SCIENTIFIC DISCUSSION

Name of the medicinal product:	Beromun
Marketing Authorisation Holder:	Boehringer Ingelheim International GmbH Binger Strasse 173 D-55216 Ingelheim am Rhein Germany
Active substance:	Tumor necrosis factor alfa-1a
International Nonproprietary Name:	Tasonermin
Pharmaco-therapeutic group (ATC Code):	LO3AA
Therapeutic indication:	As an adjunct to surgery for subsequent removal of the tumour so as to prevent or delay amputation, or in the palliative situation, for irresectable soft tissue sarcoma of the limbs, used in combination with melphalan via mild hyperthermic isolated limb perfusion (ILP).

1. Introduction

Beromun contains the active ingredient tasonermin also known as tumour necrosis factor alfa-1a or TNFα-1a. Tasonermin is a non-glycosylated cytokine produced from *E.coli* using rDNA technology. The protein consists of three identical polypeptide chains of 157 amino acids combined to form a compact, bell-shaped homotrimer. The individual subunits have a relative molecular mass each of 17,350 daltons. Beromun is provided as a sterile, lyophilised powder in single-use vials containing 1 mg of TNFα-1a. The product is intended for reconstitution with sterile physiological saline to produce a final concentration of 0.2 mg/ml TNFα-1a.

The sequence of the 157 amino acid protein is as follows:

1	Val-Arg-Ser-Ser-Ser-Arg-Thr-Pro-Ser-Asp-
11	Lys-Pro-Val-Ala-His-Val-Val-Ala-Asn-Pro-
21	Gln-Ala-Glu-Gly-Gln-Leu-Gln-Trp-Leu-Asn-
31	Arg-Arg-Ala-Asn-Ala-Leu-Leu-Ala-Asn-Gly-
41	Val-Glu-Leu-Arg-Asp-Asn-Gln-Leu-Val-Val-
51	Pro-Ser-Glu-Gly-Leu-Tyr-Leu-Ile-Tyr-Ser-
61	Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-
71	Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile-
81	Ser-Arg-Ile-Ala-Val-Ser-Tyr-Gln-Thr-Lys-
91	Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-
101	Cys-Gln-Arg-Glu-Thr-Pro-Glu-Gly-Ala-Glu-
111	Ala-Lys-Pro-Trp-Tyr-Glu-Pro-Ile-Tyr-Leu-
121	Gly-Gly-Val-Phe-Gln-Leu-Glu-Lys-Gly-Asp-
131	Arg-Leu-Ser-Ala-Glu-Ile-Asn-Arg-Pro-Asp-
141	Tyr-Leu-Asp-Phe-Ala-Glu-Ser-Gly-Gln-Val-
151	Tyr-Phe-Gly-Ile-Ile-Ala-Leu

Tasonermin = homotrimer

The clinical indication for Beromun is - as an adjunct to surgery for subsequent removal of the tumour so as to prevent or delay amputation, or in the palliative situation, for irresectable soft tissue sarcoma of the limbs, used in combination with melphalan via mild hyperthermic isolated limb perfusion (ILP).

The posology and method of administration for Beromun in conjunction with melphalan is:

Beromun:

Upper limb: 3 mg total dose by isolated limb perfusion

Lower limb: 4 mg total dose by isolated limb perfusion

Melphalan:

Melphalan dosage should be calculated according to the litre-volume method of Wieberdink (*Dosimetry in isolation perfusion of the limbs by assessments of perfused tissue volume and grading of toxic tissue reactions. Eur J Cancer Clin Oncol 1982; 18: 905-910)* to a maximum dose of 150 mg.

13 mg/litre perfused upper limb volume

10 mg/litre perfused lower limb volume

This treatment should be undertaken in specialised centres by surgical teams experienced in the management of limb sarcomas and ILP procedure, with an intensive care unit readily available and with the facilities for continuous monitoring for drug leakage into the systemic circulation.

Beromun should be administered by mild hyperthermic isolated limb perfusion. The perfusion circuit (roller pump, oxygenator with integrated reservoir, heat exchanger, connecting tubing) should be prepared prior to surgery and primed with 700 to 800 ml of perfusate, with haematocrit of 0.25 to 0.30.

Perfusion level should be chosen to adequately encompass affected tissue (external iliac, common femoral, femoro-popliteal, popliteal, axillary and brachial being accepted routes) and catheters introduced. External heat loss from the limb should be prevented by application of thermal blankets and limb temperature continuously monitored by thermistor probes inserted into subcutaneous tissue

and muscle. Hand and foot, if not affected, should be protected by Esmarch bandages. A tourniquet should be applied to the proximal limb.

After connection of the limb to the isolated circuit, flow rate should be adjusted to 35 to 40 ml/litre limb volume/minute and leakage from limb to systemic circulation checked using a radioactive tracer technique (see section 4.4). Adjustment of flow rate and tourniquet may be required to ensure leakage from perfusion circuit to systemic circulation is stable (systemic level of radioactivity has reached a plateau) and does not exceed 10 %. Beromun should only be administered if leakage is less than 10%.

Once the temperature in the distal subcutaneous tissue of the limb has reached >38°C (but not exceeding 39°C), and pH of the perfusate is between 7.2 and 7.35, Beromun should be injected as a bolus into the arterial line of the circuit. After 30 minutes perfusion of Beromun alone, melphalan should be added as a bolus into the reservoir of the circuit, or slowly into the arterial line of the circuit. The temperature should then be increased to >39°C (but not exceeding 40°C) in two different sites of measurement in the tumour area. The duration of the perfusion including melphalan should be 60 minutes. Thus, the duration of the total perfusion should be 90 minutes.

At the end of the perfusion, the perfusate should be collected into the reservoir while washout fluid is added simultaneously to the circuit and circulated at the same flow rate of 35 to 40 ml/litre limb volume/minute. Washout should be continued until the colour of the perfusate is clear (pink, transparent).

Safety and efficacy in children under the age of 16 has not been established.

Surgical resection of the tumour remnant should be undertaken whenever possible. When necessary a second ILP can be considered 6-8 weeks after the first ILP.

The use of melphalan in combination with Beromun

The clinical indication for Beromun foresees its use in combination with melphalan. The scientific basis for the combination has been justified (see overview of clinical aspects, below). However, a parental formulation of melphalan is authorised in most but not all EU member states. In these member states a parental formulation of melphalan may be available through named patient/compassionate use schemes. The CPMP did not raise objections to the authorisation of Beromun. Isolated limb perfusion under hyperthermic conditions with melphalan plus Beromun is a treatment for specialists, and treatment units will be located in a few centres.

2. Part II: Chemical and pharmaceutical aspects

Composition, Product Development and Product Manufacture.

Beromun is provided as a sterile, lyophilised powder in single-use vials containing 1 mg of TNFα-1a. The vials contain tasonermin (1 mg), sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, human albumin, and sodium chloride (arising from the buffer used in the preparation of the active ingredient, see below). The product is intended for reconstitution with sterile physiological saline to produce a final concentration of 0.2 mg/ml TNFα-1a.

The dossier also included information on a 0.2 mg vial preparation, which was used for clinical trials but is not intended for marketing. The product is identical to the 1 mg market product in every respect apart from volume fill. Information is provided on this product strength in support of the 1 mg product. In response to a question posed by the CPMP, the applicant provided further information on the formulation used in preclinical studies. Most of the batches used in preclinical studies did not contain albumin. The analytical data provided on these batches (including on storage) was reassuring.

The above composition describes the nominal product content. In practice, the vials contain a 7% overfill which, when dissolved in 5.3 ml of saline, results in a final solution strength of 0.2 mg/ml and permits withdrawal and administration of 5 ml of product.

In response to a question posed by the CPMP, the applicant clarified the amount of sodium chloride present in the formulation, and also justified the slight hypertonicity after reconstitution.

Beromun is labelled in mg units rather than biological activity units. The Expert argues that protein determination can be carried out more precisely than can biological activity determination by

cytotoxicity assay or strength by ELISA. As the substance is well characterised by a range of physico-chemical methods, it is felt that filling of product into final containers on a mass unit basis is justified.

Active ingredient TNFα-1a is produced as a solution in phosphate buffer with sodium chloride. The choice of a lyophilised product is justified on the basis that solutions are unstable unless stored frozen. From the discussion provided, it would appear that development of a suitable lyophilised product has proved to be a formidable challenge. A stabilising agent is necessary and of the variety of agents tested, human albumin found to be best in combining acceptable stabilisation, product stability and pharmaceutical characteristics pre- and post reconstitution. Stability studies using ion exchange chromatography (HPIEC) show that higher concentrations of human albumin are necessary for acceptable stability profiles and an optimal concentration of has been chosen on the basis of superior lyophilisation cakes.

In response to a questions posed by the CPMP, the applicant provided additional data to support the pH range of the phosphate buffer, and to confirm that the level of albumin chosen as stabiliser is adequate.

Preservatives are unnecessary as the product is for single use only.

All manufacturing operations for Beromun take place at Bender and Co. GesmbH, Austria. Solvent ampoules are prepared, filled and sterilised by Nycomed Austria GmbH but labelled by Bender and Co GesmbH. Details of the manufacture of the 0.9% saline solvent ampoules are provided.

