

IL-2 is also approved in several EU member states as subcutaneous injection and continuous intravenous (IV) infusion.

Two new medicinal products have been approved for the treatment of advanced RCC in 2006:

- Sorafenib (Nexavar) is a receptor tyrosine kinase inhibitor and targets the RAS/RAF/MEK/ERK pathway as well as the c-KIT, FLT-3, PDGFR (platelet derived-growth factor receptor) and VEGFR (vascular epidermal growth factor receptor) signalling pathways. In randomised placebo-controlled trials, sorafenib showed a significantly prolonged median progression-free survival (PFS) in previously treated patients with at least one systemic treatment when compared to placebo.
- Sunitinib (Sutent) is a tyrosine kinase inhibitor which targets VEGFR, PDGFR, c-KIT and FLT-3 signalling pathways. In single-arm studies, sunitinib showed a high ORR in patients previously treated with a cytokine therapy and improved PFS as compared to IFN-alpha as a first line therapy in previously untreated patients.

Temsirolimus is a selective inhibitor of mTOR (mammalian target of rapamycin), a serine/threonine kinase involved in controlling many cellular functions such as cell proliferation, cell survival, protein synthesis and transcription. Temsirolimus binds to an intracellular protein (FKBP-12), and the protein-temsirolimus complex inhibits the activity of mTOR that controls cell division. In treated tumour cells, inhibition of mTOR activity results in a G1 growth arrest caused by the disruption of

translation of regulatory cell cycle proteins (D-type cyclins, c-myc, and ornithine decarboxylase). When mTOR is bound to the temsirolimus-FKBP-12 complex, its ability to phosphorylate and control the activity of protein translation factors that regulate cell division (4E-BP1 and S6K), is blocked. These protein translation factors are both downstream of mTOR in the P13 kinase/AKT pathway.

In addition to regulating cell cycle proteins, mTOR can regulate translation of the hypoxia-inducible factors, HIF-1 and HIF-2 alpha. These transcription factors regulate the ability of tumours to adapt to hypoxic microenvironments and to produce the angiogenic factor VEGF. The anti-tumour effect of temsirolimus, therefore, may also in part stem from its ability to depress levels of HIF and VEGF in the tumour or tumour microenvironment, thereby impairing vessel development.

Wyeth Europa Ltd. has applied for a marketing authorisation through the centralised procedure for TORISEL 25 mg/ml concentrate and diluent for an infusion solution in the treatment of advanced RCC. The recommended dose of temsirolimus is 25 mg infused over a 30- to 60-minute period once weekly. Patients should receive medication of intravenous diphenhydramine 25 to 50 mg (or similar antihistamine) approximately 30 minutes before the start of each dose of temsirolimus infusion.

2 Quality aspects

Introduction

TORISEL 25 mg/ml concentrate for solution for infusion contains a new active substance, temsirolimus, a 42-bis(hydroxymethyl) propionic acid ester derived from rapamycin (sirolimus). Rapamycin, the starting material of temsirolimus, is a macrocyclic lactone produced by fermentation. While rapamycin is intended for immunosuppression, temsirolimus is indicated for the treatment of renal cell carcinoma.

Rapamycin is already present as the active substance in the authorised product, Rapamune. Rapamune 1 mg/ml, 2 mg/ml, 5 mg/ml oral solution and Rapamune 1 mg, 2 mg and 5 mg film-coated tablets were respectively authorised through the Centralised Procedure in 2001, 2002 and 2005 (EMEA/H/C/0273/01-03, EMEA/H/C/0273/04/X01, EMEA/H/C/0273/05/X05 and EMEA/H/C/0273/06/X21).

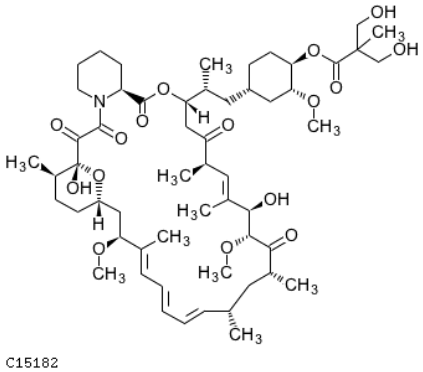
The finished product, TORISEL, is a two-vial system consisting of a concentrate solution intended for infusion containing 25 mg/ml temsirolimus (in one vial) and a specifically formulated diluent (in another vial). Before use, the temsirolimus concentrate has to be diluted with the diluent, followed by a dilution with 0.9% sodium chloride for intravenous injection.

Temsirolimus 25 mg/ml concentrate is packaged in type I glass vials containing 1.2 ml concentrate, sealed with butyl rubber stoppers. The diluent for temsirolimus concentrate 25 mg/ml is packaged in type I glass vials containing 2.2 ml of diluent, sealed with butyl rubber stoppers. A cardboard box is used as secondary packaging to protect the product from light.

This application focuses particularly on the quality by design of the active substance and the drug product as referred to in the ICH Note for Guidance Q8/Q9/Q10. Main topics were oxidative/hydrolysis degradants (including design space), the content of metal and acid in the product and issues regarding process development and monitoring using Process Analytical Technology (PAT) (including “PAT tools” for stability prediction).

Active Substance

The structure of the active substance temsirolimus (rapamycin, 42-[3-hydroxy-2 (hydroxymethyl)-2-methylpropanoate, ester of rapamycin] is disclosed hereafter.



Temsirolimus is a white to off-white non-hygroscopic powder, poorly soluble in water but very soluble in ethanol. There are two possible polymorphic forms: the crystalline form and the pseudomorph form (solvated form). In this application, only the crystalline form is obtained via the proposed commercial manufacturing process.

Regarding stereochemistry, three isomers A, B and C can exist and they interconvert in solution. Isomer B is the predominant isomer (≥ 97 %) in both solution and solid states, whereas Isomer A is only observed in solution state.

The structure of temsirolimus has been confirmed by infrared (IR), ultraviolet (UV), mass spectrometry, ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectrometry as well as by elemental analysis. Physicochemical characterisation includes data on appearance, hygroscopicity, polymorphism (X-ray diffraction, scanning calorimetry), melting point, solubility in various solvents, apparent partition coefficient and stereochemistry.

- Manufacture

The 7 steps synthesis of temsirolimus is adequately described starting from rapamycin and including the purification to yield the parenteral grade temsirolimus. The process and quality of rapamycin comply with the requirements from the Ph. Eur. monograph "Products of fermentation".

Reaction conditions (molar ratios, yields, temperatures) and the necessary in-process controls are described in detail. Specifications of starting materials and isolated intermediates including rapamycin and pre-parenteral grade temsirolimus are satisfactory. Solvents, reagents and auxiliary materials used in the synthesis have been sufficiently analysed. Analytical methods have been described and validated.

Study of the quality of drug product indicated that the following 3 factors: oxidative/hydrolysis degradant content (OD), metal content and acid content in the active substance were critical to the drug's purity and stability. Therefore the drug product has been developed using principles of Quality by Design with the objective to maximise product purity, stability, design and control limits.

A Quality by Design approach was also undertaken for the active substance. The purpose of the Quality by Design work was to identify the attributes of the active substance that were critical to the safety, quality and/or performance of the drug product.

From this knowledge, an analysis of the critical factors leading to high levels of OD, metal, and acid content in the active substance was undertaken. Critical process parameters significantly affecting the levels of OD, metal, and acid content (whether in active substance, excipients or drug product) were identified through a risk analysis of the manufacturing process. This activity was used to define a design space (for e.g. the active substance critical quality attributes). Finally, critical in-process controls were implemented in the active substance manufacturing process to ensure that the process would be maintained within the desired design space.

The applicant constructed a Quality by Design concept to avoid/reduce unfavourable factors as well as to control them. In relation to the active substance, two detailed reports pertaining to the quality by design concept (development work) were presented.

The applicant declared that during routine manufacture, the active substance and drug product will be manufactured within the control space (see for the above mentioned critical process parameters and attributes).

- Specification

The following tests were used to measure adequate specification: description, identification (IR and high pressure liquid chromatography [HPLC]), assay (HPLC), related substances (HPLC), rapamycin content (HPLC), water content (Karl-Fischer), heavy metals, residue on ignition, residual solvents (GC), optical rotation and microbiological quality.

Impurity limits were justified by toxicological studies and the level of residual solvents are in line with ICH limits. Analytical methods were satisfactorily described and validated in accordance with ICH requirements.

Certificates of analysis were submitted for 9 pre-parental grade development batches and 3 final parenteral grade active substance production-scale batches. The results are within the specification and this demonstrates the consistency of the process.

Parenteral grade temsirolimus is flushed with nitrogen and kept in triple low-density polyethylene (LDPE) bags. Dimensions and IR certificates confirm the identification of the packaging material. Certificates of analysis for the packaging materials have been provided and are in accordance with Ph. Eur.

- Stability

Studies on stability of the product were carried out under ICH conditions on 9 pre-parenteral grade batches (up to 18 months at 5°C and 25°C/60%RH and up to 6 months at 40°C/75%RH) and 3 parenteral grade batches (up to 12 months at 5°C and 25°C/60%RH and 6 months at 40°C/75%RH), including photostability studies conducted in accordance with the Note for Guidance ICH Q1B. The parameters tested during the stability studies were: appearance, impurities, assay, water content, microbial quality and bacterial endotoxins. The analytical methods were the same as the ones used for the control of the active substance. Results showed that temsirolimus remains stable at 5°C and is light-sensitive. Based on the available stability data, a re-test period of 18 months can be granted when stored in polyethylene bags inside aluminium can with desiccant under refrigerated (2°C to 8°C) conditions.

Medicinal Product

The finished product consists of 2 vials: one vial contains a concentrate of temsirolimus 25 mg/ml solution for infusion (non-aqueous, ethanolic, sterile preparation) while the second vial contains a diluent.

Temsirolimus 25 mg/ml concentrate solution for infusion

- Pharmaceutical Development

A concentrate of temsirolimus 25 mg/ml was developed using the principles of "Quality by Design" with the objective to maximise product stability and purity. Studies indicate that the critical aspects required for consistent product manufacture were primarily to control the raw materials, including the active substance (which was found to be sensitive against oxidation and hydrolysis), to detect metal and acid contents, and to shield the product from exposure to light.

Studies were performed to establish sufficient limits (design and control space).

The pharmaceutical development, including optimisation studies, was adequately described. The Concentrate and Diluent were produced by a simple aseptic process and sterile filtration. Filtration was retained and justified as the sterilisation method.

The proposed composition of the concentrate includes temsirolimus as the active substance, dl-alpha tocopherol (Vitamin E) as an antioxidant, dehydrated alcohol and propylene glycol as solvents, anhydrous citric acid as a stabiliser, and nitrogen as an inert cover in filled vials.

The excipients used for the finished product are commonly used for parenteral formulations, with some additional tests were added. The excipients are in compliance with Ph. Eur./USP and the residual solvents in excipients were in accordance with ICH Q3C note for guidance.

- Adventitious Agents

None of the excipients used for temsirolimus 25 mg/ml concentrate are derived from either a human or an animal source. However, a transmissible spongiform encephalopathies (TSE) statement concerning zinc stearate used as a processing aid for the rubber stopper was presented. Additionally, a statement is presented for rapamycin (used as a starting material for the active substance) since bacto soytone is used during the fermentation process. This is acceptable and no TSE risk is anticipated.

- Manufacture of the Product

The manufacture of temsirolimus 25 mg/ml concentrate solution for infusion can be summarised in the following steps: 1) preparation of the non-sterile bulk formulation, 2) product transfer, 3) aseptic filtration and 4) filling. The manufacture of the concentrate solution for infusion meets the current standards of pharmaceutical technology. Facilities, equipment and materials were described in detail.

The following critical steps were identified: filtration, filling and sealing. Appropriate controls were established. The manufacturing process was satisfactorily validated on 3 consecutive batches and demonstrated consistency and reproducibility of the process. The aseptic process was also validated.

- Product Specification

The parameters used for the product specification of the concentrated solution for infusion include: appearance, identification (HPLC and UV), assay of the active substance, degradation products, uniformity of dosage units (Ph. Eur.), particulate matter (Ph. Eur.), sterility (Ph. Eur.) and bacterial endotoxins (Ph. Eur.).

Analytical methods were adequately described and validated.

Batch data were presented for 16 batches and all the results were in compliance with the proposed specification.

Temsirolimus 25 mg/ml concentrate solution for infusion is packed in type I glass vials, with butyl rubber stoppers. Appropriate information has been presented for the sterilisation of the packaging components (such as vials, stoppers). Certificates of analysis of the vials and the stoppers have been provided and are in compliance with Ph. Eur. requirements for packaging materials.

- Stability of the Product

The stability of the concentrated temsirolimus solution was demonstrated in stability studies carried out on 3 pilot batches kept in the commercial packaging under ICH conditions (inverted vials, up to 24 months at 5°C and 6 months at 25°C/60% RH). Since out-of specification results were observed under 25° C/60% RH storage conditions, no extrapolation of the shelf-life was allowed.

In addition, stability results on production scale batches after 6 months were presented. The following test parameters were monitored during stability: appearance, description, strength, purity, water content, particulate matter, sterility, bacterial endotoxin, oxygen in headspace, ethanol content and d-alpha-tocopherol content. The analytical methods used during the stability studies were identical to the methods used for the drug product.

Furthermore, studies in accordance ICH Option 2 light stability studies were also conducted. Stability studies performed on the reconstituted product (concentrate+diluent) showed that it remains stable for up to 24 hours when stored at room temperature.

Stability studies of the diluted temsilorimus reconstituted solution in 0.9% sodium chloride for infusion were carried out. The solution for infusion remains stable up to 6 hours at room temperature.

Based on the stability data, the proposed shelf-life can be granted when stored in the commercial packaging under the conditions specified in the SPC before and after reconstitution.

- Comparability Exercise for Drug Product (to be changed in the EPAR to "Medicinal Product")

Studies demonstrating the compatibility of the container with the finished product were satisfactorily conducted, including extractable studies, fragmentation and self-sealing of the stopper after puncture. These were in line with section 3.2.9 of the Ph. Eur.

Additionally, compatibility studies with the reconstituted product before infusion were conducted in several intravenous administration settings. It was shown that polyvinyl chloride PVC should not be used and the material needs to be diethylhexylphthalate (DEHP)-free. The recommended materials include materials composed of glass or polyolefins.

Diluent for temsirolimus 25 mg/ml concentrate solution for infusion

The primary diluent for temsirolimus 25 mg/ml concentrate is a sterile, non aqueous solution. The resulting solution is further diluted in a 0.9% sodium chloride solution for intravenous injection.

- Pharmaceutical Development

The pharmaceutical development of the diluent was well described. The objective was to select water-miscible co-solvents that increase the solubility of the concentrated drug and, at the same time, that provide adequate chemical stability.

The diluent contains polysorbate, polyethylene glycol, dehydrated alcohol and nitrogen. Polysorbate was selected as a surfactant because it prevents the precipitation of temsirolimus in aqueous solutions, polyethylene glycol increases solubility and anhydrous ethanol can act as a diluent facilitating the filtration and filling of the container. Nitrogen is used as a headspace gas.

All the excipients comply with the requirements of the Ph. Eur. and USP/NF monographs. A certificate of analysis is presented for each excipient. Residual solvent levels are in accordance with the requirements of the ICH guideline Q3C.

The diluent for temsirolimus 25 mg/ml concentrate is packaged in a type I glass vials, containing a volume of 2.2 ml of diluent, sealed with butyl rubber stoppers.

- Adventitious Agents

None of the excipients used for the diluent is derived from human or animal sources. Nevertheless, as for the concentrate, a statement regarding TSE for the manufacture of the stoppers was provided, and is satisfactory.

- Manufacture of the Product

The diluent for temsirolimus 25 mg concentrate is manufactured by aseptic processes. The process can be summarised by the following 3 steps: 1) preparation of the bulk diluent, 2) sterile filtration and 3) aseptic filling of the container. The manufacture of the diluent meets the current standards of pharmaceutical technology and the equipment used is well described.

The following critical steps in the manufacturing process were identified: filtration, filling and sealing. Appropriate controls were established. Based on the process validation data of 3 consecutive batches,

it can be concluded that the manufacturing process for the diluent produces a product of adequate and reproducible quality. The aseptic process was also validated.

- Product Specification

The parameters used for the product specification of the diluent include: appearance and description, sterility (Ph. Eur.), bacterial endotoxins (Ph. Eur.), identity of polysorbate (Ph. Eur.), particulate matter (Ph. Eur.) and extractable volume (Ph. Eur.). Analytical methods were described and validated.

Batch data were presented for pilot and production scale batches. All the results are in compliance with the proposed specification.

The diluent is packed in a type I glass vials, with butyl rubber stoppers. Appropriate information was presented for the sterilisation of the packaging components (such as vials, stoppers). Certificates of analysis for the vials and stoppers have been provided and are in compliance with Ph. Eur. requirements.

- Stability of the Product

The stability of the diluent was demonstrated in stability studies carried out on 3 pilot batches kept in the commercial packaging under ICH conditions (inverted vials, up to 24 months at 5°C and at 25°C/60%RH). ICH Option 2 light stability studies were also conducted. The following test parameters were monitored: appearance and description, identity (polysorbate), sterility, bacterial endotoxins, oxygen in headspace, ethanol content, particulate matter, extractable volume and water content. The results showed that the diluent remained stable under all tested conditions and was not light sensitive.

Based on the stability data, the proposed shelf-life can be granted when stored in the commercial packaging under the conditions defined in the SPC. Since temsirolimus 25 mg/ml concentrate solution for infusion requires storage under refrigerated conditions, the refrigerated storage of the diluent will accommodate the co-packing of diluent with the temsirolimus concentrate.

Discussion on chemical, pharmaceutical and biological aspects

Information on the development, manufacture and control of the drug substance and drug product were presented in a satisfactory manner, especially in relation to the Quality by Design concept.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics in a satisfactory manner. Therefore, the product should have a satisfactory and uniform performance in the clinic.

3 Non-clinical aspects

Introduction

Non-clinical studies with temsirolimus were conducted to support multiple indications which included oncology. Specific to the oncology programme, nude mouse xenografts using various tumour types were used as pharmacologic animal models and toxicity studies have been conducted in rats and monkeys using IV administration (once-daily, cyclic, or once-weekly dosing regimens) or oral administration (cyclic dosing regimen) to support the clinical development trials. In addition, the effect of tumour growth inhibition by temsirolimus has been studied in combination with a number of cytotoxic agents including vincristine, topotecan, cisplatin, melphalan, carmustine (BCNU), gemcitabine, 5-fluouracil (5-FU), doxorubicine and paclitaxel *in vitro* and *in vivo* (data not shown).

