London, 17 December 2009
Doc.Ref.: EMA/CHMP/55233/2010

CHMP ASSESSMENT REPORT

FOR

Tepadina

International Nonproprietary Name: thiotepa

Procedure No. EMEA/H/C/001046

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

TABLE OF CONTENTS

1.   BACKGROUND INFORMATION ON THE PROCEDURE

1.1   Submission of the dossier

The applicant ADIENNE S.r.l. submitted on 4 July 2008 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Tepadina, which was designated as an orphan medicinal product EU/3/06/424 on 29 January 2007. Tepadina was designated as an orphan medicinal product in the following indication: conditioning treatment prior to haematopoietic progenitor cell transplantation (HPCT). The calculated prevalence of this condition was 0.5 per 10,000 EU population.

The applicant applied for the following indication: conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult and paediatric patients.

The legal basis for this application refers to:

Article 10(a) of Directive 2001/83/EC, as amended - Well-established use application.

Information on Paediatric requirements

Not applicable

Information relating to Orphan Market Exclusivity

Similarity
Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the application contained a critical report addressing the possible similarity with the authorised orphan medicinal product Busilvex (busulfan).

Market Exclusivity

Not applicable.

Protocol Assistance:
The applicant did not seek Protocol Assistance at the CHMP.

Licensing status:
The product was not licensed in any country at the time of submission of the application.

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

Rapporteur: Pierre Demolis

Co-Rapporteur: Jens Ersbøll

1.2   Steps taken for the assessment of the product

- The application was received by the EMEA on 4 July 2008.
- The procedure started on 23 July 2008.

- The Rapporteur's first Assessment Report was circulated to all CHMP members on 13 October 2008 The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 15 October 2008
- During the meeting on 17-20 November 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 21 November 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 March 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 3 July 2009
- During the CHMP meeting on 20-23 July 2009, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant
- The applicant submitted the responses to the outstanding issues on 19 October 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the outstanding issues to all CHMP members on 30 October 2009
- The Rapporteurs circulate an updated Joint Assessment Report on the applicant's responses to the outstanding issues to all CHMP members on 13 November 2009
- During the meeting on 14-17 December 2009, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Tepadina on 17 December 2009. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 10 December 2009
- The CHMP adopted a report on similarity of TEPADINA with Busilvex on 22 January 2009.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Conditioning is the preparatory treatment for Haematopoietic Stem Cell Transplantation (HSCT, also referred to as Haemopoietic Progenitor Cell Transplantation-HPCT). Transplant refers to any procedure where haemopoietic stem cells of any donor type and any source are given to a recipient with the intention of repopulating and replacing the haemopoietic system in total or in part. Stem cells can be derived from bone marrow (BMT) or peripheral blood (PBSCT).

There are different conditions treated with HSCT such as acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), amyloidosis, acquired severe aplastic anaemia, autoimmune disorders, myeloproliferative syndromes (MPS), myelodysplastic syndromes (MDS), multiple myeloma (MM), Hodgkin's lymphoma (also known as Hodgkin's disease-HD), non-Hodgkin's lymphoma (NHL), genetic diseases (primary immunodeficiencies, metabolic disorders, hemgloninopathies, congenital aplasia), paroxysmal nocturnal hemoglobinuria (PNH), severe aplastic anaemia and solid tumours.

The conditioning is generally considered the most important step in the HSCT procedure and it includes myeloablative, reduced-intensity myeloablative, and nonmyeloablative regimens. Its purpose is to help eradicate the patient's disease prior to the transplant, to create marrow space for the donor cells and to suppress the host's immune system to prevent graft rejection. This could be achieved by chemotherapy drugs and radiation because both damage cellular DNA.

The aims of conditioning are:

- Creation of "space" for donor stem cells. Under physiological conditions hemopoietic stem cells circulate in peripheral blood. Stem cells cross the blood–bone marrow barrier, settle in empty bone marrow niches, and initiate hemopoiesis. Without conditioning most bone marrow niches are occupied and do not accept stem cells.

- Prevention of Bone Marrow rejection. Hematopoietic stem cells are rejected by as yet not fully elucidated immunological mechanisms.

- Prevention of reciprocal interference. This is a term used to describe lymphocyte interactions between host and donor in cases of allogeneic tranplantation, which may have three different

outcomes: a) the host immune system wins. The majority of immune cells of the recipient will reject the transfused immune cells of the donor without noticeable side effects, b) host and donor immune systems live peacefully together ever after (incomplete chimerism), or c) donor wins and rejects the host. Graft versus host disease (GvHD) is an immune reaction in which donor lymphocytes react against host tissue.

- Eradication of immunological memory. Autoreactive B and T lymphocytes and memory cells cause autoimmune disease. For example the success of a BM transplant for patients with aplastic anaemia (AA) depends on the effective eradication of immunological memory (sensitization).

Thiotepa is a cell cycle-phase independent, non-specific alkylating antineoplastic agent, related chemically and pharmacologically to the nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals that, similar to irradiation therapy, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position that severs the linkage between the purine base and the sugar and liberates alkylated guanines.

Thiotepa undergoes complex metabolism with TEPA as the major metabolite. Both thiotepa and TEPA are active structures. TEPA is assumed to interact differently with DNA, but is also thought to produce DNA lesions.

The use of thiotepa is invariably associated with haematological toxicity, while extra-haematological toxicity is absent or mild in the commonly used posologies. Myelosuppression is the dose-limiting toxicity in its use in standard chemotherapy.

The Applicant proposed an indication for Tepadina as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult and paediatric patients.

Autologous or allogenic stem-cell transplantation takes advantage of the specific bone-marrow toxicity and the lack of dose limiting extra-medullary toxicity of thiotepa. The compound is frequently given in combination with cyclophosphamide and busulfan or cyclophosphamide and carboplatin, as well as in other combinations of high-dose chemotherapy regimens. The use of thiotepa at high dosage as part of conditioning treatments prior to haematopoietic progenitor cell transplantation dates back to the late 1980s. In this context, thiotepa has been used in patients with a wide variety of disorders, from leukaemia to solid tumours including CNS tumours, as it crosses the blood-brain-barrier and reaches the same concentration in the cerebrospinal fluid as in plasma, and from thalassemia to autoimmune disorders.

Orphan Drug Designation was granted on 29 January 2007 by the European Commission for Tepadina in Conditioning treatment prior to haematopoietic progenitor cell transplantation (EU/3/06/424), based on the prevalence of the condition estimated to be in the range of 0.5 per 10,000 of population.

2.2 Well-established medicinal use

A full bibliographical dossier was submitted according to Article 10a of Directive 2001/83/EC as amended relying on a "well established use" of Tepadina in the EU for more than 10 years. The application was based on extensive published scientific literature, which makes it possible to replace results of pharmacological and toxicological tests and of clinical trials by detailed references to published studies. Under the terms of Article 10a of Directive 2001/83/EC, the non-clinical and clinical evidence of safety and efficacy were based on the provision of expertly evaluated bibliographical references.

The claim of 'well-established medicinal use' of thiotepa has been based on the time over which the substance has been used, on quantitative aspects of the use of the substance, the degree of scientific interest in the use of the substance, and the coherence of scientific assessments.

Overview of the development of thiotepa

The clinical development of thiotepa started in the middle of the 1950s and the agent has been used in the treatment of different neoplastic disease including Chronic Leukemia, Non Hodgkin's Lymphoma, Hodgkin's Disease, adenocarcinoma of the breast and the ovary, melanoma, paediatric and germinal tumours.

Approved thiotepa therapeutic indications include the palliation of a variety of neoplastic diseases. The more consistent results have been seen in the following tumours:

- Adenocarcinoma of the breast.

- Adenocarcinoma of the ovary.
- For controlling intracavitary effusions secondary to diffuse or localized neoplastic diseases of various serosal cavities.
- Superficial papillary carcinoma of the urinary bladder.

While now largely superseded by other treatments, thiotepa has been effective against some lymphomas, such as lymphosarcoma and Hodgkin's disease.

This indication was approved in the USA in March 1959 and thiotepa was re-registered in the USA in 2001. Thiotepa has been registered in over 60 countries worldwide, including European countries.

Applicant argumentation on the fulfillment of the criteria for 'well-established use'

In the last thirty years, haematopoietic stem cell transplantation has become a standard therapy in patients with advanced haematological malignances and malignant solid tumors resistant to standard chemotherapy.

The use of thiotepa showed that the compound is accompanied by haematological toxicity, but extra-haematological toxicity was mild or absent. For this reason, already at the end of the 1980s, thiotepa was considered an ideal drug to use at high dosage in conditioning treatments prior to haematopoietic progenitor cell transplantation. High dose thiotepa has been systematically used in this indication in European hospitals for over 10 years.

The consensus of scientific assessment of the use of thiotepa in the proposed indication relied on the general idea of a correlation between dose intensity and cytotoxic effect (Terenzi, 1993; Porrata, 2001). The dose-response relationship and the knowledge of resistance patterns led the investigators to look for possibilities of overcoming the biological limitation of bone marrow toxicity.

In the last twenty years, thiotepa become a standardized and systematically used agent in the indication of conditioning treatment prior to haematopoietic progenitor cell transplantation for a wide variety of disorders from solid tumors (Bregni, 2002; Pession, 1999, Nitz, 2005) to leukemia (Bacigalupo, 1996; Zecca, 1999), from thalassemia (La Nasa, 2005) to autoimmune disorders.

In the late 1980s, the Perugia group (Italy) first introduced thiotepa in the conditioning regimen for haematopoietic stem cells transplantation in patients affected by haematological malignancies, obtaining very good results in term of efficacy and safety (Aversa, 1998).

Similar results as engraftment and survival free from disease were recorded by the Memorial Hospital Group of New York (Papadopoulos, 1998).

Following the Perugia Group experience, thiotepa has been adopted by other hospitals and it is now a standardized and systematically used agent in the indication of conditioning treatment prior to haematopoietic progenitor cell transplantation.

Good penetration into the CSF and minor organ toxicity qualified thiotepa as one of the first classical drugs to be employed in CNS tumours (Heideman, 1989). In fact, in paediatric brain tumours the possibility to increase intracerebral DNA-alkylating activity without applying highly toxic brain irradiation (Gutierrez-Delgado, 2003; Gutierrez-Delgado, 2001) makes high dose thiotepa one of the first drugs used in this context in pre-irradiated or very young children.

A broad base of clinical experience with thiotepa and different combination partners in multiple complex contexts is available. Thiotepa can certainly be regarded as well established and systematically used in the dose-intensive treatment of paediatric brain tumours.

Thiotepa is covering all patient ages, from pediatric to elderly, giving the opportunity to physicians to apply dose escalating treatment options.

Over 20 years of use in the indication of conditioning treatment prior to haematopoietic progenitor cell transplantation, thiotepa has shown:
- potent immuno-myeloablative effect;
- significant contribution to engraftment, also across the HLA barrier;
- significant contribution to eradication of leukemia/lymphoma;
- very low extra-haematological toxicity even at high doses, also in combination with TBI or other agents (no veno-occlusive disease-VOD);
- no age restriction;
- use in many different diseases from haematological malignancies to solid tumours, from thalassemia to autoimmunity disorders;
- ease of combination with TBI or many other alkylating agents (CY, Busulfan, Treosulfan, TBI, FLU, Melphalan).

In preparation of the initial submission, a PubMed search for thiotepa produced 2895 hits, 1060 of which in the conditioning treatment prior to bone marrow transplantation. Considering the literature available between 1999 and 2008, it is estimated that 15.500 patients were treated with conditioning regimens including thiotepa.

Discussion on 'well-established medicinal use'

Thiotepa has been used in conditioning treatment prior to haematopoietic progenitor cell transplantation in the EU for over 10 years. Moreover, it should be emphasized that:
1) An overview of all published bibliographical documentation with regard to the first documented time of use of the substance as a medicinal product in the EU is described and sufficiently addressed.
2) The extent over which the active substance intended for authorisation has been regularly used in patients (i.e. quantitative aspects of the use of the substance) is sufficiently addressed. The demonstration of the well-established use of the substance (incl. time and extent of use) is specifically related to the use in a part of the claimed indication. The use of thiotepa as single agent is not well documented and therefore not included in the indication.
3) The scientific literature is considered coherent with respect to the clinical efficacy and safety of the product, *i.e.* both the quality of the data in the publications and the consistency between different studies.
4) The degree of scientific interest in the use of the substance (reflected in the published scientific literature) is sufficiently addressed (e.g. high number and frequent publications on the use of the substance).

2.3   Quality aspects

Introduction

Tepadina is a powder for concentrate for infusion for intravenous use containing thiotepa.
Tepadina is available in two presentations: 15 mg of thiotepa and 100 mg of thiotepa per vial. The medicinal product is reconstituted with 1.5 ml (15 mg presentation) or 10 ml (100 mg presentation) sterile water for injections to the final concentration of 10 mg thiotepa per ml.

The medicinal product contains no other ingredients apart from the active substance and it is packed in glass vials.

Drug Substance

Thiotepa is the INN for the chemical substance 1,1',1''-phosphinothioylidynetris-Tris-(1-aziridinyl)phosphine sulphide. The molecular formula of thiotepa is $C_6H_{12}N_3PS$, the relative molecular mass 189.23 g/mol, with the following structural formula:
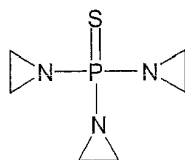
The active substance is well known and has been satisfactorily characterised. It forms fine, white crystalline flakes freely soluble in water, very soluble in organic solvents like acetonitrile, acetone, methanol and only sparingly soluble in n-hexane. No evidence of polymorphism was found during the characterisation studies performed.

- Manufacture

Thiotepa is supplied by one manufacturer only. An Active Substance Master File was submitted to support the quality of active substance. A detailed description of the manufacturing process, including process flow diagram and in-process controls were provided, and there is evidence that the process is under control and delivers an active substance of consistent quality.

- Specification

Thiotepa is controlled according to relevant tests with justified limits with regards to assay, related substances, heavy metals, clarity, particle count, bacterial endotoxins, microbiological quality and residual solvents. Adequate validation data was presented for the in-house analytical methods. Satisfactory batch analysis results were provided for seven batches.

- Stability

ICH compliant stability studies at accelerated (25 °C/60 % RH) and long-term storage conditions (2 °C - 8 °C) have been performed for up to 6 months and 36 months, respectively. The following parameters were investigated: description, water content, assay, related substances, clarity, microbiological quality and bacterial endotoxins. The stability data support the proposed re-test period of 18 months at 2 °C - 8 °C when stored in the commercial container closure system.

Drug Product

- Pharmaceutical Development

The 15 mg presentation of thiotepa was first authorised in the Europe in 1971 and is now authorised in several European Countries. The freeze-dried powder of thiotepa with no excipients was first proposed in 1995. The improved stability profile of the lyophilisate formulation was preferred to the initially developed solution of thiotepa. The 100 mg presentation of thiotepa does not have any previous marketing authorisation.
The container closure system consists of colourless 3 ml vials (15 mg presentation) or 10 ml vials (100 mg presentation), a siliconised gray bromobutyl freeze drying stopper and a planar blue (15 mg) or red (100 mg) silver "flip top" cap.
Satisfactory data are provided to justify the proposed manufacturing process of the finished product.

- Adventitious Agents

The active substance is not of human or animal origin, therefore there is no TSE/BSE risk.
This medicinal product does not contain excipients.

- Manufacture of the Product

Heat sterilisation is not possible due to the thermolability of the active substance. Consequently, the drug product is manufactured by aseptic processing, including dilution of the active substance in water, sterilisation by filtration, filling into sterile pyrogen free vials and lyophilisation under aseptic conditions.

A detailed description of the manufacturing process was provided. Process validation data are provided for three commercial size batches of the 15 mg presentation and three pilot scale batches and one commercial size batch of the 100 mg presentation; the applicant commits to perform process validation in the first two commercial size batches of the 100 mg presentation after authorisation. The critical steps of the manufacturing process of both presentations have been identified and validated.

- Product Specification

The finished product specifications include the following parameters: appearance, identity, reconstitution time, clarity of solution, pH, water content, related substances, particulate contamination, insoluble substances, sterility, bacterial endotoxins, assay and uniformity of dosage units. The specifications are adequate to control the medicinal product.

All analytical methods used to test the drug product have been properly described. All non-pharmacopoeial methods were validated.

Batch results for three batches of both product presentations are enclosed. Analytical results of commercial production batches are presented for the 15 mg formulation, whereas for the 100 mg formulation results are presented for validation batches. The results comply with the specification and confirm consistency of the product.

• Stability of the Product

Satisfactory accelerated and long-term stability data are provided for up to 6 months and 18 months, respectively. The stability studies were performed under ICH conditions.

A shelf-life of 18 months is granted for the 15 mg formulation and 100 mg formulation with the special precautions for storage "Store and transport refrigerated (2 °C – 8 °C)".

The in-use stability of the product when reconstituted with sterile water for injections and further diluted with 0.9 % saline solution has been demonstrated for up to 7 days when kept at 25 °C and for up to 21 days when stored at 2 °C – 8 °C, however since the product is not preserved it should be used immediately after reconstitution and dilution.

• Comparability Exercise for Drug Product

Compatibility of the formulation with the proposed container closure system is demonstrated by the ongoing stability studies.

Discussion on chemical, pharmaceutical and biological aspects

The active substance and the medicinal product have been appropriately characterised and generally satisfactory documentation has been provided. The results indicate that both can be reproducibly manufactured.

At the time of the CHMP opinion, there were minor unresolved quality issues which have no impact on the Benefit/Risk ratio of the product. The applicant gave a Letter of Undertaking and committed to resolve it as a Follow-up Measures after the opinion, within an agreed time-frame.

2.4 Non-clinical aspects

Introduction

The non-clinical dossier submitted by the applicant was based exclusively on literature data. None of the submitted studies were conducted for regulatory purposes. GLP compliance is therefore not expected, nor was Protocol Assistance by the CHMP or National Competent Authorities sought on the non-clinical dossier.

Pharmacology

• Primary pharmacodynamics

Mechanism of action. The applicant mainly refers to the review published by van Maanen et al (2000) about the chemistry, pharmacology and pharmacokinetics of thiotepa. According to these authors, the interaction of thiotepa with DNA can follow two different pathways:
- Formation of cross-links between thiotepa and DNA: Reaction of thiotepa with DNA leads to alkylation of guanine and adenine. This produces inter- and intrastrand cross links, which are responsible for the inactivation of DNA and for the cytotoxicity. Interstrand cross-links have been observed upon incubation of murine L1210- (lymphoblastic leukaemic) and human MCF-7- (breast carcinoma) cells with thiotepa.
- Thiotepa acting as a prodrug for aziridine: The released aziridine can react with DNA, resulting in the formation of a stable guanidine adduct, imidazole ring opening and DNA chain scission.
In addition, thiotepa is metabolized in other compounds with alkylating properties:
- TEPA: according to van Maanen et al (2000), its interaction with DNA is assumed to be different from that of thiotepa, but it produces DNA lesions similar to the ones induced by a mono-functional alkylating agent. The therapeutic effectiveness of TEPA in the treatment of transplantable rat tumours (sarcoma 231 and Flexner-Jobling carcinoma) was demonstrated by Crossley et al (1953). Teicher et al (1989) showed in MCF-7 cells that TEPA is less cytotoxic than thiotepa.
- Monochloro-TEPA and thiotepa-mercapturate, whose mechanism of action remains to be elucidated. The presence of other metabolites with alkylating activity of unknown structure is well known (van Maanen et al, 2000).

In vitro studies. Investigation on the cellular pharmacology of thiotepa and TEPA in the murine leukaemia cells P388 showed that both substances inhibit DNA, RNA and protein synthesis. Thiotepa and TEPA both showed activity against cell growth, but thiotepa appeared to be twice as active (IC50 thiotepa: 1.5μM; IC50 TEPA: 2.8μM). Inhibition of radiolabelled macromolecular precursor incorporation resulted in a dose-dependent inhibition of DNA, RNA and protein synthesis for thiotepa and TEPA. The effect of inhibition of [³H]-uridine and [³H]-leucine incorporation was much smaller than the [³H]-thymidine (Miller et al, 1988).

In vivo studies. The bone marrow and tumour toxicity of thiotepa was investigated in tumour-bearing mice by Teicher et al (1990). The surviving fractions of bone marrow (CFU-GM) cells and of tumour cells were determined 24 hours after intraperitoneal treatment with thiotepa (10, 15, 20, 30, 40 and 50 mg/kg, given either as a single dose or as 3 doses at 4.5-hour intervals).

At the lowest dose range, thiotepa on a single-dose schedule was more toxic to bone marrow cells than to tumour cells. On the multiple-dose schedule, thiotepa was always more toxic toward tumour cells than toward bone marrow cells to an extent ranging from 2- to 13-fold over the dosage levels examined. At the lowest dose of thiotepa, single-dose and multiple-dose schedules produced the same level of bone marrow toxicity; however, 30 mg/kg of thiotepa on the multiple-dose schedule killed about 5 times the bone marrow CFU-GM of the single dose regimen.

Three studies involving murine transplantation models were submitted.

Terenzi et al (1990) studied the permissive effect of thiotepa on engraftment in a murine (C3H/HeJ) model of allogenic bone marrow transplantation. C3H/HeJ mice were transplanted with mismatched (donor: C57Bl/6) T cell-depleted bone marrow following conditioning with TBI alone or in combination with cyclophosphamide, busulfan or thiotepa.

Chimerism analysis was then carried out by cytofluorimetry (using anti-H2Kb and anti-H2Kk monoclonal antibodies) to investigate the effect of conditioning combination on donor-type chimerism. The effect of TBI on engraftment was enhanced by additional treatment of the host with thiotepa or busulfan, but not when cyclophosphamide was used (data not shown). It should be noted that lethality was greater in mice undergoing conditioning with TBI and thiotepa (administered one day prior to BMT) than in the other groups. Subsequent experiments conducted by the authors showed that survival was significantly improved when the interval between thiotepa treatment and stem cell transfusion was increased. The applicant concluded that busulfan and thiotepa have comparable myeloablative effects, and this is relevant in the setting of stem cell transplantation, as well as in patients with myeloid malignancies.

Down et al (1998) investigated the haematological properties of thiotepa with respect to the survival of different progenitor and stem cell subsets in the mouse bone marrow by enumerating cobblestone area-forming-cell (CAFCs) subsets. The CAFC assay was performed on harvested bone marrow pooled from groups of three to four mice 24 hours after treatment. The influence of the combination of thiotepa (20mg/kg i.p. day −1) and TBI (5Gy day −2) on donor engraftment was tested in both syngenic and allogenic BMT models.

It was shown that thiotepa is toxic to committed progenitor populations (early developing CAFCs) and it can promote early donor engraftment. Primitive stem cells (late developing CAFCs) remained relatively resistant to this drug, which may lead to long term host marrow repopulation and disappearance of donor engraftment. TBI led to a high level of short term engraftment, too, and it was capable of producing sustained levels of chimerism through depletion of primitive CAFCs in the host. Addition of thiotepa to TBI did not significantly improve long-term engraftment of syngenic marrow, while this combination had an effect in allogenic BMT by preventing allograft rejection. The authors concluded that the effectiveness of thiotepa in allogenic graft tolerance is due to its immune suppressive properties rather than to depletion of the host primitive stem cell pool.

Abdul-Hai et al (2007) evaluated the use of thiotepa in a murine model simulating autologous stem cell transplantation with or without additional agents. Between 1 and 11 days following inoculation of BALB/c mice with B-cell leukaemia (BCL1) cells (simulating pre-transplant leukaemia loads), each group received an 'induction-like' irradiation and/or cytotoxic regimen. Animals were either followed without treatment, or an adoptive transfer (AT) was performed to untreated BALB/c mice. Without follow-up AT, high-dose thiotepa did not change the time to appearance of leukaemia. Nevertheless, in the AT experiments, thiotepa as a single agent showed better anti-leukaemic activity than busulfan. Cyclophosphamide-containing regimens were the most effective, and the thiotepa-cyclophosphamide combination was as effective as the commonly used busulfan-cyclophosphamide combination, and

more effective than the busulfan-thiotepa combination. Moreover, a synergistic effect was seen in the thiotepa-cyclophosphamide combination (none of the animals developed leukaemia, whereas 4/10 animals in the cyclophosphamide-TBI group developed leukaemia). The authors concluded that in spite of a moderate effect of thiotepa against BCL1 leukaemia when used alone, its combination with cyclophosphamide is promising and should be tested further in allogeneic murine models and clinical studies.

- Secondary pharmacodynamics

No studies considered by the CHMP as relevant were submitted (see discussion on non-clinical aspects).

- Safety pharmacology programme

No conventional safety pharmacology data are available. Of the cited publications, the following can be considered of relevance:
Rzeski et al (2004) addressed the issue of CNS safety pharmacology and toxicity. They reported morphological changes consistent with neurotoxicity *in vitro* and *in vivo*. In cortical neuronal cultures prepared from 18-day-old Wistar-rat foetuses, thiotepa (0.1-500 µM) caused concentration-dependent neuronal death. NMDA (MK 801: 10µM) and AMPA receptor antagonists (GYKI 52466; NBQX: 10µM) and also a pancaspase inhibitor (Ac-DEVCHO:1nM) ameliorated neurotoxicity. *In vivo*, thiotepa (15, 30, 45mg/kg) was injected intraperitoneally to 7-day-old Wistar rats. Electronic microscopy of different brain regions showed oedematous swelling of dendritic processes. Taking *in vitro* and *in vivo* results together, it was presumed that excitotoxic mechanisms and caspase-mediated cell death contribute to the neurotoxicity of thiotepa.

- Pharmacodynamic drug interactions

A number of publications were cited. There is evidence that drug interactions may occur with succinylcholine, cyclophosphamide and phenobarbital, but it is unclear if the interactions are of pharmacodynamic nature in addition to the recognised pharmacokinetic (see non-clinical and clinical pharmacokinetics).
Pharmacodynamic drug interaction *in vitro* has been demonstrated by determining the surviving fraction of cells after treatment with cyclophosphamide and thioTEPA. Simultaneous exposure of murine MCF-7 cells to a combination of thioTEPA and cyclophosphamide (administered as 4-hydroxycyclophosphamide), resulted in supra-additive tumour cell death (Teicher et al, 1988, 1990). However in vivo infusion of daily 5 mg/kg thioTEPA for 6 days as maximum tolerated dose and cyclophosphamide on alternate-day schedule for three infusions (100 mg/kg x3) to EMT-6 tumour bearing mice produced about 25 days of tumour growth delay. This delay was not significantly different from the expected additive individual drug effects. Combination treatment of EMT6 cells in vivo with 10 or 15 mg/kg thioTEPA and various doses of cyclophosphamide produced additive tumour cell death paralleled by sub-additive bone marrow toxicity in some cases (Teicher et al, 1988).

Pharmacokinetics

Analytical methods. Various analytical methods to determine thiotepa in plasma and urine were described to support pharmacokinetic studies. These were either chemical methods, based on the ability of ring cleavage (Titration of thiosulphate in urine samples; Colorimetric assay to determine the alkylating activity; Fluorimetric assay based on the S-alkylation of the ethyleneimine groups of thiotepa and TEPA), or physical methods, chromatographic and spectrometric (Gas chromatography: most applied method for PK studies of thiotepa in biological fluids; High pressure liquid chromatography (HPLC): rarely used for measuring thiotepa; Liquid chromatography with mass spectrometric detection (LC-MS): used for the determination of thiotepa-mercapturate in urine samples; Nuclear magnetic resonance (NMR) spectrometry: accurate and precise, but not frequently described).

Absorption. The applicant summarized absorption data obtained in various species using the oral, intraventricular or intraperitoneal routes. In some studies, the IV route was used as a reference.

Distribution. In general, distribution of thiotepa is rapidly followed by fast elimination from the plasma compartment. Craig et al (1959) found at 2 mins after intravenous administration of 2mg/kg only 10% and 14% of the radioactivity of 32P-labelled thiotepa in the blood of mice and rat. Mellet et al (1960, 1962) reported low levels of thiotepa already 2 hours after administration of the drug either orally or intravenously in dogs.
According to distribution studies in animals (Dairman, 1966; Mellet et al, 1960; Ruddon et al, 1964; Strong et al, 1986), both thiotepa and TEPA penetrated into cerebrospinal fluid. As early as 15 mins after an i.v. dose of thiotepa, CSF levels equilibrated with those in the plasma and resulted in identical plasma and CSF levels (Strong et al, 1986).
Binding to plasma proteins for thiotepa and TEPA in dogs is about 0-10% (Mellet et al, 1960). Selective retention of labelled material was found in the red cells of the rat, located in the globin part of haemoglobin (Craig et al, 1959).

Metabolism. Metabolism has been investigated in various species (mice, rat, rabbit, dog and human). In the mouse, thiotepa is metabolised to inorganic phosphate as the only product. In other species, TEPA was found to be the main metabolite of thiotepa (Maanen et al, 2000). The conversion of thiotepa to TEPA is catalysed by specific cytochrome isoenzymes. Although the specific P450 isoform involved in the metabolism is not yet clear, evidence from animal and in vitro studies suggest that thiotepa is metabolised in rats by CYP2B1 and CYP2C11 (Chang et al, 1995) and in human by CYP 2B6 and CYP3A4 (Jacobson et al, 2002).
In vitro, thiotepa is able to form conjugates with glutathione after incubation with glutathione and glutathione S-transferase. Glutathione conjugation is followed by subsequent removal of glutamyl and glycine residues to form thiotepa-cysteinate which is then N-acetylated to thiotepa-mercapturate. This metabolite has been found in vivo in humans too, accounting for 11% (range 6.3-22.5) of the administered dose.
A third metabolite of thiotepa identified in the urine is monochloroTEPA (N``2-chloroethylphosphoramide). The conversion depended on pH and chloride concentration, and could be formed in vivo but also ex vivo in the urine. The amount of monochloroTEPA excreted was 0.5% (range: 0.3-0.8%) of the administered dose.

Excretion. In general, thiotepa and its metabolites are predominantly excreted via urinary excretion. Dependent on investigated species, methods of detection and documented sampling time, between 50% and 90% of the total radiolabelled thiotepa was recovered in the urine (Craig et al, 1959; Dairman, 1966; Mellet et al, 1962). The mean urinary recovery occurred within the first 8-18 hours after administration.
According to the metabolic pathway of thiotepa in the different species, more than 99.5% of thiotepa was metabolised and only small amounts (0.3-0.7%) of unchanged drug were detectable in the beginning of urinary excretion (Craig et al, 1959; Dairman, 1966; Mellet et al, 1960 and 1962). The main urinary metabolite was TEPA, accounting for almost 8-15% of the recovered radioactivity. Noteworthy was the absence of thiotepa and TEPA in mouse urine, except in a single urine probe 8 h after oral administration (Dairman, 1966).

Pharmacokinetic drug interactions. Because thiotepa is extensively metabolised via cytochrome P450 enzymes and glutathione conjugation, it might interfere with several drugs. Apart from demonstrating the influence of phenobarbital and cyclophosphamide on thiotepa pharmacokinetics in humans, so far only limited studies of drug interactions with thiotepa have been reported in animals.
The lethal dosage of thiotepa decreased when it was administered to mice concomitantly with non-lethal dosages of pentobarbital. The LD_{50} determined 1 hour after infusion decreased from >1000 mg/kg without pentobarbital to 630 mg/kg with additional pentobarbital. The LD_{50} after 24 hours of observation fell from 400 mg/kg to 250 mg/kg respectively (Munson et al, 1974).
In rats, metabolism of thiotepa to TEPA by uninduced microsomes was found to be catalysed by CYP2C11, a constitutively expressed male specific isoform. Depletion of hepatic CYP2C11 by cisplatin led to 70% reduction of TEPA formation catalysed by the isolated liver microsomes on day 7 after cisplatin administration. In addition, treatment of rats with clofibrate and phenobarbital as

CYP2B1 inducers accelerated thiotepa clearance by increasing metabolism to TEPA (Ng and Waxman, 1991).

In 9L gliosarcoma bearing rats pretreated with phenobarbital (80 mg/kg i.p. for 4 days) and then administered thiotepa, the tumour growth delay effect was reduced and body weight loss was ameliorated. In the same study, the effect of SKF-525A (CYP2C11 inhibitor) was tested; it was shown to reduce the clearance of thiotepa as expected, enhance the tumour growth delay and increase body weight loss and lethality (Chang et al, 1995).