TNFα-1a bulk solution is diluted to the required concentration with a dilution buffer consisting of phosphate buffer, human albumin and WFI. A placebo solution is prepared and passed through the 0.22 µm sterilising filter. The formulated bulk solution is then filtered through this same 0.22 µm membrane filter and filled immediately into sterile vials. The vials are lyophilised, sealed and stored at 2° to 8°C.

In response to questions posed by the CPMP, the applicant provided a more detailed description of the manufacturing method particularly in relation to the in-process controls, equipment used, conditions for thawing of the unformulated bulk, and the method of calculation of the amount of buffer salts. The applicant also tightened the bioburden limit for the pre-filtration solution.

Validation data was provided on filling accuracy and precision, aseptic media fills, validation of cleaning of filtration equipment, container-closure integrity and validation of bacterial-retention capabilities of the sterile filters. In response to questions posed by the CPMP, the applicant provided further information on the lyophilisation process and the sterilisation by filtration step.

Control of Starting Material

The active ingredient is presented as a filtered phosphate buffered solution. Several analytical techniques are used to test the active ingredient. The techniques employed include chromatographic methods and electrophoretic methods. A bioassay is employed.

In response to questions posed by the CPMP, the applicant improved the criteria for acceptance in the SDS-PAGE test. The limits of the specifications were reviewed and tightened.

Development Genetics

The assembly of the plasmid expression construct and the *E.coli* production strain has been described in sufficient detail.

MCB and WCB have been tested for genetic stability after storage at -60°C or below. Results show that MCB and WCB meet specifications after 5 and 3 years storage respectively. Due to the very limited supply, no more direct testing will be performed on MCB. It is intended that expiration dating of the MCB will be extended whenever the WCB is analysed and released again. Given the identical specifications for both cell banks, the proposal can be accepted.

Genetic stability was tested during production. Samples from MCB, WCB, from the end of a standard fermentation and from the end of an extended fermentation have been investigated. Genetic stability of the production strain and expression construct have been investigated by DNA agarose gel analysis, restriction mapping, DNA sequence analysis and determination of plasmid copy number. Plasmid alterations or losses were not observed at any stage. In addition, average growth rates and

fermentation yields for the extended fermentation run gave comparable results to the standard production run.

Details of the preparation of the MCB have been provided. Details of the preparation of WCB from MCB have been performed at Bender & Co. The anticipated rate of usage of the WCB is at least 20 years. In response to a question form the CPMP, additional information was provided on the acceptance criteria for cell banks.

Fermentation

One fermentation batch = harvest from one fermentation, which yields the required quantity of cell paste to be used for purification. No blending occurs at any stage of the process.

In-process controls are described for the various fermentation steps and both upper and lower limits given. Growth profiles for typical fermentations together with extended fermentation run are provided showing consistency of the process. A description of major processing and associated equipment is provided and cleaning procedures are briefly described.

A comprehensive list of all raw materials used in fermentation operations together with specifications is provided. The materials are compendial grade where appropriate and sample certificates of analysis are provided for non-compendial substances. In the case of animal-derived materials, it is considered that the Commission Decision of 30.7.97 on the prohibition of the use of material presenting risks as regards transmissible spongiform encephalopathies as well as the requirements of the Note for Guidance on Minimising the risk of transmitting animal spongiform encephalopathy via medicinal products CPMP/BWP/877/96 are met.

In response to questions posed by the CPMP, the applicant provided further information on the rationale, performance and control of the fermentation process. The equipment used was described in more detail.

Down-Stream Processing

The purification process is multistep. Pooled samples obtained after the final gel permeation column step are diluted with deionised water, filtered and stored. It is stated that after lysis and homogenisation of the cell paste, the majority of the DNA and unwanted proteins are precipitated by the addition of polyethyleneimine. Thereafter, the five different chromatographic steps remove different impurities based on the properties of hydrophobicity and/or charge and/or size. In the original dossier submitted an in-depth discussion for the rationale for inclusion of each of the various steps is not provided (Expert justifies this on the basis that standard purification techniques are being used). In response to questions posed by the CPMP the applicant provided further information on the rationale for the purification strategy with reference to the protein structure and properties of tasonermin. In the initial dossier submitted in-process controls for the purification process were not clearly laid out. In response to questions posed by the CPMP, the applicant provided further information on in-process controls. Limits for microbial bioburden were clarified. The conditions for the reuse of some chromatography columns have been given. In addition, the applicant provided further details of the equipment used during down-stream processing.

Reprocessing does not occur at any stage of the purification process. This is considered acceptable.

Characterisation and Analytical Development

Natural TNFα consists of a primary structure of 157 amino acids with a disulphide bridge occurring between cysteine at positions 69 and 101. The secondary structure reflects the all-β protein class. The protein exhibits high thermodynamic stability and unfolded subunits can fold and associate to form native, biologically active TNFα. The 3D structure of the molecule is a bell-shaped trimer with each subunit in the trimer forming a "sandwich" structure of the two β-pleated sheets each composed of antiparallel β-strands. TNFα is not glycosylated.

The recombinant TNFα-1a has undergone an extensive battery of characterisation tests. The results presented support the conclusion that the recombinant variant is identical to TNFα obtained from HL-60 cells and as described in the literature. In response to a question from the CPMP the applicant provided further information on the pI of tasonermin. As TNFα-1a contains no methionine amino acid residues and is not glycosylated, the possible post-translational modifications are related to the

single disulphide bond, deamidation of asparagine and glutamine, proteolytic degradation, N-terminal heterogeneity and TNFα-1a forms with uncleaved leader sequences. To date no peptide with the cyclic imide isoform or TNFα-1a forms with uncleaved leader sequences have been found.

Biological and immunological characterisation of TNFα is quite widely quoted in the literature and references given in the dossier. The major biological activity is regression and necrosis of transplanted tumours in mice as well as cytotoxicity for several in vitro cell lines e.g. rat embryo fibroblasts, murine L929, L-M-, WEHI/3, EMT-6 and FELC cells. The company has chosen a bioassay using murine connective tissue L-M cells in serum-free medium based on the ability to increase sensitivity to TNFα-1a through use of actinomycin D. The ELISA method for immunogenicity testing employs highly specific antibodies, which are capable of discriminating between TNFα and TNFβ and show minimal cross-reactivity.

Justification has been provided for the choice of analytical methodologies and the validation documentation has addressed suitability of the various methods for use for both unformulated bulk drug substance as well as formulated finished product. The finished product is formulated with HSA, which, by masking peaks in LC techniques and bands in IEF and PAGE testing, can severely hamper the testing of molecular integrity and stability. The results of the validation testing show indeed that HSA interferes in HPSEC and SDS-PAGE determinations and these methods are only suitable for testing of the bulk drug substance. The methods are retained however in the finished product specification for identification testing. HPIEC on the other hand does not show interference from HSA and is shown suitable for finished product release and stability testing.

In response to a question posed by the CPMP, the applicant provided further information on the analytical methodologies employed to characterised the active ingredient used in preclinical and clinical trials. The applicant has agreed to review the specifications, as further experience is gained, as a follow-up measure.

In 1994, the international standard Tumour Necrosis Factor alpha, human IS 87/650 became available against which the previous internal company standard was calibrated in the bioassay method. This resulted in the following relation 4.9×10^7 U/mg = 4.2×10^7 IU/mg. Present and future internal company standards will be recalibrated at least every two years against the international standard.

Process Validation and Impurities

Validation of the fermentation process is demonstrated through the provision of data for several production runs together with 1 extended production run, showing that yields at harvest, yields of cell paste, cell density at harvest and TNFα-1a yields at harvest are consistent.

In response to questions from CPMP the applicant provided additional data on the consistency of the yields during down-stream processing.

Concerning host cell DNA removal, the applicant provided supplementary information to show the consistency of the level of removal of DNA, and clarified the methodology used to detect DNA

In response to a question posed by the CPMP, the applicant provided supplementary information to demonstrate the clearance of endotoxinand host cell protein.

In response to questions posed by the CPMP, the applicant provided further batch analysis data and provided additional information on batches of active substance produced at Bender and Genentech.

Excipients

All excipients are of Ph.Eur. grade and are stated to be additionally tested for endotoxin content.

The information provided on human albumin was considered satisfactory.

Packaging Material

Vials and stoppers comply with Ph.Eur specifications. Specifications for vials, stoppers and overseals are presented. The information is acceptable.

Control Tests on the Finished Product

The applicant has provided specifications for the finished product. In response to questions posed by the CPMP, the applicant clarified the acceptance limits and reviewed the limits in the light of batch analyses.

Stability of the Active Substance

Stability data have been provided for six batches of unformulated bulk TNFα-1a.

Based on these data, the proposed storage periods of 3 months at 2-8°C or 24 months at -70°C are acceptable.

Stability of the Finished Product

The proposed shelf-life is 36 months at 2-8°C.

Data for 6 batches of 0.2 mg/container product and 6 batches of 1 mg/container product manufactured by Bender have been presented.

In response to questions from the CPMP, the applicant provided further information to their investigations to remove HSA prior to performing analytical tests. However, this was not feasible. Further information the stability after reconstitution was presented. Information was presented to explain some anomalous results in the bioassay and the levels of a deamidated species in some batches.

Freeze-thaw and 4°C to 20°C cycling studies indicate no adverse effects under the storage conditions tested although very small changes are noted in the HPIEC profiles. The studies will continue until 3 years are collected.