Pharmacology

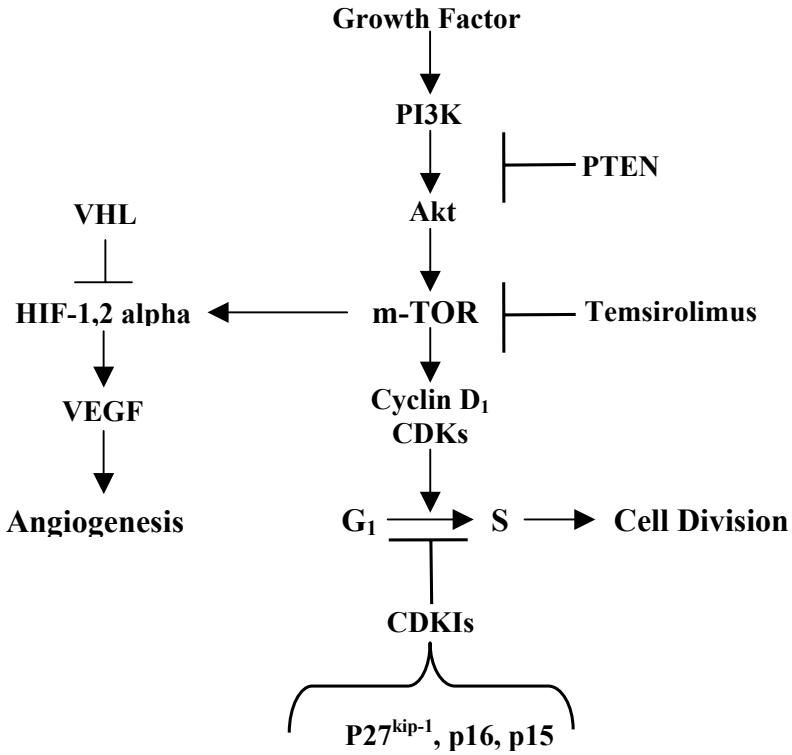
- Mechanism of action

Temsirolimus is a specific inhibitor of the mammalian target of rapamycin (mTOR), a serine/threonine kinase that regulates a signalling cascade that controls growth factor induced cell proliferation (Figure 1). Temsirolimus exerts its effect on cell proliferation by inhibiting mTOR dependent protein

translation induced by growth factor stimulation of cells. The net effect of this class of compound on the cell cycle is to block the $G_1 \rightarrow S$ phase transition by inhibiting the expression of Cyclin D_1 and activation of cyclin dependent kinases (CDKs).

Temsirolimus binds reversibly to the intracellular cytoplasmic FK506-binding protein (FKBP12). Sirolimus, the major metabolite of temsirolimus, also binds to FKBP12. Both sirolimus and temsirolimus have been shown to block the activity of mTOR.

Figure 1. Temsirolimus inhibition of tumour cell proliferation and angiogenic pathways



CDK = Cyclin dependent kinase; HIF = Hypoxia inducible transcription factor; mTOR = Mammalian target of rapamycin; PI3K = Phosphatidylinositol-3 kinase; PTEN = Phosphatase and tensin homolog tumor suppressor gene; VEGF = Vascular endothelial cell growth factor; VHL = Von Hippel Lindau tumor suppressor gene.

In addition to cell-cycle proteins, the translation of other classes of protein is selectively regulated by mTOR. Thus, inhibition of mTOR by temsirolimus can impair tumour growth indirectly through inhibition of micro-environmental factors (e.g. VEGF) that support tumour growth. Approximately 80% of RCC have lost the von Hippel-Lindau tumour suppressor (VHL) gene, a negative regulator of hypoxia inducible transcription factor (HIF-1,2α), which results in increased levels of HIF and subsequent increased transcription of VEGF. Therefore, RCC may be particularly sensitive to treatment with temsirolimus. Other supporting elements, such as the formation of tumour stroma that require growth factors, can also be blocked by inhibiting mTOR with temsirolimus.

- Primary pharmacodynamics

The antiproliferative effect of temsirolimus was tested *in vitro* against number of other human tumour lines. However, no *in vitro* studies on the antiproliferative effects of temsirolimus in renal cancer cells were provided. The mean IC_{50} for all cells tested was 1.4 μM. The most sensitive cell lines were prostate, breast cancer, CNS and T cell leukaemia lines.

Temsirolimus has shown activity *in vivo* in a broad range of human tumour mouse xenograft models including RCC models. A498 cells are human RCC cells that lack the VHL gene and also show evidence of constitutive Akt activation (a kinase that controls mTOR activation through regulation of intermediates directly upstream of mTOR). Temsirolimus administered to nude mice grafted with A498 cells demonstrated sustained inhibition of the growth of the A498 cells. In addition, combination

of temsirolimus with IFN-alpha administration in this same model caused substantial tumour regression.

Tumour inhibition by temsirolimus was also shown on xenografts of breast and glioblastoma cancers. In particular, several IV dosing regimes were studied to determine the *in vivo* responsiveness of tumour cell growth to temsirolimus in nude mice human glioblastoma U87MG xenografts. Each dosing regimes (5 days a week, alternate days or weekly) was effective in slowing tumour growth.

- Secondary pharmacodynamics

Secondary pharmacodynamic studies were conducted to investigate effects of temsirolimus on T lymphocyte function by measuring the delayed type hypersensitivity (DTH) response in mice. Temsirolimus induced marked, but transient immunosuppression in mice by prolonging the time to DTH.

No secondary pharmacodynamics studies were performed *in vitro* in order to identify additional target receptors for temsirolimus.

- Safety pharmacology programme

Safety pharmacology studies were conducted in rats, to investigate effects of temsirolimus on the central nervous system (CNS), respiratory function and cardiovascular function after a single-dose IV administration. Cardiovascular function was also investigated using oral administration. Monkeys were used to investigate cardiovascular function following escalation of single IV doses of temsirolimus. No biologically significant effects on CNS or respiratory function were observed in rats administered temsirolimus at dosages up to 5 mg/kg. There were no temsirolimus-related effects on cardiovascular function in either species at the highest dosages administered.

- Pharmacodynamic drug interactions

The effect on tumour growth inhibition by temsirolimus has been studied in combination with a number of cytotoxic agents including vincristine, topotecan, cisplatin, melphalan, carmustine (BCNU), gemcitabine, 5-fluouracil (5-FU), doxorubicine and paclitaxel *in vitro* and *in vivo* (data not shown).

Pharmacokinetics

Pharmacokinetic studies with temsirolimus were conducted to support IV and oral administration. Analytical methods were validated for the quantification of temsirolimus in whole blood or plasma from rats and monkeys and for quantification of temsirolimus and sirolimus in whole blood from mice, rats, rabbits and monkeys.

Studies on short-term stability of temsirolimus in whole blood and/or plasma from mice, rats, monkeys and humans showed that temsirolimus was unstable in the plasma of each species. In whole blood, temsirolimus was most stable in humans, with decreasing stability in monkeys, rats and mice. Therefore, assessments of pharmacokinetics were based on concentrations in whole blood and not plasma. Seco-temsirolimus (M4) appeared to be the only temsirolimus degradation product present in whole blood and plasma of rat, monkey and human. Seco-temsirolimus (M4) in addition to sirolimus and seco-sirolimus were present in mouse whole blood and plasma.

The absorption and pharmacokinetics of temsirolimus or [^{14}C]temsirolimus were evaluated in mice, rats and monkeys after single-dose IV and oral administration (only in rats) as part of an IV 4-cycle study. After IV administration of temsirolimus to mice, rats or monkeys, whole blood CL_T values increased with increasing dose and were considered to be low to moderate as compared with hepatic blood flow, in mice and rats (0.685 to 2.35 l/h/kg and 0.644 to 1.73 l/h/kg, respectively) and considered low in monkeys (0.065 l/h/kg). In whole blood, the Vd_{SS} values were high (compared with total body water) in mice and rats, ranging from 4.06 to 18.7 l/kg and moderate in monkeys, ranging from 0.821 and 0.869 l/kg. After a single oral dosage of [^{14}C]temsirolimus in rats (1.5 mg/kg) or monkeys (7.5 mg/kg), [^{14}C]temsirolimus-derived radioactivity was rapidly absorbed, with t_{max} values in whole blood of 1.5 hours in rats and between 1 and 2 hours in monkeys. Comparison of the pharmacokinetics between whole blood and plasma from rats and monkeys suggested that the AUC

and $t_{1/2}$ were greater in whole blood than in plasma and the CL_T was greater in plasma than in whole blood.

The pharmacokinetics in repeat-dose toxicity studies was determined. Based on a comparison of IV and oral exposure data (AUC) from 4-cycle toxicity studies in rats and monkeys, the oral bioavailability in rats and monkeys were estimated to be approximately 5% and 22% in rats and monkeys, respectively.

Tissue distribution was evaluated after a single IV or oral dose in male rats. Temsirolimus was extensively distributed in tissues. Concentrations after IV administration were higher in the adrenal glands, large intestine, liver, pituitary, stomach, thymus and thyroid glands than in other tissues. After oral administration, the highest concentrations were observed mainly in the large intestine, liver pituitary, small intestine, spleen, stomach, thymus and thyroid. The tissues with the lowest observed concentration were the brain, eye and testes after either oral or IV route of administration. The clearance of temsirolimus from tissues was slower compared to blood. Some tissues such as the brain, eyes, lymph nodes, testes and thymus, while never achieving high concentrations of temsirolimus, had persistent radioactivity. Temsirolimus has low affinity to melanin-containing tissues. Temsirolimus concentrations were slightly higher in melanin-containing tissues after IV administration compared to oral administration.

[^{14}C]temsirolimus protein binding *in vitro* in male human plasma was evaluated using an erythrocyte partitioning method which provides a more stable matrix for temsirolimus than plasma. Temsirolimus at concentrations between 10 and 100 ng/ml was moderately bound (85 and 87%, respectively) to male human plasma proteins.

In vitro blood:plasma distribution of [^{14}C]temsirolimus was evaluated in mouse, rat, monkey and human whole blood samples. The *in vitro* blood:plasma partitioning ratios were as follows: humans (3.4), monkeys (3.2), rats (0.8) and mice (0.6). These species differences may be due to species differences in the concentration of FKBP12 in formed blood elements. The efflux of temsirolimus from formed blood elements appeared to be low.

Placental transfer after single oral administration of [^{14}C]temsirolimus was evaluated in gravid rats on Gestation Day GD 9 and 16. On GD 9, [^{14}C]temsirolimus-derived radioactivity was found in the embryos and declined slowly with time indicating preferential retention of [^{14}C]temsirolimus and its metabolites in the embryos. On GD 16, [^{14}C]temsirolimus-derived radioactivity found in the placenta but not in the foetus or amniotic fluid. Radioactivity from the placenta declined slowly. No studies on excretion of temsirolimus into milk of lactating animals were performed. Sirolimus, a main metabolite of temsirolimus in humans, is excreted in the milk of lactating rats.

The ability of temsirolimus to act as a substrate or inhibitor of P-gp was evaluated *in vitro* using CACO-2 cells. Temsirolimus was the subject of P-gp-mediated efflux and was found to be an inhibitor of P-gp having the potential to alter the transport of P-gp substrates.

The metabolism of temsirolimus or [^{14}C]temsirolimus was evaluated *in vivo* in rats (including biliary excretion), monkeys and humans after single-dose IV and oral administration and *in vitro* in mouse, rat and human liver microsomes.

In rats and monkeys, the primary metabolites of temsirolimus are hydroxy-temsirolimus (M10) and seco-temsirolimus (M4). Oral administration of temsirolimus resulted in an extensive number of circulating metabolites. In humans, temsirolimus is readily metabolised to sirolimus and the major compounds found in human whole blood were temsirolimus, sirolimus and their oxidative metabolites. In all species tested, including humans, temsirolimus was metabolised to sirolimus. The level of sirolimus in whole blood (AUC) of rats and monkeys was lower than temsirolimus whereas, in mice and humans, the exposure to sirolimus (AUC) was higher than temsirolimus when temsirolimus was administered. More extensive metabolism was observed in the faeces of rats compared with blood and plasma, suggesting further biotransformation and/or degradation during elimination. β-glucoronidase treatment of whole blood, plasma and urine samples from rats or, whole blood and urine samples from

monkeys did not reveal the presence of hydrolysable glucuronide conjugates of temsirolimus or its metabolites.

In *in vitro* studies in human liver microsomes and dexamethasone-induced rat microsomes further metabolites appeared, including various demethylated and hydroxylated isomeric forms of temsirolimus and sirolimus and the seco (ring opened) form of both. In addition, reduced seco-temsirolimus, hydroxy reduced temsirolimus and hydroxy temsirolimus were formed in mouse liver microsomes.

Cytochrome P450 (CYP) isozymes are present in microsomes derived from human lymphoid cells and in human liver microsomes. The major CYP isozyme responsible for the metabolism of temsirolimus is CYP3A4 and, to a lesser extent, CYP2E1. Induction of enzyme expression and enzyme inhibition studies were performed and included evaluation of hepatic enzyme effects after oral administration in rats, *in vitro* CYP3A4 induction and CYP isozymes inhibition in human liver microsomes. Temsirolimus did not induce expression of CYP3A4 but metabolism of temsirolimus might be altered by inhibitors and inducers of the CYP3A4 enzyme system. Temsirolimus did not inhibit CYP1A2 or CYP2C19 activity but inhibited CYP2C, CYP2D6 and CYP3A4/5 activity and may have the potential to inhibit the metabolic clearance of drugs that are substrates for CYP3A4/5 or CYP2D6 but not of CYP2C9 or CYP2C8.

Excretion of temsirolimus or [^{14}C]temsirolimus was evaluated in rats, monkeys and humans after single-dose IV and oral administration. The primary route of excretion in all species was via the faeces and urinary excretion accounted for < 5%. Studies using bile duct cannulated rats showed that the bile is a major pathway for excretion of absorbed temsirolimus and its metabolites.

Potential drug-drug interactions were evaluated *in vitro* in human liver microsomes with temsirolimus in combination with other oncologic agents paclitaxel, doxorubicin and letrozole. Paclitaxel and doxorubicin inhibited the metabolism of temsirolimus. Letrozole, a CYP3A4 substrate, did not inhibit the metabolism of temsirolimus.

Toxicology

Toxicity studies with temsirolimus were conducted to support IV and oral administration. The toxicity of temsirolimus was assessed in different regimens: single-dose IV and oral studies in mice and rats, repeat-dose toxicity studies in mice after oral administration (once-daily dosing), repeat-dose toxicity studies in rats and monkeys after IV administration (once-daily, cyclic and once-weekly dosing) and after oral administration (gavage) (once-daily and cyclic dosing). Additional toxicity evaluations were conducted *in vitro* and *in vivo*, including genotoxicity assays, a dose-ranging carcinogenicity study in neonatal mice, fertility dose-ranging studies in male and female rats and development toxicity dose-ranging in rabbits. *In vitro* and *in vivo* impurity qualification studies were also performed. The toxicokinetic of temsirolimus was evaluated in whole blood of mice, rats, rabbits and monkeys as part of repeat-dose and reproductive toxicity studies.

- Single-dose toxicity

The single-dose toxicity of temsirolimus was assessed in IV and oral studies in mice and rats and also after the first dose in repeat-dose toxicity studies in monkeys. Based on the results of 2 IV studies in rats, the median lethal dosage was approximately 50 mg/kg. However, no evidence of lethality was found in mice after IV administration of 50 mg/kg of temsirolimus. After oral administration of 100 mg/kg, there was no mortality in either species. After the first dose in repeat-dose toxicity studies in monkeys, there was no mortality and temsirolimus was well tolerated at IV dosages up to 2.5 mg/kg and oral dosages up to 7.5 mg/kg (the highest dosages administered by each route).

- Repeat-dose toxicity (with toxicokinetics)

Repeat-dose toxicity was evaluated in mice following oral administration (once-daily dosing up to 3 months) and in rats and monkeys after IV administration (once daily for 2 weeks, 4 cycles and once weekly for 6 or 9 months, respectively). Repeat-dose toxicity was also evaluated IV studies (doses up

to 2.5 mg/kg) and the oral cyclic studies (doses up to 7.5 mg/kg) were conducted to support the oncology programme.

The toxicity profiles were similar following either IV or oral administration. Toxicity related to target organs is outlined below.

Haematology
The increase observed in parameters related to erythrocytes and the decrease in reticulocytes was suggestive of an increase in the lifespan of red blood cells. This would be consistent with immunosuppression. However, these effects may also be associated with haemoconcentration secondary to the diabetogenic state of the patient.

The observed decrease in platelets and WBC was primarily attributed to a decrease in lymphocyte numbers, a direct consequence of the immunosuppressive activity of temsirolimus. An increase in neutrophils and fibrinogen was indicative of inflammation, an event considered secondary to immunosuppression.

Lymphoid tissues
Atrophy of the thymus and of lymphoid tissues was seen in mice, rats and monkeys after temsirolimus administration and was associated with a decrease in peripheral blood lymphocytes in some studies. Bone marrow hypocellularity was observed in rats. Reversibility of haematologic parameters was demonstrated during the interval between dosing cycles in the 4-cycle IV studies in rats and monkeys.

Pancreas, hyperglycaemia and related diseases
Hyperglycaemia and pancreatic islet cell vacuolation were observed in rats. In addition, studies in rats demonstrated that other findings maybe associated with hyperglycaemia, such as cataracts, hepatocellular vacuolation and renal tubular vacuolation. Hyperglycaemia and pancreatic islet cell vacuolation were not observed in monkeys after administration of temsirolimus.

Testes
Small testes, decreased testis weights, testicular tubular degeneration, testicular tubular giant cells and/or hypospermia were observed in mice, rats and monkeys. Also observed in rats was a decrease in the weight of prostates, small seminal vesicles, epididymides and the presence of immature spermatocytes in the epididymides.

Testosterone levels have not been determined in monkey studies with temsirolimus and sirolimus. However, decreases in testicular testosterone levels were noted in studies with sirolimus in rats and were partially attributed to sirolimus-induced suppression of testicular mitochondrial steroid side-chain cleavage activity.

Skeletal system
Lameness, with or without evidence of bone fracture, was observed in rats (primarily males) administered with temsirolimus. Although the specific cause is unknown, sirolimus is known to induce lameness associated with osteopenia and bone fracture in male rats as a consequence to decrease in testosterone.

