Treosulfan, Fludarabine and Cytarabine as a Conditioning Regimen for Allogeneic Haematopoietic Stem Cell Transplantation in Patients with Acute Myeloid Leukaemia, Myelodysplastic Syndrome and Myeloproliferative Neoplasms

Von der Fakultät für Medizin und Gesundheitswissenschaften
der Carl von Ossietzky Universität Oldenburg

zur Erlangung des Grades einer

Doktorin der Medizin (Dr. med.)

angenommene

Dissertation

von

Frau Samantha Henderson

geboren am **25.01.1987** in **Epsom, Großbritannien**

Betreuer
Erstbetreuer: Professor Dr. med. Claus-Henning Köhne
Zweitbetreuer: Professor Dr. med. Jochen Casper

Institution/Klinik
Universitätsklinik für Innere Medizin – Onkologie und Hämatologie, Klinikum Oldenburg

Tag der mündlichen Prüfung: 10. Dezember 2020

Contents

Abbreviations

aGvHD	Acute graft-versus-host disease
ALL	Acute lymphoblastic leukaemia
ALT	Alanine transaminase
AML	Acute myeloid leukaemia
ARDS	Acute respiratory distress syndrome
AST	Aspartate transaminase
ATG	Antithymocyte globulin
BBB	Blood-brain barrier
BM	Bone marrow
cGvHD	Chronic graft-versus-host disease
CI	Confidence interval
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CR	Complete remission
CSA	Cyclosporine A
CTCAE	Common terminology criteria for adverse events
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein-Barr-Virus
ET	Essential thrombocythaemia
FLAG	Salvage therapy for AML consisting of fludarabine, cytarabine and G-CSF
FLAMSA-RIC	Conditioning regimen containing fludarabine, amsacrine and cytarabine and followed by a reduced intensity conditioning
FLT3	*fms*-like tyrosine kinase 3
G-CSF	Granulocyte-colony stimulating factor
GvHD	Graft-versus-host disease
GvL	Graft-versus-leukaemia (or malignancy) effect
Gy	Gray (Système Internationale unit of absorbed dose of ionizing radiation)
HLA	Human leucocyte antigen
HSCT	Haematopoietic stem cell transplantation
Ida	Idarubicin
JAK2	Janus kinase 2
MAC	Myeloablative conditioning
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
mMUD	Mismatched unrelated donor
MPN	Myeloproliferative neoplasms
MRD	Matched related donor
MTX	Methotrexate
MUD	Matched unrelated donor
NHL	Non-Hodgkin lymphoma
NMC	Non-myeloablative conditioning
NPM1	Nucleophosmin 1
NRM	Non-relapse mortality
OS	Overall survival
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PGF	Primary graft failure
PIF	Primary induction failure
PMN	Polymorphonuclear leucocytes

PTLD	Post-transplant lymphoproliferative disease
PV	Polycythaemia vera
RFS	Relapse-free survival
RIC	Reduced intensity conditioning
SGF	Secondary graft failure
S-HAM	Salvage therapy for AML consisting of sequential high dose cytarabine and mitoxantrone
SOS	Sinusoidal obstruction syndrome (previously known as VOD – hepatic veno-occlusive disease)
TBI	Total body irradiation
TKI	Tyrosine kinase inhibitor
Treo/Flu	Conditioning regimen containing treosulfan and fludarabine
Treo/Flu/AraC	Conditioning regimen containing treosulfan, fludarabine and cytarabine
WHO	World Health Organisation

Abstract

Background: Allogeneic haematopoietic stem cell transplantation (HSCT) remains the only long-term curative treatment option for selected patients with haematological malignancies including acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and the myeloproliferative neoplasms (MPN). Those who undergo transplantation do so because they are unlikely to achieve permanent remission after receiving standard treatment. This is due to the aggressive and chemotherapy-resistant nature of their disease, or due to a high risk of disease relapse. For many years, patients of advanced age and those deemed medically infirm were excluded from undergoing allogeneic HSCT due to the high non-relapse mortality (NRM) rates of the original myeloablative conditioning (MAC) regimens. Reduced intensity conditioning (RIC) regimens were therefore developed to afford an acceptable degree of toxicity for patients who would otherwise not be able to undergo such intensive treatment. However, it has been observed that patients undergoing RIC have a higher rate of disease relapse than patients undergoing MAC. The success of RIC relies more heavily on the graft-versus-malignancy effect (GvL) than MAC, without specifically aiming at the reduction of residual leukaemic burden before allogeneic HSCT. Regimens are required that provide effective conditioning for advanced and aggressive disease, reducing the risk of relapse, whilst remaining tolerable to those unsuited for MAC. The combination of treosulfan and fludarabine (Treo/Flu) is already an established toxicity-reduced conditioning regimen for patients with a range of malignant and non-malignant haematological conditions. Previous studies have used treosulfan at doses between 10 and 14 g/m^2. The aim of the addition of cytarabine to this regimen, one of the most effective chemotherapeutic agents against myeloid malignancies, is to reduce the leukaemic disease burden prior to transplantation. This, coupled with the use of a higher dose of treosulfan (14 g/m^2), has the potential to reduce the risk of malignant disease relapse, whilst maintaining an acceptable NRM and level of toxicity. This study aims to assess the feasibility, tolerability and effectiveness of Treo/Flu/AraC as a conditioning regimen.

Methods: The outcomes of 77 patients who received an allogeneic HSCT between July 2009 and August 2018 with conditioning according to the treosulfan, fludarabine and cytarabine (Treo/Flu/AraC) regimen were retrospectively analysed. The median age of the population was 54 years (range, 18-69 years). A total of 80 transplantations were evaluated. Three patients were transplanted twice using the Treo/Flu/AraC regimen. Patients were treated for AML, MDS or MPN. Only 28 % of patients were in the first complete remission (CR1) at the time of transplantation. The standard regimen consisted of treosulfan 14 g/m^2 intravenously from day -6 to day -4 or day -4 to day -2, fludarabine 30mg/m^2 intravenously from day -6 to day -2 and cytarabine 2000 mg/m^2 intravenously on day -6 to -5. For patients receiving grafts from unrelated donors, rabbit-derived antithymocyte globulin (ATG) 10 mg/kg was given intravenously from day -4 to day -2. The prophylaxis regimen against graft-versus-host disease

(GvHD) consisted of cyclosporine A in combination with either methotrexate or mycophenolate mofetil. The primary outcome was relapse-free survival (RFS). Secondary outcomes were overall survival (OS), NRM, cumulative incidence of relapse, neutrophil and platelet engraftment, chimaerism, acute and chronic GvHD and occurrence of toxicities or adverse events.

Results: Median follow-up time was 1161 days (3.2 years, range, 13 days-9.8 years). One-, two- and three-year RFS rates were 47.5 %, 40.7 % and 37.3 %, respectively. One, two- and three-year OS rates were 59.3 %, 49.3 % and 45.4 %, respectively. Cumulative incidences of NRM were 10 % (95 % confidence interval (CI), 5 %-18 %) at 100 days, 18.8 % (95 % CI, 11 %-28 %) at 1 year and 20.1 % (95 % CI, 12 %-30 %) at 2 years. The one- and three-year cumulative incidences of relapse were 34 % (95 % CI, 24 %-44 %) and 41 % (95 % CI, 30 %-52 %), respectively. The day 28 cumulative incidence of engraftment of neutrophils was 85 % (95 % CI, 75 %-91 %). By day +37 all patients had achieved neutrophil engraftment. The day 28 cumulative incidence of platelet engraftment was 82.5 % (95 % CI, 72 %-89 %). By day 100, this had increased to 85 % (95 % CI, 75 %-91 %). The cumulative incidence of complete donor-type chimaerism was 84 % (95 % CI, 74 %-90 %) on day +28. Day 100 cumulative incidences of grade I - IV, II - IV and III - IV acute GvHD were 38 % (95 % CI, 27 %-48 %), 22 % (95 % CI, 13 %-33 %) and 6 % (95 % CI, 2 %-14 %). Acute grade IV GvHD of the liver led to the death of one patient. The cumulative incidence of mild to severe chronic GvHD at two years was 15 % (95 % CI, 8 %-24 %). Mucositis was an important complication occurring in 41 % of patients at grade I-IV. Sinusoidal obstruction syndrome (SOS) was diagnosed in three patients. One patient ultimately died from liver failure caused by this condition. There was only one case of grade 3-4 nephrotoxicity. A rise in bilirubin reached grade 3-4 in 16 % of cases and an alanine transaminase (ALT)/aspartate transaminase (AST) rise in 22 %. All organ toxicities except for the one case of grade 3-4 nephrotoxicity were reversible.

Conclusion: The Treo/Flu/AraC regimen provides feasible, tolerable and effective conditioning for patients with AML, MDS or MPN even in advanced disease states. The incidence of NRM is acceptable in this heavily pre-treated patient population. A prospective randomised controlled clinical trial comparing this regimen with Treo/Flu and/or FLAMSA-RIC conditioning needs to be carried out to confirm the non-inferiority and/or superiority of the Treo/Flu/AraC regimen. The patients who would benefit the most from the application of this conditioning should be identified.

Zusammenfassung

Hintergrund: Eine allogene hämatopoetische Stammzelltransplantation (HSCT) ist die einzige kurative Behandlungsmöglichkeit für viele Patienten mit den malignen hämatopoetischen Erkrankungen akute myeloische Leukämie (AML), myelodysplastisches Syndrom (MDS) und die myeloproliferativen Neoplasien (MPN). Patienten werden für eine Transplantation selektiert, falls unter einer Standardbehandlung keine langfristige Remission erreicht werden kann. Bei älteren Patienten und die mit zahlreichen Komorbiditäten, konnten früher keine Stammzelltransplantationen durchgeführt werden. Die therapiebedingte Mortalität (non-relapse mortality, NRM) der ursprünglichen myeloablativen Konditionierungsschemata (myeloablative conditioning, MAC) war inakzeptabel hoch. Für solche Patienten wurden Protokolle mit einer reduzierten Intensität zur Konditionierung (Reduced Intensity Conditioning, RIC) entwickelt, um die Toxizität auf ein akzeptables Maß zu senken. Leider führte der Einsatz von RIC zu einer höheren Rate von Rezidiven als unter einem MAC-Regime. Der Erfolg von RIC beruht zum Teil auf dem Graft-versus-Malignancy-Effekt. So besteht weiterhin ein Bedarf an verbesserten Konditionierungsschemata, die gegen fortgeschrittene und aggressive maligne hämatopoetische Erkrankungen wirksam sind. Die Schemata müssen aber eine gute Verträglichkeit und akzeptable NRM-Rate haben. Die Kombination von Treosulfan und Fludarabin (Treo/Flu) ist bereits ein etabliertes Behandlungsschema mit reduzierter Toxizität für Patienten mit einer Reihe von hämatologischen bösartigen und nicht bösartigen Erkrankungen. Eine retrospektive Beobachtungsstudie sollte zeigen, ob die Zugabe von Cytarabin zu diesem Protokoll, und die Verwendung einer höheren Dosis von Treosulfan (14g/m^2 anstelle von 10g/m^2), das Risiko eines Rückfalls bei bösartigen Erkrankungen verringert, ohne dass Toxizität und NRM ansteigen. Diese Studie zielt darauf ab, die Durchführbarkeit, Verträglichkeit und Wirksamkeit von Treo/Flu/AraC als Konditionierungsschema zu bewerten.

Methoden: Ergebnisse von 77 Patienten im Alter von 18 bis 69 Jahren (medianes Alter 54 Jahre), die zwischen Juli 2009 und August 2018 eine allogene HSCT mit Konditionierung nach Treosulfan, Fludarabin und Cytarabin (Treo/Flu/AraC) erhielten, wurden retrospektiv analysiert. Insgesamt wurden 80 Transplantationen ausgewertet. Drei Patienten wurden zweimal unter Verwendung des Treo/Flu/AraC-Protokolls transplantiert. Die Patienten wurden wegen AML, MDS oder MPN behandelt. Nur 28 % der Patienten befanden sich zum Zeitpunkt der Transplantation in der ersten vollständigen Remission (CR1). Das Regime bestand aus Treosulfan 14 g/m^2 intravenös von Tag -6 bis Tag -4 oder Tag -4 bis Tag -2, Fludarabin 30 mg/m^2 intravenös von Tag -6 bis Tag -2 und Cytarabin 2000 mg/m^2 intravenös am Tag -6 bis -5. Bei Patienten, die Transplantate von nicht verwandten Spendern erhielten, wurde zusätzlich von Kaninchen stammendes Antithymozytenglobulin 10 mg/kg von Tag -4 bis Tag -2 intravenös verabreicht. Die Prophylaxe gegen der Graft-versus-Host-Reaktion (graft-

versus-host disease, GvHD) bestand aus Cyclosporin A in Kombination mit Methotrexat oder Mycophenolatmofetil. Das primäre Ergebniskriterium war das rezidivfreie Überleben (RFS). Sekundäre Endpunkte waren das Gesamtüberleben (OS), die NRM, die kumulative Rezidivinzidenz, die kumulative Inzidenz des Neutrophilen- und Thrombozytenengraftments (Einwachsen des Transplantats) und des Chimärismus, die kumulative Inzidenz der akuten und chronischen GvHD sowie das Auftreten von Toxizitäten oder unerwünschten Ereignissen.

Ergebnisse: Die mediane Nachbeobachtungszeitraum umfasste 1161 Tage (3,2 Jahre, Spannweite: 13 Tage–9,8 Jahre). Die ein-, zwei- und dreijährigen RFS-Raten betrugen 47,5 %, 40,7 % bzw. 37.3 %. Die ein-, zwei- und dreijährigen OS-Raten betrugen 59.3 %, 49,3 % bzw. 45,4 %. Die kumulative Inzidenz von NRM betrug 10 % (95 % Konfidenzintervall (CI), 5 %-18 %) nach 100 Tagen, 18,8 % (95 % CI, 11 %-28 %) nach ein Jahr und 20,1 % (95 % CI, 12 %-30 %) nach zwei Jahren. Die kumulative Inzidenz des Neutrophilenengraftments erreichte 85,0 % (95 % CI, (75 %-91 %) am Tag +28. Am Tag +37 hatten alle Patienten ein erfolgreiches Neutrophilenengraftment erreicht. Die kumulative Inzidenz des Thrombozytenengraftments erreichte 82,5 % (95 % CI, 72 %-89 %) am Tag +28. Bis zum Tag +100 war dieser Wert auf 85 % (95 % CI, 75 %-91 %) angestiegen. Der vollständige Spenderchimärismus erreichte 84,0 % (95 % CI, 74 %-90 %) am Tag +28. Die kumulative Inzidenz der aGvHD bis Tag +100 betrugen 38 % (Grad I-IV, 95 % CI, 27 %-48 %), 22 % (Grad II-IV, 95 % CI, 13 %-33 %) und 6 % (95 % CI, 2 %-14 %). Akute Leber-GvHD Grad IV verursachte den Tod eines Patienten. Die kumulative Inzidenz von leichter bis schwerer chronischer GvHD nach zwei Jahren betrug 15 % (95 % CI, 8 %-24 %). Mukositis stellte eine schwere Komplikation bei 41 % der Patienten dar. Bei drei Patienten wurde eine venöse okklusive Leberkrankheit (auch als hepatic sinusoidal obstruction syndrome (SOS) bekannt) diagnostiziert. Ein Patient starb an dieser Komplikation. Bei einem Patienten (1,3 %) trat eine Nephrotoxizität 3-4 Grades auf. Ein Anstieg des Bilirubins und des ALT/AST Wertes erreichte Grad 3-4 in etwa 22 % der Fälle. Alle Organtoxizitäten mit Ausnahme des einen Falls von Nephrotoxizität 3-4^{o} waren letztendlich reversibel.

Fazit: Das Treo/Flu/AraC-Schema stellt eine durchführbare, tolerable und wirksame Konditionierung für Patienten mit AML, MDS oder MPN, auch bei fortgeschrittenen Krankheitsverläufen, dar. Die NRM ist in dieser intensiv vorbehandelten Patientenpopulation akzeptabel. Weitere Studien wären erforderlich, um diese Ergebnisse zu überprüfen und Patienten zu identifizieren, die am meisten von der Therapie mit erhöhter antileukämischer Aktivität profitieren könnten. Eine prospektive randomisierte kontrollierte klinische Studie, in der dieses Schema mit Treo/Flu und FLAMSA-RIC verglichen wird, wäre hilfreich um die Non-Inferiorität oder Superiorität der Treo/Flu/AraC-Konditionierung zu überprüfen.

Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for a variety of haematological disorders, both malignant and benign. Less commonly, it is employed for the treatment for solid tumours and autoimmune diseases (1). The success of an allogeneic HSCT relies on many factors. The preparation begins long before admission to hospital. After a patient gives their consent to the procedure, a battery of tests will be conducted to assess their suitability and to identify if any underlying infections or health problems need to be treated beforehand. A search begins for a suitable donor, either from within the family or in the form of an unrelated donor. The donor should be as good an HLA-match to the patient as possible. Every nucleated cell in the human body expresses glycosylated cell surface proteins called human leucocyte antigens (HLA). They are an important part of the adaptive immune system. T-lymphocytes can only recognise an antigen if it is associated with one of these molecules. The HLA proteins are coded for by a gene complex, also known as the major histocompatibility complex (MHC), on chromosome six. Three of the genes (HLA-A, HLA-B and HLA-C) code for the class I MHC proteins. The HLA-D (HLA-DP, HLA-DR and HLA-DQ) loci codes for the class II MHC proteins. Patients and donors are typically typed based on HLA-A, -B, -C, -DP and -DQ alleles. A fully matched donor and recipient will have identical alleles of these five genes. If a donor´s cell express different HLAs to the recipient, an immune response may be initiated by the donor cells in the recipient. This is known as graft-versus-host disease (GvHD). Therefore, the success of an allogeneic HSCT depends, in part, on the matching of the donor´s and patient´s HLAs. Once a donor is found, the conditioning regimen for the patient can be discussed and chosen. This decision will be based on the individual´s characteristics including diagnosis, comorbidities, age and disease stage and sensitivity to previous treatments. Much research has been conducted in to the safety and efficacy of various regimens, with an emphasis on reducing both medullary and extra-medullary toxicity. Conditioning regimens should reduce malignant disease burden. The graft-versus-malignancy (GvL) effect can be exploited so that pre-transplant conditioning need not completely eradicate all malignant cells in the body. Sufficient immunosuppression should also be provided by the regimen in order to encourage engraftment and reduce the risk of graft rejection and GvHD. This immunosuppressive effect can be, for example, provided by low dose total body irradiation (TBI) with 2 Gray (Gy) (2, 3). Additionally, prior to the infusion of the donor stem cells, the patient begins receiving immunosuppressive drugs to further reduce the incidence of GvHD and rejection. Antithymocyte globulin (ATG) is also given prior to the administration of stem cells to reduce the risk of GvHD. The immunosuppressive drugs are continued for some months after the transplant and are tapered off in the absence of GvHD. The immunosuppressive regimen must also allow for the development of the graft-versus-leukaemia (GvL) effect, whereby the donor cells reduce the risk of relapse of the malignant

condition by killing the residual leukaemic or malignant cells. Balancing the level of immunosuppression is difficult and requires an experienced clinician and regular monitoring of disease activity and donor-host chimaerism. From beginning to end, this process is fraught with potential complications. The immune system and the interaction between the host and the graft is complex. Even with current advances in our knowledge, the outcomes seen after allogeneic HSCT, though far better than they were 50 years ago, are still not optimal for every patient. The aim of current research is to understand the factors that limit the success of a transplant and how to use that knowledge to improve the outcomes for our patients.