The results obtained for the finished product reflect those seen with bulk active substance TNFα-1a. Overall the results are supportive of the shelf-life proposal. The applicant has agreed to provide further stability data as a follow-up measure to confirm and support the shelf life.

Stability of the reconstituted solution has been examined by HPIEC. Samples examined after 1, 6, 21 or 48 hours showed no deterioration, but were degraded after 1 week.

Solvent Ampoules

The solvent provided for reconstitution of BEROMUN is 0.9% saline presented in 5 ml glass ampoules and manufactured by Hafslund Nycomed Pharma AG, Austria. In response to a question posed by CPMP a shelf life for the solvent ampoules was set. The shelf-life for the saline ampoules is set at a maximum of 5 years. For the purposes of combining saline ampoules with Beromun, which has a shelf-life of 3 years, only those saline ampoules, which are less than 2 years old, will be used in the combined packs.

Conclusion

The applicant has agreed to some follow-up measures (letter dated 17 November1998). The quality of Beromun is considered to be acceptable.

3. Part III: Toxico-pharmacological aspects

The non-clinical documentation is primarily based on studies originally undertaken to support systemic multi-dose therapy of malignant diseases. Only few studies are carried out to investigate the intended combination treatment.

Pharmacodynamic effects related to the proposed indication are documented in a vast number of publications. TNFα-1a has a direct cytotoxic action, it is toxic to the tumour vasculature and activate unspecific and specific immune responses. Combination of TNFα with cytotoxic drugs or hyperthermia improves the efficacy. The primary pharmacodynamic effect, i.e. tumour necrosis, is shown in a clinically relevant model, i.e. ILP. In respect to the clinical documentation, it may be considered that no studies are presented to examine the expected optimum ratio and dose of the combination.

TNFα-1a induces a number of secondary pharmacological effects in animals, of which the changes in the cardiovascular systems are considered to be the most important. Qualitatively, the safety

pharmacological effects observed were in line with those observed after systemically administration of TNFα to man. No secondary pharmacology studies are carried out with the combination. Hypotension is regarded as the main cardiovascular side effect. Dopamine is proposed as a vasopressor agent; no studies are carried out on the effects of dopamine or other vasopressor agents on TNFα-1a induced hypotension.

Single iv dose toxicity of TNFα-1a was examined in mice, rats, dogs and Rhesus monkeys. Hypotension was the main effect in Rhesus monkeys. Repeated iv dose toxicity of TNFα-1a up to 13 weeks has been evaluated in rats and Rhesus monkeys. Haematological changes as well as alterations of liver and kidney functions were the main adverse effects observed following repeated administration of TNFα. Neither single dose nor repeated dose toxicity studies are presented to show the effects of the combination.

No reproduction toxicity studies were carried out. TNFα-1a has been assessed in the Ames test, micronucleus test in mice, and in UDS test in rat hepatocytes *in vitro*. No evidence of mutagenic potential was observed in these tests. TNFα-1a is given in combination with melphalan, which is a mutagenic and teratogenic alkylating agent.

In response to questions posed by the CPMP the applicant provided further explanations for the relevance of the preclinical studies already performed to support the optimum ratio and dosages of tasonermin and melphalan in the clinic. The applicant also provided further information on the significance of the different biological activities of murine and human TNF alfas in respect to general pharmacodynamics and toxicology findings. In addition, the applicant provided further information to clarify the pharmacodynamic interaction between TNF alfa and drugs (such as NSAIDs) affecting prostaglandin synthesis.

The inherent problems in extrapolation of animal model data of human TNFα to the human situation should be taken into account, and it is considered that the safety documentation of the combination therapy relies on the clinical documentation.

In terms of the ecotoxicity and the environmental risk, tasonermin (a protein) is not considered to generate any concerns.

4. Part IV: Clinical aspects

Epidemiology and treatment of Soft tissue sarcoma (STS) of the limb

STS is an uncommon malignancy, comprising about 0.6% of newly diagnosed cancers and the estimated, age adjusted incidence is approximately 1-2/100 000. In about 60% of the patients, the tumour occurs in the extremities. At the time of diagnosis, about 90% of the patients are free from metastatic spread. About 10% of patients with STS of the limb are destined for amputation or severely debilitating surgery. Based on these figures (2/100 000 x 60% x 10%), the estimated amputation rate would be about 0.1/100 000. It is therefore considered reasonable to conclude that the proposed indication for use, ”limb salvage in patients with STS”, is rarely encountered. Besides being uncommon, STS has a heterogeneous histology with 15 main entities, and prognosis is supposed to depend on several factors, e.g. tumour size, histologic grade, site (proximal vs. distal), depth of penetration, presence of necrosis. The rarity of the indication put certain restraints on possible clinical developmental programs. To document convincingly in clinical trials the ”added value” of all three components (hyperthermia, TNF alpha and melphalan), and more than the feasibility of the chosen schedules would simply not be possible.

Surgery (with or without (neo)adjuvant radiation) is the principle mode of treatment. Wide local excision in conjunction with adjuvant radiation therapy results in local control results in the range of 80% or greater. Radiation alone is an alternative in medically inoperable patients or in-patients refusing recommended surgery. In-patients with large tumours, doses of more than 70 Gy are needed. STS is moderately sensitive to conventionally administered (combination) chemotherapy and in advanced disease the overall response rate is about 20-50%.

CLINICAL PHARMACOLOGY

Pharmacodynamics

It is well recognised that TNFα might induce haemorrhagic necrosis in tumours but the exact mechanism of action is not known. Endothelial damage, cytokine release, effects on adhesion molecules, and immunological effects as well as a direct cytotoxic effect to tumour cells are all mechanisms that may contribute to the overall effect on some tumours. Some data suggest that the microvasculature of tumours is the main target of TNF and is the first event to take place.

The applicant has included a selection of unpublished reports and bibliographic publications to describe the pharmacodynamics of TNFα relevant for the sought indication, STS. Also included are a number of dose-finding studies with IV administration, which could be relevant as systemic leakage during ILP might be responsible for serious adverse events.

Phase I/II trials with TNFα used systemically in-patients with cancer (IV, SC, IM routes)

Seven trials have been submitted (6 with TNF alone and one combining TNF with IFNγ). Antitumour activity was minimal with no response or progressive disease as the most common outcome in-patients with different advanced solid tumours. Some minimal responses were observed in single trials. None of these trials included patients with STS.

In all studies TNFα had a transient dose-dependent effects on WBC counts which dropped significantly 30 minutes to 2 hours after administration and returned to normal within the first 24-48 hours. This effect was suggested to be caused by release of leukocyte adhesion molecules causing adhesion to endothelial walls. Immunological effects included increase in β2-microglobulin, neopterin, IFNγ levels and some acute phase reactants. Mild anaemia was seen after repeated dosing.

The highest IV dose used was 400 μg/m^2 as a 30 min infusion twice a day for 5 days every second week for a total of 8 weeks (Eur J Cancer 1991; 27:856-63, Volume5, IV.A). With this regimen 150 μg/m^2 was well tolerated. Chills and fever were seen in almost all patients at all dose levels (10-400 μg/m^2). Other symptoms were headache, myalgia, fatigue, local skin reactions and anorexia. Hypotension was dose-dependent in most studies and this symptom is dose limiting. At the extreme the hypotensive effect might mimic the septic shock syndrome.

With a daily SC regimen the maximum tolerated dose is 50-75 μg/m^2. Asthenia was dose limiting. The MTD for IV or IM administration is 150-200 μg/m^2.

The combination of TNFα (50 μg/m^2 IV) and IFNγ (100 μg SC) is more toxic. Two patients out of 16 with advanced colorectal cancer had serious AE, one had acute renal failure, and one had severe thrombocytopenia. Leukopenia, anaemia, and thrombocytopenia were common laboratory findings.

It was considered that these findings are well known characteristics of the toxicity profile for TNFα. The data may be of limited informative value for the posology and safety sections of the SPC. Leakage from the ILP system might produce some of these above-mentioned symptoms. For efficacy the value of these data is doubtful. From the information provided it appears that TNFα as single agent had no antitumour effect that could be exploited clinically. This is in keeping with textbooks on medical oncology.

Pharmacokinetics

From older studies investigating the pharmacokinetics of TNF after systemic administration (SC, IM and IV) there are indications of non-linear kinetics with increasing AUC and decreasing clearance with increasing doses.

In the pivotal study (U97-2049), TNF alpha concentrations in human serum were measured by a two-step sandwich solid phase enzyme linked immunosorbent assay (ELISA). This method uses affinity-purified polyclonal rabbit anti-TNF-α antibodies as solid phase capture antibodies and peroxidase labelled monoclonal murine anti-TNF-α antibodies as detector antibody. The precision (Between-assay: 5.3% at 1000 pg/ml and 13.5% at 50 pg/ml) and accuracy (-2% to 2%) of the method were within acceptable limits. The limit of quantitation, 31.6 pg/ml, has been established based on precision and accuracy. Three freeze-thaw cycles did not effect the stability of TNF-α. Samples diluted in foetal bovine serum (FBS) to assay range (LOQ up to 2000 pg/ml) can be stored at -20°C up a year. TNF-α

is not stable at 4, 20 or 37°C. Cross-reactivity was not observed with 74 µg/ml TNF-β, 1 mg/ml hGH, 100 µg/ml interferon-γ and 4.5 mg/ml *E. coli* lysate. The method has been acceptably described and validated.