Ovary
Decreased ovary weights and microscopic atrophy of the ovaries, uterus and cervix and/or luteal or follicular cysts were observed in rats in repeat-dose toxicity studies. In addition, functional effects (decreased corpora lutea) were observed in a rat female fertility in a dose-ranging study.

Gastrointestinal tract
Inflammation of the caecum/colon and faecal alterations (diarrhoea, soft or unformed stools or mucoid and/or liquid faeces) were observed in monkeys. Clinical and pathologic changes (increased fibrinogen and neutrophils) consistent with mild inflammatory changes in the caecum and colon were observed in monkeys. In clinical trials, diarrhoea was reported as a temsirolimus-related event.

Skin
Abrasions, inflammation and/or ulcerations of the skin were observed in rodents and were consistent with the antiproliferative effect of temsirolimus on regenerating tissue and the immune system. Clinical and pathologic changes (increased fibrinogen and neutrophils and decreased albumin and increased globulin) consistent with mild inflammation were observed in mice and rats. In the IV 9-month study in monkeys, persistent rashes were attributed to a chronic antiproliferative effect and were not considered to be a toxic effect of temsirolimus. In clinical trials, rashes have been observed in patients administered with temsirolimus.

Heart
An increase in the incidence and severity of myocardial degeneration (sometimes described as spontaneous rat cardiomyopathy) was observed in rats. Myocardial degeneration occurs spontaneously in untreated laboratory rats and progresses in incidence and severity with age, particularly in male rats. In rats given temsirolimus, this progression was seen earlier than in age matched controls. Because the myocardial degeneration observed in these studies was an exacerbation of a naturally occurring condition in rats, and because this lesion did not occur as a temsirolimus-related effect in mice or monkeys, myocardial degeneration was not considered an adverse reaction. In a study investigating the mechanism of sirolimus, myocardial degeneration was attributed to the activation of latent parvovirus in the hearts of immunosuppressed rats.

Lung
Increased numbers of pulmonary alveolar macrophages were observed in rats without appreciable inflammatory changes and were consistent with phospholipidosis. These changes were not reversible. Accumulation of phospholipids in rat pulmonary alveolar macrophages is observed with a variety of different compounds and is generally not predictive of adverse findings in humans. Increased pulmonary alveolar macrophages with phospholipid accumulation did not occur in mice or monkeys given temsirolimus. The appearance and aetiology of the changes in the lung seen in rats are distinct from interstitial pneumonitis (observed in clinical studies with temsirolimus) and a relationship between the two conditions is considered unlikely.

Cholesterol
An increase in cholesterol levels was observed in mice, rats, and monkeys. Although the increases were generally of low magnitude, and not considered adverse, hypercholesterolemia and hyperlipidaemia have been demonstrated in humans administered with temsirolimus.

Liver
Histological changes of the liver, like mononuclear cell inflammation and necrosis, were seen in the 6-month study in rats (RPT-43567). The low incidence and severity of these changes may be the result of a temsirolimus-related decrease in immune function followed by a slight increase in inflammation with associated hepatocellular necrosis, caused by an increase in the numbers of bacteria in the enterohepatic circulation. The relevance of these finding in humans is not known.

- Genotoxicity

Temsirolimus was tested in a standard battery of *in vitro* and *in vivo* genotoxic tests. Neither *in vitro* nor *in vivo* study results provided evidence for a biologically relevant genotoxic potential of temsirolimus.

- Carcinogenicity

Carcinogenicity studies with temsirolimus were not conducted. The omission of carcinogenicity studies is accepted due to the short live expectancy of the patient population.

- Reproduction toxicity

The reproductive toxicity of temsirolimus was evaluated in fertility studies in rats and developmental toxicity studies in rats and rabbits. In male rats treated with temsirolimus, a decreased or absent fertility accompanied by testicular tubular degeneration, decreased sperm concentration and motility, decreased reproductive organ weights and prostate atrophy was observed. These effects were also seen in repeat-dose toxicity studies in mice, rats and monkeys. In developmental toxicity studies in rats and

rabbits, there was increased embryo/foetal mortality and decreased foetal growth (decreased foetal weight and delayed skeletal ossification). In addition, treated rabbits showed an increased incidence of intestinal protusion through the abdomen (omphalocele). The majority of the reproductive toxicity studies were conducted using the oral route of administration.

- Toxicokinetic data

Comparisons of dosage or exposure to temsirolimus (plus sirolimus) at the no-observed-adverse-effect levels (NOAEL) in animals and those at the proposed therapeutic dosage in humans resulted in ratios that were generally less than 1.

- Local tolerance

Separate local tolerance studies were not conducted with temsirolimus. Examination of the injection site in the repeat-dose IV studies showed that temsirolimus did not produce clinical or pathological signs of local damage to the vein or the surrounding tissues.

- Other toxicity studies

Impurity qualification studies (consisting of an *in vivo* rat study, bacterial reverse mutation assay and chromosome aberration assay) were conducted to qualify the toxicity of higher levels of impurities in the drug substance or drug products. Impurities and degradation products occurring in temsirolimus preparations were qualified by testing the batches 7636-126 (modified synthesis), 2001B0205 (heat stressed IV formulation) and L24300-016 (degraded granulation formulation) that were specifically modified to contain higher levels of impurities/degradants. The toxic effects observed in the temsirolimus batches prepared for impurities qualification were consistent with the effects observed in the temsirolimus product containing lower concentrations of the impurities, in essence qualifying the impurities.

Ecotoxicity/environmental risk assessment

The marketing authorisation application of the medicinal product temsirolimus was started in October 2006 before the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (CHMP/SWP/4447/00) come into force. Therefore the environmental risk assessment is based on the draft Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (CHMP/SWP/4447/00 draft January 2005) stating the Orphan Medicinal Products do not need to be accompanied by an environmental risk assessment. Moreover since the medicinal product has been designed as an Orphan Medicinal Product with a prevalence of 35 per 100,000 EU inhabitants, the environmental risk is considered likely to be minimal.

4 Clinical aspects

Introduction

An IV formulation of temsirolimus was developed for the treatment of advanced RCC. The clinical development programme for IV temsirolimus includes 21 clinical studies (16 Phase I, 3 Phase II and 2 Phase III studies) that were conducted in a broad demographic population from 23 countries. Six studies (including the 2 Phase III studies) were still ongoing at the time for the data cut-off date for this application (30 May 2006). The pivotal Phase III study for temsirolimus in advanced RCC is being conducted worldwide, as well as a Phase III study in mantle cell lymphoma (MCL).

In addition to studies on the IV formulation of temsirolimus, clinical studies for an oral formulation of temsirolimus in oncology (breast and prostate), multiple sclerosis and rheumatoid arthritis indications have been conducted. The oral formulation is currently not being developed in these indications because of insufficient efficacy observed in the trials.

GCP

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.

A routine GCP inspection of the pivotal study was conducted at the sponsor site in the US and two investigational sites in Poland and Germany in February/March 2007. FDA also performed a GCP inspection.

While the overall performance of the sponsor can be considered as GCP compliant, the quality of the two inspected sites was very different. The main concerns were 1) in one of the two inspected investigational sites, the quality of data documentation for the secondary endpoints, e.g. assessment and documentation of progression for calculation of PFS was regarded as inadequate, however, the quality of data documentation for the primary endpoint was adequate and 2) the handling of protocol violations by the sponsor was considered inadequate ("critical"), although a re-analysis with more stringent rules related to protocol deviations was provided and finally did not show major differences with the original analyses.

Pharmacokinetics

The pharmacokinetics (PK) of IV temsirolimus was investigated in 95 healthy subjects from 5 studies and 481 cancer patients in 9 studies. In healthy subjects, PK assessments were made following a single dose with or without potentially interacting agents. In patients, concentrations were measured following both single- and multiple-dose treatment in studies that tested temsirolimus both as a single agent and in combination with other chemotherapeutic agents (Table 1). The PK parameters of temsirolimus and its active metabolite, sirolimus, were obtained using a combination of compartmental, non-compartmental analysis or population PK methods.

Table 1. Studies in the Clinical Pharmacology Summary

Study Number	Study Description	Number Enrolled	IV Dose Range
Clinical Pharmacology Studies in Healthy Subjects			
3066K1-133-US	Phase 1, open-label, nonrandomized, parallel-group study to evaluate mass balance and metabolic profile of temsirolimus IV and PO routes	12	25 mg
3066K1-145-US	Phase 1, open-label study to quantify the temsirolimus exposure/response relationship using S6 ribosomal protein in blood	30	1 to 25 mg
3066K1-148-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of CYP3A4 inhibition on temsirolimus PK	17	5 mg
3066K1-149-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of temsirolimus on CYP2D6 metabolism	26	25 mg
3066K1-151-US	Phase 1, open-label, nonrandomized, 2-period sequential study to evaluate effect of CYP3A4 induction on temsirolimus PK	16	25 mg
3066K1-155-US[a]	Phase 1, single-blind, randomized, 3-period sequential study to evaluate effect of temsirolimus on the QT interval	60[b]	25 mg
Clinical Oncology Studies with a Clinical Pharmacology Component in Patients with Cancer			
3066K1-100-US	Phase 1, open-label, 2-part dose-escalation study to determine MTD in patients with advanced solid tumors (daily for 5 days every 2 weeks)	63 (part 1) 25 (part 2)	0.75 to 37 mg/m^2
3066K1-101-EU Part 1 and Part 2	Phase 1, open-label 2-part dose-escalation study to determine MTD in patients with advanced solid tumors (once weekly regimen)	24 (part 1) 16 (part 2)	7.5 to 220 mg/m^2
3066K1-103-EU	Phase 1, open-label, dose-escalation study to determine MTD in patients with advanced solid tumors receiving concomitant 5-FU/LV	28	15 to 75 mg/m^2
3066K1-104-US	Phase 1, open-label, 2-part study to compare bioavailability of IV and PO formulations and to determine MTD of PO formulation in patients with advanced solid tumors	24	5 to 20 mg
3066K1-124-US	Phase 1, open-label, dose-escalation combination study with IFN to determine MTD in patients with advanced RCC	71	5 to 25 mg
3066K1-131-JA	Phase 1, open-label, dose-escalation study to determine MTD in Japanese patients with advanced solid tumors	10	15 to 45 mg/m^2
3066K1-152-US (NCI study 6813) ongoing	Phase 1, open-label, nonrandomized, parallel-group study to evaluate the PK and PD of temsirolimus in patients with cancer and varying degrees of hepatic impairment (Interim)	14 to 66	15 to 175 mg
3066K1-200-US	Phase 2, randomized, blinded, parallel-group, dose-ranging study for efficacy, safety and population PK in patients with advanced RCC	111	25, 75, 250 mg
3066K1-203-EU Breast cancer	Phase 2, randomized, open-label, parallel-group, dose-ranging study to evaluate efficacy, safety and population PK in women with advanced or metastatic breast cancer	109	75, 250 mg

a Study ongoing, no data were included in the application
b number of patients planned, 48 were enrolled at 30 may 2006 cut-off date

From *in vitro* studies, it was determined that temsirolimus and sirolimus exhibited comparable degrees of biological activity. Temsirolimus and sirolimus share some common characteristics that include low oral bioavailability, specific immunophilin binding and extensive and qualitatively similar metabolism. Despite these common biological properties, differences in the profile of activity can be obtained with differences in dose level and dosing modality. For oncology indications, higher dose regimens have predominated, supported by non-clinical models that have indicated several distinct advantages of the IV route compared to that offered by oral administration. IV administration offers the opportunity to reach micro-molar concentrations in blood and tissues of cancer patients that are associated with growth-inhibitory effects. The intention of the intermittent (once weekly) treatment is to mitigate the effects of chronic immune-suppression that can occur with daily dosing, as observed with (oral) sirolimus.

For the fundamental PK descriptions of temsirolimus, whole blood was used to perform the experiments. In a pooled analysis of healthy subjects and cancer patients, summary PK parameters were determined following treatment with the 25 mg IV dose due to the nonlinear nature of temsirolimus pharmacokinetics.

A summary of results from the pooled PK analysis is reported in Table 2.

Table 2. Pooled pharmacokinetics summary - Single- and multiple-dose pharmacokinetic parameters of healthy subjects and patients following 25 mg IV temsirolimus

	Healthy Subjects	Patients	
	Single-dose N=51	Single-dose N=13[a]	Multiple-dose N=11[b]
Temsirolimus			
C_{max} (ng/ml)	592.4 ± 101.9	585.4 ± 83.1	443.0 ± 109.2
$t_{1/2}$ (h)	17.7 ± 4.5	17.3 ± 5.9	NC
AUC (ng·h/ml)	2276 ± 340	1627 ± 425	1349 ± 231.8
CL (l/h)	11.4 ± 2.4	16.2 ± 3.5	19.0 ± 3.0
V_{dss}	189.6 ± 55.2	172.3 ± 39.4	NC
Sirolimus			
C_{max} (ng/ml)	57.4 ± 14.3	55.4 ± 31.8	34.5 ± 19.3
$t_{1/2}$ (h)	73.3 ± 23.2	54.6 ± 1.5	NC
AUC (ng·h/ml)	5479 ± 1799	4151 ± 1600	3793 ± 1466
CL/f_m (l/h)	4.9 ± 1.2	6.9 ± 2.6	7.4 ± 2.5
Composite			
AUC_{ratio}[c]	2.44 ± 0.83	2.68 ± 1.22	2.98 ± 1.47
AUC_{sum} (ng·h/ml)	7755 ± 1874	5778 ± 1722	5141 ± 1345

Abbreviation: NC = Not calculated. No subjects provided data
a For C_{max} N=5 and for t1/2 and Vdss N=2
b For temsirolimus Cmax N=7, sirolimus C_{max} N=3
c AUC ratio denotes quotient of sirolimus: temsirolimus AUC

- Absorption

Not applicable, temsirolimus is administered intravenously.

- Distribution

Temsirolimus C_{max} and AUC in whole blood increased with increasing dose at low doses but reached a maximum level plateau at higher doses in a less than proportional manner. This was apparent in the various clinical studies that have been performed that contained a PK component.

Temsirolimus exhibits a polyexponential decline in whole blood concentrations and distribution is attributable to preferential binding to FK506-binding protein (FKBP12) in blood cells. In whole blood, temsirolimus and the major metabolite sirolimus binds to FKBP12. At lower doses, moderate binding (approximately 85%) to FKBP12 occurs. At the recommended dose of 25 mg, binding capacity of this protein becomes saturated. Due to this specific binding, at low doses, the blood-to-plasma ratio is high, whereas after saturation of the FKBP12, the blood-to-plasma ratio approaches 1. A mechanistic model describing the distribution of temsirolimus indicates that following doses of 1 to 25 mg, mean (10^{th}, 90^{th} percentile) specific binding in blood cells ($B_{max\mathit{r}}$) was 1.4 mg (0.47, 2.5 mg) and in peripheral tissues ($B_{max\mathit{t}}$) was 5.0 mg (0.94, 9.9 mg). Collectively, these data indicated that the amount of temsirolimus required to saturate central and peripheral tissue sites was approximately 6.4 mg (1.41, 12.4 mg). Consequently doses or dose regimens less than 12.4 mg may be associated with peripheral tissue exposures that are subtherapeutic.

- Metabolism

Temsirolimus is rapidly metabolised mainly via de-esterification to obtain sirolimus. In whole blood, temsirolimus and sirolimus were the major species with minor contributions of temsirolimus isomer C, seco-temsirolimus and hydroxy-temsirolimus. CYP3A4 is the major CYP isoenzyme responsible for other routes of metabolism of temsirolimus and sirolimus.

Following a single 25 mg intravenous dose in patients with cancer, sirolimus AUC was 2.7-fold that of temsirolimus AUC, due mainly to the longer half-life of sirolimus.

- Elimination

Across the different studies, clearance was shown to be dose-dependent, increasing with dose, presumably due to the saturable distribution of temsirolimus in blood and tissues.

The slightly higher value of half-life for healthy subjects may be explained by either the more extensive terminal phase sampling schedules attainable in healthy subjects as compared to cancer patients or the higher haematocrit relative to cancer patients.

With multiple temsirolimus doses and the once-weekly schedule, no appreciable degrees of accumulation of temsirolimus or sirolimus were observed in the various cancer patient studies conducted.

- Excretion

The elimination of temsirolimus from the body occurs principally through faeces. For the 25 mg IV-dose of radiolabelled temsirolimus, 83% of drug-derived radioactivity was recoverable from the body, with 78% recovered from faeces. Mean urinary excretion was low (5%) and likely indicates that renal elimination played only a minor role in the clearance of temsirolimus and the major metabolite sirolimus.

- Dose proportionality and time dependencies

Over the wide dose range investigated, dose-related increases in exposure, as measured by temsirolimus C_{max}, AUC and AUC_{sum} in whole blood, increased with dose, but significantly was not entirely proportional ($p < 0.001$) due to saturable binding of temsirolimus to FKBP12 in blood cells and peripheral compartments.

Inter-patient variability at a fixed 25 mg single dose with respect to C_{max} and AUC was 14 and 26% for temsirolimus and 25 and 36% for sirolimus, respectively.

- Special populations

Impaired renal function

Mass balance data from Study 3066K1-133-US indicated that mean urinary excretion following the 25 mg IV-dose was low (≈ 5%). Secondary data from the population PK analysis of temsirolimus and sirolimus indicated that PK disposition was not affected by differences in creatinine clearance.

Impaired hepatic function

A study in patients with hepatic impairment is currently ongoing in co-operation with the US National Cancer Institute (Study 3066K1-152-US). The study plans to enrol patients into 5 cohorts according to the degree of hepatic impairment. Up to 12 patients will be accrued in each cohort at each of 5 proposed dose levels (15–175 mg).

Preliminary results of the first 14 patients (15 and 25 mg) suggest that exposure of sirolimus in patients with hepatic impairment receiving temsirolimus 25 mg was disproportionately higher compared to patients receiving temsirolimus 15 mg. Definitive conclusions of an effect in this special population cannot be drawn at this time. Thrombocytopenia was a Dose Limiting Toxicity (DLT) for the mild dose cohorts leading to de-escalation and multiple dose delays.

Gender

No significant effects were observed in the population PK analysis for gender.

Race

Exposure and PK parameters of temsirolimus and sirolimus were specifically assessed in Japanese patients (Study 3066K1-131-JA). Data were derived from 10 patients with cancer following either 15 or 45 mg/m^2 doses of temsirolimus IV. Correction for individual patient BSA yielded conversions to mean flat doses of 24.2 mg (95% CI: 22.5, 26.0) and 75 mg (95% CI: 33.4, 117), respectively.