This work will focus on patients with acute myeloid leukaemia (AML), myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN) that were conditioned with a combination of treosulfan, fludarabine and cytarabine (Treo/Flu/AraC) before allogeneic HSCT. Here, the haematological conditions included in the study will be briefly outlined. Then follows a short summary of the history of allogeneic haematopoietic stem cell transplantation, with an emphasis on the conditioning regimens that have been trialled. The regimen that has been used in this study, and the rational for its use, will be outlined and will be followed by a short discussion on the current use of ATG in allogeneic HSCT.

Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterised by ineffective haematopoiesis. The term, ‘clonal stem cell disorder’, describes the situation in which alterations in a single haematopoietic progenitor cell can give rise to an entire disease (4). The clonal evolution of this disorder can lead to the development of acute myeloid leukaemia. MDS most commonly affects the elderly, with a median age at diagnosis of 70 years. The condition can arise *de novo,* from exposure to environmental toxins, or as a result of treatment of a primary malignancy. Cases of MDS present with dysplasia, refractory peripheral blood cytopenia (most commonly anaemia) and bone marrow that shows either normal or increased cellularity (5). Dysplasia, an abnormality in the maturation of cells, can affect one or more haematopoietic lineages. Commonly observed dysplastic changes in MDS include megaloblastoid erythroid maturation, neutrophil hypolobulation or hypogranulation and small megakaryocytes. Cytopenias, deficiencies of a particular type of blood cell, are most likely caused by dysregulated cell death pathways in haematopoietic precursors (6). In MDS, the apoptotic index of cells found in bone marrow biopsies is consistently higher than in healthy subjects and cells of all stages of maturation, ranging from blasts to terminally mature cells belonging to all three lineages are affected (7). Most sufferers will die of complications associated with their cytopenias.

There have been numerous attempts to create a classification system for MDS. The systems that have been devised rely on morphologic, cytochemical and immunophenotypic features of

the pathological cells. The World Health Organisation (WHO) classification system is the one in current use and was revised in 2016 (8). The system relies on an integrated approach tying in haematologic, morphologic, cytogenic and molecular genetic findings. Cytopenic and morphological changes have been refined along with new information that has emerged on the genetics of MDS. The number of dysplastic lineages and cytopenias, as well as the peripheral and bone marrow blast counts and the presence or absence of ringed sideroblasts, continue to make up a central part of the classification system. In the case of myeloid neoplasms, the malignant blast cells are immature precursors of granulocytes. These cells lose their ability to differentiate normally and do not respond to normal regulators of cell proliferation.

The best treatment choice for MDS is based on several factors. The goals of therapy are to ameliorate symptoms and reduce the risk of transformation to AML, thereby improving quality of life and potentially improving prognosis. As the average age at diagnosis is 70, many of those diagnosed have pre-existing comorbidities, which may limit the treatment that can be offered. At the time of diagnosis, a patient will be stratified according to the Revised International Prognostic Scoring System (IPSS-R) into very low risk, low risk, intermediate risk, high risk and very high risk (9). Patients receive a score based on their cytogenetic risk group, marrow blast count and severity of cytopenia. The IPSS-R is yet to incorporate molecular genetic information to group patients as the prognostic significance of commonly found mutations needs to be further investigated. The scoring system is also only validated for adult patients with *de novo* disease treated according to best supportive care guidelines (10). Despite these limitations, the IPSS-R score still forms a large part in the decision-making surrounding treatment options. These options range from watchful waiting to potentially curative therapy. Concurrent comorbidities and the patient´s performance status also play a sizeable role in deciding which treatments patients can reliably tolerate. Patients are assigned a performance status based on their general well-being and ability to perform normal daily activities. The most commonly used scoring system is the Karnofsky Index, which runs from 100 (perfect health) to 0 (death).

Early treatment of MDS has not been shown to reduce the risk of AML transformation or death. Therefore, observation is appropriate for asymptomatic low risk patients until they display symptoms (11). Erythropoiesis-stimulating agents with or without granulocyte colony stimulating factor (G-CSF) can improve anaemia in 40-50 % of anaemic patients with low-risk MDS (12). Blood and/or platelet transfusions can be offered for symptomatic cytopenia. After repeated blood transfusions, an iron chelator may be necessary, and this is generally offered at a ferritin level above 1000 ng/ml. The most appropriate treatment of neutropenia has not yet been established. It often does respond to myeloid growth factors, but they have not been shown to improve survival or to significantly reduce infection risk (13).

Within the revised 2016 WHO classification of myelodysplastic syndromes there is a distinct clinical entity known as MDS with isolated del(5q). This is a distinct haematological disorder, first described in 1974 (14), associated with an acquired deletion of the long arm of chromosome 5. The region that is most commonly found to be deleted is a 1.5 megabase interval at 5q32 (15). Patients with this 5q deletion syndrome tend to have a good prognosis with a longer life expectancy compared to other MDS subgroups and a lower risk of progression to AML (16). These patients may be offered therapy with lenalidomide (a derivative of thalidomide). This drug is an immunomodulator with several mechanisms of action. It has a direct effect on MDS blast cells by both inhibiting their proliferation and inducing apoptosis, as well as an immune modulatory effect by activating T- and natural killer (NK)-cells (17). For those with intermediate and high risk MDS, without the 5q deletion syndrome, and for those for whom a transplant is not planned, 5-azacytidin, a cytidine analogue and hypomethylating agent, can be administered. Treatment with 5-azacytidin has been shown to improve overall survival and prolong time to AML transformation (18). A once daily administration over seven continuous days has been found to be the most effective dosing schedule (19). Those with intermediate to high risk disease may be offered an allogeneic HSCT if they are deemed suitable, and if they so choose. This is the only treatment to offer the chance of a long-term cure. It is not clear if bridging therapy as an attempt at reducing the disease burden, also known as cytoreduction, prior to allogeneic HSCT is beneficial in MDS. A recent review on the matter concluded that in those patients with more than 10 % blasts, cytoreductive therapy could be considered, especially if reduced intensity conditioning is planned (20). Those with less than 10 % blasts should proceed straight to transplantation. However, the results of randomised controlled trials are still awaited.

Acute Myeloid Leukaemia

Like the myelodysplastic syndromes, acute myeloid leukaemia is a complex and heterogeneous malignant disease, affecting the myeloid cell lineage. The condition is characterised by an increase in the number of immature myeloid cells (blasts) in the bone marrow (21). It may arise *de novo,* be secondary to MDS/MPN or secondary to exposure to cytotoxic drugs used to treat other malignancies. The median age at diagnosis is 70 years. After chronic lymphocytic leukaemia, it is the most commonly diagnosed leukaemia in adults (22). It still has a very poor prognosis and is fatal in around 80 % of patients. According to a large study performed by the Swedish Acute Leukaemia Registry, outcomes were strongly dependent on age and performance status (23). Other factors affecting overall survival and relapse-free survival include: existing comorbidities, disease characteristics including cytogenetics and available treatment options. AML presents with a range of signs and symptoms including those associated with bone marrow failure (fatigue, bleeding, increased number and severity of infections and atypical infections) and organ infiltration with leukaemic

cells (splenomegaly, lymphadenopathy, gingival hyperplasia and, rarely, pericarditis and myopericarditis (24)). Signs and symptoms caused by infiltration of the brain and lung with leukaemic cells have also been reported (25, 26). Blood results may reveal a bi- or pancytopenia, or indeed a leucocytosis with or without circulating blasts.

Like MDS, the classification of AML has undergone a revision since the last WHO update in 2008. There is an increased focus on significant cytogenetic and molecular genetic subgroups, reflecting the advances in research in this field (8). Treatment of AML is based partially on the risk profile afforded by the specific mutations found in the leukaemic blast cells (see Table 1).

Table 1: *2017 European LeukemiaNet (ELN) risk stratification by genetics. Reproduced from [23]*

Risk Category	Genetic Abnormality
Favourable	t(8;21)(q22;q22.1); RUNX1-RUNX1T1 inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 Mutated NPM1 without FLT3-ITD or with FLT3-ITDlow* Biallelic mutated CEBPA
Intermediate	Mutated NPM1 and FLT3-ITDhigh* Wild-type NPM1 without FLT3-ITD or with FLT3-ITDlow* (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); MLLT3-KMT2A Cytogenetic abnormalities not classified as favourable or adverse
Adverse	t(6;9)(p23;q34.1); DEK-NUP214 t(v;11q23.3); KMT2A rearranged t(9;22)(q34.1;q11.2); BCR-ABL1 inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1) 25 or del(5q); 27; 217/abn(17p) Complex karyotype, monosomal karyotype Wild-type NPM1 and FLT3-ITDhigh* Mutated RUNX1, ASXL1 or TP53

*Low: low allelic ratio (<0.5), high: high allelic ratio (≥0.5)

A mutation in the nucleophosmin (*NPM1*) gene is the most commonly acquired molecular abnormality in AML cells (27). NPM1 is expressed ubiquitously and is predominantly localised in the nucleolus. It shuttles between the nucleus and cytoplasm and has been implicated in multiple cell functions, including ribosomal protein assembly and transport and control of centrosome duplication (28). Mutations in exon 12, the part of the gene that encodes for the nucleolar localisation sequence, seem to be the most commonly associated with AML (29). An *NPM1* mutation in leukaemic cells with a normal karyotype and no further genetic abnormalities provides a good prognosis. At the other end of the spectrum leukaemic cells with a complex karyotype and a 5q deletion provide a poor prognosis for the patient. However, the course of the disease and response to standard treatment is also a big factor in considering the best treatment to offer the patient. There is a significant survival advantage for those who achieve complete remission (CR) within 100 days of starting standard therapy (30). The most commonly employed treatment protocol consists of two rounds of induction therapy followed

by three to four rounds of consolidation therapy. Induction consists of, for example, a seven-day course of a low-dose continuous infusion of cytarabine combined with a three-day course of daunorubicin, a DNA intercalating agent. Consolidation therapy consists generally of three to four courses of high dose cytarabine. If, on day 15 after the first induction therapy, there are still more than 5 % myeloid blasts in the bone marrow, the initiation of a salvage or rescue therapy should be considered. Frequently used regimens include FLAG (fludarabine, cytarabine, G-CSF), with or without idarubicin (Ida), or S-HAM (sequential high dose cytarabine and mitoxantrone). Although idarubicin and mitoxantrone stem from different drug classes, they both prevent DNA replication by interfering with the action of the enzyme topoisomerase II. Suitable patients with a high-risk leukaemia should be informed early on of their option to undergo an allogeneic HSCT. A transplantation may be performed as early as following the first course of induction.

As we learn more about the molecular biology of acute myeloid leukaemia, more targeted therapies are being developed and approved. One therapy of note is for patients with mutations in the gene *FLT3* (f*ms*-like tyrosine kinase). This gene encodes a receptor tyrosine kinase that regulates haematopoiesis and the mutations that lead to AML cause a constitutive activation of this enzyme. Midostaurin, a tyrosine kinase inhibitor, has been approved for use in patients with *FLT3* mutations based on the international phase III RATIFY study (31). Midostaurin was given orally and combined with standard chemotherapy, induction and consolidation, and patients received midostaurin as maintenance therapy for a year after completion of standard treatment. The placebo-controlled study showed a significant improvement in overall and event-free survival for patients treated with midostaurin. Whilst research is ongoing in to specific drug targets in acute myeloid leukaemia (22), for many patients with high risk disease characteristics, an allogeneic HSCT remains the only option to induce long term remission.

Myeloproliferative Neoplasms

The myeloproliferative neoplasms (MPN) are a collection of seven pathological entities that occur due to the clonal expansion of one or more haematopoietic cell lineages in the bone marrow. The diagnosis is based on bone marrow histology and mutational analysis of cells. The conditions are classified as one of the following: polycythaemia vera, essential thrombocythaemia, primary myelofibrosis, chronic myeloid leukaemia (CML), chronic neutrophilic leukaemia, chronic eosinophilic leukaemia (not otherwise specified) and MPN unclassifiable. The diagnosis still depends heavily on bone marrow morphology, but mutational screening and analysis is becoming a bigger part of the diagnostic process. The classification system for MPN was also updated in 2016 by the WHO (32). Changes partially reflected the advances in the understanding of the molecular biology of this group of diseases and the advances regarding the characterisation and standardisation of morphological bone marrow features. Patients present with features specific to the condition, for example, unprovoked

thromboses in essential thrombocythaemia. In the case of CML, 30-50 % of those diagnosed will have been asymptomatic at diagnosis, and the condition will have been picked up on a routine blood test (33). Symptomatology is associated with the phase of presentation, of which there are three (defined by the WHO): chronic stable, accelerated and blast crisis (8). Symptomatic presentations of CML include nonspecific symptoms of weight loss and fatigue and symptoms related to hepato- or splenomegaly such as early satiety and abdominal pain. Those in the accelerated or blast crisis phase may present with symptoms of bone marrow failure such as bleeding and increased frequency and severity of infections.

Treatment of CML is also based on the individual condition and genetic mutations found within the aberrant cells. The Philadelphia chromosome, created by a translocation between chromosome 9 and 22, leads to the production of the fusion gene *BCR-ABL*. This gene encodes for a constitutively active tyrosine kinase and is present in almost all cases of CML. Several targeted tyrosine kinase inhibitors (TKIs) are available to treat CML. Their discovery was seen as the first successful example of targeted therapy for a malignant disease. Prior to the introduction of TKIs, allogeneic HSCT was a standard treatment for CML (34). In the TKI era, there are still several indications for transplantation, although the timing of transplantation remains controversial (35). TKI therapy may not be possible due to the absence of the Philadelphia chromosome (Ph^- CML). If TKI therapy is indicated, it may become ineffective due to the development of resistant mutations such as T315I or cannot be tolerated. In these cases allogeneic HSCT may be indicated. Patients presenting in the accelerated phase or in blast crisis also benefit from transplantation (36).

The constitutively activating V617F mutation of the JAK2 (Janus Kinase 2) is found in 97 % of all cases of polycythaemia vera (PV) (37). JAK2 is a member of a family of tyrosine kinases involved in cytokine receptor signalling. The main treatment of PV is venesection to maintain the haematocrit level under 45 %. If this is unsuccessful, then hydroxyurea may be considered. For those patients who do not respond to either of these two therapies, a JAK1/2-inhibitor is available. Ruxolitinib was approved, based on a phase 3 open label study called RESPONSE, which showed that in these patients, ruxolitinib was superior to standard therapy in controlling haematocrit, reducing spleen volume and improving symptoms associated with the disease (38).

Essential thrombocythaemia (ET) will be found to have the JAK2-V617F-mutation in 55 % of cases. Other mutations that are commonly found are in the genes encoding calreticulin (*CALR*, 25 %), additional sex combs-like 1 (*ASXL1*, 11 %) and myeloproliferative leukaemia virus oncogene (*MPL*, 3 %). Therapy involves reducing the risk of thromboembolism, by keeping the platelet count under 400,000/µl and leukocytes under 10,000/µl. Hydroxyurea and anagrelide (inhibitor of platelet maturation from megakaryocytes) are both options based on patient age,

comorbidities and clinician preference. The use of ruxolitinib in patients with JAK2 mutations is not routine, due to the publication of two conflicting studies in 2017. The MAJIC trial, a randomized trial comparing ruxolitinib to best available therapy in a total of 110 patients, did not show superiority of ruxolitinib to current second line treatments for ET (39). However, an open label phase 2 trial including 39 patients, conducted by the MD Anderson Cancer Centre in Texas, concluded that patients who were refractory to or intolerant of hydroxyurea could achieve clinically meaningful and durable reductions in platelet and leucocyte counts and improvements in disease-related symptoms under ruxolitinib (40). The results of these trials are difficult to compare as the trial structures, sizes and follow-up times are radically different. Median follow-up during the MAJIC trial was 31.3 months, whereas the MD Anderson group followed patients for 82.8 months. Certainly, large randomised controlled trials are required to assess the effectiveness of ruxolitinib.

Advanced primary myelofibrosis is often treated palliatively with erythrocyte and thrombocyte transfusions and the administration of erythropoietin. In 60 % of patients, the JAK2 mutation will be present. Ruxolitinib is approved for the treatment of myelofibrosis (primary and secondary to PV and ET) to improve splenomegaly and disease-related symptoms (41). The United States Food and Drug Administration (FDA) has recently approved a second JAK2 inhibitor, Fedratinib, for use in myelofibrosis based on the phase III JAKARTA trial (42). An allogeneic HSCT should also be considered for suitable patients with a poor prognosis. Several scoring systems are available to assess prognosis in primary and secondary myelofibrosis including IPSS, DIPSS, MIPSS70 (primary myelofibrosis) and MYSEC-PM (secondary myelofibrosis) (43). A failure to respond to ruxolitinib is a further indication to proceed to transplantation.

A Brief History of Allogeneic Haematopoietic Stem Cell Transplantation

The first report of an allogeneic HSCT performed in humans was published in 1957 by E. Donnall Thomas (44). Patients were treated with chemotherapy and radiation and then infused with bone marrow harvested from an unmatched donor. Only two of the six patients engrafted, and all died within a hundred days of the transplant. The first allogeneic HSCTs attempted with matched donors were performed in the late 1960s (45, 46). These transplants were performed in patients who were suffering from severe inherited immunodeficiency disorders. However, it was already clear from animal studies, that an immune competent host would need to be immunosuppressed in order to achieve engraftment (47). Engraftment is the process by which the stem cells within the graft find their way into bone marrow niches and begin to proliferate. By the 1970s, allogeneic HSCTs were being performed in patients with acute leukaemias and aplastic anaemia, as well as inherited immunodeficiency disorders. Conditioning regimens at the time included high dose cyclophosphamide (45-50 mg/kg over 4 days)(48, 49) and total body irradiation (TBI, total doses generally between 10 Gray and 16 Gray) (50).

Due to the relatively poor tolerability of regimens containing high-dose TBI, researchers were spurred on to find alternatives. Animal studies in the 1970s led to the study and introduction of busulfan in conditioning regimens for humans (51, 52). Busulfan is an alkylating agent that causes crosslinks between strands of DNA - inhibiting DNA replication. One of the early studies in the 1980s combined busulfan with cyclophosphamide in patients with Wiskott-Aldrich syndrome, and showed that they were able to achieve engraftment without the use of TBI (53). It was postulated by some at the time, that only TBI could provide satisfactory ablation of host haematopoiesis to allow for engraftment. Three years later, a study comparing a regimen containing TBI to conditioning using busulfan and cyclophosphamide in patients with congenital bone marrow disorders, showed that the regimens were comparable for rates of engraftment. However, transplant related mortality was higher in those treated with TBI (54). In the years following these studies, busulfan was established as one of the mainstays in conditioning regimens, alongside the established cyclophosphamide and TBI. Over the years, researchers have attempted to alter the doses and application of these three treatments to reduce toxicity or improve relapse-free survival (55, 56). Some have combined these treatments with other substances such as etoposide for acute lymphoblastic leukaemia (57), thiotepa for genetic diseases including β-thalassaemia (58) or melphalan for AML (59).