The estimate of the volume of distribution varies with dose. Following an intravenous bolus dose of 35 and 150 µg/m^2 the mean estimates were 55 l and 17 l, respectively. There is no information on the elimination pathways of TNF alpha. Following intravenous administration of 35 and 150 µg/m^2 clearance values were estimated to approximately 2 and 0.5 l/min. The terminal half-life is fairly short, being around 20-30 minutes at 150 µg/m^2 and shorter at lower doses. The data indicate that there is a non-linear component in the pharmacokinetic characteristics, resulting in a systemic exposure that increases more than in proportion to dose.

For assessment of systemic exposure, 51 patients were analysed. Blood samples were collected from the systemic circulation both during ILP and after restoration of circulation to the isolated limb and from the ILP circulation circuit. Detection of TNF during ILP in the systemic circulation indicated leakage. Two methods were used to assess leakage, radiolabelled albumin added to the ILP circuit and the ratio of AUC in the systemic circulation/AUC in the circuit at the end of ILP for TNF. The relation between leakage and AUC (ng.h/ml) is summarised in table below:

		In perfusion circuit	**In plasma during ILP**	**In plasma after ILP**
	N	Mean (SE)	Mean (SE)	Mean (SE)
Leak < 2%*	31	5133 (332)	8.2 (5.1)	19.3 (9.1)
Leak ≥ 2%	20	3883 (412)	172 (109)	104 (32.3)
Leak ≥ 10%	4	2916 (439)	631(531)	252 (119)
All patients	51	4643 (270)	72 (43.7)	52 (15)

* radiolabelled albumin method

These AUC values could be compared with an old study investigating the pharmacokinetics after SC IM or IV systemic administration of TNF. The study also used an ELISA technique but it can be not be evaluated whether the assays are identical. Therefore, the results may not be directly comparable.

It was concluded that although pharmacokinetics are limited but most importantly the data show that all patients will be exposed to systemic effects of TNF, and that AE will be more frequent and severe with increasing level of leakage during ILP. The albumin method might underestimate the level of leakage but the clinical data indicate that the ILP procedure is fairly well tolerated even if the dose of TNF is high. The derivation of the leakage values based on the albumin method was not completely clear. A time component together with the actual raw data (radioactivity measurements) was therefore requested to explain and justify the method.

CLINICAL EXPERIENCE

EFFICACY

One important characteristic for all 4 clinical trials, submitted in the original dossier, is highlighted by the Independent Review Committee: *Approximately 20% of the patients included in the ILP programme were not considered as candidates for imminent amputation or other debilitating limb surgery.* Therefore, the individual trial efficacy data are interpreted with caution and that the **Report from Independent Review and integrated summary of efficacy** (see below) should constitute the "pivotal" efficacy data set.

Description of ILP technique with TNFα, Melphalan and mild hyperthermia

The technique is described. The dose of melphalan should preferably be based on both the body weight and limb volume methods. Based on body weight the upper limb should perfused with 0.6-1.0 mg/kg BW, and the lower limb with 1.0-1.4 mg/kg (maximum dose for the arm 70 mg and for the leg 140 mg). The dose may be underestimated in muscular (skinny) persons, and overestimated in fat persons. Limb volume may either be calculated directly (immersion of the limb in a water tank) or indirectly by a computer program. The recommended dosage is 13 ml/l perfused upper limb volume, and 10 ml/l lower limb volume. The volume method may overestimate the dose in-patients with peripheral obesity (mostly women). The TNF dose is a total of 3 mg/arm or 4 mg/leg. It recommended that the patient receives antipyretic medication with paracetamol prior to ILP. The procedure is performed in general anaesthesia with mechanical ventilation. The patient should be heparinized with

200 IU/kg prior to surgery and occlusion of the vessels. The isolated limb is perfused with a flow rate of 35-40 ml/l limb volume/min at 40^{o} on the arterial side. The oxygen saturation should be kept above 60%. Beromun is given first as a bolus into the arterial line, 30 minutes later melphalan is injected into the integrated reservoir of the perfusion circuit or slowly into the arterial line. After a total of 90 minutes of perfusion, the wash-out procedure is started (1-2 litres for the arm, and 3-6 litres for the leg) and is continued until the colour of the perfusate is clear in appearance. During the wash out period the oxygen supply to the limb is interrupted. The maximum duration of limb anoxia should not exceed 20 minutes.

Leakage into systemic circulation is monitored with precordial scintillation probe (131-I-albumin, 99-Tc-albumin or 99-Tc-erythrocytes are the methods that have been used in the ILP-programme; iodinated albumin is preferred).

After ILP anticoagulation should be continued for 48 hours. The patient is taken to the ICU and closely monitored for at least 48 hours post ILP. Adequate fluid hydration and small doses of dopamine as infusion is continued.

If a tumour response is seen it occurs with a maximum 4-6 weeks after ILP and resections of tumour remnants is performed 12-16 weeks after ILP.

Studies with melphalan +TNFα + IFNγ

Study 152.63

This pilot study was open-labelled non-randomised. Patients received IFNγ 0.2 mg s.c for two days prior to ILP. The dosage and ILP procedure were identical to what is recommended in the posology section of the SPC (TNF 3000 µg upper limb, 4000 µg lower limb, L-PAM 13 mg/l perfused limb (upper limb) and 10 mg/l perfused limb (lower limb), except for the addition of IFNγ 0.2 mg). The total perfusion time was 90 min (30 min with TNF + IFNγ followed by 60 min of L-PAM). The arterial blood temperature was maintained at 40^{o} during ILP.

IFNγ was included because of its in-vitro upregulating effect on TNF receptors and a synergistic effect in animal models. The study was initiated in 1989 before the implementation of GCP Guidelines in the EU. The exclusion criteria were the same as in the IOR programme (see below). Included were patients with high grade STS and good performance status. All patients received dopamine infusion plus fluid during the ILP and for the first 48 hours after ILP. All patients were monitored in an ICU for 48 hours after ILP. Paracetamol 500 mg was used as prophylaxis against fever, chills and myalgias.

Thirty-nine patients were screened and consented to participate in the trial. Of these, 20 patients completed a 1-year follow-up after ILP. 13 patients experienced recurrence or progression of the disease, two patients were excluded due to protocol violation, two received other chemotherapy or radiotherapy, one patient died and one patient was lost to follow-up. The median follow-up from ILP was 846 days (84-1472 days). Two patients did not met the inclusion criteria (one was aged 13 years and one was not staged according to S-criteria), 5 patients were included despite of other cancers, one patient did not meet the vascular disease exclusion criteria, 1 did not meet the renal disease exclusion criteria.

Protocol violations occurred during the ILPs. In particular there were deviations in the doses of melphalan (see also IOR programme). The median dose administered was 95 mg (33-150 mg). Deviations of 2.5 mg or less were recorded in 9 patients, between 2.5 and 10 mg in 6 patients, between 10 and 37.6 mg in 14 patients. Four patients received less TNF than planned.

Other protocol violations included excessive leakage to the systemic circulation (16, 11 and 25%), lacking distal temperature measurement in 7 patients, ILPs administered outside the approved centres in 2 patients.

Efficacy population: All 39 patients were evaluated for efficacy. Ten patients underwent amputation giving a limb salvage rate of 29/39 (74%) during the follow-up period. The 10 amputations occurred 13-910 days after ILP (median 475 days). Responses were as follows:

Final response	n	%
Complete Response (CR)	11	28.2
Partial Response (PR)	13	33.3
No Change (NC)	9	23.1
Progressive Disease (PD)	4	10.3
Non Assessable (NA)	2	5.1
Total	39	

A number of prognostic factors for response were assessed. No fixation to neurovascular bundle, smaller tumour size, only one lesion implied a higher response rate. There was some centre effect in a multivariate analysis (P=0.11).

17 patients suffered local recurrence or progression with a median duration of 888 days.

This was the first study to investigate TNF (tasonermin) in both STS and malignant melanoma. The high response rate prompted the initiation of the **IOR programme** by the same investigators as well as other perfusionists.

The SPC for Beromun is specific for the calculation of the dose, as it is apparent from the clinical trials performed that the calculation of the dose was not straightforward.

Study 152.62

This was an open label non-randomised multicentre study. After two days of IFNγ 0.2 mg s.c/day the ILP procedure was initiated. The dosage and ILP procedure were identical to what is recommended in the posology section of the SPC (TNF 3000 µg upper limb, 4000 µg lower limb, L-PAM 13 mg/l perfused limb (upper limb) and 10 mg/l perfused limb (lower limb), except for the addition of IFNγ 0.2 mg). The total perfusion time was 90 min (30 min with TNF + IFNγ followed by 60 min of L-PAM). The arterial blood temperature was maintained at 40^{o} during ILP.

The inclusion criteria and exclusion criteria were identical to those used in **Study 152.66** (see below) except for including some patients with metastatic disease (S4). Fluid and dopamine prophylaxis, observations in ICU and monitoring leakage into systemic circulation regimens were identical. Criteria for ILP retreatment were the same.

23 patients were included. All met the inclusion and not the exclusion criteria. 17 protocol violations were due to dosage deviations during ILP. Two patients received 50% and 16% less melphalan than planned. Nine patients had deviations that did not exceed 2.5 mg, and 4 had deviations of 10%. One patient received 3,000 µg TNF instead of 4,000 µg. Two patients had excessive systemic leakage (16% and 20%), two patients received their ILP outside the approved centres.