No significant effects were observed in the population PK analysis for race.

Weight

No significant effects were observed in the population PK analysis for weight.

Elderly
According to the results from population PK modelling, no major difference between elderly and younger patients was found.

Children
A Phase I/II dose-escalation study (Study 3066K1-139-US) to determine maximum tolerated dose (MTD) in a paediatric population is currently ongoing.

- Pharmacokinetic interaction studies

In vitro
Based on *in vitro* data, clinically relevant inhibition of CYP2D6 and CYP3A4/5 by temsirolimus is possible. However, the likelihood of relevant inhibition of CYP2C9 and CYP2C8 by temsirolimus appears remote. Inhibition of CYP2B6 and CYP2E1 was not investigated *in vitro*. *In vitro* data do not suggest that temsirolimus has enzyme inducing activity.

In vivo
Drug interaction potential of temsirolimus was formally tested through specific studies in healthy subjects. Factors examined included temsirolimus as an inhibitor of CYP2D6 or CYP3A4 metabolism and temsirolimus as a substrate for CYP3A4 metabolism.

Temsirolimus as substrate
When the potent CYP3A4 inhibitor ketoconazole was administered concomitantly with 5 mg temsirolimus IV, temsirolimus C_{max} and AUC were unaffected. However, sirolimus mean C_{max} and AUC increased 2.2-fold and 3.1-fold, respectively. However, the relative free temsirolimus and sirolimus blood concentration available for metabolism is higher at a temsirolimus dose of 25 mg. Therefore, the impact on the inhibition of CYP3A4 may be more pronounced at this higher dose.

The potent inducer rifampicin did not influence temsirolimus C_{max} or AUC but decreased sirolimus C_{max} to 36% (90% CI: 31% to 41%) and decreased sirolimus AUC to 44% (90% CI: 39% to 49%) compared to temsirolimus alone.

Temsirolimus as inhibitor
The potential of temsirolimus to act as a CYP2D6 inhibitor was tested in healthy subjects. Using desipramine as a substrate of CYP2D6, results indicated that the 25 mg IV-dose of temsirolimus did not have a clinically relevant effect on desipramine or 2-hydroxy-desipramine pharmacokinetics.

Co-administration of IFN-α does not significantly affect temsirolimus and sirolimus pharmacokinetics.

- Pharmacokinetics using human biomaterials

In addition to pharmacokinetic studies investigating plasma-protein binding, hepatic metabolism and drug interactions, two pharmacokinetic studies have been conducted using human biomaterials to study the transport and inhibition of P-gp activity (main results reported in the non-clinical section).

Pharmacodynamics

- Primary and secondary pharmacology

S6RP is a cytosolic protein substrate immediately distal to the p70S6 kinase target of mTOR signalling in the PI3 kinase pathway. Inhibition of lymphocyte phosphorylation of S6RP by temsirolimus was chosen as a pharmacodynamic marker of mTOR modulation. The analytical method to measure phospho-S6RP activity demonstrated adequate performance characteristics during method validation. The biochemical target of phospho-S6RP demonstrated robust changes in response to the presence of temsirolimus in non-clinical models. An indirect model to measure cellular response was used in which the inhibition of response was measured in both CD3+ and CD19+ lymphocytes. Following single IV doses ranging from 1 to 25 mg, inhibition of signalling was measured in lymphocytes of healthy subjects from Study 3066K1-145-US.

In Study 3066K1-145-US, following the 25 mg IV dose in healthy subjects, the mean (10^{th}, 90^{th} percentile) total capacity for specific binding of temsirolimus to FKBP12 in the body (B_{max}) was estimated to be 6.4 mg (1.4 to 12.4 mg). This dose range is the minimal amount of drug required to saturate specific binding sites in the blood and tissues (lower doses would exhibit lower free concentrations in the peripheral tissues than expected from linear extrapolations since specific binding is operative within this range). Doses above 12.4 mg would exceed maximum binding capacity; therefore, 12.4 mg is considered a minimum dose to be administered to optimise tissue penetration and to maximise clinical benefit for the majority of patients.

From Study 3066K1-145-US, the mean dissociation constant of specific binding (K_d) was determined to be 5.1 ng/ml. A similar K_d value was also identified in the population PK analysis of 158 µg or 15.9 ng/ml. These values are comparable to active concentrations (< 10 nM ≈10 ng/ml) studied in non-clinical models for tumour growth inhibition with temsirolimus.

Maximal receptor occupancy was achieved during the infusion period and dropped off rapidly following cessation of infusion.

Clinical efficacy

The list of clinical studies included in the development programme of temsirolimus in RCC is shown in Table 3.

Table 3. Development programme – List of clinical studies

Protocol No.	Study design and key objective	Regimen(s)	Study population	No. of enrolled subjects[a], ***Demography***
3066K1-100-US Completed	Phase 1, open-label, 2-part dose-escalation study to determine MTD	Temsirolimus: once daily for 5 days every 2 weeks Part 1: 0.75 to 24 mg/m^2 Part 2: 15 to 37 mg/m^2	Part 1: Adults with advanced solid tumours and not taking anticonvulsants Part 2: Adults with recurrent gliomas and taking CYP450 inducers	Total 88 Part 1: 63, *39/24 19-79 years* Part 2 : 25, *17/8 27-66 years*
3066K1-101-EU Completed	Phase 1, open-label, 2-part dose-escalation study to determine MTD	Temsirolimus: once weekly Part 1: 7.5 to 220 mg/m^2 Part 2: 220 mg/m^2	Adults with advanced solid tumours	Total 40 Part 1: 24, *14/10 30-63 years* Part 2: 16, *8/8 29-70 years*
3066K1-124-US Completed	Phase 1, open-label, dose-escalation study to determine MTD	Temsirolimus 5 to 25 mg once weekly plus IFN 3 times weekly	Adults with advanced renal cell carcinoma who had received 0 to 2 prior therapies	71, *54/17 35-80 years*
3066K1-200-US Completed	Phase 2, randomised, blinded, parallel-group, dose-ranging study to evaluate efficacy and safety	Temsirolimus 25, 75 or 250 mg once weekly	Adults with advanced renal cell carcinoma who had received prior therapy or who had not received prior therapy but were not eligible for IL-2	111, *77/34 17-81 years*
3066K1-304-WW Ongoing	Phase 3, randomised, open-label, parallel-group, pivotal study to evaluate efficacy and safety	Arm A: IFN 3 times/week Arm B: Temsirolimus 25 mg once weekly Arm C: Temsirolimus 15 mg once weekly plus IFN 3 times/week	Adults with first-line, poor-prognosis advanced renal cell carcinoma	626, *432/194 23-86 years*

a. Number of study subjects enrolled as of 30 May 2006

- Dose response study(ies)

Study 3066K1-101-EU investigated escalating doses of temsirolimus with a once weekly schedule in patients with advanced solid tumours.

Methods

The primary objective of Study 3066K1-101-EU Part 1 was to determine the safety, tolerability and maximum tolerated dose (MTD) of IV temsirolimus administered once weekly to patients with advanced solid tumours who were not taking anticonvulsant therapy.

In a Part 2 of the study, 16 patients with brain metastases or primary brain tumours were included at MTD to evaluate the safety, tolerability and effect of concomitant enzyme-inducing anticonvulsant agent medication on the pharmacokinetic (PK) profile of temsirolimus (results not reported).

Patients with a histologic diagnosis of advanced solid tumours, who were refractory to or were inappropriate candidates for standard therapy, were eligible as long as they were not taking cytochrome P450-inducing anticonvulsant medication.

Dosing steps were not fixed in advance. The selected starting dose was set at 7.5 mg/m^2 in a 30 minute IV-infusion. A modified continuous reassessment method was used to decide on the dose escalation scheme estimating the MTD after each patient completed the first 3 infusions. This method was chosen to minimise the number of patients treated at inefficacious low doses. The MTD was predefined as the dose level at which 33% of the patients would have unacceptable toxicity. Unacceptable toxicity for the purpose of determining MTD was defined as drug-related National Cancer Institute (NCI) Grade 3 non-haematologic toxicity (excluding alopecia, untreated nausea and vomiting, serum triglycerides if < 16.95 mmol/l and with recovery within 1 week), Grade 4 thrombocytopenia, neutropenia lasting > 5 days, febrile Grade 4 neutropenia requiring hospitalisation or treatment delay of > 2 weeks as a result of unresolved toxicity and drug-related death due to toxicity that occurred during the first 21 days of treatment.

Results

The MTD was determined to be 220 mg/m^2 (2 out of 6 patients (33%) who had drug-related unacceptable toxicity during the first 21 days of treatment: Grade 3 stomatitis and asthenia).

Study 3066K1-124-US was a multicentre, Phase I study to study the safety, tolerability and anti-tumour activity of escalating doses of IV temsirolimus given once weekly with increasing doses of IFN-alpha administered subcutaneously 3 times weekly.

Methods

The primary objective of the study was to establish the MTD of temsirolimus in patients with advanced RCC for whom concomitant IFN-alpha therapy was appropriate.

Temsirolimus (5, 10, 15, 20 or 25 mg) was administered as a 30 minute IV-infusion, on a weekly basis beginning in Week 2, on a non-IFN-alpha day. IFN-alpha 6 MU was combined with each of the temsirolimus doses with the intention to reach the MTD of temsirolimus. Then the dose level of IFN-alpha was to be escalated to the 9 MU dose level in combination with temsirolimus one dose level below the MTD. Premedication with IV diphenhydramine 25 to 50 mg before each temsirolimus infusion and acetaminophen (2 tablets or 650 mg) with or without a non-steroidal anti-inflammatory agent (NSAID) prior to IFN-alpha administration was mandatory to minimise for side effects.

Patients, aged 35 to 80 years, with a histologic diagnosis of advanced (metastatic or locally advanced) RCC whose disease was progressing were eligible. Patients may have received a total of 0, 1 or 2 prior immunotherapy, chemotherapy or other systemic therapy regimens for their disease.

Results

In total six dose levels were tested. The dose combination of temsirolimus 15 mg/IFN-alpha 6 MU was determined to be the MTD.

Grade 3 or 4 drug-related TEAE were most frequently haematologic (leucopenia [32%], anaemia [14%] and thrombocytopenia [7%]) or metabolic (hypophosphataemia [20%], hyperlipaemia [14%], hyperglycaemia [11%] and hyponatraemia [6%]).

Study 3066K1-200-US is a multicentre, randomised, double-blind, dose-comparing, Phase II study of IV temsirolimus administered on a weekly basis to patients with advanced RCC.

Methods

Patients with a histological diagnosis of advanced RCC, who had documented disease progression and had prior therapy for advanced disease or no prior therapy for advanced disease, and were not eligible to receive high-dose IL-2 therapy, were included in the study. Bidimensionally measurable target tumour lesions were required. Patients should present with an ECOG performance status 0 to 1 and a life expectancy of at least 12 weeks.

Eligible patients were randomised in a 1:1:1 ratio to receive 25, 75 or 250 mg of IV temsirolimus once weekly until evidence of disease progression. These doses were based on Phase I monotherapy study results where tumour response/stabilisation was observed at 15 and 45 mg/m^2/week dose levels. The fact that patients in the Phase I trial had received much higher doses (up to 220 mg/m^2) provided the basis for a higher dose level to be administered. Flat doses were used to simplify dosing. The rationale for the weekly dosing schedule was to minimise the immunosuppressive effects from temsirolimus. Patients could remain on study until there was evidence of disease progression, and as long as the treatment was well tolerated.

The primary objective of the study was to evaluate the safety and efficacy of 3 dose levels of temsirolimus when administered to previously treated patients with advanced RCC or to previously untreated patients who were not appropriate candidates to receive high-dose IL-2 therapy.

The primary efficacy endpoint was objective response (CR or PR). Tumour measurements were made approximately every 8 weeks using radiographic methods. For bidimensionally measured tumour lesions, the longest diameter and its perpendicular were measured. Objective tumour response was determined by the sum of the products of all measurable lesions (bone lesions were not considered measurable disease because of the inability to assess the response in this tissue). Evaluation of tumour response was performed using 2 different definitions of disease progression: the original protocol definition (based on WHO criteria) and the sum change definition (modified WHO criteria that are consistent with RECIST guidelines). Analysis of tumour response was performed according to both definitions.

The primary efficacy analyses were based on 2 patient populations, the intent-to-treat (ITT) population (all patients randomly assigned) and the evaluable population (patients who met eligibility requirements and completed 8 weekly doses without discontinuing treatment because of disease progression or adverse events).

Results

The results for tumour response are presented in Tables 4 and 5.

Table 4. Study 3066K1-200-US - Tumour response rates by protocol definition, ITT population

	25 mg n=36	75 mg n=38	250 mg n=37	Total N=111
Complete response	0	0	0	0
Partial response	2 (5.6)	1 (2.6)	3 (8.1)	6 (5.4)
Minor response	5 (13.9)	14 (36.8)	10 (27.0)	29 (26.1)
Stable disease[a]	13 (36.1)	11 (28.9)	9 (24.3)	33 (29.7)
Progressive disease	13 (36.1)	10 (26.3)	11 (29.7)	34 (30.6)
Unknown / no data	3 (8.3)	2 (5.3)	4 (10.8)	9 (8.1)

a Duration of SD had to be at least 8 weeks ± 1 week.

Table 5. Study 3066K1-200-US - Tumour response rates by sum change definition, ITT population

	25 mg n=36	75 mg n=38	250 mg n=37	Total N=111
Complete response	0	0	0	0
Partial response	5 (13.9)	13 (34.2)	11 (29.7)	29 (26.1)
Minor response	20 (55.6)	11 (28.9)	11 (29.7)	42 (37.8)
Stable disease[a]	6 (16.7)	9 (23.7)	7 (18.9)	22 (19.8)
Progressive disease	3 (8.3)	2 (5.3)	5 (13.5)	10 (9.0)
Unknown / no data	5 (13.9)	13 (34.2)	11 (29.7)	29 (26.1)

a Duration of SD had to be at least 8 weeks ± 1 week.

The differences in tumour response were not statistically significant between the 3 treatment groups. ORR, OS and PFS results for the different doses are displayed in Table 6.

Table 6. Study 3066K1-200-US – Overall survival, progression-free survival, objective response rates and clinical benefit, ITT population

	25 mg n=36	75 mg n=38	250 mg n=37	Total N=111
Median OS in months (95% CI)	13.8 (9.0-18.7)	11.0 (8.6-18.6)	17.5 (12.0-24.6)	15.0 (10.4-18.3)
Median PFS in months (95% CI)	6.3 (3.6-7.8)	6.7 (3.5-8.5)	5.2 (3.7-7.4)	5.8 (3.8-7.2)
OOR (sum definition) in % (95% CI)	5.6 (0.7-18.7)	7.9 (1.7-21.4)	8.1 (1.7-21.9)	7.2 (3.2-17.7)
Clinical benefit for 24 weeks in % (95% CI)	52.8 (35.5-69.6)	55.3 (38.3-71.4)	43.2 (21.7-60.5)	50.5 (40.8-60.1)

For the Phase III study, 25 mg dose of temsirolimus as single agent was chosen as the lowest dose associated with activity, on the basis that patients randomised to receive 25, 75 or 250 mg of temsirolimus IV exhibited comparable clinical activity. While the incidence of TEAE, NCI Grades 3 or 4 TEAE or SAE was not significantly different among the different doses, there was a trend of a reduction in the number of doses with the increase in dose. Causes for delaying administration of a dose were typically related to the appearance of thrombocytopenia and mucositis.

- Main study(ies)

Study 3066K1-304-WW was conducted as a pivotal, confirmatory, clinical study in advanced RCC with the following title: "A Phase III, three-arm, randomized, open-label study of interferon alfa alone, CCI-779 alone and the combination of interferon alfa and CCI-779 in first-line poor-prognosis subjects with advanced renal cell carcinoma".

Methods

Study Participants

This was a multicentre study conducted at 148 investigational sites in 23 countries.

Main inclusion criteria were:

- histologically or cytologically confirmed Stage IV (AJCC) RCC who have not received prior systemic therapy for their disease
- presence of at least 3 of the following 6 prognostic factors:
  - < 1 year from time of initial RCC diagnosis to randomisation
  - Karnofsky performance status (KPS) of 60 or 70
  - haemoglobin less than the lower limit of normal (ULN)
  - hypocalcaemia
  - LDH > 1.5 times the upper limit of normal
  - > 1 metastatic site of disease
- KPS ≥ 60
- at least 1 measurable lesion that can be accurately measured in at least 1 dimension

Main exclusion criteria were:

- subjects with central nervous system (CNS) metastases

- history of other prior malignancy in past 5 years, other than basal cell carcinoma, squamous cell carcinoma of the skin or cervical carcinoma in situ
- not recovered from prior surgery and/or surgery or radiation therapy within 4 weeks of randomisation

Treatments
The eligible patients were allocated to one of the three treatment arms:

- IFN-alpha alone: IFN-alpha 2a subcutaneous (s.c.) 3-times weekly starting with 3 MU and escalating to the highest tolerable dose, maximum IFN-alpha dose of up to 18 MU 3 times weekly
- temsirolimus alone: temsirolimus once weekly 25 mg in an IV-infusion over 30 to 60 minutes
- combination of IFN-alpha and temsirolimus: temsirolimus 15 mg IV once weekly with IFN-alpha 2a s.c. 3 times weekly. Dosing of IFN-alpha started with 3 MU 3 times weekly without temsirolimus in the first week, however, all subsequent IFN-alpha doses were 6 MU 3 times weekly. It was recommended that temsirolimus be administered on non-IFN-alpha days.

Premedication with an antihistamine (e.g. 25-50 mg diphenhydramine) was mandatory 30 minutes prior to each temsirolimus infusion and premedication with paracetamol with or without another NSAID was required 1-2 hours prior to IFN-alpha.

In case of Grade 3 and 4 toxicity, the next treatment should be withheld until recovery (for maximum of 3 weeks) and dose reductions for all further doses for temsirolimus i.e. 20, 15 and 10 mg, for IFN-alpha i.e. 9 MU, 6 MU, 4.5 MU and 3 MU. Recovery for ANC is defined as $\geq 1.0 \times 10^9$/l and for platelet count $\geq 75 \times 10^9$/l.