In dogs pre-treated with SKF-525A prior to thiotepa administration, plasma TEPA levels were reduced and a significant delay was noted in the appearance and disappearance of TEPA in the plasma compared to controls (Mellet et al, 1960).

Toxicology

- Single dose toxicity

Three published single dose toxicity studies were submitted which are summarised in the following table:

Table 1: Single Dose toxicity studies

Reference	Species/ Sex/Number/ Group	Route/dose mg/kg	LD_{50} mg/kg	Major findings
Sloboda et al, 1960	Mouse (C3H ♀)	i..p/12-39 p.o./30-63 i.p. 6x (daily)/4-8	24.0 44.5 5.1	Only death recorded
Sloboda et al, 1960	Rat (wistar ♀)	i.p/8-16 p.o./35-72 i.p. 6x (daily)/2.83-5.76	9.4 54.5 3.48	Only death recorded
Sloboda et al, 1962	Mouse (C3H) sex unknown	i.p. p.o.	24 45	Only death recorded
Sloboda et al, 1962	Rat (wistar) sex unknown	i.p. p.o.	9.4 55	Only death recorded
Halliday et al, 1958	Mice – swiss ♂	p.o./6.25-50 i.p./1.25-10 p.o. x5 daily/3.1-25 i.p. x5 daily/2.5-40	46 8.4 35 18	p.o. x5: ↓ white blood cells at low dose >5 ↓ red blood cells, haemoglobin No other information
Halliday et al, 1958	Rat – wistar ♂	i.p./10-40 p.o. (vehicle water)/? p.o. (NaHCO₃) i.p. x5 daily/4, 8	8.4 26.5 14.5 3.9	i.p. prior to death: diarrhea, weight loss, dyspnea, bloody masks p.o.: N.A.
Halliday et al, 1958	Dog	Intravenous/0.25-1 Oral/0.5-2	Between 1 and 5 >2	haematocrit↓ peripheral lymphocytes↓ prothombin time ↓ coagulation time↑

- Repeat dose toxicity (with toxicokinetics)

Repeat dose toxicity data were derived primarily from 3 published studies summarised below:

Table 2: Repeat dose toxicity studies

Species Réf.	Route, dose, duration	Major findings
Mouse (Swiss) 10 M/ group[a] Halliday et al, 1958	Oral 2.5, 5, 10, 20, 40 mg/kg/day 5 days	LD_{50} = approx. 35mg/kg/day (multiple oral doses) No gross or microscopic pathology was conducted.
Mouse (Swiss) 10 M/ group[b] Halliday et al, 1958	IP 3.1, 6.25, 12.5, 25 mg/kg/day 5 days	LD_{50} = 18 mg/kg/day (multiple ip doses) No gross or microscopic pathology was conducted.
Mouse (Swiss) 10 M/ group Halliday et al, 1958	Oral 5, 10, 18.75, 37.5, 75 mg/kg/day 4 – 46 days[c] (5 days/ week)	No mortality was seen at 5 mg/kg/day. At $\geq$ 10 mg/kg/day death generally occurred between the 4th and the 26th day. Doses $\geq$18.8 mg/kg/day were lethal to all mice. Five animals initially surviving the 10 mg/kg/d and all mice surviving 5 mg/kg/d were examined for effects of thiotepa on haematology on day 31 after first doses. No significant effect was noted except for a significant reduction in circulating white blood cells.
Rat (Wistar) 10F/ group Sloboda et al, 1960	IP 2.83-5.76 mg/kg/day 6 days	LD_{50} = 3.5 mg/kg/day
Rat (Wistar) M (number not reported) Halliday et al, 1958	IP 5 days (doses not mentioned)	LD_{50} = 3.9 mg/kg/day (multiple ip doses)
Rat (Wistar) M (number not reported) Halliday et al, 1958	IP 4 mg/kg/day 5 days	Mortality occurred 2-3 days after the final injection. At gross examination, bone marrow and intestinal contents were fluid. At microscopic examination, the nucleated elements of the bone marrow showed 95% depletion, the thymus involuted, and changes (unspecified) were noted in all parts of intestinal tract.
Rat (Wistar) 6M/ time point Halliday et al, 1958	IP 5 mg/kg/day 4 days	Haematological, gross and histopathological examinations were performed on day 2, 4 and 7 after treatment completion (+predose for haematology). Haematology: $\downarrow$ platelet, $\downarrow$ WBC, $\downarrow$ polymorphonuclear neutrophils, $\downarrow$ hematocrit, $\downarrow$ reticulocytes Gross examination: fluid marrow, small thymus, , haemorrhages in lymph nodes and serosa of the bowel Microscopic examination: depletion of nucleated elements in bone marrow, thymus involution, $\downarrow$ lymphocytes in lymph nodes and splenic follicles
Rat (albino) 6M/ group Cappiello et al, 1960	SC 0, 0.5, 1 mg/kg/day	$\downarrow$ body weight gain, $\downarrow$ food consumption ($\geq$ 0.5 mg/kg) Organ weights: $\downarrow$ thymus weight ($\geq$ 0.5 mg/kg), $\downarrow$spleen weight ($\geq$ 0.5 mg/kg), $\downarrow$ liver weight (1 mg/kg), $\downarrow$ kidney weight (1 mg/kg), $\downarrow$

Species Réf.	Route, dose, duration	Major findings
	14 days	lungs weight (1 mg/kg), ↓testes weight (1 mg/kg)
		Haematology: ↓ WBC, ↓ lymphocytes (1 mg/kg[d])
		Microscopic examination: slight atrophy of lymph nodules, lack of erythromyelosis in the spleen, kidney changes (tubular dilatation, tubular vacuolization), ↓ nucleated cells and ↑in bone marrow (1 mg/kg)
Dog 1-2/sex/group Halliday et al, 1958	Oral 0.5, 1, 2 mg/kg/day 14 days (5 days/ week)	Mortality: 4/4 on days 9-11 at 2 mg/kg, 1/2 on day 14 at 1 mg/kg
		Clinical signs: anorexia, weight loss, hyperthermia, diarrhea, vomiting (1 and 2 mg/kg), corneal opacity exudate from the eyes (2 mg/kg)
		Haematology (performed in the high dose group only): near disappearance of WBC (all types) and platelets, virtually total depletion of the bone marrow
		Biochemistry (performed in the high dose group only): ↑ sulfobromophthalein retention, ↑ ALP ⇒ slightly impaired liver function. ↓ blood glucose, which may be a consequence of liver inadequacy
		Gross examination (performed in the high dose group only): widespread haemorrhages (lymph nodes, GI tract, lungs, heart, peritoneal surfaces), ulcerations in oral cavity
		Histopathological examination (performed in one high dosed dog only): Changes in the intestinal tract, lymph nodes, spleen and tonsil were essentially related to the haemorrhages noted above (sections of bone marrow were not present). In the lungs, in addition to the haemorrhages, some of the alveoli contained edema fluid, fibrin and karyorrhectic debris, but without inflammatory cells ("acellular pneumonia").
Dog 1-2/sex/group Halliday et al, 1958	IV 0.25, 0.5, 1 mg/kg/day 14 days (5 days/ week)	Mortality: 4/4 on days 9-10 at 1 mg/kg
		Clinical signs: anorexia, hyperthermia, diarrhea, epistaxis, rectal bleeding (1 mg/kg)
		Haematology (performed in the high dose group only): see oral study
		Biochemistry (performed in the high dose group only): see oral study
		Gross examination (performed in the high dose group only): see oral study
		Histopathological examination (performed in one high dosed dog only): Virtually 100% depletion of nucleated elements in bone marrow which contained only congested vessels filled with red cells The duodenum and remainder of small intestines showed villous haemorrhages, denuded villi, slight atypis of the deep glandular epithelial elements, occasional dilated glands, ↑ mononuclear cells in the mucosa. The large intestine showed only haemorrhages. The lymph nodes contained many red cells in the sinuses as well as areas of haemorrhage. Virtually all lymphocytes had disappeared and only large mononuclear and plasma cells remained. The splenic follicles were reduced in size and perifollicular haemorrhages were present. The tonsils were ulcerated and contained necrotic debris and numerous bacterial colonies. The only inflammatory cells were

Species Réf.	Route, dose, duration	Major findings
		mononuclear cells. Bacterial colonies were also nioted in the lung, one lymph node, and the heart, but no cellular reaction or necrosis was associated with them.

[a] 9 M in the group dosed at 10 mg/kg/day

[b] 5 M in the group dosed at 3.1 mg/kg/day

[c] treatment duration varied according to death rate; treatment duration amounted to 46 days in groups dosed at 5 and 10 mg/kg/day, 37 days in the group dosed at 18.75 mg/kg/day, and 4 days in the groups dosed at 37.5 and 75 mg/kg/day

[d] on days 7 and 14 of treatment

- Genotoxicity

The genotoxicity of thiotepa is well established based on numerous studies. These were reviewed by the International Agency for Research on Cancer (IARC, 1990), while the Applicant additionally submitted some key publications (Bochkov et al, 1981; Breau et al, 1984, Rao et al, 2005).

According to IARC (1990), thiotepa induced chromosomal aberrations in germ cells, sperm abnormalities and dominant lethal mutation in mice *in vivo*. It induced micronuclei in the bone marrow of rats and mice, chromosomal aberrations in bone marrow cells and liver cells of mice and in peripheral lymphocytes of rabbits and rhesus monkeys. It also caused sister chromatid exchange in bone-marrow cells of mice *in vivo*.

Thiotepa induced DNA damage in chick embryos. It induced chromosomal aberrations in cloned hamster cells, in Chinese hamster cells and in human cells, sister chromatid exchange in human, mouse, Chinese hamster and rabbit cells, gene mutations in Chinese hamster cells and unscheduled DNA synthesis in human peripheral lymphocytes *in vitro*. It induced cell transformation in mouse cells. Thiotepa induced sex-linked recessive lethal mutations in *Drosophila* and sister chromatid exchange and chromosomal aberrations in *Vicia faba*. It induced gene mutations in *Aspergillus nidulans* and *Salmonella typhimurium*.

Bochkov et al (1981) reviewed and compared the mutagenicity of 3 alkylating compounds, including thiotepa and its metabolite TEPA. Mutagenic and genotoxic effects are reported in a number of *in vitro* and *in vivo* models.

Comparing the mutagenic activity in the Ames assay with antineoplasitc activity against different human cell lines, Breau et al (1984) assumed that growth was inhibited at concentrations that induced mutagenicity.

Recently, Rao et al (2005) evaluated in non human primates the extent to which genotoxicity of thiotepa was dependent on the schedule of administration by giving the drug as either a bolus or a 96-hour continuous infusion. They quantified the chromosomal aberrations in bone marrow cells on days 1, 2, 5 and 28 of the study. They showed that thiotepa produced more abnormal metaphases following bolus administration compared to a 96 h infusion. So, they concluded that infusion of thiotepa is less genotoxic to normal bone marrow cells than is bolus administration.

- Carcinogenicity

The IARC (1990) classified thiotepa as being carcinogenic to humans (group 1) based on sufficient studies in humans that indicate a causal relationship between exposure to thiotepa and human cancer. The review of human carcinogenicity data showed that several cases of leukaemia following treatment with thiotepa alone have been reported. One case-control study showed a strong combination between risk for leukaemia and treatment with thiotepa.

The findings in humans were supported by carcinogenicity studies in mice and rats. The National Cancer Institute (1978) concluded that thiotepa is carcinogenic in both Sprague-Dawley rats and B6C3F1 mice under the experimental conditions used (intraperitoneal administration of up to 2.3 mg/kg in mice and 2.8 mg/kg in rats, 3 administrations per week for up to 52 weeks). In the rats, it induced squamous cell carcinoma of the skin or ear canal in both males and females, and haematopoietic neoplasms in the males. In the mice, it induced lymphoma or lymphocytic leukaemia in both sexes and squamous-cell carcinoma in the skin and associated glands of males.

In particular mice strains (A/J and A/He) where increased rates of pulmonary tumours are common, intraperitoneal administration of thiotepa (3x/ week for 4 weeks) was shown to further increase the

rate of lung tumours and to shorten their induction time (Shimkin et al 1966; Stoner et al 1973).
Rats treated with thiotepa by intravenous infusion (1x/week for 52 weeks) developed benign and malignant tumours at multiple sites, including the abdominal cavity, mammary gland, blood vessels, bone marrow, lymphatic system, salivary glands, adrenal gland and testes (Schmahl et al. 1970).

- Reproduction Toxicity

It is well established that thiotepa may cause impaired fertility in animals and humans (NCI, 1979; NIH, 2002).
In the studies by Evenson et al (1986) and Meistrich et al (1982), impaired fertility and interference with spermatogenesis were observed in male mice.
Adams et al (1961) investigated the effects of thiotepa in early embryonic development in rabbits. Rabbits were given thiotepa in parenteral doses of 3-10 mg/kg, during the blastocyst stage and implantation was determined. Thiotepa given on days 3, 4, 5 and 6 affected all blastocysts. The histological appearance of the 6.5-day embryo depended on the timing of treatment: the earlier it was given, the more drastic the effect. Intervals of 9 hours allowed the recognition of the specific blastocyst stage. After treatment on day 3 or 4, only small thin embryonic discs containing a few granules remained. The effect seemed to be exerted mainly on the embryonic disc rather than on the trophoblast. Moreover, lethality of low doses (3 mg/kg on day 6-8) of thiotepa to rabbit foetuses was observed without the need for special experiments.
Teratogenic effects of thiotepa were investigated in mice by Tanimura et al (1968). The lethal effects on the foetus were dose dependent above 7.5 mg/kg. The dose required to kill all foetuses was 10 mg/kg on day 8.5 of gestation and increased to 20 mg/kg on day 10.5 of gestation. In addition, growth suppression under treatment was also demonstrated.
Examination of the cleared specimens revealed additional skeletal abnormalities. First, malformations of the skeleton, especially the axial, were numerous. In the vertebrae, fusion of the arches and deformations of the bodies were found. Early infusion (day 6.5) affected mainly the superior cervical vertebrae, at later infusion until day 10.5 malformation descended caudally. Rib anomalies such as fusion and waviness occurred with marked stage specificity. In general, some of the same anomalies listed above can also be caused by other teratogens.
Infusion in the early post-implantation stage (day 4.5-5.5) of 7.5 mg/kg was not found to alter significantly the implantation rate, foetal mortality and incidence of malformed foetuses. Survivors were found to have considerably reduced body and placental weights.
Repeated infusions of 0.5 mg/kg from day 0.5-12.5 were not found to cause significant differences concerning the implantation site or foetal mortality.

- Toxicokinetic data

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

- Local tolerance

No studies were submitted.

- Other toxicity studies

No studies were submitted.

Ecotoxicity/environmental risk assessment

The $PEC_{surface\ water}$ was calculated taking into account that in Europe there are 25000 Haematopoietic Progenitor Cells Transplants each year.

The Fpen was found to be $6.85.10^{-5}$ µg/L (which is lower than the limit value of 0.01 µg/L).

Discussion on the non-clinical aspects

There is consistent evidence from years of numerous studies and clinical practice that thiotepa possesses anti-tumour activity by alkylating activity. The metabolites TEPA, monochloro-tepa and thiotepa-mercaptoturate also have such an activity. Both thiotepa and tepa bind to DNA, RNA and presumably proteins and inhibit DNA, RNA and protein synthesis with IC_{50} values in the low micromolar range, thiotepa being the most active.

Published data were submitted to support the therapeutic indication proposed for Tepadina, *i.e.* conditioning treatment prior to conventional haematopoietic progenitor cell transplantation. The myeloablative effect of thiotepa was studied in mice undergoing allogenic bone marrow transplantation. In a first study, the combination of total body irradiation (TBI) and thiotepa (10 mg/kg, i.v.) was shown to have similar effect in terms of donor engraftment as the combination of TBI and busulfan (20 mg/kg, oral). However, the interval between thiotepa administration and stem cell transfusion had to be increased (at least 2 days) to obtain similar recipient survival. Other authors suggested that the effectiveness of thiotepa in allogenic graft tolerance is due to its immune suppressive properties rather than to depletion of the host primitive stem cell pool.

No conventional safety pharmacology data is available. This is acceptable due to the significant experience available in humans with thiotepa. For the same reason, pharmacological drug-drug interactions should be directly handled clinically.

In terms of pharmacokinetics, the submitted studies were conducted since the 1960s using analytical methods available at that time, which were non-specific, insensitive, and not in conformity with current regulatory expectations. Results did not allow making a reliable estimation of pharmacokinetic parameters currently required by the ICH S3 guidelines. Recently, modern assay methods have been developed including GC, HPLC, LC-MS, NMR, but these were not validated.

There was no integration between pharmacokinetic studies and the toxicology studies in the form of toxicokinetic investigations. The metabolism of thiotepa was almost complete and metabolites were partially characterized in published studies. In general, thiotepa and its metabolites were predominantly excreted via urinary excretion; however there was no balance study. It is not clear that data from pharmacokinetic studies conducted in animals are similar to those obtained in patients.

Overall, the whole pharmacokinetic section relies heavily on the review published by Van Maanen et al, 2000. In this review, it is concluded that limited data exist on the pharmacokinetic properties of thiotepa, especially when high doses are administered. It is impossible to extrapolate from animal to human and estimate any safety margin. However, due to the toxicity of thiotepa and its specific therapeutic use, it can be considered that the concept of safety margin does not make sense in this case, since only the expected clinical benefit could justify the use of such a drug.

Single dose toxicity studies submitted to support the MAA of Tepadina showed that the LD_{50} of thiotepa obtained in rodents (intraperitoneal route) and dogs (intravenous route) are in the range of the therapeutic dose proposed for Tepadina. Death was noted a couple of days after administration. Besides clinical findings, haematological toxicity was recorded. No unexpected toxicity occurred. However, neither histopathological nor toxicokinetic data were available in single or repeat-dose studies. The sparse distribution data suggest that thiotepa distributes evenly to other organs where toxicity would be expected due to the reactive nature of thiotepa. Furthermore, the reversibility of toxic effects is unknown.

Only one repeat-dose study is available with the intended route of administration, i.e a 14-day dog study (Halliday *et al,* 1958). Strong haematological and bone marrow toxicity were observed and slightly impaired liver function was noted. Death occurred at 1 mg/kg/day. In rats treated subcutaneously at 1 mg/kg/day for 14 days, severe effects on the haematological system, bone marrow, body weight, as well as decreases in thymus, spleen and liver weight were reported. It is noted that these studies do not meet the current standards, which is not unexpected taking into account that they were performed approximately 50 years ago.

The very limited animal safety database regarding the long-term effects of thiotepa is accepted. Indeed, it is acknowledged that further non-clinical studies would provide only limited additional information regarding the long-term toxicity of thiotepa. Furthermore, conclusions obtained from clinical safety data are complicated by the co-administration of other drugs and the highly variable therapeutic context. Section 5.3 of the SPC correctly reflects the lack of information regarding the toxicity of thiotepa.

Thiotepa was shown to be genotoxic in various in vitro and in vivo tests. It was also carcinogenic in studies performed in rodents. Thiotepa was classified as carcinogenic to humans by the International Agency for Research on Cancer; a strong combination between risk for leukaemia and treatment with thiotepa was shown in one case-control study. Those results are not unexpected in view of the alkylating properties of the drug.

Thiotepa was shown to impair fertility and interfere with spermatogenesis in male mice and to impair ovarian function in female mice. It was teratogenic in mice and in rats, and foeto-lethal in rabbits. These effects were seen at doses lower than the recommended therapeutic dose. As a result, thiotepa is contraindicated during pregnancy and lactation. Women of childbearing potential have to use effective contraception during treatment and a pregnancy test should be performed before treatment is started, while male patients should seek for sperm cryopreservation before therapy is started and should not father while treated and during the year after cessation of treatment.

The $PEC_{surface\ water}$ value for Tepadina is approximately 150-fold lower than the limit value of 0.01µg/L. To fulfil the Phase 1 assessment, the applicant still has to determine experimentally an octanol-water partition coefficient value in accordance with the current OECD guidelines.

2.5 Clinical aspects

Introduction

For this bibliographical application, the Applicant has submitted clinical studies performed in adult and paediatric patients based on the published literature.

The Applicant proposed an indication for Tepadina as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult and paediatric patients. This was modified during the assessment procedure and the finally approved indication was as follows:

In combination with other chemotherapy medicinal products:

1) with or without total body irradiation (TBI), as conditioning treatment prior to allogeneic or autologous haematopoietic progenitor cell transplantation (HPCT) in haematological diseases in adult and paediatric patients;

2) when high dose chemotherapy with HPCT support is appropriate for the treatment of solid tumours in adult and paediatric patients.

The posology of Tepadina is variable and it depends on age, disease setting, type of transplant (autologous vs allogeneic) used and type of chemotherapy regimen to which Tepadina is incorporated.

GCP

The applicant has not conducted as yet any additional clinical studies. Scientific data were cited if published in peer-reviewed journals. As a rule, however, this conveys no statement on the degree of GCP-compliance of such investigations and trials.

Pharmacokinetics

The following tables report the main pharmacokinetic characteristics of thiotepa in adult populations after intravenous administration at the dosages of 11.8-900 mg/m^2 and in paediatric populations after intravenous administration at the dosage of 25-300 mg/m^2.

Table 3: Summary of thiotepa pharmacokinetic characteristics in adults

Dose mg/m^2	Patients (N)	Vd (L/m^2)	CL (L/h/m^2)	T$_{1/2}$ (h)	References
12.0	21	26.6 [a]	11.2	2.1	Cohen BE et al, 1986
12.5 [a]	6	31.0 [a]	15.5	1.4	Hagen B et al, 1987
12.5	6	30.9	13.9	1.6	Hagen B et al, 1990
11.8	6	31.8	12.0	1.9	Hagen B et al, 1988
17.6	6	28.2	10.9	1.8	
37.5	13	nr	16.7	2.4	Hagen B et al, 1991
50.0	13	nr	15.7	2.3	
30.0	5	65.9	34.9	1.3	O'Dwyer PJ et al, 1991

40.0	3	72.0	43.7	1.5	
55.0	3	47.3	46.7	1.7	
65	5	68.6	22.9	2.2	
75	5	64.4	18.9	2.1	
45-270	16	60.0 [a]	16.2	2.6	Lazarus HM et al, 1987
80-120	35	40.8	19.0	1.5	Huitema AD et al, 2000/ 2001
67.5 [a]	3	58.5 [a]	14.3	2.3	Ackland SP et al, 1988
216 [a]	3	68.2 [a]	14.6	2.3	
288 [a]	5	75.0 [a]	22.9	2.3	
420 [a]	3	53.8 [a]	7.8	4.1	
180	5	nr	23.2	nr	Henner WD et al, 1987
360	5	nr	17.9	nr	
500	2	nr	13.4	nr	
700	3	nr	11.3	nr	
900	3	nr	11.4	nr	

[a]Parameter estimated from mean body weight 60 kg/surface 1.6 m^2

Table 3: Summary of thiotepa pharmacokinetic characteristics in paediatric patients

Dose mg/m^2	Patients (N)	Vd (L/m^2)	CL (L/h/m^2)	$T_{1/2}$ (h)	References
25	3	18.4	28.6	1.7	Heideman RL et al, 1989
50	4	29.6	15.7	1.8	
65	5	22.0	15.4	1.3	
75	5	25.0	11.9	2.0	
300	10	19.4	11.3	1.3	Kletzel M et al, 1992

- Absorption

No studies were submitted (see discussion on clinical pharmacology). The Applicant stated that thiotepa is unreliably absorbed from the gastrointestinal tract: acid instability prevents thiotepa from being administered orally.

- Distribution

Thiotepa is a highly lipophilic compound. According to the Applicant, after intravenous administration, plasma concentrations of the active substance fit a two compartment model with a rapid distribution phase.
In adults, the volume of distribution was calculated at 40-75 l/m² at doses between 30 and 420 mg/m². In children, the volume of distribution at steady state was calculated to be 18-29 l/m² at doses between 25 and 75 mg/m² (Heideman et al, 1989). At 300 mg/m², the volume of distribution was within the same range (Kletzel M et al, 1992).
The Applicant claimed that the apparent volume of distribution of thiotepa appears independent of the administered dose. The fraction unbound to proteins in plasma is 70-90%; insignificant binding of thiotepa to gamma globulin and minimal albumin binding (10-30%) has been reported.
Thiotepa and the main metabolite, TEPA, penetrate very well into the CSF; CSF and plasma concentrations are comparable with a delayed CSF increase of TEPA (Heideman et al, 1989).

- Elimination

Thiotepa undergoes intensive hepatic metabolism, which in humans is known to be catalysed by the cytochrome P450 isoenzymes 2B6 and 3A4 (Jacobson et al, 2002). The low rate of urinary recovery, however, indicates substantial metabolism by other pathways as well. The major active metabolite is TEPA, which therefore has been co-investigated in most of the pharmacokinetic investigations. A second metabolite is the mercapturic acid conjugate of thiotepa which is formed via glutathione conjugation. A third metabolite, monochloro-TEPA, was identified in the urine (reviewed in van Van Maanen et al., 2000).

According to the Applicant, the total excreted amount of thiotepa and its identified metabolites accounts for 54-100% of the total alkylating activity, indicating the presence of other alkylating metabolites. During conversion of GSH conjugates to N-acetylcysteine conjugates, GSH, cysteinylglycine, and cysteine conjugates are formed. These metabolites are not found in urine, and, if formed, are probably excreted in bile or as intermediate metabolites rapidly converted into thiotepa-mercapturate.

Reports on clearance rates vary in that some claim dose-dependence while others found clearance rates to be independent of the dose. The total clearance of thiotepa ranged from 11.4 to 23.2 l/h/m^2. The elimination half-life varied from 1.5 to 4.1 hours.

- Dose proportionality and time dependencies

Different pharmacokinetic investigations of thiotepa reported dose-independent (Hagen et al, 1987; Hagen et al, 1988; Hagen, 1991, Ackland et al, 1988) as well as dose-dependent (Heideman et al, 1989) kinetics of this drug. The population pharmacokinetic study, which suggested a 2-compartment model, gave no indication for non-linearity, granted that the doses (up to 120 mg/m²/d) were not very high (Huitema et al, 2001).

There was only one investigation looking at the continuous infusion of high dose thiotepa (Henner et al, 1987). The authors' pharmacokinetic estimations showed no significant difference from values obtained with the more intensively investigated bolus or short term infusion; they did, however, find plasma levels to be inconsistent with any standard pharmacokinetic model. The levels of thiotepa decreased continuously during the 96 hour infusion.

- Special populations

There are no pharmacokinetic data on the use of thiotepa during pregnancy or lactation, in elderly patients and in patients with significant pre-existing hepatic or renal insufficiency (see discussion on clinical pharmacology).

- Pharmacokinetic interaction studies

Generally, thiotepa contributes to the cumulative immunosuppressive effect of high dose chemotherapy schedules. In this context, vaccination with a live virus vaccine can result in severe and fatal infections.

As a result of the claimed acid instability mentioned before, thiotepa should not be mixed with other medicinal products except those mentioned in section 6.6 of the SPC.

Alkylating chemotherapeutic agents, including thiotepa, inhibit plasma pseudocholinesterase 35% to 70%. The action of succinyl choline can be prolonged by 5 to 15 minutes. Thiotepa is an irreversible inhibitor of plasma cholinesterase activity (Drugdex(R) System).

The interaction with cyclophosphamide is well-known and argues for a CYP2B6 inhibition process. Using human liver microsomes and recombinant P450 enzymes, Rae et al (2002) were able to identify thiotepa as a potent and specific inhibitor of CYP2B6. As CYP2B6 may play an important role in the 4-hydroxylation of cyclophosphamide, inhibition of this enzyme by thiotepa is the likely mechanism by which thiotepa inhibits the activation of cyclophosphamide. Huitema et al (2000) altered the sequence of infusion of cyclophosphamide (1000-1500 mg/m²/d), carboplatin (265 or 400 mg/m²/d), and thiotepa (80 or 120 mg/m²/d) and analysed the pharmacokinetics of cyclophosphamide and thiotepa. Administration of thiotepa one hour before cyclophosphamide, as compared to the reverse sequence, reduced the maximum concentration and the area under the curve of the active metabolite 4-hydroxy-cyclophosphamide by -62% and -26%. In a more recent publication implementing a hypothetical metabolic enzyme rather than investigating the effect of application sequence, the same group described a modest induction of thiotepa metabolism by cyclophosphamide; however, in the complex area of active metabolites these authors are the first to estimate the clinical relevance of increased thiotepa clearance (de Jonge et al, 2004).

A direct pharmacokinetic interaction has been reported for the antiemetic drug aprepitant (de Jonge et al., 2005a). Interactions of this moderate CYP3A4 inhibitor were investigated in 8 patients on a conditioning regimen of thiotepa, cyclophosphamide, and carboplatin using the population pharmacokinetic model based on 49 patients at the time. Aprepitant co-medication reduced the rate of

cyclophosphamide autoinduction by 23% and TEPA formation by 33%. The authors regarded this effect as small considering the overall variability of the metabolism of these drugs; they proposed CYP3A4 and 2B6 inhibition to be the mechanistic pathway, but they introduced the discrimination between inducible and non-inducible clearance into their population pharmacokinetic model.

- Pharmacokinetics using human biomaterials

No studies were submitted (see discussion on clinical pharmacology).

Pharmacodynamics

- Mechanism of action

Thiotepa is an alkylating agent chemically and pharmacologically related to nitrogen mustard and its clinically utilised derivatives. Thiotepa undergoes complex metabolism with TEPA as the major metabolite. Both thiotepa and TEPA are active compounds. The cellular reaction of thioTEPA with DNA primarily leads to the alkylation of guanine and adenine, producing inter- and intra-strand cross-links which in turn are responsible for the inactivation of DNA and for cytotoxicity. TEPA is assumed to interact differently with DNA, but is also thought to produce DNA lesions. Monochloro-TEPA and thiotepa-mercapturate (the other important metabolites) show alkylating activity as well (reviewed in van Maanen et al., 2000).

- Primary and Secondary pharmacology

The primary pharmacodynamic action of thiotepa is to damage the DNA and cellular structure of malignant cells. With regards to the sought indication, myelotoxicity of thiotepa and its metabolites in general can be considered of relevance. A number of published phase I and II studies were submitted, in which the MTD of thiotepa was investigated; reported toxicities included myelotoxicity (studies not described).
Secondary to DNA damage, tumour cell toxicity and effects on the haematopoietic system, other pharmacologic effects have also been observed. They encompass the complete range of unintended effects which are always linked to alkylating drugs including alopecia, gastrointestinal toxicity, mutagenicity, carcinogenicity, and impairment of fertility. No studies addressing the well-known mechanisms of these secondary pharmacologic effects were submitted.

Discussion on clinical pharmacology

The principal pharmacokinetic data tabulated above show the variability of the main PK parameters (Vd, CL, AUC, $T_{1/2}$) of thiotepa over the wide range of doses used in the published studies and that the elimination time of thiotepa is independent of the dosage.
Absorption data are not considered relevant for the current application due to the suggested intravenous administration of thiotepa.
The metabolism and elimination of thiotepa can be considered sufficiently well characterised.
Pharmacokinetic data in special populations are not available. The absence of information in hepatic and renal insufficiency patients is reflected in the SPC. Relevant contraindications or precautions with regard to fertility, pregnancy and lactation are included in the relevant sections of the SPC. As few elderly patients (> 65 years old) are expected to undergo HPCT, the lack of PK data in elderly patients is accepted and the information provided in the SPC is considered satisfactory.
All relevant interactions with other medicinal products are reflected in section 4.5 of the SPC. A contraindication for the concomitant use with the yellow fever vaccine and with live virus and bacterial vaccines is included in section 4.3 of the SPC and warnings related to drug-drug interactions are included in the SPC, section 4.4.
The mechanism of action of thiotepa is well-known. Thiotepa belongs to the alkylating substances for which clinical experience dates back roughly 50 years.

