

SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Busilvex. For information on changes after approval please refer to module 8.

1. Introduction

Pierre Fabre Médicament submitted a Marketing Authorisation Application for a medicinal product containing the active substance busulfan in a concentrate for solution for infusion. This application has been submitted as so-called "bibliographical application", in accordance with directive 65/65/EEC, article 4.8 (a) ii.

Hematopoietic progenitor cell transplantation

Autologous stem cell transplantation involves the reinfusion of the patient's own stem cells, previously harvested and cryopreserved in liquid nitrogen, to reconstitute the haematopoietic cell function after the administration of high-dose therapy. The infused stem cells are obtained from the patient's bone marrow or peripheral blood at a time when the marrow displays minimal or no disease infiltration. Therefore, immunologic transplantation barriers do not exist but, theoretically, the tumour contamination of the graft may increase the relapse rate.

Allogeneic transplantation entails the transfer of stem cells from a donor to another person. This differs from autologous transplantation in that the potential exists for immune rejection of the infused stem cells by the recipient (host-*versus*-graft effect) and the immune attack of the donor's cells against some target tissues in the recipient (graft-*versus*-host disease). However, it has become apparent that in the allogeneic transplant setting, the immunocompetent cells infused with the stem cells, or arising from them, exert a potent graft-*versus*-tumour (GVT) effect independent of the effects of the high-dose therapy.

Cure rates vary, but they range from as high as 80% for patients with chronic myeloid leukaemia treated during chronic phase, to only 15-20% for patients with acute leukaemia refractory to conventional chemotherapy. Additionally, patients with non-malignant disorders such as severe combined immunodeficiency (SCID), inborn errors of metabolism, and haemoglobinopathies may also benefit from allogeneic haematopoietic stem cell transplantation (HSCT).

The last published European Blood and Marrow Transplantation (EBMT) survey on transplant activity in 1999 recorded a total of 21,430 transplants, with a rate of 6.6/100.000 inhabitants, performed by 580 teams in 35 European countries. Of these transplants, 18,720 were first transplants, (31% allogeneic and 69% autologous), 905 were second transplants (53% allogeneic and 47% autologous) and 1,805 double or triple transplants (5% allogeneic and 95% autologous). Ninety-five percent of the autologous transplants and 45% of the allogeneic transplants were peripheral blood stem cell transplants. A total of 103, respectively 1,8% of the allogeneic transplants was cord blood cell transplants.

The age of the patient is a compelling factor in the decision to perform a transplant. Patients over 50 years of age are less likely to have a favourable outcome after transplantation. Indeed, the results are best in children and become less favourable with each advancing decade.

The success of HSCT as curative therapy for patients with malignancy is limited, in part by transplant-related morbidity and mortality. However, the main cause of treatment failure is disease relapse, owing to the inability of conditioning regimens to eradicate malignancy.

Conditioning regimens

Conditioning regimens used for HSCT must accomplish two goals, depending on the patient's disease and the source of progenitor cells. Since the majority of autologous and allogeneic HSCT are performed for the treatment of malignant disease, the regimen must provide tumour cytoreduction and, ideally, disease eradication. In the case of allogeneic HSCT, the regimen must be sufficiently immunosuppressive to overcome graft rejection of the donor stem cells.

Early conditioning regimens were based on total body irradiation (TBI), where high doses of radiation were required to provide adequate immunosuppression. However, in an effort to decrease the toxicity of the regimen, modified TBI-based conditioning regimens were introduced where a reduced or fractionated dose of TBI was administered in combination with cyclophosphamide as immunosuppressant (and in some cases the addition of other cytotoxic agents). TBI disadvantages and the high technical requirements of the irradiation prompted consideration for effective regimens based on chemotherapy alone.

Over the past two decades, the combination of high dose oral busulfan (1 mg/kg every 6 hours x 16 doses, over 4 days) with cyclophosphamide (120 mg/kg), so-called "Bu/Cy2", or with cyclophosphamide (200 mg/kg), so-called "-Bu/Cy4" with/without other chemotherapeutic agents and/or radiotherapy has been extensively used as a preparative regimen for malignant haematological diseases, non malignant diseases, as well as genetic diseases, childhood metabolic disorders and paediatric solid tumours. In both autologous and allogeneic transplantation, the Bu/Cy regimen is the most commonly used non-TBI-based pre-transplant conditioning treatment. In this preparative regimen, the primary purpose of busulfan is to eradicate malignant cells (myeloablation) and for cyclophosphamide is to induce immunosuppression in the recipient so that rejection is prevented and engraftment of the donor haematopoietic system is permitted.

At present, although a number of different agents have been used for their antitumor effects in haematological malignancies, in general, they can be divided into two categories: TBI-based and chemotherapy-based. The decision guiding which preparative regimen to use is often based on the predicted tumour-specific activity of that regimen, its potential toxicity, or the availability of the radiotherapy facilities. In general, radiation- and chemotherapy-based regimens are associated with a similar probability of achieving long-term disease free survival.

A regimen with busulfan (16 mg/kg) and melphalan (140 mg/m²) followed by autologous or allogeneic HSCT demonstrated efficacy in patients with myeloid malignancies and multiple myeloma. In patients with thalassemia, busulfan at 14 mg/kg and cyclophosphamide at 120-200 mg/kg was a well-tolerated and effective regimen for allografting. A conditioning regimen including busulfan (12 mg/kg), melphalan (100 mg/m²) and thiotepa (500 mg/m²) following by autologous HSCT have shown good results in a variety of haematological malignancies and solid tumours. Regimens involving busulfan, cyclophosphamide and thiotepa or etoposide has been utilised prior to autografting and allografting.

Transplant procedure

The conditioning regimen is given during the days before transplant. The precise duration and sequence of administration depends on the specific conditioning regimen. Severe prolonged myelosuppression follows the intensive conditioning regimen with declining in peripheral blood cell counts that require platelet and red blood cells transfusion support and special measures to avoid lethal infections. Two to five weeks after the transplant, the engraftment of donated cells becomes apparent by the appearance of normal white cells, platelets and reticulocytes in the blood. Engraftment is usually defined as the number of days from transplant to reach >500 neutrophils/ μ L and >20.000 platelets/ μ L without transfusion support. However, it takes 6 to 12 months (or somewhat longer) to recover the immune cell function. Toxicity of the high-dose chemo-radiotherapy may also affect other organs or tissues such as the gastrointestinal, lung or liver, causing life-threatening problems.

Graft rejection (immune destruction of the graft by host residual cells) or graft failure due to insufficient-abnormal stem cells in the graft or anomalies in the microenvironment is an infrequent but serious complication.

Graft *versus* host disease (GVHD) results when the donor's immune cells, especially the T lymphocytes, sense that the host cells are different from themselves and, attack them if they find significant variations. GVHD is manifested primarily by symptoms and signs associated with the skin, gastrointestinal system and liver. This immune reaction also has the potential to recognise and attack the malignant cells of the recipient (GVT) and has been associated with a less likely disease recurrence. GVHD can be divided into two somewhat distinct clinical entities: acute GVHD occurring in the first 90-100 days after transplantation, and chronic GVHD developing after the third month post-transplant. GVHD may be life threatening in the severe forms of the disease. Patients undergoing allogeneic progenitor cell transplantation typically receive prophylactic treatment for acute GVHD.

Most patients undergoing transplantation require blood cell replacement, nutritional support and treatment for GVHD. The conditioning treatment prior to transplant can impair any system that is dependent on replacement by stem cells. The immunosuppression also leads to a very high risk of infections by bacteria, fungi, viruses or other parasites.

Peripheral blood mobilised stem cells determines a more rapid engraftment as compared to bone marrow stem cells. Haematopoietic growth factor such as granulocyte colony-stimulating factor (G-CSF) can be used after transplant to stimulate the production of neutrophils and shorten the period of neutropenia.

Busulfan

Busulfan is an alkylating agent with myeloablative properties and activity against non-dividing marrow cells and, possibly, non-dividing malignant cells. Its use has been well established in the treatment of haematological malignancies, particularly in patients with chronic myeloid leukaemia and other myeloproliferative syndromes. High-dose oral busulfan is also used in combination with cyclophosphamide as preparative (conditioning) regimens for haematopoietic progenitor cell transplantation. Oral busulfan at high dose of 1 mg/kg every 6 hours over 4 days has been authorised and marketed in France, in Austria, and in Spain.

Nowadays, only an oral form of busulfan (2 mg tablets) is marketed in the European Union. It is currently licensed since more than ten years in twelve European countries for the palliative treatment of chronic myeloid leukaemia. The use of oral busulfan, administered at high doses (*i.e.*, 1 mg/kg x 16 doses, administered over 4 days) for the preparative treatment before HSCT is current practice in Europe and elsewhere, although this particular indication has been authorised only in some Member States.

Busulfan-based conditioning therapy produces high anti-tumour efficacy. However, these high doses (up to 280 mg/day) currently require patients to swallow large numbers of tablets (30 tablets every 6 hours, 120 tablets a day). This method of drug delivery is unpredictable as it is often compromised by vomiting, variation of dietary intake, concurrent drugs and inherent biological variability in intestinal absorption among patients. Also, oral busulfan is poorly water-soluble leading to unpredictable systemic exposure (Grochow 1993; Hassan, Ehrsson et al. 1996). The steady-state busulfan concentration has been correlated with graft rejection and disease relapse. Some studies also suggest a direct relationship between the severity of conditioning regimen toxicities, especially veno-occlusive disease (VOD) of the liver, and high plasma levels of busulfan (Grochow, Jones et al. 1989; Slattery, Sanders et al. 1995; Dix, Wingard et al. 1996).

Rationale for intravenous high dose busulfan

It is recognised that as a result of these factors, the oral administration of high-dose busulfan is associated with an inherent safety problem both from the risk for lethal toxicities from inadvertent overdosing and the potential for recurrent or persistent malignancy after transplantation as a result of under dosing.

Consequently, the main benefits expected from an i.v. form are:

- To provide a well-controlled dose to the patient, avoiding problems of compliance with the oral form due to, e.g., vomiting.
- To reduce inter-individual variability on blood exposure by administering a 100% bioavailable dose to every patient at every administration. A lower inter-patient variability would result in a less frequent over- or under-exposure.
- To reduce the high hepatic concentration of busulfan resulting from hepatic first pass through the liver, which is suspected to be involved in the risk of hepatic VOD.
- To offer a more comfortable way of administering to patients having difficulties in swallowing numerous pills, such as elderly people or children (500 to 600 pills for an adult over 4 days), or to those with anticipatory nausea.

The scope of the current application involves a pharmaceutical form of busulfan for intravenous use, exclusively for high-dose conditioning treatment. Concerning the traditional low-dose indication(s), such as treatment of chronic myeloid leukaemia and other myeloproliferative disorders, these are

outside the scope of the current application, and a positive benefit-risk ratio is not claimed in these conditions.

2. Chemical, pharmaceutical and biological aspects

Composition

The product consists of the active substance; busulfan dissolved in a vehicle of polyethylene glycol 400 (macrogol 400) and dimethylacetamide in a ratio of 2:1. It appears as a clear solution in a 10ml, single use, glass ampoule. It is presented as a concentrate (6mg / ml) which should be diluted to approx. 0.5mg/ml prior to use as stated in the Summary of Product Characteristics (SPC).

Active substance

Busulfan INN PhEur is 1,4-di(methylsulfonyl)butane, an established pharmacopoeial substance

It is a white crystalline solid synthesised from the addition of methanesulfonyl chloride to 1,4-butanediol in triethylamine and THF. The crude product is purified by recrystallisation from acetone and water. The simple structure of busulfan has been confirmed by spectroscopic evidence; it is achiral. Solid-state properties of the active substance are not relevant as the final product is a solution.

Specification of the active substance

The specification is in line with the PhEur specification and includes tests for identity, assay (high performance liquid chromatography - HPLC and potentiometric titration), and related substances by HPLC, water content and residual solvents etc., as well as microbial contamination. Analytical methodology has been validated and the results of recent batch analyses (n=12) indicate satisfactory uniformity and compliance with specification.

Stability of the active substance

Four batches of drug substance were included in stability studies following the CPMP stability guideline. All controls carried out in these studies were stability demonstrating.

From the results presented, a 3-year re-test period could be authorised without any special storage conditions.

Other ingredients

The other ingredients are Macrogol 400 PhEur and dimethylacetamide controlled to the pharmeuropa monograph. It was concluded that the aforementioned materials are of satisfactory quality and pose no concern of risk in the context of TSE.

Product development and finished product

The objective of the formulation work was to develop a busulfan dosage suitable for intravenous infusion. A potency of 6 mg/ml was desired for the parenteral formulation. Due to the poor aqueous solubility of busulfan, (0.1 mg/ml) a non-aqueous system was necessary to prepare a concentrate. Also, as the main mechanism of busulfan degradation was hydrolysis, aqueous compositions were rejected.

Some solubilising compositions were evaluated, and different busulfan concentrations in solution were achieved. Taking into account the high solubility of busulfan in DMA and since PEG 400 appeared to be a physiologically acceptable co-solvent; various ratios of both were evaluated to obtain the desired 6-mg/ml potency. Keeping in mind the need to avoid the leaching from a rubber stopper, an ampoule made of type I glass was chosen as the primary packaging. The chemical resistance to interactions with the components of glass containers was also an important consideration.

The labelled volume is 10.0 ml. although overfilling was necessary to compensate for the viscosity of the liquid and to make possible the total extraction of the volume.

Concerning development of the manufacturing process, busulfan is known to be sensitive to heat, therefore heat sterilisation was discounted in favour of sterilisation by filtration (0.2 micron hydrophobic polyvinylidene fluoride membrane). The process was qualified in terms of:

- Filter integrity test
- a bacterial retention study performed with various values of filtration parameters such as flow rates and differential pressures which simulate a worst-case-scenario.
- a quantitative estimation and qualitative characterisation of materials that are extracted from the sterilising filters by the product, and their biological effects.

Added sterility assurance is given by the fact that, due to the nature of busulfan and the vehicle, microorganisms do not proliferate in the drug product, which in fact has a biocidal effect, even against *A. niger*.

The manufacturing process is straightforward with mixing, filter sterilisation and filling under nitrogen.

Product Specification

The release specification for the product includes relevant tests and acceptance criteria for assay, degradation products, water, endotoxins, sterility, etc. Batch analytical results indicate satisfactory compliance with the agreed specification.

Stability of the Product

The results of forced degradation studies (excessive heat, light and oxidative stress) indicate that the product is not sensitive to light, peroxide or oxygen; no change in colour and no degradation was observed. In acidic, basic and heat conditions, the product is sensitive to hydrolysis and the stability is directly dependent on solution moisture levels.