Efficacy population: Three patients underwent amputation following ILP resulting in a limb salvage rate of 20/23 (87%) for a median observation time of 555 days. The 3 amputations occurred 239,248 and 692 days after ILP. One of these patients had received a very small dose of melphalan (50% of scheduled dosage). Three patients died without amputation during the first year after ILP.

The response rates for an OR of 82.6% (95 CI 61.2-95.1%) were as follows:

	n	**(%)**
Complete Response (CR)	3	13.0
Partial Response (PR)	16	69.6
No Change (NC)	3	13.0
Progressive Disease (PD)	1	4.3
	23	100.0

Factors that influenced response were centre (p=0.008), gender (p=0.02), and Sarcoma Stage (p=0.15) as assessed by stepwise logistic regression.

The Kaplan-Meier estimate for median survival was 770 days after ILP (6 deaths during the follow up period). The median duration until local progression/recurrence exceeds 237 days.

Except for survival there was a strong centre effect on response rate, progression-free survival, and overall limb recurrence-free survival.

This trial is small sized as most other studies in the application. The limb salvage rate is high with only 3 amputations during the follow-up period.

Studies with melphalan and TNFα alone

Study 152.66

This was an open label non-randomised multicentre study (three centres in the Netherlands and one centre in Switzerland). The dosage and ILP procedure were identical to what is recommended in the posology section of the SPC (TNF 3000 µg upper limb, 4000 µg lower limb, L-PAM 13 mg/l perfused limb (upper limb) and 10 mg/l perfused limb (lower limb). The total perfusion time was 90 min (30 min with TNF followed by 60 min of L-PAM). The arterial blood temperature was maintained at 40^{o} during ILP.

23 patients took part in this study (22 received one treatment, one patient received two treatments). The trial was conducted according to EU GCP guidelines. Only high risk factor patients with STS aged between 12 and 80 years and S1-S3 risk grades were included. Exclusion criteria as in the IOR programme (se below). Potential systemic leakage was monitored with radioactive albumin. Perfusion was terminated if systemic leakage exceeded 10%. Fluid and dopamine prophylaxis was administered to all patients prior to TNF and for 48 hours after ILP. Patients were to spend 48 hours in the ICU after ILP. Immediate TNF side effects treated prophylactically with paracetamol or indomethacin.

A second ILP was allowed 28-42 days after first ILP in-patients with stable disease or partial response and still unresectable tumours and haematological recovery and normal renal function.

23 patients were included of whom 7 completed the observation period of one year. All other patients (14) had progression of the disease or recurrence. One patient had amputation and one was lost to follow-up. 19 patients met both inclusion and exclusion criteria. One patient was aged 82 years, two had concurrent malignancy, and one patient was known to use vasopressor agents at the time of inclusion.

Again, the main reason for protocol violation was deviation from the planned dosage of melphalan (n=15). However, 9 patients had small deviations ($\leq$ 2.5 mg), 5 had deviations of 10%, and one patient received 36% (14 mg) less than prescribed. Hyperthermia was not obtained in two patients.

Efficacy: 4 patients underwent amputation with a median observation time of 421 days for all patients. Limb salvage was thus achieved in 19/23 (83%). This should be compared with 6 deaths within the first year without amputation. CR and PR was achieved in 6 patients (26%) and 7 (30%) respectively. There were important centre differences but the numbers are small. OR varied from 0% to 85% among centres. The centre effect could not be found in a logistic regression analysis that identified Sarcoma Stage (S-status) and tumour size as the only significant factors.

10 patients died after ILP with a median survival time of 551 days for the entire group. The time to local recurrence/progression was > 348 days.

The follow-up period was relatively short, and the patients included seemed to have more aggressive sarcomas than patients included in the pilot trial with 10 deaths during the first 18 months after ILP. The limb sparing effect was in the same range as reported in the other trials.

The Investigators' Own Responsibility Study (152.12)

This was an open-labelled non-randomised multicentre study investigating the efficacy and safety of one or two treatments with TNF and melphalan by mild hyperthermic ILP in-patients with STS. Efficacy endpoints have been described earlier in this AR.

103 patients received one treatment (90 patients), two treatments (12 patients) or three treatments (one patient). The decision to give more than one treatment depended on the clinical response (i.e. either insufficient response to allow limb sparing surgery or subsequent progression after initial CR).

The dosage and ILP procedure were identical to what is recommended in the posology section of the SPC (TNF 3000 µg upper limb, 4000 µg lower limb, L-PAM 13 mg/L perfused limb (upper limb) and 10 mg/L perfused limb (lower limb). The median total dose was 80 mg (0-180 mg). The total

perfusion time was 90 min (30 min with TNF followed by 60 min of L-PAM). The arterial blood temperature was maintained at 40^{o} during ILP.

The study continues to accrue patients. As of July 1997, 207 patients have been included. The results for the first 103 patients entered until October 1995 are presented in an interim report. The cut-off date is January 1997.

The inclusion criteria was patients STS of the extremities with a good performance status (ECOG 0-1) and high risk factor graded from S1 to S4: Irresectable STS grade II-III, grade I (> 8 cm) or any grade II-III tumour that can only be resected at the cost of unacceptable functional morbidity, patients with local recurrence (grade I > 8 cm, grade II-III) if treated before by surgery and/or radiotherapy or ILP with chemotherapy alone; patients with metastatic disease at the time of presentation of the primary STS if it can be resected only by amputation or unacceptable morbidity. There were a large number of exclusion criteria including significant cardiovascular disease, significant renal dysfunction (s-creatinine > 150 mmol/l or creatinine clearance < 50 ml/min), inability to tolerate vasopressor agents, WBC < 2,500 and platelets < 60,000, clinically relevant pulmonary dysfunction etc., patients requiring anticoagulants, antithrombotic agents, systemic corticosteroids and NSAIDs. Previous chemotherapy and/or radiotherapy within 4 weeks.

Overall efficacy results: 84 patients (82%) achieved the target limb salvage for a median post-ILP observation time of 372 days. In terms of response rate, 17 CR and 50 PR (OR 65%, 95% CI 55-74%) were observed. The median survival has not been reached but is actually in excess of 2 years. The median time to local recurrence exceeds 1 year. 19 patients underwent amputation during the follow-up period

Prognostic factors for response rate evaluated by logistic regression included centre (p=0.001), age (p=0.049), fixation to neurovascular bundle, multiple lesions, and tumour size did not attain statistical significance (p> 0.10 and < 0.20). These factors were selected from univariate analysis. For survival the S-status was the most important variable.

A number of *protocol violations* occurred. During ILP particularly the melphalan dosage deviated from the planned dosage. On centre did not record limb volumes, and melphalan deviations for 38 ILPs could not be calculated. For another 23 patients the differences from protocol dosage did not exceed 2.5 mg. The deviation was > 2.5 mg and < 10 mg in 12 patients, and from 10 to 158 mg in 14 patients. In two of these cases the deviations of 84.5 mg and 110 mg respectively could be explained by TNF leakage and interruption of the ILP. Hence, melphalan was not given to these patients. TNF doses deviated in 5 patients. All received lower doses than planned. Distal skin temperature was not available for 31 ILPs and it was below 38^{o} for 5 patients. Eight occurrences of excessive leakage into systemic circulation were recorded as protocol violation.

Two patients received IFNγ.

The IOR programme was uncontrolled and a number of protocol violations occurred. However, the efficacy results were comparable to whose achieved in the pilot trial and the two small "pivotal" clinical trials with a limb salvage rate of 82% and a high overall tumour response rate.

Report from Independent Review and integrated summary of efficacy

The Independent Review Committee received all CRF, histology reports, radiological assessment etc. from the 4 open label studies. 188 cases were submitted to the committee, 6 patients were found to have other tumours than STS, 2 patients had insufficient data for review, and one patient had no measurable tumour pre-ILP. The committee had to reach agreement with respect to diagnosis, destiny (amputation or not), and outcome. In case of disagreement, an arbitrator should be consulted. In no case was it necessary to use the arbitrator. None of the members of the committee had any direct involvement in the conduct of the clinical trials and two of three members (two surgeons with expertise in STS plus one radiologist) came from countries that had not participated in the TNFα programme. A third surgical oncologist acted as arbitrator in cases where agreement was not reached among the members.

All clinical source material was blinded so the reviewers were unaware of the patient's identity, centre or investigator. Meetings were restricted so that no investigator or monitor could attend.

Surgical outcome: The committee considered 145 of 179 (81%) assessable patients destined to be amputated or resected with significant loss of function. These patients were "Review Positive", the remaining 34 patients "Review Negative".

The post ILP outcome was evaluable in 166/179 patients. 13 patients could not be allocated to any response category because of early death, complication of treatment or lack of follow-up data. These 13 patients were analysed as if they required amputation. Using this method the committee identified 97/179 (54%) who achieved a better response than predicted, 77/179 (43%) achieved their predicted outcome, and 5/179 did worse than expected:

Limb salvage without resection	31/179 (17%)
Resection without significant loss of function	90/179 (50%)
Resection with loss of function	4/179 (2%)
Amputation	41/179 (23%)
Outcome not assessable considered as amputation	13/179 (7%)

Considering the *target population* (i.e. 145 patients destined to be amputated or resected with significant loss of function) the following results were obtained: 62% had a better outcome than predicted (66% if one counts the 4 patients who underwent resection with loss of function but was destined to be amputated):

Limb salvage without resection	27/145 (19%)
Resection without significant loss of function	63/145 (43%)
Resection with loss of function	4/145 (3%)
Amputation	39/145 (27%)
Outcome not assessable considered as amputation	12/179 (8%)

Time-to-event data: 47 "Review positive" and 4 "Review negative" patients died during the follow-up period. For the "Review positive" group the median survival was approximately 3.2 years. The time to amputation or loss of function was close to 3 years for patients surviving for more than one year post ILP (n=113).