Clinical efficacy

The Applicant submitted clinical studies performed in adult and paediatric patients based on the published literature. The data are presented separately for adults and paediatric populations, and for autologous and allogeneic HPCT.

THIOTEPA IN ADULTS

Autologous HPCT	**Allogeneic HPCT**

Haematological Diseases — Autologous HPCT
- Lymphoma
- CNS Lymphoma
- Multiple Myeloma

Solid Tumours — Autologous HPCT
- Breast Cancer
- CNS Tumours
- Ovarian Cancer
- Germ Cell Tumours

Haematological Diseases — Allogeneic HPCT
- Lymphoma
- Multiple Myeloma
- Not otherwhise specified
- Leukemia
- Thalassemia
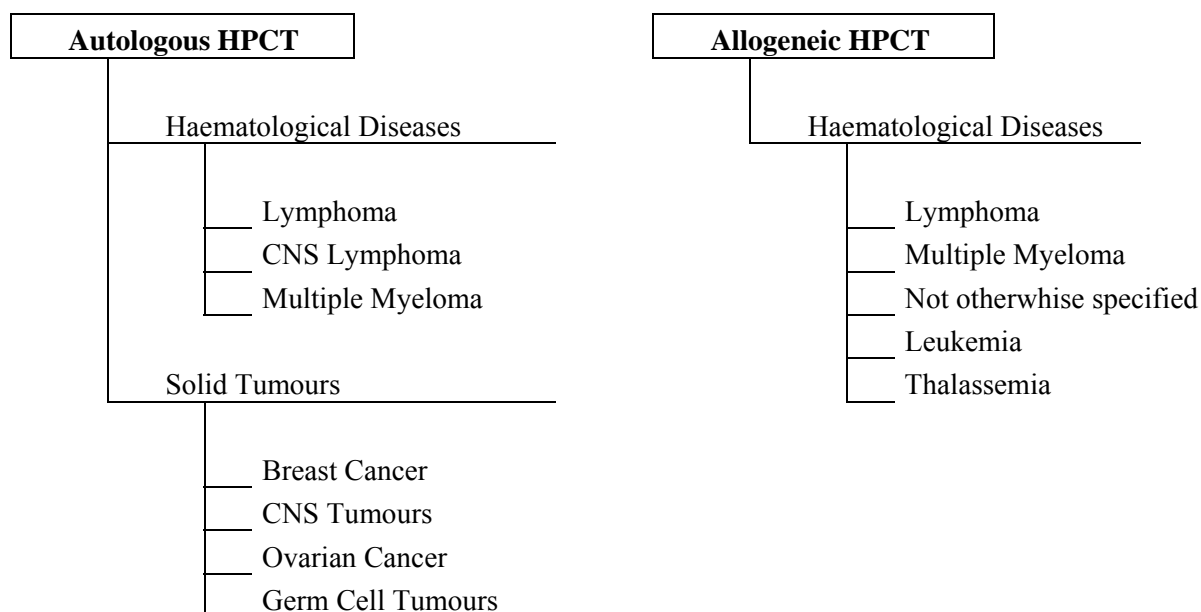
Autologous HPCT in adult patients

A total of 4501 patients with haematological diseases (N=826) and with solid/CNS tumours (N=3675) received thiotepa i.v. as conditioning regimen before autologous HPCT.

Table 5: Autologous HPCT in adult patients

DISEASE	CHARACTERISTIC	CONDITIONING TREATMENT	N PTS	AUTHORS
Haemato-logical diseases	Lymphoma	TT/BU/MEL	204	Gopal et al, 2001; Gutierrez-Delgado et al, 2001; Gutierrez-Delgado et al, 2003
		TT/BU/CY	34	Przepiorka et al, 1995
		TT/MEL/CARB	42	Demirer et al, 2004
		TT/MITOX/CARB	125	Waheed et al, 2004; Glossmann et al, 2005
		TT/CARB/VP16	31	Papadopoulos et al, 2005
		TT/VP16/CY	29	Cumpston et al, 2007
		TT/VP16	65	McCoy et al, 2004
	CNS Lymphoma	TT/CARM	23	Illerhaus et al, 2006
		TT/BU	16	Montemurro et al, 2007
	Multiple Myeloma	TT/BU/CY	257	Dimopoulos et al, 1993; Shimoni et al, 2001; Anagnostopoulos et al, 2004
Sub-total			826	
Solid tumours	Breast cancer	TT/CY	566	Tallman et al, 2003; Leonard et al, 2004

		TT/CY/CARB	2119	Weaver et al, 1997; Stemmer et al, 2001; Stemmer et al, 2003; Rodenhuis et al, 2003; Rodenhuis et al, 2006; Schrama et al, 2003; Coombes et al, 2005
		TT/CY/CARM	341	Wong et al, 2003; Cheng et al, 2004
		TT/CY/EPIR	201	Nitz et al, 2005
		TT/CY/MITOX	150	Zander et al, 2004
		TT/ MITOX/CARB	28	Yalamanchili et al, 2008
		TT alone	52	Rose et al, 2000
	CNS tumours	TT/VP16/CARM	15	Papadopoulos et al, 1998
		TT/VP16/CARB	3	Papadopoulos et al, 1998
		TT alone	59	Cairncross et al, 2000; Abrey et al, 2006
		TT/CARB	21	Chen et al, 2004
		TT/CARM	10	Gill et al, 2008
	Ovarian cancer	TT/BU/MEL	31	Holmberg et al, 1998
		TT alone	17	Tiersten et al, 2006
	Germ Cell tumours	TT/CARB/VP16	62	Rick et al, 2001
Sub-total			3675	
TOTAL			**4501**	

BU=busulfan; CARB=carboplatin; CARM=carmustine; CISPL=cisplatin; CY=cyclophosphamide; EPIR=epirubicine; MEL= melphalan; MITOX= mitoxantrone; TT = thiotepa; VP16=etoposide

Haematological diseases
Adults with haematological diseases (N=826) comprised 569 patients with lymphomas and 257 patients with multiple myeloma.
- In **Gopal *et al*, 2001, Gutierrez-Delgado *et al*, 2001** and **Gutierrez-Delgado *et al*, 2003,** lymphoma patients (N=204) received thiotepa in combination with busulfan and melphalan.
Engraftment at 92%, OS at 42%, EFS at 39% and 5-year relapse at 42% were reported in Gutierrez-Delgado *et al*, 2001. Engraftment at 92%, estimated OS at 52%, EFS at 34% and 5-years relapse at 32% were shown in Gutierrez-Delgado *et al*, 2003. EFS at 45% and relapse at 33% were reported in Gopal *et al*, 2001. In these three studies most patients were at high risk because of their very advanced disease or prior dose-limiting radiation therapy.
- In **Przepiorka *et al*, 1995**, lymphoma patients (N=34) received thiotepa in combination with busulfan and cyclophosphamide. This was a dose finding study combining thiotepa (from 450 to 750 mg/m^2), busulfan (from 12 to 10 mg/kg) and cyclophosphamide (from 120 to 150 mg/kg). The combination of thiotepa at 450 mg/kg or 750 mg/kg, cyclophosphamide and busulfan provided good efficacy in term of OS and DFS despite the advanced status of disease (data not shown).
- In **Demirer *et al*, 2004,** lymphoma patients (N=42) received thiotepa in combination with melphalan and carboplatin. This conditioning treatment was given to patients with intermediate-grade NHL or HD, with HD representing 45% of patients. OS, EFS, relapse estimated at 2 years were reported at 65%, 60% and 21%, respectively.
- In **Waheed *et al*, 2004** and **Glossmann *et al*, 2005**, lymphoma patients (N=125) received thiotepa in combination with mitoxantrone and carboplatin. 5-year DFS was estimated at 43% in high risk patients (**Waheed *et al*, 2004**). This conditioning treatment was also evaluated in autologous tandem transplantation making the sequential high-dose chemotherapy feasible with the BEAM treatment in patients who could receive both transplants (**Glossmann *et al*, 2005**).
- In **Papadopoulos *et al*, 2005**, lymphoma patients (N=31) received thiotepa in combination with carboplatin and etoposide. The regimen was a tandem HD chemotherapy represented by melphalan and mitoxantrone followed by thiotepa, carboplatin and etoposide. Tandem regimen was administered to only 31 patients. The results in term of efficacy are comparable with standard regimens used in

HPCT to treat lymphomas, but the number of patients enrolled in this study is too small to be compared with other strategies.

- In **Cumpston** *et al*, **2007**, lymphoma patients (N=29) received thiotepa in combination with cyclophosphamide and etoposide. This conditioning regimen showed DFS at 66% and OS at 76%.

- In **McCoy** *et al*, **2004,** lymphoma patients (N=65) received thiotepa in combination with etoposide. This aggressive high-dose thiotepa-etoposide regimen used in high risk patients was accompanied at 3 years by an OS of 40% and an EFS of 32%.

- In **Illerhaus** *et al*, **2006**, CNS lymphoma patients (N=23) received thiotepa in combination with carmustine. The survival rate (87%) at 5 years was 87% and it was associated with a relapse mortality rate of 8.7%, which suggests that this protocol may be curative in a substantial proportion of patients. High doses of the alkylating agents carmustine and thiotepa were chosen as they are able to penetrate the intact blood brain barrier due to their lipophilic properties.

- In **Montemurro** *et al*, **2007**, CNS lymphoma patients (N=16) received thiotepa in combination with busulfan. This approach was accompanied with good response rates and no severe neurotoxicity.

- In **Dimopoulos** *et al*, **1993, Shimoni** *et al*, **2001** and **Anagnostopoulos** *et al*, **2004,** multiple myeloma patients (N=257) received thiotepa in combination with busulfan and cyclophosphamide. All three studies suggested that a regimen including thiotepa, busulfan and cyclophosphamide has comparable efficacy with the standard regimen including high dose melphalan. The different thiotepa doses used in these studies permitted decreases especially in the dose of busulfan while maintaining the same efficacy as the melphalan scheme in terms of engraftment and response of the disease.

Solid tumours

Adults with solid tumours (N=3675) comprised patients with breast cancer (N=3457), CNS tumours (N=108), ovarian cancer (N=48) and germ cell tumours (N=62).

- In **Tallman** *et al*, **2003** and **Leonard** *et al*, **2004**, breast cancer patients (N=566) received thiotepa in combination with cyclophosphamide. Disease Free Survival (DFS) and Overall Survival (OS) at 6 year were 49% and 58%, respectively.

- In **Weaver** *et al*, **1997, Stemmer** *et al*, **2001 , Stemmer** *et al*, **2003, Rodenhuis** *et al*, **2003, Rodenhuis** *et al*, **2006, Schrama** *et al*, **2003** and **Coombes** *et al*, **2005,** breast cancer patients (N=2119) received thiotepa in combination with cyclophosphamide and carboplatin.
Engraftment was 98% in **Weaver** *et al*, **1997**. In **Rodenhuis** *et al*, **2006,** OS and RFS at 5 years were 73% and 64.3%, respectively. In **Coombes** *et al*, **2005**, OS was 66% and RFS was 59%. In **Stemmer** *et al*, **2001**, OS and DFS with a median follow up of 51 months was 81% and 72% respectively. RFS was 65% in **Rodenhuis** *et al*, **2003**. High-dose alkylating therapy improves relapse-free survival among patients with stage II or III cancer and 10 or more positive lymph nodes. This benefit may be confined to patients with HER2/neu-negative tumours (**Rodenhuis** *et al*, **2003**). Early radiotherapy after intensive adjuvant treatment including high-dose chemotherapy with autologous stem cell transplantation is feasible and effective for patients with high-risk locally advanced breast carcinoma (**Stemmer** *et al*, **2001**). Adjuvant chemotherapy followed by high-dose chemotherapy and autologous cell stem transplantation is feasible in patients with multinode positive stage II breast carcinoma and outcome is promising (**Stemmer** *et al*, **2003**). The results suggest that a subgroup of breast cancer (HER2/neu-negative) is specifically sensitive to high-dose alkylating agents (**Rodenhuis** *et al*, **2006**).

- In **Wong** *et al*, **2003** and **Cheng** *et al*, **2004**, breast cancer patients (N=341) received thiotepa in combination with cyclophosphamide and carmustine. With a median follow up of 63 months, OS and RFS were 68% and 62% respectively (**Cheng** *et al*, **2004**). OS and RFS at a median follow up of 44.3 months were 74.7% and 59.3% (**Wong** *et al*, **2003**). High-dose chemotherapy with thiotepa, cyclophosphamide and carmustine with autologous haematopoietic stem cell transplantation is feasible with comparable efficacy to other high dose chemotherapy regimens.

- In **Nitz** *et al*, **2005,** breast cancer patients (N=201) received thiotepa in combination with cyclophosphamide and epirubicine. Patients with at least nine positive nodes were randomly assigned to two courses of accelerated conventionally dosed epirubicin and cyclophosphamide followed or not by two courses of high-dose chemotherapy (epirubicin, cyclophosphamide and thiotepa supported by peripheral-blood progenitors). The primary endpoint was event-free survival.

403 patients were enrolled; 201 were assigned high-dose chemotherapy and 202 conventional treatment. The mean number of positive nodes was 17.6, and median follow-up was 48.6 months. In high dose group OS was 87%, 78% and 73% at 2, 3 and 4 years, respectively. EFS was 71%, 60% and 52% at 2, 3 and 4 years, respectively. 4 year event-free survival was 60% (95% CI 53-67) in the high-

dose chemotherapy group and 44% in the control group (p=0.00069). The corresponding overall survival was 75% vs 70% (p=0.02).

- In **Zander** *et al*, **2004**, breast cancer patients (N=150) received thiotepa in combination with cyclophosphamide and mitoxantrone. OS and EFS at 4 years were 70% and 52%, respectively. There was a trend in favor of high-dose chemotherapy with respect to EFS.

- In **Yalamanchili** *et al*, **2008,** breast cancer patients (N=28) received thiotepa in combination with mitoxantrone and carboplatin. The purpose of this study was to determine the efficacy of high-dose chemotherapy (HDCT) with thiotepa, mitoxantrone and carboplatin (TMJ regimen). Between 1991 and 1998, twenty-eight patients with stage IIIB inflammatory breast cancer underwent an autologous stem cell transplant after undergoing chemotherapy, surgery and/or radiation. Progression-free survival and overall survival was assessed over a 15-year period. At the time of last follow-up in May 2007, sixteen patients had relapsed. The median overall survival was 49.5 months. The median progression free survival was 40 months.

- In **Rose** *et al*, **2000**, breast cancer patients (N=52) received thiotepa alone. This was considered a valid strategy for patients with reduced left ventricular ejection fraction undergoing therapy with high-dose chemotherapy and autologous HPCT support, when cardiotoxic agents, such as cyclophopshamide, should be avoided.

- In **Papadopoulos** *et al*, **1998**, CNS tumours patients (N=15) received increasing doses of thiotepa in combination with etoposide and carmustine. Median survival was 12 months with a time to tumour progression of 7 months.

- In **Papadopoulos** *et al*, **1998,** CNS tumours patients (N=3) received thiotepa in combination with etoposide and carboplatin. Median survival was 12 months with a time to tumour progression of 7 months.

- In **Cairncross** *et al*, **2000** and **Abrey** *et al*, **2006,** CNS tumours patients (N=59) received thiotepa alone. Median survival was 49 months, significantly longer than 12 months for comparable CNS patients who did not proceed to high-dose chemotherapy (median survival time 49 months vs 12 months, p=0.002) (**Cairncross** *et al*, **2000**). In **Abrey** *et al*, **2006** relapse was 46% with a median of 33,5 months and 7 patients of 59 (12%) died of relapse. Median PFS was 78 months.

- In **Chen** *et al*, **2004**, CNS tumours patients (N=21) received thiotepa in combination with carboplatin. Mean survival was 34.5 +/- 5.5 months (range 9-94 months). The Kaplan-Meier estimate for the PFS was 47% +/- 11 at 24 months.

- In **Gill** *et al*, **2008**, CNS tumours patients (N=10) received thiotepa in combination with carmustine. At 1 year, 6/10 patients achieved complete response (CR). Median survival time was 3.47 years. 5/10 patients were alive at 2.9 years. 4/5 alive patients were without disease progression. Time to disease progression was 1.25 years.

- In **Holmberg** *et al*, **1998**, ovarian cancer patients (N=31) received thiotepa in combination with busulfan and melphalan. In this study the actuarial survival, EFS and relapse probability at 18 months was 57%, 30% and 63%, respectively. Overall, the efficacy in patients autografted with relapse or persistent disease was similar to results published with other high-dose chemotherapy regimens.

- In **Tiersten** *et al*, **2006** ovarian cancer patients (N=17) received thiotepa alone. 17 patients were enrolled for investigating a novel high-dose chemotherapy (HDCT) regimen with peripheral blood progenitor cell (PBPC) support in patients with pretreated advanced ovarian cancer. Patients received three consecutive cycles of HDCT with PBPC support. Cycle 1 HDCT included carboplatin with an area under the concentration curve of 20 mg/m^2 and paclitaxel at 250 mg/m^2, respectively. Cycle 2 included topotecan starting at 5 mg/m^2, dose escalated in 2 mg/m^2 increments, and etoposide 600 at mg/m^2. Cycle 3 included thiotepa at 500 mg/m^2. The response rate was 50%, including 5 CRs and 2 partial responses. Stable disease was noted in four (29%) patients, two of who had minimal responses. One patient progressed and went off the study after cycle 1. At a median follow-up of 11.7 months, the median progression-free survival was 7 months, and the median overall survival was 18 months.

- In **Rick** *et al*, **2001** germ cell tumour patients (N=62) received thiotepa in combination with etoposide and carboplatin. 41% of patients responded to the high dose chemotherapy, 32% had stable disease or tumour progression. Survival estimated at 3 years was 30%.

Allogeneic HPCT in adult patients

A total of 1771 patients with haematological diseases received thiotepa i.v., in combination with TBI and other chemotherapeutic drugs as conditioning treatment before allogeneic HPCT.

Table 6: Allogeneic HPCT in adult patients

DISEASE	CHARACTERISTIC	CONDITIONING TREATMENT	N PTS	AUTHORS
Haemato-logical diseases	Lymphoma	TT/CY/FLU	187	Corradini et al, 2004; Corradini et al, 2007
	Multiple Myeloma	TT/FLU/MEL	53	Majolino et al, 2007
	Haematological disease not otherwise specified	TT/CY	625	Bacigalupo et al, 2007a; Bacigalupo et al, 2007b; Bacigalupo et al, 2009; Raiola et al, 2000; di Grazia et al, 2001
		TT/CY/FLU	150	Corradini et al, 2005
		TT/FLU	93	Alessandrino et al, 2001; Alessandrino et al, 2004; Grullich et al, 2008; Picardi et al, 2004
		TT/FLU/MEL/OKT 3	10	Bethge et al, 2006
		TT/TBI/FLU	52	Jakubowski et al, 2007
	Leukemia	TT/CY	78	Bacigalupo et al, 1996; Bacigalupo et al, 2007c
		TT/CY/BU	30	Rosales et al, 1999
		TT/FLU/MEL/ATG	14	Lacerda et al, 2003
		TT/TBI/CY	81	Rigden et al, 1996; Papadopoulos et al, 1998
		TT/TBI/CY/ATG	107	Aversa et al, 1994; Aversa et al, 1999; Aversa et al, 2001
		TT/TBI/FLU/ATG	276	Aversa et al, 1998; Aversa et al, 2001; Aversa et al, 2002; Aversa et al, 2005
	Thalassemia	TT/CY/BU	15	La Nasa et al, 2005
TOTAL			**1771**	

ATG=anti-tymocytes rabbit immunoglobuline; BU=busulfan; CY=cyclophosphamide; FLU=fludarabine; MEL=melphalan; OKT3=murine monoclonal antibody; TBI=total body irradiation; TT = thiotepa;

Adult patients with haematological diseases undergoing allogeneic HPCT comprised patients with lymphomas (N=187), multiple myeloma (N=53), haematological diseases not otherwise specified (N=930), leukemia (N=586) and thalassemia (N=15).
- In **Corradini et al, 2004** and **Corradini et al, 2007** lymphoma patients (N=187) received thiotepa in combination with fludarabine and cyclophoshamide. The majority of patients were treated with allogeneic transplantation for very high risk lymphomas. The majority of patients had received several lines of chemotherapy and at least one autologous transplant. The results in term of efficacy reported in all studies confirm that the conditioning regimens including thiotepa, at doses greater than 10 mg/kg, are myeloablative in different types of transplant. Engraftment was 100%, estimated OS at 3 year was 81% and relapse was 12% with estimated PFS at 3 year of 64% (**Corradini et al, 2004**). Engraftment was 100% (84% full chimerism and 16% mixed), OS estimated at 3 years was 62% and 1–3 year cumulative relapse was 31% and 41%, respectively (**Corradini et al, 2007**). In conclusion, several types of reduced-intensity conditioning (RIC) regimens provided a high rate of engraftment with a significant decrease in organ toxicity, making SCT a feasible procedure in patients considered not eligible for conventional transplantation because of age, comorbid conditions, or extensive previous therapies.
- In **Majolino et al, 2007**, multiple myeloma patients (N=53) received thiotepa in combination with fludarabine and melphalan. Because Multiple Myeloma is associated with high risk of relapse, the conditioning regimens used prior to HPCT generally favour graft versus tumour effect over immunosuppression. For this reason, high risk of GvHD and high incidence of TRM is expected. In this study, a RIC regimen with thiotepa, fludarabine and melphalan was used with the aim of

achieving myeloablation with low TRM, mainly in old patients or patients with comorbidity. The study achieved the primary end point in terms of engraftment, OS and relapse. Estimated relapse at 3 years was 32% considering that 82% of patients had advanced disease. This result is comparable with other results obtained with different myeloablative regimens characterized by high TRM.

- In **Raiola et al, 2000**, **Di Grazia et al, 2001**, **Bacigalupo et al, 2007a**, **Bacigalupo et al, 2007b**, and **Bacigalupo et al, 2009**, patients with haematological diseases not otherwise specified (N=625) received thiotepa in combination with cyclophosphamide. Across these studies a reduced intensity regimen (thiotepa = 10 mg/kg) was compared with a conventional regimen (thiotepa > 10 mg/kg) (**Bacigalupo et al, 2009**) or with an intensified regimen (thiotepa/cyclophosphamide supplemented with Melphalan or TBI) (**Bacigalupo et al, 2007**a and **Di Grazia et al, 2001**).

The haematological diseases encountered were AML, ALL, CML, CLD, MDS, NHL, HL and MM. Engraftment ranged from 97% to 99%. Event free survival was reported in only two studies: 61% in **Raiola et al, 2000** with 2 years of follow up and 89% in **Bacigalupo et al, 2007b** with 10 years of follow-up.

Across all studies, the relapse and the deaths to relapse were different in early and in late phases of disease. Patients in chronic phase or first complete remission (CR1) were considered as early disease, while all others (CR > 1) were late disease. Patients beyond first complete remission had a higher risk of relapse (mean of 36%) than those in CR1 (mean 17.5%). The second variable that affected the relapse rate is the stem cell source: for bone marrow graft the relapse was higher than for peripheral blood graft (67% vs 33%). With 2 years of follow up (**Raiola et al, 2000**) overall survival was 72%, whereas with 10 years of follow up (**Bacigalupo et al, 2007a**, **Bacigalupo et al, 2007b** and **Di Grazia et al, 2001**) overall survival was 48%. Relapse depended on the phase of disease (higher in CR1 (67%) than in >CR1 (43%)) and on stem cell source (BM 87% vs PB 67%, at 2 years). For the long follow up (10 years) the different survival in BM and PB tends to equalize (BM 58% vs PB 51%).

- In **Corradini et al, 2005**, patients with haematological diseases not otherwise specified (N=150) received thiotepa in combination with cyclophosphamide and fludarabine. Engraftment rate was 99%.

The 5-year OS and PFS rates for indolent lymphomas were 66% and 73%, respectively. Aggressive NHL had estimated 5-year OS and PFS rates of 72% and 59%. Acute Myeloid Leukemia and MDS were analysed together; the estimated OS and PFS rates at 5 years were 32% and 38%, respectively. Multiple Myeloma had estimated OS and PFS rates of 70% and 30% at 5 years, respectively.

Patients who undergo transplant are considered old when they are > 55 years. Overall, OS at 5 years was 66% in younger patients and 61% in older patients. There were no statistical differences in terms of efficacy concerning OS, PFS, NRM and relapse between the two age groups.

- In **Alessandrino et al, 2001, Alessandrino et al, 2004, Picardi et al, 2004** and **Grullich et al, 2008,** patients with haematological diseases not otherwise specified (N=93) received thiotepa in combination with fludarabine.

The reduced intensity regimen based on thiotepa and fludarabine was introduced for the treatment of patients who are ineligible for myeloablative conditioning but considered to be at risk of relapse. Transplant related toxicity and mortality are high with conventional allografts, particularly in patients with advanced age, comorbidities, poor performance status and a previous regimen and autologous/allogeneic transplantation. Following a preliminary study including six patients (**Alessandrino et al, 2001**), **Corradini et al, 2004** evaluated the combination of thiotepa and fludarabine given as a preparative regimen in patients with haematological malignancies with a poor performance status. This regimen led to engraftment in all treated patients with rates of acute GvHD and chronic GvHD comparable to those reported in other studies. The 1-year relapse rate was 19 %. In **Grullich et al, 2008**, the conditioning regimen including thiotepa was used to treat failures of an autologous or an allogeneic transplant. These types of patients require an appropriate conditioning regimen to ensure an adequate myeloablative activity combined with a good safety profile; the patients had indeed multiple comorbidities. The thiotepa/fludarabine regimen led to engraftment in all 46 evaluable patients. Donor chimerism was evaluable in 43 of 49 patients, 38 of 43 (88%) achieved complete donor chimerism at one month and no graft failure occurred. The overall incidence of severe acute GVHD was 24% and chronic GVHD developed in 17 of 39 evaluable patients (7 extensive). The estimated probability of relapse/progression at 1 year was 55.1%. At 1 year the estimated survival for all patients was 42.6% and the estimated event free survival was 38.1%. Similar results were reported also in **Picardi et al, 2004**. The cohort of patients with advanced haematologic disease achieved both complete chimerism and absence of rejection of 95%. The 6-year cumulative incidence of relapse was

56% and the Overall Survival and Disease Free Survival rates at 74 months were 31.7 % and 23%, respectively.

- In **Bethge *et al*, 2006**, patients with haematological diseases not otherwise specified (N=10) received thiotepa in combination with fludarabine, melphalan and OKT3. Haploidentical haematopoietic cell transplantation (HHCT) might be especially suited for patients with relapsed or treatment-refractory disease even after preceding HCT. Initially, however, trials of HHCT were complicated by a high incidence of engraftment failure, GvHD, and infectious complications resulting in an unacceptable treatment-related morbidity and mortality. This study aimed to improve engraftment by using CD3/CD19 depletion of PBMC and reduce the high treatment-related toxicity by a dose-reduced conditioning regimen. Engraftment was rapid and full donor chimerism was achieved after 2 weeks in all patients. Overall survival was 50% (5/10 patients) with 4 patients in CR and a median follow-up of 435 (range, 229–814) days. These are comparable with OS expected after HCT in standard risk patients.

- In **Jakubowski *et al*, 2007**, patients with haematological diseases not otherwise specified (N=52) received thiotepa in combination with TBI and fludarabine. Engraftment was 100%, OS was 62% and relapse was 17% with an estimated DFS at 3 year of 61%. The relapse rates, comparable with those of unmodified grafts, reaffirmed the findings of prior studies with respect to the anti-malignancy potential of the T-cell depletion transplantation strategy. Furthermore, this study suggested the curative potential of this treatment approach even in older patients.

- In **Bacigalupo *et al*, 1996** and **Bacigalupo *et al*, 2007c**, leukemia patients (N=78) received thiotepa in combination with cyclophosphamide. In **Bacigalupo *et al*, 1996**, 94% of patients achieved engraftment. OS was 61% with a relapse incidence of 16%. **Bacigalupo *et al*, 2007c** reported rapid and good engraftment with 5-year survival of 64%. The cumulative risk of relapse was 45% with a median follow up of 4.6 years. These results suggest that the combination of thiotepa and cyclophosphamide was comparable to busulfan i.v., fludarabine and ATG.

- In **Rosales *et al*, 1999,** leukemia patients (N=30) received thiotepa in combination with cyclophosphamide and busulfan. In order to reduce the risk of relapse and maintain the therapeutic potential of stem cell allografting while avoiding GvHD, the conventional conditioning regimen for T-cell depleted grafts consisting of BU and CY was intensified by the addition of thiotepa. The effect of this intensified regimen was investigated in a total of 30 patients who underwent T-cell depleted BMT from HLA identical sibling donors. Engraftment rate was 100% with 82% of evaluable patients achieving a full-donor chimerism; estimated EFS rate at 60 months was 50%, estimated OS rate at 60 months was 50% and relapse rate was 13%. The results were within the range observed in other studies on allogeneic BMT in acute leukemia..

- In **Lacerda *et al*, 2003,** leukemia patients (N=14) received thiotepa in combination with fludarabine, melphalan and ATG. All patients achieved engraftment with full-donor chimerism. Estimated event-free survival and OS at 4 years was 42%. 5 patients relapsed and the estimated relapse-related mortality at 4 years was 38.1%.

- In **Rigden *et al*, 1996** and **Papadopoulos *et al*, 1998**, leukemia patients (N=81) received thiotepa in combination with TBI and cyclophosphamide. 58 patients underwent allogeneic marrow transplantation from matched sibling donors and 23 from unrelated donors. Rejection and non-engraftment were reported in 1% of unmanipulated sibling and 3% of unrelated allogeneic BMT (**Rigden *et al*, 1996**). Engraftment rate was 95% in **Rigden *et al*, 1996** and 100% in **Papadopoulos *et al*, 1998**. Event-free survival and overall survival was 38% and 50% in **Rigden *et al*, 1996**; 63% and 64% in **Papadopoulos *et al*, 1998**. The relapse rate was 9% and 15%, respectively.

- In **Aversa *et al*, 1994**, **Aversa *et al*, 1999** and **Aversa *et al*, 2001**, leukemia patients (N=107) received thiotepa in combination with TBI and cyclophosphamide and ATG.

Clinical results of T-cell-depleted HLA-matched transplants in acute leukemia have varied greatly because of differences in T-cell depletion methods and conditioning regimens. Eliminating GvHD by ex vivo T-cell depletion improves disease-free survival only if graft rejection is prevented and the antileukemic effect is maintained. The purpose of the above studies was to test whether the conditioning regimen based on thiotepa, TBI, cyclophosphamide and antithymocyte globulin facilitates engraftment of an extensively T-cell–depleted transplant. In **Aversa *et al*, 1994**, 17 patients received this regimen while in refractory end-stage disease. Engraftment rate was 94% and the relapse was 12% (2 patients). In **Aversa *et al*, 2001**, acute leukemia patients received this regimen while in remission (18) or relapse (18). Twenty-nine patients (80%) with advanced end-stage acute leukemia achieved primary sustained engraftment. In **Aversa *et al*, 1999**, all 52 patients achieved primary and
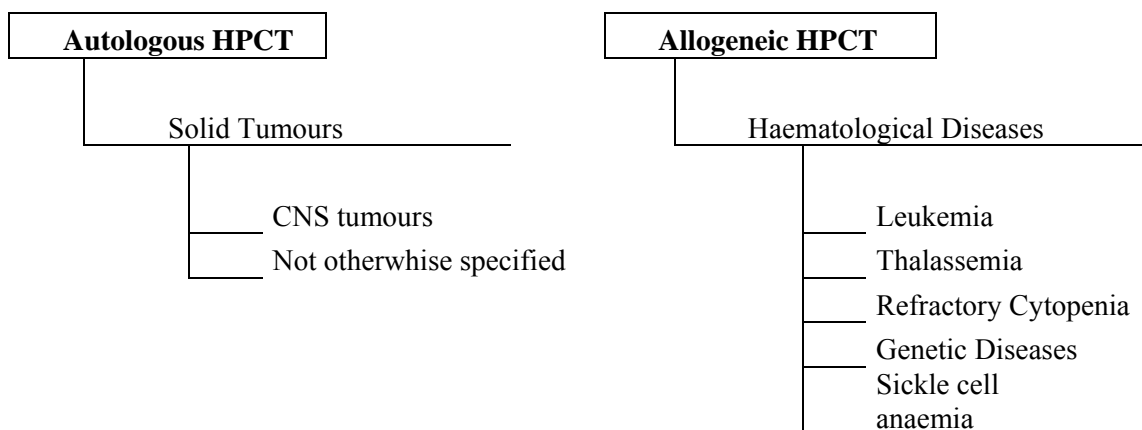
sustained engraftment with full donor-type chimerism. After the conditioning regimen with thiotepa neither graft rejection nor GvHD occurred. The probability of relapse was 12% for patients with acute myeloid leukemia and 28% for patients with acute lymphoblastic leukemia who received transplants at the first or second remission. Furthermore, at a median follow-up of 6.9 years (minimum follow-up, 4.9 years), event-free survival for patients who received transplants while in remission was 74 % for acute myeloid leukemia patients and 59% for acute lymphoblastic leukemia patients. These results are comparable with 2-year leukemia-free survival rates of 57% and 45% in first-remission patients with acute leukemia reported by the International Bone Marrow Transplant Registry. Moreover, a significant interaction effect on relapse between AML and thiotepa ($P = 0.02$) was found in this study when the study group patients were compared with historical group of 30 patients who underwent BMT before the clinicians included thiotepa.