Stability results deciding the shelflife were derived from 4 production batches stored under ICH and temperature-cycling conditions for up to two years. Since the product is intended to be stored in the refrigerator these were: accelerated (25°C / 60% RH) and long term (refrigerated, 2 - 8°C). As expected, the decrease in busulfan assay in accelerated conditions was very pronounced, > 10% loss after 6 months, correlating with a pronounced increase of degradation products. In long-term conditions (2 – 8 °C) a better stability profile has been observed. Appearance, colour of the solution and water content do not change, and particulate matter results are acceptable.

An appropriate shelf-life specification was defined, which is larger than the release specification for assay and impurities. At certain stability time points the particulate matter has also been verified.

In summary the results support the product shelf life and storage conditions as defined in the SPC.

Stability during use, after dilution

The stability of busulfan has been examined after dilution of the product in 5% dextrose and in 0.9 % sodium chloride for injection (saline), Busulfan was found stable after dilution at the required final concentration (0.5 mg/ml), in PVC perfusion bag, not protected from light, for:

- 8 hours after dilution in 5% dextrose or 0.9% sodium chloride (saline) for injection, stored at 20°C ± 5°C
- 12 hours after dilution in 0.9% sodium chloride (saline) for injection stored at 2°C-8°C followed by 3 hours stored at 20° C ± 5°C

From a microbiological point of view, the product should be used immediately after dilution. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than the above-mentioned conditions when dilution has taken place in controlled and validated aseptic conditions.

Concerning the loss of busulfan by interaction with plastic infusion materials, busulfan solutions prepared at 0.1 mg/ml in both infusion media showed that there is no significant loss when the product is stored in PVC bags for up 8 hours at room temperature or when passed through an i.v. administration set.

A study of extractables has been conducted to determinate the compatibility of busulfan diluted and undiluted with PVC bag/tubing/polypropylene syringe and syringe filters after 12 hours storage at 25°C. Extractables detectable by UV were not observed in the samples.

Discussion on chemical, pharmaceutical and biological aspects

The purity of busulfan active substance, and the control of the manufacturing process and specification for the finished product indicate reliable *in vitro* reproducibility of this medicinal product. The filtration sterilisation process has been well validated and the intrinsic biocidal properties of the composition indicate no concerns relating to microbiological safety. Stability of the product has also been shown to be satisfactory. Furthermore, when diluted in accordance with directions in the SPC the *in-use* stability has been investigated and found to be maintained for several hours.

In general, the information provided in the Quality dossier suggests this is a product that should have satisfactory and uniform quality characteristics from batch to batch.

At the time of the opinion, there were a number of unresolved minor pharmaceutical issues having no impact on the benefit/risk balance of the product. The applicant committed to resolve these by means of post-opinion Follow Up Measures within an agreed timeframe.

3. Toxicopharmacological aspects

The dossier includes a literature review of the non-clinical aspects of busulfan and the vehicle for injection N, N-dimethylacetamide (DMA) and polyethylene glycol 400. The applicant according to GLP undertook additional non-clinical toxicology studies of the new formulation for intravenous use.

Pharmacodynamics

The primary molecular action of busulfan is alkylation of intracellular nucleophiles. Both proteins and nucleic acids are affected. With regard to DNA, busulfan reacts with guanine residues to form a four-carbon di-guanine DNA cross-linkage with release of methylsulfonate (Tong and Ludlum 1980; Douglas 1984; Buggia, Locatelli et al. 1994). The DNA cross-linkage causes misreading of the DNA code and single-strand breakage. The degree of DNA cross-linkage has been shown to be proportional to the dose and cytotoxicity of the compound (Chaney and Sancar 1996). Busulfan-induced cross-linkages of DNA to nuclear proteins may also occur and is considered a cytotoxic mechanism (Buggia, Locatelli et al. 1994). Busulfan has also been reported to esterify phosphate groups of chromosomal DNA, accounting for the fragmentation of chromosomes seen in various cell types after treatment (Buggia, Locatelli et al. 1994). Chromosomal damage further contributes to the overall cytotoxic effect.

Studies of cloned tumour and normal cells indicate that busulfan interrupts certain parts of the cell mitotic cycle. While busulfan can act on cells at any stage of mitosis, similar to other alkylating agents, *in vitro* studies with tumour cell lines indicate that replicating cells are most sensitive to busulfan in the late G1 phase and that progression through the cell cycle is blocked in the G2 phase. Cultured cells in the S-phase, which involves DNA synthesis, are not as affected because the DNA repair systems are active at this stage (Buggia, Locatelli et al. 1994). Clinically, busulfan is cytotoxic to proliferating tissues, especially granulopoietic cells in the bone marrow.

Studies of busulfan resistant strains of cultured tumour cells support the idea that the DNA cross-linkage is a primary toxic action of busulfan. Resistant cells can eliminate the DNA cross-linkage produced by the drug within six hours after a busulfan exposure. In contrast, DNA cross-linkages were unchanged in similarly treated cultures of non-resistant, busulfan sensitive cells, even after twenty-four hours (Vendrik, Bergers et al. 1992). Busulfan enters cells by passive diffusion, so an alteration of transport is not involved as a mechanism of drug resistance (Fox, Boyle et al. 1991). *In vitro* studies of cultured marrow cells indicate that the lower range of cytotoxic concentrations of busulfan is relatively selective for granulocytes as compared to cells giving rise to other blood elements, such as platelets and lymphocytes (Buggia, Locatelli et al. 1994). As the dose of busulfan is increased, cultured cells from all three haematopoietic cell lines are affected (Black and Livingston 1990; Buggia, Locatelli et al. 1994).

The selectivity of busulfan for granulocytes has been related to differences in the cellular permeability of granulocytes to busulfan relative to other cells (Buggia, Locatelli et al. 1994). Alternatively, other authors suggest that the relative susceptibility of haematopoietic stem cells to busulfan is because they

remain in the G1 phase of the cell mitotic cycle longer than the cells that spend significant time in the S-phase (Dunn 1974; Bishop and Wassom 1986). This latter explanation fits with the effects of busulfan on other tissues, such as reproductive stem cells or the ocular lenticular cells, which also spend a relatively extended time in the G1 phase of the cell mitotic cycle. The susceptibility of these cells to busulfan supports the view that busulfan is cytotoxic to slowly proliferating stem cell compartments (Dunn 1974; Bishop and Wassom 1986).

- *In vivo* studies

The acute effects of busulfan involve the reduction of the number of haematopoietic precursor or stem cells found in bone marrow. Oral doses of 29 to 80 mg/kg have, in the mouse, been shown to produce an acute 10% cell loss [D₁₀] sixteen to twenty-four hours after treatment. The slower reproducing haematopoietic stem cells (identified as spleen colony forming units in assays) were reduced following acute exposure to busulfan. The more differentiated precursor cells developing into granulocytes and erythrocytes were reduced to a lesser extent (Dunn 1974). In addition to reducing the number of stem cells, there is evidence that a proportion of those stem cells surviving acute busulfan exposure have an altered capacity to produce differentiated progeny as a result of genetic damage (Dunn 1974). Longer-term studies suggest that busulfan induces a defect in the ability of haematopoietic cells to replicate and differentiate in the bone marrow. Eighty percent of the mice treated orally with busulfan doses of 20 mg/kg eventually experienced complete marrow loss accompanied by aplastic anaemia and death when the group was followed for 240 days post-exposure. The marrow failure was associated with a marked reduction (*i.e.*, less than 1% of pre-treatment values) in the numbers of both spleen and agar colony forming marrow progenitor cells (Morley and Blake 1974).

The biological effect of busulfan has been studied in the rat by Dunn (Dunn and Elson 1970; Dunn 1973). In the rat, single doses of busulfan are reported to produce a delayed decrease in mature haemopoietic cells that can not be explained by a delayed absorption of busulfan. Further studies (Dunn and Elson 1970; Dunn 1973; Dunn 1974) have indicated that busulfan has a relatively selective effect on spleen colony forming units (CFU), initially sparing the mature haemopoietic cells, which decline later in time. In one such study (Dunn 1974), a single 10 mg/kg i.p. dose of busulfan in rats produced a maximal 90% decline in the survival of CFU by 2 days after injection, followed by a slow steady recovery of normal levels over the ensuing 3 weeks. Increasing the dose of busulfan beyond 10-mg/kg i.p. was reported to cause no further effect, in spite of the fact that radiation or other cytotoxic drugs were able to kill additional CFU. This phenomenon has led to the postulation CFU may exhibit a cell cycle phase resistance to the toxic actions of busulfan, especially during the DNA-synthetic period (S period) of the cell cycle. Additional studies have supported the view that busulfan is differentially toxic to CFU in the G₁ or G₀ state of the cell cycle (Dunn 1974). In conclusion, it appears that busulfan inhibits bone marrow stem cells.

In dogs, busulfan is profoundly myelotoxic (Phadungpojna, Andersson et al. 1996). Four consecutive i.v. daily doses of 4 mg/kg/day over a two-hour infusion every six-hours produced bone marrow depletion in the rib and femur by day five after the first day of treatment. Lower doses resulted in decreased platelet and white cell counts on day five. Historically, high repeated oral doses of busulfan (doses not given) have been used in dogs as preparation for grafting bone marrow from MHC identical litter mates (Storb, Weiden et al. 1976). Deeg and coauthors carried out a more recent dose escalation study in a canine model with an intravenous busulfan preparation (Deeg, Schuler et al. 1999). The goal was to determine a marrow-ablative dose and to test the ability of autologous marrow to reconstitute hematopoiesis in dogs so treated. Marrow ablation was achieved at 20 mg/kg given either as a single dose or in four daily increments of 5 mg/kg each. Four dogs were given busulfan at 20-mg/kg followed 30 hours later by infusion of autologous marrow, and all showed prompt and complete hematopoietic reconstitution.

- *Pharmacodynamic drug interactions*

No pharmacodynamic interaction study or literature reference with busulfan and other possible co-administered drugs have been submitted.

- *General and safety pharmacology programme*

Few general acute pharmacological effects of busulfan are reported in the literature. Doses of busulfan in the myelotoxic range produce emesis and myoclonic seizures in animals and man, but treatment with anticonvulsant drugs successfully prevents the seizures and droperidol and other antiemetics have been useful in treating emesis.

Neuropharmacology

As a small lipophilic molecule busulfan has been determined to easily cross the blood brain barrier. High doses of busulfan have been reported to cause acute convulsions and death in mice (Fitzsimmons, Ghalie et al. 1990). Seizures, and the resulting death of the animal, are prevented by pretreatment with either phenytoin (i.p) or phenobarbital (i.p) (Fitzsimmons, Ghalie et al. 1990). Valproic acid was not effective in preventing the convulsions or death produced by busulfan in mice.

Cardiovascular

Acute i.v. administration of myelotoxic doses of busulfan has been reported to cause flushing in Rhesus macaques but no other acute cardiovascular events are mentioned (Dix, Bucur et al. 1995). In man, treatment with high myeloablative doses of busulfan (14 mg/kg orally) and cyclophosphamide during conditioning for bone-marrow transplant was reported to cause sudden cardiac tamponade in 2% of thalassaemic patients (Angelucci, Mariotti et al. 1992). This effect was caused by the accumulation of a massive volume of clear fluid in the pericardial cavity. It appears to be related to systemic iron overload and haemochromatosis rather than cardiac dilation, microthrombi, focal artery wall necrosis, or myocardial cell necrosis (Angelucci, Mariotti et al. 1992). Reports of cardiac tamponade, or similar actions in animals were not found in the literature. Also, there were no reports of acute changes in cardiovascular function after busulfan treatment.

Respiratory

In man, use of busulfan for bone marrow transplantation has caused subclinical histopathological changes in both pulmonary epithelial cells (Israel-Biet, Labrune et al. 1991) and proliferating type II alveolar cells (Hook and DiAugustine 1976; Pietra 1991). These changes were dose-related (Comis 1985). The nuclear constituents of the affected cells were characterised by large, bizarre hyperchromatic nuclei that contained coarse chromatic granules. Such cells are found not only in tissue, but also in sputa and bronchoalveolar lavage fluid and serve as a reliable marker of the degree of drug toxicity. Large numbers of these cells can signal impending pulmonary fibrosis (Pietra 1991). Fibrotic changes in lung tissue after busulfan administration begin with accumulation of cellular debris from dead and dying alveolar cells. The cellular debris becomes infiltrated with fibroblasts and organised with the placement of mature fibrotic tissue. Eventually, large fibrous areas occur in the lung, giving rise to «busulfan lung» (Littler, Kay et al. 1969; Filipek 1979).

Gastrointestinal

Busulfan doses of 100 mg/m², 150mg/m², or 200mg/m² i.v. on two consecutive days resulted in a 37% incidence of emesis in Rhesus monkeys (Dix, Bucur et al. 1995). In man, the lower doses of busulfan used in cancer chemotherapy are virtually devoid of gastrointestinal activity while the higher cytotoxic doses of oral busulfan used in transplant programs cause nausea, vomiting and stomatitis, liver function abnormalities and hepatic VOD (Buggia, Locatelli et al. 1994). Hepatic VOD is a recognized complication of conditioning therapy prior to transplant. Dogs appear to be somewhat more sensitive to myelotoxicity of busulfan than humans (Epstein, Min et al. 1992). VOD is the most serious liver toxicity observed after high-dose busulfan-containing regimens (Vassal 1994). In man, high oral doses of busulfan (1 mg/kg) administered every six hour for four days have been reported to deposit busulfan crystals in liver cells (Buggia, Locatelli et al. 1994) which has resulted in non-thrombotic obstruction of intrahepatic veins (Vassal 1994). The pathophysiology of VOD is multifactorial involving cytotoxic, immunologic, inflammatory and hemostatic events that are not mutually exclusive.

Spermatogenesis

Busulfan blocks spermatogenesis in rats and other species by acting on early spermatogonia and germ cells to prevent mitosis; this may ultimately result in sterility (Dunn 1974). When given prior to

puberty, doses of 10 mg/kg or higher produce sterility, destruction of seminiferous epithelium and abnormal gonadal development and maturation (Dunn 1974; Bishop and Wassom 1986). Single oral doses of busulfan as low as 10 mg/kg can cause infertility in male rats by killing type A1 spermatogonia. Higher doses (20 to 60 mg/kg) or longer exposure to lower doses (2 mg/kg i.p. at two-week intervals over 150 days) killed all A1 spermatogonia (Bishop and Wassom 1986).

Immunoreactivity

Studies in mice have indicated that busulfan depresses the immune system (Dunn 1974; Bishop and Wassom 1986).