Patients were allowed to continue treatment until disease progression or treatment withdrawal. Reasons for withdrawal included progressive disease as determined by the investigator, adverse event, patients request, death and symptomatic deterioration.

Any concurrent treatment was to be documented. Prohibited concurrent treatment included chemotherapy, biologic anticancer therapy, radiation therapy, resection of metastases, hormonal treatment for RCC and thalidomide.

Objectives
The primary study objective was to compare the OS in patients treated with temsirolimus alone or temsirolimus in combination with IFN-alpha with control patients receiving IFN-alpha alone.

The secondary study objectives included the comparison of PFS, response rates, clinical benefit rate, duration of overall response and time-to-treatment failure.

Outcomes/endpoints
Primary endpoint was OS as defined as the time between the randomisation date and the time of death. Patients were followed by regular clinical visits during the treatment period and by telephone contacts every 2 months during the follow-up period. At the request of the independent data monitoring committee (IDMC), patients were contacted prior to the interim analyses. The specific date and reason of death were documented.

The secondary endpoints were:

- PFS was defined as the interval from the date of randomisation until the earlier date of progression or death, censored at the last tumour evaluation date
- Response rate (CR and PR)
- Clinical benefit rate (CR/PR/SD $\geq$ 24 weeks)
- Duration of overall response
- Time-to-treatment failure (TTF) as defined as the interval from the date of randomisation until the earliest date of progression or death (any cause), withdrawal from treatment owing to

adverse event, patient refusal, loss to follow-up (censored at the last contact date) or further anti-cancer therapy before documented progression (whichever occurred first)
- Safety comparison of temsirolimus and the combination arm with IFN-alpha alone
- Responses across all three treatment arms were evaluated based on screening of proteins involved in the Akt-mTor pathway in the tumours (i.e. Akt phosphorylation, PTEN expression) and the vascular endothelial growth factor (VEGF) pathway (HIF1-α and HIF2-α).

Tumour assessments were to be obtained approximately every 8 weeks. The measurability of a tumour was defined by the RECIST criteria and tumour response assessments were done accordingly. CT and MRI were the recommended methods for tumour assessment. The same method of assessment and the same technique should have been used to characterise each identified and reported tumour lesion at baseline and during follow-up. Only superficial lesions were regarded as measurable by clinical examination (may be supplemented by ultrasound).

Progression was determined by each investigator and by independent radiology review.

Sample size
Two null hypotheses were to be tested:
- The first null hypothesis for primary efficacy comparison was that overall survival between temsirolimus monotherapy and IFN-alpha monotherapy groups was the same. The first alternative hypothesis was that overall survival is not the same.
- The second null hypothesis for primary efficacy comparison was that overall survival between temsirolimus/IFN-alpha combination and IFN-alpha monotherapy groups was the same. The second alternative hypothesis is that overall survival is not the same.

To detect a hazard ratio of 1.40 (assuming 4.9 months median survival for IFN-alpha alone versus 6.86 months for temsirolimus containing arms with 80% power using a 2-sided log-rank test at the 2.5% significance level) 504 deaths were required to be observed. With a sample size of 600 patients (200 in each group), the accrual time was to be approximately 20 months.

The original protocol was designed with one single interim analysis using the O'Brien-Fleming boundary for superior efficacy to guide the decision to declare early success/futility based on conditional power. A protocol amendment was implemented that added a second interim analysis.

In order to preserve an overall 5% significance level, the critical p-value needed to be adjusted. If the p-value is ≤ 0.0211 for the primary analysis, then the null hypothesis of treatment difference would have to be rejected.

Randomisation
Approximately 600 patients were planned for enrolment and randomisation in a 1:1:1 ratio. Patient randomisation was stratified by prior nephrectomy status within each of the 3 geographic regions, resulting in 6 strata. The regions were: Region 1: US; Region 2: Western Europe, Canada, Australia; and Region 3 Asia, Eastern Europe, Africa, South America and other countries.

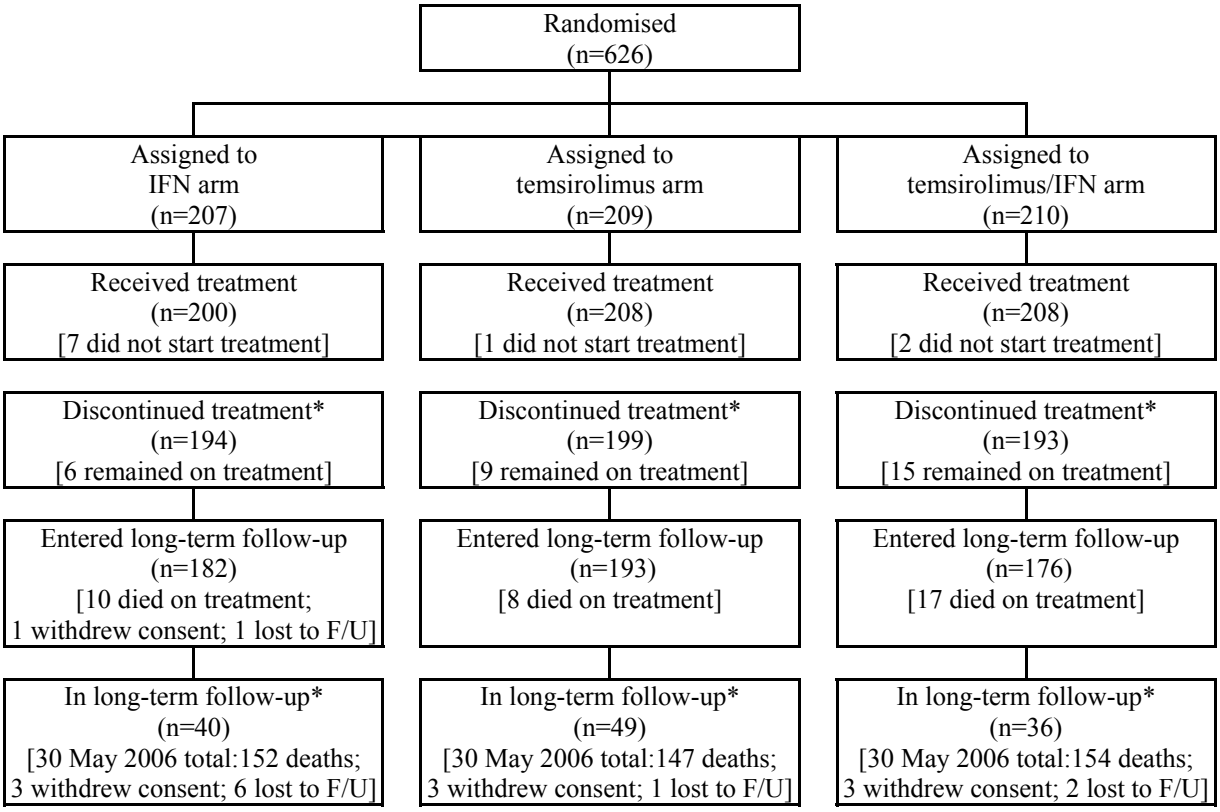
Blinding (masking)
The study was not blinded for logistical reasons (efficient masking with study treatments requiring different pre-medication and with very different safety profiles would have been meaningless).

Statistical methods
The primary efficacy analysis of all efficacy endpoints was conducted using the ITT population which included all patients who were randomised into the study. The primary analysis for the primary efficacy endpoint was carried out by using a stratified (on prior nephrectomy and geographic region) log-rank test at the 2.5% significance level, 2-sided for each primary comparison. Hazard ratio (HR) and the corresponding 95% CI between the 2 treatment groups were estimated using the stratified (on prior nephrectomy and geographic region) Cox proportional hazard model.

Results

Participant flow
Patient flowchart is displayed in Figure 2 (as of 30 May 2006).

Figure 2. 3066K1-304-WW – Study flowchart and patient disposition



* Status as of 30 May 2006

Recruitment
In the accrual period from June 2003 to April 2005, a total of 626 patients were randomly assigned to 3 treatment arms. Overall, there were 207 patients in the IFN-alpha arm, 209 in the temsirolimus arm and 210 in the combination arm. A total of 148 centres enrolled patients, with the range of 1-26 patients per centre.

Conduct of the study
The study consists of two phases: a treatment phase and a follow-up phase. In the treatment phase, patients visited the clinic weekly to receive the treatment and to be monitored for safety on a routine basis. Tumour assessments were scheduled every 8 weeks. In the follow-up phase, patients were followed for survival every 2 months.

The original study protocol was amended 4 times including changes in the selection criteria, sample size, prognostic factors.

An IDMC reviewed the study conduct and the 2 unmasked interim analyses. The IDMC unmasked and reviewed safety data approximately every 6 months.

In terms of protocol deviation, a total of 71 patients (11%) failed to meet the study entry criteria. Reasons for not meeting entry criteria included (patients could be in more than 1 category):

- protocol-specified laboratory values not met (26 patients)
- fewer than 3 specified poor prognosis factors at the time of randomisation (23 patients), brain metastases with symptoms (6 patients)
- radiation therapy or surgery not within the timeframe set forth in the protocol (4 patients)

- prior systemic therapy (4 patients)
- KPS criteria not met (3 patients)
- active infection (4 patients)
- history of other prior malignancy within the last 5 years (1 patient)
- prior investigational therapy/agents with 4 weeks of randomisation (1 patient)
- no histological or cytological confirmation of advanced RCC.

Twenty-six patients (4%) who had progressive disease remained on treatment for at least 30 days after disease progression: 1 patient in the IFN-alpha arm, 13 patients in the temsirolimus arm and 12 patients in the combination arm.

Thirty-eight patients (6%) had anticancer therapy (medication, radiation or surgery) other than study treatment prior to the discontinuation of study treatment. Medications were either megestrol acetate (16 patients) or corticosteroids (17 patients). In addition, 5 patients received palliative radiation therapy concomitant with study treatment.

Baseline data
Baseline demographic and disease characteristics are displayed in Tables 7 and 8, respectively.

Table 7. Study 3066K1-304-WW – Baseline demographic characteristics, ITT population

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210	Total n=626
Age, years				
Mean	59.2	58.7	59.3	59.1
SD	10.4	10.0	9.8	10.1
Median	60.0	58.0	59.0	59.0
Min, Max	23.0, 86.0	32.0, 81.0	32.0, 82.0	23.0, 86.0
≥ 65 years	65 (31.4)	64 (30.6)	57 (27.1)	186 (29.7)
Sex (n, %)				
Female	59 (28.5)	70 (33.5)	65 (31.0)	194 (31.0)
Male	148 (71.5)	139 (66.5)	145 (69.0)	432 (69.0)
Geographic distribution				
Region 1	61 (29.5)	61 (29.2)	62 (29.5)	184 (29.4)
Region 2	43 (20.8)	44 (21.1)	42 (20.0)	129 (20.6)
Region 3[a]	103 (49.8)	104 (49.8)	106 (50.5)	313 (50.0)
Race (n, %)				
White	191 (92.3)	186 (89.0)	193 (91.9)	570 (91.1)
Asian	4 (1.9)	6 (2.9)	3 (1.4)	13 (2.1)
Black	8 (3.9)	9 (4.3)	8 (3.8)	25 (4.0)
Other	4 (1.9)	8 (3.8)	6 (2.9)	18 (2.9)
Karnofsky score (n, %)				
< 60	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.2)
60-70	171 (83.0)	168 (80.4)	177 (84.3)	516 (82.6)
> 70	34 (16.5)	41 (19.6)	33 (15.7)	108 (17.3)
Unknown	1	0	0	1

a Eastern Europe in Poland (n = 101), Russia (n = 63) and the Ukraine (n = 43)

Table 8. Study 3066K1-304-WW – Baseline disease characteristics, ITT population

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210	Total n=626
Stage of disease at baseline				
Stage IV	201 (97.1)	200 (95.7)	205 (97.6)	606 (96.8)
Recurrent Stage II	1 (0.5)	1 (0.5)	2 (1.0)	4 (0.6)
Recurrent Stage III	5 (2.4)	8 (3.8)	3 (1.4)	16 (2.6)
Primary cell type[a]				
Clear	170 (82.5)	169 (82.0)	163 (79.1)	502 (81.2)
Indeterminate	23 (11.2)	24 (11.7)	35 (17.0)	82 (13.3)
Non clear	13 (6.3)	13 (6.3)	8 (3.9)	34 (5.5)
Unknown[a]	1	3	4	8
No. of metastatic organ sites[b]				
0	1 (0.5)	4 (1.9)	1 (0.5)	6 (1.0)
1	41 (19.8)	39 (18.7)	41 (19.5)	121 (19.3)
≥ 2	165 (79.7)	166 (79.4)	168 (80.0)	499 (79.7)
Baseline tumour burden, mm				
< 100 mm	64 (30.9)	67 (32.1)	70 (33.3)	201 (32.1)
≥ 100 mm	143 (69.1)	142 (67.9)	140 (66.7)	425 (67.9)
Metastasis in liver				
Yes	54 (26.1)	60 (28.7)	61 (29.0)	175 (28.0)
Metastasis in lung				
Yes	155 (74.9)	159 (76.1)	155 (73.8)	469 (74.9)
Metastasis in lymph nodes				
Yes	100 (48.3)	106 (50.7)	100 (47.6)	306 (48.9)
Metastasis in bone[a]				
Yes	87 (42.9)	80 (40.0)	91 (44.6)	258 (42.5)
Unknown[a]	4	9	6	19
Metastasis in brain[a]				
Yes	7 (3.4)	11 (5.3)	10 (4.8)	28 (4.5)
Unknown[a]	4	2	3	9
Dominant site of disease[a, c]				
Bone	14 (7.0)	15 (7.4)	13 (6.3)	42 (6.9)
Soft tissue	6 (3.0)	15 (7.4)	8 (3.9)	29 (4.8)
Visceral	180 (90.0)	174 (85.3)	184 (89.8)	538 (88.3)
Unknown[a]	7	5	5	17
Time from initial diagnosis to first metastasis[a]				
< 1 year	172 (83.5)	182 (89.2)	185 (89.4)	539 (87.4)
Unknown[a]	1	5	3	9
Time from initial diagnosis to randomisation				
< 1 year	164 (79.2)	174 (83.3)	179 (85.2)	517 (82.6)
Nephrectomy				
Yes	139 (67.1	139 (66.5)	141 (67.1)	419 (66.9)

a For characteristics that were "unknown" for some patients, percentages are based on the number of patients with known characteristics
b Patients are classified according to the investigator-determined assessment of tumour sites
c Patients are classified according to the independent radiologist-determined assessment of dominant site of disease

Numbers analysed

The primary efficacy analysis of all efficacy endpoints was conducted using the ITT population defined as all patients randomised. Secondary analyses of all efficacy endpoints were conducted using the 'Evaluable for Efficacy' population, as defined by the number of patients that remained at least 8 weeks on treatment, unless progression or death occurred, and who had no major protocol deviations (Table 9).

Table 9. Study 3066K1-304-WW – Study patient populations

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210	Total n=626
ITT	207	209	210	626
Evaluable for efficacy	185	196	188	569
Evaluable for safety	200	208	208	616

Outcomes and estimation

Overall Survival

The first interim analysis was conducted on 30 March 2005 after 239 deaths had been observed. Results for OS presented in Table 10.

Table 10. Study 3066K1-304-WW – Overall survival, ITT population (first interim analysis)

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210
Median OS (months)	7.3	11.3	8.4
95% CI (months)	5.7 - 9.6	8.5 – 12.7	6.7 - 11.8
p-value		0.0007	0.2923

The IDMC recommended that the study continue as planned.

The second interim analysis, which is the basis for the application, was conducted after 442 deaths had been observed. The IDMC advised that the protocol-specified analysis of the primary endpoint (OS) had crossed the O'Brien-Fleming boundary for the comparison of temsirolimus alone versus IFN-alpha and recommended the release of the current study data to the Applicant.

The cut-off date for the OS was 15 March 2006 and any alive patients beyond this date were censored on 15 March 2006.

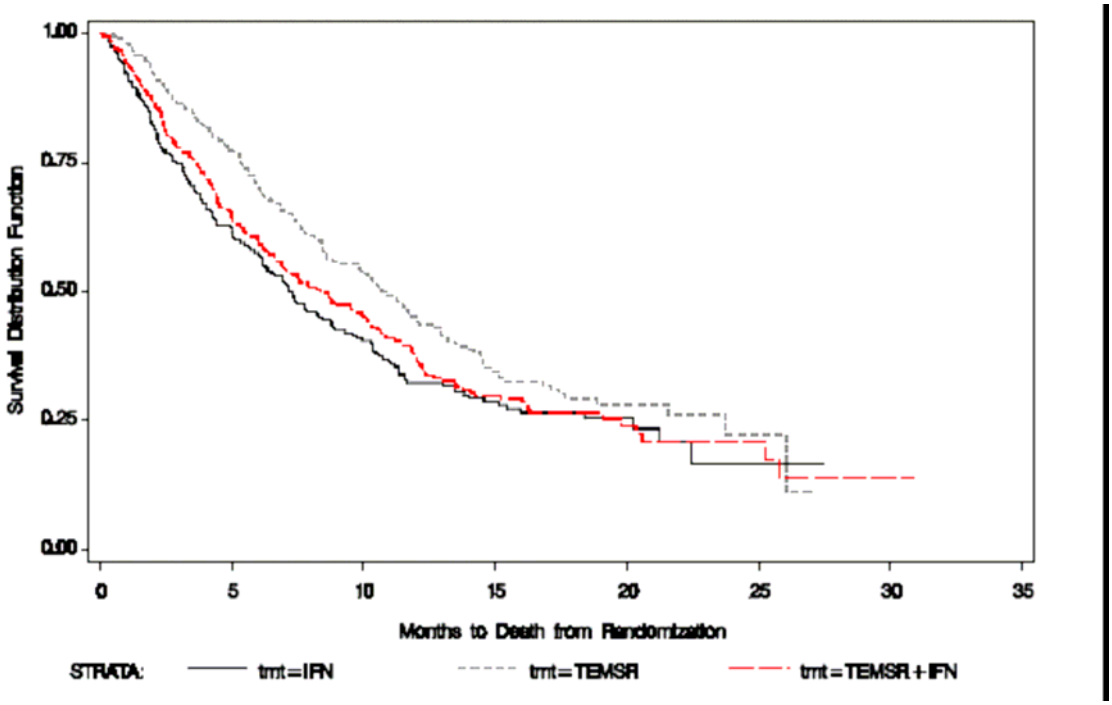
As of 15 March 2006, 446 (71%) of the 626 patients in the ITT population had died, 37 patients (6%) were on treatment, 125 patients (20%) were in follow-up and 18 patients (3%) were lost to follow-up. The results of survival analysis for the ITT population are summarised in Table 11a and Figure 3a.