- In **Aversa *et al*, 1998**, **Aversa *et al*, 2001**, **Aversa *et al*, 2002** and **Aversa *et al*, 2005**, leukemia patients (N=276) received thiotepa in combination with TBI and fludarabine and ATG.

The main limitations of bone marrow transplantation from donors who are matched with the recipient for only one HLA haplotype are GvHD and graft failure. A serious problem in the transplantation of T-cell–depleted bone marrow is an increased risk of relapse after transplantation due to lack of the GvHD related graft-versus-leukemia effect. Only 16% of the present haploidentical recipients relapsed when transplanted in remission after total-body irradiation and thiotepa-based conditioning and no post-transplantation immunosuppression. AML relapse rate was 10% and 13% in **Aversa *et al*, 1998** and **Aversa *et al*, 2002**, respectively. ALL relapse rate was 48% and 88% in **Aversa *et al*, 1998** and **Aversa *et al*, 2002**, respectively. In the studies considered, relapses occurred mainly in patients with ALL, particularly those in relapse at the time of transplantation (48% vs 10% in **Aversa 1998**, 88% vs 13% in **Aversa 2002**). Cumulative relapse rates of 25% and 30% were reported in **Aversa *et al*, 2005** and **Aversa *et al*, 2001**, respectively. These results suggest that T-cell–depleted mismatched transplants trigger unique graft-versus-leukemia effector mechanisms. The probability of event-free survival was significantly better in AML patients whose transplant included donor vs recipient NK cell alloreactivity than in those whose transplant did not. Overall, EFS compared favourably with what is reported in patients at the same stage of disease who receive transplants from matched donors.

- In **La Nasa *et al*, 2005**, thalassemia patients (N=15) received thiotepa in combination with cyclophosphamide and busulfan. This study is the first report on the combination of busulfan, thiotepa and cyclophosphamide in thalassemia patients. The 27 patients with a median age of 22 years were treated with two different conditioning regimens: 15 patients were treated with busulfan, thiotepa and cyclophosphamide, the other 12 were treated with busulfan and cyclophosphamide. Engraftment was 96%. Thalassemia-free survival and OS was 70%. These results suggest that the conditioning regimen has a good efficacy and that Unrelated Donor-BMT in adult class 3 thalassemia patients, with donors selected through high-resolution molecular typing, may offer a success rate similar to that historically reported in patients with similar prognostic characteristics transplanted from HLA identical siblings.

THIOTEPA IN PAEDIATRIC POPULATION

Autologous HPCT	**Allogeneic HPCT**
Solid Tumours	Haematological Diseases

Autologous HPCT:
- CNS tumours
- Not otherwhise specified

Allogeneic HPCT:
- Leukemia
- Thalassemia
- Refractory Cytopenia
- Genetic Diseases
- Sickle cell anaemia

Autologous HPCT in paediatric patients

A total of 476 patients with CNS tumours (N=378) and solid tumours not otherwise specified (N=98) received thiotepa i.v., as conditioning treatment before autologous HPCT.

Table 7: Autologous HPCT in paediatric patients

DISEASE	CHARACTERISTIC	CONDITIONING TREATMENT	N PTS	AUTHORS
Solid tumours	CNS tumours	TT/CARB/VP16	106	Dunkel et al, 1998; Mason et al 1998; Broniscer et al, 2004; Dhall et al, 2008; Grodman et al, 2009
		TT/CARM/VP16	57	Finlay et al, 1990; Grovas et al 1999; Papadakis et al, 2000
		TT/VP16	94	Finlay et al, 1996; Bouffet et al 1997,; Fagioli et al, 2004
		TT alone	31	Massimino et al, 2005; Massimino et al, 2006
		TT/BU	90	Grill et al, 1996; Dupuis-Girod et al 1997; Valteau- Couanet et al, 2005; Ridola et al, 2007
	Solid tumours not otherwise specified	TT alone	40	Lucidarme et al, 1998; Lafay-Cousin et al, 2000
		TT/CY/MEL	21	Chan et al, 1997
		TT/CARB/TOPOTE	21	Kushner et al, 2001
		TT/BU/MEL	16	Hawkins et al, 2000
TOTAL			476	

BU=busulfan; CY=cyclophosphamide; CARB=carboplatin; CARM=carmustine; MEL=melphalan; TOPOTE=topotecan; TT = thiotepa; VP16=etoposide.

- In **Dunkel *et al*, 1998**, **Mason *et al*, 1998**, **Broniscer *et al*, 2004**, **Dhall *et al*, 2008** and **Grodman *et al*, 2009**, CNS tumour patients (N=106) received thiotepa in combination with etoposide and carboplatin. Engraftment was achieved in all studies.

In **Dunkel *et al*, 1998**, tumours recurred in 13 patients (57%) at a median of 7 months post HPCT (range 3 to 36 months). Median survival after recurrence was 6 months (range 1-31 months). The Kaplan-Meier estimates of OS were 46% ± 11% at 36 months post transplant. These results suggest that an aggressive regimen including the use of high-dose chemotherapy in conjunction with HPCT may provide long-term survival for some patients with recurrent medulloblastoma. High-dose chemotherapy with HPCT is expected to be more effective against a smaller residual tumour burden which suggests that aggressive therapy with neurosurgical resection, irradiation, and/or conventional chemotherapy after detection of relapse and before high-dose chemotherapy with HPCT and early detection of relapse, perhaps via surveillance scanning, may be an important component in the salvage of patients with recurrent medulloblastoma.

In **Mason *et al*, 1998**, for the entire cohort, the 1-, 2- and 3-year OS rate was 63%, 48% and 40%, respectively. 1-, 2- and 3-year OS estimates following HPCT for 37 children who received consolidation chemotherapy were 84%, 73% and 62%. A significant proportion of children with malignant brain tumours can avoid radiotherapy and prolong maintenance chemotherapy.

In **Bronisher *et al*, 2004**, 10 patients (59%) experienced tumour relapse at a median of 160 days and all succumbed to their disease. Five patients with conical primitive neuroectodermal tumours (PNETs) remained alive and disease-free with a median follow-up of 8.3 years. A subset of patients with recurrent non-cerebellar PNETs can be salvaged utilizing a multi-modality approach including high-dose regimen based on two (thioteta-etoposide) or three (thiotepa-etoposide-carboplatin) drugs. These patients should be considered for myeloablative chemotherapy as consolidation only when there is either minimal or no residual disease prior to HPCT.

In **Dhall *et al*, 2008**, 7 relapses have been reported. 4 patients relapsed locally, 2 patients had both local and metastatic relapse and 1 patient had metastatic disease only at relapse. All relapses were observed within 12-26 months from initial diagnosis. 5-year overall survival was 79% ± 11%. The

majority of survivors (71%) avoided irradiation completely. This strategy of brief intensive induction chemotherapy based on high-dose thiotepa, etoposide and carboplatin for young children with non-metastatic medulloblastoma could eliminate the need for craniospinal irradiation and preserve quality of life and intellectual functioning.

In **Grodman *et al*, 2009**, four out of eight patients remained in complete remission at 62–164 months following treatment. Three died of progressive disease after 10–32 months. The 2- and 5-year survival rate was 75% and 50%, respectively. None of the survivors required additional salvage irradiation. Although most of the patients in this study were high-risk patients who had relapsed after standard chemotherapy and maximum irradiation therapy, the strategy with ablative carboplatin and thiotepa with stem cell rescue showed promising survival.

- In **Finlay *et al*, 1990**, **Grovas *et al*, 1999** and **Papadakis *et al*, 2000**, CNS tumour patients (N=57) received thiotepa in combination with etoposide and carmustine. Engraftment was achieved in all studies.

In **Finlay *et al*, 1990**, the responses were as follows: 2 CR, 1 PR, 1 SD and 1 non-evaluable patient. The two patients in CR remained without evidence of tumour progression more than 15 months from marrow reinfusion. The patient in PR and the non-evaluable patient had relapsed.

In **Grovas *et al*, 1999**, 3/11 patients (27%) were alive and remained disease free 2.9, 3.9 and 5.1 years after HPCT. 6/11 patients (55%) died of relapse. For these 11 patients, the overall survival rates at 1- and 2- years were 73% ± 13% and 46% ± 14%, respectively. The progression-free survival rates at 1- and 2-year were 64% ± 14% and 46% ± 14%, respectively.

In **Papadakis *et al*, 2000**, thirty-three patients were evaluable for response to treatment. Six patients demonstrated CR following ABMR and eight patients remained in CCR for a median of 54.9 months (range, 3.5–86.3) and 20.2 months (range, 4.6–110.2), respectively. Fifteen patients remained alive with stable disease for a median of 5.7 months (range, 1.0–64.4).

Overall survival (OS) was 36% at 1 year, 24% at 2 years and 17% at 3 years, with median OS of 9.5 months. Patients treated for recurrent disease fared worse than patients with newly diagnosed disease, with median survivals of 5.0 and 10.7 months, respectively.

- In **Finlay *et al*, 1996**, **Bouffet *et al*, 1997** and **Fagioli *et al*, 2004**, CNS tumour patients (N=94) received thiotepa in combination with etoposide. Engraftment was achieved in all studies. Only in **Finlay *et al*, 1996** did five patients die before engraftment within 30 days from HPCT.

In **Bouffet *et al*, 1997**, the overall response rate was 29%. 17 patients died of progression or recurrent disease. The median survival and PFS were 9 months (range: 1-65 months) and 5 months (range: 0-65 months), respectively. 3 patients were alive and progression free 54, 60 and 65 months after BMT.

In **Fagioli *et al*, 2004**, twelve patients had newly diagnosed high-risk brain tumours and 15 patients had recurrent brain tumours. 11 patients achieved CR after HPCT including 9 patients in continuing complete remission; 1 patient achieved PR and there was only 1 case of disease progression. 15/27 patients died of disease while 11 patients were alive including 7 with no evidence of disease (time from 7.3 months to 127.1 months). All 11 patients attended school after HDC. OS rate at 3 years was 44.6% after a median follow up of 13.9 months (range: 1–127 months). The 3-year-OS rates were 77.1% and 27.5% for patients who underwent HPCT while experiencing CR and patients with measurable disease at the time of HPCT, respectively (*P*=0.03). There was no statistically significant difference in OS and EFS rates between patients who underwent HPCT at diagnosis and patients who underwent HPCT at the time of recurrence. The median time to disease recurrence/progression was 6.2 months (range, 1.9 –32.0 months).

In **Finlay *et al*, 1996**, survival rates of 40% at 6 months, 33% at 1 year and 16% at 2 years were reported. 5 out of 18 patients (28%) with high-grade gliomas survived more than 39, 44, 49, 52 and 59 months post ABMR. All survivors are within the group of high-grade gliomas.

These preliminary results in patients with high grade astrocytoma are notable for the rapidity of tumour regression (as evaluated by MRI or CT scan on day 28 post-BMR) and the high frequency of responses in glioblastoma multiforme.

- In **Massimino *et al*, 2005** and **Massimino *et al*, 2006**, patients with CNS tumours (N= 31) received thiotepa alone. Engraftment was positive in all studies.

In **Massimino *et al*, 2005**, with a median follow-up of 57 months, overall survival was 43% and PFS at four years was 46% with a relapse of 57%; death upon relapse was 92%. The authors concluded that thiotepa does not have a "consolidation" role in high grade glioma, but it can be a further tool for obtaining remission and possibly cure of this ominous disease.

In **Massimino *et al*, 2006**, OS was 87.5% ± 12% at 3 years and PFS was 70% ± 18% with a relapse of 40%. 83% of the patients that relapsed died at a median of 13 months. The authors concluded that myeloablative phase with thiotepa improved the prognosis in supratentorial primitive neuroendocrine tumours (S-PNET).

- In **Grill *et al*, 1996**, **Dupuis-Girod *et al*, 1997**, **Valteau-Couanet *et al*, 2005** and **Ridola *et al*, 2007**, CNS tumour patients (N= 90) received thiotepa in combination with busulfan. Engraftment was achieved in all studies.

In **Dupuis-Girod *et al*, 1997**, patients with relapsed medulloblastoma had a median follow up of 39.5 months post HPCT (range 21-92 months) with a 75% response rate. The relapse rate was 35% (7/20 patients).

In **Grill *et al*, 1996**, the TT/BU combination was evaluated in refractory or relapsed ependymoma. Of 15 evaluable patients, 9 achieved a significant response 1-2 months after HPCT. Eleven patients experienced relapse. There were 3 patients free of disease at 15, 25 and 27 months after HPCT. The latest relapse occurred 5 years after transplantation.

In **Valteau-Couanet *et al*, 2005**, 15 children with relapsing medulloblastoma after conventional therapy were treated with TT/BU. Tumour response was evaluable in 7/10 patients and consisted in two CR, three PR and two NR. Two patients were alive with no evidence of disease 158 and 135 months post HPCT, respectively, and eight patients had died of disease progression.

In **Ridola *et al*, 2007**, the 5-year estimated OS and EFS were 68.8% (95% CI, 53–81.2%) and 61.5% (95% CI, 45.9–75.1%), respectively. Treatment failures occurred in 13 patients within the first 2 years of salvage therapy and the median delay of disease recurrence was 10 months (range, 7–24 months). Among the 37 evaluable patients, disease recurrences were observed in 1 out of 18 patients who were in CR and in 12 out of 19 patients who were not in CR.

The cognitive outcome associated with the current strategy, with a median FSIQ of 71.8 at 5.8 years of follow-up, is an improvement on previously reported results after CSI-based salvage therapy (median FSIQ, 62 at 4.8 years of follow-up in children aged <4 years at diagnosis).

Collectively, these studies show that high dose chemotherapy with TT/BU followed by HPCT is efficacious in relapsed medulloblastoma, inducing responses even in heavily pretreated relapsing medulloblastoma patients.

- In **Lucidarme *et al*, 1998** and **Lafay-Cousin *et al*, 2000**, patients with solid tumours not otherwise specified (N=40) received thiotepa alone. Engraftment was achieved in both studies.

In **Lucidarme *et al*, 1998**, 22 children with refractory solid tumours entered a phase II study of high-dose thiotepa (900 mg/m2) followed by stem cell transplantation (SCT). Tumour types were rhabdomyosarcoma (8), osteosarcoma (7), neuroblastoma (3), Ewing's sarcoma (3) and Burkitt's lymphoma (1). Partial remission was observed in 50% (11/22) of the patients. Four out of 22 had stable disease (SD) and 7/22 progressive disease (PD). Of the seven patients with osteosarcoma, four achieved PR; there were two cases of SD and one of PD. Three were alive with no evidence of disease (NED) more than 25, 48 and 51 months post transplantation, respectively. Four PRs were obtained in patients with rhabdomyosarcoma, disease was stable in two patients and progression was seen in the two remaining ones. One was alive with NED 33 months post BMT. Of the three children with Ewing's sarcomas, two achieved PR and one had PD. One was alive with NED 28 months after the first transplant. One PR and two cases of PD were observed in the three patients with neuroblastoma. Five patients were alive with NED with a follow-up of 35, 38, 43, 58 and 61 months.

In **Lafay-Cousin *et al*, 2000**, all patients were evaluable for tumour response. Of the 18 patients, one achieved CR, five achieved PR, and all the others were considered as NR. Six out of 18 patients responded following high dose thiotepa, attaining a response rate of 33% (95%CI: 11% to 55%).

Among the 12 non-responders, 10 died of progressive disease despite various post-transplantation treatments over a median of 4 months (range 2–31). The overall median time to relapse was 4 months (range 1–21) with a median follow-up of 8 months (range 2–63). This is the first large series evaluating the anti-tumour activity of hig-dose thiotepa in malignant mesenchymal tumours. All children who achieved a significant response had metastatic disease and half of the responders were experiencing a second or further relapse, i.e. these patients had particularly poor prognosis.

- In **Chan *et al*, 1997**, patients with solid tumours not otherwise specified (N= 21) received thiotepa (TT) in combination with cyclophosphamide and melphalan. All patients achieved the engraftment. 9 patients were evaluated for tumour response. 5 showed a decrease in residual tumour while the other four showed no change. Upon follow up, 12 patients developed progressive disease at a median of 7

months from HSC reinfusion. 6 patients were alive in continuous remission 5-50 months (median 36) after transplantation.

- In **Kushner *et al*, 2001**, patients with solid tumours not otherwise specified (N=21) received thiotepa in combination with carboplatin and topotecan. Engraftment occurred at the expected time. Only 2 patients received second infusion of bone marrow on day 21 because of slow engraftment. Relapse was seen in 5/21 patients with a follow up of 6-32 months after transplantation. In the neuroblastoma group, 10/11 patients relapsed (follow-up: 6-16 months after transplantation) while in the non-neuroblastoma group, 6/10 patients relapsed (follow-up: 9-32 months after transplantation). All 21 patients were alive with a median observation time of 11.8 months (range: 6 to 32 months).

The arguments for treatment intensification in such patients using thiotepa, carboplatin, and topotecan included the steep dose–response anti-tumour effect of alkylators, the evidence that alkylators are non-crossresistant, the enhancing effect of topotecan on alkylator cytotoxicity and the activity of each agent against a broad range of cancers including brain tumours. All three agents are active against neuroblastoma and their combined use aimed at overcoming two vexing problems of this embryonal neoplasm, i.e. minimal residual disease and CNS relapse.

- In **Hawkins *et al*, 2000**, Ewing's sarcoma family tumour (ESFT) patients (N=16) received thiotepa in combination with busulfan and melphalan. Patients with an adequate PBSC collection to support a second myeloablative course and who had not received prior dose-limiting radiotherapy were eligible to receive total marrow irradiation (TMI) following recovery from TT/BU/MEL. The median TMI dose was 12 Gy, with a range of 10.5–15 Gy. PBSC were infused 24 hr after the final dose of TMI. Six out of nine patients that received TT/BU/MEL+TMI remain in CR at a median of 42 months (range 27-66) and three developed recurrent disease at 6.3–17 months and succumbed. None of the seven patients that only received TT/BU/MEL survived. Overall, the progression-free survival with a median follow-up of 42 months is similar to the 2-year EFS of 21-45% following other HPCT regimens for poor-risk ESFT.

Allogeneic HPCT in paediatric patients

A total of 426 patients with haematological diseases received thiotepa i.v, in combination with other chemotherapeutic drugs and TBI as conditioning treatment before allogeneic HPCT.

Table 8: Allogeneic HPCT in paediatric population

DISEASE	CHARACTERISTIC	CONDITIONING TREATMENT	N PTS	AUTHORS
Haematological diseases	Leukemia	TT/TBI/CY	97	Zecca et al, 1999; Locatelli et al, 2009
		TT/CY/ALG/TBI	41	Locatelli et al, 2009
		TT/FLU/ATG/TBI	21	
		TT/MEL/TBI	18	
		TT/FLU/TBI	19	
		TT/MEL/ALG/TBI	15	
		TT/FLU/TREO	10	
		TT/CY/ATG/TBI	8	
	Thalassemia	TT/BU/CY	28	La Nasa et al, 2002
		TT/TREO/FLU	30	Bernardo et al, 2008
		TT/BU/FLU	65	Locatelli et al, 2009
		TT/BU/FLU/ALG	16	
	Refractory Cytopenia	TT/FLU/ATG	19	Strahm et al, 2007
	Genetic Diseases	TT/BU/CY	26	Rosales et al, 1999
	Sickle cell anaemia	TT/FLU/BU	14	Locatelli et al, 2009
TOTAL			**426**	

ATG= antithymocyte globuline; ALG= antilymphocyte globuline; BU=busulfan; CY=cyclophosphamide; FLU= fludarabine; MEL=melphalan; TBI= total body irradiation; TREO=treosulfan; TT= thiotepa

The paediatric population with haematological diseases receiving allogeneic HPCT comprised patients with leukemia (N=228), thalassemia (N=139), refractory cytopenia (N=19), genetic diseases (N=26) and sickle cell anaemia (N=14).

- In **Zecca** *et al*, **1999** and **Locatelli** *et al*, **2009**, lymphoblastic leukemia patients (N=97) received thiotepa in combination with TBI and cyclophosphamide.

The success of allogeneic HPCT in eradicating leukaemia depends on the following factors: the ability of the chemoradiotherapy administered as myeloablative treatment to reduce or, at best, to eliminate clonogenic malignant cells, and the graft-versus-leukemia effect.

The study by **Zecca** *et al*, **1999** (N=40) is the first prospective study to evaluate the tolerability and efficacy of a new conditioning regimen based on the combination of TBI, thiotepa and CY in a homogeneous groups of children with ALL. The engraftment rate was 98%, OS at 36 months was 65% and the relapse related death at 36 months was 15%. 56% DFS documented in the 27 patients who underwent transplantation in second CR is noteworthy for two main reasons. First, only three out of 27 patients underwent the transplantation procedure after a previous isolated CNS relapse, and the DFS was not modified by their exclusion from the analysis. Second, the escalation of intensity of the last generation first-line chemotherapy protocols that were administered to the great majority of the patients (23 of 27 in second CR and 11 of 13 in first CR) may have led to an unfavorable selection of relapsed patients, who were more likely to harbor refractory disease and more prone to develop severe toxicity after an aggressive pre-transplantation conditioning regimen.

Locatelli *et al*, **2009** reported the results of 57 ALL patients treated with TT/TBI/CY. With an average follow up of 121 months, 37 (65%) patients are alive. The relapse rate was 25% and the DFS was 60%. The addition of thiotepa to the TBI-CY schedule minimized the risk of a previously acquired drug resistance of the leukemic blasts. Moreover, owing to its effect on enhancing engraftment of donor stem cells, thiotepa could be of particular value in critical situations characterized by a high risk of graft failure, such as cord-blood, unrelated donor or mismatched donor transplantation.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=40) received thiotepa in combination with TBI, cyclophosphamide and ALG. OS was 55%. Relapse and DFS reported was 35% and 45% respectively.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=21) received thiotepa in combination with TBI, fludarabine and ATG. The engraftment was achieved in the expected time. 16/21 (76%) of patients were alive with a follow up of 21.2 months. 6 patients (28.5%) had relapse. The DFS was 67%.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=18) received thiotepa in combination with TBI and melphalan. 11/18 patients were alive with a follow up of 42.3 months. Relapse and DFS reported was 44% and 50%, respectively.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=19) received thiotepa in combination with TBI and fludarabine. OS was 68% with a follow up of 42 months. 7 patients had relapse (37%), 3 of whom died of disease progression. DFS was 47%.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=15) received thiotepa in combination with TBI, melphalan and ALG. All patients experienced engraftment in the expected recovery time. OS was 60% (9/15). Relapse was 40% and it was cause of death in 4/6 patients. DFS was 53%.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=10) received thiotepa in combination with fludarabine and treosulfan. OS was 50%. 3 patients relapsed. Progression of disease was cause of death in 2 patients. DFS was 40%.

- In **Locatelli** *et al*, **2009**, leukemia patients (N=8) received thiotepa in combination with TBI, cyclophosphamide and ATG. 75% of patients were alive and disease-free with a follow up of 41 months. 2 patients relapsed and died of disease progression.

- In **La Nasa** *et al*, **2002**, thalassemia patients (N=28) received thiotepa in combination with busulfan and cyclophosphamide. This is the first large series of consecutive thalassemia patients with transplants from unrelated donors reported.

In this cohort, rejection and mortality rates were 12.5% and 19%, respectively. 69% of the patients were alive with sustained engraftment of donor hematopoiesis, this leading to a projected thalassemia-free survival of 66%. Only one death was observed among 15 class I and class II patients (7%), the other 5 occurring among 17 class III patients (29%). 2 of the 4 cases of rejection occurred in the first 4 patients conditioned with the standard BU-CY regimen. However, since in adult thalassemia patients increasing the dosage of BU carried the risk of increased mortality and toxicity, in the second series of patients thiotepa was added. Thiotepa at a dose of 10 mg/kg was shown to intensify both the myeloablative and immunosuppressive effect of the conditioning regimen, without a significant

increase of extramedullary toxicity. Thiotepa added to BU/CY decreased rejection to 7% (2/28). The OS rate was 93% in class I-II pts and 69% in the cumulative population.

- In **Bernardo *et al*, 2008** and **Locatelli *et al*, 2009**, thalassemia patients (N=30) received thiotepa in combination with treosulfan and fludarabine.

In **Bernardo *et al*, 2008**, all patients (N=20) engrafted. Two patients (both transplanted from unrelated donors) experienced secondary graft failure. The overall cumulative incidence of graft failure was 11%. 17 patients were transfusion independent. 2 year estimated transfusion-free survival (TFS) was 85% and OS at 2 years was 95%.

In **Locatelli 2009**, the results are comparable with the previous report. All patients engrafted in the expected time. With a follow up of 25.6 months, 9 (90%) patients were alive and disease-free.

- In **Locatelli 2009**, thalassemia patients (N=65) received thiotepa in combination with busulfan and fludarabine. The engraftment occurred at the expected time. With a follow up of 131 months 97% (63/65) patients were alive. There was only 1 relapse at day +633 from transplantation. DFS was 95% (62/65). Thalassemia represents a particular disease because the risk of graft failure is higher than other haematological diseases. As reported in **La Nasa *et al*, 2002**, TT added to BU-CY decreased rejection to 7% (2 on 28 pts).

- In **Locatelli *et al*, 2009**, thalassemia patients (N=16) received thiotepa in combination with busulfan, fludarabine and ALG. 81% of patients were alive. DFS was 81% and no patient died from progression of disease.

- In **Strahm *et al*, 2007**, refractory cytopenia patients (N=19) received thiotepa in combination with fludarabine and ATG. Sustained neutrophil engraftment was achieved in all but three patients. Patients that achieved the engraftment had full chimerism. Sixteen out of the 19 patients are alive with a median follow-up of 13 months. The estimated OS and EFS at 3 years was 0.84 (0.68–0.99) and 0.74 (0.54–0.94), respectively.

- In **Rosales *et al*, 1999**, genetic diseases patients (N=26) received thiotepa in combination with busulfan and cyclophosphamide. The aim of this study was to evaluate the effectiveness of the addition of 10 mg/kg thiotepa to the standard BU-CY conditioning regimen for T cell depleted BMT in genetic diseases. The engraftment was achieved in 96% of the patients. There was only 1 case of relapse. The overall survival and DFS survival rates were both 71% at 60 months.

- In **Locatelli *et al*, 2009**, sickle cell anaemia patients (N=14) received thiotepa in combination with busulfan and fludarabine. With a follow up of 51 months all 14 patients were alive without signs of disease (DFS = 100%). No case of relapse was reported.

- Dose response study(ies)

No studies were submitted.

- Main study(ies)

No studies were submitted.

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

No pooled analyses and no meta-analysis were submitted.

- Clinical studies in special populations

No studies were submitted.

- Supportive studyies

No studies were submitted.

- Discussion on clinical efficacy

In autologous HPCT, efficacy depends almost exclusively on the conditioning treatment which must provide cytoreduction and ideally disease eradication. Thiotepa has marrow ablation as its dose-

limiting toxicity thus allowing significant dose escalation. In allogeneic HPCT, efficacy depends both on the conditioning treatment and on the allogeneic cells from the donor, more specifically the donor immune system. The conditioning treatment must be sufficiently immunosuppressive and myeloablative to overcome rejection of the graft by the host. On the other hand, the allogeneic cells of the graft determine the percentage of GvHD (Graft vs Host Disease) and GvL (Graft vs Leukaemia) effects. GvHD is a major complication of allogeneic transplants, whereas the GvL effect plays a crucial role in the control of disease.

Autologous HPCT in adult patients

Efficacy in haematological diseases following Autologous HPCT
The efficacy of thiotepa in combination with other chemotherapeutic drugs as conditioning treatment prior to autologous HPCT was evaluated in 826 adult patients with haematological diseases. These comprised 569 patients with lymphomas and 257 patients with multiple myeloma.
In combination with other chemotherapeutic drugs the dose of thiotepa in haematological diseases ranged from 125 mg/m^2/day (3.38 mg/kg/day) to 300 mg/m^2/day (8.10 mg/kg/day) as a single daily infusion, administered from 2 up to 4 consecutive days before autologous HPCT, depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 900 mg/m^2 (24.32 mg/kg) during the time of the entire conditioning treatment.
Engraftment was achieved at 96% in low risk patients (Papadopoulos 2005) and from 92 % to 99% in patients at high risk for their advanced, recurrent and relapsed disease at the time of tranplantation. This is a potential benefit for all high risk patients and in particular for those patients with an history of prior dose-limiting radiation and for whom a chemotherapy alone based conditioning regimen was the only option (Gutierrez-Delgado *et al,* 2001, Gutierrez-Delgado *et al,* 2003).
Disease-free survival (DFS), overall survival (OS) and relapse are the most relevant efficacy endpoints for conditioning regimens prior to conventional HPCT.
In IBMTR and EBMT Registry data (Bierman *et al,* 2003) DFS estimated at 5 years was < 50% and overall survival was 45%. In some conditioning regimens including thiotepa, DFS at 5 years was 43% (Waheed *et al,* 2004) and DFS at 44 months was 66% (Cumpston *et al,* 2007).
Across all submitted studies, OS ranged from 29% to 87% with a follow up that ranged from 22 up to 63 months. At 5 years follow up, OS ranged from 42 to 52% in lymphoma patients (Gutierrez-Delgado *et al,* 2001 and 2003) and 29% in multiple myeloma patients (Shimoni *et al,* 2001). In the study by Illerhaus *et al* (2006), patients with CNS lymphoma had 87% survival at 5 years.
Relapse at 5 years ranged from 34 to 42% in lymphoma patients (Gutierrez-Delgado *et al,* 2001 and 2003). Relapse related death at 5 years in CNS lymphoma (Illerhaus *et al,* 2006) was 8.7%. In addition, event free survival (EFS) at 5 years ranged from 34 to 42% in lymphomas (Gutierrez-Delgado *et al,* 2001 and 2003).
The recommended dose in adults with haematological diseases undergoing autologous HPCT ranges from 125 mg/m^2/day (3.38 mg/kg/day) to 300 mg/m^2/day (8.10 mg/kg/day) as a single daily infusion, administered from 2 up to 4 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 900 mg/m^2 (24.32 mg/kg), during the time of the entire conditioning treatment.
LYMPHOMA
The recommended dose ranges from 125 mg/m^2/day (3.38 mg/kg/day) to 300 mg/m^2/day (8.10 mg/kg/day) as a single daily infusion, administered from 2 up to 4 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 900 mg/m^2 (24.32 mg/kg), during the time of the entire conditioning treatment.
CNS LYMPHOMA
The recommended dose is 185 mg/m^2/day (5 mg/kg/day) as a single daily infusion, administered for 2 consecutive days before autologous HPCT, without exceeding the total maximum cumulative dose of 370 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.
MULTIPLE MYELOMA
The recommended dose ranges from 150 mg/m^2/day (4.05 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day) as a single daily infusion, administered for 3 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the

total maximum cumulative dose of 750 mg/m^2 (20.27 mg/kg), during the time of the entire conditioning treatment.