Ocular

Busulfan treatment has been reported to produce lenticular changes and cataracts in both animals and man (Grimes and Von Sallmann 1966; Dunn 1974; Vizek and Oster 1982; Fraunfelder and Meyer 1983) corneal thinning has been reported after bone marrow transplantation preceded by high-dose oral busulfan treatment.

Pharmacokinetics

Absorption

For the proposed intravenous route of administration of Busilvex immediate and full absorption into the systemic circulation is expected.

The pharmacokinetics of i.v. busulfan has been explored in rats and dogs in comparison to oral busulfan. For both routes of administration, AUC-dose proportional increase was observed.

The pharmacokinetics of busulfan following single intravenous administration was linear over the dose range administered and less variable than following oral administration to rats. The drug was eliminated from plasma, following intravenous and oral dosing, in a first-order fashion with mean elimination half-lives of 2.2 and 2.0 h, respectively. The pharmacokinetics parameters were less variable with the i.v. formulation than for the oral tablet. The route of administration did not affect half-life, clearance and volume of distribution.

Distribution

Distribution data in mice and in rat were supported by literature on i.v. busulfan (Bishop and Wassom 1986). Additional data on distribution in monkey were presented

It has been demonstrated that whilst busulfan is cleared rapidly from the blood of mice and rabbits following i.v. injection, with only 10% of the administered dose being present after 5 or 10 minutes (Bishop and Wassom 1986) a low level of drug remains in the plasma for 24 to 48 hours.

Rapid tissue distribution was confirmed in a study using monkeys (Hassan, Oberg et al. 1992) Busulfan was labelled in the alkylating portion of the molecule ($1\text{-}^{11}\text{C}$). The authors administered i.v. ^{11}C -busulfan and determined the distribution of the radioactivity by positron emission tomography. The liver was found to accumulate increasing amounts of busulfan throughout a 45-minute observation period. In addition, significant drug levels were measured in the lungs and brain. In all three organs, distribution occurred within minutes. The radioactivity accumulated in the liver was 9-fold higher than in the brain at the end of the experiment. The compound showed also a tendency to accumulate in the lungs with time. Nevertheless, the peak of radioactivity reached in the brain within 2-4 min subsequently declined (approximately 50%). No data is available on the distribution in other tissues.

Comparison of the distribution of busulfan was studied in rats following single and multiple dosing by intravenous and oral routes of administration (0.6-0.75mg/kg). Six major organs were harvested at each of four time points from 5 to 360 minutes post-dose and busulfan levels quantified using HPLC assay. The levels peaked 5 minutes after i.v. single or multiple dose with a descending order of heart $0.829\mu\text{g/g}$ > brain > lung > kidney = liver > spleen ($0.316\mu\text{g/g}$). The levels were found to have declined significantly after 6 hours and that after 16 doses there was no apparent accumulation (Phadungpojna, Gong et al. 1996)

In another distribution study, ¹⁴C-labelled busulfan was administered i.p. at a dose of 15 mg/kg to rats (Hassan, Ehrsson et al. 1988). Plasma and brain tissue samples were collected for up to 24 hours. Concentration curves for brain and plasma were found to be remarkably similar and confirmed rapid tissue distribution. These curves remained parallel throughout an eight-hour collection time; the levels in the brain were only slightly lower than those in the plasma. The elimination half-lives in plasma and brain were also similar and calculated to be approximately three hours for intact busulfan and 8 hours for total ¹⁴C-radioactivity. Plasma protein binding was low, 9.2 ± 4.4%. Plasma and brain samples collected revealed at least three metabolites: 3-hydroxysulfolane, tetrahydrothiophene-1-oxide, and sulfolane.

Metabolism

Data on metabolism were provided from literature review. Relevant information was available on the metabolism pathway of busulfan in rat and from a cytotoxicity study on the metabolites (Hassan and Ehrsson 1987) after oral administration.

[¹⁴C] busulfan was administered i.p. (15 mg/kg) to 5 male Sprague-Dawley rats (Hassan and Ehrsson 1987). For 72 hours, the urinary recovery of ¹⁴C was approximately 70% of the total dose, while the fecal excretion was within the range 1.5-2%. The pattern of the urinary metabolites of busulfan was studied by HPLC in combination with radioactivity detection of the pooled urine. At least eight radioactive fractions could be separated. Three major metabolite peaks were identified by GC/MS and NMR spectroscopy: 3-hydroxysulfolane (39% of total urine radioactivity), tetrahydrothiophene 1-oxide (20%), and sulfolane (13%). Busulfan (6%) and tetrahydrofuran (2%) were also identified. A sulfonium ion glutathione conjugate was hypothesised as another metabolite, but was not isolated because it was very unstable. However, another compound was observed. This metabolite co-eluted with the sulfonium ion obtained of the reaction of busulfan with N-acetyl-L-cysteine and produced tetrahydrothiophene when hydrolyzed. Finally, busulfan and the three main metabolites were tested for cytotoxicity on Chinese V79 hamster cells *in vitro*. Cell toxicity was induced only by busulfan, which indicates that the cytotoxicity *in vivo* is mediated by the parent compound, as expected.

In another experiment, the liver was perfused and the common bile duct cannulated (Hassan and Ehrsson 1987). Busulfan was added to the perfusate and bile was collected for four hours. HPLC analyses of the bile indicated only parent compound and one metabolite, the sulfonium ion of glutathione, or γ -glutamyl- β -(S-tetrahydrothiophenium) alanyl-glycine. When GSH-transferase was inhibited by the addition of ethacrynic acid, the amount of the sulfonium ion excreted in the bile significantly decreased and the amount of busulfan excreted increased significantly. These data support the previous study in which the presence of the glutathione conjugate was only hypothesised.

In a similar experiment, Marchand et al administered busulfan i.v. at a dose of 1 mg/kg via a jugular cannula to male rats and bile was collected for 8 hours (Marchand, Rimmel et al. 1988). Peak biliary excretion occurred at 90 minutes and declined thereafter, but an increase in the amount of administered dose excreted in the bile was observed over the 8-hour time period. Twenty-six percent of the dose was the glutathione-sulfonium metabolite.

Pharmacokinetic drug interactions

As stated in the SPC, all patients should be premedicated with anticonvulsant drugs to prevent seizures reported with the use of Busilvex. Phenytoin and phenobarbital effectively prevented acute neurotoxicity in 60 and 68% of mice respectively (Fitzsimmons, Ghalie et al. 1990). Aroclor did not, and it is possible that this is due to the fact that only a single dose Aroclor was administered.

Studies in murine models suggest that an inducible enzyme system such as cytochrome P450 or glutathione S-transferases plays an important role in the metabolic conversion of busulfan. Concomitant administration of enzyme inducing anticonvulsants such as phenytoin and phenobarbital, or inhibiting, may affect the myeloablative efficiency of high-dose busulfan.

Toxicology

Most of the toxicology studies in the application were based on the literature of oral busulfan. Busulfan is the drug of preference in treatment of chronic myelogenous or granulocytic leukemia because its cytotoxic activity results in primary damage or destruction of hematopoietic cells.

Additional effects resulting from the cytotoxicity of busulfan in hematological and other tissues, as documented by both human and animal model studies, include lethality, sterility, teratogenicity, and alteration of immune function. Busulfan has been shown to be mutagenic to microorganisms, mammalian cells in culture, *Drosophila*, and rodents. This agent is also considered potentially carcinogenic to humans. Various tissue hyperplasia and preneoplastic cells have been observed in animal model studies with busulfan, and case reports on human patients implicate busulfan as the causative agent in induction of secondary malignancies (Bishop and Wassom 1986).

Busulfan is genotoxic (Bishop and Wassom 1986) and it is classified as a carcinogenic agent in humans (Group 1) according to the International Agency for Research on Cancer (IARC 1982).

The applicant has conducted a single dose toxicity study in rat, and a repeated dose toxicity studies in dogs (dose range-finding and definitive toxicity studies) following the recommended human schedule of administration to evaluate the safety of the finished product administered intravenously.

Single dose toxicity study with busulfan injection formulation

Acute lethality studies of busulfan have been extensively conducted in rats and mice (Bishop and Wassom 1986). The authors reported an LD₅₀s ranging from 30 to 125 mg/kg for mice and from 14 to 25 mg/kg for rats. Delayed death due to the myelotoxic effects of busulfan is reported (Morley and Blake 1974) to result in LD₅₀ values in mice between 15 and 30 mg/kg i.p.

The applicant has conducted an acute intravenous toxicity study in rats to qualify impurities. Single doses of 1, 5, 10, 20 or 40 mg/kg of two lots of the busulfan formulation were administered, one lot representing a standard batch and one lot that underwent forced degradation. The degraded busulfan formulation was less toxic than the reference formulation, possibly due to a reduced quantity of active drug in this formulation. At the higher dose (40mg/kg) the early deaths were probably a consequence of acute CNS effects of DMA (DMA dose 2164mg/kg compared to published LD50 value for DMA by the intravenous route in rats of 2640mg/kg and the therapeutic human exposure of 166mg/kg/day) whereas at 20mg/kg (and a reduced administered dose of DMA) the typical delayed toxicity of busulfan (exaggerated pharmacological response) was evident.

Repeated dose toxicity study with busulfan injection formulation

A dose range-finding study and definitive toxicity study were conducted in male and female dogs. Busulfan was administered as a two-hour infusion at a rate of 0.5 mL/kg/hr every 6 hours (*i.e.*, q.i.d.) for four days followed by a saline flush.

In the range-finding study, five i.v. dose groups (saline control, vehicle control DMA and PEG, 0.25, 1, and 4 mg/kg) and one oral dose group (1 mg/kg) were used. Blood samples were collected for toxicokinetic evaluation. Animals were monitored for 28-days post-dosing for clinical signs and other treatment-related effects.

Two high-dose animals have been euthanised *in extremis* by Day 7. Bone marrow depletion, hepatocellular necrosis and a variety of lung lesions (indicative of sepsis) were believed to contribute to the deaths. Haematology effects such as decreased mean white blood cell counts were observed in the surviving animals in the 1-mg/kg i.v. and oral groups. Decreased mean platelet levels were reported in all treatment groups.

In the definitive toxicity study, busulfan was administered intravenously to four dogs/sex/group at doses of 0.25, 0.5, or 1 mg/kg as a two-hour infusion at a rate of 0.21 mL/kg/hour q.i.d. for four days followed by a saline flush. Saline and vehicle (*i.e.*, DMA and PEG 400) control groups also received the same infusion regimen. Following cessation of treatment, three dogs per group were sacrificed. The remaining one animal/sex/group was allowed to recover for 28 days

The toxic effects of administration on haematological parameters (decreases in white blood cells and platelet counts) observed in dogs were dose related. Myelotoxicity is the sought effect of the conditioning regimen with busulfan. Hyperbilirubinemia, alkaline phosphatase increase, alanine aminotransferase and gamma glutamyltransferase elevations observed in the high dose group, suggesting liver damage. These altered enzyme levels returned to normal during the recovery period. Vehicle administration (at a concentration equivalent to the 1.0 mg/kg q.i.d. group) caused cytoplasmatic vacuolation and fibrosis in liver. This hepatotoxicity is consistent with the toxicity of

DMA described in the scientific literature these findings were also observed in the animals groups that received busulfan at the doses of 0.5 and 1.0 mg/kg. The incidence was similar. The dose of DMA administered in the vehicle group and in the high dose intravenous group is inferior to the dose of DMA in patients at the recommended posology of intravenous busulfan (on a mg/m² basis). The applicant concluded that 0.25 mg/kg q.i.d. was the no-observable-effect-level (NOEL).

Reproductive and developmental toxicity

Busulfan produces sterility, as determined in male and female hamsters, rats and mice and is teratogenic in mice, rats and rabbits (Bishop and Wassom 1986).

Local tolerance

No specific study of local tolerance has been undertaken with busulfan injection. In the repeated dose toxicity study in dog, cellular changes in the vessels at the sites of injection were observed. The histopathological data for the injection site (the site immediately surrounding the catheter tip) suggested that reactions were related to the catheter and not to the vehicle or busulfan. The recovery data indicated that most changes were reversible.

Studies on excipients and impurities

SPF-NMRI mice (n = 5/sex/group) and Sprague-Dawley rats (n = 5/sex/group) received single intravenous, intraperitoneal or oral doses of DMA and the lethal doses were determined. For mice, LD₅₀ values of 3.2, 3.4 and 4.9 ml/kg were reported following administration by the three respective routes. In rats, LD₅₀ values of 2.8, 3 and 5.4 ml/kg were reported for intravenous, intraperitoneal and oral dosing, respectively (Bartsch, Sponer et al. 1976).

Effects resulting from single DMA exposures have been summarized by Kennedy review (Kennedy 1986). Rats treated orally with 2.25 g DMA/kg or greater (lethal doses) exhibited pulmonary edema and congestion, hepatic necrosis and congestion, and renal tubule swelling. In other study in rats, an oral LD₅₀ of 3.5 g/kg was described. Death occurred within 24 h due to respiratory failure. Autopsy revealed generalized haemorrhage in several organs with degeneration of isolated nerve cells and necrosis of the liver and kidney. Following intravenous injection in cats and dogs, 95 mg/kg produced no blood pressure effects, 236 mg/kg produced mild hypotension in both species and 472 mg/kg was lethal to cats. LD₅₀ (i.v.) in both species was approximately 0.24 g/kg. Ocular instillation resulted in mild, but reversible corneal injury in rabbits.

In mice, most deaths from a single dose of DMA (intravenous, intraperitoneal, or oral) occurred following depression and coma during the first day posttreatment (Kim 1988). Some deaths were delayed as long as 2 weeks. The pattern of mortality in rats was dose related. At the high dose levels the mortality in rats occurred between 5-10 min to 26 h, whereas at the lower lethal dose levels the mortality was protracted, occurring up to 11 days.

DMA is teratogenic in rats and may impair fertility. Repeated doses of DMA produce signs of liver toxicity, the first being increases in serum clinical enzymes followed by histopathological changes in the hepatocytes. Higher doses can produce hepatic necrosis and liver damage can be seen following single high exposures. The ICH Note for Guidance on Impurities: Residual Solvents (CPMP/ICH/283/95) classifies DMA as a level 2 solvent, the use of which should be limited due to its potentially significant toxicity. The limit for permitted daily exposure (PDE) is 10.9 mg/day. In the intravenous busulfan injection formulation, DMA will exceed this exposure level (166 mg/kg/day of DMA), resulting in a total daily dose of approximately 12.3g. This also corresponds to approximately 42% of the maximum-tolerated dose on a mg/kg basis, as established in a phase I trial in man, exploring DMA as a potential anticancer agent (Weiss, Jackson et al. 1962). Reviews on the toxicity of DMA agree upon the fact that the main target organ of DMA by administration of single or repeated doses in dogs is the liver. The dose-limiting toxicities of DMA in the phase I trial were hepatic toxicity and CNS effects (Weiss, Jackson et al. 1962). The earliest recognizable signs, generally noted on the second or third day of treatment, were depression, lethargy, and occasionally confusion and disorientation. Lethargy and confusion became very severe in 5 patients.