Today, conditioning regimens are often grouped in to three categories: myeloablative conditioning (MAC), reduced intensity conditioning (RIC) and nonmyeloablative conditioning (NMC) (60). These classifications are based on the duration of the cytopenia that the regimen causes and whether stem cell support is needed or not. At the far end of the spectrum, MAC causes irreversible cytopenia with a requirement for stem cell support to resume normal haematopoiesis. At the other end, nonmyeloablative regimens do not require stem cell support and cause minimal cytopenia. Reduced intensity conditioning does not fit in to either of these categories, as the cytopenia may be reversible, but stem cell support is generally required. Reduced intensity regimens rely on the GvL effect to reduce the risk of relapse, rather than the cyto-reductive effect of the conditioning therapy. Conditioning regimens containing TBI (≥ 5 Gy single dose or ≥ 8 Gy fractionated dose) and busulfan (> 8mg/kg) are considered myeloablative. Within the past 25 years, the use of RIC for patients not deemed suitable for MAC, has become popular. Many of the RIC regimens contain fludarabine, a purine analogue, which was introduced into treatment protocols in the late 1990s (61). Fludarabine is often combined with another agent such as busulfan or melphalan in reduced doses or with reduced dose TBI. The use of fludarabine in conditioning regimens will be discussed in more detail in the next section.

In an attempt to tackle the problem of poor relapse rates in high-risk AML, a regimen known as FLAMSA-RIC, was introduced in 1999. The FLAMSA regimen contains fludarabine, amsacrine and cytarabine. Amsacrine is an acridine derivative first described in the 1970s (62).

Although its exact mechanism of action remains to be elucidated, it is thought to intercalate with DNA and inhibit the activity of topoisomerase II (63). It has been employed in the treatment of acute myeloid leukaemia and may be able to induce remission in patients refractory to daunorubicin and cytarabine (64). The first FLAMSA-based protocol was combined with a TBI and cyclophosphamide RIC regimen (65). The rationale behind this regimen was to reduce the leukaemic burden in high-risk AML patients by giving a short intensive course of chemotherapy and then RIC before transplantation. The reduction of leukaemic disease burden gives GvL time to develop and eradicate remaining malignant cells. Several iterations of RIC portion of the FLAMSA-RIC protocol have been trialled including; treosulfan/cyclophosphamide (66), busulfan/cyclophosphamide (67) and busulfan/fludarabine (68).

Across almost all studies, comparing outcomes based on conditioning intensity, the greater the intensity, the lower the risk of relapse, but the higher the NRM (69). This balancing act between reducing the risk of relapse, promoting GvL and safety, is extremely challenging.

The number of allogeneic HSCTs performed yearly has increased dramatically since the 1970s (70, 71). In 2016, according to the European Society for Blood and Marrow Transplantation (EBMT), 16,507 allogeneic haematopoietic stem cell transplants were performed by 679 centres in 49 countries (1). The list of indications for performing a transplantation has lengthened since its inception over 50 years ago. Whilst great progress has been made in developing the regimens used for conditioning, there is still much room for improvement. Protocols should be tailored to the conditions and individuals being treated, taking in to account disease biology as well as patient history and comorbidities. The search for a regimen with more intensive anti-leukaemic activity, but with limited toxicity, is what has driven the development of the combination of drugs used in this study.

Conditioning with Treosulfan, Fludarabine and Cytarabine

This section will be structured as an introduction to the development of the conditioning regimen containing treosulfan and fludarabine, followed by an explanation as to the reasons behind the addition of cytarabine.

Treosulfan is a bifunctional alkylating agent that has been approved in several European countries since the late 1990s for use in ovarian cancer. It is a hydrophilic analogue of busulfan. Within its use as a treatment for ovarian cancer it has been seen to be well tolerated, especially in elderly patients, with toxicities being classified as moderate (72, 73). The highest tolerated dose before haematological toxicity becomes unacceptable has been found to be 10 g/m^2 (74). Whilst the use of busulfan at doses required for successful MAC is often limited by toxicities, such as interstitial pneumonitis, convulsions, sinusoidal obstruction syndrome (SOS), mucositis and haemorrhagic cystitis (75), treosulfan has been seen to have an improved extra-medullary toxicity profile at doses up to 46 g/m^2 (76). Furthermore, treosulfan demonstrates

pronounced *in vitro* committed (unipotent) and non-committed (pluripotent) haematopoietic stem cell toxicity (77). This is in contrast to busulfan, which preferentially depletes non-committed stem cells (78). Furthermore, it has also been demonstrated to have improved anti-leukaemic activity when compared with busulfan in an *in vitro* analysis of leukaemic cells from paediatric patients (79). These properties made treosulfan a good candidate for use in conditioning prior to allogeneic HSCT.

In 2004, an initial study was published assessing the safety, tolerability and efficacy of a conditioning regimen containing treosulfan and fludarabine (Treo/Flu) in 30 patients who were not otherwise eligible for standard conditioning (80). The haematological malignancies included in the study were AML, MDS, CML, multiple myeloma, non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL). Only 10 % of the patients were in CR1 at the time of transplantation. Patients received fludarabine at a dose of 30 mg/m^2 intravenously from day -6 to day -2 (day 0 defined at the day the transplant takes place) and treosulfan at a dose of 10 g/m^2 from day -6 to day -4. Patients receiving an unrelated donor transplant were given ATG (10 mg/kg, day -4 to day -2). Extramedullary toxicity was generally mild and sinusoidal obstruction syndrome, cardiac and pulmonary toxicity were not observed. SOS is an obliterative venulitis of the terminal hepatic venules caused by, among other things, cytoreductive therapy prior to allogeneic HSCT (81). The mechanism of injury is believed to be damage to endothelial cells in the liver causing their necrosis and extrusion into the terminal hepatic venules and sinusoids (liver capillaries), leading to obstruction and congestion. It is a feared complication because of its high risk of mortality. Overall survival- and treatment-related mortality rates of the Treo/Flu regimen compared favourably to existing regimens and given the selection of patients in this small study, the results were very promising. Since that first study was published, several other small studies have been published looking at this regimen to treat conditions including AML, MDS, CML and multiple myeloma (82-86). Other studies have combined treosulfan with other agents, including cyclophosphamide, thiotepa and replacing TBI in the FLAMSA-RIC protocol (66, 87). The regimen has also been studied in non-malignant diseases such as chronic granulomatous disease, sickle cell disease and β-thalassaemia (88, 89). A large-scale retrospective multicentre analysis, using registry data from the EBMT, comparing the outcomes of patients with *de novo* or secondary AML, treated with busulfan or treosulfan-based conditioning regimens, was published in 2017 by Shimoni *et al.* (90). This showed that the outcomes were similar for both regimens but that treosulfan can be administered in older patients with lower rates of GvHD. There was also the suggestion that the outcomes after using treosulfan are better in patients who were not transplanted in remission. In 2018, a further large analysis, again using registry data of the EBMT, comparing the use of treosulfan and fludarabine with two FLAMSA (fludarabine, amsacrine and cytarabine) based protocols (FLAMSA/TBI and FLAMSA/busulfan) in patients with AML was

published (91). The multivariate analysis showed that those conditioned with FLAMSA/TBI had a decreased risk of relapse and better leukaemia-free survival rates compared to treosulfan and fludarabine. However, rates of acute GvHD (aGvHD) were significantly higher in those treated with FLAMSA/TBI. Overall survival, non-relapse mortality and chronic GvHD (cGvHD) were not significantly different between the groups.

A recently published open-label, randomised, non-inferiority, phase III clinical trial, compared the outcomes of patients with AML (in CR) or MDS undergoing allogeneic SCT with either Treo/Flu or Bu/Flu conditioning. A non-inferiority trial is designed to determine if a new treatment is not worse than an established treatment by a predetermined amount with a given degree of confidence (92). This study has demonstrated the non-inferiority of Treo/Flu compared to Bu/Flu as a conditioning regimen (93). Two-year overall survival, transplant-related mortality and non-relapse mortality were all significantly better in the Treo/Flu group compared to those conditioned with Bu/Flu. Event-free survival was also better in the Treo/Flu group, but the difference did not achieve the rigid significance level set for superiority. During the implementation of this trial, two important observations with regard to the design of the Treo/Flu protocol came to light. Firstly, the dose of treosulfan in the initial trial design was 14 g/m^2. Due to concerns about prolonged neutropenia and related infectious complications in a planned interim analysis, the dose of treosulfan was reduced to 10 g/m^2. Secondly, due to the same concerns, the first dose of treosulfan was administered on day -4 instead of day -6. Again, the aim was to reduce the length of the neutropenic pre-engraftment phase.

Fludarabine, a purine analogue, enters cells via active transport as the free nucleoside 9-β-Darabinosyl-2-fluoroadenine (F-ara-A). Inside the cell it is phosphorylated to form the 5′-triphosphate, F-ara-ATP. F-ara-ATP is required for the cytotoxic effect of fludarabine. The principal mechanism of action is via the inhibition of DNA synthesis, although it is also able to inhibit RNA synthesis (94). One of its main indications is in the treatment of chronic lymphocytic leukaemia (95), however, with the advent of the new tyrosine kinase inhibitors, such as ibrutinib, its place is being questioned. Fludarabine is also incorporated into the FLAG (Fludarabine, cytarabine, G-CSF) salvage regimen for the treatment of refractory or relapsed AML. Fludarabine has recently replaced cyclophosphamide in many conditioning regimens. A large meta-analysis, published in 2016, comparing busulfan/fludarabine and busulfan/cyclophosphamide regimens concluded that both regimens have similar efficacy, but that toxicity was lower with the fludarabine containing regimen (96).

In the two centres in this study, treosulfan and fludarabine have been combined with high-dose cytarabine (2000 mg/m^2). Cytarabine, a pyrimidine analogue, is one of the chemotherapeutic backbones in the treatment of haematologic malignancies. It is included in regimens for: AML, acute lymphoblastic leukaemia (ALL), aggressive non-Hodgkin lymphoma, indolent non-

Hodgkin lymphoma and primary central nervous system (CNS) lymphoma. It has been used in AML for over four decades and is one of the most effective drugs in the arsenal against this condition (97). It has been shown to be effective even in salvage therapies in the treatment of refractory AML (98). Resistance to cytarabine has been documented, and the underlying mechanism is thought to be caused by a number of factors including reduced levels of activating enzymes and increased levels of inactivating enzymes. This ultimately leads to a reduction in the intracellular levels of the active metabolite ara-CTP in the leukaemic cells (99). When fludarabine is infused prior to the administration of cytarabine, there is evidence to suggest that the intracellular accumulation of ara-CTP is potentiated (100). The combination of fludarabine and cytarabine could, therefore, sensitise leukaemic cells that were previously resistant to cytarabine.

Based on the evidence discussed above, the combination of cytarabine with fludarabine and treosulfan should provide a tolerable regimen with improved antileukaemic activity compared to standard Treo/Flu conditioning. The addition of cytarabine, with the synergistic effects of fludarabine, and potent stem and leukaemic cell toxicity of treosulfan, should make this a good regimen for patients transplanted with active disease and those with a high risk of relapse. When compared to the long pre-transplant conditioning phase of FLAMSA-RIC (12- 13 days), the shorter duration of this regimen could result in a reduction of infectious complications.

Antithymocyte Globulin and Its Use in Allogeneic HSCT

In the two centres involved in this study, rabbit ATG was given to all patients who received an unrelated donor transplant. ATG is the purified polyclonal IgG fraction of sera from rabbits, horses or, rarely, goats that have been immunized with thymocytes or T-cell lines. It is given in allogeneic HSCT to deplete the T-lymphocytes in the graft. Additionally, it depletes host T-lymphocytes that have survived the conditioning and thereby reduces the risk of graft rejection. It is thought to deplete T-lymphocytes through a combination of complement-dependent lysis, T-cell activation and apoptosis. However, there is evidence that ATG does more than just deplete T-lymphocytes and it is thought to have, through various mechanisms, an immunomodulatory effect as well (101). Mechanisms that have been suggested include; the modulation of key cell surface molecules that mediate leukocyte/endothelial interactions; apoptosis induction in B-cell lineages; interference with the function of dendritic cells; and induction of T-regulatory and natural killer T cells.

Use of ATG goes back over 40 years in allogeneic HSCT, but its use continues to be controversial. It has been reported to significantly reduce the incidence of acute and chronic GvHD (102). However, there are some conflicting reports about the risk reduction of aGvHD following the administration of ATG (103). Reducing the risk of GvHD is especially important in unrelated donor transplants and peripheral blood stem cell transplants, both of which are

associated with a higher risk of the condition (104). ATG does not, however, according to a Cochrane analysis, affect overall survival, relapse rate or non-relapse mortality (105). However, a large retrospective study that looked at the use of ATG in patients undergoing RIC, found that the use of *in vivo* T-cell depletion led to a higher incidence of disease relapse (106). This effect was deemed likely due to the reduced GvL effect following T-cell depletion. ATG has also been linked to an increased incidence of cytomegalovirus (CMV) and Epstein-Barr-virus (EBV) reactivation and a significant delay in neutrophil engraftment (107, 108).

Whilst ATG has classically been used in patients receiving a transplant from an unrelated donor, the use in patients receiving stem cells from a sibling donor has also been examined. A small early study of 56 patients with haematological malignancies receiving MAC showed no significant toxicity but also no beneficial effect of giving ATG (109). In contrast, a recent prospective, multicentre, open-label, randomised phase III study in 168 patients receiving MAC and a transplant from a related donor showed a significantly lower rate of chronic GvHD in those receiving ATG (110). Survival rate was similar and, perhaps surprisingly, relapse-free survival was also higher in those receiving ATG.

A recent discussion in the journal, Blood Advances, examined the current opinion on the use of ATG in Europe compared with that in the USA. In this article, Andreas Bacigalupo argued strongly for the continued use of ATG in unrelated donor transplants as well as those from related donors (111). This was based on a number of randomised trials that show no increased risk of disease relapse related to the use of ATG (112, 113). However, the counter argument presented by Kekre and Antin from the USA and Canada, respectively, spoke against the blanket use of ATG and called for a more measured approach (114). They recommended the use of ATG to be considered on an individual patient basis. Those who are at an increased risk of relapse, including those at an advanced disease stage, those who have a poor cytogenetic profile or those receiving RIC, should not be given ATG due to the reduction in the GvL effect. As mentioned earlier, an increased risk of disease relapse was seen with the use of ATG after RIC (106). The authors referenced a recent American prospective, randomised, double-blind, phase III clinical trial encompassing 254 patients undergoing MAC and transplants from unrelated donors, suggesting that the use of ATG did not improve moderate to severe cGvHD-free survival, but the rate of moderate to severe cGvHD was lower in those receiving ATG. Worryingly however, progression-free survival and overall survival were worse in those patients receiving ATG (115). Why this study did not reproduce the findings of previous trials, in which progression-free survival and overall survival were not affected by the administration of ATG, is not clear. The authors offer some suggestions, which included; the dosing of ATG used (50 % higher in this study than in others), difference in the donors used (unrelated vs. sibling) and the impact of the conditioning regimen. There is no clear-cut answer

as to whether the use of ATG in allogeneic HSCT is appropriate for every patient and more research is needed to define the patients who will benefit the most.

Conclusion and Goal of the Study

Allogeneic HSCT is an important treatment modality for haematological malignancies. In an era where ever more targeted therapies are being developed, the conditioning of patients prior to allo-HSCT should also aim to target the underlying disease being treated. This should not only make the treatment more effective by reducing the risk of relapse, but also improve non-relapse mortality. For the reasons described in the previous sections, the addition of cytarabine may improve the antileukaemic activity of the Treo/Flu protocol. Furthermore, the shorter duration of Treo/Flu/AraC (six days) in comparison to FLAMSA-RIC (twelve-thirteen days) may improve NRM over the FLAMSA-RIC protocol, due to a potential reduction of the length of the neutropenic phase. The goal of this retrospective analysis is to observe the feasibility of the regimen and outcomes of patients conditioned with Treo/Flu/AraC. Questions regarding the superiority of this regimen over existing Treo/Flu and FLAMSA-RIC regimens can only be definitively answered by prospective randomised controlled trials, but a rough comparison of the outcomes observed here will be made with those of existing trials in similar patient populations.

Patients and Methods

A retrospective analysis of patients conditioned with a regimen containing treosulfan and fludarabine in combination with cytarabine before allogeneic HSCT was performed. Data was collected on patients between July 2009 and August 2018 from two centres, University Hospital Oldenburg and University Hospital Jena in Germany. Patients were followed up until June 2019.

Patients

In total 77 patients with AML, MDS and MPN who were between 18 and 69 years of age (median age 54 years) were conditioned before allogeneic HSCT using treosulfan and fludarabine in combination with cytarabine. In total, 80 transplantations were evaluated.

The decision to give this regimen was made by an experienced clinician and based on, amongst other considerations: comorbidities, previous response to therapy and disease status. All patients gave written informed consent to the treatment. Patient characteristics are listed in Table 2. The number of pre-treatments were defined as one or more cycles of chemotherapy or tyrosine kinase inhibitors that were intended to induce or consolidate remission. Salvage chemotherapy, given due to insufficient response or relapse, was considered to be a further line of treatment. Prior allogeneic HSCT was also counted as one line of treatment.

The disease status of patients prior to transplantation was defined according to the Center for International Blood and Marrow Transplant Research (CIBMTR) (116).

A note on the HCT-CI: the haematopoietic cell transplantation-specific comorbidity index (HCT-CI) was devised by Mohamed Sorror et al. and published in 2005 (117). It is also known as the Sorror score. Scores are given based on the presence of comorbidities (for a full list and a tutorial on how to score patients, please see (118)). It provides valuable prognostic information and predictions for non-relapse mortality and overall survival rates following allogeneic HSCT. The original study examined the outcomes of patients with a range of haematologic conditions, both malignant and non-malignant. There have been some criticisms of the validity of the score with one single-centre study by Birninger *et al.*, published in 2011, who concluded that it had no predictive value in patients with high-risk AML (119).

Table 2: *Characteristics of all 77 patients*

Patient Characteristic	**Number (%)**
Median patient age (years)	54 (range, 18-69)
Male	52 (68)
Female	25 (32)
Diagnosis at time of transplantation	
Primary acute myeloid leukaemia	33 (43)
Secondary acute myeloid leukaemia	25 (32)
Myelodysplastic syndrome	6 (8)
Myeloproliferative neoplasm	13 (17)
Primary AML ELN risk stratification by genetics (120)	
Favourable	5 (15)
Intermediate	20 (61)
Adverse	8 (24)
Status at transplantation	
CR1	20 (25)
CR2	13 (16)
≥CR3	3 (4)
First or second partial response	12 (15)
Relapse	6 (7.5)
Progressive disease	14 (18)
First or second chronic phase	5 (6)
First blast crisis	1 (1)
Stable disease	6 (7.5)
CR1 (breakdown of diagnoses within this group)	
Secondary AML	9
Primary AML	8
• Adverse genetic risk (ELN)	4
• Intermediate genetic risk (ELN) *	4
High-risk CML (ELTS score)	3
Treatment before transplantation (no. of lines)	
None	2 (3)
One	25 (31)
Two	32 (40)
>Two	21 (26)
Prior allogeneic HSCT	10 (13)

Patient Characteristic	Number (%)
Median interval from diagnosis to transplantation	147.5 days (range, 26–818 days)
CMV status (patient/donor)	
Negative/negative	26 (32.5)
Negative/positive	9 (11)
Positive/negative	18 (22.5)
Positive/positive	27 (34)
Haematopoietic cell transplantation-specific comorbidity index (HCT-CI)	
0	48 (60)
1-2	20 (25)
>2	12 (15)

*These patients with intermediate genetic risk AML (ELN) failed to respond to standard induction and required salvage therapy.