Tumour Response (secondary variable): Overall response rate was 64% (27CR+66PR) in the "Review Positive" group and 71% (7CR + 17PR) in the "Review Negative" group.

As regards the hard endpoint "amputation or resection with significant loss of function" more than half of the population assessable for the Independent Review had a better outcome than predicted. If one only considers the true target population, namely the 145 patients in whom the Independent Review Committee felt sure that major surgery was unavoidable, 62% had a limb sparing effect of the ILP procedure.

An Independent Review is highly recommendable and the methodology used was conservative. In the absence of comparative trials this approach strengthen the credibility of the results obtained in 4 rather weak clinical trials with a number of deficiencies (poor protocol adherence and large centre differences in the IOR programme, the inclusion of IFNγ in two studies, a continuously decreasing target population in order to achieve homogeneity). The Independent Review did not comment on the data quality.

The inclusion of IFNγ in two trials did change the results. However, the number of patients are small.

Other information on efficacy

The applicant has submitted a report comparing the survival of patients treated with ILP (melphalan plus TNF) with a retrospective series of 632 patients treated conventionally (amputation/large resections with or without radiotherapy) from the STS Database of the Musculoskeletal Tumor Center University Hospital, Lund, Sweden. The test population comprised 97 patients (54 with primary tumour and 43 with local recurrence) derived from 126 patients who received the proposed regimen and who were judged as being destined for amputation by the Independent Review. As there was no difference in-patients with either primary tumour or local recurrence, both groups were included in the analysis. In a multivariate adjustment further 6 ILP patients were excluded due to incomplete data. The Cox proportional hazards model revealed a RR for a tumour related death for ILP treated patients of 1.5 (90% CI 1.0-2.3). At the 5% level, this RR was not different from the RR of controls (p=0.08).

The matched analysis was performed on 114 matched patients (57 + 57). The Cox model now revealed a RR of 1.4 for ILP patients. At the 5% level there was no difference between the RRs (p=0.4931).

Although the survival for ILP treated patients could be slightly worse than for patients treated with conventional therapy, the difference was not significant. Such matched analysis should be interpreted critically. The main finding is that ILP - and more limb preservation - does not appear to have detrimental effects on patients' tumour-related survival.

SAFETY

The AE seen in the 4 individual ILP studies have been provided. However, these are not very detailed. Also provided was a table derived from the textual summary describing the results for 445 patients that have received the ILP procedure for either STS or other tumours (mostly malignant melanoma). There have been 4 procedure/drug-related deaths in these 445 patients. Two was caused by wound infections, one death was a result of severe hypotension, renal failure and ARDS (not an STS patient) after ILP, and one patient had cardiac arrest and tension pneumothorax caused by a central venous catheter.

The systemic toxicity of the ILP procedure is well documented and not different from the expected for TNF and Melphalan. In addition the ILP procedure in itself might give local reactions, mainly pain in the perfused limb but also more serious AE such as nerve injury, severe tissue damage, thrombotic complications and wound infections.

Studies with melphalan +TNFα + IFNγ

Study 152.63

Systemic toxicity consisted of fever (100%), chills (51%), cardiac rhythm disturbances (62%), nausea and vomiting (31%), fatigue (26%). Serious adverse events consisted of liver toxicity (18%), ARDS (13%), thrombocytopenia (10%). Impairment of cardiac function (8%), acute renal failure (8%). Regional toxicity consisted mainly in pain (67%). Nerve injury (10.3%) and venous thrombosis (7.7%) were other AE that could be related to the ILP procedure. No deaths could be attributed directly to ILP.

Blood pressure < 100/60 mmHg occurred in at least 14 patients, HR varied between 55 and 200 bpm.

A special section deals with AE in patients with excessive leakage into systemic circulation defined as greater than 10% of leakage corresponding to a theoretical TNF exposure of 300-400 μg, e.g. in the range of systemic MTD. This occurred in 2 patients (3 ILPs). In one patient with a leakage of 16% severe thrombocytopenia and leukopenia occurred on the day of ILP (resolved by day 29)

The other patient with an 11% leakage during the first ILP developed cardiac rhythm disturbance, ARDS and liver toxicity. During the second ILP the leakage reached 24% during the first 60 minutes, but the perfusion was not terminated prematurely. Subsequently, the patient developed infection, cardiac function disturbance, venous thrombosis, wound infection, serious loss of consciousness, ARDS and ARF. The patient recovered completely within 6 weeks following ILP.

A number of laboratory variables were changed after ILP. Unfortunately, many data are missing. Direct bilirubin, LDH, ALAT, ASAT tended to increase. Haemoglobin, leukocytes and platelets decreased during the day of ILP most values returned to normal within 2 weeks. Hypoalbuminaemia was seen in 9 patients post ILP.

Most of the AE are expected after some systemic exposure of the two cytokines and melphalan.

Study 152.62

All 23 patients were evaluated for safety. Whereas the dosage of TNF and IFN in the vast majority of the cases was the one specified in the protocol, melphalan dosage varied considerably. The median dose was 80 mg (range 28-140 mg).

Fever, chills, nausea and vomiting, pain in the perfused limb, cardiac rhythm disturbances, liver toxicity as assessed by increased bilirubin and transminase levels were the most common AE as in the other clinical trials.

No patient died during the first two weeks after ILP. All 6 deaths were due to progressive STS.

Two patients experienced excessive leakage to the systemic circulation (16 and 20%). The first patients had an uncomplicated post ILP course with prolonged fever as the most characteristic symptom. The other patient had infection and proteinuria on day 2 followed by shock for 2 days. Thrombocytopenia and anaemia was also recorded. The symptoms resolved completely.

With respect to laboratory parameters, the effects on haematology and liver function parameters the findings were similar to those observed in the other clinical trials: Early and transient fall in WBC and platelets, increases in transaminases, s-bilirubin and LDH, decreases in total s-protein and albumin. Some patients had increases in creatinine kinase levels.

5/23 had systolic BP < 110 mmHg with rapid recovery.

Studies with melphalan and TNFα alone

Study 152.66

Adverse Events (AE) were reported. Fever (78%), nausea and vomiting (35%), and cardiac rhythm disturbances (26%) were the most common AE. Two patients underwent amputation within the first 14 days after ILP and are therefore considered as having an AE. Local toxicity included pain in the infused limb (70%), nerve injury (17%), arterial thrombosis (13%). All 10 deaths were due to fulminant progression of the malignant disease (4-18 months after ILP). Serious AE were few, two patients with liver toxicity and one patient with thrombocytopenia.

No patient had systemic TNF leakage in excess of 10%.

A number of laboratory changes were recorded. Even if both leukocyte and platelets decreased after ILP this was of no serious clinical concern. Platelets were below 100 giga/l in 4 patients days 1-2, and WBC below 4 giga/l in 4 patients. Bilirubin increased in 5 patients, of whom 2 were recorded as having liver toxicity (elevated LDH and transaminase values as well). Low s-albumin was seen in 11 patients. Creatinine kinase levels were elevated in 11/13 assessable patients.

5 patients had BP below 100/45 mmHg. Increased pulse rate was in most instances related to elevated body temperature, 3 patients had very high pulse rates (160, 170, and 190 bpm).

There were no serious systemic leakages. The toxicity except for local toxicity was the expected for melphalan and TNF-α

The Investigators' Own Responsibility Study (152.12)

Safety monitoring for this trial included TNF leakage by two methods (radioactive albumin or radio-labelled erythrocytes). All patients received appropriate fluid challenge for the prophylaxis of shock. If indicated dopamine infusion was initiated at an infusion rate of 3 μg/kg/min and continued for up to 48 hours after ILP. Patients were closely monitored for 48 hours in an intensive care unit. If indicated a Swan-Ganz catheter was used to monitor wedge pressure and PAP. The chills, fever, and myalgias were treated with paracetamol 500 mg orally or by suppository (indomethacin 100 mg suppository was used as an alternative).

All 103 patients were evaluated for safety regardless of the above mentioned protocol violations. Overall 116 ILP procedures were performed in these 103 patients. Nearly all patients had at least one AE (97.1%). Fever was the most common AE (75%) followed by nausea/vomiting (33%), fatigue (19.4%), chills (13.6%), serious liver toxicity assessed by clinical chemistry (12.6%). Thrombocytopenia (7.8%), impaired cardiac function (3.9%), shock (2.9%), acute respiratory failure (1%). Table 10.2.2. (Appendix A) shows all recorded AE.

Laboratory changes of potential concern included: haemoglobin < 5 mmol/l (n=7), platelets < 100 giga/l (n=17), WBC < 4 giga/l (n=16), increased bilirubin (> 30 μmol/l for 20 patients), elevated transaminases (more than x2 upper limit for 45 patients), elevated LDH (more than x2 upper limit for 8 patients). Vital signs were poorly recorded and no information is available after the pre-ILP evaluation.