Triethylamine

Triethylamine (a potential impurity that could be present in busulfan drug substance when manufactured by the commercial process proposed) was not detected using a gas chromatographic method (the detection and the quantitation limits were determined respectively at 0.008% and 0.025% respectively).

Ecotoxicity/environmental risk assessment

The Applicant has provided an estimate of the potential environmental exposure (PEC) in the aquatic compartment, assuming all unchanged drug is excreted in the urine, and there is no biodegradation, adsorption, or other removal of the product from waste. Based on the data submitted, no adverse environmental effects are predicted from intravenous busulfan.

Busulfan is toxic in contact with skin, and if swallowed or inhaled. Overexposure to busulfan in the occupational setting may result in the same adverse effects which have been observed in experimental studies or when this substance is used medicinally. Acute exposure in sufficient dose may result in anaemia, leukopenia, and thrombocytopenia. Therefore, procedures for proper handling and disposal of cytotoxic medicinal products should be followed. Any unused product or waste should be disposed of in accordance with local requirements for cytotoxic drugs (see SPC, section 6.6).

Discussion on toxico-pharmacological aspects

The mechanism of action of busulfan is similar to that of other alkylating agents. It produces DNA cross-linking and chromosomal damage that can be lethal to rapidly dividing cells. At the low end of the active dose range, busulfan causes a selective depression of granulocytogenesis. Increasing doses lead to progressive general myelotoxicity culminating in marrow ablation due to cell death.

While intravenous administration circumvents problems associated with variable absorption after oral administration, it does not prevent individual variability related to drug elimination and other processes.

Studies in murine models suggest that an inducible enzyme system such as cytochrome P450 or glutathione S-transferases plays an important role in the metabolic conversion of busulfan. Therefore, concomitant administration of enzyme inducing or inhibiting substances may affect the myeloablative efficiency and modify the profile of toxicity (neurotoxicity, hepatotoxicity) of busulfan.

The duration of Repeated dose intravenous toxicity study in Dog, is considered too short. Nevertheless, taking into account the clinical studies performed with intravenous and the clinical experience with high dose oral busulfan, longer-term repeated-dose studies are not considered necessary.

The CPMP Note for Guidance on the Pre-clinical evaluation of anticancer medicinal products (CPMP/SWP/997/96) would require that repeated dose studies in both rodent and non-rodent species are conducted. No repeated dose toxicity study in rodents has been provided. However, it is considered that the extensive reports of high-dose oral busulfan toxicity in clinical use and the repeated dose toxicity study in dogs provide adequate information. Concerning the latter, one cannot eliminate the possibility that the hepatic toxicity findings are related to the excipient and a potential risk of liver toxicity in humans due to the DMA content in Busilvex.

Busulfan is genotoxic. With regard to carcinogenicity, the human data are sufficient to establish busulfan as a human carcinogen. As the SPC states in section 4.4, the increased risk of a second malignancy should be explained to the patients.

Busulfan produces sterility, as determined in male and female hamsters, rats and mice and is teratogenic in mice, rats and rabbits. In view of the preclinical information and the absence of data from adequate, well-controlled patient studies, patients should be made fully aware of the potential hazards to reproduction of busulfan injection. Adequate information has been included in the SPC (section 4.4) and package leaflet in relation to infertility and teratogenicity. Also, busulfan is contraindicated during pregnancy.

Overall, the main toxicity of busulfan is neurotoxicity and damage to the liver. Thus, the risk of hepatic toxicity due to DMA was of concern. However, based on the clinical data, i.v. busulfan did not

appear to be associated with additional toxicity compared to oral busulfan. Comparison of liver function data for i.v. busulfan and oral busulfan did not show major differences between the two formulations (see Clinical Documentation). This is reflected in the SPC (section 4.8). It is not considered necessary to carry out additional local tolerance testing.

4. Clinical aspects

*The overall clinical development program for Busilvex in adult patients consists of two pharmacokinetic studies (OMC-BUS-2 and Amendment 4 to OMC-BUS-3/OMC-BUS-4) and two phase II studies (OMC-BUS-3, OMC-BUS-4) (Table 1). Results from two supportive phase II studies in adults (OMC-BUS 6 and OMC-BUS 7) and one study in paediatric patients (OMC-BUS-5) also became available.

Table 1. Summary of clinical pharmacokinetics studies with Busilvex

Study	Phase	Study design	Patient diagnosis	Sampling
OMC BUS 2	I	Dose 1: I.V. doses 2 through 16: oral	HSCT	dose 1, 5 (rich data)
OMC BUS 3	II	Doses 1 through 16: I.V.	Autologous HSCT	dose 1, 9 (rich data) dose 13 (LS)
OMC BUS 4	II	Doses 1 through 16: I.V.	Allogeneic HSCT	dose 1, 9 (rich data) dose 13 (LS)
Amendment 4 of OMC BUS 3&4	II	Dose 1: oral doses 2 through 16: I.V.	Autologous and Allogeneic HSCT	dose 1, 9 (rich data) dose 13 (LS)

Rich data: complete pharmacokinetic profile (9 samples)

LS: Limited sampling, pre-dosing (C_{min}) and end of infusion (C_{max})

Clinical pharmacology

Pharmacodynamics

Specific pharmacodynamic investigations with i.v. busulfan have not been submitted. The pharmacodynamic profile of busulfan is well known due to the experience with the oral formulation.

Pharmacokinetics

Pharmacokinetics have been investigated in 4 trials (Table 1). OMC BUS 2 was the first phase I trial performed with i.v. busulfan. "Amendment 4 of OMC BUS 3&4" is a mainly pharmacokinetic sub-study performed after conduct of OMC BUS 3 and 4. Two studies (OMC-BUS 2, and Amendment 4 to OMC-BUS 3&4) include an intra-patient comparison of the pharmacokinetics of Busilvex with oral busulfan.

Pharmacokinetic data with Busilvex were also obtained during phase II efficacy and safety trials (OMC-BUS 3 and OMC-BUS 4) as conditioning therapy prior to HSCT at the proposed dose and schedule of administration (equivalent to oral busulfan standard high-dose). These data provided primarily population pharmacokinetics investigations of intra-patient variability and phase effects of the i.v. administration of busulfan.

Absorption

Busilvex as a liquid formulation for intravenous administration has a bioavailability of 100%. Busilvex was administered as a 2-hour infusion every 6 hours, mimicking the pharmacokinetic profile of the oral formulation where the absorption kinetics indicated a T_{max} at about 2 hours (Hassan, Oberg et al. 1989; Hassan, Oberg et al. 1991).

From OMC-BUS 2 (see further), the dose-normalised ratio of i.v. AUC at first dose, v. oral AUC at steady-state was $90 \pm 29\%$ (3 patients). From amendment 4 of OMC-BUS 3&4 (see further), the ratio of oral AUC at first dose, v. i.v. AUC at steady-state was $96 \pm 12\%$ (9 patients). Others have reported

the bioavailability of oral busulfan to be in the range of 70-80%, $75 \pm 27\%$, and 69% (40 - 94%)(Hollis, Marcellus et al. 1998; Schuler, Ehrsam et al. 1998; Hassan, Ljungman et al. 2001). Based on further historical data presented during the application (data not shown), mean (s.d.) oral and i.v. AUC at first dose was 1718 (868) and 1144 (313) in one series, and 1102 (411) and 1130 (312) $\mu\text{Mol}\cdot\text{min}$ in a second series, respectively. At steady state the corresponding values were 1252 (509) and 1183 (228), respectively.

The applicant selected median bioavailability of 80% to develop Busilvex at a dose regimen of 0.8 mg/kg in order to be equivalent to the oral dose regimen at 1 mg/kg.

Distribution

The mean volume of distribution (V_z) for i.v. busulfan ranged from 0.62 to 0.85 L/kg across the four studies (OMC-BUS 2, 3, 4 studies and amendment 4, see further). The reported volume of distribution of oral busulfan is 66% of body weight (Grochow, Jones et al. 1989), which was equivalent to that obtained with Busilvex.

Busulfan achieves concentrations in the cerebrospinal fluid (CSF) approximately equal to those in plasma (Hassan, Oberg et al. 1989; Vassal 1994). The substance also distributes freely into erythrocytes (Ehrsson and Hassan 1984). Reversible plasma protein binding is low, $7.4\% \pm 4.9\%$, over the range of 350 to 2750 ng/mL as determined by equilibrium dialysis at 25°C (Hassan, Oberg et al. 1989). Plasma concentrations in this range are achieved with a 1-mg/kg oral dose. Irreversible binding to plasma elements, primarily albumin, has been estimated to be $32.4 \pm 2.2\%$ (Ehrsson and Hassan 1984).

Metabolism

Information on metabolism was obtained from literature review on oral busulfan. The metabolites identified in the human are identical to those identified in the rat (Hassan, Oberg et al. 1989). All metabolites arise from the initial conjugation of busulfan with glutathione in a glutathione S-transferase (GST) catalysed reaction. The most active human GST catalysing this reaction is GSTA1-1 (it is the major form of GST in the liver). Little work has been done on the identification of circulating metabolites in blood, although it is likely that the metabolites identified do circulate. A thiophenium ion, with an estimated half-life of 5 hours has been assayed in plasma (Gibbs, Murray et al. 1997). The clinical significance of this is unclear. None of the metabolites are thought to contribute significantly to either efficacy or toxicity because busulfan is a more potent non-specific alkylating agent (Hassan and Ehrsson 1987; Commandeur, Sijntjes et al. 1995).

Elimination

Mean absolute CL values for intravenous busulfan were consistent across studies submitted, ranging from 2.25 mL/min/kg to 2.74 mL/min/kg. There was no significant difference in CL between dose 1 and dose 9 for oral busulfan. Clearance has been described as constant whatever the age except in very young children (≤ 4 years) who presented higher values (Vassal, Gouyette et al. 1989; Grochow, Krivit et al. 1990).

In human, approximately 45% and 60% of a sulphur (^{35}S) radiolabelled dose was excreted into urine within 48 hours when administering busulfan I.V. and orally respectively (Nadkarni, Trams et al. 1959). When using a carbon-radiolabelled dose, approximately 30% were excreted in urine by both routes. Negligible amounts of radiolabelled drug were recovered in faeces. Discrepancies between sulphur and carbon labelling are most likely due to elimination of the sulphur atom from the molecule when busulfan was metabolised (forming methane sulphonic acid), whereas the labelled carbon remained in the butane chain of busulfan. The lack of complete recovery in urine may be due to long-lived metabolites production or more likely to non-specific alkylation of macromolecules.

Only 1 to 2% of an oral dose of busulfan is eliminated unchanged in human urine (Ehrsson, Hassan et al. 1983; Hassan, Oberg et al. 1989).

Following intravenous administration, the mean half-life of busulfan ranged from 2.83 hours to 3.90 hours in the four OMC studies. Oral busulfan had a mean half-life of 3.87 hours in OMC-BUS 2 and a mean half-life of 3.55 hours in Amendment 4 to OMC-BUS 3&4. For the doses with full blood sampling (rich data), pharmacokinetics was always performed over 6 hours, including

the 2 h-infusion. This was necessary to comply with the established administration schedule of dosing every 6 hours.

Pharmacokinetic Linearity

The linearity of the pharmacokinetics of oral busulfan has been shown in a single dose-escalation study. In this study, doses of 2, 4, and 6 mg were evaluated. No dose dependence was observed (Ehrsson, Hassan et al. 1983).

Bioanalytical methods

Two analytical methods were used during the clinical development of Busilvex for the quantification of plasma busulfan concentrations.

- A HPLC method with ultraviolet detection (HPLC-UV). The HPLC-UV method was validated over the range of 50 to 10000 ng/mL of busulfan. This method was used in study OMC-BUS 2.
- A gas chromatography with mass spectrometry detection (GC-MSD) was validated over the range of 65.5 to 3750 ng/mL. The GC-MSD was used in studies OMC-BUS 3, OMC-BUS 4, and in the amendments to studies OMC-BUS 3, OMC-BUS 4.

The two methods (HPLC and GC-MSD) were not cross-validated.

Pharmacokinetic studies

OMC-BUS 2

Study OMC-BUS 2 was a phase open label, multicentre safety, pharmacokinetic study and I. The i.v. dosing of Busilvex (dose 1 for all patients) was escalated from 0.08 to 0.8 mg/kg, with 3 patients in each of the four steps, except for the 0.8 mg/kg dose that was administered to 6 patients (N=15). The individual oral dose of busulfan ranged from 0.86 to 1 mg/kg (Doses 2 to 16). Busulfan treatment was followed by 2 cycles of cyclophosphamide (60 mg/kg i.v.) at day's -3 to -2. After a day of rest bone marrow transplantation or peripheral haematopoietic progenitor/stem cell (re-)transfusion/transplantation was performed on day 0.

Results

Only 6/15 patients were included in the pharmacokinetic analysis. Five patients had broken or missing bioassay samples, 2 vomited their fourth oral dose and 2 had sampling methodology errors. The intrapatient ratio of oral busulfan plasma levels at steady state vs. plasma levels after first dose i.v. was 90%. Of the 6 patients evaluable for the pharmacokinetic analysis 3, received the i.v. dose of Busilvex finally selected for further evaluation (AUC values were 943, 1124, 1472, target 2000 ± 1200 $\mu\text{mol}\cdot\text{min}/\text{L}$).

Amendment 4 of studies OMC-BUS 3 and OMC-BUS 4

Protocol amendment 4 to trials OMC-BUS 3 and 4 was intended to document the plasma pharmacokinetics of an initial high dose of oral busulfan (1 mg/kg) followed by 15 i.v. doses of Busilvex (0.8 mg/kg). An additional objective was to investigate whether the repeated i.v. administration of 0.8 mg/kg of Busilvex were adequate for targeting an $\text{AUC} < 1500$ $\mu\text{Mol}\cdot\text{min}$, which has been considered by the applicant to be associated with a reduction of the risk of hepatic VOD after oral busulfan. Rich pharmacokinetic samplings were collected over 6 hours at dose 1 (oral) and 9 (i.v.). Sparse pharmacokinetic samplings (trough and peak concentrations) were obtained at dose 13 (i.v.). Busulfan dose per administration was based on ideal body weight (IBW) in all but one patient.