Donors and Grafts

Donor and graft characteristics are listed in Table 3.

***Table 3**: Donor and graft characteristics*

Donor or Graft Characteristic	Number (%)
Donor	
Matched related donor (MRD)	21 (26)
Matched unrelated donor (MUD)	49 (61)
Mismatched unrelated donor (mMUD)	10 (13)
Stem cell source	
Bone marrow	2 (2.5)
Mobilised peripheral blood stem cells	78 (97.5)
Donor blood type	
Identical	35 (44)
Blood group major incompatibility only	24 (30)
Blood group minor incompatibility only	12 (15)
Both minor and major incompatibility	9 (11)
Sex (donor)	
Male	55 (69)
Female	25 (31)
Female donor for male recipient	17 (21)

The majority of patients received either a matched related donor (MRD, 26 %) or a matched unrelated donor transplant (MUD, 61 %). Ten patients received an HLA-mismatched unrelated donor transplant (mMUD, 13 %). Two patients were transplanted with unmanipulated bone marrow (BM) and 78 patients with G-CSF-mobilised peripheral blood stem cell (PBSC) grafts.

A median number of 4 million/kg body weight (range, 0.8 million-8.3 million/kg body weight) CD34+ cells were transplanted. Thirty-five patients received a transplant from a donor with an identical blood group, twenty-four with a blood group major incompatibility only, twelve with a minor incompatibility only and nine with both major and minor incompatibilities. Thirty-four patients received a graft from a donor of a different gender. Seventeen male patients received a graft from a female donor.

HLA-Typing

HLA- typing was performed on patients to identify a suitable donor. Typing was based on high-resolution testing of class I and class II HLA antigens. Twenty-one patients had an MRD, forty-nine an MUD and ten an mMUD. Three of the mismatched transplants had an HLA-A incompatibility, four had an HLA-B incompatibility, one an HLA-DRB1 incompatibility, one an HLA-DQB1 incompatibility and one an HLA-DQB1 and an HLA-DRB1 incompatibility (8/10). High resolution matching of the HLA-A, -B, -C and -DRB1 alleles has been shown to be associated with the best clinical outcomes (121). Those who received a transplant from an mMUD were informed and consented to the increased risks of mortality and morbidity including the increased risk of GvHD and graft failure (122).

Conditioning Regimen

Fifty-one patients received 14 g/m^2 treosulfan (Medac, Hamburg, Germany) intravenously over two hours from day -6 to day -4. Twenty-eight patients received the same dose of treosulfan on day -4 to -2. One patient received treosulfan on day -5 to -3. The reasoning behind giving treosulfan later in the regimen was to potentially reduce the duration of neutropenia that the patients experienced (93). Fludarabine (Schering, Berlin, Germany) was given at a dose of 30 mg/m^2 intravenously over 30 minutes from day -6 to day -2. Cytarabine was administered at a dose of 2000 mg/m^2 once daily over three hours on day -6 to -5. Since July 2009, cytarabine has been supplied by a number of different manufacturers including; Hospira Ltd, Accord-UK Ltd and Fresenius Kabi AG. For patients receiving grafts from unrelated donors, rabbit ATG (various manufacturers including Fresenius) 10 mg/kg was given intravenously from day -4 to day -2.

Supportive Care

Patients were treated in high-efficiency particulate air (HEPA) filtered rooms. All patients received cotrimoxazole as prophylaxis against *Pneumocystis jirovecii* infection. Antiviral prophylaxis with aciclovir and antifungal prophylaxis with fluconazole was given routinely.

Erythrocyte and platelet transfusions were given to maintain haemoglobin levels above 80 g/L and platelet counts above 10 x 10^9/L in patients without fever or bleeding and above 20 x 10^9/L in those patients with fever or signs of bleeding. Blood products were irradiated and CMV status was matched.

Febrile neutropenia was treated with broad-spectrum antibiotics based on local hospital guidelines. Sepsis was treated empirically unless a pathogen was isolated from blood cultures. Antibiotic treatment was then changed according to sensitivity of the isolated pathogen. Patients who experienced delayed granulocytic reconstitution were given G-CSF at the discretion of the treating clinician.

Prophylaxis against GvHD consisted of cyclosporine A (CSA) 1.5 mg/kg every 12 hours starting on day -1. The dose of CSA was then adjusted accordingly to maintain whole-blood steady-state trough concentrations between 100 ng/ml and 200 ng/ml. Dosing was reduced as clinically indicated for nephrotoxicity. CSA was given initially intravenously and then converted to oral dosing before discharge. The dose of CSA was tapered and discontinued after discharge in the absence of GvHD. Methotrexate (MTX) as prophylaxis against GvHD was also administered intravenously at a dose of 15 mg/m^2 on day +1, and at a dose of 10 mg/m^2 on day +3, +6 and +11. Doses were omitted as clinically indicated for hepatotoxicity. Five patients were given mycophenolate mofetil (MMF) instead of MTX as GvHD prophylaxis according to local practice. Two patients were changed to tacrolimus from CsA, one due to an unsatisfactory blood concentration and the other due to intractable nausea and vomiting under the immunosuppressant.

Chimaerism

Chimaerism was evaluated from BM aspirates or peripheral blood using polymerase chain reaction (PCR) to amplify previously identified microsatellites. Analysis was performed by the MLL Münchner Leukämielabor GmbH. Complete chimaerism was defined as ≥98 % of donor type chimaerism.

Chimaerism analysis was performed on day +28, day +100 and day +180 as standard. Further analyses were performed as clinically indicated.

Definition of Primary and Secondary Endpoints

The primary endpoint was relapse-free survival (RFS). This was defined as the length of time in days after day 0 that the patient survived without any cytological, histological or molecular evidence of disease relapse. Day 0 is the day on which the donor cells were infused into the patient.

Secondary endpoints included overall survival (OS), non-relapse mortality (NRM), cumulative incidence of relapse, cumulative incidence of engraftment and graft failure (primary or

secondary), cumulative incidence of chimaerism, cumulative incidence of aGvHD and cGvHD and toxicities/adverse events as defined by the common terminology criteria for adverse events (CTCAE) version 5.0. Overall survival was the length of time in days from day 0 until death due to any cause. Non-relapse mortality was the probability of dying without previous occurrence of a relapse. This also included deaths due to transplant-related complications. The relapse of a haematological malignancy was defined by the standard cytological or molecular criteria in use at the time of the event. When relapse occurred, it was deemed to be the primary cause of death, irrespective of other events. Engraftment of neutrophils was defined as the first of three consecutive days where neutrophil counts exceed 0.5 x 10^9/l. Engraftment of platelets was defined as the first of three days where the platelet count exceeded 20 x 10^6/l. This number had to be independent of platelet transfusions. Primary graft failure (PGF) was the failure to achieve engraftment by day +28 after allogeneic HSCT with the addition of a failure to achieve complete chimaerism without evidence of disease relapse (123). It is important here to distinguish PGF from poor graft function. Poor graft function was defined as failure to achieve the criteria set out in the definition of engraftment by day +28, but in the presence of complete donor chimaerism and in the absence of severe GvHD and relapse (124). Secondary graft failure was defined as loss of donor cells after initial engraftment (122). Ideally, other causes of pancytopenia will have been excluded, for example infections (parvovirus, CMV, HHV-6), drug toxicity, GvHD and hypersplenism. Acute and chronic GvHD were grading according to standard criteria (125, 126). Toxicities and adverse events were graded according to CTCAE version 5.0. They were recorded during conditioning and the first 28 days following transplantation.

Although not considered an endpoint, the duration and neutropenia that patients experienced was also recorded. Neutropenia was defined as an absolute neutrophil count of less than 1000/µl.

Statistical Analysis

The primary endpoint was RFS (see definitions under ´Definition of Primary and Secondary Outcomes´). Patients alive and free of disease at their last follow-up were censored. RFS was considered to be the time from the day of infusion of donor stem cells to death or disease progression/relapse. Analysis was performed using the Kaplan-Meier method, with day 0 being the starting point.

Secondary endpoints included OS, NRM, relapse, engraftment, chimaerism, graft failure (primary or secondary), acute and chronic GvHD and toxicities as defined by the CTCAE version 5.0. Overall survival was analysed using the Kaplan-Meier method. Patients still alive at their last follow-up were censored. Non-relapse mortality, relapse, engraftment, chimaerism, acute and chronic GvHD were estimated using cumulative incidence analysis considering

competing risks. In the case of NRM, a relapse was considered a competing event. In the case of relapse, NRM was considered a competing event. For engraftment, death without recovery was a competing event and patients who did not engraft on or by day 28 were censored. For chimaerism, death before day +28, +100 or +180 (control points) were considered a competing event. Those who did not achieve complete chimaerism at the control points, but were still alive, were censored. In the case of GvHD, death without the development of GvHD prior to the cut-off of 100 days for aGvHD and two years for cGvHD was considered a competing event. Those who were alive at the cut-offs without development of GvHD were censored. Relapse or progression were not considered as a competing event in this case. In the case of graft failure, another proven cause for pancytopenia was a competing event. The incidence of adverse events was calculated as the number of patients who experienced at least one adverse event of a certain CTCAE category as the percentage of the total number of patients.

For exploratory purposes, outcome data (RFS, OS, NRM and cumulative incidence of relapse) were stratified by type of donor (MRD vs. MUD vs. mMUD), remission status at time of transplantation (CR1 vs. all other statuses) and age (<50 years old vs. ≥ 50 years old at time of transplantation). In the univariate analysis comparing groups, log-rank Mantel-Cox tests were used in Kaplan-Meier analyses and Gray's test was applied to cumulative incidence curves. To allow a better comparison between the patient group presented here and those observed in the FLAMSA-RIC studies, a subgroup analysis of patients with a diagnosis of AML, both primary and secondary, was performed. The Mann-Whitney *U* test (two-tailed) was used to compare the length of neutropenia between patients who became neutropenic after day -7 and those who were neutropenic before day -7.

Statistical analysis using the Kaplan-Meier method was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) kindly provided by the University of Oldenburg on Windows platform. Cumulative incidence curves with competing risk analysis and 95 % confidence intervals were performed using R version 3.5.3, kindly provided by the R Foundation. The method for the univariate analysis was provided by Scrucca *et al* (127, 128). The level of statistical significance was defined at 0.05.

Ethics and Data Protection

The ethics commissions of the Universities of Oldenburg and Jena approved the study in its current form (reference number University of Oldenburg: 2018-106, University of Jena: 2019-1316-BO, see Appendix II).

Analysis was performed on pseudonymised data and in accordance with the Declaration of Helsinki.

Results

Patients

A total number of 77 patients with AML, MDS or MPN underwent allogeneic HSCT according to the protocol described under ´Conditioning Regimen`. Three patients underwent an allogeneic HSCT twice with the same protocol, one after a late relapse of AML, two after an early relapse of secondary AML. An early relapse was defined as cytological or molecular evidence of disease relapse, prior to, or six months after day 0. The median follow-up time was 1161 days (3.2 years, range, 13 days-9.8 years). This was calculated using the method described by Schemper and Smith, published in 1996 (129).

Table 2 summarises patient and disease characteristics. The median age of subjects included in the study was 54 years (range, 18–69 years). In all, 25 % of the patients were in CR1, 15 % in CR2 and 4 % in CR3 at the time of transplantation. Fifty-four percent were not in remission at the time of transplantation either with partial remission, refractory or stable disease, or following disease relapse. Cytogenetic characteristics according to predefined categories for AML (33 patients, 43 %) were adverse in 24 %, intermediate in 61 % and favourable in 15 % of patients. Thirty-two percent of all patients had a secondary AML (43 % of all patients with a diagnosis of AML included in the study). The remaining 25 % of patients had either a diagnosis of MDS or MPN at the time of transplantation. Of the six patients transplanted with a diagnosis of MDS, one was classed as very high-risk, four were classed as high-risk and one as intermediate-risk according to the IPSS-R. Of the 13 patients diagnosed with an MPN, ten had CML (four Philadelphia chromosome negative, six in blast crisis). Two subjects had a diagnosis of myelofibrosis with intermediate-II risk according to the DIPSS and a failure to respond to JAK2 inhibitor therapy with ruxolitinib. The final patient was diagnosed with an MDS/MPN overlap syndrome with bone marrow fibrosis and splenomegaly (Ph negative, JAK2 negative).

Of those patients transplanted in CR1, 17 were diagnosed with AML. Of those 17 patients, nine had sAML. Of the remaining eight with a *de novo* AML, four had an adverse genetic risk profile according to the ELN risk profile and four were classified as intermediate risk. All four patients classified as intermediate risk had a failure to respond to standard induction therapy and required salvage therapy (either FLAG-Ida or S-HAM) to achieve complete remission. The remaining three patients transplanted in CR1 were high risk CML patients (according to the EUTOS-Long Term Survival (ELTS) score (130)).

Remission Status Following Immune Reconstitution

Three patients either did not achieve a complete remission directly following transplantation or relapsed within 30 days. All three patients had a diagnosis of AML. The first was diagnosed with primary AML with an MLL-rearrangement and a t(1;9;11) translocation and was transplanted in CR2 (borderline remission, blasts 5 % in cytology at the time of transplantation).

This patient appeared to achieve engraftment (neutrophils on day +20, platelets on day +16), however, a persistence of the previously diagnosed AML was discovered on day +29. Analysis revealed a mixed chimaerism with 41 % of all peripheral leucocytes originating from the host. Following the relapse, the patient died on day +38 due to progression of the disease. The second patient, diagnosed with secondary AML M2, was found to have a BM blast count of 10-12 % on day +28. This patient, however, had a blast count of 24 % with progressive disease prior to transplantation. Immunosuppressive drugs were stopped, and complete remission was achieved on day +58. This patient relapsed on day +386 and died +416 days following the transplantation. The third patient, who had a diagnosis of AML M7, refractory to standard conditioning, was transplanted in CR1 (cytology showed 3.1 % blasts and a cell poor smear) after salvage therapy with FLAG. The third patient was found to have a relapse on day +28 with a mixed chimaerism (24 % host). Immunosuppression was stopped in order for the GvL effect to halt the progression of disease. However, he subsequently died on day +35.

Relapse-Free Survival

As the median follow-up was 3.2 years, RFS and OS were measured at one-, two and three-year cut-offs. Relapse-free survival was measured from day 0 until relapse, or in the case that the patient did not experience a relapse, until last follow-up. Patients who died from non-relapse causes were censored. The median relapse-free survival was 1,098 days.

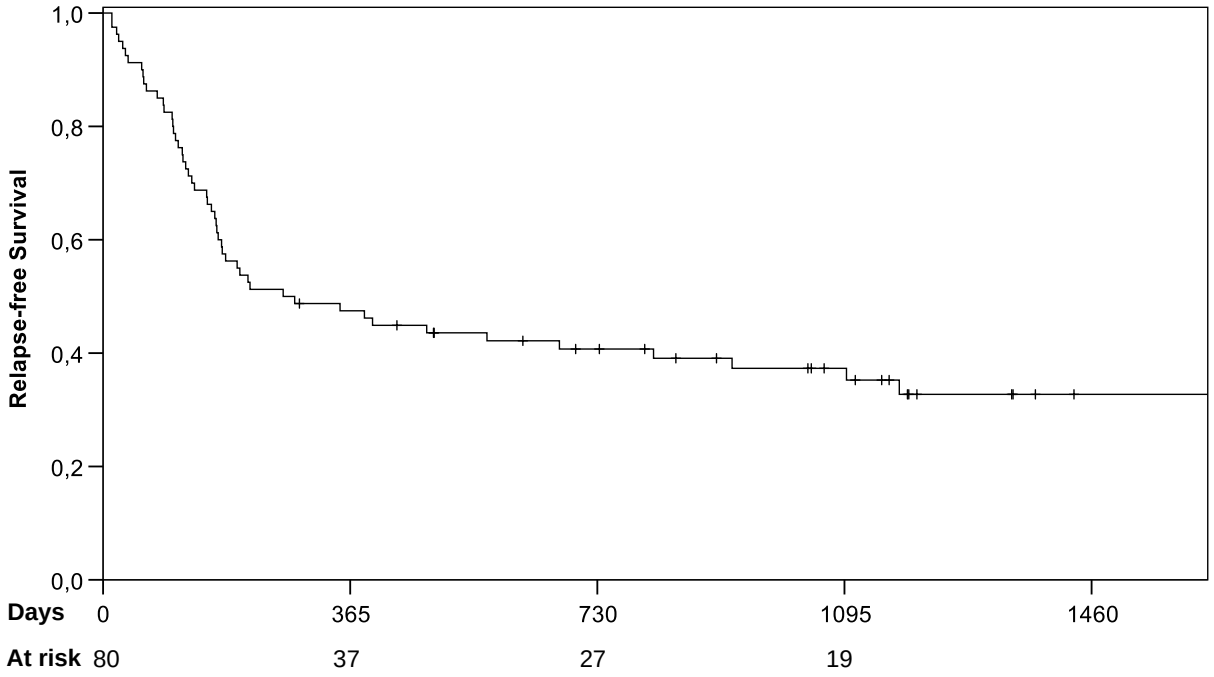

***Figure 1**: Relapse-free survival estimates, calculated using the Kaplan-Meier method, for all 77 patients conditioned with Treo/Flu/AraC. Crosses denote a censored event. At risk refers to the number of patients at risk of relapse.*

One-, two- and three-year relapse free survival rates were 47.5 %, 40.7 % and 37.5 %, respectively (see Figure 1 and see Table 3 for an overview of all results). For patients who

received a transplant from an MUD these figures were 46.9 %, 38.0 % and 35.4 %, respectively. For those receiving a transplant from an MRD these figures were 52.4 %, 47.1 % and 39.3 %, respectively. For patients receiving a transplant from an mMUD, RFS was 40 % at one year and remained at this level throughout follow-up. There was no significant difference between RFS rates between the three groups (p = 0.76, log-rank Mantel-Cox test).

Those patients transplanted in CR1 had a one-, two and three-year RFS of 45.0 %, 40.0 % and 33.3 %, respectively. The figures for those transplanted in all other states were 48.3 %, 41.0 % and 38.7 %, respectively. Again, there was no significant difference in RFS between these two groups (p = 0.978, log-rank Mantel-Cox test).

Those patients receiving a transplant at or over the age of 50 had a one-, two- and three-year RFS of 49.1 %, 43.1 % and 40.2 %, respectively. The figures for those transplanted under the age of 50 was 44.4 %, 37.0 % and 33.3 %, respectively. Again, there was no significant difference in RFS between these two groups (p = 0.969, log-rank Mantel-Cox test).

One-, two- and three-year RFS rates for the AML subgroup analysis were 43.3 %, 38.1 % and 33.5 % (see Figure 2).