AE in-patients experiencing excessive systemic leakage were described for 8 patients. The symptoms were typical for TNF and melphalan systemic toxicity with fever, hypotension, myelosuppression, nausea and vomiting, liver toxicity and renal toxicity. No mortality was seen in this subset.

Except for the patients with excessive systemic leakage, which should be avoided, toxicity profile was the expected from previous experience with melphalan and TNFα. The laboratory and clinical monitoring show that a number of data sets are missing.

Conclusion (clinical) of the CPMP on the Original Dossier Submitted.

Due to the lack of controlled clinical studies and a conservative estimate of the limb sparing effect of 60% in patients with soft tissue sarcomas of the extremities, the CPMP posed a number of questions to the applicant regarding reanalysis of the efficacy data. Also pertinent for a final analysis of the risk/benefit ratio is a more clear discussion of the exact role of the various components of the treatment procedure, i.e. *hyperthermia, melphalan*, and *the optimal dose relation between* TNFα and melphalan.

Isolated limb perfusion under hyperthermic conditions with melphalan + Beromun is a treatment for subspecialists and treatment units will be located in a few centres with thorough knowledge of the ILP technique, ICU facilities, close monitoring of TNF leakage etc. The risk must be viewed in that context. However, there is concern on the risk of systemic leakage of large doses of TNFα and the optimal methodology for monitoring leakage during isolated limb perfusion. Also important for risk/benefit is the potential adverse effect on overall survival that may be a consequence of limb sparing.

Responses to questions posed by CPMP on clinical aspects

The CPMP questions were divided into four main areas: 1) *Methodology related issues* (i.e. justification for the lack of comparative trials, request for more mature clinical data from ongoing clinical trials, the need for external control data to substantiate the risk-benefit ratio, and the data behind the claim, that about 10% of patients with soft tissue sarcoma (STS) are destined for amputation or severely debilitating surgery); 2) *Issues related to the clinical pharmacology* of TNF-α and melphalan and the isolated limb perfusion (ILP) procedure; 3) *Issues related to efficacy* of the treatment and 4) *Issues related to safety*.

New Data

The original Dossier has been expanded with more efficacy and safety data mainly because the applicant has updated the **Report from Independent Review and integrated summary of efficacy** presented in the initial application. The target population has been increased to *196 patients destined to be amputated or resected with significant loss of function*. With more mature data in a larger population the efficacy results are confirmed and the Independent Review Board consistently have considered approximately 80% of the patients to be destined for amputation. The most pertinent changes in numbers are shown in Table below. Considering the target population (i.e. 196 patients destined to be amputated or resected with significant loss of function) the following results were obtained: 60.7 % had a better outcome than predicted (63.3% if one counts 5 patients who underwent resection with loss of function but was destined to be amputated, after a median follow-up time of more than 500 days).

	Original Dossier	**Updated for Response to CPMP**
Patients - submitted	188 (100%)	260
- assessable	179 (95%)	246 (95%)
Pre-ILP Destiny	**179**	**246**
- amputation	124 (69.3%)	164 (66.7%)
- resection with function loss	21 (11.7%)	32 (13%)
- resection w/o function loss	34 (19%)	50 (20.3%)
Post-ILP outcome 'review pos.'	**145**	**196**
- limb salvage w/o resection	27 (18.6%)	37 (18.9%)
- resection w/o function loss	63 (43.4%)	82 (41.8%)
- resection with function loss	4 (2.8%)	5 (2.6%)
- amputation	39 (26.9%)	56 (28.6%)
- not assessable	12 (8.3%)	16 (8.2%)
Post-ILP outcome 'review neg'	**34**	**50**
- limb salvage w/o resection	4 (11.8%)	8 (16%)
- resection w/o function loss	27 (79.4%)	29(78%)
- resection with function loss	-	-
- amputation	2 (5.9%)	2 (4.0%)
- not assessable	1 (2.9%)	1 (2.0%)

Justification of the lack of comparative trials

The applicant has again stressed the fact that Beromun is only intended for the treatment of the 10% of patients with STS that are destined to be amputated or resected with significant loss of limb function. The 10% figure seems well substantiated by data from the literature and major Swedish and French databases. Major arguments for an application based on phase II data only are listed: 1). Literature review supports the conclusion that truly irresectable patients are rarely subjected to adjuvant radiotherapy or radiotherapy alone. An extensive and exhaustive literature review 1988-98 has not revealed other relevant treatment options that should have been included in a control arm. 2). Disease severity is the key issue and distinguishing feature in the ILP series of this application (55% primary tumours, 45% recurrent tumours, 22 multifocal tumours, 46% tumor size $\geq$ 10 cm, 13% previous radiotherapy, and 15% previous chemotherapy). No comparable series have been reported in the literature. 3) A comparison of ILP versus radiotherapy is difficult to conduct because of patient eligibility problems. The investigators and the Independent Committee had considered all standard treatment options, including all forms of radiotherapy before designating the patient as *irresectable* and destined for amputation/debilitating surgery. Therefore, according to the applicant, radiotherapy was considered *not any more* to be a treatment option (i.e. adjuvant radiotherapy would not make the patient resectable or radiotherapy alone would not ensure local tumour control). In some patients prior irradiation precluded curative dosimetry, in others the tumour area was too large or multifocal. 4) The applicant has considered the possibility to include patients with less severe disease into a comparative trial, but since this population does well with surgery plus adjuvant radiotherapy and thus does not need ILP it would not be justified to enroll patients into a randomised trial. 5). Another issue is the choice of radiotherapy. The most comparable regimen to ILP would be adjuvant radiotherapy prior to surgery. The application has reviewed the literature for such trials in a comparable population. The conclusion was that the assessment of patient's benefit is impossible due to

heterogeneous population, retrospective collection of patients from single institutions, and poorly defined criteria for resectability 6). Brachytherapy (internal radiotherapy) plus marginal resections requires patients with nearly resectable tumours. Moreover, it is only recommended to use brachytherapy in-patients with negative margins. Adjuvant radiotherapy postoperatively plus intralesional resections has a high local recurrence rate 7). The applicant, finally, refers to the current Note for Guidance on Evaluation of Anticancer Medicinal Products in Man (CPMP/EWP/205/95) claiming that TNF+melphalan by ILP has *outstanding anticancer activity* in a population with no other real therapeutic options, that the regimen has *acceptable and characterised toxicity profile*, that clinical benefit in terms of *limb salvage* has been demonstrated, that the classical *tumour response rate* is consistently high. Thus, the criteria for not performing controlled trials are present.

Issues related to the clinical pharmacology and ILP procedure

The choice of *melphalan* as the preferred drug for ILP is justified. The dosing regimen is also justified. Prerequisites for the use in ILP are that the drug is active without prior systemic metabolisation and that the loco-regional toxicity is manageable. Thus, ifosfamide, cyclophosphamide and dacarbazine (DTIC) are unsuitable for ILP use. Doxorubicin is an active drug in STS but the risk of local toxicity is high when used for ILP purpose. Actinomycin D has radiation sensitising effects, which may last for several months. Moreover, the combination of melphalan and Actinomycin D does not seem to be more effective than melphalan alone in ILP settings. Finally, cisplatin is one of the best-documented drugs for ILP but not for the STS indication, since most of the experience has been gathered from melanoma trials. From the limited data it appears that cisplatin is not very active in STS and that cisplatin is bound to limb tissues for a prolonged period of time after ILP. This property makes cisplatin less suitable for combination with TNF-α, which rapidly targets and destroys tumour vasculature. One major area of concern was the systemic leakage of TNF and the potential risk of serious adverse events. This issue is not completely resolved but the clinical experience supports that the apparent high systemic exposure to TNF is poorly correlated with safety. Since the radiotracer technique appears to function in the clinical situation and no better alternatives are available the rapporteurs support that this method remains in the SPC text. The methodology for measurement of leakage is sufficiently described and justified (the albumin method and Indium-111 labelled erythrocytes).

Another area of concern was a potential interaction between TNF and NSAIDs and corticosteroids. Total patients exposed to either medication pre- or post-ILP amounts to 51/260. These 51 patients are compared with non-exposed patients for efficacy assessment. Complete response plus partial response rate was 51% (95%CI 37.3%-64.7%) for exposed versus 68% (95%CI 61.6%-74.3%) for non-exposed. As regards the whole distributions of response categories, (i.e. complete response plus partial response, no change, progressive disease), however, there is no statistically significant difference. The corresponding values for limb salvage were 71% and 79% (difference not significant). Regarding safety comparing data for 51 exposed with 209 non-exposed patients, there were differences in type and number of AE. Cases of acute renal failure were not reported. The applicant believes it is unnecessary to include a warning in the SPC on the concomitant use of NSAIDs during ILP with melphalan and TNF.

Efficacy issues

Justification for designation as a candidate for amputation or resection with loss of limb function was evaluated by the Independent Review Committee and the reasons are tabulated below for the target population of 196 patients. Major subgroups could not be defined, and even if they could be defined, the alternative therapy for these patients is still amputation or resection with loss of limb function. Other therapies that have been reported in the literature remain experimental as none have been proven in properly conducted prospective clinical trials.