Results

Twelve patients enrolled in studies OMC-BUS 3 and 4 (3 patients of OMC-BUS 3 and 9 patients of OMC-BUS 4) were enrolled under amendment 4. Three patients were excluded from the pharmacokinetic analysis. Two of them did not reach C_{max} after the end of the dosing interval after oral busulfan, and in another the 6-hour plasma sample was drawn after the start of the next i.v. dose. Eleven out of 12 (92%) patients for i.v. busulfan and 6 out of 9 (67%) patients for oral busulfan presented AUC below the targeted 1500 $\mu\text{Mol}\cdot\text{min}$. Peak and trough values at doses 9 and 13 allowed

to conclude that steady state had been reached at dose 9 and that there seems not to be time-dependent pharmacokinetics with Busilvex. The results are summarised in Table 2.

The intra-patient ratio of oral busulfan plasma levels at first dose v. plasma levels at steady state at dose 9 i.v. was near 100% .

Table 2. Summary of results from amendment 4 of studies OMC-BUS 3 and 4 (mean, coefficient of variation, and range).

Dose mg/kg	C _{max} µg/mL	T _{max} hr	T _½ hr	AUC _{inf} (oral) AUC _{ss} (i.v.) µMol.min	CL or CL/F mL/min/kg	V _z or V _z /F L/kg	Absolute F
Oral dose 1 1.0	0.87 ± 30% (0.61-1.32)	2.76±46% (1.50-4.25)	3.55±33% (2.41-5.66)	1396±24% (1021-1951)	2.50 ± 18% (1.62-2.92)	0.73 ± 17% (0.58-1.00)	0.96 ± 12% (0.79-1.16)
i.v. dose 9 0.8	1.17 ± 12% (1.00-1.41)	1.99±10% (1.83-2.50)	3.11±10% (2.64-3.59)	1156±14% (965-1404)	2.36 ± 13% (1.86-2.91)	0.64 ± 17% (0.53-0.81)	N/A

C_{max}: Maximal plasma concentration after oral busulfan or at the end of the i.v. administration.

T_{max}: Time of occurrence of C_{max}.

T_½: The terminal half-life

AUC_{inf}: The area under the concentration v. time curve from time 0 to infinity for dose 1, calculated from AUC_{last} + (C_{last}/λ_z), where AUC_{last} is the AUC from time 0 to the last sampling time post-dosing using the linear trapezoidal rule, C_{last} is the concentration at the last quantifiable point, and λ_z is the elimination rate constant, calculated by log-linear regression analysis of the terminal phase of the plasma busulfan concentration v. time curve.

AUC_{ss}: The AUC from time 0 to the last sampling time post-dosing (6 hour dosing interval) at steady state, calculated by the linear trapezoidal rule.

V_z or V_z/F: The terminal volume of distribution of busulfan. Apparent volume of distribution for oral route.

F: The bioavailability factor of oral busulfan, calculated as (AUC_{inf} oral/AUC_{inf} i.v.) x (Dose i.v./Dose oral) x 100

OMC-BUS 3 and OMC-BUS 4

These were two open, multicentre trials to assess the efficacy and safety of Busilvex as conditioning treatment of patients being prepared for autologous (OMC-BUS 3) or allogeneic (OMC-BUS 4) HSCT (see Main studies, under Clinical Efficacy).

Methods

Blood sampling for full PK characterisation was done at doses 1 and 9. Additionally, peak and trough samplings were also done at dose 13. The applicant defined an optimal efficacy and tolerability therapeutic window for AUC ranging from 900 to 1500 µMol.min, on the basis of published data.

Results

The results are summarised in Table 3. Overall, 98/103 patients (39/42 in OMC-BUS 3 and 59/61 in OMC-BUS 4) had complete blood samples available for both doses. In 22 /98 patients (8 in OMC-BUS 3 and 14 in OMC-BUS 4), through samples at dose 1 were drawn after the dose 2 infusions had been initiated. These concentrations were excluded from the pharmacokinetic analysis. In 22 patients, (7 in OMC-BUS 3 and 15 in OMC-BUS 4), the dose 9 pre-dose blood samples were drawn either more than 5 minutes before or more than 5 minutes later than the due time. These concentrations were included in the pharmacokinetic analysis. In 28 patients, (10 in OMC-BUS 3 and 18 in OMC-BUS 4), trough samples at dose 9 were drawn after the next dose infusion had been initiated. The observed concentrations were replaced by extrapolated concentration values in order to calculate the AUC_{ss}.

The target AUC below 1500 µMol.min was reached since the first dose in 90% and 87% of patients in OMC-BUS 3 and OMC-BUS 4 respectively. The same exposure was maintained through dosing without adjustment in 90% and 93% of patients in OMC-BUS 3 and OMC-BUS 4 at dose 9, respectively.

In OMC-BUS 3 and OMC-BUS 4 studies (103 patients) the incidence of HVOD was not increased in the shorter interval (< 7 h v. 7-15h) between the last busulfan dose and start of cyclophosphamide treatment.

Table 3. Summary of pharmacokinetics results from OMC-BUS-3 and OMC-BUS-4 (mean, coefficient of variation, and range).

Study		C _{max} µg/mL	T _{max} hr	T _½ hr	AUC _{inf} (dose 1) AUC _{ss} (dose 9) µMol.min	CL or CL/F mL/min/kg	V _z or V _z /F L/kg
OMC-BUS 3	Dose 1	0.89 ± 21% (0.44-1.41)	2.09±23% (0.75-4.25)	3.20±33% (1.93-7.33)	1194±27% (607-2190)	2.37 ± 26% (1.20-4.31)	0.62 ± 18% (0.43-0.98)
	Dose 9	1.28 ± 23% (0.94-2.26)	2.10±12% (1.58-2.53)	3.47±39% (2.07-9.42)	1225±18% (857-1711)	2.25 ± 20% (1.40-3.13)	0.68 ± 50% (0.34-2.18)
OMC-BUS 4	Dose 1	0.95 ± 25% (0.42-1.77)	2.16±23% (0.25-4.20)	2.83±27% (1.69-6.81)	1106±29% (413-2511)	2.74 ± 30% (1.28-6.00)	0.64 ± 16% (0.38-1.02)
	Dose 9	1.22 ± 18% (0.5-1.68)	2.07±12% (1.75-3.00)	2.99±19% (2.11-5.05)	1167±20% (556-1673)	2.52 ± 25% (1.49-4.31)	0.64 ± 31% (0.37-1.70)

Population pharmacokinetics

A retrospective population pharmacokinetic analysis was carried out on patients with complete Busilvex treatment (OMC-BUS 3 and OMC-BUS 4). A total of 102 patients were included in the database representing 337 administrations with either complete pharmacokinetic profiles (doses 1 and 9) or limited sampling (doses 2, 8 or 13). In fact, sampling on doses 2 and 8 were not planned in the protocols but inappropriate sampling time at dose 1 (later than 6 hours) or at dose 9 (early pre-dose) provided complementary information. The study was aimed at quantifying the inter-patient variability, intra-patient variability and at determining demographic covariates that might impact those sources of variability. From the ANOVA analysis, combined with Bayesian calculation on doses 1 and 9 only, the inter- and intra-patient variability on AUC were 20% and 10%, respectively. From the population pharmacokinetic analysis, the final model led to an inter- and intra-variability on clearance of 15% and 14%, respectively.

The covariates influencing pharmacokinetics were body surface area (BSA) and actual body weight (ABW). BSA was better than ABW in reducing pharmacokinetic variability. Clearance normalised by BSA or weight showed no difference on variability. ABW, adjusted ideal body weight (AIBW), or ideal body weight (IBW) and BSA account for some of the inter-patient difference in clearance. Based on this analysis, the use of ABW in normal patients, but AIBW in obese patients respectively was proposed.

Age, gender and race did not show any influence. Diagnosis of CML also accounted for some inter-patient variability. No effect of the hepatic status on busulfan clearance was observed.

Renal function was not evaluated since all patients had normal or clinically non-significantly abnormal serum creatinine levels. It has been reported that less than 2% of a busulfan dose is excreted unchanged via the kidneys (Ehrsson, Hassan et al. 1983; Hassan, Oberg et al. 1989).

Potential drug interactions

Anti-fungal drugs are frequently inhibitors of isoform CYP 3A4. This enzyme is likely to be involved in the metabolism pathway of busulfan, but at a late step. One study observed that itraconazole, but not fluconazole, increased busulfan plasma concentrations, and markedly affected the pharmacokinetics of busulfan (Buggia, Zecca et al. 1996).

A case report stated a possible interaction between ketobemidone (opioid analgesic) and busulfan during myeloablative treatment in a patient with AML (Hassan, Svensson et al. 2000). Therefore, special care is recommended when combining this drug with busulfan. In one report a longer (24-50h v. 7-15 h) interval between the last busulfan dose and start of cyclophosphamide treatment has been associated with decreased HVOD occurrence (Hassan, Ehrsson et al. 1996).

Paracetamol less than 72 hours prior to Busilvex, or used concurrently, might result in modified busulfan clearance because paracetamol is known to decrease glutathione levels in blood and tissues (Shulman and Hinterberger 1992).

Phenytoin increased the clearance of busulfan by 10% or more in one series (Hassan, Oberg et al. 1993). However, the impact of important co-medications known to interact with busulfan, as phenytoin (virtually all patients had been pre-treated) and itraconazole (only one patient received it concomitantly), could not be assessed from the data. 5-HT3 antagonists might explain a small percentage (< 2%) of the inter-patient variability in CL. However, from a clinical point of view, the effect of ondansetron or granisetron is unlikely to be of any significance. Fluconazole had no relevant influence.

Relationship between PK parameters and HVOD

The relationship between Busilvex pharmacokinetics and the incidence of HVOD was also assessed in an exploratory multivariate analysis (logistic regression) on pooled data from OMC-BUS 3 and 4. Covariates included drug exposure; clearance, C_{max}, half-life, T_{max} and V_d. Patient covariates included pre-transplant laboratory parameters (alkaline phosphatase, SGTP and creatinine), gender, race, type of transplant and previous therapy. The covariates associated with an increased risk for HVOD were: high C_{max}, higher AUC than the median value (1126 µMol.min), low pre-treatment serum creatinine and prior haematopoietic progenitor cell transplantation.

Discussion on Clinical Pharmacology

Intravenous busulfan, by definition, has a bioavailability of 100%. No new toxicity was identified from dosing with the intravenous formulation or has any toxicity been more severe than with the oral formulation. This supported the assumption that Busilvex is not metabolised in a significantly different way from that of the oral formulation.

The amendment 4 to studies OMC-BUS 3 and 4 can be considered as the source of pivotal data for this application with regard to the pharmacokinetic comparability between the oral and i.v. regimens of busulfan as conditioning treatment for bone marrow transplantation.

More patients on Busilvex (92%) than on oral busulfan (67%) presented AUC under the value of 1500 µMol.min, which the applicant considered to be associated with a reduced incidence of hepatic VOD. However, firm conclusions cannot be drawn, also due to the small number of patients included in the analysis. Furthermore, AUC_{inf} values for oral busulfan were unreliable and AUC_{ss} values after oral dosing have not been determined taking into account that the experimental terminal half-life has been shown to be around 4 hour and sampling has been done during a 6-hour period.

Pharmacokinetic comparability would have been better evaluated in a randomised crossover or parallel study design assessing both formulations at steady state. The data presented from amendment 4 of OMC-BUS 3 and 4 do not allow to assess this. Nevertheless, it seems reasonable to assume a lower variability and a higher predictability of busulfan pharmacokinetics after i.v. compared to oral administration.

The applicant has not performed specific pharmacokinetic studies in special populations, although some results are available from the population pharmacokinetic analysis. The lack of comprehensive information for special populations has been reflected in the SPC.

In patients with hepatic dysfunction there is concern that high-dose busulfan therapy may result in a higher likelihood of transplant patients developing VOD. The use of busulfan/cyclophosphamide preparation regimen requires special caution in patients with hepatic dysfunction such as active chronic hepatitis and this has been reflected in the SPC. It is unlikely that the pharmacokinetics of Busilvex in patients with renal dysfunction would be very different from that of patients with normal renal function. Less than 2% of a busulfan dose is excreted unchanged via the kidneys, according to the literature presented. Also, busulfan clearance was not different between the day with or without haemodialysis in two patients with severe renal impairment (Masauzi, Higa et al. 1998; Ullery, Gibbs et al. 2000).

Body size measures can account for some of the inter-patient differences in busulfan clearance. Thus, adjusting the dose of Busilvex on the basis of ABW or AIBW seems justified.

Phenytoin (and busulfan itself) has been shown to be a busulfan metabolism inductor, and was associated with a significant decrease of busulfan AUC between dose 1 and dose 16 and an increase in apparent clearance (Hassan, Oberg et al. 1993). No pharmacokinetic interaction was observed with

diazepam (Hassan, Oberg et al. 1993). Others did not observe this interaction with either of the two anticonvulsant agents (Embree, Heggie et al. 1997). From the data presented, there is no experience on the co-administration of intravenous busulfan with antiepileptic drugs other than phenytoin.

The substitution of phenytoin by other anticonvulsant could change plasma Busulfan levels, and modify the efficacy and the toxicity profile of i.v. busulfan, and this has been reflected in the SPC. The applicant has committed to conduct a phase I study using benzodiazepine prophylaxis to study the impact on the pharmacokinetics profile of busulfan, to be conducted as a follow-up measure.

Other potential drug interactions related to itraconazole, ketobemidone, paracetamol, and those related to the time interval between busulfan and cyclophosphamide have been adequately reflected in the SPC.

Clinical efficacy

HSCT for adult patients with advanced haematological malignancies. Two additional supportive efficacy and safety studies have been completed in adult patients (OMC-BUS 6 and OMC-BUS 7 A study in paediatric patients (OMC-BUS 5) has also been completed. All clinical trials were conducted according to GCP.

Main studies

Patients and methods

The **OMC-BUS-3 and OMC-BUS-4** protocols were prospective, multi-centre, open-label, uncontrolled Phase II studies to investigate the safety and efficacy of Busilvex in combination with cyclophosphamide as a conditioning regimen for patients undergoing autologous (OMC-BUS-3) or allogeneic (OMC-BUS-4) HSCT (marrow or peripheral blood-derived progenitor cells) for haematological malignancies. The two protocols were similar with the exception of the source of stem cells and the management of GVHD. In these trials, Busilvex 0.8 mg/kg was administered intravenously over two hours via a central venous catheter every six hours for a total of 16 doses (BMT day-7 through day -4). Cyclophosphamide 60 mg/kg/day was administered intravenously via a central venous catheter (BMT day -3 through day -2). The dose of Busilvex was adjusted according to ideal body weight (IBW) or actual body weight (ABW), whichever proved smallest. No dose adjustment was done over the 4-day treatment. The main objectives of the studies were to demonstrate that this regimen was myeloablative and supportive of engraftment, and to determine the safety, and the plasma pharmacokinetics profile of intravenous busulfan.