Table 11. Study 3066K1-304-WW – Overall survival, ITT population

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210
Number of deaths (n, %)	149 (72.0)	143 (68.4)	154 (73.3)
Median OS in months (95% CI)	7.3 (6.1, 8.8)	10.9 (8.6, 12.7)	8.4 (6.6, 10.3)
% Change in median OS from IFN		49%	15%
Hazard ratio[a] (95% CI)		0.73 (0.58, 0.92)	0.96 (0.76, 1.20)
p-value[b]		0.0078	0.6965

a Compared with IFN alone based on Cox proportional hazard model stratified by prior nephrectomy and region.
b Compared with IFN alone based on log-rank test stratified by prior nephrectomy and region.

Figure 3. Study 3066K1-304-WW – Overall survival, ITT population



The final study report was provided which confirmed the results of the interim report.

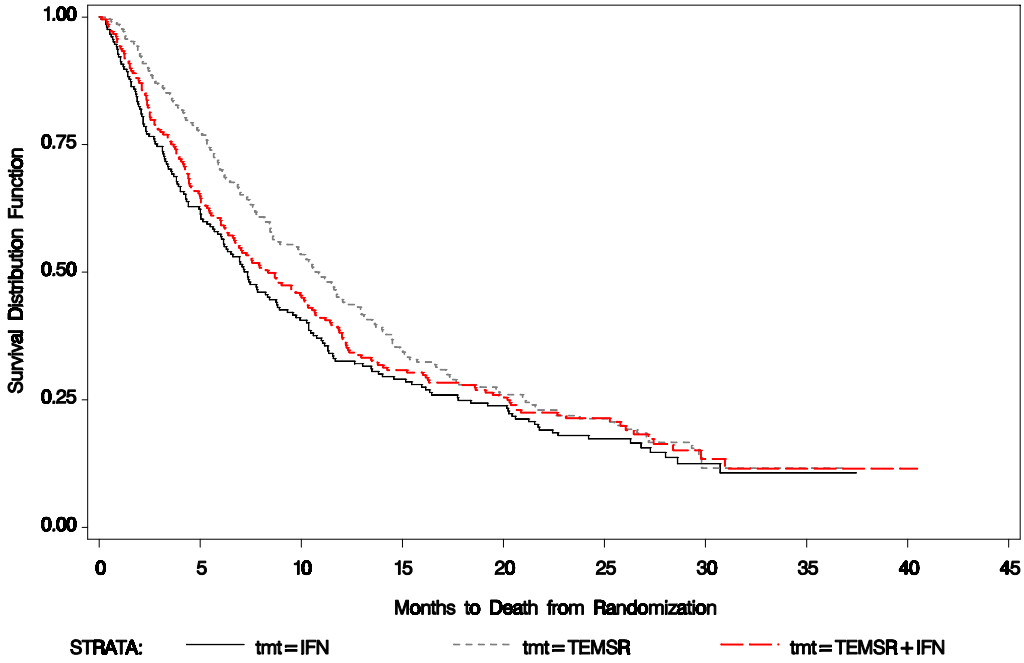
As of 07 March 2007 (the database cut-off date for this report), 13 patients (2.1%) remained on treatment (2 [1.0%] in the IFN-alpha arm, 5 [2.4%] in the temsirolimus arm and 6 [2.9%] in the combination arm) and 25 were in the follow-up period. Most of the remaining patients had died: there were 514 documented deaths (82.1% of patients) and 74 patients (11.8%) were lost to follow-up. The results of survival analysis for the ITT population are summarised in Table 12 and Figure 4.

Table 12. Study 3066K1-304-WW – Overall survival, ITT population

	IFN n=207	**Temsirolimus 25 mg n=209**	**Temsirolimus 15 mg/IFN n=210**
Number of deaths (n, %)	172 (83.1)	171 (81.4)	171 (81.4)
Median OS in months (95% CI)	7.3 (6.1, 8.8)	10.9 (8.6, 12.7)	8.4 (6.6, 10.3)
% Change in median OS from IFN		49%	15%
Hazard ratio[a] (95% CI)		0.78 (0.63, 0.97)	0.93 (0.756, 1.15)
p-value[b]		0.0252	0.4902

a Compared with IFN alone based on Cox proportional hazard model stratified by prior nephrectomy and region.
b Compared with IFN alone based on log-rank test stratified by prior nephrectomy and region.

Figure 4. Study 3066K1-304-WW – Overall survival, ITT population



Progression-free survival

Progression was determined by each investigator and by an independent radiology review. There was an imbalance in the number of patients without post-baseline tumour assessments. For the investigator assessments, post-baseline tumour assessments were absent for 21.7% of the IFN-alpha arm, 5.7% in the temsirolimus arm and 17.6% in the combination arm. The numbers were even higher for independent assessment. Several factors may have contributed to the difference in tumour assessment between the treatment arms: the greater number of early treatment discontinuations due to symptomatic deterioration, disease progression, AE or deaths in the IFN-alpha and combination arms and the greater number of patients in the IFN-alpha arm who were never treated or who discontinued early due to patient request.

Factors contributing to the difference between the investigator and independent assessment could be that investigators based their assessments on symptomatic deterioration and/or radiographic data, whereas the independent radiologists' assessments were based on radiographic data only. In addition, the investigator could have obtained unscheduled scans to confirm suspected PD, which were sometimes not part of the independent radiologist documentation.

Table 13, Figures 5 and 6 summarise these PFS results.

Table 13. Study 3066K1-304-WW – Progression-free survival, ITT population

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210
Independent assessment			
No of patients with post-bas. tumour assessment (n, %)	154 (74.4)	195 (93.3)	171 (81.4)
Median PFS in months (95% CI)	3.2 (2.2, 4.0)	5.6 (3.9, 7.2)	4.9 (3.9, 6.0)
% Change in median PFS from IFN		75%	53%
Hazard ratio[a] (95% CI)		0.74 (0.60, 0.91)	0.76 (0.62, 0.94)
p-value[b]		0.0042	0.0107
No. patients with PD or who died (n, %)	186 (89.9)	191 (91.4)	186 (88.6)
No. censored patients (n, %)	21 (10.1)	18 (8.6)	245 (11.4)
Investigator's assessment			
No of patients with post-bas. tumour assessment (n, %)	162 (78.3)	197 (94.3)	173 (82.4)
Median PFS in months (95% CI)	1.9 (1.9, 2.2)	3.8 (3.6, 5.2)	3.7 (3.1, 5.2)
% Change in median PFS from IFN		100%	95%
Hazard ratio[a] (95% CI)		0.749 (0.60, 0.90)	0.78 (0.63, 0.95)
p-value[b]		0.0028	0.0129
No. patients with PD or who died (n, %)	196 (90.3)	200 (95.7)	201 (95.7)
No. censored patients (n, %)	11 (5.3)	9 (4.3)	9 (4.3)

a Compared with IFN alone based on Cox proportional hazard model stratified by prior nephrectomy and region.
b Compared with IFN alone based on log-rank test stratified by prior nephrectomy and region.

Figure 5. Study 3066K1-304-WW – Progression-free survival, ITT population (independent assessment)

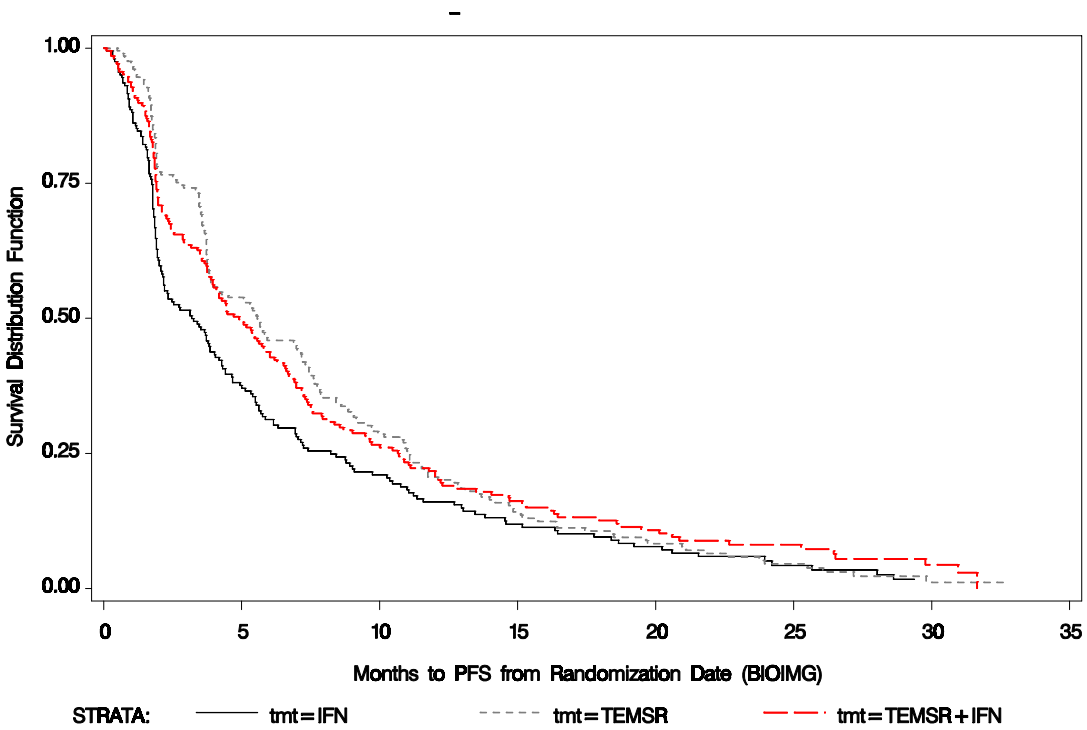
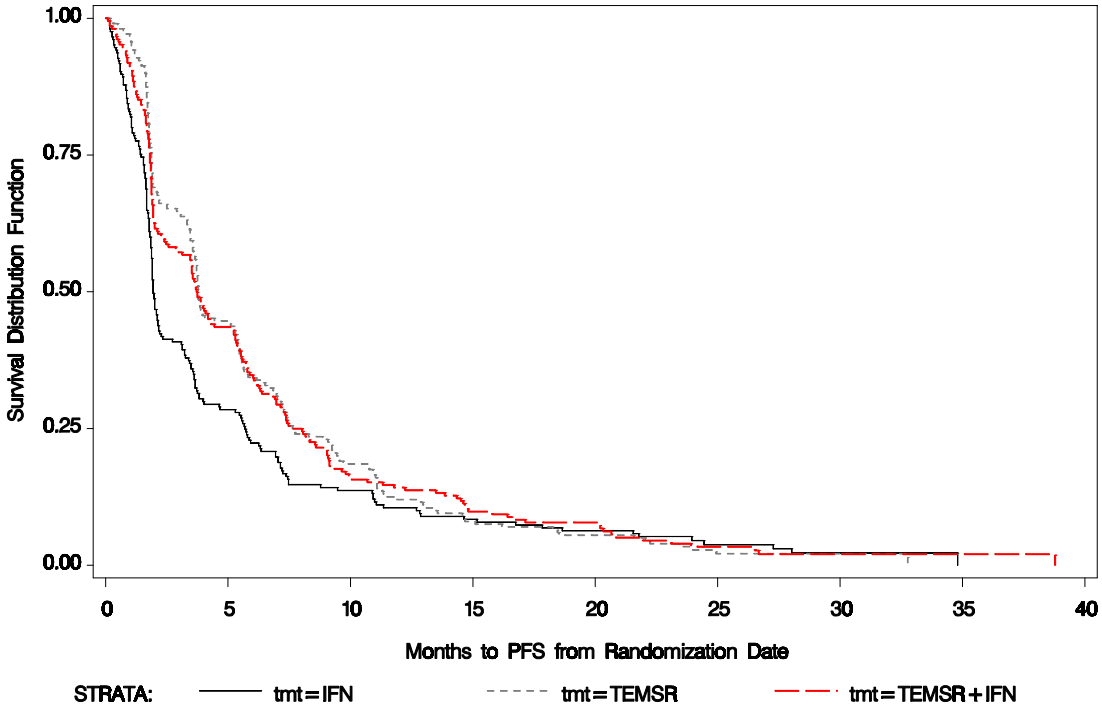


Figure 6. Study 3066K1-304-WW – Progression-free survival, ITT population (investigator assessment)



Response rate

The objective response rate (ORR) was defined as the percentage of patients who had a confirmed complete or partial response (CR or PR) as their best response to treatment. Response was confirmed by a second assessment at least 4 weeks thereafter. ORR was assessed by each investigator and by independent radiology review. These results are displayed in Table 14.

Table 14. Study 3066K1-304-WW – Progression-free survival, ITT population

	IFN n=207	Temsirolimus 25 mg n=209	Temsirolimus 15 mg/IFN n=210
Independent assessment			
Complete response (n, %)	-	-	-
Partial response (n, %)	11 (5.3)	19 (9.1)	20 (9.5)
Stable disease (n, %)	80 (38.6)	133 (63.6)	109 (51.9)
Progressive disease (n, %)	60 (29.0)	41 (19.6)	38 (18.1)
Indeterminate[a] (n, %)	3 (1.4)	2 (1.0)	4 (1.9)
No post-baseline tumour assessment (n, %)	53 (25.6)	14 (6.7)	39 (18.6)
No of patients with CR or PR (n, %)	11 (5.3)	19 (9.1)	20 (9.5)
95% CI	(2.3, 8.4)	(5.2, 13.0)	(5.6, 13.5)
p-value[b]		0.1361	0.1062
Investigator's assessment			
Complete response (n, %)	3 (1.4)	0 (0)	0 (0)
Partial response (n, %)	14 (6.8)	18 (8.6)	25 (11.9)
Stable disease (n, %)	64 (30.9)	121 (57.9)	94 (44.8)
Progressive disease (n, %)	78 (37.7)	57 (27.3)	52 (24.8)
Indeterminate[a] (n, %)	3 (1.4)	1 (0.5)	2 (1.0)
No post-baseline tumour assessment (n, %)	45 (21.7)	12 (5.7)	37 (17.6)
No of patients with CR or PR (n, %)	17 (8.2)	18 (8.6)	25 (11.9)
95% CI	(4.5, 12.0)	(4.8, 12.4)	(7.5,16.3)
p-value[b]		0.8881	0.2273

a Had assessment of stable disease or unconfirmed response prior to 8 weeks after randomisation.
b Compared with IFN alone based on Cochran-Mantel-Haenszel test stratified by prior nephrectomy and region.

There was no statistically significant difference between the temsirolimus or combination arm and the IFN arm in terms of ORR, using either investigator or independent assessment. There was no statistically significant difference in duration of response between temsirolimus-containing arm and the IFN-alpha arm using either investigator or independent assessment.

Ancillary analyses
More patients in the temsirolimus arm received anti-cancer therapies during long-term follow-up. This was mainly due to the use of IFN at the end of the study medication in 15 patients in the IFN-alpha arm and 48 patients in the temsirolimus arm. The use of other anticancer therapies during follow-up was relatively similar across the treatment arms.

Treatment interaction test for nephrectomy status or geographical region showed no difference between the relative efficacies of temsirolimus, IFN-alpha or the combination with respect to OS.

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

No analysis on pooled data from different studies or meta-analysis has been performed.

- Clinical studies in special populations

The effect of hepatic impairment on PK and safety parameters for temsirolimus is currently being evaluated in cancer patients with mild, moderate or severe hepatic impairment (**Study 3066K1-152-US**). Initial safety and PK data available for 14 patients showed that thrombocytopenia was a dose-limiting factor for patients with mild hepatic impairment, leading to de-escalation and multiple dose delays. Therefore, caution should be exercised when administering temsirolimus to patients with hepatic impairment. Use of temsirolimus in patients with severe hepatic impairment is not recommended.

Data from the integrated population PK analysis of temsirolimus and sirolimus indicated that PK disposition was not affected by differences in creatinine clearance. It is therefore likely that renal elimination plays only a minor role in the clearance of temsirolimus and sirolimus. Until definitive data are available, temsirolimus should be used with caution in patients with severe renal impairment.

The exposure and PK parameters of temsirolimus and sirolimus were specifically assessed in a study of Japanese patients (**Study 3066K1-131-JA**). This study suggested that following the 15 and 45 mg/m^2 doses of temsirolimus IV, exposures between Japanese and non-Japanese patients were similar and do not justify an alteration of dose or regimen for this patient population.

A Phase I/II study in paediatric patients with cancer (**Study 3066K1-139-US**) is currently being conducted and includes a PK assessment.

- Supportive study(ies)

Table 15 provides an overview of efficacy results (OS, PFS, ORR and clinical benefit) obtained from the 3 studies conducted in patients with RCC.

Table 15. Studies 3066K1-304-WW, 3066K1-200-US and 3066K1-124-US – Efficacy results, ITT population

Protocol number	Treatment group	Number of patients	Median OS months (95% CI)	Median PFS months (95% CI)	ORR (sum definition) % (95% CI)	Clinical benefit rate % (95% CI)
3066K1-304-WW	Temsirolimus 25 mg	209	10.9 (8.6-12.7)	5.5 (3.9-7.0)	8.6 (4.8-12.4)	32.1 (25.7-38.4)
	Temsirolimus 15 mg/IFN	210	8.4 (6.6-10.3)	4.9 (3.9-5.9)	8.1 (4.4-11.8)	28.1 (22.0-34.2)
	IFN	207	7.3 (6.1-8.8)	3.1 (2.2-3.8)	4.8 (1.9-7.8)	15.5 (10.5-20.4)
3066K1-200-US	Temsirolimus 25 mg	36	13.8 (9.0-18.7)	6.3 (3.6-7.8)	5.6 (0.7-18.7)	52.8 (35.5-69.6)
	Temsirolimus 75 mg	38	11.0 (8.6-18.6)	6.7 (3.5-8.5)	7.9 (1.7-21.4)	55.3 (38.3-71.4)
	Temsirolimus 250 mg	37	17.5 (12.0-24.6)	5.2 (3.7-7.4)	8.1 (1.7-21.9)	43.2 (21.7-60.5)
	All patients	111	15.0 (10.4-18.3)	5.8 (3.8-7.2)	7.2 (3.2-17.7)	50.5 (40.8-60.1)
3066K1-124-US	Temsirolimus 15 mg/IFN	39	22.1 (11.0-26.0)	7.6 (5.5-11.0)	7.7 (1.6-20.9)	43.6 (27.8-60.4)
	All patients	71	18.8 (15.0-25.0)	9.1 (6.2-13.0)	11.3 (5.0-21.0)	46.5 (34.6-58.7)

Clinical safety

The dataset supporting the safety evaluation of temsirolimus is based on 12 clinical studies (Table 15). This represents the majority of the 1072 temsirolimus-treated patients and healthy subjects who received IV temsirolimus who were enrolled in 18 clinical studies (408 temsirolimus-treated patients in the Phase III advanced RCC study, 179 temsirolimus-treated patients in 2 other advanced RCC studies, 178 temsirolimus-treated patients in three studies of other cancers [breast, prostate and MCL], 215 temsirolimus-treated patients with advanced solid tumours in seven Phase I studies and 92 healthy subjects in five Phase I studies).