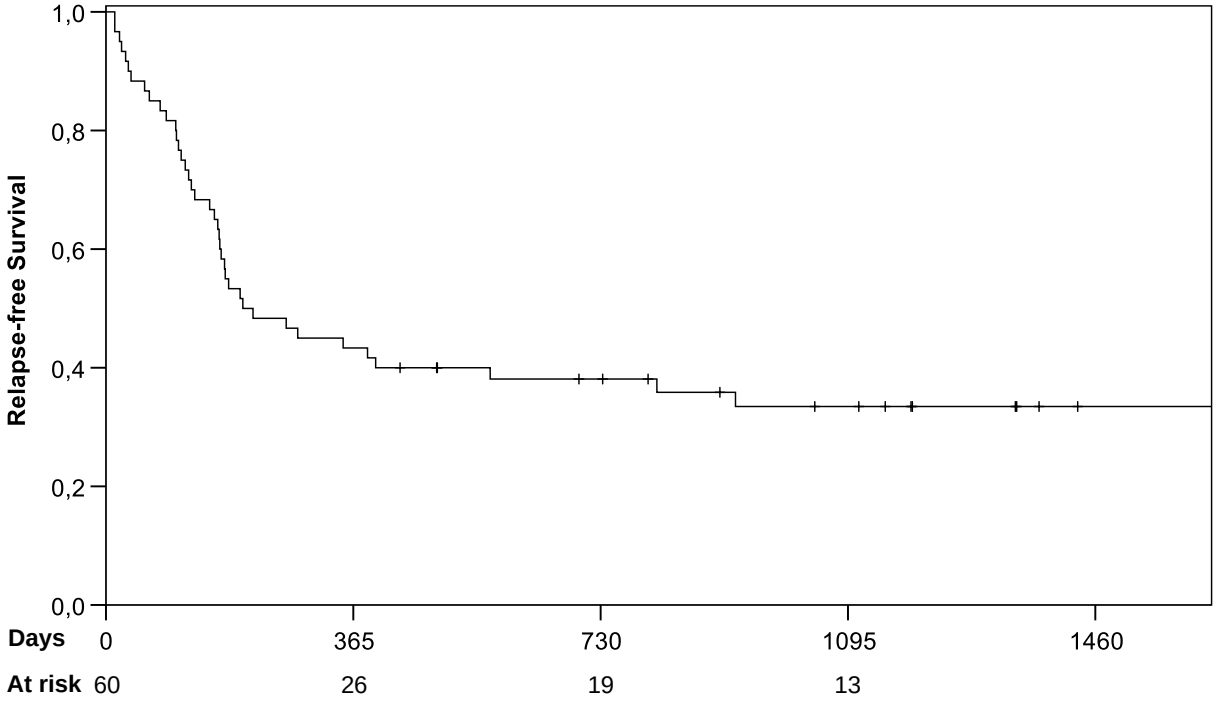

Figure 2: *Relapse-free survival estimates, calculated using the Kaplan-Meier method, for all 58 patients with a diagnosis of acute myeloid leukaemia. Crosses denote a censored event. At risk refers to the number of patients at risk of relapse.*

Table 4: *Overview of the RFS rates for all patients and group analyses*

Patient Groups (number of transplants/patients)	**Relapse-Free Survival (%)**			***P*-value (log-rank test)**
	One-year	Two-year	Three-year	
All patients (77)	47.5	40.7	37.3	
MUD (49)	46.9	38.0	35.4	0.76
MRD (21)	52.4	47.1	39.3	
mMUD (10)	40.0	40.0	40.0	
CR1 (20)	45.0	40.0	33.3	0.978
All other statuses (60)	48.3	41.0	38.7	
<50 years (27)	44.4	37.0	33.3	0.969
≥50 years (53)	49.1	43.1	40.2	
AML patients (58)	43.3	38.1	33.5	

Overall Survival

Overall survival is the length of time in days from day 0 to death due to any cause. Those who were still alive at their last follow-up were censored.

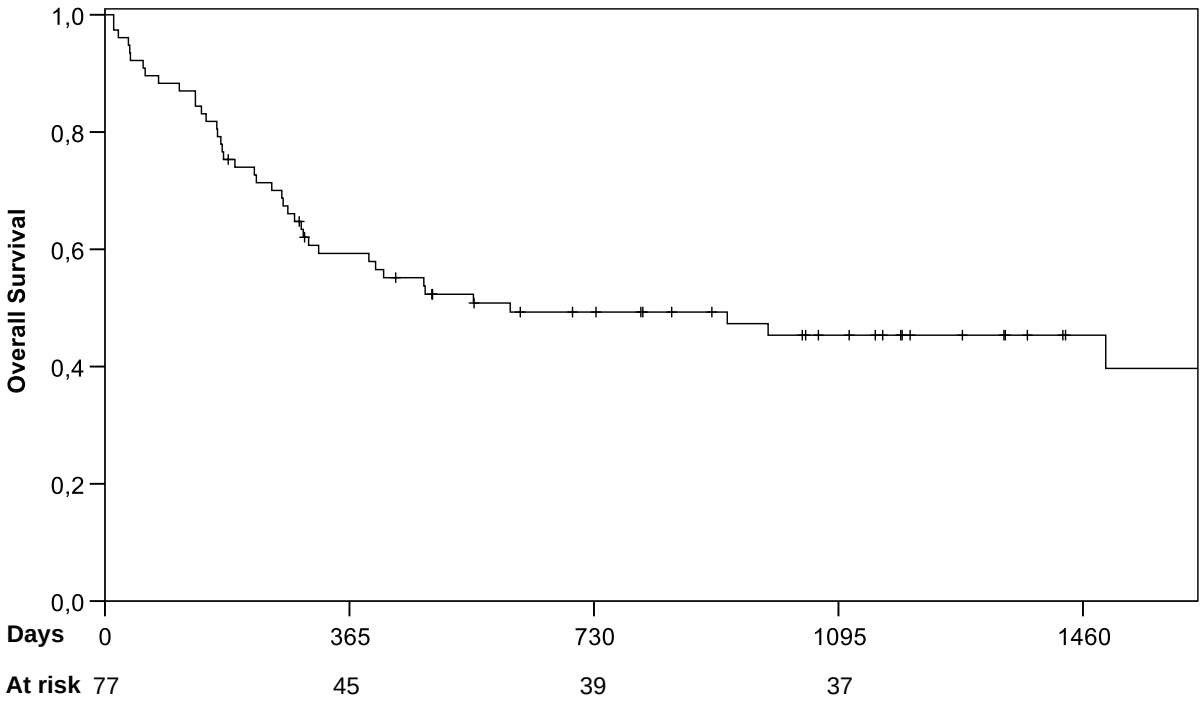

Figure 3: *Overall survival estimates, calculated using the Kaplan-Meier method, for all 77 patients conditioned with Treo/Flu/AraC. Crosses symbolise a censored event. At risk refers to the number of patients at risk of mortality.*

One, two- and three-year OS rates were 59.3 %, 49.3 % and 45.4 %, respectively (see Figure 3 and see Table 4 for an overview of all overall survival results). One, two and three-year OS for those transplanted with an MRD were 70.0 %, 48.1 % and 40.1 %, respectively. The same figures for those receiving a transplant from an MUD were 56.8 %, 49.9 % and 46.7 %, respectively. The one-year OS for those receiving an mMUD was 48 %. The difference between the three groups was not statistically significant (p = 0.95, log-rank Mantel-Cox test).

For those patients transplanted in CR1 the one-, two- and three-year OS rates were 60.0 %, 60.0 % and 52.5 %, respectively. For those not transplanted in CR1 these figures were 59.7 %, 47.1 % and 42.1 % respectively. The difference between the two groups did not reach statistical significance (p = 0.825, log-rank Mantel-Cox test).

Thirty-four percent of the patients were aged under 50 years at the time of transplantation. The one-, two- and three-year OS for patients aged under 50 were 58.1 %, 54.2 % and 44.4 %, respectively. Those rates for patients aged 50 and over were 60.3 %, 47.4 % and 44.5 %, respectively. There was no significant difference between the two groups (p= 0.785, log-rank Mantel-Cox test).

The one-, two- and three-year OS of the patients with AML (both primary and secondary) were 56.5 %, 48.9 % and 43.5 % (see Figure 4).

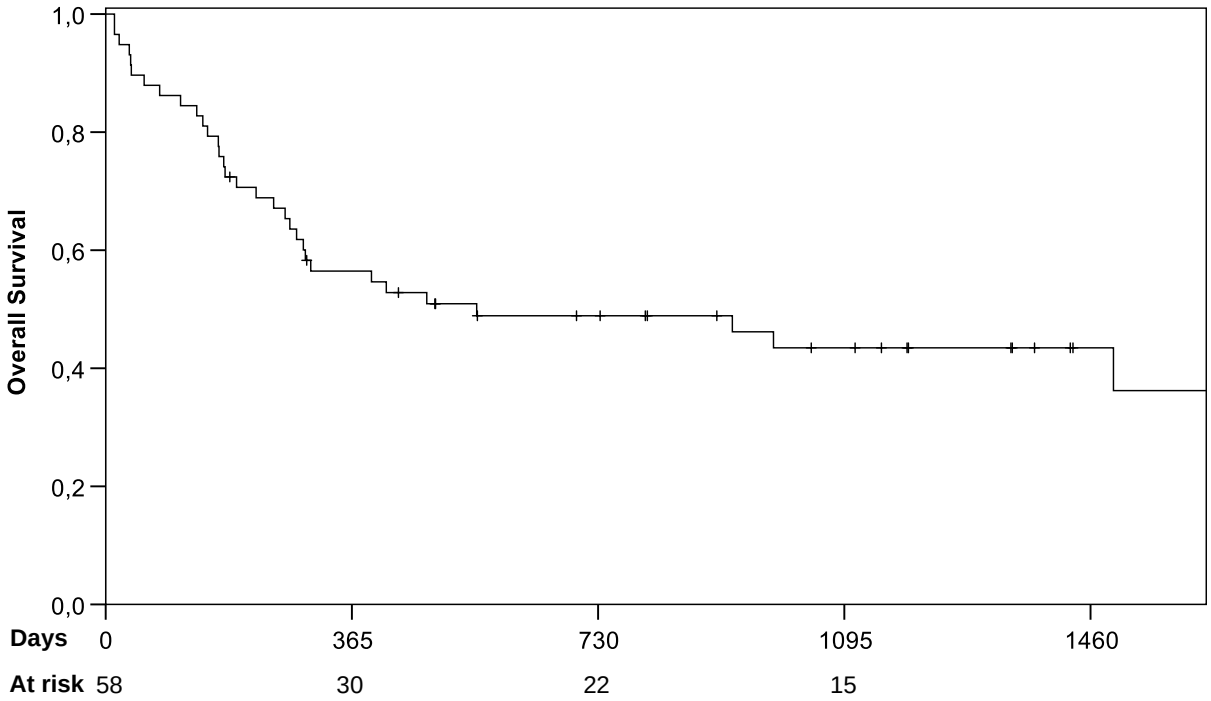

Figure 4: *Overall survival estimates, calculated using the Kaplan-Meier method, for all 58 patients with a diagnosis of acute myeloid leukaemia. Crosses denote a censored event. At risk refers to the number of patients at risk of mortality.*

Table 5: *Overview of the OS rates for all patients and group analyses*

Patient Groups (number of transplants/patients)	**Overall Survival (%)**			***P*-value (log-rank test)**
	One-year	Two-year	Three-year	
All patients (77)	59.3	49.3	45.4	
MUD (49)	56.8	49.9	46.7	0.95
MRD (21)	70.0	48.1	40.1	
mMUD (10)	48.0	48.0	48.0	
CR1 (20)	60.0	60.0	52.5	0.825
All other statuses (60)	59.7	47.1	42.1	
<50 years (27)	58.1	54.2	44.4	0.785
≥50 years (53)	60.3	47.4	44.5	
AML patients only (58)	56.5	48.9	43.5	

Non-relapse Mortality

Eighteen patients were neutropenic before the start of conditioning and eight began conditioning with radiological evidence of fungal pneumonia. A total of eighteen patients died without previous relapse of their disease. Four patients died within 28 days of transplantation. The first two died on day +13. One due to SOS and the other as a result of sepsis. Both patients had received a transplant from an mMUD. The third patient died on day +20 of sepsis. This patient, with AML M4, had been transplanted with a peripheral blast count of 50 % after not responding to standard or salvage chemotherapy. The fourth patient died of pneumonia on day 23. This patient was one of the three patients in the study who had previously received a transplant following Treo/Flu/AraC conditioning. Following the first transplant he suffered a relapse of his disease on day +160.

The causes of death for the remaining fourteen patients were sepsis/infection including pneumonia (7), acute respiratory distress syndrome (ARDS, 2), intracerebral bleed (1), post-transplant lymphoproliferative disorder (PTLD, 1) and grade IV aGvHD of the liver (1). In the case of two patients the cause was unknown as they were lost to follow-up.

Cumulative incidences of NRM were 10.0 % (95 % CI, 5 %-18 %) at 100 days, 18.8 % (95 % CI, 11 %-28 %) at one year and 20.1 % (95 % CI, 12 %-30 %) at two years (see Figure 5). Only two patients died of non-relapse related causes more than two years following transplantation. The exact cause of death of these patients is unknown as they were lost to follow-up. It seems in this patient population that the critical period for NRM are the first two years following transplantation. This is largely in line with what has been found in large long-term follow-up studies for AML patients ((131), (132)).

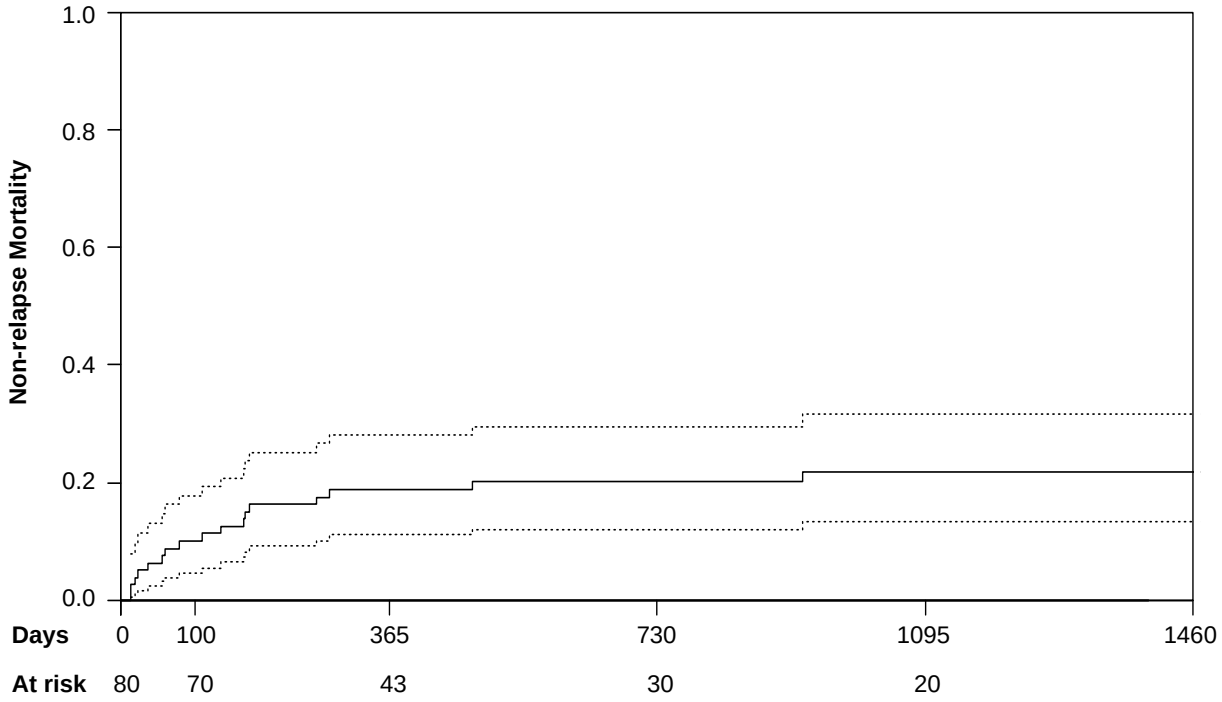

Figure 5: *Cumulative incidence of non-relapse mortality for all patients (full line) with 95 % confidence intervals (broken line). At risk refers to the number of patients at risk of NRM.*

There was no significant difference in NRM between patients receiving an MUD, MRD or mMUD (*p*=0.31, Gray's test). For those receiving an MUD the NRM at 100 days, one- and two-years was 12 % (95 % CI, 5 %-23 %), 16 % (95 % CI, 7.5 %-28 %) and 16 % (95 % CI, 7.5 %-28 %), respectively. For those receiving an MRD transplant these figures were 0 %, 19 % (95 % CI, 6 %-38 %) and 24 % (95 % CI, 8 %-45 %), respectively. For those receiving an mMUD these figures were 20 % (95 % CI, 27 %-49 %), 30 % (95 % CI, 6 %-60 %) and 30 %, respectively.

There was no significant difference in NRM between patients receiving a transplant in CR1 or any other status (*p*=0.27, Gray's test). For those receiving a transplant in CR1 the NRM at 100 days, one- and two-years was 10.0 % (95 % CI, 2 %-28 %), 25.0 % (95 % CI, 9 %-46 %) and 25.0 % (95 % CI, 9 %-46 %), respectively. For those receiving a transplant in any other disease status these figures were 10 % (95 % CI, 4 %-19 %), 16.7 % (95 % CI, 8 %-27 %) and 18.5 % (95 % CI, 10 %-29 %), respectively.

There was no significant difference in NRM between patients receiving a transplant at or above the age of 50 and those receiving a transplant below the age of 50 (*p*=0.077, Gray's test). For those receiving a transplant under the age of 50 the NRM at 100 days, one- and two-years was 0 %, 11 % (95 % CI, 3 %-26 %) and 11 %, respectively. For those receiving a transplant at or over the age of 50, these figures were 15 % (95 % CI, 7 %-26 %), 23 % (95 % CI, 12 %-35 %) and 25 % (95 % CI, 14 %-37 %), respectively (see Table 5 for an overview of all results).

For those patients with a diagnosis of AML, 100 day, one- and two-year NRM was 11.7 % (95 % CI, 6 %-21 %), 21.7 % (95 % CI, 12 %-33 %) and 21.7 % (95 % CI, 12 %-33 %), respectively (see Table 6 for an overview of all results).

Table 6: *Overview of the NRM rates for all patients and group analyses*

Patient Groups (number of patients/transplants)	**Non-Relapse Mortality (%)**			***P*-value (Gray's test)**
	100 days	One-year	Two-year	
All patients (77)	10.0	18.8	20.1	
MUD (49)	12.0	16.0	16.0	0.31
MRD (21)	0.0	19.0	24.0	
mMUD (10)	20.0	20.0	30.0	
CR1 (20)	10.0	25.0	25.0	0.27
All other statuses (60)	10.0	16.7	18.5	
<50 years (27)	0.0	11.0	11.0	0.077
≥50 years (53)	15.0	23.0	25.0	
AML patients only (58)	11.7	21.7	21.7	

Cumulative Incidence of Relapse

The one- and three-year cumulative incidences of relapse were 34 % (95 % CI, 24 %-44 %) and 41 % (95 % CI, 30 %-52 %), respectively (see Figure 6).

There was no significant difference in the cumulative incidence of relapse for patients transplanted using an MRD, MUD or an mMUD with a *p*-value of 0.25 (Gray's test). For MRD the one- and three-year rates of relapse were both 29 % (95 % CI, 11 %-49 %). For MUD the one- and three-year rates of relapse were 37 % (95 % CI, 23 %-50 %) and 48 % (95 % CI, 33 %-62 %), respectively. For mMUD these both one- and three-year figures were 30 % (95 % CI, 6 %-60 %).