Selection criteria

Patients with the following diseases were eligible to enter the studies:

- Acute leukaemia past first remission, in first or subsequent relapse, in first remission (high-risk) or induction failures.
- Chronic myelogenous leukaemia in chronic phase, accelerated phase or blast crisis.
- Primary refractory or resistant relapsed Hodgkin's disease (HD) or malignant lymphoma.
- Myelodysplastic syndromes.

In addition, patients had to meet the following criteria:

OMC-BUS 3: All autologous transplantation patients were required to have $\geq 1 \times 10^8$ marrow derived mononuclear cells/kg with $\geq 1 \times 10^4$ CFU-GM/kg and/or $\geq 0.7 \times 10^6$ CD34+ cells/kg harvested and cryopreserved while in remission, as documented by bilateral aspirates and biopsies within four weeks prior to harvest or $> 4 \times 10^8$ peripheral mononuclear blood cells/kg with $\geq 4 \times 10^4$ CFU-GM/kg and/or $\geq 4 \times 10^6$ CD34+ cells/kg.

OMC-BUS 4: For allogeneic transplantation: $> 1 \times 10^8$ marrow mononuclear cells/kg from normal, HLA-matched related donors. The marrow should not be T-cell depleted. The marrow collections must contain at least 2×10^8 nucleated cells/kg or 1×10^6 CD34+ cells/kg. The marrow from the normal donor may be processed per routine procedure for major or minor ABO-incompatibilities.

As an alternative to marrow, filgrastim-mobilised peripheral blood progenitor cells (PBPCs) may have been collected from HLA-matched related donors in 1-4 apheresis and cryopreserved. The apheresis collections had to contain at least 1.1×10^9 nucleated cells/kg or 4×10^6 CD34+ cells/kg.

To be eligible, patients also had to meet the following screening criteria: Physiological age 15 to 55 years; ZUBROD performance status <2; Left ventricular ejection fraction $\geq 50\%$; no uncontrolled arrhythmias or symptomatic cardiac disease; Forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), and diffusion capacity of carbon monoxide (DLCO) $\geq 50\%$ of expected, corrected for haemoglobin; no symptomatic pulmonary disease; Serum glutamic pyruvic transaminase (SGPT) ≤ 3 times upper limit of normal, serum bilirubin, alkaline phosphatase (optional) and serum creatinine within accepted laboratory standard normal limits or considered clinically non-significant; no evidence of chronic active hepatitis or cirrhosis (any positive hepatitis serology was discussed with the study chairman and a liver biopsy was considered); no effusion or ascites ≥ 1 L prior to drainage; Life expectancy not severely limited by concomitant illness and expected to be >12 weeks; HIV-negative; Patient was not pregnant; Patient or patient's legal representative, parent(s) or guardian was able to sign informed consent; and had central venous access secured through an indwelling catheter; Patients should not be eligible for a chemotherapy preparation protocol of higher institutional priority; Patients should not have received any other investigational drugs within 30 days of planned administration of busulfan.

Concomitant therapy

For Busilvex injection administration, phenytoin and anti-emetics were to be given to all patients prior to the first dose of Busilvex and continued on a fixed schedule through 12-24 hours after the last dose of cyclophosphamide. For cyclophosphamide administration, I.V. fluid with bicarbonate, anti-emetics and mesna were recommended per institutional guidelines. Each clinical centre was allowed to follow their institutional guidelines for HSCT.

Recommended supportive care included: G-CSF 5 mcg/kg/d subcutaneous from BMT day 0 until ANC > 3500/mcL for three or more days or according to institutional guidelines, cytosine arabinoside for CNS prophylaxis where appropriate, starting at the time of haematological recovery, defined as ANC > 1500/mcL off G-CSF and platelet count > 50000/mcL. Allopurinol, menstrual suppression, prophylactic antibiotics, empirical antibiotics, I.V. hyperalimentation, etc., and GVHD prophylaxis were allowed according to institutional guidelines.

Efficacy endpoints included short-term (myeloablation, median time of engraftment) and long-term end points (disease-free survival, relapse, survival and transplant-related mortality).

Endpoint definitions

Myeloablation was defined as any one or the combination of the following: neutropenia (ANC < $0.5 \times 10^9/L$), lymphopenia (ALC < $0.1 \times 10^9/L$), thrombocytopenia (platelet count of < $20 \times 10^9/L$) or bleeding requiring platelet transfusion. Engraftment was recorded as the day the ANC exceeded $0.5 \times 10^9/L$. Non engraftment was defined for the purpose of these studies as the failure to reach an ANC > $0.5 \times 10^9/L$ within 100 days after transplantation. In addition, patients who engrafted at this time, but who developed an ANC < $0.5 \times 10^9/L$ thereafter were classified as late rejection or late graft failure. The clinical engraftment was confirmed by demonstration of donor cells in T-lymphocyte and haematopoietic lineage (chimerism). Relapse was defined as the day of detection of disease recurrence. Disease free survival (DFS) was defined as the documented day of death or relapse.

Statistical considerations and sample size

There was no pre-specified statistical hypothesis. Sample size was not pre-specified in the original protocols. Amendment 1 set the upper limit of enrolment to a total of 45 patients and amendment 3 increased the maximum number of patients to 100.

Results

42 and 62 patients were enrolled respectively in OMC-BUS 3 and OMC-BUS 4 studies. The summary of patient characteristics is shown in Table 4. **OMC-BUS 3:** A total of 42 patients were enrolled in this study and were distributed among five study centres. All 42 received the full 16-dose regimen of Busilvex and are included in the analyses. **OMC-BUS 4:** A total of 62 patients were enrolled in this

study and were distributed among seven study centres. 61 of these patients received the full 16-dose regimen of Busilvex and are included in the analyses. One patient withdrew before receiving study medication due to the inability to have blood samples drawn for pharmacokinetic analysis. No additional data were collected for this patient and this patient was excluded from analysis of safety and efficacy. Additionally, two patients were discontinued due to acute mortality during the study period (BMT Day -7 through BMT Day +28). Data for these two patients are included in the study report and analysis. Overall, 95 out of 103 (92%) patients were considered high-risk for relapse with poor outcome following HSCT in both OMC-BUS 3 and OMC-BUS 4. 63/103 (61%) patients met one or more of the following high-risk criteria: ≥ 3 prior chemotherapy regimens 49 (48%); at least one prior radiation regimen including TBI 38 (37%); prior transplant 11 (11%)]. A total of 81/103 (79%) patients were transplanted whilst in active disease, and 49/103 (48%) patients were both heavily pre-treated and transplanted with active disease.

At the cut-off for analysis, 34 out of 103 patients (33% patients enrolled in the studies (11 in OMC BUS 3 and 23 in OMC BUS 4) continued to be followed, with a median time of follow-up of 812 days (range 723-1113 days).

Table 4. Summary of patient characteristics for OMC-BUS 3 and OMC-BUS 4

Characteristics		OMC-BUS 3 N = 42	OMC-BUS 4 N = 62
Age mean (range)		37.8 (18.0-60.0)	39.5 (20.0-63.0)
Gender No. (%)	Male	24 (57)	37 (60)
	Female	18 (43)	25 (40)
Weight (kg) mean (range)		82.0 (40.9-122.1)	78.7 (40.9-133.2)
Height (cm) mean (range)		170.4 (148.0-190.0)	172.7 (149.0-198.0)
Disease No. (%)	Acute leukaemia	7 (17)	26 (43)
	Chronic myelogenous Leukaemia	0	17 (28)
	HD	24 (57)	3 (4.8)
	Malignant lymphoma	11 (26)	6 (9.7)
	Myelodysplastic syndromes	0	9 (14.5)
Disease status No. (%)	Active	35 (83)	46 (75)
	Remission	7 (17)	15 (25)
Prior chemotherapy regimens No. (%)	0-2	14 (33)	35 (56)
	>3	28 (67)	21 (34)
	Data not available	0	6 (9.7)
Prior radiation regimens No. (%)	0	23 (55)	36 (58)
	>1	19 (45)	20 (32)
	Data not available	0	6 (9.7)
Prior transplants No. (%)	0	39 (93)	53 (85)
	>1	3 (7)	8 (13)
	Data not available	0	1 (1.6)

Myeloablation

In OMC-BUS 3 myeloablation occurred in all patients (42/42). The median time to neutropenia was 4 days (range -7 to +6). The median duration of neutropenia and lymphopenia was 6 days (range 2 - 13 days) and 3 days (range 1 - 7 days) respectively. For allogeneic transplantation (OMC-BUS 4), all patients experienced profound myelosuppression (61/61). The median time to neutropenia was 4 days

(range -7 to +5). The median duration of neutropenia was 9 days (range 1- 28 days) and for lymphopenia was 4 days (range 1-19 days). In this study, donor-derived haematopoiesis (chimerism) was further documented by cytogenetic markers, and/or Fluorescent in Situ Hybridization (FISH) and/or Restriction Fragment-Length Polymorphism (RFLP) analysis in 43 of the 60 evaluable patients at 1 and/or 3 months post-transplant (6 patients did not have tests performed, 11 patients had indeterminate results due to a lack of an appropriate marker). Molecular analysis showed that all 43 (100%) patients had documented evidence of donor cell engraftment; 38/43 patients (88%) achieved full chimerism. Five out of 43 (12%) had mixed chimerism (RFLP) with host-type DNA contributed by persistent or recurrent leukaemic marrow cells. No patient had autologous recovery post-transplantation, nor did anyone later revert to host-derived haematopoiesis unless the underlying malignancy recurred. No patient with chimerical evidence of allogeneic engraftment suffered a later loss of the allogeneic graft.

The main results in terms of engraftment are summarised in Table 5. The effect of factors affecting engraftment was explored (methotrexate use, stem cell source, G-CSF use). No major effect on engraftment was observed for the parameters examined except for a trend toward a shorter time to engraftment for PBPC transplants ($p=0.0725$) and a possible widening in the range of time to engraftment for those patients receiving methotrexate (although the median value was not affected).

Table 5. Summary of time to engraftment for OMC-BUS 3 and OMC-BUS 4

Group	Time to Engraftment	OMC-BUS 3	OMC-BUS 4
		(n = 42)	(n = 61)
Patients who engrafted	No.	42	60*
	Mean BMT days (SD)	11 (3)	15 (4)
	Range	8-19	9-29
Patients who received bone marrow transplants**	No.	4	27
	Mean BMT days (SD)	15 (3)	16 (4)
	Range	11-16	11-29
Patients who received peripheral blood progenitor cell transplants**	No.	35	33
	Mean BMT days (SD)	11 (2)	14 (3)
	Range	8-19	9-22
Patients who received G-CSF supplementation	No.	39	47
	Mean BMT days (SD)	11 (3)	14 (3)
	Range	8-19	9-22
Patients who did not receive G-CSF supplementation	No.	3	13
	Mean BMT days (SD)	11 (1)	18 (4)
	Range	10-11	13-29

BMT = bone marrow transplant; G-CSF = granulocyte colony stimulating factor; Max . = maximum; Min . = minimum; SD = standard deviation

* One patient died on BMT Day +20 from fungal pneumonia before engraftment could be documented; therefore, this patient is not included in this summary of engraftment.

** Three patients received both bone marrow and peripheral blood progenitor cells; data from these patients are not included in this table

Relapse and survival

At the cut off date (January 9, 1998) 98% and 97% of the patients had follow-up data for post-transplantation day +28, 74% and 77% had been observed for the short term follow up (day +28 - +100), and 43% and 33% of the patients had follow up data for more than +100 days in trial OMC-BUS 3 and 4 respectively. An update of long term follow up with a cut-off date of June 2000 was also provided. Relapse and survival data according various cut-offs are provided in Table 6. Additional analyses in selected disease categories, and a literature review on high dose oral busulfan by transplant group and disease classification were also submitted (data not shown).

Table 6. Summary of Relapse Data of Trial OMC-BUS 3 and OMC-BUS 4

	OMC-BUS 3 n=42		OMC-BUS 4 n=62	
	January 1998	June 2000	January 1998	June 2000
No. (%) of patients who had relapse	7 (17%)	29 (69%)	6 (10%)	26 (43%)
Median (range) time to relapse (days)	105 (13-184)	181 (13-716)	87 (34-255)	201.5 (36-701)
No. (%) of patients who had died	2 (5%)	9 (21%)	10 (16%)	20 (33%)
Median (range) time to death (days)	217 (214-220)	220 (124-528)	52 (20-275)	164 (20-831)

Comparison with oral busulfan

Two series of high-dose *oral* busulfan were presented and compared retrospectively to the intravenous data. The first series consisted of patients that received *oral* busulfan and cyclophosphamide as a preparative regimen for autologous peripheral blood progenitor cell transplantation for NHL. Data for 17 patients were available. 11/17 (65%) was defined as high-risk for transplant-related morbidity (at least one of the following: ≥ 3 chemotherapy regimens; prior radiation; prior transplant). All patients received oral BuCy2 with a dose of 1mg/kg busulfan administered. These were compared with the data from the combined i.v. busulfan autologous group (n=47) which consisted of patients with NHL (n=11), HD (n=29) or AML (n=7). I.V. busulfan had similar short-term outcome when compared to oral busulfan in a similar high-risk patient population (data not shown). The second series consisted of patients that received oral busulfan and cyclophosphamide used as a preparative regimen for allogeneic marrow or peripheral blood progenitor cell transplantation for hematologic malignancies (Kashyap, Wingard et al. 2002). This series was compared to the patients treated by i.v. busulfan (n=61, OMC-BUS-4). Patient demographics were similar between the two groups with 93% and 95% (oral vs i.v., respectively) of patients being at high-risk for transplant related morbidity and/or mortality. For oral busulfan, 28 (93%) patients were evaluable for engraftment ($ANC > 0.5 \times 10^9/L$). Engraftment was similar between the two groups with 100% of evaluable patients engrafting in both studies. The probability estimate for overall survival at day +100 was significantly higher in patients treated with Busilvex than with oral busulfan.

Special populations

Clinical studies investigating the efficacy and safety of Busilvex in special populations have not been submitted. A reduced number of patients between the ages of 55-63 were treated with Busilvex and no special findings were reported. Oral busulfan as conditioning therapy for HSCT is being used in children, however, no information was provided.