Efficacy in solid tumours following Autologous HPCT
The efficacy of thiotepa as conditioning treatment prior to autologous HPCT has been evaluated in 3675 adult patients with solid tumours. These comprised patients with breast cancer (N=3457), CNS tumours (N=108), ovarian cancer (N=48) and germ cell tumours (N=62).
In combination with other chemotherapeutic drugs, the dose of thiotepa in solid tumours ranges from 120 mg/m^2/day (3.24 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day), divided in once or twice daily infusions administered from 2 up to 5 consecutive days before autologous HPCT, depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 800 mg/m^2 (21.62 mg/kg) during the time of the entire conditioning treatment.
The number of haematopoietic stem cell transplantation for treating solid tumours seems constant in recent years as reported from EBMT (European Group for Blood and Marrow Transplantation, Gratwohl *et al,* 2009, 2008, 2007).
In the EBMT Group and in IBMTR (International Bone Marrow Transplantation) there are many Commissions aiming to evaluate the different transplantation strategies for treating different diseases.
Ljungman *et al* (2009) published a special EBMT report regarding the current practice of allogeneic and autologous transplantation to treat all haematological and non haematological diseases.
The existence of a dose-response effect in epithelial tumours (breast, ovarian) is still a matter of investigation. However, the benefit of high-dose chemotherapy (HDCT) in selected subgroups of patients has become clearer. The role of autologous HPCT for primary breast cancer at high risk of recurrence (at least four involved axillary lymph nodes) was assessed in a meta-analysis of individual patient data from 15 known randomized trials comparing HDCT with standard-dose chemotherapy (Ueno *et al*, 2009). It was shown that HDCT prolonged disease-free survival when used as adjuvant therapy and showed a benefit on breast cancer-specific survival and OS. In the context of metastatic breast cancer, HDCT seems to be effective in stage IV patients rendered free of macroscopic disease by previous therapy and in patients with oligometastatic disease. High-dose chemotherapy for germ cell tumours is considered a clinical option for sensitive relapse and as standard therapy for refractory disease.
Regarding ovarian cancer, the results obtained with high dose chemotherapy followed by autologous HPCT did not achieve statistically significant difference in progression- free survival or OS compared with standard-dose chemotherapy (Ljungman *et al,* 2009).
Weaver *et al* (1997) published an analysis of 1000 consecutive patients who underwent ASCT with different conditioning regimens with a follow up over a 5-year period. 1000 patients were enrolled between 1989 and 1994. The aim of the study was to evaluate the treatment relate mortality (TRM) of different conditioning regimens. On a total of 1000 patients, 713 with breast cancer were treated with thiotepa (167 mg/m^2 for three days), carboplatin and cyclophosphamide. The remaining patients were treated with other regimens, such as BEAC, BU/CY, TT/BU/MEL and ICE. Lower TRM at 100 days was observed in the TT/CARB/CY group, 1.8%, *vs* 7.2% in patients receiving other preparative regimens (*P* = 0.0001).
The first publications documenting experience with thiotepa, carboplatin and cyclophosphamide date to 1992 (Rodenhuis *et al,* 1992) and 1995 (van der Wall *et al,* 1995).
Fifteen articles were submitted comprising a total of 3457 patients with breast cancer. Seven articles out of fifteen are comparative as reported by the authors, for a total of 1944 patients treated with thiotepa in combination with other chemotherapeutic drugs as conditioning regimens before ASCT. Three articles out of seven, for a total of 1085 patients treated with thiotepa regimens, show a significant difference in efficacy in terms of relapse or OS or EFS of high-dose chemotherapy including thiotepa when compared with conventional chemotherapy (Nitz *et al,* 2005, Rodenhuis *et al,* 2003 and Rodenhuis *et al,* 2006). Tallman *et al* (2003) and Zander *et al* (2004), for a total of 420 patients treated with thiotepa regimens, reported a better trend in term of relapse and EFS, respectively, but without statistical significance.
High dose chemotherapy followed by ASCT can be considered a clinical option only if used in a very specific subgroups of patients with solid tumours (e.g., sensitive relapse and refractory germ cell tumours, CNS tumours, etc).
The recommended dose in adults with solid tumours undergoing autologous HPCT ranges from 120 mg/m^2/day (3.24 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day) divided in one or two daily

infusions, administered from 2 up to 5 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 800 mg/m^2 (21.62 mg/kg), during the time of the entire conditioning treatment.

BREAST CANCER

The recommended dose ranges from 120 mg/m^2/day (3.24 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day) as a single daily infusion, administered from 3 up to 5 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 800 mg/m^2 (21.62 mg/kg), during the time of the entire conditioning treatment.

CNS TUMOURS

The recommended dose ranges from 125 mg/m^2/day (3.38 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day) divided in one or two daily infusions, administered from 3 up to 4 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 750 mg/m^2 (20.27 mg/kg), during the time of the entire conditioning treatment.

OVARIAN CANCER

The recommended dose is 250 mg/m^2/day (6.76 mg/kg/day) as a single daily infusion, administered in 2 consecutive days before autologous HPCT, without exceeding the total maximum cumulative dose of 500 mg/m^2 (13.51 mg/kg), during the time of the entire conditioning treatment.

GERM CELL TUMOURS

The recommended dose ranges from 150 mg/m^2/day (4.05 mg/kg/day) to 250 mg/m^2/day (6.76 mg/kg/day) as a single daily infusion, administered for 3 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 750 mg/m^2 (20.27 mg/kg), during the time of the entire conditioning treatment.

Adequate data to demonstrate the well established use of thiotepa in the EU over 10 years as a single agent prior to conventional autologous HPCT for adult patients with solid tumours were not submitted.

Allogeneic HPCT in adult patients

A total of 1771 patients with haematological diseases received thiotepa i.v., in combination with TBI and other chemotherapeutic drugs as conditioning treatment before allogeneic HPCT. These comprised patients with lymphomas (N=187), patients with multiple myeloma (N=53), patients with haematological diseases not otherwise specified (N=930), patients with leukemia (N=586) and patients with thalassemia (N=15).

In combination with other chemotherapeutic drugs the dose of thiotepa in haematological diseases ranged from 185 mg/m^2/day (5 mg/kg/day) to 481 mg/m^2/day (13 mg/kg/day) divided in one or two daily infusions administered from 1 up to 3 consecutive days before allogeneic HPCT, depending on the combination with other chemotherapeutic medicinal products and without exceeding the total maximum cumulative dose of 555 mg/m^2 (15 mg/kg) during the time of the entire conditioning treatment.

The engraftment was achieved with success rates of 83% to 100%. All the conditioning treatments evaluated assured a low incidence of acute GvHD grade III-IV (from 2% to 32%, Aversa *et al*, 1998; Bacigalupo *et al*, 2007a).

Disease Free Survival (DFS) was reported with an estimated value of 12 to 74 months. DFS ranged from 37% to 64% at 36 months (Majolino *et al*, 2007; Corradini *et al*, 2004) and it was 59% at 60 months (Corradini *et al*, 2005) and 23% at 74 months (Picardi *et al*, 2004).

Relapse at 3 years ranged from 12% to 41% (Corradini *et al*, 2004; Corradini *et al*, 2007) and it was 56% at 6 years (Picardi *et al*, 2004).

OS ranged from 31% to 81% with a follow up ranging between 7.3 and 120 months.

OS at 3 years ranged from 62% to 81% (Corradini *et al*, 2007; Corradini *et al*, 2009); at 5 years it ranged from 50% to 66% (Rosales *et al*, 1999; Corradini *et al*, 2005); at 6 years it was 31% (Picardi *et al*, 2004) and at 10 years it was 55% (Bacigalupo *et al*, 2007b).

The recommended dose in adults with haematological diseases undergoing allogeneic HPCT ranges from 185 mg/m^2/day (5 mg/kg/day) to 481 mg/m^2/day (13 mg/kg/day) divided in one or two daily infusions, administered from 1 up to 3 consecutive days before autologous HPCT depending on the

combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 555 mg/m^2 (15 mg/kg), during the time of the entire conditioning treatment.

LYMPHOMA

The recommended dose in lymphoma is 370 mg/m^2/day (10 mg/kg/day) divided in two daily infusions before allogeneic HPCT, without exceeding the total maximum cumulative dose of 370 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

MULTIPLE MYELOMA

The recommended dose is 185 mg/m^2/day (5 mg/kg/day) as a single daily infusion before allogeneic HPCT, without exceeding the total maximum cumulative dose of 185 mg/m^2 (5 mg/kg), during the time of the entire conditioning treatment.

LEUKEMIA

The recommended dose ranges from 185 mg/m^2/day (5 mg/kg/day) to 481 mg/m^2/day (13 mg/kg/day) divided in one or two daily infusions, administered from 1 up to 2 consecutive days before allogeneic HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 555 mg/m^2 (15 mg/kg), during the time of the entire conditioning treatment.

THALASSEMIA

The recommended dose is 370 mg/m^2/day (10 mg/kg/day) divided in two daily infusions, administered before allogeneic HPCT, without exceeding the total maximum cumulative dose of 370 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

Autologous HPCT in paediatric patients

A total of 476 patients with CNS tumours (N=378) and solid tumours not otherwise specified (N=98) received thiotepa i.v. as conditioning treatment before autologous HPCT.
In combination with other chemotherapeutic drugs, the dose of thiotepa in solid tumours in paediatric patients ranged from 150 mg/m^2/day (6 mg/kg/day) to 350 mg/m^2/day (14 mg/kg/day) as a single daily infusion administered from 2 up to 3 consecutive days before autologous HPCT, depending on the combination with other chemotherapeutic medicinal products and without exceeding the total maximum cumulative dose of 1050 mg/m^2 (42 mg/kg) during the time of the entire conditioning treatment.
Engraftment was achieved with all evaluated conditioning regimens including thiotepa.
Disease Free Survival (DFS) was reported in the evaluated conditioning treatments with an estimated value of more than 12 months. From 24 months to 57 months, DFS ranged from 46% to 70% (Grovas *et al,* 1999; Massimino *et al,* 2005).
The relapse rates, from 12 up to 57 months, ranged from 33% to 57% (Massimino *et al,* 2005; Dhall *et al,* 2008).
OS ranged from 17% to 84% with a follow up ranging from 12.3 to 99.6 months. OS at 36 months was 44.6% (Fagioli *et al,* 2004) and 87.5% (Massimino *et al,* 2006); OS at 57 months was 43% (Massimino *et al,* 2005); OS at 60 months was 70% (Dhall *et al,* 2008).
Considering the high-risk, poor prognosis patients included, the OS results achieved with conditioning regimens including thiotepa are satisfactory.
Thiotepa used as a single agent prior to conventional autologous HPCT seems effective in some specific solid tumours (malignant mesenchymal tumours, osteosarcoma, Ewing tumours, neuroblastoma) in paediatric patients. However, as no solid comparative data are provided to support the specific and exceptional use as single agent, this information could not be included in the indication.
The recommended dose in paediatric patients with solid tumours undergoing autologous HPCT ranges from 150 mg/m^2/day (6 mg/kg/day) to 350 mg/m^2/day (14 mg/kg/day) as a single daily infusion, administered from 2 up to 3 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 1050 mg/m^2 (42 mg/kg), during the time of the entire conditioning treatment.

CNS TUMOURS

The recommended dose ranges from 250 mg/m^2/day (10 mg/kg/day) to 350 mg/m^2/day (14 mg/kg/day) as a single daily infusion, administered for 3 consecutive days before autologous HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the

total maximum cumulative dose of 1050 mg/m^2 (42 mg/kg), during the time of the entire conditioning treatment.

Allogeneic HPCT in paediatric patients

A total of 426 patients with haematological diseases received thiotepa i.v, in combination with other chemotherapeutic drugs and TBI as conditioning treatment before allogeneic HPCT. These comprised patients with leukemia (N=228), patients with thalassemia (N=139), patients with refractory cytopenia (N=19) patients with genetic diseases (N=26) and patients with sickle cell anaemia (N=14).

In combination with other chemotherapeutic drugs the dose of thiotepa in haematological diseases in paediatric patients ranged from125 mg/m^2/day (5 mg/kg/day) to 250 mg/m^2/day (10 mg/kg/day), divided in once or twice daily infusions administered from 1 to 3 consecutive days before allogeneic HPCT, depending on the combination with other chemotherapeutic medicinal products and without exceeding the total maximum cumulative dose of 375 mg/m^2 (15 mg/kg) during the time of the entire conditioning treatment.

Engraftment ranged from 96% to 100% in all evaluated conditioning regimens including thiotepa.

Disease Free Survival (DFS) ranged from 40% to 75% at timepoints greater than 1 year (Locatelli *et al*, 2009). In acute lymphoblastic leukemia (ALL), thiotepa, TBI and cyclophosphamide ensure a DFS of 60% at 121 months (Locatelli *et al*, 2009). In thalassemia, DFS was 66% (La Nasa *et al*, 2002) and 95% (Locatelli *et al*, 2009).

Relapse rates of 25% (Zecca *et al*, 1999) and 44% (Locatelli *et al*, 2009) were reported. The relapse-related death at 36 months was 15% (Zecca *et al*, 1999). The relapse in thalassemic and genetic disease patients was very low as 1.5% (Locatelli *et al*, 2009) and 3.8% (Rosales *et al*, 1999), respectively.

OS ranged from 50% to 100% with a follow up ranging between 9.4 and 121 months. In patients who underwent allogeneic transplantation to treat acute lymphoblastic leukaemia OS at 41 months was 75%, and at 121 months it was 65% (Locatelli *et al*, 2009).

Overall, thiotepa was used in several different hematological malignant and non malignant diseases with different allogeneic transplant settings (unmanipulated, T cell depleted, CD34 selected, cord blood, myeloablative, intensified, reduced intensity).

The recommended dose in paediatric patients with haematological diseases undergoing aloogeneic HPCT ranges from 125 mg/m^2/day (5 mg/kg/day) to 250 mg/m^2/day (10 mg/kg/day) divided in one or two daily infusions, administered from 1 up to 3 consecutive days before allogeneic HPCT depending on the combination with other chemotherapeutic medicinal products, without exceeding the total maximum cumulative dose of 375 mg/m^2 (15 mg/kg), during the time of the entire conditioning treatment.

LEUKEMIA

The recommended dose is 250 mg/m^2/day (10 mg/kg/day) divided in two daily infusions, administered before allogeneic HPCT, without exceeding the total maximum cumulative dose of 250 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

THALASSEMIA

The recommended dose ranges from 200 mg/m^2/day (8 mg/kg/day) to 250 mg/m^2/day (10 mg/kg/day) divided in two daily infusions, administered before allogeneic HPCT without exceeding the total maximum cumulative dose of 250 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

REFRACTORY CYTOPENIA

The recommended dose is 125 mg/m^2/day (5 mg/kg/day) as a single daily infusion, administered for 3 consecutive days before allogeneic HPCT, without exceeding the total maximum cumulative dose of 375 mg/m^2 (15 mg/kg), during the time of the entire conditioning treatment.

GENETIC DISEASES

The recommended dose is 125 mg/m^2/day (5 mg/kg/day) as a single daily infusion, administered for 2 consecutive days before allogeneic HPCT, without exceeding the total maximum cumulative dose of 250 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

SICKLE CELL ANAEMIA

The recommended dose is 250 mg/m^2/day (10 mg/kg/day) divided in two daily infusions, administered before allogeneic HPCT, without exceeding the total maximum cumulative dose of 250 mg/m^2 (10 mg/kg), during the time of the entire conditioning treatment.

Clinical safety

- Patient exposure

Thiotepa has been in clinical use for several decades, in combination with other chemotherapeutic drugs as follows prior to conventional HPCT in haematological diseases and solid tumours. The applicant has presented bibliographic data for a total of 6588 adult patients and 902 paediatric patients. The number of patients by type of disease and type of chemotherapeutic regimen received, as well as the relevant publications serving as the source of data, are summarised in the efficacy tables 5, 6, 7 and 8. Additionally, 316 adult patients receiving autologous HPCT were included in the safety analysis. These included 39 breast cancer patients receiving TT/CY (Ando 2000), 251 breast cancer patients receiving TT/CY/MITO (Kroger 2003) and 26 ovarian cancer patients receiving TT/CC/CISPL (Stiff 2004).

- Adverse events/Serious adverse event/deaths/other significant events

Clinical data on Adverse events grade I-II, Adverse events grade III-IV (collected at +100 days), Regimen Related Mortality (RRM) and Treatment Related Mortality (TRM) have been evaluated by the Applicant in the different conditioning treatments including thiotepa with or without TBI for adult and paediatric patients undergoing autologous and allogeneic HPCT for haematological diseases and solid tumours. The data are presented separately for adults and children and for autologous and allogeneic HPCT.

Autologous HPCT in adult patients

In autologous HPCT the safety database consisted of a total of 4817 patients with haematological diseases (N=826) and with solid tumours (N=3991). These patients received thiotepa i.v. in combination with other chemotherapeutic drugs as conditioning treatment before autologous HPCT.

Haematological diseases
- In **Gopal *et al*, 2001, Gutierrez-Delgado *et al*, 2001** and **Gutierrez-Delgado *et al*, 2003**, lymphoma patients (N=204) received thiotepa in combination with busulfan and melphalan.
At the time of autologous transplantation, patients with advanced/refractory Hodgkin's disease have usually been heavily pretreated to be kept in clinical remission; consequently, they are at high risk for toxicity including VOD and multiorgan failure syndrome.
In **Gutierrez-Delgado *et al*, 2001** and **Gutierrez-Delgado *et al*, 2003**, low rates of TRM were reported at 1% and 16%, respectively. These results are even more important considering that a high number of patients with prior dose limiting radiation therapy were enrolled (in literature these patients are characterized by a high morbidity and toxicity).
- In **Przepiorka *et al*, 1995**, lymphomas patients (N=34) received thiotepa in combination with busulfan and cyclophosphamide. The safety results were encouraging considering the advanced status of the diseases and that increases in the dose of thiotepa allowed decreases of the doses of busulfan and cyclophosphamide without increasing the overall regimen toxicity.
- In **Demirer *et al*, 2004**, lymphoma patients (N=42) received thiotepa in combination with melphalan and carboplatin. This conditioning treatment showed a good safety profile.
- In **Waheed *et al*, 2004** and **Glossmann *et al*, 2005**, lymphoma patients (N=125) received thiotepa in combination with mitoxantrone and carboplatin. The regimen was well tolerated with only 4 and 5 transplant-related deaths, respectively. Unlike recipients of BEAM therapy, which is usually associated with high rates of pulmonary toxicity due to the overlapping effects of BCNU, those receiving the thiotepa-based conditioning had only 6% grade III-IV pulmonary toxicity and, as for late toxicity, 5% of second malignancies were observed (**Waheed *et al*, 2004**).
- In **Papadopoulos *et al*, 2005** lymphoma patients (N=31) received thiotepa in combination with carboplatin and etoposide. The regimen was a tandem HD chemotherapy represented by melphalan and mitoxantrone followed by thiotepa, carboplatin and etoposide. The RRT was 9.7% and this very intensive strategy could be applied as rescue therapy based on the acceptable results in term of safety.

- In **Cumpston et al, 2007**, lymphoma patients (N=29) received thiotepa in combination with cyclophosphamide and etoposide. This study reported 3 deaths of infections in patients with advanced NHL suggesting good safety profile with TRM = 10,3%.

- In the **study McCoy 2004,** lymphoma patients (N=65) received thiotepa in combination with etoposide. Although all these patients had received several courses of chemotherapy, the high-dose thiotepa-etoposide regimen was safe and only one patient died of regimen related mortality.

There were 3 secondary malignancies (etoposide is a risk factor for the second haematological malignancies): 1 AML after 29 days from ASCT at 7 months after transplant, one carcinoma of the bladder (but the patients remained free of lymphoma after 82 months of follow-up) and one MDS 6 years after transplant (patient who received four pre transplant therapeutic regimens and one cycle of fludarabine for relapse 5 years post transplant).

- In **Illerhaus et al, 2006**, CNS lymphoma patients (N=23) received thiotepa in combination with carmustine. Patients tolerated induction therapy and HDT had a very good profile of safety with a TRM = 0. The very low relapse mortality (8.7%) associated with the high survival rates (87%) at 5 years suggests that this conditioning treatment may be curative.

- In **Montemurro et al, 2007**, CNS lymphoma patients (N=16) received thiotepa in combination with busulfan. This combination of high dose thiotepa and busulfan for PCNSL showed good tolerability and it is a promising approach without severe neurotoxicity when WBRT is avoided.

- In **Dimopoulos et al, 1993, Shimoni 2001 et al,** and **Anagnostopoulos et al, 2004**, multiple myeloma patients (N=257) received thiotepa in combination with busulfan and cyclophosphamide.

These studies were designed as an alternative regimen for optimizing cytoreduction prior to transplant in anticipation of better disease control. Each drug used in this combination has a different spectrum of extra-medullary toxicity. To decrease the relative high incidence of RRT the authors decided to introduce thiotepa in order to reduce the busulfan dose. High dose of thiotepa showed anti-myeloma activity. Early TRM was comparable with melphalan/TBI or non-TBI regimens. The comparative study **Anagnostopoulos et al, 2004** between thiotepa, busulfan and cyclophosphamide vs high dose of melphalan showed the same efficacy but more toxicity (6 RRT vs 1 RRT) for the TT/BU/CY regimen. In conclusion, positive safety profile is reported in two studies (**Shimoni 2001 and Dimopoulos 1993**) while the comparison study (**Anagnostopoulos 2004**) suggests that the tolerability of high dose of melphalan is better although the results are the same in terms of efficacy.

Solid tumours
Adults with solid tumours (N=3991) comprised patients with breast cancer (N=3747), CNS tumours (N=108), ovarian cancer (N=74) and germ cell tumours (N=62).

- In **Ando et al, 2000, Tallman et al, 2003** and **Leonard et al, 2004**, breast cancer patients (N=605) received thiotepa in combination with cyclophosphamide.

The increased time to recurrence suggests that the long-term outcome may improve if transplantation-related mortality can be avoided (**Tallman et al, 2003**). Because of the low incidence of treatment-related mortality, the results appear to reflect a genuine equivalence in efficacy and in safety of the two treatments (conventional and high-dose) (**Leonard et al, 2004**). A possible etiology of heart block is the physical damage to the conduction system secondary to microangiopathy or transient spasm caused by CY-induced injury of capillary endothelium. Such complication has never been reported with thiotepa (**Ando et al, 2000**).

- In **Weaver et al, 1997, Stemmer et al, 2001, Stemmer et al, 2003, Rodenhuis et al, 2003, Schrama et al, 2003, Rodenhuis et al, 2006** and **Coombes et al, 2005**, breast cancer patients (N=2119) received thiotepa in combination with cyclophosphamide and carboplatin.

High-dose alkylating chemotherapy with cyclophosphamide, thiotepa and carboplatin was reasonably safe in patients with primary stage III breast cancer (**Rodenhuis et al, 2006**).

High-dose cyclophosphamide, thiotepa and carboplatin was associated with low risk of mortality compared to other regimens (p=0.0001). Thus, high-dose chemotherapy and autologous PBPC support can be performed in community cancer centres with relative safety (**Weaver et al, 1997**).

The HDC regimen was associated with an increase in grade III-IV toxicities of various types, but there was similar occurrence of treatment-related deaths in both study arms (HDC and conventional) (**Coombes et al, 2005**). High-dose chemotherapy was associated with a moderate mainly reversible toxicity. The toxic death rate was only 1% (**Schrama et al, 2003**). The RRT mortality rate (1%) was less than the rate (7.4%) previously reported for the regimen of cisplatin, cyclophosphamide and carmustine. In addition, there were no significant differences in the quality of life between treatment

groups (**Rodenhuis *et al*, 2003**). Locoregional radiotherapy after high-dose chemotherapy and autologous stem cell transplantation appears to be feasible and can be delivered safely within 10 weeks from transplantation in patients with high-risk stage II-III and locally advanced breast carcinoma (**Stemmer *et al*, 2001**). The combined approach of doxorubicin followed by high-dose chemotherapy and stem cell support followed by locoregional radiotherapy was safe and effective in patients with multinode positive stage II breast cancer (**Stemmer *et al*, 2003**).

- In **Wong *et al*, 2003** and **Cheng *et al*, 2004**, breast cancer patients (N=341) received thiotepa in combination with cyclophosphamide and carmustine. The HDC regimen thiotepa-cyclophosphamide-carmustine was well tolerated with a very low TRM in both studies. IPS seems to be associated with the high dose of carmustine. The reduction in the dose of carmustine-based regimens may improve rates of lung toxicity and improve patient outcomes (**Wong *et al*, 2003**).

- In **Nitz *et al*, 2005**, breast cancer patients (N=201) received thiotepa in combination with cyclophosphamide and epirubicine. Absence of TRM and a single patient death suggest that this high-dose chemotherapy regimen is safe and feasible.

- In **Kroger *et al*, 2003** and **Zander *et al*, 2004**, breast cancer patients (N=401) received thiotepa in combination with cyclophosphamide and mitoxantrone. The HDC tolerability was as expected (**Zander *et al*, 2004**). MDS/AML after mitoxanrone-based HDC is a possible but rare complication in breast cancer patients (**Kroger *et al*, 2003**).

- In **Yalamanchili *et al*, 2008**, breast cancer patients (N=28) received thiotepa in combination with mitoxantrone and carboplatin. The HDC regimen was considered safe and effective.

- In **Rose *et al*, 2000**, breast cancer patients (N=52) received thiotepa alone as conditioning treatment. Because the cardiac toxicity was acceptable, breast cancer patients with impaired left ventricular function could be treated with HDC.

- In **Papadopoulos *et al*, 1998**, CNS tumours patients (N=15) received thiotepa in combination with etoposide and carmustine. The mortality after HDC and autologous stem cell transplantation was low (3%) and does not differ significantly from the mortality associated with conventional-dose chemotherapy.

- In **Papadopoulos *et al*, 1998**, CNS tumours patients (N=3) received thiotepa in combination with etoposide and carboplatin. TRM rate was 0%; however,this does not differ significantly from the mortality associated with conventional-dose chemotherapy.

- In **Cairncross *et al*, 2000** and **Abrey *et al*, 2006**, CNS tumours patients (N=59) received thiotepa alone. Previous brain irradiation may have contributed to severe toxic reactions because in an ongoing companion study neither irreversible encephalopathy nor prolonged anorexia was observed in a series of newly diagnosed patients with anaplastic oligodendrogliomas treated with I-PVC chemotherapy and high-dose thiotepa instead of radiation (**Cairncross *et al*, 2000**). In **Abrey *et al*, 2006**, the protocol was designed to replace radiotherapy with myeloablative dose of thiotepa in an effort to eradicate microscopic disease and provide non neurototoxic antitumour therapy.

- In **Chen *et al*, 2004**, CNS tumours patients (N=21) received thiotepa in combination with carboplatin. High-dose chemotherapy with thiotepa and carboplatin with concomitant autologous stem cell transplant may be used safely to treat patients with malignant astrocytomas.

- In **Gill *et al*, 2008**, CNS tumours patients (N=10) received thiotepa in combination with carmustine for the treatment of embryonal CNS tumours. No toxic deaths and excellent tolerance were reported.

- In **Stiff *et al*, 2004,** ovarian cancer patients (N=26) received thiotepa in combination with cyclophosphamide and cisplatin. The non-hematopoietic toxicity of this CTC regimen was higher when compared with CARB/MITOX/CY (CMC) group, primarily as a result of higher rate of severe nephrotoxicity and GI toxicity (including significant stomatitis).

- In **Holmberg *et al*, 1998**, ovarian cancer patients (N=31) received thiotepa in combination with busulfan and melphalan. The regimen including TT/BU/MEL was characterized by a good tolerability considering mainly the status of disease at the time of ASCT. 3% of patients died of regimen related toxicity (RRT) and 6% of transplant related mortality (TRM). TRM occurred only in patients with advanced disease such as stage IV breast cancer, advance lymphomas or in heavily pre treated patients. TRM rate was 0% in patients with stage II-III breast cancer or less advanced lymphoma. In conclusion, the regimen TT/BU/Mel was well tolerated in a subset of patients with low and intermediate disease status.

- In **Tiersten *et al*, 2006**, ovarian cancer patients (N=17) received thiotepa alone. The non hematological toxicity was limited and the three-cycle regimen of multiagent HDC with PBPC support in patients with advanced ovarian cancer can be considered well tolerated.

- In **Rick** *et al*, **2001**, germ cell tumour patients (N=62) received thiotepa in combination with etoposide and carboplatin. Treatment with conventional-dose chemotherapy followed by high-dose chemotherapy was feasible in patients with relapsed or refractory germ cell tumours, but peripheral nervous toxicity in approximately one third of patients is a disadvantage of this salvage strategy.

Allogeneic HPCT in adult patients

A total of 1771 patients with haematological diseases received thiotepa i.v. in combinations with other chemotherapeutic drugs and TBI as conditioning treatment before allogeneic HPCT.
- In **Corradini** *et al*, **2004** and **Corradini** *et al*, **2007**, lymphoma patients (N=187) received thiotepa in combination with fludarabine and cyclophoshamide. The studies showed the good results in terms of safety achieved using a reduced intensity regimen (RIC) with thiotepa, fludarabine and cyclophosphamide to treat advanced lymphoma with allogeneic transplantation in patients with significant comorbidity. TRM was very low in both studies: 6% and 13%, respectively.
- In **Majolino** *et al*, **2007**, multiple myeloma patients (N=53) received thiotepa in combination with fludarabine and melphalan. Acute GvHD of grade III/IV occurred in 5% in contrast with high incidence of chronic GvHD (64%) probably due to a short course of CsA (diagnosed at a median of 191 days coinciding with CsA withdrawal). TRM of 13% compares favourably with the very high incidence of mortality of the standard myeloablative regimens.
These results obtained with RIC including thiotepa, fludarabine and melphalan show that this regimen could be safer than myeloalative conditioning regimens.
- In **Raiola** *et al*, **2000, Di Grazia** *et al*, **2001, Bacigalupo** *et al*, **2007a, Bacigalupo** *et al*, **2007,** and **Bacigalupo** *et al*, **2009**, patients with haematological diseases not otherwise specified (N=625) received thiotepa in combination with cyclophosphamide.
In all studies, a reduced intensity regimen was used with a dose of thiotepa = 10 mg/kg and it was often compared with a conventional regimen thiotepa > 10 mg/kg (**Bacigalupo** *et al*, **2009**) or with a intensified regimen (thiotepa + cyclophosphamide supplemented with melphalan or TBI) (**Bacigalupo** *et al*, **2007a, Di Grazia** *et al*, **2001**). The most common adverse events were: infections, leukaemias, acute and chronic GvHD. Hepatitis, multi-organ failure, second malignancy and hemorrage were less frequent. In **Bacigalupo** *et al*, **2009**, leukemia was the main common cause of death. After more than 5 of follow-up, the rate of leukemia was below 3% with any conditioning regimen. Rate of deaths due to GvHD with thiotepa regimens in the first year was 9% with the 10 mg/kg regimen, while it was 2.3% with 15 mg/kg. In all five studies, reduced intensity conditioning with TT+CY was associated with a low transplant-related mortality (mean 23.8%). In **Raiola** *et al*, **2000** and **Bacigalupo** *et al*, **2007b**, bone marrow graft recipients showed a low incidence of chronic GvHD (35% vs 75%) and of TRM (6% vs 29%) than peripheral blood ones. In **Bacigalupo** *et al*, **2009** and in **Raiola** *et al*, **2000**, TRM was lower with a reduced intensity preparative regimen than with conventional or intensified ones.
These 5 studies show that the thiotepa-based conditioning regimens are associated with low toxicity, when used in combination with cyclophosphamide or fludarabine alone, especially in patients with early disease; they produce encouraging long term survival, with a low incidence of GvHD; the addition of melphalan or low dose TBI reduces relapse-related deaths, but increases significantly transplant-related mortality. Disease phase and source remains a major predictor of outcome. Advanced age appears not to be associated with increased risk of non relapse mortality in patients that used thiotepa in conditioning regimens.
- In **Corradini** *et al*, **2005**, patients with haematological diseases not otherwise specified (N=150) received thiotepa in combination with cyclophosphamide and fludarabine. A reduced intensity TT+CY+FLU regimen (RIC) was used. It was thought that RIC could lower the incidence of acute GVHD because of a reduction of tissue and mucosal barrier damage but, at present, the incidence of acute GVHD after RIC remains a cause of concern. The presence of an adverse prognostic factor, such as a previously failed autograft, increases significantly the toxicity of RIC transplantations. This is the first study which shows that age greater than 55 years is not a risk factor per se when the TT+CY+FLU RIC regimen is used. Nevertheless, in the older age category, a previously failed autograft is a risk factor affecting NRM and OS. This implies that correct timing of allogeneic SCT can limit its toxicity. In addition, a major improvement in RIC programs should concern the reduction of severe acute GVHD.