One- and three-year cumulative incidences of relapse for patients transplanted in CR1 were 30 % (95 % CI, 12 %-51 %) and 35 % (95 % CI, 15 %-56 %), respectively. For those patients not transplanted in CR1, these figures were 35 % (95 % CI, 23 %-47 %) and 42.8 % (95 % CI, 30 %-55 %), respectively. Although there was a trend to a lower rate of relapse for those transplanted in CR1, there was no significant difference between the two groups (p= 0.23, Gray's test). Performing the same analysis on patients transplanted in any complete remission and comparing it to patients transplanted not in complete remission also yielded no significant difference (p= 0.73, Gray's test). The figures for one- and three-year relapse rates were for any complete remission 32 % (95 % CI, 18 %-48 %) and 41 % (95 % CI, 25 %-56 %),

respectively, and for no remission/active disease, 35 % (95 % CI, 21 %-49 %) and 41 % (95 % CI, 25 %-56 %), respectively.

With regards to age there was also no significant difference in the relapse rate for patients transplanted at or over the age of 50 and those transplanted under the age of 50 (p = 0.08, Gray's test). The one- and three-year relapse rates for those transplanted over at, or over, the age of 50 were 28 % (95 % CI, 17 %-40 %) and 32 % (95 % CI, 20 %-45 %), respectively. Those figures for those transplanted under the age of 50 were 44 % (95 % CI, 25 %-62 %) and 55 % (95 % CI, 34 %-72 %), respectively.

For those patients with a diagnosis of AML one- and three-year relapse rates were 35.0 % (95 % CI, 23 %-47 %) and 42.5 % (95 % CI, 29 %-55 %), respectively (see Table 6 for an overview of all results).

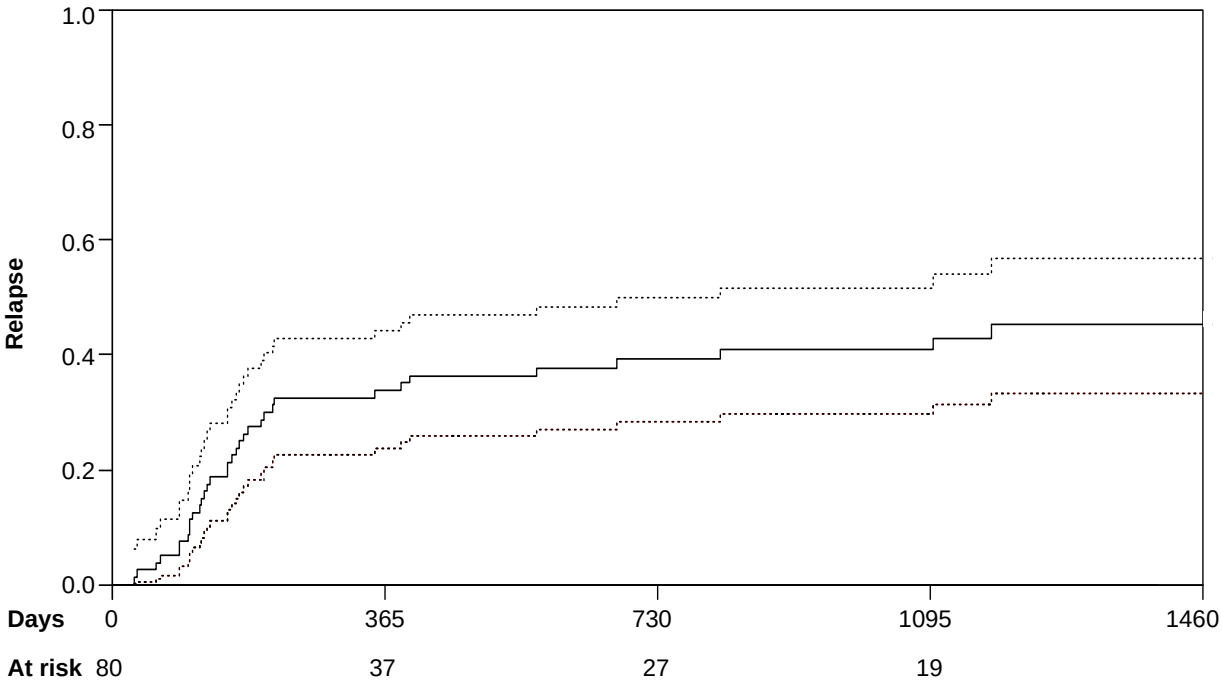

Figure 6: *Cumulative incidence of relapse for all patients (full line) with 95% confidence intervals (broken line). At risk refers to the number of patients at risk of relapse.*

Table 7: *Overview of the relapse rates for all patients and group analyses*

Patient Groups (numbers of transplants/patients)	**Rate of Relapse (%)**		***P*-value (Gray's test)**
	One-year	Three-year	
All patients (77)	34.0	41.0	
MUD (49) MRD (21) mMUD (10)	37.0 29.0 30.0	48.0 29.0 30.0	0.25
CR1 (20) All other statuses (60)	30.0 35.0	35.0 42.8	0.41
<50 years (27) ≥50 years (53)	44.0 28.0	55.0 32.0	0.08
AML patients only (58)	35.0	42.5	

Engraftment, Graft Failure and Chimaerism

CTCAE grade IV neutropenia, leukocytopenia and thrombocytopenia occurred in all patients. Three patients failed to achieve neutrophil or platelet reconstitution, two having died on day 13 (SOS and sepsis, respectively) and the other from sepsis on day 20. One patient achieved primary engraftment of neutrophils on day 11, but then died on day 23 from pneumonia before platelet engraftment was achieved. All other patients achieved primary engraftment of neutrophils and platelets.

The day 28 cumulative incidence of engraftment of neutrophils (or polymorphonuclear leucocytes [PMN]) reached 85 % (95 % CI, 75 %-91 %). By day +37 all patients had achieved successful PMN engraftment. The day 28 cumulative incidence of platelet engraftment reached 82.5 % (95 % CI, 72 %-89 %). By day +100, this had increased to 85 % (95 % CI, 75 %-91 %). Two patients achieved successful platelet engraftment after day +100, the first on day +117 and the second on day +174.

The median time to neutrophil engraftment was 20 days (range, 9–37 days). The median time to platelet engraftment was also 20 days (range, 11–174 days). No primary or secondary engraftment failure was documented. There were nine documented cases of poor graft function with regards to neutrophil engraftment and twelve cases with regards to platelet engraftment.

The cumulative incidence of complete donor-type chimaerism was 84 % (95 % CI, 74 %-90 %), 66 patients were complete chimaeras) on day +28. By day +100, 80 % of patients were

found to have complete donor-type chimaerism (55 subjects). By day +180 this figure was 68 % (41 subjects).

Acute and Chronic Graft-Versus-Host Disease

Two patients experienced grade IV aGvHD. The first patient received a histologically confirmed diagnosis of grade IV aGvHD of the liver on day +29. The same patient was diagnosed with grade II-III intestinal aGvHD. The second patient developed a late-onset of grade IV aGvHD of the liver after ending immunosuppression on day +188. Cyclosporine A was restarted, then changed to tacrolimus after a poor response to this drug. Unfortunately, this could not reverse the process and the patient died on day +266 as a consequence of aGvHD. This was the only patient in the study to die as a direct result of aGvHD.

Day +100 cumulative incidences of grade I - IV, II - IV and III - IV acute GvHD were 38 % (95 % CI, 27 %-48 %), 22 % (95 % CI, 13 %-33 %) and 6 % (95 % CI, 2 %-14 %). Five patients developed late-onset aGvHD after day +100, on day +108, +176, +188, +267 and +284. The first patient, who developed aGvHD on day +108, was diagnosed with keratoconjunctivitis sicca, with an infective conjunctivitis as the differential diagnosis. This was assessed as being a manifestation of aGvHD and not cGvHD (133). The second patient (day +176) was diagnosed with a late onset aGvHD of the liver (grade II). The third patient (day +188) was diagnosed with grade I aGvHD of the skin and grade IV of the liver for an overall grade of IV. The fourth patient (day +267) developed skin aGvHD (grade II). The fifth and final patient (day +284) developed intestinal aGvHD (grade I). These late manifestations of aGvHD were likely as a result of a reduction in the immunosuppressive medication.

The cumulative incidence of mild to severe cGvHD at two years was 15 % (95 % CI, 8 %-24 %). There were three cases of severe cGvHD, two affected the skin (day +879, day +1755) and one affected the gastrointestinal tract (day +199).

Of the ten patients who received an mMUD transplant, known to be a risk factor for the development of acute and chronic GvHD (134), only three developed either of these conditions. The first developed skin and intestinal aGvHD grade I on day +16. They did not go on to develop cGvHD and were still alive at the time of writing. The second developed aGvHD of the skin, liver and intestine with an overall grade of II. He went on to develop mild cGvHD of the skin and was still alive at the time of writing. The third patient developed grade I aGvHD of the skin. This patient experienced an early relapse of his disease on day +64 but was still alive at the time of writing.

Toxicities and Adverse Events

Every patient experienced chemotherapy-related myelosuppression that was expected after the application of the conditioning regimen. Every patient required the transfusion of blood products prior to or following the transplantation as a direct consequence of the conditioning chemotherapy. In some cases, myelosuppression was also caused by the underlying malignant condition or previous bridging or salvage therapy given prior to the start of conditioning.

The diagnosis of SOS (grade 3) was suspected in two patients and they were successfully treated with defibrotide. A third patient is thought to have died due to SOS (grade 5).

One patient developed PTLD diagnosed on day +98 following allogeneic HSCT. He presented with manifestations in the liver and cervical, mediastinal, hilar and axillary lymph nodes. The BM was not affected. They were treated with three courses of R-CHOP (rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone), but unfortunately succumbed to the illness on day +167.

The toxicities and adverse events that were recorded for all patients from day -6 to day +28 following allogeneic HSCT are summarised in Table 8. Further adverse events included haemorrhagic colitis (1), neutropenic colitis (1), pericardial effusion with haemodynamic relevance (2), NSTEMI (1), pulmonary embolism (1) and cardiac decompensation (2).

Two of the patients developed a solid tumour subsequent to the allogeneic HSCT. Whether these malignancies were related to the transplantation cannot be definitively said. The first developed a palliative gallbladder cancer three years following an mMUD transplantation for AML M5. The second developed a ductal carcinoma *in situ* (ER negative, PR positive, Ki67 30 %, pTis, pN0, R0, G3) just over two and a half years following MUD transplantation for CML.

Table 8: *Toxicities and adverse events that occurred during conditioning up until day +28 following transplantation, graded according to the CTCAE version 5.0*

Adverse Event	**Number of Patients (%)**
Oral mucositis	
Grade 1-2	14 (18 %)
Grade 3-4	18 (23 %)
Creatinine rise	
Grade 1-2	34 (44 %)
Grade 3-4	1 (1.3 %)
Sinusoidal obstruction syndrome	
Grade 3-4	2 (2.6 %)
Grade 5	1 (1.3 %)
AST/ALT rise	
Grade 1	33 (43 %)
Grade 2	22 (29 %)
Grade 3-4	17 (22 %)
ALP rise	
Grade 1-2	45 (58 %)
Grade 3-4	3 (3.9 %)
Bilirubin rise	
Grade 1-2	52 (68 %)
Grade 3-4	12 (16 %)
Febrile neutropenia	
Grade 3-4	48 (62 %)
Sepsis	
Grade 3-4	32 (42 %)
Grade 5	2 (2.6 %)
Lung infection	
Grade 3-4	27 (34 %)
Grade 5	1 (1.3 %)

Duration of Neutropenia

The median length of neutropenia was 24 days (range, 11–137 days). Eighteen patients were neutropenic before the start of the conditioning regimen due to disease progression or prior chemotherapy used to induce remission. Fifty-one patients received treosulfan from day -6 to day -4. Twenty-eight patients received treosulfan from day -4 to -2 and one patient received treosulfan on day -5 to -3. The median length of neutropenia when excluding patients who were neutropenic prior to the start of conditioning was 23 days (range, 11–50 days). There was no significant difference in the length of neutropenia between the patients who first received treosulfan on day -6 and those who first received it on day -4 (*p*-value = 0.79) (see Table 9). Patients who were neutropenic prior to the start of conditioning (day -7) were excluded from this analysis.

Table 9: *Median duration of neutropenia for all patients not neutropenic at the start of conditioning (divided in to two groups based on the first day of treosulfan application).*

Number of Patients	First Day of Treosulfan	Median Duration of Neutropenia (Days)
36	-6	22.5 (range, 11–50)
26	-4	23 (range, 14–36)

For those patients who were neutropenic prior to day -7, the median length of neutropenia was 47 days (range, 23–137 days). Those who became neutropenic after day -7 this figure was 23 days (range, 11–50 days). There was a significant difference in the duration of neutropenia between these two groups of patients (*p* = <0.0001).

The OS, RFS, NRM and cumulative incidence of relapse of patients who were neutropenic prior to day -7 and those who became neutropenic after day -7 were compared. Those who were neutropenic prior to day -7 had one-, two- and three-year OS rates of 44.4 %, 27.8 % and 22.2 %, respectively. These figures for those who became neutropenic during conditioning were 64.1 %, 56.8 % and 51.6 %, respectively. There was a significant difference in the OS between these two groups (*p* = 0.028, log-rank Mantel-Cox test, see Figure 7).

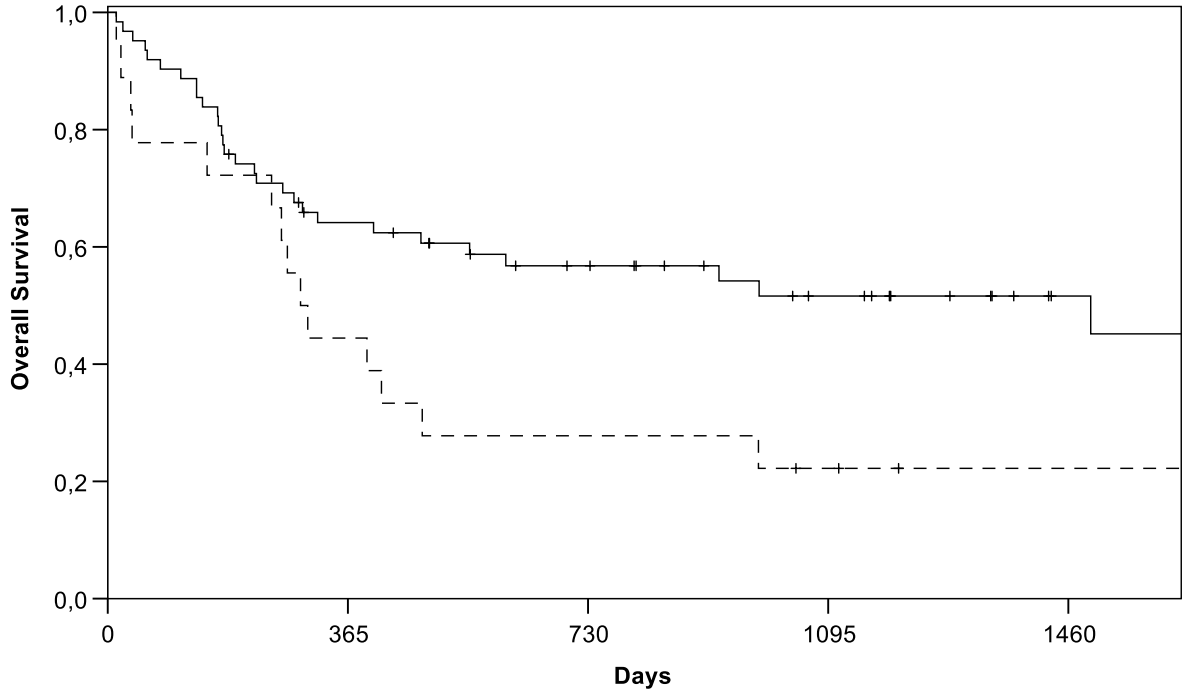

Figure 7: *Overall survival estimates, calculated using the Kaplan-Meier method, comparing patients who became neutropenic after day -7 (full line) with those who were neutropenic before day -7 (broken line). Crosses denote a censored event. P-value = 0.028, log-rank Mantel-Cox test.*

Of the eighteen patients who were neutropenic prior to day -7, four were alive at the time of writing. Two died of pneumonia, one of sepsis, one due to ARDS secondary to a fungal pneumonia and one due to VOD. The remaining nine patients died as a result of a relapse of their underlying disease.

There was no significant difference in the RFS (p = 0.14, log-rank Mantel-Cox test), NRM (p = 0.89, Gray's test) and relapse rates (p = 0.24, Gray's test) between the two groups of patients (see Table 10 for a summary of results).

Table 10: *NRM and relapse rates comparing patients who were neutropenic before day -7 with those who became neutropenic after day -7. Abbreviation: n – number of patients*

	Neutropenic Before Day -7 (n= 18)			**Neutropenic After Day -7 (n= 62)**		
RFS	*One year*	*Two years*	*Three years*	*One year*	*Two years*	*Three years*
	38.9 %	27.8 %	22.2 %	49.9 %	44.5 %	42.0 %
NRM	*Day 100*	*One year*	*Two years*	*Day 100*	*One year*	*Two years*
	16.7 %	16.7 %	22.2 %	8.1 %	19.3 %	19.3 %
Relapse	*One year*		*Three years*	*One year*		*Three years*
Rate	44.4%		55.6%	30.7%		36%

Discussion

The results of the current study show that treosulfan and fludarabine, in combination with cytarabine, provides a feasible and tolerable conditioning regimen prior to allogeneic HSCT for patients with AML, MDS or MPN. The combination of treosulfan and fludarabine has already been shown to be a promising conditioning regimen in phase II and more recently phase III studies (135) for the treatment of primary MDS (136), acute myeloid and lymphoid leukaemias (137) and CML (138). The addition of cytarabine to the Treo/Flu regimen has the potential to further increase its antileukaemic activity with the aim of reducing the risk of relapse, especially in high-risk patients. As previously discussed, fludarabine and cytarabine are shown to work synergistically to increase their individual antileukaemic effects. Treosulfan has also been found to have significant antileukaemic activity in its own right. It is also seen as a good alternative to busulfan due to its better toxicity profile (78). The advantage of Treo/Flu/AraC conditioning over FLAMSA-RIC is the reduced length of the regimen (six days instead of twelve-thirteen days), and corresponding potential reduced length of hospital stay, reduced transfusion requirement, reduced duration of pancytopenia, and associated infectious complications.

When examining the outcomes presented here, it is important to consider the retrospective nature of this trial and the inherent biases and problems with data collection that are associated with this type of study. Furthermore, it is must be remembered that there are a group of variables that have been shown to have prognostic value in all predictive models, independent of the conditioning regimen employed. These are as follows: age, performance status, disease status, type of donor (MRD, MUD or mMUD), HLA compatibility, CMV serology, interval between diagnosis and HSCT (most relevant in CML), comorbidities (Sorror score, HCT-CI), iron overload due to previous blood transfusions and/or hereditary haemochromatosis and the experience of the centre (139). The median age of patients in this study was 54 (range, 18–69). When comparing this to that of the Treo/Flu and FLAMSA-RIC studies, this number sits in the middle of the range (45–60 years). The patients in this study were, as a group, heavily pre-treated with 66 % having received two or more previous lines of treatment. Thirteen percent of the patients had previously undergone an allogeneic HSCT. The degree of pre-treatment of the patient will impact negatively on their condition and iron overload status prior to transplantation. It may, however, improve engraftment due to increased host immunosuppression.