A study in paediatric patients (OMC-BUS 5) has been completed. Preliminary results were provided. In this study, Busilvex was administered over 4 days (at dose 0.8 and 1.0 mg/kg ABW every six hours according to age > 4 years and ≤ 4 years, respectively) followed by cyclophosphamide administered over 4 days (50 mg/kg in 200 ml of D5W over one hour) as a preparative regimen BuCy4 for allogeneic bone marrow or blood progenitor cell transplantation. The target AUC range of 900-1350 $\mu\text{Mol}\cdot\text{min}$ ($\pm 5\%$) was achieved in 21/23 (91%) evaluable patients at steady state. Clinical evidence of myeloablation occurred in 100% (24/24) of the patients and engraftment was observed in 100% patients (23/23) taking into account that one patient died before engraftment could be evaluated. Engraftment was established at a median of 13 days post-transplant. The estimated probability of survival at the time of median follow-up (305 days) was 0.82 (95% CI: 0.67 to 0.98).

Supportive studies

OMC-BUS 6 and OMC-BUS 7. Both supportive trials were prospective, single-centre open-label uncontrolled studies of Busilvex in patients undergoing autologous (OMC-BUS 6) or allogeneic (OMC-BUS 7) transplantation in the treatment of haematological malignancies.

All 23 (100%) patients treated with Busilvex engrafted as demonstrated by $ANC > 0.5 \times 10^9/L$. The time to patient engraftment ranged from BMT Day +9 to BMT Day +10 for the two autologous transplant recipients while median time for patient engraftment was BMT Day +13 (range BMT Day +10 to BMT Day +20) for the 21 allogeneic transplant recipients. Patients were discharged from the hospital in a median of 15 days post-transplant (range 10-27). 19/19 evaluable patients showed evidence of donor cell engraftment. 17/19 was donor cell only, for at least one of the evaluations. 8/17 (47%) had stable sustained engraftment for three months or greater. Fourteen of these 17 (82%) patients remained donor cell only at the last observation (median follow-up 91 days; range 32-412). As of the date of data cut-off (June 24, 1999), no late graft failures had been reported.

As of the clinical cut-off date for this study (June 24, 1999), the median follow-up for all patients was 190 days (range 11 to 412 days). At the last available follow-up, 87% (20/23) of patients were alive (N=2, autologous and N=18, allogeneic) and 13 of these 20 (56%) surviving patients remained disease-free. 7/23 patients had relapsed on or before the data cut-off date (June 24, 1999).

Discussion on Clinical Efficacy

The efficacy and safety of Busilvex as conditioning treatment in allogeneic and autologous HSCT has been investigated in two pivotal phase II trials. The trials were conducted concurrently and were virtually identical with the exception of the source of stem cells and concomitant therapy for prophylaxis of GVHD.

The protocols have considered patients with haematological malignancies that are candidates for HSCT. Selection criteria were similar for both studies, with the exception of the source of the progenitor cells to be transplanted (autologous v. allogeneic). This selected different populations, as in clinical practice the indications for autologous and allogeneic transplantation are not the same (predominance of lymphoma patients in BUS 3 and leukaemia patients in BUS 4).

Concerning short-term efficacy, all patients showed engraftment and this occurred at the expected time. Although, it is difficult to relate this outcome of the transplant exclusively to busulfan therapy, this result can be considered positive and an indirect measure of the myelosuppressive activity of Busilvex.

Disease-free survival, and overall survival are the most relevant efficacy endpoints for conditioning regimens prior HSCT. The sparse number of patients enrolled in these trials (n = 103) and the short follow-up do not allow a thorough evaluation of such outcomes. Although a high rate of disease recurrence was observed in the autologous group, these results were expected. The number of patients was too limited as to allow for a disease-specific analysis.

No comprehensive information with regard to Busilvex pharmacokinetics in children has been submitted. The applicant has committed to provide the full data package in children as soon as it becomes available.

In summary, concerning clinical efficacy, the clinical data submitted are supportive of the efficacy of Busilvex as conditioning regimen in patients undergoing HSCT. Prospective randomised comparative data of intravenous v. oral busulfan would have been preferable. However, in view of the nature of the dossier (new formulation of a known substance), and the well-established use of oral busulfan as conditioning regimen for HSCT, the demonstration of the pharmacokinetic comparability of both formulations together with supportive efficacy data is sufficient.

Clinical safety

Patient exposure

The safety database for Busilvex in combination with cyclophosphamide as a conditioning regimen with the proposed dose schedule for progenitor cell transplantation consisted of a total of 103 patients,

aged 18 to 63 years, enrolled in studies OMC-BUS 3 (N=42) and OMC-BUS 4 (N=61). Data on serious adverse events, other adverse events and toxicity grades 3 or 4 were collected up to 100 days post-transplant. A summary of post-marketing adverse drug experience with Busilvex in USA were also provided. In addition, results from OMC-BUS 6 & 7 have been included in this application. Study OMC-BUS 2 (only the first of 16 doses was intravenous busulfan) was excluded.

All 103 patients included in the primary safety database received a total of 16 doses of Busilvex injection administered via a central venous catheter. Each dose was delivered over two hours every six hours for four consecutive days. Six patients experienced a delay in receiving a dose due to either technical difficulties associated with the pump and/or administrative error (n=5) or evaluation of an adverse event (n=1). Doses were calculated to deliver 0.8mg/kg body weight. Dosage calculations were based upon either actual (n=25), ideal (n=65), or adjusted ideal (n=11) patients body weight per individual institutional guidelines. Total doses of Busilvex injection ranged from 33 mg to 76 mg with the average total dose for the safety population being 56 mg per 6-hour dosing interval. No dosage adjustment was required.

Following Busilvex injection treatment, patients received cyclophosphamide at a dose of 60 mg/kg administered intravenously over one hour on two consecutive days (BMT Day -3 and BMT Day -2). The minimum total dose of cyclophosphamide was 2454 mg and the maximum dose administered was 6300 mg.

BMT Day -1 was a day of rest (no chemotherapy) prior to infusion of haematopoietic progenitor cells (either autologous OMC-BUS 3 or allogeneic OMC-BUS 4) on BMT Day 0. Only supportive care procedures were provided from BMT Day +1 through BMT Day +28.

No patients prematurely discontinued study drug; however, three patients discontinued from OMC-BUS 3 and OMC-BUS 4 prior to the completion of 36-day study period (BMT Day -7 through BMT Day +28). One patient (OMC-BUS 3) requested withdrawal from the study on BMT Day +15 due to disease progression requiring an alternative course of chemotherapy. Two patients (OMC-BUS 4) died on BMT Day +27 and BMT Day +20 respectively. Causes of death were pneumonia (with pulmonary haemorrhage) and respiratory failure secondary to aspergillus pneumonia, respectively

Adverse events and serious adverse event/deaths

All 103 patients experienced at least one AE during the 36-days study period. Most of the reported adverse events were already well known events for oral busulfan, commonly encountered following myeloablative-conditioning therapy, including TBI, high-dose busulfan and alternative chemotherapy combinations. No patients discontinued the study drug due to an AE.

Adverse events were analysed by gender, age, race, diagnosis and pre-treatment subgroups. With a threshold for differences between subgroups, a greater number of AEs were more commonly reported in females (allergic reaction, hypotension, constipation, rhinitis, cough increase, pharyngitis, pruritus, and pain ear) than in males (infection, hypocalcemia and SGPT increase) (Frequency of occurrence differed by $\geq 15\%$) and a greater number were more commonly reported in patients over 50 years old than in younger patients. No other subgroup differences were observed.

The majority of the AEs were mild or moderate. The most frequently reported AEs, during the defined study period, were nausea (97%), stomatitis (96%), leucopenia (96%), thrombocytopenia (94%), vomiting (91%), anaemia (88%), fever (87%), anorexia (80%), diarrhea (80%) and insomnia (80%).

Invasive infections (39%) were more common in patients having undergone an allogeneic transplant. Other adverse events included capillary leak syndrome (one case), and oedema, hypervolemia or weight increase (71%); GVHD (15%); lung toxicity; seizures; (one case), mild or moderate pain (93%), chills (47%) asthenia (56%) (one G3), allergic reactions (32%), inflammation at an injection site (23%), abdominal enlargement (16%), ascites (2%).

Cardiovascular toxicity included tachycardia (50%), arrhythmia (3%), atrial fibrillation (2%), ventricular extrasystoles (1%) and bradycardia (1%), hypotension (17%) and hypertension (25%), thrombosis (27%), cardiomegaly (n=3%), ECG abnormality (n=1%); pericardial effusion (n=1%); decreased ejection fraction (n=1%), and pericarditis (n=1%). Toxicity to the digestive system included: dyspepsia (40%) and constipation (31%), rectal disorders (24%), jaundice (8%), ileus (7%)

hepatomegaly (5%), pancreatitis (2%). Splenomegaly (4%). For the metabolic and nutritional system this included potentially clinically significant elevations in glucose, and electrolyte abnormalities.

VOD was observed in 6 patients (serious in 5 patients and fatal in two). For five patients liver dysfunction was a part of multi-organ failure preceding death.

Musculoskeletal toxicity occurred in less than 20% of the patients. Other toxicity involved the nervous system: insomnia (80%, one severe), anxiety (65%), dizziness (26%) and depression (20%); G3 serious delirium, G4 coma and G4 cerebral haemorrhage, the respiratory system: rhinitis (44%) and cough (36%) epistaxis (23%), hyperventilation (5%), asthma (7%), atelectasis (3%), pleural effusion (3%) and hypoxia (1%); the skin and appendages: rash (50%), pruritus (30), urticaria (n=3), alopecia (n=15), blisters (n=8), maculopapular rashes (n=7) and purpura rash (n=1); the special senses (less than 20% of the patients); and the urogenital system: haematuria (n=9, 1 G3), haemorrhagic cystitis (n=2, 1 severe), moderate renal insufficiency (n=2%).

Serious adverse events (SAE)

In the combined studies OMC-BUS 3 and OMC-BUS 4, 26/103 (25%) had a single serious adverse event (SAE), 10/103 (10%) two SAE, 3/103 (3%) three SAE, and one patient (1%) four SAE; 63/103 (61%) patients experienced no SAE (Table 7). Two SAEs (acute delirium and hypotension) were unexpected. Both occurred (in different patients) prior to transplant; treatment was not interrupted for the delirium but the second cyclophosphamide dose was substituted with melphalan for hypotension.

Table 7. Summary of Serious Adverse Events from OMC-BUS 3 and OMC-BUS 4 Combined (n = 103)

Serious Adverse Event	BMT Day -7 through BMT Day +28	BMT Day +29 through BMT Day +100
	Infection or signs of infection	11
Liver toxicity	9	0
VOD	5	0
Elevated bilirubin or SGPT	4	0
GVHD	1	3
Haemorrhagic cystitis	2	3
Other:		
Alveolar haemorrhage	1	2
Cerebral haemorrhage	1	0
Gastrointestinal haemorrhage	0	1
Ileus	1	0
Thrombosis	1	0
Ureteral obstruction with hydronephrosis	0	1
Allergic reaction	1	0
Delirium	1	0
Arrhythmia	0	1
ARDS	0	1
Hypotension	1	0
Respiratory failure	1	1
Total SAEs	31	28

Hepatic Venous-Occlusive Disease

VOD is a well recognised and a potentially serious complication of high dose chemotherapy and irradiation administered to patients prior to transplant. Two patients had VOD rated moderate in severity, two had life-threatening VOD, and two had fatal VOD. Five of the six events were reported as serious. VOD and high-dose busulfan correlation has been analysed in the literature reviewing the use of oral busulfan. Specifically, the literature analysis reports that VOD occurs with a frequency of approximately 20% in patients who receive high-dose busulfan-based conditioning regimens prior to HSCT.

In the Busilvex studies, the diagnosis of VOD was based upon clinical examination and laboratory findings and verified retrospectively using the Jones' Criteria by an independent central reviewer. In the combined OMC-BUS 3 and OMC-BUS 4 studies, the overall incidence of VOD and VOD-related

mortality was 5.8% (6/103) and 2% (2/103), respectively (Table 8). VOD was confirmed by liver biopsy in 3/6 (50%) patients. Five of these events were reported as serious. Four patients out of the six who presented VOD were transplanted with active malignancy, and all met at least one criterion for extensive prior therapy (i.e. ≥ 3 prior chemotherapy regimens, prior irradiation, and/or prior transplant). Twenty-six out of 103 (25%) patients, had baseline evidence of liver abnormalities. Two out of these 26 patients developed VOD. None of the other 24 patients with evidence of pre-transplant liver impairment developed VOD.

Table 8. Listing of VOD and busulfan AUC for patients (OMC-BUS 3 and OMC-BUS 4)

Disease Status at Transplant	Dose 1 AUC ($\mu\text{Mol.min}$)	Prior Therapy	Outcome
Lymphoma (active)	1032	C	Resolved
HD	1256	R, C, T	Resolved
AML	1106	C	Death
MDS (active)	1202	R	Resolved
MDS (active)	1644	R, C, T	Death
CML (active)	1567	R	Resolved

C= > 3 prior chemotherapy ; R= prior radiation ; T= prior transplant

Risk factors for hepatic VOD

The association between high-dose busulfan and hepatic VOD is well known (Kennedy 1986; Grochow, Jones et al. 1989; Slattery, Sanders et al. 1995; Dix, Wingard et al. 1996; Ljungman, Hassan et al. 1997). Hepatic VOD occurs with a frequency of approximately 20% in patients who receive high-dose busulfan-based conditioning regimens prior to HSCT. High exposure to busulfan may increase its incidence by up to 10 fold (Dix, Wingard et al. 1996).

AUC of 1500 $\mu\text{Mol.min}$ has been described as a relatively sharp cut-off level above, which there is very high risk of hepatic VOD. Subsequently, the possibility of AUC guided dose adjustment has been proposed (Grochow, Jones et al. 1989; Grochow 1993).

A number of treatment-related and patient-related factors have been identified as predisposing patients to the development of hepatic VOD after HSCT: autologous *v.* comparably conditioned allogeneic recipients, second transplants preceded by second courses of high-dose chemotherapy, prior radiation therapy, and the elevation of liver enzymes before transplantation.

In patients undergoing first transplant, hepatic VOD was observed in 4/92 (4.3%, including one fatal case) cases, compared to 2/11 (18%, including one fatal case) cases for patients previously transplanted.

Hepatic VOD was observed in 5/61 (8.2%) allogeneic compared to 1/42 (2.5%) autologous transplant recipients. Both cases of fatal hepatic VOD occurred in allogeneic patients.

Concerning disease subgroups, hepatic VOD was observed in 1/16 (6.2%) lymphoma patients and 1/17 (5.7%) CML patients.

A complementary analysis on pharmacokinetic / pharmacodynamic relationship about hepatic VOD has been performed through multivariate analysis of pooled data (OMC-BUS 3 and OMC-BUS 4). High busulfan C_{max} and higher than median AUC (1126 $\mu\text{Mol.min}$), prior HSCT, and low pre-treatment serum creatinine were associated with increased risk of hepatic VOD. In allotransplanted patients only (OMC-BUS 4), prior radiation therapy, high pre-treatment SGPT, low pre-treatment serum creatinine, higher than median AUC and low plasma busulfan clearance were associated with hepatic VOD.