- In **Alessandrino et al, 2001, Alessandrino et al, 2004, Picardi et al, 2004** and **Grullich et al, 2008**, patients with haematological diseases not otherwise specified (N=93) received thiotepa in combination with fludarabine.

In **Alessandrino et al, 2004**, mild nausea and vomiting (grade I) occurred in two patients. In three cases, a slight increase of amylases (grade I) was noticed. No evidence of renal or liver toxicity was found. No evidence of mild or severe mucositis was noted. Four patients died of nonrelapse causes and five of relapse. TRM was 19% at 1 year after transplant which compares favourably with that of myeloablative approaches.

In **Picardi et al, 2004**, four patients experienced a toxicity grade > II. Mucositis was of grade III in 2 patients and of grade II in 7. Grade I renal toxicity was observed in 4 patients (18%). No veno-occlusive disease of the liver was observed. One patient died on day +27 post-transplant of cardiac toxicity and one patient died of multiorgan failure (MOF) after transplant. One additional patient died of cerebral aneurysm and respiratory distress. Six patients died of infectious complications occurring within 6 months from transplant. The 6-year cumulative incidence of TRM was 38%.

In **Grullich et al, 2008**, 9 patients (18%) suffered 14 severe nonhaematological toxicity events (grade IV/V) with fatal outcome in two of them. There were seven cases of severe renal toxicities, one with fatal outcome; one case of severe cardiac toxicity with fatal outcome; five cases of severe pulmonary toxicity and one case of severe neurological toxicity. Severe toxicities were attributable to metotrexate in three patients who received metotrexate for GVHD prophylaxis. The estimated Non Relapse Mortality at 1 year is 29%. This is a very promising result considering that other investigators reported an NRM rate ranging between 50 and 80% for patients receiving a conventional high dose chemotherapy regimen for second transplantation after the failure of a previous auto- or allograft.

- In **Bethge et al, 2006**, patients with haematological diseases not otherwise specified (N=10) received thiotepa in combination with fludarabine, melphalan and OKT3. This regimen is of low toxicity with maximal CTC grade II/III mucositis and moderate degree of nausea as most pronounced side effects, enabling its use even in an older or heavily pretreated patient population. Neurotoxicity was observed in 4 patients treated with 200 mg/m^2 fludarabine. The dose was reduced to 150 mg/m^2 with no further cases of neuropathy thereafter.Treatment-related mortality in the first 100 days was 30%, with one death each due to idiopathic pneumonia syndrome, GVHD, and CMV disease. Reflecting the fast engraftment and low toxicity of the conditioning regimen containing thiotepa, median hospital stay after HCT was 24 (range, 19–101) days.

- In **Jakubowski et al, 2007**, patients with haematological diseases not otherwise specified (N=52) received thiotepa in combination with TBI and fludarabine. The study addressed the challenge of facilitating T-cell recovery and decreasing regimen-related toxicity with several modifications to a standard TBI-based transplantation regimen. A major benefit observed in the current study relates to the T-cell recovery, measured by CD4 counts and PHA responses in vitro, and its apparent impact on OIs. In fact, only 2% engrafted patient died of OIs compared to the incidence of 5% to 23 % of fatal OIs reported in literature. The removal of ATG from this regimen reduced the incidence of EBV-LPD: only two cases (4%) occurred in 49 patiens.

19 (35%) of the 52 patients died and very early bacterial infections accounted for at least half the infectious deaths. In literature, the incidence of bacteremia in patients receiving TBI-containing regimens is reported at 35%, especially in those including fludarabine.

- In **Bacigalupo et al, 1996** and **Bacigalupo et al, 2007c**, leukemia patients (N=78) received thiotepa in combination with cyclophosphamide. 75 of the patients underwent allogenic marrow transplantation from an HLA identical sibling. In these studies TRM associated with thiotepa and cyclophosphamide regimen was very low (13% and 29%, respectively).

Bacigalupo et al, 2007 highlight the comparable TRM values in patients treated with thiotepa- or busulfan-based regimens.

In **Bacigalupo et al, 1996**, TRM was higher with the higher CY dose confirming that CY is associated with significant toxicity in recipients of HPCT, particularly when transplanted in advanced stages of disease or in advanced ages (>50 years). This study reported acceptable short term side effects of grade III-IV (the most frequent being mucositis). Despite the fact that it was an allogeneic setting with large numbers of T cells and elderly patients, the liver toxicity was acceptable. Acute and chronic GvHD was observed in both studies. In **Bacigalupo et al, 1996**, 87% of patients developed no or mild acute and chronic GvHD. **Bacigalupo et al, 2007c** reported a comparable incidence of chronic GvHD in patients receiving TT and CY or BU and CY; the lower incidence of acute GvHD in patients treated with BU and CY is probably due to the dose ATG administered only to this group of patients.

- In **Rosales *et al*, 1999**, leukemia patients (N=30) received thiotepa in combination with cyclophosphamide and busulfan. In order to reduce the risk of relapse and maintain the therapeutic potential of stem cell allografting while avoiding GvHD, the conventional conditioning regimen for T-cell depleted grafts, consisting of BU and CY, has been intensified by the addition of thiotepa.

TRM was 40%. Overall rates of organ toxicity were low with predominant complications being grade I-II mucositis and gastrointestinal toxicities. The incidence of liver, pulmonary and CNS toxicity was substantially lower than generally reported. No acute skin toxicity or cardiotoxicity were observed. A rather high incidence of GvHD has been observed. This could be due to incomplete T-cell depletion, the administration of donor peripheral blood lymphocytes (DLI), or to the intensification of the pre-BMT conditioning regimen.

- In **Lacerda *et al*, 2003**, leukemia patients (N=14) received thiotepa in combination with fludarabine, melphalan and ATG followed by standard doses of immunoselected CD34+ peripheral blood cells from haploidentical donors. Most conditioning regimens in haplotype-disparate transplantations have included total body irradiation, which is highly immunosuppressive, because of the significant risk of graft failure after the infusion of T cell-depleted grafts. This conditioning regimen was composed of chemotherapy alone. The results of this study demonstrate a minimal toxicity of TT/FLU/ATG/MEL regimen. The major adverse events were viral and bacterial infections (71% and 50%). 3 patients developed a GvHD grade III-IV (5 patients developed acute GvHD grade I-II) and 2 patients developed chronic GvHD. No instance of hepatic veno-occlusive disease was observed.

- In **Rigden *et al*, 1996** and **Papadopoulos *et al*, 1998,** leukemia patients (N=81) received thiotepa in combination with TBI and cyclophosphamide. 58 patients underwent allogenic marrow transplantation from matched sibling donors and 23 from unrelated donors. The curative potential of an allogeneic BMT is often been mitigated by the risks of transplant-associated morbidity and mortality, particularly due to GvHD and its complications. An important result of these 2 studies was the low incidence of GvHD observed. Significant acute GvHD grade III-IV occurs generally in 10-30% of sibling-matched recipients and 36-61% of MUD recipients; chronic GvHD usually develops in 30-60% of sibling and 60-70% of MUD recipients (**Rigden *et al*, 1996**). In these 2 studies only 6 of 81 patients developed acute GvHD, all in MUD recipients (**Rigden *et al*, 1996**). A total of 8 patients developed chronic GvHD. The studies further suggested that the conditioning regimen (TBI/TT/CY) is well tolerated and associated with little therapy-related toxicity. Fungal and viral infections, and lymphoproliferative disorders (a consequence of the in vivo T-cell depletion effect exerted by the ATG) were observed with a low incidence. Clinical veno-occlusive disease was not seen in these patients, witch contrasts favorably with the 24% incidence reported by the National Marrow Donor Program (**Rigden *et al*, 1996**).

- In **Aversa *et al*, 1994, Aversa *et al*, 1999** and **Aversa *et al*, 2001**, leukemia patients (N=107) received thiotepa in combination with TBI, cyclophosphamide and ATG. The purpose of these studies was to test whether the conditioning regimen based on thiotepa, antithymocyte globulin, cyclophosphamide and TBI facilitates engraftment of an extensively T-cell–depleted transplant in patients with advanced refractory leukemia. The regimen was associated with low extrehaematologic toxicities. In **Aversa *et al*, 1994**, a TRM of 53% was reported. In **Aversa *et al*, 1999**, there were 13 nonleukemic deaths in 16.6% of patients who underwent BMT while in remission and in 38.8% of patients who underwent BMT while in relapse. These figures compare favourably with the 21% transplant-related mortality rate reported in literature. The cumulative incidence of fatal infections was 8.3% in the 36 patients who underwent BMT while in first or second remission and 23.5% in the 17 patients who underwent BMT while in relapse. The low incidence of infectious deaths was probably because no posttransplant immunosuppressive therapy was given, no acute or chronic GVHD occurred in any patient, and, consequently, their posttransplant immunologic recovery was not seriously delayed. Indeed, no patient died of infection after posttransplant day 150. In conclusion, prevention of GVHD without increased transplant-related mortality should enable patients who rarely were considered suitable candidates for unmanipulated transplants because of advanced age or advanced-stage disease or both to have the option of this strategy.

- In **Aversa *et al*, 1998, Aversa *et al*, 2001, Aversa *et al*, 2002** and **Aversa *et al*, 2005**, leukemia patients (N=276) received thiotepa in combination with TBI and fludarabine and ATG.

These studies were designed to test a conditioning regimen that would facilitate engraftment of an extensively T-cell–depleted mismatched transplant without excessive non-hematologic toxicity, which has been a considerable problem in previous clinical trials. Substituting fludarabine for cyclophosphamide, **Aversa *et al*** did not reduce the degree of immunosuppression (even at the lower

dosage of fludarabine): this is indicated by the lack of clonable T cells in peripheral blood at the end of the conditioning regimen and by the excellent engraftment. Furthermore, non hematologic toxicity was minimal: there was no veno-occlusive disease, and the incidence of severe mucositis was low, even in advanced stage, heavily pretreated patients. Despite the rapid neutrophil recovery, recipients of mismatched transplant tended to remain susceptible to opportunistic infections and in fact, many of the non-leukemic deaths were caused by infections. The high incidence of infectious complications was probably due to the delay in the recovery of T cells.

However, the fact that the majority of patients had been heavily pre-treated and their long history of disease leading to bacterial and fungal colonization before transplant, must be kept in mind. Indeed, irrespective of the conditioning regimen used, a history of infections and colonization at transplant were the most significant factors for infection-related deaths.

A singular finding was the low incidence of GVHD in these patients. The ATG that was administered during conditioning might have helped prevent GVHD by a cytotoxic effect against donor T lymphocytes. The average overall TRM of 38% needs to be viewed in light of the clinical characteristics of this cohort of patients. Most were at high risk of TRM because of advanced disease status at transplantation. Although TRM was mainly infection-related, no fatal infection occurred after the first year post-transplantation, indicating that there is no risk of late infection-related death in the absence of chronic GvHD and immunosuppressive therapy.

- In **La Nasa *et al*, 2005**, thalassemia patients (N=15) received thiotepa in combination with cyclophosphamide and busulfan.

This study suggested that the outcome of unrelated donor BMT in adult, class 3 thalassemia patients is similar to that reported in the literature for patients belonging to the same class of risk transplanted from an HLA-identical sibling donor.

A low incidence of grade I-II GvHD (22% acute GvHD in 22% and 15% chronic GvHD) and and of grade III-IV GvHD (15% acute GvHD and 7,4% chronic GvHD) was observed. No other types of toxicity were reported.

This relatively low incidence of GVHD can probably be attributed to the careful immunogenetic selection of the donor/recipient pairs. Overall, GvHD, particularly in the severe form, was reduced in patients who shared at least one extended HLA haplotype with the donor. The mortality due to transplant is 30% and the major causes of death are organ impairment, CMV infection and occurrence of GvHD. Although the difference between patients treated with BU+TT+CY and with BU+CY was not statistically significant, a higher number of deaths was observed in patients conditioned with the protocol including TT (6/15=40% vs 2/11=17%). A conditioning regimen with three drugs (BU-TT-CY) appeared to be too toxic for patients with organ impairment due to iron overload or hepatitis infection. Although the only patient who experienced graft failure was prepared with the combination of BU-CY, this regimen seems to be better indicated in adult patients with heavy iron overload.

Autologous HPCT in paediatric patients with solid tumours

Paediatric patients (infants and toddlers, children and adolescents) with solid tumours (N = 476) comprised patients with CNS tumours (N=378) and solid tumours not otherwise specified (N=98).

- In **Dunkel *et al*, 1998, Mason *et al*, 1998, Broniscer *et al*, 2004, Dhall *et al*, 2008** and **Grodman *et al*, 2009** CNS tumour patients (N=106) received thiotepa in combination with etoposide and carboplatin.

In **Mason *et al*, 1998**, toxicity from induction chemotherapy was generally well tolerated, predictable and primarily haematologic. For children who completed induction therapy (cisplatin, vincristine, etoposide and cyclosphosphamide), the incidence of cisplatin-related high-frequency hearing loss was high but similar to results of other trials that incorporated this drug for brain tumours in children. 37/42 children were stable or improved at the completion of induction chemotherapy and proceeded to consolidation with TT/CARB/VP16 before ABMR, upon which all patients became severely neutropenic and thrombocytopenic. No patient died due to failure of engraftment. 3 patients (8%) died of acute complications of consolidation chemotherapy with ABMR. Apart from the three patients no children suffered significant organ toxicities. Infections were common and the mortality rate was similar to that reported in other studies of ABMR in children with CNS neoplasm.

Broniscer *et al*, 2004 reported significant acute toxicity, similar to that described with regimens of myeloablative chemotherapy. All patients experienced pancytopenia and oral mucositis. Infections

were documented in nine patients (41%). Non-hematologic grade 3 and 4 toxicities were seen in six patients (35%). Treatment-related death rate was 11% (2/17 patients).

In **Dhall et al, 2008**, the use of TT/CARB/VP16 before HPCT in young children with non-metastatic medulloblastoma obviated the need of craniospinal irradiation in 52% of patients. Regimen related mortality was 14%. None of the 21 patients had any evidence of leukoencephalopathy on brain MRIs during or after treatment. The EFS was achieved with preservation of QoL and intellectual functioning albeit with a higher toxic mortality rate as compared to the regimens using upfront irradiation or intra-ventricular methotrexate.

In **Dunkel et al, 1998**, regimen related deaths occurred in three patients (13%) within 21 days of ASCR. This was because of multiorgan system failure in two patients, and Aspergillus infection/veno-occlusive disease in the third patient.

Grodman et al, 2009, treated high risk patients with CNS germinoma and recurrent medulloblastoma. All patients had anemia, thrombocytopenia, neutropenia and oral mucositis. 1 patient died of sepsis. Neurotoxicity in two of these heavily pretreated surviving patients is the most concerning long-term issue for this group.

In conclusion, these studies show that TT/CARB/VP16 regimen is characterized by a satisfactory safety profile considering that all patients enrolled were high risk patients with advanced status of various CNS tumours.

- In **Finlay et al, 1990, Grovas et al, 1999** and **Papadakis et al, 2000**, CNS tumours patients (N=57) received thiotepa in combination with etoposide and carmustine.

In **Finlay et al, 1990**, thiotepa, etoposide and carmustine were administered to 5 patients with high grade astrocytomas. All patients developed febrile neutopenia, oro-pharyngeal mucositis and erythema/desquamation/hyperpigmentation secondary to high dose chemotheraphy. One patient died of salmonella sepsis within 3 days of receiving a platelet transfusion contaminated with salmonella from an asymtomatic donor.

Grovas et al, 1999 reported that the most commonly occurring toxicity was pulmonary and the next common severe toxicity was neurotoxicity. Carmustine given at high dose has been associated with pulmonary toxicity and leukoencephalophathy. TRM was 18% (2/11 patients).

In **Papadakis et al, 2000**, nine deaths were observed (21%) within the first 60 days post transplantation. All patients suffered predictable grade IV pancytopenia. Pulmonary toxicity was significant and possibly related to the nitrosurea treatment (BCNU). Fifteen patients experienced 22 episodes of neurotoxicity; 17 events were reversible. TRM was 15%.

- In **Finlay et al, 1996, Bouffet et al, 1997** and **Fagioli et al, 2004**, CNS tumour patients (N=94) received thiotepa in combination with etoposide.

In **Finlay et al, 1996**, none of the five long term survivors display any sequelae attributable to the therapy. The use of this thiotepa based regimen at an earlier stage in the course of the disease may reduce the susceptibility to veno-occlusive disease of the liver and the multiorgan system failure syndrome.

In **Bouffet et al, 1997**, two patients died of treatment related toxicities. 14 patients developed thiotepa-related cutaneous toxicities with erytromatous rash and subsequent desquamation. Early neurotoxicity was observed in 11 patients. Increased peritumoral oedema was observed in 4 patients, and intracranial hemorrhage in one. All these symptoms were transient and resolved within two weeks. Since the onset of interstitial pneumonitis in one patient occurred early after high-dose chemotherapy, this complication was more likely due to a previous treatment with carmustine.

In **Fagioli et al, 2004**, pancytopenia was noted in all patients. Mucositis occurred in all patients except one. TRM was observed in 1 patient (3.6%), who died of Candida pneumonia. All 11 patients attended school after HDC.

- In **Massimino et al, 2005** and **Massimino et al, 2006** patients with CNS tumours (N= 31) received thiotepa alone. In **Massimino et al, 2005**, all 21 children who were administered high-dose thiotepa experienced grade 3–4 neutropenia followed by fever. Grade 2 stomatitis was present in 77% of patients; in 33% of cases, 24 to 48 h after the end of thiotepa infusion, a mild mood depression was evident and low oral doses of amitriptyline were administered with success. No TRM was observed.

Massimino et al, 2006, enrolled 10 patients with sPNET tumours. All patients experienced grade 3–4 hematologic toxicity and grade 3 mucositis after high-dose thiotepa. Grade 4 infections occurred in 3 patients. No patients died due to the treatment.

- In **Grill et al, 1996, Dupuis-Girod et al, 1997, Valteau-Couanet et al, 2005** and **Ridola et al, 2007**, CNS tumour patients (N= 90) received thiotepa in combination with busulfan. In all four studies profound myelosuppression was reported.

In **Grill et al, 1996**, GI toxicity including mucositis was severe and frequent. Other organ toxicities were mild and reversible. Only one patient died of treatment-related toxicity on day 50 following ABMT. Toxicity, mainly digestive and cutaneous, was severe but manageable. Toxicity was similarly high but manageable in the study by **Dupuis-Girod et al, 1997**.

Valteau-Couanet et al, 2005 reported that digestive toxicity and especially severe mucositis were the most common extra-hematological complications. Four patients died as a result of toxicity. The incidence of toxic deaths was significantly higher in previously irradiated patients (P=0.01). More specifically, previous craniospinal irradiation appeared to be a major risk factor for increased incidence and severity of toxicity.

In **Ridola et al, 2007**, acute toxicity was manageable but characterized mainly by a significant percentage of hepatic VOD in 33% of patients. None of the children died of liver toxicity. This reported liver toxicity is caused mainly by busulfan but it could be modified by refinement of the use of busulfan or by the prophylactic use of defibrotide. Regimen related mortality was 5% (2/39 patients).

Delayed toxicities from this treatment strategy (TT/BU) involved mainly the gonads and the central nervous system. Major toxicity includes the risk of irreversible ovarian failure resulting from the use of high-dose busulfan. Neurotoxicity was observed as a delayed side effect characterized by transient radiologic anomalies in the irradiated fields in 23% of patients. In conclusion, the immediate toxicity of TT/BU is manageable and may be improved with more specific treatments of VOD; however, delayed neurologic and gynecologic side effects remain main concerns and warrant treatment refinements.

- In **Lucidarme et al, 1998** and **Lafay-Cousin et al, 2000**, patients with solid tumours not otherwise specified (N=40) received thiotepa alone. **Lucidarme et al, 1998** studied 22 paediatric patients with a median follow up of 61 months. All patients experienced an expected profound myelosuppression. All organ toxicities (GI, liver, skin, CNS) were of grade I-II. No toxicity-related deaths occurred. In **Lafay-Cousin et al, 2000**, the median follow up was 63 months. Myelotoxicity was the most common toxicity observed. 2/18 patients experienced acute renal toxicity (tubular pathology and a decrease in creatinine clearance). These complications were transient and recovery was complete and rapid. No adverse events were life-threatening and no toxicity related deaths occurred in the study.

- In **Chan et al, 1997**, patients with solid tumours not otherwise specified (N= 21) received thiotepa (TT) in escalating doses in combination with cyclophosphamide and melphalan. 750 mg/m^2 thiotepa and 180 mg/m^2 melphalan was tolerated by most of the children with advanced solid tumours. The most significant toxicities were oral mucositis, skin toxicity, diarrhea and other gastrointestinal side effects. The treatment related mortality in this series was 14.3% (three of 21 patients).

- In **Kushner et al, 2001** patients with solid tumours not otherwise specified (N=21) received thiotepa in combination with carboplatin and topotecan. Toxicity was severe but manageable. No patients died of TRM and/or relapse regimen mortality.

- In **Hawkins et al, 2000** solid tumours patients (N=16) received thiotepa in combination with busulfan and melphalan. Patients with an adequate PBSC collection to support a second myeloablative course and who had not received prior dose-limiting radiotherapy were eligible to receive TMI following recovery from BU/MEL/TT. The median TMI dose was 12 Gy, with a range of 10.5–15 Gy. PBSC were infused 24 hr after the final dose of TMI.

Following BU/MEL/TT one patient receiving allogeneic bone marrow as the HSC source died of regimen-related toxicity. Grade 2 or 3 gastrointestinal toxicity was observed only in patients who received pelvic irradiation prior to BU/MEL/TT.

Allogeneic HPCT in paediatric patients with haematological malignancies

The paediatric patients (infants and toddlers, children and adolescents) with haematological diseases (N=426) comprised patients with leukemia (N=228), thalassemia (N=139), refractory cytopenia (N=19), genetic diseases (N=26), and sickle cell anaemia (N=14).

- In **Zecca et al, 1999** and **Locatelli et al, 2009** leukemia patients (N=97) received thiotepa in combination with TBI and cyclophosphamide.

Zecca et al, 1999 is the first prospective study to evaluate the tolerability and efficacy of a new conditioning regimen based on the combination of TBI, TT, and CY in a homogeneous group of children with ALL. The addition of thiotepa to the TBI-CY regimen was well tolerated, as suggested by the overall TRM of 15%. The RRM was 2.5% (1/40 patients). The only organ toxicity of grade III-IV affected the lung and it was reported in one patient.

Locatelli et al, 2009 confirmed the previous results. In 57 paediatric patients with ALL and a mean follow up of 121 months, the incidence of liver and pulmonary toxicity of grade III-IV was only 1 and 3%, respectively. TRM was 15.7%.

These safety results suggest that is possible to add thiotepa at TBI-CY to increase the engraftment and the GvL effect without increasing the toxicity of the regimen.

- In **Locatelli et al, 2009**, leukemia patients (N=40) received thiotepa in combination with cyclophosphamide, antilymphocyte globulin and TBI. The most common adverse event was mucositis of grade I-II. Toxicities of grade III-IV involved heart, bladder, kidney, liver and CNS and their incidence was 2.5%. The pulmonary toxicity was 5%. TRM was 20%.

- In **Locatelli et al, 2009** leukemia patients (N= 21) received thiotepa in combination with fludarabine, anti-thymocyte globulin and TBI. No organ toxicity of grade III-IV occurred. The TRM was 5% (1/21).

- In **Locatelli et al, 2009**, leukemia patients (N= 18) received thiotepa in combination with melphalan and TBI. Mucositis of grade I-II occurred in all patients. The organ toxicity concerned only the heart and bladder level (5.5% in both organs). Only one patient died of TRM. This safety profile is encouraging considering that thiotepa and TBI were combined with melphalan.

-In **Locatelli et al, 2009**, leukemia patients (N= 19) received thiotepa in combination with fludarabine and TBI. With a follow up of 42 months, adverse events of grade III-IV concerned the liver (5%; 1/19), CNS (10,5%; 2/19) and GI tract (5%; 1/19). TRM was 16%.

- In **Locatelli et al, 2009**, leukemia patients (N=15) received thiotepa in combination with melphalan, antilymphocyte globulin and TBI. Mucosal toxicity of grade I-II was reported in all patients. There was only 1 patient with adverse event of grade III-IV at bladder level. No toxicity occurred in the other organs. Only 1 patient died for TRM.

- In **Locatelli et al, 2009** leukemia patients (N=10) received thiotepa in combination with fludarabine and treosulfan. Mucositis was the common adverse event. Bladder toxicity and gastrointestinal toxicity were reported in 1 and 2 patients, respectively. No organ class toxicity of grade III-IV occurred in these 10 patients with relapsed ALL who underwent allogeneic HPCT with TT/TREO/FLU. TRM was 30%. Considering the very high risk patients in terms of disease but also in term of possible comorbidities this regimen including TT/TREO/FLU can be considered safe.

- In **Locatelli et al, 2009** leukemia patients (N=8) patients received thiotepa in combination with cyclophosphamide, anti-thymocyte globulin and TBI. All 8 patients experienced mucositis of grade I-II. Only 1 patient reported hepatic toxicity of grade I-II. No other organs toxicity of was observed.

- In **La Nasa et al, 2002** thalassemia patients (N=28) received thiotepa in combination with busulfan and cyclophosphamide. This was the first large series of consecutive thalassemia patients receiving transplants from unrelated donors reported. 2 of the 4 cases of rejection occurred in the first 4 patients conditioned with the standard BU-CY regimen. Thiotepa at a dose of 10 mg/kg was shown to intensify both the myeloablative and immunosuppressive effect of the conditioning regimen, without a significant increase of extramedullary toxicity. In this cohort, mortality rate was 19%. There were 4 deaths that occurred among 11 adults (age > 16 years) and 2 among 21 pediatric patients. Only one death was observed among 15 class I and class II patients (7%), the other 5 occurring among 17 class III patients (29%). The protocol BU-TT-CY was well tolerated. The results show that thiotepa can be added to other alkylating drugs like busulfan without increase the general non haematological toxicity but also the characteristic toxicity of every single drugs.

- In **Bernardo et al, 2008** and **Locatelli et al, 2009**, thalassemia patients (N=30) received thiotepa in combination with treosulfan and fludarabine. **Bernardo et al, 2008**, reported that the combination thiotepa/treosulfan/fludarabine is a good myeloablative regimen but with a very mild extramedullary toxicity for allogeneic HPCT. The regimen was well tolerated, with limited organ toxicity. In detail, no VOD, no lung, no heart and CNS toxicity was recorded. The only toxic effect frequently observed was mucositis, occurring in six (30%) of the 20 enrolled patients. Only one patient died of transplantation-related complications (acute GvHD). The cumulative incidence of TRM was 5%. Similar safety results were seen by **Locatelli et al, 2009**. Myeloabaltion with thiotepa, treosulfan and fludara-bine might be a suitable elective application in patients or in those with poor performance

status and/or organ dysfunction, who have a high risk of life-threatening complications if busulfan is used.

- In **Locatelli *et al*, 2009** thalassemia patients (N=65) received thiotepa in combination with busulfan and fludarabine. The follow up was 133 months. TRM was only 3%. Only 1 patient experienced liver toxicity of grade III-IV while in the other organs (heart, kidney, lung, bladder, CNS and GI) no toxicity occurred. The most common toxicity of grade I-II was mucositis (32%) following by liver toxicity (7,7%). These results confirm the very good safety profile of the conditioning regimen including thiotepa considering the long term follow up and the particular type of thalassemic patients.

- In **Locatelli *et al*, 2009,** thalassemia patients (N=16) received thiotepa in combination with busulfan, fludarabine and ALG. Mucositis of grade I-II was the most common side effect. Only bladder toxicity was reported in 12.5% of patients. No toxicity grade III-IV occurred at organ levels.

- In **Strahm *et al*, 2007**, refractory cytopenia patients (N=19) received thiotepa in combination with fludarabine and ATG. HPCT is the only curative treatment for refractoy cytopenia (RC). Myeloablative preparative regimen is associated with a low probability of relapse but considerable transplant-related mortality. The reduced intensity chemotherapy regimen of thiotepa nad fludarabine may be able to not only reduce the risk of transplant-related life-threatening complications but also the incidence of late effects. Infections were the most frequently observed complication. TRM was 16%. No deaths from regimen related toxicities were reported. In conclusion, this RIC regimen with thiotepa and fludarabine permits to obtain the same results using a myeloablative regimen but with a good safety profile in comparison with a myeloablative regimen.

- In **Rosales *et al*, 1999**, patients with genetic diseases (N=26) received thiotepa in combination with busulfan and cyclophosphamide. In this study, mucositis and gastrointestinal tract (GIT) toxicity were the predominant complications. The only organ toxicities reported were at hepatic and pulmonary level: 7.6% (2/26) and 3.8% (1/26), respectively. The relatively low incidence of both VOD and pulmonary toxicity in the present series of patients may indicate that thiotepa does not play a major role in these complications. TRM was 15.5% and no deaths from RRM were reported. In conclusion, TTP-BU-CY achieved good results in terms of safety in T cell depleted BMT in genetic diseases.

- In **Locatelli *et al*, 2009**, sickle cell anaemia patients (N=14) received thiotepa in combination with busulfan and fludarabine. With a follow up of 51 months all patients were alive and free of disease. Except moderate mucositis (14.3%) no other adverse event of grade I-II and III-IV has been reported in this group of patients.

Overall, the most frequently adverse events reported in the different conditioning treatments including thiotepa are: infections, cytopenia, acute GvHD and chronic GvHD, gastrointestinal disorders, haemorragic cystitis and mucosal inflammation.

The adverse reactions considered at least possibly related to conditioning treatment including thiotepa, reported in adult patients as more than an isolated case, are listed below by system organ class and by frequency. Within each frequency grouping, undesiderable effects are presented in order of decreasing seriousness. Frequencies are defined as: very common ($\geq$1/10), common ($\geq$1/100 to < 1/10), uncommon ($\geq$1/1,000 to <1/100), rare ($\geq$1/10,000 to <1/1,000) very rare (<1/10,000), not known (cannot be estimated from the available data).