Safety results from ongoing studies which were initiated shortly before 30 May 2006 (cut-off date for this application), including the Phase I study in healthy subjects assessing the effect of temsirolimus on the QT/QTc interval, is not described in this application.

- Patient exposure

Safety evaluation is based on a total of 823 cancer patients and 92 healthy volunteers who received IV temsirolimus, and 200 patients who received IFN-alpha alone (Table 16). However the temsirolimus safety description results relate mainly to Study 3066K1-304-WW, since this study is based on controlled data.

Table 16. Safety population – List of studies

Population	Studies	Temsirolimus dose(s) and regimen	No. treated subjects
Advanced RCC patients treated with single-agent temsirolimus	3066K1-304-WW 3066K1-200-US	25 mg once weekly	244
Advanced RCC patients treated with temsirolimus in combination with IFN	3066K1-304-WW 3066K1-124-US	15 mg once weekly with IFN 6 MU or 9 MU 3 times weekly	253
Advanced RCC patients treated with single-agent IFN	3066K1-304-WW	18 MU 3 times weekly	200
Advanced RCC, prostate cancer and breast cancer patients treated with higher temsirolimus doses	3066K1-200-US 3066K1-201-US 3066K1-203-EU	75 mg or 250 mg once weekly	199
Patients with advanced solid tumours treated with temsirolimus on alternate dosing schedules	3066K1-100-US 3066K1-101-EU	< 15 mg/m^2, 15 mg/m^2 or > 15 mg/m^2 once weekly or once daily for 5 days every 2 weeks[a]	128
Healthy subjects who received single temsirolimus doses	3066K1-133-US 3066K1-145-US 3066K1-148-US 3066K1-149-US 3066K1-151-US	< 25 mg or 25 mg	92

a A 15 mg/ m^2-dose corresponds to approximately a 25-mg dose, using a conversion factor of 1.8 m^2 for average adult body surface area

The demographic and baseline characteristics for the Phase III advanced RCC study are presented in the Clinical efficacy section.

Patients with advanced RCC, prostate cancer or breast cancer received high temsirolimus doses (75 or 250 mg) in 3 Phase II studies. Prostate cancer patients were slightly older (median 71 years, range 51-87), while breast cancer patients were slightly younger (median 56 years, range 30-79). These patients were mainly Caucasian (96% and 95%, respectively). Most patients had good performance status (ECOG PS 0-1 or KPS 80-100), with a minority of patients having poor performance status.

In Study 3066K1-304-WW, patients had higher exposure to temsirolimus when it was administered alone rather than in combination with IFN-alpha. Median duration of temsirolimus treatment for patients who received temsirolimus 25 mg or temsirolimus 15 mg/IFN-alpha was 17 weeks (1-126) and 15 weeks (1-138), respectively. Sixty patients in the temsirolimus and 43 patients in the combination

arm were treated for at least 32 weeks. The median relative dose intensity was 0.99 (range 0.43-1.2) and 0.75 (range 0.08-1.0) for patients in the temsirolimus alone and combination arms, respectively.

In the IFN-alpha arm, the median actual dose was 28.5 MU per week (3.0-53.2) as compared with an expected maximum dose of 54 and 18 MU per week. In the combination arm, the median actual dose for the IFN-alpha arm was 13.5 MU per week (5.0-19.8 MU).

The median duration of treatment was 8.0 weeks (1-124) for IFN-alpha alone and 12.0 weeks (1-138) for IFN-alpha in the combination with temsirolimus. Median duration of temsirolimus treatment in Study 3066K1-200-US was longer (26 weeks, 1-193) in the temsirolimus 25 mg dose group. In the Study 3066K1-124-US, median duration of both temsirolimus and IFN-alpha treatment was also longer (24 weeks, 4- 136). This difference in exposure was likely due to the more advanced disease stage of patients in the Phase III studies.

Although relative dose intensity was lower at higher temsirolimus doses, patients were able to tolerate single-agent temsirolimus at a dose almost 10 times higher than that tested in the Phase III study. For patients who received 75 and 250 mg the median dose intensities were 54.0 mg/week and 170.8 mg/week, respectively, whereas the median treatment duration decreased with higher doses from 22 to 16.5 weeks, respectively.

- Adverse events

All but one patient in the study population of the pivotal study experienced one or more TEAE. Table 17 displays the TEAE per system class organ (SOC) and then by decreasing incidence for temsirolimus 25 mg treatment group.

Table 17. Studies 3066K1-304-WW - Number (%) of patients reporting TEAE (≥ 10%)

Body System Adverse Event	IFN n=200	Temsirolimus n=208	Temsirolimus/IFN n=208
Any adverse event	199 (99.5)	208 (100)	208 (100)
Body as a whole	184 (92.0)	179 (86.1)	196 (94.2)
Asthenia	129 (64.5)	106 (51.0)	130 (62.5)
Pain	30 (15.0)	58 (27.9)	42 (20.2)
Fever	99 (49.5)	51 (24.5)	126 (60.6)
Abdominal pain	34 (17.0)	46 (22.1)	36 (17.3)
Back pain	30 (15.0)	43 (20.7)	32 (15.4)
Chest pain	18 (9.0)	35 (16.8)	23 (11.1)
Headache	30 (15.0)	32 (15.4)	47 (22.6)
Infection	11 (5.5)	29 (13.9)	31 (14.9)
Chills	59 (29.5)	17 (8.2)	72 (34.6)
Flu syndrome	23 (11.5)	7 (3.4)	28 (13.5)
Cardiovascular system	64 (32.0)	68 (32.7)	83 (39.9)
Digestive system	151 (75.5)	165 (79.3)	171 (82.2)
Nausea	83 (41.5)	77 (37.0)	84 (40.4)
Anorexia	87 (43.5)	66 (31.7)	79 (38.0)
Diarrhoea	39 (19.5)	57 (27.4)	56 (26.9)
Constipation	36 (18.0)	42 (20.2)	40 (19.2)
Stomatitis	7 (3.5)	41 (19.7)	44 (21.2)
Vomiting	57 (28.5)	40 (19.2)	61 (29.3)
Mucositis	10 (5.0)	39 (18.8)	48 (23.1)
Haemic and lymphatic system	113 (56.5)	116 (55.8)	172 (82.7)
Anaemia	84 (42.0)	94 (45.2)	128 (61.5)
Thrombocytopenia	16 (8.0)	28 (13.5)	78 (37.5)
Neutropenia	26 (13.0)	15 (7.2)	57 (27.4)
Leucopenia	34 (17.0)	13 (6.3)	65 (31.3)
Lymphopenia	17 (8.5)	11 (5.3)	27 (13.0)
Metabolic and nutritional	138 (69.0)	159 (76.4)	172 (82.7)
Hyperlipaemia	29 (14.5)	57 (27.4)	80 (38.5)
Peripheral oedema	16 (8.0)	55 (26.4)	36 (17.3)
Hyperglycaemia	22 (11.0)	53 (25.5)	36 (17.3)
Hypercholesteremia	9 (4.5)	51 (24.5)	55 (26.4)
Weight loss	50 (25.0)	40 (19.2)	66 (31.7)
Creatinine increased	21 (10.5)	30 (14.4)	41 (19.7)
Alkaline phosphatase increased	15 (7.5)	20 (9.6)	31 (14.9)
Hypophosphataemia	4 (2.0)	17 (8.2)	21 (10.1)
AST increased	29 (14.5)	17 (8.2)	43 (20.7)
Hypoproteinaemia	19 (9.5)	12 (5.8)	21 (10.1)
Hypocalcaemia	21 (10.5)	11 (5.3)	31 (14.9)
Dehydration	20 (10.0)	10 (4.8)	23 (11.1)
Musculoskeletal system	67 (33.5)	61 (29.3)	61 (29.3)
Arthralgia	29 (14.5)	38 (18.3)	28 (13.5)
Myalgia	30 (15.0)	16 (7.7)	22 (10.6)
Nervous system	110 (55.0)	96 (46.2)	113 (54.3)
Insomnia	30 (15.0)	25 (12.0)	35 (16.8)
Dizziness	25 (12.5)	19 (9.1)	27 (13.0)
Anxiety	12 (6.0)	16 (7.7)	23 (11.1)
Somnolence	21 (10.5)	14 (6.7)	15 (7.2)
Confusion	20 (10.0)	9 (4.3)	10 (4.8)
Depression	26 (13.0)	9 (4.3)	26 (12.5)
Respiratory system	94 (47.0)	132 (63.5)	134 (64.4)
Dyspnoea	48 (24.0)	59 (28.4)	55 (26.4)
Cough increased	29 (14.5)	54 (26.0)	48 (23.1)
Epistaxis	7 (3.5)	25 (12.0)	27 (13.0)
Pharyngitis	3 (1.5)	25 (12.0)	27 (13.0)
Skin and appendages	51 (25.5)	140 (67.3)	86 (41.3)
Rash	11 (5.5)	77 (37.0)	34 (16.3)
Pruritus	16 (8.0)	40 (19.2)	24 (11.5)
Nail disorder	1 (0.5)	28 (13.5)	6 (2.9)
Dry skin	14 (7.0)	22 (10.6)	13 (6.3)
Acne	2 (1.0)	21 (10.1)	7 (3.4)
Special senses	31 (15.5)	66 (31.7)	36 (17.3)
Taste perversion	13 (6.5)	31 (14.9)	18 (8.7)
Urogenital system	48 (24.0)	53 (25.5)	61 (29.3)
Urinary tract infection	10 (5.0)	16 (7.7)	22 (10.6)

Temsirolimus 25 mg was associated with a lower overall incidence of Grade 3 or 4 TEAE (66.8% vs. 77.5%, respectively) compared with IFN-alpha. The most common Grade 3 or 4 TEAE reported for temsirolimus 25 mg included anaemia (19.7% vs. 21.5%), asthenia (11.1 vs. 26%) and hyperglycaemia (10.6% vs. 1.5%). Severe anaemia and asthenia were reported more frequently in the IFN-alpha treated group. The incidence of certain Grade 3 or 4 metabolic abnormalities was significantly higher with temsirolimus 25 mg compared to IFN-alpha, including hyperglycaemia, hypophosphataemia (5.3% vs. 0.5%) and hypokalaemia (3.4% vs. 0%).

The combination of temsirolimus with IFN-alpha was associated with highest overall incidence of Grade 3 or 4 TEAE (86.5% vs. 77.5% for IFN-alpha and 66.8% for temsirolimus 25 mg). Body systems most frequently affected following administration of temsirolimus 15 mg/IFN-alpha included haemic and lymphatic (56.7%), body as a whole (44.2%), metabolic and nutritional (37.5%), digestive system (28.8%) and respiratory system (20.7%).

Based on previous clinical experience with temsirolimus, certain categories of AE with special interest are presented below.

Hypersensitivity and acute reactions
Allergic reactions occurring on the dosing day were reported for 1 (0.5%) patient in the IFN-alpha arm, 10 (4.8%) patients in the temsirolimus arm and 11 (5.3%) patients in the combination arm. Most of these events were of low grade. Two patients in the combination arm were discontinued due to allergic reactions on dosing day. All but one patient who experienced allergic reactions had received premedication with an antihistamine prior to temsirolimus administration according to the protocol.

For 4 patients in the temsirolimus and in the combination arms, re-exposition to the drug led to a repeat experience of allergic reactions. Indicators of possible hypersensitivity were observed in 2 patients in the temsirolimus arm and 3 in the combination arm (allergic reaction plus oedema, vasodilatation, dizziness and dyspnoea).

Bleeding and thrombotic events
Bleeding events were observed in 17% of patients in the IFN-alpha arm, 24.5% in the temsirolimus arm and 25% in the combination arm. Minor bleeding (epistaxis) was the most frequent bleeding event and had at least 3-times the incidence in either the temsirolimus or combination arm as compared to the IFN-alpha arm (3.5%, 12.0% and 13.0% in the IFN-alpha, temsirolimus and combination arm, respectively).

Haematologic events
Anaemia was the most frequent haematologic event, with reported incidences of 41.5% in the IFN-alpha arm, 45.2% in temsirolimus arm and 61.1% in the combination arms.

The incidences of neutropenia, leucopenia and lymphopenia were lower in the temsirolimus arm than in the IFN-alpha arm. Thrombocytopenia and neutropenia had at least twice the incidence in the combination arm as in either the IFN-alpha or temsirolimus arm.

Metabolic events
Hypercholesterolaemia, hypophosphataemia, hyperlipaemia, hyperglycaemia and diabetes mellitus had at least a 2-fold increase in incidence in patients in at least one temsirolimus-containing arm compared to the IFN-alpha arm. Hypocalcaemia had approximately half the incidence in the temsirolimus arm as in the IFN-alpha arm.

Infection-related events
Infection-related events were reported for 14.5, 27.4 and 33.7% in the IFN-alpha, temsirolimus and combination arms, respectively. “Infection” was the most frequent term in this category and had more than 3-times the incidence in either the temsirolimus or in the combination arm as in the IFN-alpha arm. The incidence of pneumonia was nearly twice as high in the temsirolimus-containing arms

compared to IFN-alpha arm. Respiratory infections were the most frequent type of infections. No case of systemic fungal infections or *Pneumocystis carinii* was reported.

Cardiovascular events
Cardiovascular TEAE had a similar incidence in the treatment arms (41.5, 45.2 and 46.2% for IFN-alpha, temsirolimus and combination, respectively). Cardiovascular TEAE reported in at least 5% of patients in the temsirolimus arm were chest pain (18.3%), dizziness and hypokalaemia (9.1% each), hypertension and somnolence (6.7% each). Of these events, hypokalaemia and chest pain were at least twice as frequent in the temsirolimus arm as compared to the IFN-alpha arm.

Respiratory events
"Dyspnoea" and "cough increased" were the most often reported events, both occurred more often in the temsirolimus arm compared to the IFN-alpha arm, with a statistically significant difference for "cough increased". About one third was considered treatment related by the investigator. Pneumonitis was reported for 4 patients in the temsirolimus arm.

Renal events
Renal TEAE reported for at least 5% of patients in the temsirolimus arm were peripheral oedema, increased creatinine and oedema. Peripheral oedema and oedema had more than twice the incidence in the temsirolimus arm as compared to the IFN-alpha arm, while dehydration and hyperkalaemia had approximately half the incidence in the temsirolimus arm as compared to the IFN-alpha arm. There were very few patients with Grade 3 or 4 peripheral oedema (5 patients, 2.4% in the temsirolimus arm only) or oedema (1 patient, 0.5%, in each treatment arm).

Thirteen patients had acute kidney failure: 6 patients in the IFN-alpha arm, 3 in the temsirolimus arm and 4 in the combination arm.

Renal-related events had approximately twice the incidence in either temsirolimus-containing arm (24.5 and 24.0% in the temsirolimus and combination arms, respectively) as in the IFN-alpha arm (12.0%). Creatinine increase was the most frequent renal-related event and it had over 3-fold incidence in the temsirolimus and the combination arm (11.1% in each) as in the IFN-alpha arm (3.5%).

Rash-related events
Both rash and acne had more than twice the incidence in the temsirolimus or combination arm as in the IFN-alpha arm. Rashes had a higher incidence in the temsirolimus arm than in the combination arm, so it could be possibility related to the administered dose.

Of note, the incidences of maculopapular rash and pustular rash were low (2.9% and 1.4%, respectively). All cases of acne and most cases of rash were of low grade. Grade 3 or 4 rashes were reported for 0% of patients in the IFN-alpha arm, 2.4% of patients in the temsirolimus arm and 1.0% of patients in the combination arm.

The pattern of AE observed in 36 patients included in the Study 3066K1-200-US receiving temsirolimus 25 mg/week, was similar to what has been previously reported. The incidences of back pain, infection, diarrhoea, stomatitis, mucositis, vomiting and rash were substantially higher, the incidence of anaemia was lower.

In total 199 cancer patients were treated with higher doses of temsirolimus (75 and 250 mg/week). Dose-dependency was found for incidences of the following TEAE (incidence $\geq$ 10% for any dose group and $\geq$ 2-fold higher incidence at higher doses) in the 25, 75 and 250 mg dose groups, respectively:

Gastrointestinal system
- stomatitis (24.6%, 51.0%, 50.5%)
- dry mouth (7.0%, 10.6%, 17.9%)
- mouth ulceration (2.9%, 13.5%, 11.6%).

Skin and appendages

- acne (12.3%, 29.8%, 30.5%)
- maculopapular rash (4.9%, 11.5%,10.5%)
- alopecia (2.9%, 7.7%, 15.8%).

Nervous system

- insomnia (11.1%, 20.2%, 21.1%)
- somnolence (5.7%, 18.3%, 21.1%)
- depression (5.3%, 12.5%, 23.2%).

For the following TEAE, there was also a dose-dependent trend for the incidence: infection, flu-like syndrome, urinary tract infection, thrombocytopenia, leucopenia and epistaxis. While the incidence of TEAE increased with dose, the overall incidence of Grade 3 and 4 TEAE was similar. For some Grade 3 and 4 TEAE, a dose-dependent increase was observed in the 25, 75 and 250 mg dose groups: stomatitis (1.2 %, 2.9%, 6.3%), thrombosis (0.4, 2.9 and 5.3%) and depression (0.4, 1.0 and 7.4%).

- Serious adverse event/deaths/other significant events

The number of deaths within 30 days of the first dose of study drug was 35 for all treatment arms in Study 3066K1-304. The lowest was in the temsirolimus arm (4/35) compared with IFN-alpha (18/35) or the combination (13/35). Two deaths in the temsirolimus arm were deemed related to study drug (hyperkalaemia and acute renal failure in one patient each), compared with 1 in the IFN-alpha arm (cerebral vascular disorder) and 7 in the combination arm (renal failure [2], adult respiratory distress syndrome and acute renal failure [1], acute myocardial infarction and renal failure [1], cardiorespiratory insufficiency [1], cerebral stroke [1], bronchiolitis obliterans [1]).