Only 25 % of the patients in the current study were in CR1 at the time of transplantation. An allogeneic HSCT in CR1 offers the best outcomes for patients. Those in CR1 are most often the patients who are evaluated in studies for new medications and regimens. The definition of CR1, given by the CIBMTR, includes the statement, *'Include recipients meeting the above CR*

criteria regardless of how many courses of therapy were required to achieve CR'. The patients in the current study, who met the criteria of a CR1, were nonetheless at very high risk of relapse. This was due to a number of different factors including: a diagnosis of sAML, having required salvage chemotherapy to achieve remission and having a high risk MPN (see Table 2). Many studies do not specify the exact status of their CR1 patients, instead relying on an inclusion criterion such as, 'indication for allogeneic HSCT according to institutional policy'. The definition of CR1 hides a wide spectrum of differently responsive leukaemic blasts, making comparisons between studies difficult.

Additionally, further complicating the comparison between allogeneic HSCT studies specifically, the definition of CR1 according to the CIBMTR explicitly states that, '*In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment. In this case, CR should still be reported as the status at transplant since it represents the "best assessment" prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks'*. Patients who experience a relapse less than four weeks after achieving CR have different disease biology to those who have been able to maintain a durable remission beyond this time point. However, if transplantation is carried out in the four weeks after achieving CR, it would have been impossible to ascertain if a patient would have relapsed or not. This could mean the relapse risk of their malignant disease is underestimated.

To avoid confusion, the current study will be referred to as the Treo/Flu/AraC study throughout the discussion.

Remission Status Following Immune Reconstitution

Three patients did not achieve complete remission directly following transplantation. All three patients had a diagnosis of AML, but their risk factors for not achieving a complete remission were drastically different. Table 11 provides an overview of patient and transplant characteristics.

The first patient, diagnosed with primary AML with an MLL-rearrangement and a translocation (1;9;11) and transplanted in a borderline complete remission (CR2), had already experienced an early relapse of his disease following standard induction therapy. He had not responded to the first salvage therapy (FLAG-Ida) and achieved only a borderline remission following second line salvage therapy (S-HAM). The outcomes of patients in first relapse of AML following standard induction therapy are partially dependent on the cytogenetics of their disease and the length that they were in remission (140). According to the study by Weltermann *et al.* (140), published in 2004, this patient with intermediate risk cytogenetics and an early relapse, would have a poor probability of long-term survival with a three-year OS of 18 %.

The second patient, diagnosed with secondary AML M2, was found to have a BM blast count of 10-12 % on day +28. This patient had a blast count of 24 % with progressive disease prior to transplantation as a result of primary induction failure (PIF). Patients transplanted after PIF have much poorer outcomes than those transplanted in complete remission (141). Several studies have looked at the outcomes of patients with AML who were not in complete remission at the time of transplantation. The studies have shown a three-year leukaemia-free survival rate of between 21 % and 31 % in this group (142-144). Some studies suggest that 8-30 % of patients who fail to respond to initial induction therapy can be salvaged by early allogeneic HSCT, although large trials are lacking (145, 146). So even though outcomes are poor, allogeneic HSCT has the potential to offer a long-term cure.

Patient three, who had a diagnosis of AML M7 refractory to standard induction and transplanted in CR1 after salvage therapy with FLAG, was found to have a relapse on day +33 with a mixed chimaerism (24 % host). Immunosuppression was stopped in order for the GvL effect to halt the progression of disease. Unfortunately, he died subsequently on day +35 as a result of the relapse. AML M7, or acute megakaryocytic leukaemia, is a rare subtype of leukaemia and makes up about 1 % of cases diagnosed in adults. Reports suggest that the risk of relapse of this disease after standard induction and consolidation therapy are higher than with other AML subtypes (147). Although allogeneic HSCT offers a good chance of long-term survival, relapse rates are also still high after this intervention (148).

Table 11: *Characteristics of the three patients who did not achieve full remission directly following transplantation*

	Patient One	**Patient Two**	**Patient Three**
Diagnosis	1º AML with MLL-rearrangement and t(1;9;11)	2º AML M2, NPM1 wild type	AML M7
Age	18	56	55
Treatment lines before allo-HSCT	Four	One	Two
Status Before Transplantation	Borderline CR2	Progressive disease, blast count 24 %	CR1 (salvage therapy required)
Donor Type	MUD	MUD	MUD
Engraftment (days)			
Neutrophils	20	18	20
Platelets	16	19	19
OS (days)	38	416	35

Primary Outcome: Relapse-free Survival

At the time of writing, there has been no other study reporting the outcomes of patients following Treo/Flu/AraC conditioning for allogeneic HSCT. The median age of our population was 54 years (range, 18-69 years). Table 15 (Appendix I) summaries the studies in which patients with haematological malignancies including AML, MDS or CML were conditioned with Treo/Flu prior to allogeneic HSCT. It can quickly be gleaned from this summary, that the patient populations observed in these studies are extremely heterogeneous with regards to diagnoses, disease characteristics, remission status and risk factors for relapse. The results that were reported were also drastically different. Comparing the results of the Treo/Flu/AraC study to the outcomes of similar patient groups treated with a combination of treosulfan and fludarabine should be done with caution. The patient population of the Treo/Flu/AraC study is also very heterogeneous as they were selected to receive Treo/Flu/AraC conditioning on an individual treatment basis (see Table 2). Twenty-five percent of all patients were in CR1, 15 % in CR2 and 4 % in CR3 at the time of transplantation. As discussed above, CR1 hides a potentially wide range of different disease characteristics and risk of relapse. Fifty-four percent were not in remission at the time of transplantation either with partial remission, refractory or stable disease, or following disease relapse. Cytogenetic characteristics according to predefined categories for AML (33 patients, 43 %) were adverse in 24 %, intermediate in 61 % and favourable in 15 %. Thirty-two percent of all patients had a secondary AML (43 % of all patients with a diagnosis of AML included in the study). The remaining 25 % of patients had either a diagnosis of MDS or MPN at the time of transplantation (see Table 2).

Taking in to account the characteristics of the group of patients treated in the Treo/Flu/AraC study and comparing their outcomes with those of patients conditioned with Treo/Flu, we can form an idea of what can be achieved or what is expected in similar patient populations. FLAMSA-RIC is employed almost exclusively in high-risk AML patients, either in remission or with active disease. Therefore, a comparison of the RFS results with the FLAMSA-RIC protocol will be discussed later under the AML subgroup analysis.

One-, two- and three-year relapse-free survival rates in the Treo/Flu/AraC study were 47.5 %, 40.7 % and 37.3 %, respectively. In contrast to the patients included in this study, the majority of prospective Treo/Flu studies to date have selected AML patients in complete remission or MDS/CML patients with a low relapse risk (93, 136, 149, 150). A prospective nonrandomised phase II study observing the outcomes of 75 patients with AML in complete remission (CR1 80 %, CR2 17 %, CR3 3 %) with a median age of 45 years demonstrated a two-year relapse free survival of 55 % (149). This result is better than the one achieved in the Treo/Flu/AraC study, however, the patients in the phase II study were younger and in complete remission at the time of transplantation. A separate small prospective study including 31 medically infirm

patients (median age 55 years, HCT-CI >2) with AML, MDS or treatment-related AML showed an impressive disease-free survival of 79 % at 20 months (151). Again, these patients were all transplanted without active disease. A further small prospective study examining the outcomes of 26 patients, median age 60 years, with advanced MDS or sAML (47 % CR1) observed a two-year relapse-free survival of 34 % (85). These patients were older than the group in the Treo/Flu/AraC study, however, the disease characteristics were similar between the two studies. This could suggest that in high risk patients Treo/Flu/AraC provides an improved RFS. From this small overview of the studies of Treo/Flu conditioning, it can be seen that there is a large variation in the RFS rates following allogeneic HSCT. The results of the Treo/Flu/AraC study sit within the range of those observed in the published Treo/Flu studies.

Following the analysis of the whole patient population conditioned with Treo/Flu/AraC, patients were split into groups, based on the type of donor, remission status at transplantation and age. Patients who received a transplant from an MUD were found to have a one-, two- and three-year RFS of 46.9 %, 38.0 % and 35.4 %, respectively. For those receiving a transplant from an MRD, these figures were 52.4 %, 47.1 % and 39.3 %, respectively. Patients receiving a transplant from an mMUD had an RFS of 40.0 % at one year and remained at this level throughout follow-up. Although there was a trend to better RFS after MRD transplantation, this was not statistically significant in the univariate analysis. It is still accepted that the standard donor should be an HLA-matched sibling. That said, prospective (152, 153) and retrospective studies (154, 155) have indicated that outcomes after MRD and MUD HSCT are comparable. No randomised trial has compared outcome of transplants from different donors. The results of a large retrospective study, published in 2010 by Woolfrey *et al.*, concluded that patients with high-risk disease displayed equivalent overall survival and disease-free survival rates after receiving an MRD transplant as compared to an MUD transplant. However, those with intermediate-risk disease had a worse overall survival after receiving an MUD transplant than after an MRD transplant (156). This could be explained by the added importance of the GvL effect in disease with a higher risk of relapse. In contrast, another large retrospective study of patients with AML, ALL or CML, published in 2009 by Ringdén *et al*., concluded that there is a similar GvL effect between MRD and MUD transplants (157). In the Treo/Flu/AraC study, due to the homogeneity of the diagnoses and remission status at transplantation, a multivariate analysis controlling for risk of relapse, could not be meaningfully performed. However, from the results of the univariate analysis, it can be said that the type of donor used in this patient group did not affect the outcome of the transplantation with regards to RFS.

Transplants from mMUDs have been associated with an increased risk of GvHD and a related increase in NRM with poorer OS (121, 158). The success of a transplant from an mMUD is dependent on the level of mismatch (9/10, 8/10) and exactly which loci are mismatched (121). The deleterious effects on NRM and GvHD have to be weighed against the potential for an

improved RFS rate due to the increased GvL effect in patients with advanced and high-risk disease (159). In this study, three of the ten patients who received a graft from an mMUD experienced an early relapse of disease (< 6 months following allogeneic HSCT). However, no patient experienced a late relapse. Indeed, this may have been due to the enhanced GvL effect in these high-risk patients.

Those patients who were transplanted in CR1 did not achieve significantly better RFS rates than those transplanted in CR2, CR3 or other non-remission states. There was a trend to better RFS at one year, but by two years there was no difference in RFS and at three years this trend had reversed. The difference between the two groups was not significant (p = 0.839). Most large randomised studies of potential new conditioning regimens take patients in CR1 as they have the best rates of RFS and OS (160). A large study using registry data by Nagler *et al.*, examined the outcomes of AML patients conditioned with Treo/Flu and found a significantly improved leukaemia-free survival for patients transplanted in CR1 compared to those with active/advanced disease (161). The finding that there was no significant difference in the RFS of the two patient groups in the Treo/Flu/AraC study could suggest that the addition of cytarabine makes this regimen more effective against those transplanted with high-risk or active disease. This could be a significant finding and could indicate a positive effect of cytarabine on RFS. However, as discussed previously, even patients in CR1 have widely varying risks of disease relapse. The patients in the Treo/Flu/AraC study who were transplanted in CR1 were, despite initial response to therapy, still at a very high risk of relapse and this too, could explain the result observed here.

Those patients receiving a transplant at the age of 50 or over had a one-, two- and three-year RFS of 49.1 %, 43.1 % and 40.2 %, respectively. The figures for those transplanted under the age of 50 were 44.4 %, 37.0 % and 33.3 %, respectively. Again, there was no significant difference in RFS between these two groups (p = 0.969). Age has been shown, however, to be a poor prognostic indicator in predicting outcomes, especially RFS, following allogeneic HSCT (162). This observation matched the findings of this study.

Whether a patient will experience a relapse or not is governed by the interplay of many factors. By increasing the antileukaemic activity of the Treo/Flu protocol with the addition of cytarabine and the use of the higher dose of treosulfan (14 g/m^2 instead of 10 g/m^2), it was hoped that the Treo/Flu/AraC protocol would reduce the risk of disease relapse in high-risk patients. Prospective randomised studies against established conditioning regimens such as Treo/Flu and FLAMSA-RIC are required to prove this assumption.

Secondary Outcomes

Overall Survival

One, two- and three-year OS rates were 59.3 %, 49.3 % and 45.4 %, respectively. As with the RFS results, there was no significant difference in the OS between patients transplanted with MRD, MUD or mMUD, no difference in those transplanted in CR1 and all other disease statuses and no difference with regards to those transplanted aged 50 or above and those transplanted below the age of 50.

Comparison with other studies is hampered by the fact that authors report RFS and OS at different time points depending on the length of follow-up. Table 15 (Appendix I) summarises the studies observing the outcomes of patients with AML, MDS and/or MPN who were conditioned with Treo/Flu. Studies report overall survival rates at a range of time points from one year to five years.

A small retrospective study evaluating the outcomes of patients with MDS undergoing treosulfan-based conditioning with a median age of 48.3 years at transplantation saw one- and three-year overall survival of 74 % and 57 % respectively (82). A prospective nonrandomised phase II trial of AML patients in complete remission (median age 45) undergoing Treo/Flu conditioning observed a two-year overall survival of 61 % (149). The results of these two studies are somewhat better than that of the Treo/Flu/AraC study, however, the median age of patients included was lower and in the AML study, all patients were in complete remission at the time of transplantation. A small prospective study of patients by Kröger *et al.* with advanced MDS or secondary AML (7/15 in CR1, 6/15 in ≥CR2, 2/15 untreated or refractory) with a median age of 60 undergoing Treo/Flu conditioning observed a two- year OS of 36 % (85). A larger registry data study by Nagler *et al*., analysing the outcomes of patients with AML treated with a treosulfan-based conditioning regimen found a five-year overall survival of 38 % (161). In the Nagler study, 43% of all patients were in CR1, compared to 25% of the patients in the Treo/Flu/AraC study. Extrapolating the results of the Treo/Flu/AraC study to the five-year mark gives an overall survival of 39.7 %. The results of the Treo/Flu/AraC study demonstrate potentially better OS compared to the Kröger and Nagler studies, when the number of patients in CR1 are taken in to account.

Even from looking at this small group of studies, the overall survival rates, like the RFS rates for patients with AML, MDS or MPN following allogeneic HSCT, vary greatly. However, the results reported in the Treo/Flu/AraC study are acceptable in this high-risk population.

Non-relapse Mortality

Cumulative incidences of NRM in this study were 10 % (95 % CI, 5 %-18 %) at 100 days, 18.8 % (95 % CI, 11 %-28 %) at one year and 20.1 % (95 % CI, 12 %-30 %) at two years. After two years there were only two patients who died without a known relapse of their disease. Again, as with the RFS and OS rates, the cumulative incidence of NRM varies greatly between the Treo/Flu studies. A treosulfan dose escalation study, published in 2010 by Casper *et al.*, analysed the outcomes of 56 patients with various haematological malignancies conditioned with Treo/Flu (median age 50 years). They found an NRM of 13 % at day 100 and 20 % at two years (83). A study by Claudiani *et al.*, published in 2016, observed the outcomes of 14 patients with myelofibrosis with a median age of 57 years and found an NRM at two years of 39 % (163). The authors suggest a number of reasons for this high NRM including; high median age of patients, a heavy pre-HSCT transfusion history and splenomegaly ≥ 20cm in all patients. A further study by Casper *et al*., published in 2012, examined the outcomes of 75 patients with AML after Treo/Flu conditioning and showed an NRM of 11 % at two years (149). The reason for this low NRM compared to that of the Treo/Flu/AraC study is likely the strict exclusion criteria for participants including therapy-related secondary AML, previous allogeneic HSCT, active infectious disease and impaired renal function and the median age of the study participants was 45 years old. The NRM of the Treo/Flu/AraC study is acceptable, in the heavily pre-treated population studied, and comparable to that of a number of the Treo/Flu studies. To gather better quality data for comparison of the regimens, a prospective, randomised controlled trial would need to be carried out.

As with the results of RFS and OS, there was no significant difference in NRM between patients receiving different donor types and disease status at transplantation. Studies in the myeloablative setting have suggested that patients receiving grafts from MUDs have a higher NRM due to the increased risk of GvHD (157, 164). However, in the nonmyeloablative setting (2Gy TBI +/- fludarabine) there seems to be no significant difference in NRM between different donors (165). In the Treo/Flu studies where this analysis was performed, there also appears to be no significant difference in NRM between donor types (136, 149), in agreement with the results of the Treo/Flu/AraC study.

Traditionally, the high risk of NRM is a major obstacle in transplanting patients with active neoplastic haematological disease. Historical trials using busulfan- or TBI-based regimens report an NRM of 30-40 % at day 100 in patients not transplanted in remission (142, 166). An advanced or active disease status at transplantation was also found to be an independent risk factor for NRM in the large registry-based analysis of 520 patients with AML conditioned with Treo/Flu (161). The authors of this registry-based analysis did not suggest a reason for this finding. In the univariate analysis of the Treo/Flu/AraC study, there was no significant difference in the NRM between patients transplanted in CR1 and those in other remission

statuses. The findings of the Treo/Flu/AraC study could be explained by the number of previous lines of treatment that were required by patients to achieve CR1. Patients transplanted in CR1 and those transplanted in other remission statuses had a median of two previous lines of chemotherapy. Therefore, the factors contributing to NRM were likely to have been similar between the two groups.

Age is also said to be a factor in the incidence of NRM following allogeneic HSCT (161, 167), and in our study the difference almost reached significance ($p = 0.077$, with younger age being the positive prognostic factor). This suggests that age may play a role in the rate of NRM in patients conditioned with Treo/Flu/AraC. However, some studies have not found any significant difference in NRM based on age in both the myeloablative and reduced intensity conditioning setting (149, 168). Indeed, within the HCT-CI devised by Sorror *et al.* age has been shown to be a poor prognostic factor and is assigned only one point (162). A dual centre retrospective study from the UK, observing the outcomes of patients with AML, MDS, ALL and CML undergoing RIC, concluded that age, disease status at transplantation and HCT-CI, taken alone, did not have an impact on NRM. However, patients with two or more of these adverse factors had worse NRM than those with none or one of them (169). The role that age alone plays in the incidence of NRM is not clear, and with improvements in supportive care and reduced intensity conditioning regimens, patients should not be excluded from allogeneic HSCT based solely on age.

Cumulative Incidence of Relapse

The one- and three-year cumulative incidences of relapse in the Treo/Flu/AraC study were 34 % (95 % CI, 24 %-44 %) and 41 % (95 % CI, 30 %-52 %), respectively. These results are on the higher end of the range that was reported in the Treo/Flu studies (see Table 15, Appendix I) and reflects the highly aggressive nature of the diseases included in the Treo/Flu/AraC study. The exact risk an individual has of experiencing a relapse is dependent on many factors. The presence and severity of aGvHD and cGvHD has been found to be related to a reduced risk of AML, CML and MDS relapse following allogeneic HSCT (170), (171)). In the Treo/Flu/AraC study, the cumulative incidence of cGvHD was much lower than expected and this may also have contributed to the higher incidence of relapse. Severe aGvHD (grades III-IV) was also only recorded in 6 % of patients. However, the strength of the correlation between GvHD and graft-versus-malignancy effect differs between AML, CML and MDS. AML is the least sensitive disease to this effect, MDS has an intermediate sensitivity and CML is the most sensitive malignancy (172). Therefore, the cumulative incidence of relapse observed in this study cannot solely be explained by the low incidence of GvHD.

The other major factors affecting the risk of relapse are the individual disease characteristics (specific diagnosis, cytogenetic and mutational analysis), conditioning intensity and donor

choice. The patients selected for treatment with the Treo/Flu/AraC regimen were at an exceptionally high risk of disease relapse and this is another contributing factor to the cumulative incidence of relapse observed here.