Table 9: Adverse drug reactions in adults

System organ class	Very common	Common	Uncommon
Infections and infestations	Infection susceptibility increased Sepsis		Toxic shock syndrome
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		Treatment related second malignancy	
Blood and lymphatic system disorders	Leukopenia Thrombocytopenia Febrile neutropenia Anaemia Pancytopenia Granulocytopenia		

Immune system disorders	Acute graft versus host disease Chronic graft versus host disease	Hypersensitivity	
Endocrine disorders		Hypopituitarism	
Metabolism and nutrition disorders	Anorexia Decreased appetite Hyperglycaemia		
Psychiatric disorders	Confusional state Mental status changes	Anxiety	Delirium Nervousness Hallucination Agitation
Nervous system disorders	Dizziness Headache Vision blurred Encephalopathy Convulsion Paraesthesia	Intracranial aneurysm Extrapyramidal disorder Cognitive disorder Cerebral haemorrhage	
Eye disorders	Conjunctivitis	Cataract	
Ear and labyrinth disorders	Hearing impaired Ototoxicity Tinnitus		
Cardiac disorders	Arrhythmia	Tachycardia Cardiac failure	Cardiomyopathy Myocarditis
Vascular disorders	Lymphoedema Hypertension	Haemorrhage Embolism	
Respiratory, thoracic and mediastinal disorders	Idiopathic pneumonia syndrome Epistaxis	Pulmonary oedema Cough Pneumonitis	Hypoxia
Gastrointestinal disorders	Nausea Stomatitis Oesophagitis Vomiting Diarrhoea Dyspepsia Abdominal pain Enteritis Colitis	Constipation Gastrointestinal perforation Ileus	Gastrointestinal ulcer
Hepatobiliary disorders	Venoocclusive liver disease Hepatomegaly Jaundice		
Skin and subcutaneous tissue disorders	Rash Pruritus Alopecia	Erythema	Pigmentation disorder Erythrodermic psoriasis
Musculoskeletal and connective tissue disorders	Back pain Myalgia Arthralgia		
Renal and urinary disorders	Cystitis haemorrhagic	Dysuria Oliguria Renal failure Cystitis Haematuria	
Reproductive system and breast disorders	Azoospermia Amenorrhoea	Menopausal symptoms	Infertility female Infertility male

	Vaginal haemorrhage		
General disorders and administration site conditions	Pyrexia Asthenia Chills Generalised oedema Injection site inflammation Injection site pain Mucosal inflammation	Multi-organ failure Pain	
Investigation	Weight increased Blood bilirubin increased Transaminases increased Blood amylase increased	Blood creatinine increased Blood urea increased Gamma-glutamyltransferase increased Blood alkaline phosphatase increased Aspartate aminotransferase increased	

The adverse reactions considered at least possibly related to conditioning treatment including thiotepa, reported in paediatric patients as more than an isolated case, are listed below by system organ class and by frequency. Within each frequency grouping, undesiderable effects are presented in order of decreasing seriousness. Frequencies are defined as: very common ($\geq$1/10), common ($\geq$1/100 to < 1/10), uncommon ($\geq$1/1,000 to <1/100), rare ($\geq$1/10,000 to <1/1,000) very rare (<1/10,000), not known (cannot be estimated from the available data).

Table 10: Adverse drug reactions in paediatric patients

System organ class	Very common	Common	Uncommon
Infections and infestations	Infection susceptibility increased Sepsis	Thrombocytopenic purpura	
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		Treatment related second malignancy	
Blood and lymphatic system disorders	Thrombocytopenia Febrile neutropenia Anaemia Pancytopenia Granulocytopenia		
Immune system disorders	Acute graft versus host disease Chronic graft versus host disease		
Endocrine disorders	Hypopituitarism Hypogonadism Hypothyroidism		
Metabolism and nutrition disorders	Anorexia Hyperglycaemia		
Psychiatric disorders	Mental status changes	Mental disorder due to a general medical condition	
Nervous system disorders	Headache	Ataxia	

	Encephalopathy Convulsion Cerebral haemorrhage Memory impairment Paresis		
Ear and labyrinth disorders	Hearing impaired		
Cardiac disorders	Cardiac arrest	Cardiovascular insufficiency Cardiac failure	
Vascular disorders	Haemorrhage	Hypertension	
Respiratory, thoracic and mediastinal disorders	Pneumonitis	Idiopathic pneumonia syndrome Pulmunary haemorrage Pulmonary oedema Epistaxis Hypoxia Respiratory arrest	
Gastrointestinal disorders	Nausea Stomatitis Vomiting Diarrhoea Abdominal pain	Enteritis Intestinal obstruction	
Hepatobiliary disorders	Venoocclusive liver disease	Liver failure	
Skin and subcutaneous tissue disorders	Rash Erythema Desquamation Pigmentation disorder		
Musculoskeletal and connective tissue disorders	Growth retardation		
Renal and urinary disorders	Bladder disorders	Renal failure Cystitis haemorrhagic	
General disorders and administration site conditions	Pyrexia Mucosal inflammation Pain Multi-organ failure		
Investigation	Blood bilirubin increased Transaminases increased Blood creatinine increased Aspartate aminotransferase increased Alanine aminotransferase increased	Blood urea increased Blood electrolytes abnormal Prothrombin time ratio increased	

- Laboratory findings

Clinical laboratory evaluations were not reported in detail in the published studies and therefore any important findings regarding laboratory results were described in the analyses of adverse events.

- Safety in special populations

Studies in renally impaired patients have not been conducted. As thiotepa and its metabolites are poorly excreted in the urine, dose modification is not recommended in patients with mild or moderate renal insufficiency. However, caution is recommended.

Thiotepa has not been studied in patients with hepatic impairment. Since thiotepa is mainly metabolized through the liver, caution needs to be observed when thiotepa is used in patients with pre-existing impairment of liver function, especially in those with severe hepatic impairment. When treating such patients it is recommended that serum transaminase, alkaline phosphatase and bilirubin are monitored regularly following transplant, for early detection of hepatotoxicity.

The administration of thiotepa has not been specifically investigated in elderly patients. However, in clinical studies, a proportion of patients over the age of 65 received the same cumulative dose as the other patients. No dose adjustment was deemed necessary.

- Safety related to drug-drug interactions and other interactions

Specific interactions with thiotepa
Live virus and bacterial vaccines must not be administered to a patient receiving an immunosuppressive chemotherapeutic agent and at least three months must elapse between discontinuation of therapy and vaccination.

Thiotepa appears to be metabolised via CYP2B6 and CYP3A4. Co-administration with inhibitors of CYP2B6 (for example clopidogrel and ticlopidine) or CYP3A4 (for example azole antifungals, macrolides like erythromycin, clarithromycin, telithromycin, and protease inhibitors) may increase the plasma concentrations of thiotepa and potentially decrease the concentrations of the active metabolite TEPA. Co-administration of inducers of Cytochrome P450 (such as rifampicin, carbamazepine, phenobarbital) may increase the metabolism of thiotepa leading to increased plasma concentrations of the active metabolite. Therefore, during the concomitant use of thiotepa and these medicinal products, patients should be carefully monitored clinically.

Thiotepa is a weak inhibitor for CYP2B6, and may thereby potentially increase plasma concentrations of substances metabolised via CYP2B6, such as ifosfamide, tamoxifen, bupropion, efavirenz and cyclophosphamide. CYP2B6 catalyzes the metabolic conversion of cyclophosphamide to its active form 4-hydroxycyclophosphamide (4-OHCP) and co-administration of thiotepa may therefore lead to decreased concentrations of the active 4-OHCP. Therefore, a clinical monitoring should be exercised during the concomitant use of thiotepa and these medicinal products.

Contraindications of concomitant use:
Yellow fever vaccine: risk of fatal generalized vaccine-induced disease.
More generally, live virus and bacterial vaccines must not be administered to a patient receiving an immunosuppressive chemotherapeutic agent and at least three months must elapse between discontinuation of therapy and vaccination.

Concomitant use not recommended:
Live attenuated vaccines (except yellow fever): risk of systemic, possibly fatal disease. This risk is increased in subjects who are already immunosuppressed by their underlying disease.
An inactivated virus vaccine should be used instead, whenever possible (poliomyelitis).
Phenytoin: risk of exacerbation of convulsions resulting from the decrease of phenytoin digestive absorption by cytotoxic medicinal product or risk of toxicity enhancement and loss of efficacy of the cytotoxic medicinal product due to increased hepatic metabolism by phenytoin.

Concomitant use to take into consideration:
Ciclosporine, tacrolimus: excessive immunosuppression with risk of lymphoproliferation.
Alkylating chemotherapeutic agents, including thiotepa, inhibit plasma pseudocholinesterase by 35% to 70%. The action of succinyl-choline can be prolonged by 5 to 15 minutes.
Thiotepa must not be concurrently administered with cyclophosphamide when both medicinal products are present in the same conditioning treatment. Tepadina must be delivered after the completion of any cyclophosphamide infusion.

The concomitant use of thiotepa and other myelosuppressive or myelotoxic agents (i.e. cyclophosphamide, melphalan, busulfan, fludarabine, treosulfan) may potentiate the risk of haematologic adverse reactions due to overlapping toxicity profiles of these medicinal products.

Interaction common to all cytotoxics
Due to the increase of thrombotic risk in case of malignancy, the use of anticoagulative treatment is frequent. The high intra-individual variability of the coagulation state during malignancy, and the potential interaction between oral anticoagulants and anticancer chemotherapy require, if it is decided to treat the patient with oral anticoagulants, to increase the frequency of the INR (International Normalised Ratio) monitoring.

- Discontinuation due to adverse events
No studies were submitted.

- Post marketing experience
No studies were submitted

- Discussion on clinical safety

Autologous HPCT in adult patients

In autologous HPCT the safety database consisted of a total of 4817 patients with haematological diseases (N=826) and with solid tumours (N=3991). These patients received thiotepa i.v., in combination with other chemotherapeutic drugs as conditioning treatment before autologous HPCT.

Haematological diseases
The safety profile of thiotepa has been evaluated in 826 patients. The analyzed population is a very high risk group considering the disease phase, comorbidity, previous treatments and prior limited dose of radiation.
Regimen Related Mortality (RRM) ranging from 2.5% to 29% was reported in the analysed studies. Transplant Related Mortality (TRM) ranged from 0% to 21% at 1 year. OS ranged from 29% to 87% with a follow up of 22 to 63 months.
The most important fatal events registered in the whole population evaluated were infections (1.5% to 14.7%), VOD ($\leq$5.7%), idiopathic pneumonia syndrome ($\leq$6.8%), cardiac toxicity ($\leq$2.4%), MOF (1.5% to 4%), respiratory toxicity (1.25% to 1.5%) and hepatobiliary toxicity ($\leq$1.5%). Deaths from second malignancies ranged from 1.5% to 5%.

Solid tumours
The safety profile of thiotepa has been evaluated on 3991 patients. Regimen Related Mortality (RRM) ranged from 0% to 2% and TRM ranged from of 0% to 7.4%. OS ranged from 30% to 87% with a follow up of 11.7 to 87 months.
The most common fatal events observed in the population treated with conditioning regimens including thiotepa were as follows: pulmonary toxicity (0.2% to 3%), cardiac toxicity (0.4% to 0.6%), VOD (1.4% to 3%), MOF ($\leq$0,4%), CNS toxicity($\leq$2%) and hepatic failure ($\leq$0.5%).
Only one study reported fatal renal failure in 2/26 patients (8%). Deaths from second malignancies ranged from 0.5% to 0.94%.

Allogeneic HPCT in adult patients

In allogeneic HPCT, the safety is determined mainly by the conditioning treatment, by the allogeneic cells from the donor, by GvHD prophylaxis and by prior transplant and chemotherapy. A total of 1771 patients with haematological diseases received thiotepa i.v. in combinations with other chemotherapeutic drugs and TBI as conditioning treatment before allogeneic HPCT.
The results are comparable with the analysis of EBMT (European Group for Blood and Marrow Transplantation) on 14403 patients and IBMT (International Bone and Marrow Transplant) registry on 6691 patients regarding the toxicities after allogeneic HPCT.

Deaths from the studies analysed versus deaths in the EBMT and IBMT registries are analysed in the following table 11:

Table 11: Causes of death in thiotepa studies vs EBMT and IBMTR registries in adult patients undergoing allogeneic HPCT

	Thiotepa	EBMT	IBMTR
aGvHD	0,5%-20%	25%	31%
Infection/Sepsis	2%-20%	10%	39%
MOF	1.2%-5.5%	/	6%
Second cancer	0.5%	/	65

In the EBMT registry, the incidence of bronchiolitis obliterans (BO) in 6257 patients at 2 years was reported at 1.7%. Patients treated with conditioning regimens including thiotepa have not reported BO. In the EBMT registry, among the 1652 enrolled patients, 631 underwent allogeneic transplantation. The incidence of VOD in this group was 8.7%. In the thiotepa group the incidence of VOD ranged from 2% to 12% and the incidence of second malignancies ranged from 0.5% to 4.8%. The incidence of acute GvHD grade III-IV in all conditioning regimens evaluated ranged from 4% to 24%. Fatal heart failure occurred at a rate of 3.7%, TRM ranged from 2% to 53% and OS ranged from 40% to 81% with a follow up ranging from 7.2 to 120 months.

Autologous HPCT in paediatric patients with solid tumours

The safety profile of thiotepa has been evaluated in 476 patients. The analyzed population is a very high risk group considering the disease phase, comorbidity, previous treatment including many chemotherapy cycles and eventually post autologous transplant radiotherapy.
OS ranged from 17% to 84% with a follow up from 12.3 to 99.6 months. TRM ranged from 0% to 18% and RRM from 0% to 26.7%.
The most important fatal events registered in the evaluated population treated with conditioning regimens including thiotepa are represented by MOF (3% to 21.4%) and respiratory toxicity (maximum incidence of 9%).
Severe/fatal events have been observed as VOD (4.4% to 14.9%) and sepsis (2.6% to 15.4%). Cardiac failure and renal failure, not associated to death, were respectively registered with a maximum of 4.5% and of 6.3% respectively. Secondary malignancies have been reported with a maximum frequency of 9%. The good safety long term profile in children population is satisfactory.
These data with high rate of OS show the positive safety profile of the conditioning regimen including thiotepa considering the advanced, relapsed or recurrence diseases treated with autologous transplantation in the most part of patients with poor prognosis.
The results confirm that conditioning regimens including thiotepa in combination with other chemotherapeutic medicinal products are safe and well tolerated in paediatric patients with solid tumours who underwent autologous HPCT.

Allogeneic HPCT in paediatric patients with haematological malignancies

The safety profile of thiotepa has been evaluated in 426 patients. The results show that thiotepa can be considered as safe and they are comparable with those of the EBMT (European Group for Blood and Marrow Transplantation) report published by Uderzo in 2007.
Although the EBMT report focused on lung and heart impairment, it included other important safety information. OS at 5 years was 77% and transplant related mortality (TRM) was due to multiorgan failure (MOF, 6.2%), Acute respiratory distress syndrome (ARDS, 2%), infections (2%) and non viral acute pneumonia (0.6%). No patients suffered or died from bronchiolitis obliterans. The lung and heart impairment was 35% and 26%, respectively.
All conditioning regimens evaluated by the Applicant showed an incidence of acute GvHD grade III-IV ranging from 0% to 20%. Chronic GvHD ranged from 0% to 26.6% (limited) or between 0% and 19% (extensive). Considering that the acute GvHD represents an important cause of death, the conditioning regimens with thiotepa have demonstrated a good profile of safety. Considering that chronic GvHD have a negative impact on the quality of life of patients, the results obtained confirm

that conditioning regimens including thiotepa are safe. TRM ranged from 0% to 30%.

The data reported in the conditioning treatment regimens containing thiotepa are comparable with those of the EBMT registry. The maximum incidence of infections and MOF deaths was 3.8% and 2.5%, respectively. Fatal VOD and fatal liver failure was reported with maximum frequency of 5% and 3%, respectively. The lung and heart severe toxicity ranged from 1.5% to 10% and from 2.5% to 5.5%, respectively. The incidence of other severe organ toxicities was very low: CNS toxicity ranged from 2.5 to 10.5%, gastrointestinal occurred with maximum frequency of 5.2%, hepatobiliary disorders ranged from 1% to 7.6% and renal toxicity occurred with maximum frequency of 2.5%.

The consequence of treatment with thiotepa at the recommended dose and schedule is profound myelosuppression, occurring in all patients. Severe granulocytopenia, thrombocytopenia, anemia or any combination thereof may develop. Frequent complete blood counts, including differential white blood cell counts, and platelet counts need to be performed during the treatment and until recovery is achieved. Platelet and red blood cell support, as well as the use of growth factors such as Granulocyte-colony stimulating factor (G-CSF), should be employed as medically indicated. Daily white blood cell counts and platelet counts are recommended during therapy with thiotepa and after transplant for at least 30 days.

Prophylactic or empiric use of anti-infectives (bacterial, fungal, viral) should be considered for the prevention and management of infections during the neutropenic period.

Thiotepa has not been studied in patients with hepatic impairment. Since thiotepa is mainly metabolized through the liver, caution needs to be observed when thiotepa is used in patients with pre-existing impairment of liver function, especially in those with severe hepatic impairment. When treating such patients it is recommended that serum transaminase, alkaline phosphatase and bilirubin are monitored regularly following transplant, for early detection of hepatotoxicity.

Patients who have received prior radiation therapy, greater than or equal to three cycles of chemotherapy, or prior progenitor cell transplant may be at an increased risk of hepatic veno-occlusive disease (see section 4.8 of the SPC).

Caution must be used in patients with history of cardiac diseases, and cardiac function must be monitored regularly in patients receiving thiotepa.

Caution must be used in patients with history of renal diseases and periodic monitoring of renal function should be considered during therapy with thiotepa.

Thiotepa might induce pulmonary toxicity that may be additive to the effects produced by other cytotoxic agents (busulfan, fludarabine and cyclophosphamide) (see section 4.8 of the SPC).

Previous brain irradiation or craniospinal irradiation may contribute to severe toxic reactions (e.g. encephalopathy).

The increased risk of a secondary malignancy with thiotepa, a known carcinogen in humans, must be explained to the patient.

Safety information regarding drug-drug interactions has been reflected in sections 4.4 and 4.5 of the SPC.

2.6 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan

Table Summary of the risk management plan

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine and additional)

	(routine and additional)	
Important identified risks		
Myelosuppression	Routine pharmacovigilance: • Collect all reported adverse reactions of , but not limited to, neutropenia, anaemia, leukopenia, thrombocytopenia • Attempt to determine prior exposure to myelotoxic agents • Follow the frequency and severity of reported events to monitor changes • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	As indicated in 4.4 of the proposed SPC for Tepadina: "The consequence of treatment with thiotepa at the recommended dose and schedule is profound myelosuppression, occurring in all patients. Severe granulocyto-penia, thrombocytopenia, anemia or any combination thereof may develop. Frequent complete blood counts, including differential white blood cell counts, and platelet counts need to be performed during the treatment and until recovery is achieved. Platelet and red blood cell support, as well as the use of growth factors such as Granulocyte-colony stimulating factor (G-CSF), should be employed as medically indicated. Daily white blood cell counts and platelet counts are daily recommended during therapy with thiotepa and after transplant for at least 30 days". All adverse reactions related to myelosuppression (neutropenia, anaemia, thrombocytopenia, leukopenia, febrile neutropenia) are listed in section 4.8 of the proposed SPC.
Hypersensitivity reactions	Routine pharmacovigilance: • Collect all adverse events of hypersensitivity reactions including signs and symptoms consistent with reactions • Investigate to determine if there was prior cytotoxic agent exposure • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	Contraindication outlined in SPC section 4.3. All adverse reactions related to hypersensitivity reactions (included also in other SOCs, i.e Skin and Subcutaneous Tissue Disorders) are listed in section 4.8 of the proposed SPC.
Infection	Routine pharmacovigilance: • Collect all adverse events of infection • Attempt to determine the etiology of all serious infections • Follow the frequency of reported infections to monitor for changes • Analysis of all ADRs within the required	Warning outlined in SPC section 4.4: "Prophylactic or empiric use of anti-infectives (bacterial, fungal, viral) should be considered for the prevention and management of infections during the neutropenic period". All adverse reactions related to Infections are listed in section 4.8 of the proposed SPC.

	• Provide specific update in the PSURs	
Second primary malignancy treatment related	Routine pharmacovigilance: • Collect, where possible, information on the incidence of second cancers and overall survival • Monitor adverse events associated with second cancers • Provide specific update in the PSURs	Healthcare professionals should advice patients of the potential risk of a second malignancy as outlined in section 4.4 of the SPC. Treatment related second malignancy is also listed in the section 4.8 of the proposed SPC.
Graft Versus Host Disease	Routine pharmacovigilance: • Collect, where possible, information on the incidence of aGVHD and cGVHD • Monitor adverse events associated with GVHD • Provide specific update in the PSURs	Characteristics on GvHD (graft versus host disease) and results of conditioning treatments are reported in section 5.1 (Pharmacodynamic properties) of the proposed SPC. Although not directly related to conditioning regimen, Acute Graft Versus Host Disease and Chronic Graft Versus Host Disease are listed in section 4.8 of the SPC as potential risks of the allogenic HPCT.
Mucositis	Routine pharmacovigilance: • Collect all adverse events of mucositis • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	Mucosal inflammation is listed in section 4.8 of the proposed SPC.
Confusion	Routine pharmacovigilance: • Collect all adverse events of confusion and other psychiatric events • Follow the frequency of reported cases to monitor for changes • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	Confusional state and mental status changes are listed in section 4.8 of the proposed SPC.
Veno-occlusive disease	Routine pharmacovigilance: • Collect all adverse events of veno-occlusive disease including signs and symptoms consistent with reactions • Monitor adverse events associated with veno-occlusive disease • Investigate to determine baseline hepatic function	Warning outlined in SPC section 4.4: "Patients who have received prior radiation therapy, greater than or equal to three cycles of chemotherapy, or prior progenitor cell transplant may be at an increased risk of hepatic veno-occlusive disease." Veno-occlusive disease is listed in section 4.8 of the proposed SPC.

	• Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	
Pulmonary toxicity	Routine pharmacovigilance: • Collect all adverse events of pulmonary toxicity including signs and symptoms consistent with reactions • Monitor adverse events associated with pulmonary toxicity • Investigate to determine baseline lung function • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	Advice that thiotepa might induce pulmonary toxicity additive to the effects produced by other cytotoxic agents (busulfan, fludarabine and cyclopho-sphamide) in section 4.4 of the SPC. Pulmonary toxicity (i.e, pneumo-nitis, idiopathic pneumonia syndrome) are listed in section 4.8 of the proposed SPC.
Nervous system disorders	Routine pharmacovigilance: • Collect all adverse events of nervous system disorders • Follow the frequency of reported cases to monitor for changes • Analysis of all ADRs within the required PSURs • Provide specific update in the PSURs	Nervous system disorders are listed in section 4.8 of the proposed SPC.
Important missing information		
Pregnant or lactating women	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	Pregnancy and lactation are controindications to treatment (see section 4.3 of the proposed SPC). In section 4.6 of the proposed SPC we reported: "Pregnancy: There are no data on the use of thiotepa during pregnancy. In pre-clinical studies thiotepa, as most alkylating agents, has been shown to cause embryofoetal lethality and teratogenicity. Therefore, thiotepa is contraindicated during pregnancy. Women of childbearing potential have to use effective contraception during treatment and a pregnancy test should be performed before treatment is started. Lactation: it is not known whether thiotepa is excreted in human milk. Due to its pharmacological properties and its potential toxicity for nursing infant, breast-feeding is

		contraindicated during treatment with thiotepa."
Elderly patients	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	In section 4.2 of the proposed SPC under "Special populations" we reported: "The administration of thiotepa has not been specifically investigated in elderly patients. However, in clinical studies, a proportion of patients over the age of 65 received the same cumulative dose as the other patients. No dose adjustment was deemed necessary".
Patients with clinically significant renal disease	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	In section 4.2 of the proposed SPC under "Special populations" we reported: "Studies in renally impaired patients have not been conducted. As thiotepa and its metabolites are poorly excreted in the urine, dose modification is not recommended in patients with mild or moderate renal insufficiency. However, caution is recommended" In section 4.4 of the proposed SPC we reported: "Caution must be used in patients with history of renal diseases and periodic monitoring of renal function should be considered during therapy with thiotepa". In section 5.2 of the proposed SPC we reported: "*Patients with renal dysfunction:* The effects of renal dysfunction on thiotepa elimination have not been assessed".
Patients with clinically significant hepatic disease	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	In section 4.2 of the proposed SPC under "Special populations" we reported: "Thiotepa has not been studied in patients with hepatic impairment. Since thiotepa is mainly metabolized through the liver, caution need to be exercised when thiotepa is used in patients with pre-existing impairment of liver function, especially in those with severe hepatic impairment. Dose modification is not recommended for transient alterations of hepatic parameters". In section 4.4 of the proposed SPC we reported: "Thiotepa has not been studied in patients with hepatic impairment. Since thiotepa is mainly metabolized through the liver, caution need to be observed when thiotepa is used in patients with pre-existing impairment of liver function, especially in those with severe hepatic impairment. When treating such

		patients it's recommended that serum transaminase, alkaline phosphatase and bilirubin are monitored regularly following transplant for early detection of hepatotoxicity". In section 5.2 of the proposed SPC we reported: "*Patients with hepatic dysfunction:* The effects of hepatic dysfunction on thiotepa metabolism and elimination have not been assessed".
Patients with impaired cardiac function	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	In section 4.4 of the proposed SPC we reported: "Caution must be used in patients with history of cardiac diseases and cardiac function must be monitored regularly in patients receiving thiotepa".
Patients with impaired pulmonary function	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	In section 4.4 of the proposed SPC we reported: "Thiotepa might induce pulmonary toxicity that may be additive to the effects produced by other cytotoxic agents (busulfan, fludarabine and cyclophosphamide)".
Patients with previous brain or craniospinal irradiation	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	Warning in the SmPC section 4.4: "Previous brain irradiation or craniospinal irradiation may contribute to severe toxic reactions."
Data on ethnicity/race	Routine pharmacovigilance: • Close monitoring of eventual safety reports • Provide specific update in the PSURs	None (no special risk)

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

2.7 Overall conclusions, risk/benefit assessment and recommendation

Quality
The quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. At the time of the opinion, there were some outstanding issues which remain as follow up measures and do not have any impact on the Benefit Risk balance of the product.

Non-clinical pharmacology and toxicology
No conventional acute and repeat-dose toxicity studies were performed.
Thiotepa was shown to be genotoxic in vitro and in vivo tests, and carcinogenic in mice and rats.
Thiotepa was shown to impair fertility and interfere with spermatogenesis in male mice, and to impair ovarian function in female mice. It was teratogenic in mice and in rats, and foeto-lethal in rabbits. These effects were seen at doses lower than those used in humans.

Efficacy
The efficacy of thiotepa was reviewed in 106 published studies comprising 6272 adult patients and 902 paediatric patients.
- In adult patients with haematological malignancies undergoing autologous HPCT the following results were reported:

Engraftment: conditioning treatments including thiotepa have proved to be myeloablative.

Disease Free Survival (DFS): 43% has been reported in the evaluated conditioning treatments as positive estimated value at five years, confirming that conditioning treatment containing thiotepa following autologous HPCT are valid and effective therapeutic strategies for treating patients with haematological diseases.

Relapse: In all the conditioning treatments containing thiotepa, the relapse rates at more than 1 year have been reported as being lower than 60%, which was considered by the physicians as the reference to prove efficacy. In some of the conditioning treatments evaluated, this relapse rate lower than 60% has been reported also at 5 years.

Overall Survival (OS): OS ranged from 29% to 87% with a follow up ranged from 22 up to 63 months.

- In adult patients with solid tumours undergoing autologous HPCT the following efficacy results were reported:

Engraftment: conditioning treatments including thiotepa have proved to be myeloablative.

Disease Free Survival (DFS): has been reported in the evaluated conditioning treatments as positive estimated value at more than 1 year, confirming that conditioning treatment containing thiotepa following autologous HPCT are valid and effective choices for treating patients with solid tumours.

Relapse: In all the conditioning treatments containing thiotepa, the relapse rates at more than 1 year have been reported as being lower than 60%, which was considered by the physicians as the reference to prove efficacy. In some of the conditioning treatments evaluated, relapse rates of 35% and of 45% have been reported at 5 years and 6 years respectively.

Overall Survival: OS ranged from 30% to 87% with a follow up ranged from 11.7 up to 87 months.

- In adult patients undergoing allogeneic HPCT for haematological malignancies the following efficacy results were reported:

Engraftment: The engraftment has been positively achieved (92%-100%) in all the conditioning treatments evaluated and were considered to occur at the expected time. Therefore it can be concluded that conditioning treatments including thiotepa are myeloablative.

D*isease Free Survival (DFS):* has been reported in the evaluated conditioning treatments as positive (estimated value: more than 1 year up to 5 years), confirming that conditioning treatments containing thiotepa following allogeneic HPCT are valid and effective choices for treating patients with haematological diseases.

Relapse: In all the conditioning treatments containing thiotepa, the relapse rates at more than 1 year have been reported as being lower than 40% (which was considered by the physicians as the reference to prove efficacy). In some of the conditioning treatments evaluated, relapse rates lower than 40% have been reported also at 5 years and 10 years.

Overall Survival: OS ranged from 31% to 81% with a follow up ranged from 7.3 up to 120 months.

- In paediatric patients undergoing autologous HPCT for solid tumours the following efficacy results were reported:

Engraftment: it has been achieved with all evaluated conditioning regimens including thiotepa.

Disease Free Survival (DFS): it has been reported in the evaluated conditioning treatments as positive estimated value at more than 1 year.

From 36 to 57 months DFS range from 46% to 70%. These results are very satisfactory considering that all patients are treated for high risk solid tumours. DFS confirms that conditioning treatments containing thiotepa following autologous HPCT are valid and effective therapeutic strategies for treating paediatric patients with solid tumours.

Relapse: In all the evaluated conditioning regimens containing thiotepa, the relapse rate from 12 to 57 months range from 33% to 57%. Considering that all patients suffer of recurrence or poor prognosis solid tumours, this range proves the efficacy of conditioning regimens based on thiotepa.

Overall Survival (OS): OS ranged from 17% to 84% with a follow up ranged from 12.3 up to 99.6 months.

- In paediatric patients undergoing allogeneic HPCT for haematological malignancies the following efficacy results were reported:

Engraftment: it has been achieved with all evaluated conditioning regimens including thiotepa with a range of 96% - 100%. The haematological recovery is in the expected time.

Disease Free Survival (DFS): it has been reported in the evaluated conditioning treatments as positive range 40% - 75% at more than 1 year. DFS confirms that conditioning treatment containing thiotepa following allogeneic HPCT are valid and effective therapeutic strategies for treating paediatric patients with haematological diseases.

Relapse: In all the evaluated conditioning regimens containing thiotepa, the relapse rate is very satisfactory with a range of 15% - 44%. These data prove the efficacy of conditioning regimens based on thiotepa in all haematological diseases.

Overall Survival (OS): OS ranged from 50% to 100% with a follow up ranged from 9.4 up to 121 months.

Safety

The safety of thiotepa was examined through a review of adverse events extracted from published data of 109 clinical trials. In these studies, a total of 6588 adult patients and 902 paediatric patients received thiotepa for conditioning treatment prior to haematopoietic progenitor cell transplantation.

Serious toxicities involving the haematologic, hepatic and respiratory systems were considered as expected consequences of the conditioning regimen and transplant process. These include infections and Graft-versus host disease (GvHD) which, although not directly related, were the major causes of morbidity and mortality, especially in allogeneic HPCT.

The most frequently adverse events reported in the different conditioning treatments including thiotepa are: infections, cytopenia, acute and chronic GvHD, gastrointestinal disorders, haemorrhagic cystitis, mucosal inflammation.

From the safety database all the adverse reactions reported in the reviewed literature references have been included in the Summary of Product Characteristics.

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

- User consultation

The CHMP considered that the User testing was acceptable. The methodology was in accordance with EU requirements. The report was clear and complete and it demonstrated that the main objectives of the test were achieved. The results achieved the success criteria and the mock-up was considered satisfactory.

Risk-benefit assessment

High dose Thiotepa has been used in combination with various regimens for conditioning treatment prior to haematopoietic progenitor cell transplantation in European hospitals for over 10 years. Many experts consider Thiotepa as a useful drug in this clinical context.

The applicant provided satisfactory data supporting the use of thiotepa in conditioning treatment in combination with other drugs. No satisfactory data were provided to support the use of thiotepa alone in conditioning treatment

Thiotepa has been in clinical use for several decades, in combination with other chemotherapeutic drugs as conditioning treatment prior to conventional HPCT in haematological diseases and solid tumours. Adverse events reported in the bibliographic references have been collected according to the therapeutic indication in order to quantify toxicity due to the conditioning treatment used in autologous and allogenic HSCT, and not strictly related to thiotepa. The frequency of each important identified risk has been presented by the type of conditioning treatment and by indication.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that routine pharmacovigilance was adequate to monitor the safety of the product and that no additional risk minimisation activities were required beyond those included in the product information.

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that Tepadina is not similar to Busilvex within the meaning of Article 3 of Commission Regulation (EC) No. 847/200 (See appendix 1).