The number of deaths within 15 days of the last dose of study drug was 67 for all treatment arms in Study 3066K1-304, the lowest was in the temsirolimus arm (13/67) compared with IFN-alpha (24/67) or the combination arm (30/67). No death occurred within 15 days after the last dose in Study 3066K1-200, and only one death, considered study drug related, occurred in Study 3066K1-124. Nine and 5 deaths occurred within 30 days after the last dose in Studies 3066K1-100-US and 3066K1-101-EU, respectively. Only 1 death was considered related to study drug (intracranial haemorrhage) in Study 3066K1-101-EU.

Overall, almost half of the patients treated in Study 3066K1-304 had at least 1 SAE. The overall incidence of SAE was lowest in the temsirolimus arm (38.5%) compared with the IFN-alpha or combination arms (48.5 and 55.3%, respectively). In the temsirolimus arm, the most commonly reported SAE were anaemia (5.3%); pneumonia (4.8%); abdominal pain (3.4%); dehydration and hyperglycaemia (2.9% each); asthenia and carcinoma (2.4% each).

The incidence of individual SAE was generally similar across the 3 treatment groups, with the exception of a higher incidence of hyperglycaemia SAE in the temsirolimus arm (6 patients, 2.9%) compared with the IFN-alpha arm (0%). The type of individual SAE seen with temsirolimus was consistent with its overall TEAE profile. The overall incidence of treatment-related SAE was 17.8 and 14.0% in the temsirolimus and IFN-alpha arms respectively and was higher in the combination arm (26.0%).

In the Phase II studies, in patients receiving temsirolimus 75 or 250 mg/week, SAE that were considered drug-related occurred more often in higher doses: fever, infection, diarrhoea, nausea, vomiting and dehydration. Of note is that 4/104 patients receiving 75 mg reported interstitial pneumonia.

- Laboratory findings

Table 18 presents the overall and Grade 3 or 4 incidences of laboratory parameters considered of special interest.

Table 18. Studies 3066K1-304-WW – Laboratory parameters of special interest

Parameter, n (%)	All Grades	Grade 3/4
Any abnormality	208 (100)	162 (77.9)
Blood chemistry		
Glucose (low)	11 (5.3)	2 (1.0)
Glucose (high)	186 (89.4)	33 (15.9)
Total cholesterol/lipid (high)	181 (87.0)	5 (2.4)
Triglycerides (high)	173 (83.2)	92 (44.2)
AST (high)	79 (38.0)	5 (2.4)
Alkaline phosphatase (high)	141 (67.8)	7 (3.4)
Total bilirubin (high)	16 (7.7)	2 (1.0)
Creatinine (high)	119 (57.2)	7 (3.4)
Calcium (low)	82 (39.4)	9 (4.3)
Calcium (high)	46 (22.1)	8 (3.8)
Phosphorus (low)	102 (49.0)	38 (18.3)
Haematology		
Haemoglobin (low)	195 (93.8)	41 (19.7)
WBC (low)	67 (32.2)	1 (0.5)
Neutrophils (low)	39 (18.8)	10 (4.8)
Lymphocytes (low)	110 (52.9)	33 (15.9)
Platelet count (low)	84 (40.4)	3 (1.4)

Nearly all patients reported changes in laboratory results. Grade 3 and 4 changes were observed in > 70% of patients in all groups. The incidences of Grade 3 and 4 changes for hyperglycaemia, hypertriglyceridaemia and hypophosphataemia were higher with temsirolimus compared with IFN-alpha. Although creatinine increase and thrombocytopenia were reported more often for temsirolimus, the incidence of Grade 3 and 4 toxicity did not substantially increase. The Grade 3 and 4 incidences were lower for temsirolimus for leucopenia, neutropenia and AP increase.

QT/QTc interval

The effect of temsirolimus on the QT/QTc interval was evaluated for 31 patients in Study 3066K1-304-WW (18 and 13 patients in temsirolimus and combination arms, respectively) and 53 patients in Study 3066K1-305-WW study who received higher temsirolimus doses (27 and 26 patients in temsirolimus 175/25 mg and 175/75 mg, respectively). Analysis of within group and between-group changes in QT/QTc interval did not reveal any clinically important trends following temsirolimus treatment.

Five out of 84 patients had a clinically noteworthy value for QTc interval at 1 or more isolated post-baseline timepoints. One patient (175/25 mg) had a TEAE in association with a prolonged QTc interval which coincided with cardiac arrhythmia at the study withdrawal visit (1 week after the last dose of temsirolimus). The investigator judged the TEAE probably not related to treatment. The other four patients did not have clinical signs or symptoms in association with the QTc findings.

Analysis of ECG data for 36 healthy subjects who received a single IV temsirolimus dose ranging from 1 to 25 mg showed that no prolonged QTc interval. There were no AE reports of 'torsade de pointes' or other arrhythmia for any healthy subject.

Given the relatively modest number of patients and subjects tested, a potential effect of temsirolimus on the QT/QTc interval cannot be conclusively ruled out. A Phase I study in healthy subjects evaluating the effect of temsirolimus on the QT/QTc interval is ongoing.

- Safety in special populations

In the pivotal study, the temsirolimus 25 mg treated-group had 63 patients (30%) that were ≥ 65 years old. The incidence of TEAE in this population was generally similar for patients < 65 years old. Older patients treated with temsirolimus appear to have an increased incidence of face oedema, increased lactic dehydrogenase and pneumonia compared with younger patients.

In the temsirolimus 25 mg treated-group, 70 patients (34%) were female. The incidence of most TEAE was generally similar both for men and women. There was no consistent trend indicating increased risk for any event based on sex.

Preliminary results of Study 3066K1-152-US in patients with various degrees of hepatic impairment indicate that thrombocytopenia is a DLT for the mild dose cohorts (15-25 mg) leading to de-escalation and multiple dose delays.

- Safety related to drug-drug interactions and other interactions

Three Phase I drug-interaction studies were conducted with temsirolimus in healthy subjects. The results are described in the Pharmacokinetics section. As a general recommendation:

- Caution should be used when temsirolimus is administered with strong CYP3A4 inhibitors
- Caution should be used when administering strong CYP3A4 inducers with temsirolimus
- Co-administering temsirolimus with CYP2D6 substrates requires no dosage adjustment

- Discontinuation due to adverse events

In Study 3066K1-304, temsirolimus was associated with a lower overall incidence of TEAE leading to dose reduction or treatment discontinuation (20.2 and 18.3%, respectively) as compared with IFN-alpha (39.5 and 30.5%, respectively) or with the combination (47.6 and 36.5%, respectively). The overall incidence of TEAE leading to dose delay was similar for both temsirolimus 25 mg and IFN-alpha (51.4 vs. 47.0%, respectively) but higher in the combination arm (72.6%). Grade 3 or 4 asthenia and anaemia were the most common events leading to modification of temsirolimus treatment.

TEAE leading to treatment discontinuation reported for ≥ 1% of patients who received temsirolimus 25 mg were dyspnoea (2.4%), creatinine increased (1.9%), back pain (1.4%) and abdominal pain, anaemia, confusion and pleural effusion (1.0% each). TEAE leading to treatment discontinuation reported for ≥ 1% of patients who received IFN-alpha were mainly asthenia (10.5%) and TEAE from nervous system (7.5%).

- Post-marketing experience

None, although recently approved in the USA.

5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan.

Table Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Interstitial lung disease	Routine pharmacovigilance Development and implementation of an active query tool in Wyeth sponsored studies	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
QT interval prolongation	Routine pharmacovigilance Development and implementation of an active query tool and ECG monitoring in Wyeth sponsored studies	-
Dyspnoea	Routine pharmacovigilance Proactively collect cardiopulmonary function data at baseline in Wyeth sponsored studies	SPC section 4.8 Undesirable effects
Hyperglycaemia	Routine pharmacovigilance Proactively collect HbgA1c data at baseline and at specific time points in Wyeth sponsored studies	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Diabetes Mellitus	Routine pharmacovigilance	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Drug interactions	Routine pharmacovigilance	SPC section 4.2 Posology and method of administration SPC section 4.4 Special warnings and precautions SPC section 4.5 Interaction with other medicinal products and other forms of interaction
Off-label use	Routine pharmacovigilance Support sponsor and investigator studies Utilisation study post launch	-
Infections	Routine pharmacovigilance	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Use in hepatic impaired patients	Routine pharmacovigilance	SPC section 4.2 Posology and method of administration SPC section 4.4 Special warnings and precautions
Use in patients with significant CV disease	Routine pharmacovigilance	-
Renal impaired patients	Routine pharmacovigilance	SPC section 4.2 Posology and method of administration SPC section 4.4 Special warnings and precautions
Patients with active infection or recent surgery	Routine pharmacovigilance	SPC section 4.4 Special warnings and precautions
Intracerebral bleeding	Routine pharmacovigilance	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Abnormal wound healing	Routine pharmacovigilance	SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Hypersensitivity reaction	Routine pharmacovigilance	SPC section 4.3 Contraindications. SPC section 4.4 Special warnings and precautions SPC section 4.8 Undesirable effects
Lymphoproliferative disease/skin cancer	Routine pharmacovigilance	-

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

6 Overall conclusions, risk/benefit assessment and recommendation

Kidney cancer represents approximately 2% of all cancers worldwide and is responsible for 2% of all cancer-related deaths. Renal cell carcinoma (RCC) are tumours that are mainly of epithelial origin. Approximately 30% of RCC patients have metastases at the time of initial diagnosis. For the last 20 years, immunotherapy with cytokines has been the standard care of treatment for patients with advanced RCC. The two different cytokines used for the treatment of advanced RCC are interferon-alpha (IFN-alpha) and interleukin-2 (IL-2).

RCC, which originates in the renal cortex, accounts for up to 85% of malignant kidney tumours. Tumours in stages I-III require surgical procedures as part of standard care. It has been shown that in patients diagnosed with Stage I, 50% can be cured by nephrectomy, whereas almost 30% of patients presenting with Stage IV are incurable. Without intervention, patients with advanced RCC have a median overall survival (OS) of approximately one year and a 5-year survival rate of ≤ 10%. Surgical resection may be appropriate for selected patients, including those with isolated metastases. However, the disease often recurs, even when the primary and metastatic sites are aggressively resected. Radiation therapy can provide significant palliation of painful metastases. The use of conventional chemotherapy has generally been very limited because of low rate of responders to therapy, leading to a poor rate survival benefit.

When temsirolimus development started, the only available active comparators were IFN-alpha and IL-2. There is no clinical data comparing temsirolimus with recently approved drugs (sorafenib and sunitinib) for an indication in RCC. Moreover, comparisons of study results would be hazardous since the main criteria defining the clinical trial population are different.

Quality

Quality aspects are satisfactory. There are no unresolved quality issues which could have a negative impact on the risk/benefit balance of the product.

Non-clinical pharmacology and toxicology

Adverse reactions not observed in clinical studies, but seen in animals at exposure levels similar to or even lower than clinical exposure levels, with possible relevance to clinical use, were as follows: pancreatic islet cell vacuolation, testicular tubular degeneration, lymphoid atrophy, mixed cell inflammation of the colon/cecum and pulmonary phospholipidosis.

Diarrhoea, with mixed cell inflammation of the cecum or colon, was observed in monkeys and was associated with an inflammatory response that may have been due to a disruption of the normal intestinal flora.

General inflammatory responses, as indicated by increased fibrinogen and neutrophils and/or changes in serum protein, were observed in mice, rats and monkeys. Although in some cases these clinical pathologic changes were attributed to skin or intestinal inflammation as noted above, for some animal species?, there were no specific clinical observations or histological changes that suggested inflammation.

Temsirolimus was not genotoxic in a battery of *in vitro* (bacterial reverse mutation in *Salmonella typhimurium* and *Escherichia coli*, forward mutation in mouse lymphoma cells and chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse micronucleus) assays.

Carcinogenicity studies have not been conducted with temsirolimus, however, sirolimus, the major metabolite of temsirolimus in humans, was carcinogenic in mice and rats. The following effects were reported in mice and/or rats in the carcinogenicity studies conducted: granulocytic leukaemia, lymphoma, hepatocellular adenoma and carcinoma and testicular adenoma.

In reproduction studies in animals, temsirolimus caused embryo/foetotoxicity that manifested itself as foetal mortality and reduced foetal weights (with associated delays in skeletal ossification) in rats and rabbits. Teratogenic effects (omphalocele) were seen in rabbits. In reproduction toxicity studies in animals, decreased fertility and partly reversible reductions in sperm counts were reported in male rats. Reductions in testicular weights and/or histological lesions (e.g. tubular atrophy and tubular giant cells) were observed in mice, rats and monkeys. In rats, a decrease in weight of accessory sex organs (epididymides, prostate, seminal vesicles) was observed.

Efficacy

The clinical dossier includes a pivotal multicentre multinational randomised Phase III study involving more than 626 patients and a supportive randomised dose-comparing Phase II study involving 111 patients. The study design of the pivotal study comparing temsirolimus with IFN-alpha and the combination of both is endorsed. IFN-alpha-2a is a well known reference therapy and was considered an appropriate comparator since, at the time the study was planned, there was no other therapy leading to a survival benefit in second line RCC.

Patients with histologically or cytologically confirmed, advanced (Stage IV or recurrent disease) RCC who had not received prior systemic therapy for their disease and who presented with 3 of 6 protocol-specified prognostic factors (< 1 year from time of initial RCC diagnosis to randomisation, Karnofsky performance status of 60 or 70, haemoglobin less than the lower limit of normal (ULN), hypocalcaemia, LDH > 1.5 times the upper limit of normal, > 1 metastatic site of disease) were eligible for the pivotal study. Stratification by nephrectomy status (a well known prognostic factor) and geographical region (to cover possible imbalances by regional differences) was justified. In addition, the choice of OS for the primary endpoint was accepted.

The final results of the pivotal Phase III study showed that the median OS for temsirolimus was statistically significant and clinically meaningful longer, demonstrating an OS of 10.9 months compared to 7.3 months for the IFN-alpha alone, (HR: 0.78, 95% CI: 0.63-0.97). For the comparison between the combination of temsirolimus and IFN-alpha with IFN-alpha alone, the difference in OS was not statistically significant. At the time of the final analysis more than 80% of patients had died. The favourable treatment effect on OS of temsirolimus in comparison to IFN-alpha alone is observed across all randomisation strata (3 geographical regions and for patients with or without nephrectomy). The robustness of the results has also been shown with several sensitivity analyses (including analyses per number of poor risk factors).

The results for the secondary endpoints of temsirolimus monotherapy are consistent with the results for the primary endpoint. Median PFS from investigator assessments was 1.9 months (95% CI: 1.9-2.2) in the IFN-alpha arm, 3.8 (95% CI: 3.6-5.2) in the temsirolimus arm and 3.7 (95% CI: 3.1-5.2) in the combination arm. The 2-fold difference between temsirolimus and IFN-alpha was statistically significant. Based on the independent assessment, the objective response rate was 5.3% in the IFN-alpha arm, 9.1% in the temsirolimus arm and 9.5% in the combination arm. SD for at least 6 weeks was observed more often in the temsirolimus arm (133 patients, 63.6%) than in the IFN-alpha arm (80 patients, 38.6%) or in the combination arm (109 patients, 51.9%). Most of the remaining patients had PD or no post-baseline evaluation It is thought that the difference in PFS results obtained between investigator and independent assessment (1.9 and 3.8 months, respectively) may be because of the high number of missing post-baseline tumour assessments, the different methods used for classification of progression based on clinical deterioration and the systematic problems occurring with the independent review.

Safety

Adverse events of all grades, with a significantly higher incidence with temsirolimus 25 mg versus IFN-alpha were rash, maculopapular rash, pruritic rash, skin disorder, pruritus, exfoliative dermatitis, nail disorder, acne, hyperlipaemia, hyperglycaemia, hypercholesteremia, hypophosphataemia, hypokalaemia, oedema including peripheral oedema and face oedema, increased cough, rhinitis, upper

respiratory infection, epistaxis, pharyngitis, chest pain, gastrointestinal stomatitis, mucositis, aphthous stomatitis, pain, infection, diabetes mellitus, conjunctivitis, taste perversion, and allergic reactions.

The global safety profile of temsirolimus 25 mg is favourable compared to IFN-alpha. For temsirolimus, lower incidences of Grade 3 and 4 toxicities and serious adverse events, longer time on treatment, less treatment discontinuation due to adverse events and a lower rate of death within the first 30 days of dose were observed. In addition, an explorative analysis demonstrated that the treatment outcome for patients showing rash as an AE while treated with temsirolimus appears to be numerically better than for patients without rash.

Investigations of pharmacokinetics in patients with hepatic impairment and in paediatric population are currently ongoing and results are pending. These will be part of the requested FUM. Finally, as for any immunosuppressive agents, there is a potential risk of secondary malignancies.

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

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

- User consultation

The Patient Information Leaflet (PIL) for TORISEL 25 mg/ml Concentrate and diluent for solution for infusion (temsirolimus) has been tested in English in accordance with Articles 59(3) and 61(1) of Directive 2001/83/EC, as amended by Directive 2004/27/EC. The PIL for TORISEL 25 mg/ml Concentrate and diluent for solution for infusion (temsirolimus) was found to contain all the necessary information in a way that is accessible and understandable to those who participated in this test.

It is considered that the tested PIL meets the requirements set for User Testing.

Risk-benefit assessment

In the treatment of patients with advanced RCC who have not previously received systemic therapy and who have at least 3 of 6 prognostic risk factors, the benefit of a favourable overall survival of temsirolimus compared to IFN-alpha overweighs the risk of the treatment.

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

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

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that TORISEL is not similar to Sutent or Nexavar within the meaning of Article 3 of Commission Regulation (EC) No. 847/200 (see appendix 1) on the basis that TORISEL has a different molecular structure and mechanism of action then the other 2 medicinal products.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus/majority decision that the risk-benefit balance of TORISEL for first line treatment of patients with advanced renal cell carcinoma who have at least 3 of 6 prognostic risk factors was favourable and therefore recommended the granting of the marketing authorisation..

In addition, the CHMP, with reference to Article 8 of Regulation EC No 141/2000, considers TORISEL not to be similar (as defined in Article 3 of Commission Regulation EC No. 847/2000) to authorised orphan medicinal products for the same therapeutic indication.