In univariate analysis, combination of higher than median AUC (1084 $\mu\text{Mol}\cdot\text{min}$) and prior irradiation (most often TBI) resulted in an incidence of 4/9 hepatic VOD (44%) while the incidence of hepatic VOD in patients with no prior irradiation therapy and low AUC was 0/21 (0%). In addition, the combination of higher than median AUC and prior radiation accounted for 4/5 (80%) of the total cases of hepatic VOD in allogeneic patient group.

Deaths

The overall mortality and treatment-related mortality (TRM) rates were 28% (29/103) and 16% (16/103), respectively. The incidence of total and non-relapse mortality remains higher in the allogeneic than in the autologous transplant subgroups: 33% (20/61) and 23% (14/61) v. 21% (9/42) and 5% (2/42), respectively.

In OMC-BUS-4 (allogeneic), of the 20 patients who died, 13 (65%) were transplanted with active disease, 10 (50%) were heavily pre-treated, and 6 (20%) were > 50 years old. Patients with heavy pre-treatment died earlier than those not heavily pre-treated prior HSCT.

In OMC-BUS-3 (autologous), six of the nine (67%) patients who died were transplanted with active disease, 8 (89%) were heavily pre-treated (67% were both), and 3 (33%) were > 50 years old. Comparative data were provided from a literature review. For autologous HSCT for lymphoma, with the use of busulfan-based therapy and/or other regimens, the treatment-related mortality ranged from 3% to 7% for patients with standard risk, while it was higher above 10% for those with advanced disease. For allogeneic HSCT, the overall treatment-related mortality following busulfan-based conditioning regimen ranged from 12% to 30%, and it was higher \geq 40% in high-risk patients (advanced disease, heavily pre-treated). Most serious toxicities with high-dose busulfan involved the liver, lungs, and the nervous system.

Laboratory findings

Haematology

All subjects exhibited one or more potentially clinically significant hematology abnormalities. Hematological abnormalities were an expected consequence and desirable end-point of BuCy combination therapy. No patients discontinued the study due to clinically significant hematology abnormalities. Abnormalities included anemia, leucopenia, neutropenia lymphocytopenia and thrombocytopenia. The majority of patients (101/103, 98%) required platelet transfusion during the 36-day study period. The maximum number of transfusion required was 27.

Clinical Chemistry

All but one patient exhibited one or more potentially clinically significant blood chemistry abnormality. Abnormalities occurred with glucose, kidney function tests, electrolytes, serum proteins, serum uric acids and liver function tests.

Abnormal values reflecting liver dysfunction were recorded in 100 patients. These included elevated LDH (n=66), serum bilirubin (n=40) with associated elevations of SGPT and/or SGOT; elevations in SGPT (n=82); SGOT (n=43).

Supportive studies: OMC-BUS-6 and OMC-BUS-7

Safety data were also obtained from the supportive OMC-BUS 6 & 7 studies. All 23 patients (100%) received the complete 16-dose regimen of Busilvex (0.8 mg/kg) administered intravenously via a central venous catheter. There were no safety-related dose adjustments required for any patients. The safety profile consisted of well-described toxicities commonly encountered during HSCT. A total of 16 SAEs (four were judged possibly related to busulfan) were reported in 9 patients without unexpected SAEs; 9/16 SAEs involved infection (including fever and the episode of diffuse alveolar haemorrhage secondary to bacteremia/pneumonia); 6 SAEs were of immunologic origin and 1 SAE was for nausea resulting in prolonged hospitalisation due to the associated anorexia and weight loss. No cases of VOD were observed.

Three deaths occurred in the studies OMC-BUS 6 & 7, according to preliminary results in 23 patients. All three occurred in patients who had received allogeneic transplantation and all three were associated either directly or indirectly with infection. Two of the deaths occurred after BMT Day

+250, as a direct consequence of infection (pneumonia and sepsis). A third patient died on BMT Day +77 as a result of multi-organ system failure and acute respiratory distress syndrome, as a consequence of diffuse alveolar haemorrhage, which was secondary to pneumonia/haemoptysis. No acute mortality (\leq day 28 post transplant) was reported.

Safety in special populations

Paediatric patients (OMC-BUS 5)

All patients had episodes of vomiting and most also had nausea. 22/24 patients had liver toxicity (observed as elevation of liver enzymes or bilirubin, abdominal pain or VOD). In total, 5 patients had VOD. One case was resolved in three days and not considered SAE and the rest of VOD cases are described above (see SAEs). Other AEs were considered tachycardia (54%), hypotension (17%), hypertension (58%), and a larger number of common AEs. 16/24 patients (67%) experienced at least one SAE: infection (18), hepatobiliary disease (9), respiratory disease (4), cardiovascular disease (2), capillary leak syndrome (2), GVHD (2), to multisystem organ failure (2), seizure (1) and hemolytic uremic syndrome (1). Serious hepatobiliary toxicity was reported in five children. Three episodes of hyperbilirubinemia and three of hepatic VOD were related to the BuCy preparative regimen, 2 of these hepatic VOD cases resolved in 9 and 10 days respectively and the last episode was unresolved at death. There were 2 (8%) acute (Day-10 to BMT Day +29), and 2 (8%) short-term post study surveillance (BMT Day+29 to BMT Day +100) deaths. Three of the four patients who died had been heavily pre-treated and the last one was transfusion dependent at the time of enrolment. No patients were discontinued or withdrawn from the study due to adverse events related to Busilvex.

Post marketing survey

Since the first marketing authorisation in the US (February 1999) more than 5000 patients were treated worldwide. Seven adverse drug events (AEs) concerning 5 patients were reported to the sponsor (VOD; hepatomegaly, aspergilloma and sepsis; pulmonary haemorrhage; acute pulmonary toxicity; myocardiopathy).

Dimethylacetamide

The potential influence of dimethylacetamide (DMA) on neurological, cardiovascular and hepatic safety profiles has been investigated. Review of the i.v. busulfan data and comparison to oral busulfan data did not reveal any increased incidence of events in the i.v. busulfan group in any of the three systems of interest CNS, cardiovascular or hepatic. i. v. busulfan behaved in a similar fashion to oral busulfan in terms of CNS, cardiovascular and hepatic adverse events and did not appear to be associated with additional toxicity due to DMA compared to oral busulfan.

Discussion on Clinical Safety

Busilvex Injection is expected to enhance ease and convenience of administration of the drug, decreasing the variability in absorption, eliminating the first pass effect, and enhancing a physician's ability to maintain blood levels within an optimal therapeutic window. Although the number of patients included in the safety database was low, the applicant relies on the fact that oral busulfan is a drug with an extensive and well-established use in the target population.

The pattern of adverse events and serious adverse events reported in OMC-BUS 3 & 4 safety data was similar to that reported for high-dose oral busulfan therapy. The most frequently AE reported in OMC-BUS 3 & 4 (nausea, stomatitis, leucopenia, thrombocytopenia, vomiting, anaemia, fever, anorexia, diarrhoea and insomnia) are commonly encountered in oral busulfan literature. The majority of deaths occurring in the two pivotal studies were mainly related to disease progression, infection or complication secondary to infection.

Busulfan has been reported to produce dose-related neurotoxic effects in some patients that are clinically characterised by myoclonic movements and seizures. Seizures may occur at high myeloablative doses of oral busulfan as early as the second treatment (Buggia, Locatelli et al. 1994; Vassal 1994). Phenytoin and anti-emetics were to be given to all patients prior to the first dose of Busilvex and continued on a fixed schedule through 12-24 hours after the last dose of cyclophosphamide.

In retrospective comparisons, hepatic VOD incidence appears to be lower than that observed with oral busulfan. It would seem plausible that i.v. administration should lead to a lower variable pharmacokinetics and to a better control of exposure, leading theoretically to a lower incidence of VOD. However, as prospective comparative safety data are lacking, it is not possible to definitively confirm this hypothesis.

Patients with hepatic dysfunction, particularly if severe, may be at a higher risk of developing VOD when receiving the busulfan/cyclophosphamide preparation, and this is reflected as a warning in the SPC.

The safety profile observed over 3 years post-marketing survey did not modify that one observed during the clinical development. In conclusion, the safety profile can be considered as comparable to that of the oral busulfan, with the possibility that the incidence of some toxicity with Busilvex may be lower.

A complete analysis of potential toxicity of DMA has been provided. No significant differences have been found between i.v. and oral busulfan in terms of CNS, cardiovascular system and liver functions have been observed.

5. Overall conclusions and benefit/risk assessment

Quality

The purity of busulfan active substance, and the control of the manufacturing process and specification for the finished product indicate reliable in vitro reproducibility of this medicinal product. Stability has been shown to be satisfactory; this in turn should indicate a uniform performance in the clinic. There are no unresolved quality issues which could have a negative impact on the benefit / risk balance of the product.

Preclinical pharmacology and toxicology

Busulfan is a bifunctional alkylating agent in which two labile methanesulphonate groups are attached to opposite ends of a four-carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulphonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.

Rapid tissue of Busulfan distribution has been demonstrated in mice, rats, rabbits and monkeys. In the distribution study in monkeys, the liver was found to accumulate increasing amounts of busulfan throughout a 45-minute observation period. In this period, the compound showed also a tendency to accumulate in the lungs with time.

As a small lipophilic molecule busulfan has been determined to easily cross the blood brain barrier. Its presence in cerebrospinal fluid (CSF) has been recorded in humans at concentrations equivalent to or slightly higher than plasma levels. Distribution studies in animals showed similar results.

Busulfan is metabolized extensively and the main metabolites are the same in human and rats. Busulfan is predominantly metabolized by conjugation with glutathion, both spontaneously and by glutathione S-transferase (GST) catalysis. This conjugate undergoes further extensive oxidative metabolism probably in the liver. This oxidative metabolism probably involves the cytochrome P450 system.

The major part of the metabolites is eliminated via renal and the faecal excretion is a minor elimination route in human and rat.

Most of the toxicology studies in the application were based on the literature of oral busulfan. Overall, the main toxicity of busulfan is neurotoxicity and damage to the liver.

Taking into account the toxicological profile of DMA, a Major objection was presented in relation to the content of this excipient. Although from the preclinical data it could be concluded that hepatotoxicity due to DMA cannot be discarded, taking into account that the content of DMA in the formulation has been reasonably justified and that, according to the clinical data, Busilvex did not appear to be associated with additional toxicity compared to oral busulfan, the overall benefit/risk is

considered positive, and this issue is resolved. Several points to clarify related to local tolerance evaluation, environmental risk assessment, and other toxicological aspects, were raised. The Company has answered adequately and the questions are considered resolved.

Based on the data submitted, no adverse environmental effects are predicted from the medicinal use of Busilvex.

Efficacy

Oral busulfan is a drug widely used as a part of conditioning regimens before HSCT. Busilvex Injection is expected to enhance easy and convenient administration of the drug, decreasing the variability in absorption, eliminating the first pass effect, and enhancing physician's ability to maintain blood levels within an optimal therapeutic window. These advantages are expected to minimise the risk of toxicity (especially the risk of hepatic venoocclusive disease) and increase the probability of achieving a complete myeloablation.

Normally, considering busulfan as a drug of well-established use in the claimed indication, a pharmacokinetic study showing the bioequivalence between the proposed dosing schedule of Busilvex (0.8mg/kg) and the standard dose of oral busulfan could have been considered sufficient to substantiate the present application. As for the data provided by the Applicant the observed systemic exposure after Busilvex at 0.8 mg/kg seemed to be similar to that obtained after oral busulfan at 1.0 mg/kg. It seems also reasonable to think that a lower variability and a higher predictability of busulfan pharmacokinetics are normally expected after i.v. than after oral administration. The additional pharmacokinetic comparison of i.v. Busilvex with data coming from different historical databases of oral busulfan pharmacokinetics further allow concluding a similar pharmacokinetic profile between both formulations with a reasonable level of confidence.

Safety

A prospective comparison of the efficacy and safety of Busilvex with oral busulfan have not been carried out. Nevertheless, as mentioned above, the nature of the dossier (new formulation of a well-known substance) and the well-established use of oral busulfan as conditioning regimen for HSCT, allow to accept a proper demonstration of the pharmacokinetic comparability of both formulations altogether with the provided supportive efficacy as compatible with a positive benefit/risk assessment of the efficacy of Busilvex as a component of the Bu/Cy2 conditioning regimen pre-HSCT.

As per CPMP request, the Applicant has provided additional information on the safety of Busilvex in the target population, especially concerning with the potential DMA-related toxicity (hepatic, neurologic and cardiac toxicity). A detailed review of the safety data of Busilvex does not reveal any specific toxicity pattern suspected to be associated with the use of DMA in the Busilvex formulation.

Benefit/risk assessment

Formally, oral busulfan has not this indication in most EU countries, although the Bu/Cy2 regimen is used worldwide. Early conditioning regimens were based on TBI, and currently modified TBI-based conditioning regimens are in some cases considered as the first choice approach prior to HSCT. However, the high technical requirements of the irradiation, the associated toxicity of TBI (sometimes not reversible), and the fact that these techniques are not universally available, prompted consideration for effective regimens based on chemotherapy alone. In this regard, the Bu/Cy2 regimen is a well-established therapy as conditioning regimen for HCST, although comparative data between both approaches are inconclusive or even lacking for some haematological malignancies.

With the compiled data, the Applicant has to a reasonable extent shown that the chosen i.v. dose of 0.8 mg/kg gives an exposure comparable to that with the established oral dose of 1 mg/kg. The data also indicate a lower variability with the i.v. formulation than with PO administration, which is not unexpected. Although not sufficiently shown to e.g. make claims in the product information, this might indicate that the risk for individual very low or very high exposures is decreased with i.v. administration. Thus, Busilvex (0.8 mg/kg) can be reasonably assumed to produce a similar systemic exposure to oral busulfan (1 mg/kg). The non-comparative clinical data provided by the Applicant do

not suggest any specific safety concern associated to either the intravenous use of busulfan nor the substances contained in the Busilvex formulation (and more specifically the presence of DMA)

In conclusion, the data contained in this application show a positive benefit/risk ratio for Busilvex in the following indication: Busilvex is indicated as conditioning treatment prior to haematopoietic progenitor cell transplantation in adult patients when the combination of busulfan and cyclophosphamide (Bu/Cy2) is considered the best available option.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Busilvex was favourable for Busilvex followed by cyclophosphamide (BuCy2) as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option

Medicinal product no longer authorised