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Tepadina 'in combination with other chemotherapy medicinal products

1) with or without total body irradiation (TBI), as conditioning treatment prior to allogeneic or autologous haematopoietic progenitor cell transplantation (HPCT) in haematological diseases in adult and paediatric patients,
2) as conditioning treatment when autologous HPCT is appropriate in solid tumours in adult and paediatric patients'
was favourable and therefore recommended the granting of the marketing authorisation.

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

BIBLIOGRAPHY

Abdul-Hai et al. (2007) The effect of high-dose thiotepa, alone or in combination with other chemotherapeutic agents, on a murine B-cell leukemia model simulating autologous stem cell transplantation. Bone Marrow Transplant; 40 (9): 891-6.

Abrey et al. (2006) High-dose chemotherapy with stem cell rescue as initial therapy for anaplastic oligodendroglioma: Long-term follow-up. Neuro-Oncol; 8: 183-186.

Ackland S.P. et al. (1988) Human plasma pharmacokinetics of thiotepa following administration of high-dose thiotepa and cyclophosphamide. J Clin Oncol; 6: 1192-96.

Adams C.E. et al. (1961) The action of various agents upon the rabbit embryo. J Embryol Exp Morphol; 9: 468-91.

Alessandrino E.P. et al. (2001) Thiotepa and fludarabine (TT-FLUDA) as conditioning regimen in poor candidates for conventional allogeneic hemopoietic stem cell transplant. Ann Hematol; 80: 521-24.

Alessandrino E.P. et al. (2004) Reduced-intensity conditioning regimen with thiotepa and fludarabine followed by allogeneic blood stem cell transplantation in haematological malignancies. Bone Marrow Transplantation; 34: 1039-45.

Anagnostopoulos et al. (2004) Comparison of High-Dose Melphalan with a More Intensive Regimen of Thiotepa, Busulfan, and Cyclophosphamide for Patients with Multiple Myeloma. Cancer; 100: 2607-12.

Ando et al, Cardiac conduction abnormalities in patients with breast cancer undergoing high-dose chemotherapy and stem cell transplantation. Bone Marrow Transplantation (2000) 25, 185–189

Aversa F. et al. (1994) Successful Engraftment of T-Cell-Depleted Haploidentical ''Three-Loci'' Incompatible Transplants in Leukemia Patients by Addition of Recombinant Human Granulocyte Colony-Stimulating Factor-Mobilized Peripheral Blood Progenitor Cells to Bone Marrow Inoculum. Blood; 84: 3948-55.

Aversa F. et al. (1998) Treatment of high-risk acute l.eukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med.; 339 (17): 1186-93.

Aversa F. et al. (1999) Improved Outcome With T-Cell–Depleted Bone Marrow Transplantation for Acute Leukemia. Journal of Clinical Oncology; 17 (5): 1545.

Aversa F. et al. (2001) Haploidentical stem cell transplantation in leukemia. Blood Rev; 15: 111-19.

Aversa F. et al. (2002) Haploidentical Stem Cell Transplantation for Acute Leukemia. Int J Hematol.; 76 (Suppl 1):165-68.

Aversa F. et al. (2005) Full Haplotype-Mismatched Hematopoietic Stem-Cell Transplantation: A Phase I1 Study in Patients With Acute Leukemia at High Risk of Relapse. J Clin Oncol.; 23(15): 3447-54.

Bacigalupo et al. (1996) Thiotepa Cyclophosphamide Followed by Granulocyte Colony-Stimulating Factor Mobilized Allogeneic Peripheral Blood Cells in Adults With Advanced Leukemia. Blood; 88: 353-57.

Bacigalupo A et al. (2007a) Thiotepa based conditioning regimen in 374 patients undergoing allogeneic stem cell transplants from related or unrelated donors. Bone Marrow Transplantation; 39: S1-S343.

Bacigalupo et al. (2007b) Thiotepa-based reduced intensity conditioning regimen: a 10 year follow up. Bone Marrow Transplantation; 40(11): 1091-3. Epub 2007 Oct 1.

Bacigalupo et al. (2007c) Allogeneic hematopoietic stem cell transplantation (HPCT) after conditioning with i.v. busulfan or i.v. thiotepa containing regimens. Bone Marrow Transplantation; 39 Suppl. 1: P595.

Bacigalupo et al. (2009) 10-year survey of 154 adult patients that underwent hematopoietic progenitor cell transplantation (HPCT) in the Bone Marrow Transplantation Unit of San Martino Hospital, Genova, Italy.

Bethge et al. (2006) Haploidentical allogeneic hematopoietic cell transplantation in adults with reduced-intensity conditioning and CD3/CD19 depletion: Fast engraftment and low toxicity. Experimental Hematology; 34: 1746–52.

Bernardo et al. (2008) Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major. Br J Haematol; 143 (4): 548-51.

Bierman PJ. et al. (2003) Syngenic hematopoietic stem cell transplantation for Non Hodgkin's lymphoma: a comparison with allogeneic and autologous transplantation. The Lymphoma Working Coommittee of The International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation; JCO, Vol 21, No 20, 2003

Bochkov N.P. (1981) Comperative Mutagenicity of TEPA, thio-TEPA and METEPA. Environm Sci Res; 24: 917-42.

Bouffet E. et al. (1997) Etoposide and thiotepa followed by ABMT (autologous bone marrow transplantation) in children and young adults with high-grade gliomas. Eur J Cancer; 33: 91-95.

Breau A.P. et al. (1984) Thiono compounds. 4. In vitro mutagenic and antineoplastic activity of TEPA and thio TEPA. Cell Biol Toxicol; 1: 21-30.

Bregni M. et al. (2002) Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. Blood; 99: 4234-36.

Broniscer et al. (2004) High-Dose Chemotherapy With Autologous Stem-Cell Rescue in the Treatment of Patients With Recurrent Non-Cerebellar Primitive Neuroectodermal tumour. Pediatr Blood Cancer; 42: 261-67.

Cairncross G. et al. (2000) Myeloablative chemotherapy for recurrent aggressive oligodendroglioma. Neuro-Oncol; 2: 114-19.

Cappiello V. et al. (1960) Antineoplastics. A 14-day study in rats of CL25,477: Phosphinic amide, P,P-bis(l-aziridinyl)-N-ethyl-N 1,3,4-thiazol-2-yl. Exp Ther Res 6: 356-382.

Chan et al. (1997) High-dose sequential chemotherapy and autologous stem cell reinfusion in advanced pediatric solid tumours. Bone Marrow Transplantation; 20: 1039–43.

Chang T.K. et al. (1995) Modulation of thiotepa antitumor activity in vivo by alteration of liver cytochrome P450- catalyzed drug metabolism. J Pharmacol Exp Ther; 274: 270-75.

Chen et al. (2004) Safety and Efficacy of High-Dose Chemotherapy with Autologous Stem Cell Transplantation for Patients with Malignant Astrocytomas. American Cancer Society; 100: 2201-207.

Cheng Y.C. et al. (2004) The use of high-dose cyclophosphamide, carmustine, and thiotepa plus autologous hematopoietic stem cell transplantation as consolidation therapy for high-risk primary breast cancer after primary surgery or neoadiuvant chemotherapy. Biol Blood Marrow Transplan; 10: 794-804.

Cohen B.E. et al. (1986) Human plasma pharmacokinetics and urinary excretion of thiotepa and its metabolites. Cancer Treat Rep; 70: 859-64.

Coombes R.C. et al. (2005) High dose chemotherapy and autologous stem cell transplantation as adjuvant therapy for primary breast cancer patients with four or more lymph nodes involved: long-term results of an international randomised trial. Ann Oncol; 16: 726-34.

Corradini P et al. (2004) Graft-Versus-Lymphoma Effect in Relapsed Peripheral T-Cell Non-Hodgkin's Lymphomas After Reduced-Intensity Conditioning Followed by Allogeneic Transplantation of Hematopoietic Cells. J Clin Oncol; 22: 2172-76.

Corradini P. et al. (2005) Effect of Age and previous autologous transplantation on nonrelapse mortality and survival in patients treated with reduced-intensity conditioning and allografting for advanced hematologic malignances. J Clin Onco 2005; 23: 6690-98.

Corradini P et al. (2007) Allogeneic stem cell transplantation following reduced-intensity conditioning can induce durable clinical and molecular remissions in relapsed lymphomas: pre-transplant disease status and histotype heavily influence outcome. Leukemia; 21 (11): 2316-23.

Craig A.W. et al. (1959) Metabolic studies of 32P-labelled triethylenethio-phosphoramide. Biochem Pharmacol; 3: 42-50.

Crossley ML et al. (1953) Chemotherapy of tumors in rats with certain ethylenephosphor-amides. Proc Soc Exp Biol Med; 83: 438-47.

Cumpston A.D. et al. (2007) Outcome with a thiotepa containing regimen and autologous HSCT in patients with NHL. Biology of Blood and Marrow Transplant; 13 Suppl. 2: 37.

Dairman W. (1966) Azetepa Phosphinic Amide. A comparative evaluation of Azetepa, CL25,477 and Thio-Tepa, CL8206, in normal male C3H mice and dogs. Toxicology Evaluation III 1: 83-121.

de Jonge M.E. et al. (2004) Integrated Population Pharmacokinetic Model of both cyclophosphamide and thiotepa suggesting a mutual drug-drug interaction. J Pharmacokinet Pharmacodyn; 31: 135-56

Demirer T. et al. (2004) High-dose thiotepa, melphalan and carboplatin (TMCb) followed by autologous peripheral blood stem cell transplantation in patients with lymphoma a retrospective evaluation. Bone Marrow Transplant; 34: 781-86.

Dhall et al. (2008) Outcome of children less than three years old at diagnosis with nonmetastatic medulloblastoma treated with chemotherapy on the "Head Start" I and II protocols. Pediatr Blood Cancer; 50(6): 1169-75.

di Grazia C. et al. (2001) Conventional hematopoietic stem cell transplants from identical or alternative donors are feasible in recipients relapsing after an autograft. Haematologica; 86: 646-51.

Dimopoulos et al. (1993) Thiotepa, busulfan, and cyclophosphamide: a new preparative regimen for autologous marrow or blood stem cell transplantation in high-risk multiple myeloma. Blood; 82: 2324-28.

Down J.D. et al. (1998) Thiotepa improves allogeneic bone marrow engraftment without enhancing stem cell depletion in irradiated mice. Bone Marrow Transplant; 21: 327-30.

Dunkel I.J. et al. (1998) High-dose carboplatin, thiotepa, and etoposide with autologous stem-cell rescue for patients with recurrent medulloblastoma. Children's Cancer Group. J Clin Oncol; 16: 222-28.

Dupuis-Girod S. et al. (1997) High-dose chemotherapy in relapse of medulloblastoma in young children. Bull Cancer; 84: 264-72.

Drugdex(R) System. Thiotepa. Klasco RK (Ed): DRUGDEX® System.Thomson Micromedex, Greenwood Village, Colorado Edition 125 expires 6/2005. Ref Type: Generic.

Evenson D.P. et al. (1986) Toxicity of thiotepa on mouse spermatogenesis as determined by dual-parameter flow cytometry. Toxicol Appl Pharmacol; 82: 151-63.

Fagioli F at el. (2004) High-dose thiotepa and etoposide in children with poor-prognosis brain tumours. Cancer; 100: 2215-21.

Finlay J.L. et.al. (1990) High-dose multi-agent chemotherapy followed by bone marrow 'rescue' for malignant astrocytomas of childhood and adolescence. J Neurooncol; 9: 239-48.

Finlay J.L. et al. (1996) Pilot study of high-dose thiotepa and etoposide with autologous bone marrow rescue in children and young adults with recurrent CNS tumours. The Children's Cancer Group. J Clin Oncol; 14: 2495-503.

Gill et al. (2008) High-dose Chemotherapy With Autologous Stem Cell Transplantation in Adults With Recurrent Embryonal Tumours of the Central Nervous System. Cancer; 112: 1805-11.

Glossmann J.P. et al. (2005) Autologous tandem transplantation in patients with primary progressive or relapsed/refractory lymphoma. Ann Hematol; [14 p.].

Gopal et al. (2001) Efficacy of high-dose therapy and autologous hematopoietic stem cell transplantation for non-Hodgkin's lymphoma in adults 60 years of age and older. Bone Marrow Transplantation; 27(6): 593-9.

Gratwohl A et al. Results of the EBMT activity survey 2005 on haematopoietic stem cell transplantation: focus on increasing use of unrelated donors. Bone Marrow Transplantation (2007) 39:71-87.

Gratwohl A et al. The EBMT activity survey 2006 on hematopoietic stem cell transplantation: focus on the use of cord blood products. Bone Marrow Transplantation (2008) 41: 687-705.

Gratwohl A et al. The EBMT activity survey 2007 with focus on allogeneic HSCT for AML and novel cellular therapies. Bone Marrow Transplantation (2009) 43: 275–291.

Grill J. et al. (1996) A high-dose busulfan-thiotepa combination followed by autologous bone marrow transplantation in childhood recurrent ependymoma. A phase-II study. Pediatr Neurosurg; 25: 7-12.

Grodman et al. (2009) Outcome of Patients With Recurrent Medulloblastoma or Central Nervous System Germinoma Treated With Low Dose Continuous Intravenous Etoposide Along With Dose-Intensive Chemotherapy Followed by Autologous Hematopoietic Stem Cell Rescue. Pediatr Blood Cancer; 53: 33-36.

Grovas A.C. et al. (1999) Regimenrelated toxicity of myeloablative chemotherapy with BCNU, thiotepa, and etoposide followed by autologous stem cell rescue for children with newly diagnosed glioblastoma multiforme: report from the Children's Cancer Group. Med Pediatr Oncol; 33: 83-87.

Grullich et al. (2008) A fludarabine, thiotepa reduced toxicity conditioning regimen designed specifically for allogeneic second haematopoietic cell transplantation after failure of previous autologous or allogeneic transplantation. Bone Marrow Transplantation; 41: 845–50.

Gutierrez-Delgado et al. (2001) Autologous stem cell transplantation for non-Hodgkin's lymphoma: comparison of radiation-based and chemotherapy-only preparative regimens. Bone Marrow Transplant ; 5: 455-61.

Gutierrez-Delgado F. et al. (2003) Autologous stem cell transplantation for Hodgkin's disease: busulfan, melphalan and thiotepa compared to a radiation-based regimen. Bone Marrow Transplant; 32: 279-285.

Hagen B et al. (1987) Single and repeated dose pharmacokinetics of thio-TEPA in patients treated for ovarian carcinoma. Cancer Chemother Pharmacol; 19: 143-148.

Hagen B. et al. (1988) Pharmacokinetics of thio-TEPA at two different doses. Cancer Chemother Pharmacol; 22: 356-58.

Hagen B. et al. (1990) Long-term pharmacokinetics of thio-TEPA, TEPA and total alkylating activity following i.v. bolus administration of thio-TEPA in ovarian cancer patients. Cancer Chemother Pharmacol; 25: 257-62.

Hagen B. (1991) Pharmacokinetics of thio-TEPA and TEPA in the conventional dose-range and its correlation to myelosuppressive effects. Cancer Chemother Pharmacol; 27: 373-78.

Halliday S. et al. (1958) Antineoplastics. Studies on the antitumor activity and toxicity of CL 8206: N,N',N''-triethylenethiophosphoramide (thio-TEPA). Pharmacol Res III; 4: 556-95.

Hawkins et al. (2000) Busulfan, Melphalan, and Thiotepa With or Without Total Marrow Irradiation With Hematopoietic Stem Cell Rescue for Poor-Risk Ewing-Sarcoma-Family Tumours. Medical and Pediatric Oncology 34: 328–37.

Heideman R.L. et al.(1989) Phase I and pharmacokinetic evaluation of thiotepa in the cerebrospinal fluid and plasma of pediatric patients: evidence for dose-dependent plasma clearance of thiotepa. Cancer Res; 49: 736-41.

Henner W.D. et al. (1987) Pharmacokinetics of continuous-infusion high-dose thiotepa. Cancer Treat Rep; 71: 1043-47.

Holmberg et al, High-dose busulfan, melphalan and thiotepa followed by autologous peripheral blood stem cell (PBSC) rescue in patients with advanced stage III/IV ovarian cancer. Bone Marrow Transplantation; 22: 651–59.

Huitema A.D. et al. (2000) Reduction of cyclophosphamide bioactivation by thioTEPA: critical sequence-dependency in high-dose chemotherapy regimens. Cancer Chemother Pharmacol; 46: 119-27.

Huitema A.D. et al. (2001) Population pharmacokinetics of thioTEPA and its active metabolite TEPA in patients undergoing high-dose chemotherapy. Br J Clin Pharmacol; 51: 61-70.

IARC "Thiotepa" Monographs on the evaluation of carcinogenic risk to humans (1990); 50: 122-41.

Illerhaus G. et al. (2006) High-Dose Chemotherapy With Autologous Stem-Cell Transplantation and Hyperfractionated Radiotherapy As First-Line Treatment of Primary CNS Lymphoma. J Clin Oncol; 24: 3865-70.

Jacobson P.A. et al. (2002) Cytochrome P450 isozymes 3A4 and 2B6 are involved in the in vitro human metabolism of Thiotepa to TEPA. Cancer Chemother Pharmacol; 49: 461-67.

Jakubowsky AA et al. (2007) T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin. Blood; 110 (13): 4552-59.

Kletzel M. et al. (1992) Pharmacokinetics of high dose thiotepa in children undergoing autologous bone marrow transplantation. Bone Marrow Transplant; 10: 171-75.

Kroger N. et al. (2003) Low incidence of secondary myelodysplasia and acute myeloid leukemia after high-dose chemotherapy as adjuvant therapy for breast cancer patients: a study by the Solid Tumors Working Party of the European Group for Blood and Marrow Transplantation. Ann Oncol; 14: 554-58.

Kushner et al. (2001) Topotecan combined with myeloablative doses of thiotepa and carboplatin for neuroblastoma, brain tumours, and other poor-risk solid tumours in children and young adults. Bone Marrow Transplantation; 28: 551–56.

Lacerda J.F. et al. (2003) Haploidentical stem cell transplantation with purified CD34 cells after a chemotherapy-alone conditioning regimen. Biol Blood Marrow Transplant; 9: 633-42.

Lafay-Cousin L. et al. (2000) High dose thiotepa and hematopoietic stem cell transplantation in pediatric malignant mesenchymal tumours: a phase II study. Bone Marrow Transplant; 26: 627-32.

La Nasa G. et al. (2002) Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. Blood; 99(12): 4350-56.

La Nasa G. et al. (2005) Unrelated donor stem cell transplantation in adult patients with thalassemia. Bone Marrow Transplantation; 36: 971-75.

Lazarus H.M. et al (1987) High-dose i.v. thiotepa and cryopreserved autologous bone marrow transplantation for therapy of refractory cancer. Cancer Treat Rep; 71: 689-95.

Leonard R.C. et al. (2004) Conventional adjuvant chemotherapy versus single-cycle, autograftsupported, high-dose, late-intensification chemotherapy in high-risk breast cancer patients: a randomized trial. J Natl Cancer Inst; 96: 1076-83.

Ljungman P. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. BMT (2009), 1-16

Locatelli et al. (2009) Survey of 432 patients that underwent hematopoietic progenitor cell transplantation (HPCT) in Paediatric Onco-hematological Unit of Policlinico San Matteo Hospital, Pavia, Italy.

Lucidarme N. et al. (1998) Phase II study of high-dose thiotepa and hematopoietic stem cell transplantation in children with solid tumours. Bone Marrow Transplant; 22: 535-40.

Majolino I. et al. (2007) Reduced intensity conditioning with thiotepa, fludarabine, and melphalan is effective in advanced multiple myeloma. Leukemia & Lymphoma; 48: 759-66.

Mason W.P. et al. (1998) Intensive chemotherapy and bone marrow rescue for young children with newly diagnosed malignant brain tumours. J Clin Oncol; 16: 210-21.

Massimino M et al. (2005) Sequential chemotherapy, high-dose thiotepa, circulating progenitor cell rescue, and radiotherapy for childhood high-grade glioma. Neuro-Oncol; 7: 41-48.

Massimino M. et al. (2006) Supratentorial primitive neuroectodermal tumours (S-PINET) in children: a prospective experience with adjuvanmt intensive chemotherapy and hyperfractionated accelerated radiotherapy. Int J Radiation Oncology Biol Phys.; 64: 1031-37.

McCoy A.G. et al. (2004) Cytoreduction regimens. A novel preparative regimen for autologous transplant in non-Hodgkin's lymphoma: long-term experience with etoposide and thiotepa. Bone Marrow Transplantation; 33: 19–24.

Meistrich M.L. et al (1982) Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res; 42: 122-31.

Mellett L.B. et al. (1960) The comparative physiological disposition of thio –TEPA and TEPA in the Dog. Cancer Res; 20: 524-32.

Mellett L.B. et al. (1962) Absorption and fate of C14-labeled N,N',N''-triethylenethiophosphoramide (thio-TEPA) in humans and dogs. J Lab Clin Med; 60: 818-25.

Miller B. et al. (1988) Cellular pharmacology of N,N',N''-triethilenethiophosphoramide. Cancer Lett; 41: 157-68.

Montemurro et al. (2007) Primary central nervous system lymphoma treated with high-dose methotrexate, high-dose busulfan/thiotepa, autologous stem-cell transplantation and response-adapted whole-brain radiotherapy: results of the multicenter Ostdeutsche Studiengruppe Hämato-Onkologie OSHO-53 phase II study. Annals of Oncology; 18: 665-71.

Munson A.E. et al. (1974) Synergistic lethal action of alkylating agents and sodium pentobarbital in the mouse. Pharmacology; 11: 231-40.

NCI (1979) Bioassay of Thio-TEPA for possible carcinogenicity. Testing Program N° 58: 1-168.

Ng SF and Waxman D.J. (1991) N,N',N''-triethylenethiophosphoramide (thio-TEPA) oxygenation by constitutive hepatic P450 enzymes and modulation of drug metabolism and clearance in vivo by P450-inducing agents. Cancer Res; 51: 2340-45.

NIH "Thiotepa" Rep. Carcinog. 10 (2002) : 131-32.

Nitz UA et al. (2005) Comparison of rapidly cycled tandem high-dose chemioterapy plus peripheral blood stem cell support versus dose-dense conventional chemotherapy for ajuvant treatment of high-risk breast cancer: results of a multicentre phase III trial. Lancet; 366: 1935-44.

O'Dwyer P.J. et al. (1991) Phase I/pharmacokinetic reevaluation of thioTEPA. Cancer Res; 51: 3171-76.

Papadakis et al. (2000) High-dose carmustine, thiotepa and etoposide followed by autologous bone marrow rescue for the treatment of high risk central nervous system tumours. Bone Marrow Transplantation; 26: 153–60.

Papadopoulos K.P. et al. (1998) High-dose thiotepa and etoposide-based regimens with autologous hematopoietic support for high-risk or recurrent CNS tumours in children and adults. Bone Marrow Transplant; 22: 661-67.

Papadopoulos E.B. et al. T-cell-depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease. Blood; 91 (3): 1083-90.

Papadopoulos K.P. et al. (2005) Pilot study of tandem high dose chemotherapy and autologous stem cell trasnplatation with a novel combination of regimens in patients with poor risk lymphoma. BMT; 36: 491-97.

Pession A. et al. (1999) Phase I study of high-dose thiotepa with busulfan, etoposide, and autologous stem cell support in children with disseminated solid tumors. Med Pediatr Oncol; 33): 450-54.

Picardi A. et al. (2004) Possibility of long-term remission in patients with advanced hematologic malignancies after reduced intensity conditioning regimen (RIC) and allogeneic stem cell transplantation. Hematol J; 5: 24-31.

Porrata L.F. et al. (2001) The pharmacologic basis of high dose chemotherapy with haematopoietic stem cell support for solid tumours. Br J Cancer; 85: 484-89.

Przepiorka D. et al. (1995) A phase I-II study of high-dose thiotepa, busulfan and cyclophosphamide as a preparative regimen for autologous transplantation for malignant lymphoma. Leuk Lymphoma; 17 : 427-33.

Rae J.M. et al. (2002) Triethylenethiophosphor-amide is a specific inhibitor of cytochrome P4502B6: implications for cyclophosphamide metabolism. Drug Metab Dispos; 30: 525-30.

Rao V.K. et al (2005) The extent of chromosomal aberration induced by chemotherapy in non-human primates depends on the schedule of administration. Mutation Research; 583: 105-19.

Raiola et al. (2000) Reduced intensity thiotepa-cyclophosphamide conditioning for allogeneic haemopoietic stem cell transplants (HPCT) in patients up to 60 years of age. Br J Haematol.; 109(4): 716-21.

Rick et al. (2001) Salvage Treatment With Paclitaxel, Ifosfamide, and Cisplatin Plus High-Dose Carboplatin, Etoposide, andThiotepa Followed by Autologous Stem-Cell Rescue in Patients With Relapsed or Refractory Germ Cell Cancer. J Clin Oncol; 19: 81-88.

Ridola et al. (2007) High-dose Chemotherapy With Autologous Stem Cell Rescue Followed by Posterior Fossa Irradiation for Local Medulloblastoma Recurrence or Progression After Conventional Chemotherapy. Cancer; 110: 156-63.

Rigden JP. et al. Minimizing graft rejection in allogeneic T cell-depleted bone marrow transplantation. Bone Marrow Transplantation; 18 (5): 913-19.

Rodenhuis S. et al. (2003) High dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer. N Engl J Med; 349: 7-16.

Rodenhuis S et al. (2006) Efficacy of high-dose alkylating chemotherapy in HER2/neu-negative breast cancer. Ann. Oncol.

Rosales F. et al. (1999) The role of thiotepa in allogeneic stem cell transplantation in patients with leukemia. Leuk Res; 23: 947-52.

Rose et al. (2000) The feasibility of high-dose chemotherapy in breast cancer patients with impaired left ventricular function. Bone Marrow Transplantation; 26: 133–39.

Ruddon R.W. et al. (1964) Distribution of C14-labeled ThioTEPA (NSC-6396) and its metabolites in normal and tumor-bearing rats. Cancer Chemother Rep; 39: 7-13.

Rzeski W. et al. (2004) Anticancer agents are potent neurotoxins in vitro and in vivo. Ann Neurol; 56: 351-60.

Shimkin M.B. et al. (1966) Bioassay of 29 alkylating chemicals by the pulmonary-tumor-response in strain A mice. J Natl Cancer Inst; 36: 915-35.

Shimoni A. et al. (2001) Thiotepa, busulfan, cyclophosphamide (TBC) and autologous hematopoietic transplantation: an intensive regimen for the treatment of multiple myeloma. Bone Marrow Transplantation; 27(8): 821-28.

Shrama et al, Toxicity of the high-dose chemotherapy CTC regimen (cyclophosphamide, thiotepa, carboplatin): the Netherlands Cancer Institute experience. British Journal of Cancer; 88: 1831–38.

Sloboda A.E. (1960) Antineoplastics- Evaluation. The Effect of Polyethylene glycol 400 upon the LD50 of CL 8206 (Thiotepa) in rats and mice. Pharmacol Res; 6: 1811-18.

Sloboda A.E. et al. (1962) Comparison of two antineoplastic compounds with thioTEPA: a laboratory evaluation. Cancer Chemother Rep; 24: 7-17.

Stemmer et al. (2001) Feasibility and Low Toxicity of Early Radiotherapy after High-Dose Chemotherapy and Autologous Stem Cell Transplantation for Patients with High-Risk Stage II–III and Locally Advanced Breast Carcinoma. Cancer: 1983-91.

Stemmer et al. (2003) Adjuvant treatment of high-risk stage II breast cancer with doxorubicin followed by high-dose chemotherapy and autologous stem-cell transplantation: a single-institution experience with 132 consecutive patients. Bone Marrow Transplantation 31: 655–61.

Stiff P.J. et al. (2004) Randomized Phase II trial of two highdose chemotherapy regimens with stem cell transplantation for the treatment of advanced ovarian cancer in first remission or chemosensitive relapse: a Southwest Oncology Group study. Gynecol Oncol; 94: 98-106.

Stoner GD et al. (1973) Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmon tumor response in strain A mice. Cancer Res; 33: 3069-85.

Strahm et al. (2007) Reduced intensity conditioning in unrelated donor transplantation for refractory cytopenia in childhood. Bone Marrow Transplant; 40 (4): 329-33.

Strong J.M. et al. (1986) Pharmacokinetics of intraventricular and intravenous N,N',N''-tri-ethylenethiophosphoramide (thiotepa) in rhesus monkeys and humans. Cancer Res; 46 (Pt 1): 6101-104.

Tallman M.S. et al. (2003) Conventional adjuvant chemotherapy with or without high-dose chemotherapy and autologous stem-cell transplantation in high-risk breast cancer. N Engl J Med; 349: 17-26.

Tanimura T. (1968) Relationship of dosage and time of administration to the teratogenic effects of thio-TEPA in mice. Okajimas Folia Anat Jpn; 44: 203-53.

Teicher B.A. et al. (1989) Evidence for enzymatic activation and oxygen involvement in cytotoxicity and antitumor activity of N,N',N''-triethylenethiophosphoramide. Cancer Res 49: 4996-5001.

Teicher B.A. et al. (1990) Preclinical studies relating to the use of thiotepa in the high-dose setting alone and in combination." Semin Oncol; 17 Suppl. 3: 18-32.

Terenzi A. et al. (1990) Enhancement of T cell-depleted bone marrow allografts in mice by thiotepa. Transplantation; 50: 717-20.

Tiersten et al, Phase I/II study of tandem cycles of high-dose chemotherapy followed by autologous hematopoietic stem cell support in women with advanced ovarian cancer. Int J Gynecol Cancer; 16: 57–64.

Ueno N.T. et al. (2009) High-Dose Chemotherapy with Autologous Stem-Cell Support Versus Standard-Dose Chemotherapy for High-Risk Breast Cancer: Meta- Analysis of Individual Patient Data from 15 Randomized Adjuvant Trial. Biology of Blood and Marrow Transplantation February 2008 (Vol. 14, Issue 2, Page 91) 929-35.

Valteau-Couanet D. et al. (2005) High-dose busulfan and thiotepa followed by autologous stem cell transplantation (HPCT) in previously irradiated medulloblastoma patients: high toxicity and lack of efficacy. Bone Marrow Transplantation; 36: 939-45.

van der Wall et al., High-dose carboplatin, thiotepa and cyclophosphamide (CTC) with peripheral blood stem cell support in the adjuvant therapy of high-risk breast cancer: a practical approach. BJC (1995) 71: 857-862.

van Maanen M.J. et al. (2000) Chemistry, pharmacology and pharmacokinetics of N,N',N"-triethylenethiophosphoramide (ThioTEPA). Cancer Treat Rev; 26: 257-68.

Viby-Mogensen J. (1985) Interaction of other drugs with muscle relaxants. Sem Anesth 4.1: 52-64.

Waheed et al. (2004) High dose chemotherapy with thiotepa, mitoxantrone and carboplatin (TMJ) followed by autologous stem cell support in 100 consecutive lymphoma patients in a single centre: analysis of efficacy, toxicity and prognostic factors. Leuk Lymphoma; 45: 2253-59.

Weaver C.H. et al. (1997) Treatment-related mortality in 1000 consecutive patients receiving high-dose chemotherapy and peripheral blood progenitor cell transplantation in community cancer centers. Bone Marrow Transplant; 19: 671-78.

Wong et al. (2003) Idiopathic pneumonia syndrome after high-dose chemotherapy and autologous hematopoietic stem cell transplantation for high-risk breast cancer. Bone Marrow Transplantation; 31: 1157-63.

Yalamanchili et al. (2008) High-dose chemotherapy with autologous stem cell rescue in stage IIIB inflammatory breast cancer. Anticancer Res.; 28(5B): 3139-42.

Zander A.R. et al. (2004) High-dose chemotherapy with autologous hematopoietic stem-cell support compared with standard-dose chemotherapy in breast cancer patients with 10 or more positive lymphnodes: first results of a randomized trial. J Clin Oncol; 22: 2273-83.

Zecca et al. (1999) Total Body Irradiation, Thiotepa, and Cyclophosphamide as a Conditioning Regimen for Children With Acute Lymphoblastic Leucemia in First or Second Remission Undergoing Bone Marrow Transplantation With HLA-Identical Siblings. J Clin Oncol 17: 1838-46.