	Destiny Assessment			
	Amputation		**Resection with loss of function**	
Reason for destiny	**N**	**%**	**N**	**%**
Tumour size	71	43.3	13	40.6
Tumour location	107	65.2	25	78.1
Involvement of neurovascular bundle	86	52.4	17	53.1
Involvement of bone	71	43.3	2	6.3
Tumour type	9	5.5	1	3.1
Previous surgery	18	11.0		
Local recurrence	74	45.1	10	31.3
Previous radiotherapy	27	16.5	3	9.4
Previous chemotherapy	3	1.8		
Multiple lesions	34	20.7	4	12.5
Other	6	3.7	2	6.3
Any reason	164	100.0	32	100.0

Centre effects for *limb salvage* and *survival* were assessed by fitting logistic models with and without centre as explanatory variate. For a common parameter obtained by pooling across centres, 95% confidence intervals were established. The results are shown below:

Centre - Effect for Limb Salvage - Test for Homogeneity p= 0.7772

Estimate of Common Limb Salvage Rate and 95% Confidence Interval = 76.42% (70.72% - 81.31%).

Centre - Effect for Events with Respect to Survival - Test for Homogeneity p=0.6738

Estimate of Common Event Rate and 95% Confidence Interval = 32.1% (26.58% - 38.20%).

The width of these confidence intervals is considered as sufficiently narrow and that there is no significant centre effect with respect to the "hard" endpoints, limb salvage and survival.

The "life-time" outcome for limb salvage according to destiny prior to treatment is shown in table below: 119/196 (61%) "review positive" patients achieved a fully functioning limb post-ILP.

	Outcome after ILP											
	Limb Salvage w/o resection		Resection w/o Loss of funct.		Resection with Loss of funct.		Amputation		Not assessable		Total	
Destiny	N	%	N	%	N	%	N	%	N	%	N	%
Resection	8	16.0	39	78	0	0.0	2	4.0	1	2.0	50	100.0
Total	45	18.3	121	49.2	5	2.0	58	23.6	17	6.9	246	100.0

Figures in **bold** reflect the patients from the true target population successfully treated according to limb salvage criteria.

Safety issues

An area of concern was that patients undergoing ILP might have a poorer survival than patients treated with traditional methods. For the endpoints DFS and overall survival historical control comparison did not allow any conclusion because they were not reported in the literature. A randomised clinical trial comparing amputation or debilitating resection with ILP with melphalan + TNF was not considered feasible.

Therefore, the applicant decided to make a survival comparison based on data from the Musculoskeletal Tumor Centre of the Department of Orthopedics of the University Hospital in Lund, Sweden (Southern Swedish Health Care Region). When comparing tumour-related survival of TNF + melphalan-treated patients to the traditionally treated patients with STS of the extremities in a multivariate analysis, the estimated risk for tumour-related death was increased but (at the 5% level) not statistically significant; RR = 1.5 (90% CI 1.0-2.3, p=0.0786). The baseline prognostic factors were more negative for the ILP population than for the traditionally treated patients. In a matched pairs analysis with 19 ILP cases versus 27 controls, the RR was 1.4 (90% CI 0.6-3.0, p=0.4931) and Kaplan-Meier curves show similar survival. The applicant also refers to a NCI (U.S.A.) prospective trial comparing amputation with limb sparing surgery showing that the overall survival results were not different (Brennan MF *et al. Soft Tissue*

Sarcoma. In: Cancer. Principles and Practice of Oncology. DeVita VT, Hellmann S, Rosenberg SA (Eds). 5th Edition 1997).

The historical control comparisons have known deficiencies but it is agreed that the applicant had no other options due to lack of a randomised trial and such a trial is hardly feasible. It seems reasonable to conclude that overall survival is within the range of what has been reported for comparable patients treated traditionally with amputation or major tumour resections.

Another concern was that ILP might decrease tolerance to subsequent radiotherapy. The applicant refers to a report (Olieman *et al*. Int J Radiat Oncol Biol Phys 1998; 40:807) on the results of 60-70 Gy external beam radiotherapy for STS *after* ILP with melphalan and TNF and *after* resection of tumour remnants. Tolerance to radiotherapy which was initiated within 5 weeks after tumour resection was scored with the SOMA system (**S**ubjective, **O**bjective, **M**edical management, and **A**nalytical evaluation) evaluating skin, subcutaneous tissue, and muscle and soft tissue (Pavy *et al*. EORTC late effects working group. Late toxicity scoring: the SOMA scale. Radiotherapy & Oncology 1995; 35:11-5. Scale: 0 no impairment - 4 most serious tissue damage). Of 15 patients treated with radiotherapy post-ILP 14 had scores smaller than 1 (of a maximum 4), whereas one patient had SOMA scores 3-4. 19 patients did not receive any treatment post-ILP, 16 had scores below 1, one patient had score 2-3, and two score 3-4. All patients with scores 3-4 had to be amputated due to treatment-induced necrosis. These results are comparable to those seen after radiotherapy without ILP.

The safety database comprises 431 patients (IFN gamma excluded). Overall 9/431 (2%) patients had the ILP interrupted due to leakage > 10%, whereas 31/431 (7%) patients had leakage > 10% and therefore should have had their perfusion terminated according to the trial protocols (and the SPC proposal).

The applicant has compared the frequency of AE according to leakage > 10% or $\leq$ 10%. Patients with leakage > 10% had more cardiac rhythm AE (51.6% vs 20.8%), fatigue (45.2% vs 18.2%), chills (41.9% vs 11.9%), liver toxicity (35.5% vs 7.3%), other serious AE (29% vs 8.1%), infection (19.4% vs 8.1%), thrombocytopenia (19.4% vs 3%), cardiac function (16.1% vs 5.3%), night sweats (16.1% vs 2%) and consciousness (16.1% vs 2.5%). Excessive leakage should be avoided because of an increased number of AE. Among STS patients 18 had leakage > 10%. There appeared to be some centre effect (Centre11 had more cases with excessive leakage as 5 of the first 6 patients suffered this complication). The only other factor that could have contributed to leakage > 10% was a high Body Mass index, since 16/18 had BMI > 20.

As regards haematological toxicity 6 of 431 patients (1.4%) experienced WHO grade III or IV leukopenia, starting between 2 and 13 days post ILP. These episodes were transient and usually short-lived. Only in one patient leukopenia was confirmed repeatedly for 63 days.

Rate and grade of leukopenia are comparable to observations made after ILP with melphalan alone.

Twenty of 431 patients (4.6%) experienced WHO grade III or IV thrombocytopenia, 19 of which started during the first week after ILP. 13 had thrombocytopenia for 1-3 days, 4 had thrombocytopenia for one week, and 3 patients had more prolonged thrombocytopenia with a maximum of 64 days.

In all trials in STS, melanoma, and miscellaneous tumours without IFN-γ 14/431 (3%) had a Wieberdink (Wieberdink J *et al. Dosimetry in isolation perfusion of the limbs by assessment of perfused tissue volume and grading of toxic tissue reactions*, Eur J Cancer Clin Oncol 1982; 18:905-10) score of IV or greater (where IV is "extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances; threatening or manifest compartmental syndrome"). Two of these cases underwent fasciotomy, one due to a hypervolaemic ILP, the other presumably for compartment syndrome (cause not specified).

A total of 9/431 (2%) patients suffered amputation that was considered to be possibly related to ILP with a median time to amputation of 97 days. The applicant refers to the data of the French Sarcoma Group reporting on 9 trials enrolling 1128 patients with STS candidates and non-candidates for amputation treated with surgery, radiotherapy or chemotherapy. The amputation rate varied between 0 and 12%.

In total 41/260 (18%) patients from the updated STS database suffered neuropathy post-ILP. The date of this assessment varies by patient and reflects the latest condition of the patient prior to completion of the Additional Information Form. Many patients suffered neuropathy prior to ILP due to tumour infiltration or

prior therapies. In 26/260 (10%) patients neuropathy might have been caused by the ILP procedure. In 10 of these patients motor deficit was > 50%.

These results compare favourably with the outcome after standard therapies for resectable STS, where about 27% of the patients suffer some kind of complications of which 12% are severe (*Standard, Options et Recommendations. Sarcomes des Tissus Mous et Osteosarcomes*. Blackwell A (Eds). Centres de Lutte Contre Le Cancer: Paris, 1995;1). Neither efficacy nor safety seems to be affected upon repeated ILP. The data are, however, limited. It can be concluded that TNFα-1a so far does not induce anti-TNF antibodies.

ORAL EXPLANATION

The applicant presented at an oral explanation. The applicant presented data on the comparison of patients treated with Beromun (ILP) versus historical controls. Data on multivariate analysis and matched pairs were given. The CPMP were reassured by the data, but felt that the SPC should be updated to reflect this information. The applicant introduced the following phrase into section 5.1 of the SPC – 'However, the treatment is specifically a loco-regional treatment and is not expected to influence survival. A matched-pair survival analysis of patients treated by BEROMUN and melphalan ILP as compared to a historical control failed to demonstrate any survival difference (p=0.5).'

5. Conclusion

The quality of Beromun is considered to be acceptable. Physico-chemical-biological aspects relevant to uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

The safety of Beromun is considered to be acceptable.

Based on the clinical studies, Beromun has a favourable risk/benefit ratio for the proposed indication. The proposed indication for Beromun is - as an adjunct to surgery for subsequent removal of the tumour so as to prevent or delay amputation, or in the palliative situation, for irresectable soft tissue sarcoma of the limbs, used in combination with melphalan via mild hyperthermic isolated limb perfusion.