As with RFS, OS and NRM, there was no significant difference between the cumulative incidence of relapse between patient groups based on donor type and remission status at transplantation. As previously discussed under ´Primary Outcome: Relapse-free Survival`, the finding that there was no significant difference between rates or relapse based on donor type (MRD vs. MUD vs mMUD) is in line with the findings of several studies (154, 173). However, the large Treo/Flu registry study by Nagler *et al.*, found a significantly lower risk of relapse in patients who received a graft from an MUD or an mMUD when compared to those who received a graft from an MRD (161). This finding could be due to the increased GvL effect in patients receiving unrelated donor grafts. There is still a lot of conflicting evidence surrounding optimal donor selection for allogeneic HSCT.

With regards to remission status, most studies have found that patients transplanted in complete remission have lower incidences of relapse than those transplanted with active disease (131, 141, 161). There are, however, a number of studies in which remission status prior to transplantation has not influenced the incidence of relapse. These include a FLAMSA-RIC study (174) and a single centre experience of both MAC and RIC (170). As discussed previously, the reason for the difference between the outcomes of patients in CR1 in these studies is likely due to the heterogeneity of relapse risk of patients transplanted in CR1. The 20 CR1 patients in the Treo/Flu/AraC study were, despite achieving a complete remission, still at a very high risk of disease relapse. The same is likely true for the patients transplanted in the aforementioned FLAMSA-RIC study. This finding could, however, also mean that the increased antileukaemic activity of the Treo/Flu/AraC regimen makes it a good candidate as a conditioning regimen for patients with very high-risk disease, regardless of their remission status prior to transplantation.

The available data on age and risk of relapse is also conflicting. Some Treo/Flu studies have found that patients under the age of 50 have a lower incidence of relapse compared to those transplanted at or over the age of 50 (149), whereas others have found the risk of relapse to be independent of age (151). In the Treo/Flu/AraC study the *p*-value examining the difference in the risk of relapse between the two groups (age <50 versus ≥50) was 0.08, in favour of patients transplanted at or over the age 50. This result seems counterintuitive given that older patients tend to have poorer prognostic factors including high-risk cytogenetics and secondary AML (23, 175). That the *p*-value almost reached significance perhaps warrants further investigation and suggests that the Treo/Flu/AraC regimen may have a positive effect on the incidence of relapse for patients transplanted at or over the age of 50. However, the reason

for this result is likely to be a combination of many factors both dependent and independent of the conditioning regimen. As the goal of the study was to assess the efficacy and feasibility of the Treo/Flu/AraC regimen and not to identify general risk factors for relapse and NRM, a detailed investigation of these findings does not fit within the scope of this work.

Engraftment and Graft Failure

The source of stem cells is one of the most important factors affecting engraftment kinetics (176, 177). Haematopoietic reconstitution is fastest after transplantation with PBSCs, then BM, and slowest after umbilical cord blood transplants. Other factors that appear to affect the speed of haematopoietic reconstitution (engraftment) include; underlying disease (reconstitution in AML faster than MDS, (178)), number of $CD34^+$ cells infused ($\geq 5 \times 10^6$/kg for PMN, $\geq 2 \times 10^6$/kg for platelets) (179, 180) and graft composition (the numbers of CD8+ and CD56+ cells) (181). The effect of the conditioning regimen on engraftment kinetics is not clear. Two retrospective studies examined the outcomes of patients with AML or MDS who underwent MAC or RIC prior to allogeneic HSCT. Time to engraftment was found to be independent of the conditioning regimen employed (182, 183). In contrast, a separate phase III randomised trial, published in 2017, comparing the outcomes of 272 patients with AML and MDS who underwent RIC or MAC, found that PMN engraftment was significantly quicker after MAC than RIC (184). There was no significant difference in the time to platelet engraftment.

After allogeneic transplantation with PBSCs, it is normally expected that the neutrophil count exceeds 0.5×10^9/l between day +14 and day +21 (178, 185). A short engraftment time is important to prevent complications such as infection (186) and bleeding (187). The median time to neutrophil and platelet engraftment in the Treo/Flu/AraC study was 20 days. This is comparable to the time required for engraftment in patients with CML conditioned with Treo/Flu (138), where 21 days were required for engraftment. However, other studies examining conditioning with Treo/Flu found that the period to engraftment was shorter. In a study examining patients with primary MDS, the time to neutrophil engraftment was 17 days and 16 days for platelets (136). Even faster engraftment was found in a small retrospective study examining outcomes in 31 patients with AML or MDS conditioned using Treo/Flu. The median time to neutrophil and platelet engraftment was 10 and 12 days, respectively (151). However, the protocol in this study included giving G-CSF as standard after transplantation. This is not the case in our institutions. G-CSF has only been found to reduce the neutropenic pre-engraftment phase by a few days without reducing the number of infections or length of hospital stay (188).

In the Treo/Flu/AraC study, the day +28 cumulative incidence of engraftment for neutrophils was 84.4 % (95 % CI, 74 %-91 %). By day +37 all patients had achieved successful neutrophil engraftment. The day +28 cumulative incidence of platelet engraftment in our study was 82 %

(95 % CI, 71 %-89 %). By day 100, this had increased to 92 % (95 % CI, 83 %-97 %). Two patients achieved platelet engraftment after day +100, the first on day +117 and the second on day +174. The large registry data study by Nagler *et al.*, looking at 520 patients with AML (43 % in CR1) who underwent transplantation using treosulfan-based regimens found that neutrophil engraftment rates were 94 % at day +30 (161). Ninety-three percent of patients had platelet engraftment by day 180. In a prospective nonrandomised phase II study examining the outcomes of 75 patients with AML (80 % in CR1) conditioned with Treo/Flu, a conditional cumulative incidence of neutrophil and platelet engraftment were found to be 93 % at day 28 (149). The cumulative incidence of neutrophil and platelet engraftment observed in the Treo/Flu/AraC study compare well with the findings of other studies.

Primary graft failure is a relatively rare complication of allogeneic HSCT. One large retrospective study of 967 patients identified only 6 cases (0.6 %) of PGF (123). The same study found 48 cases (4.9 %) of secondary graft failure. Several risk factors for graft failure have been identified and these should be modified if possible, prior to transplantation. These include: HLA-mismatches, use of an MUD, lower number of CD34+ cells in the graft, conditioning regimen (TBI reduces the risk of graft failure) and drug toxicity (139). There was no case of primary or secondary graft failure observed in the Treo/Flu/AraC study. However, nine patients (11 %) experienced poor graft function. Poor graft function is an important complication following allogeneic HSCT and occurs in 5 % to 27 % of patients (124). It is associated with considerable morbidity and mortality due to the increased duration of vulnerability to infection and haemorrhage. Few allogeneic transplantation studies define or even mention poor graft function making a comparison of this outcome unfeasible.

From the results of the Treo/Flu/AraC study it can be concluded that the combination of treosulfan and fludarabine with cytarabine performs well against other regimens in preparing the BM of the host for the engraftment of donor stem cells.

Chimaerism

The assessment of chimaerism after allogeneic HSCT monitors the origin of the haematopoiesis seen in the full blood count. It may alert the clinician to the development of graft rejection and the possibility of an impending relapse of the malignant disease. In patients transplanted due to a malignant disease, a mixed chimaerism may indicate the reappearance of the malignant cells, normal host haematopoiesis or both. All, but two, of the 20 patients in the Treo/Flu/AraC study, who were found to have a degree of mixed chimaerism between day 0 and day +180, experienced a relapse of their disease at or around the time that mixed chimaerism was detected. Of the two patients who did not experience a relapse following the detection of mixed chimaerism, one had a very low level of host cells (3-4 %) and the other had a decreasing mixed chimaerism over time.

The combination of Treo/Flu conditioning regimen has been shown to provide both the stem cell toxicity and immunosuppressive features to allow engraftment and complete chimaerism in the majority of patients (189). The cumulative incidence of complete donor-type chimaerism in the Treo/Flu/AraC study was 84 % (95 % CI, 74 %-90 %) on day +28. By day +100, 80 % of the patients were found to have complete donor-type chimaerism. By day +180 this figure was 68 %. The figures for day +28 complete chimaerism seen in the Treo/Flu studies range from 72 % (149) to 100 % (151). Day +100 figures range as well from 76 % (day 84) (190) to 94 % (83). The figures presented here fit comfortably within this range. The Treo/Flu/AraC conditioning regimen allows for the development of complete chimaerism in the patient population observed here.

Acute and Chronic GvHD

Acute and chronic GvHD greatly affect the quality of life, morbidity and NRM of patients following transplantation. The incidence of aGvHD is dependent on a number of well-defined risk factors including increasing degrees of HLA-mismatching, older age, the use of female donors for male recipients, prior alloimmunisation of the donor and the nature of GvHD prophylaxis (139). Other less well-defined risk factors include increasing donor age (191), increasing intensity of the conditioning regimen (192), the use of PBSC over BM (193) and recipient seropositivity for CMV (194).

In this study the day 100 cumulative incidences of grade I - IV, II - IV and III - IV acute GvHD were 38 % (95 % CI, 27 %-48 %), 22 % (95 % CI, 13 %-33 %) and 6 % (95 % CI, 2 %-14 %), respectively. According to the EBMT Handbook 2019 the incidence of moderate to severe aGvHD occurs in around 40 % of all recipients of an allogeneic HSCT (139). This figure is, however, heavily dependent on the donors used and the prophylaxis employed to avoid GvHD. Looking at the figures from published Treo/Flu conditioning studies, the overall incidence of aGvHD grade I-IV ranges from 27 % (137) to 56 % (136). The figure published here sits in the middle of this range. Furthermore, the incidence of grade III-IV aGvHD in the Treo/Flu/AraC study was comparable to the results seen in the Treo/Flu studies.

The cumulative incidence of mild to severe chronic GvHD at two years was 15 % (95 % CI, 8 %-24 %). There were three cases of severe cGvHD, two affecting the skin (day +879, day +1755) and one affecting the gastrointestinal tract (day +199). The incidence of cGvHD in our study is very low compared to the results seen in the numerous Treo/Flu conditioning studies, where the incidence of cGvHD ranged from 24 % (137) up to 72 % (82). Risk factors for cGvHD are MUD or mMUD transplant, older donor age, female donor for a male recipient and the use of TBI. The strongest risk factor is the history and severity of aGvHD (139). All three of the patients who developed severe cGvHD had a history of aGvHD, although the severity of the

aGvHD did not exceed grade II. Two of the patients received stem cells from an MUD and the third patient received an MRD.

The low rate of cGvHD in the Treo/Flu/AraC study could be explained by several factors. Firstly, the subjects presented here received treosulfan at the higher dose of 14 g/m^2. In the 2010 dose escalation study by Casper *et al.*, there were no cases of cGvHD observed in patients treated at this dose (83). The majority of Treo/Flu studies to date have used doses of 3 x 10 g/m^2–3 x 12 g/m^2. Treosulfan has been shown to exhibit strong immunosuppressive characteristics with less proinflammatory cytokine release than busulfan or cyclophosphamide in a mouse model (195). The increased immunosuppressive effect of the higher dose of treosulfan with the addition of cytarabine may have contributed to the lower incidence of cGvHD observed here. However, this observation is somewhat counteracted by the observed cumulative incidence of cGvHD of 48.1 % at one year by Sakellari *et al (151)*. This group administered treosulfan at a dose of 14 g/m^2. The relationship between the dose of treosulfan and the cumulative incidence of GvHD is not clear. Secondly, ATG for unrelated donors was employed in the protocol described here. The use of this substance has, as discussed previously, been shown to reduce the incidence of cGvHD. Thirdly, the retrospective nature of our study may also mean that not all cases of cGvHD were captured during data collection. Given that the biggest risk factor for cGvHD is the history and severity of aGvHD, our rates of aGvHD were not unusually low. A prospective study would need to be conducted to see if the low incidence of cGvHD could be reproduced in a similar patient population following Treo/Flu/AraC conditioning.

Toxicity and Adverse Events

The Treo/Flu regimen has already been demonstrated to achieve acceptable toxicity levels when used as a conditioning protocol prior to allogeneic HSCT. A retrospective study by Remberger *et al.* examining potential treosulfan-related toxicity in 118 patients undergoing Treo/Flu conditioning for both malignant and non-malignant haematological conditions concluded that the extramedullary toxicity of this regimen is low despite similar marrow toxicities when compared to myeloablative regimens (190). In the Remberger study, SOS occurred in one patient and incidences of infections, graft failure, GvHD and NRM did not differ from reduced intensity regimens.

Three patients (4 %) in the Treo/Flu/AraC study were suspected to have SOS and treated with defibrotide. One patient died on day 13 of SOS. This patient had received a graft from an mMUD. This has been identified as a risk factor for the development of SOS (196). Neither of the other two patients died as a result of SOS. The administration of the treosulfan analogue, busulfan, in conditioning regimens for allogeneic HSCT has been also shown to be an independent risk factor for the development of SOS (197). Following stem cell transplantation,

the overall mean incidence of SOS is thought to be in the range of 8 % to 14 %. This figure, however, varies greatly and is dependent on many factors including patient characteristics, type of stem cell transplant and conditioning regimen used (198). Treosulfan-based conditioning is believed to afford a lower risk of SOS compared to busulfan-based conditioning. The incidence of 4 % reported here, falls very much under the average value for all conditioning regimens and is broadly similar to the results found in some Treo/Flu studies (84), (161). In other Treo/Flu studies no cases of SOS were reported at all (82, 199). The addition of cytarabine to the Treo/Flu protocol does not seem to have a marked effect on the incidence of SOS.

As discussed previously, treosulfan is a hydrophilic analogue of busulfan. High-dose busulfan has long been associated with severe neurotoxicity and tonic clonic seizures as its lipophilic form allows it to easily cross the blood-brain barrier (BBB) (200, 201). Studies in rats have found that treosulfan does not readily cross the BBB (202). This result corresponds with clinical studies in children and adults, where serious neurotoxicity and seizures have only been observed in children under 4 months of age, due to the immaturity of the BBB (203). In our study no patient experienced seizures during their inpatient stay.

Mucositis is a common problem experienced during treatment with chemotherapeutic agents. Its incidence varies hugely in allogeneic HSCT with one systematic review estimating that oral mucositis occurs in between 47 % and 100 % of patients undergoing the procedure (204). It is defined as the painful inflammation and ulceration of the mucous membranes lining the gastrointestinal tract and can lead to increased bacterial translocation from the gut leading to sepsis and ultimately death. The increased friability of the GI tract also increases the risk of bleeding in already thrombocytopenic patients. In total, 41 % of the patients in our study experienced some degree of oral mucositis. This compares well with the Treo/Flu studies, especially as with the addition of the S-phase-specific agent cytarabine, the incidence of mucositis might be expected to be higher (205). The retrospective nature of this study may mean, however, that the incidence of mucositis in our patients was underreported. A prospective study with mucositis as a toxicity endpoint would need to be conducted to verify this result.

Renal and hepatotoxicity were, in the vast majority of the patients, self-limiting and reversible. Renal toxicity, measured by a rise in serum creatinine, was observed at grade 3-4 in only one patient. From this result we can see that there was a low occurrence of severe nephrotoxicity during and after Treo/Flu/AraC conditioning. The combination of treosulfan and fludarabine was also found to have relatively low nephrotoxic potential with no grade 3-4 creatinine rise seen in the original phase I/II study (80). The addition of cytarabine to the regimen could potentially increase the risk of nephrotoxicity, however, renal dysfunction comes under one of

the less commonly observed side-effects following the administration of this substance (206). The one patient who was observed to develop grade 3 nephrotoxicity required haemodialysis and subsequently developed chronic kidney disease stage IIIb. This patient had chronic kidney disease stage II prior to transplantation. In addition, she was very heavily pre-treated, having had two previous allogeneic HSCTs and numerous salvage therapies.

A transaminase rise was observed in 94 % of all patients, but at grade 3-4 only in 17 %. A grade 3-4 rise in the level of alkaline phosphatase was seen only in 3 patients, but in 58 % a grade 1-2 rise was observed. A grade 3-4 rise in bilirubin was seen in 16 % of patients. These results highlight the relatively low occurrence of severe hepatotoxicity in line with the findings of several Treo/Flu studies. It might be expected that with the addition of cytarabine the regimen would lead to increased hepatotoxicity. A study comparing high-dose cytarabine with intermediate dose cytarabine in the induction and consolidation of AML found that even with those receiving intermediate doses of cytarabine, 54 % suffered grade 3-4 hepatotoxicity (206). Such high numbers were not observed in the Treo/Flu/AraC study, suggesting that the Treo/Flu/AraC regimen provides acceptable levels of hepatotoxicity.

Infectious complications occurred in a large proportion of the patients in the Treo/Flu/AraC study. Sixty-two percent of all patients experienced febrile neutropenia at some point between the start of conditioning and day +28. Sepsis affected 45 % of patients with two patients dying in the first 28 days following transplantation as a result. A lung infection was diagnosed in 28 patients. One patient died on day +23 as a consequence of pneumonia. In total, an infectious cause was solely responsible for three out of the four deaths within the first 28 days following transplantation. The one cause of death in the first 28 days that was officially recorded as SOS was likely compounded by concurrent febrile neutropenia, fungal and bronchopneumonia. Therefore, it is highly likely that an infectious cause was responsible, at least in part, for all four of the early deaths. This remains a huge problem following allogeneic HSCT, especially with the increased prevalence of multidrug-resistant bacteria. In order to reduce the incidence of infection with Treo/Flu/AraC conditioning, the timing of the administration of treosulfan and cytarabine could be moved from day -6 to -4 to day -4 to -2. This could potentially reduce the duration of neutropenia and vulnerability to infection.

It is pertinent here to make a comparison with the FLAMSA-RIC regimen. As described previously, FLAMSA-RIC has been employed in the treatment of high-risk AML and the protocol is administered over 12 to 13 days. The toxicities and adverse events recorded after these regimens are administered, will be partially dependent on the specific treatments used in the RIC. An example of this is the incidence of SOS following FLAMSA-RIC. A regimen containing busulfan reported two deaths attributed to SOS (67). Other than these two cases, no other case of SOS has been reported following FLAMSA-RIC.

Due to the length of the FLAMSA-RIC protocol, the duration of pre-engraftment neutropenia may be longer when compared to that of other regimens, including Treo/Flu/AraC. The duration of neutropenia experienced by patients is very rarely reported in the literature. In the Treo/Flu/AraC study, three (4 %) of patients died, of an infectious cause, in the first 28 days following transplantation. A further three patients died as a result of an infection in the first 100 days. According to the FLAMSA-RIC study published by Malard *et al.* in 2017, 11.7 % of the subjects died from infection, the most common cause of NRM in this study. The authors do not, however, specify in what time frame these deaths took place and, therefore, it is unclear if they are related to the conditioning regimen. Schneidawind *et al.* reported a total number of five deaths (8 %) from infection in their FLAMSA-RIC (Bu/Flu, Bu/Cy or TBI/Cy) study (68). Again, the timeframe of these deaths was not explicitly stated. The analysis of 17 high-risk AML patients conditioned with FLAMSA and treosulfan-based RIC by Cheminitz *et al.*, reported that two patients died of septic shock prior to day +100 (11.7 %) (66). In a further treosulfan-based RIC FLAMSA study by Holtick *et al.* (167), eight patients (13.8 %) died due to infectious causes. Again, the time frame of these deaths was not reported. The NRM due to infection in these studies appears to be higher than that recorded in the Treo/Flu/AraC study. Unfortunately, none of these papers reported on the incidence of sepsis, pneumonia or febrile neutropenia. One FLAMSA-RIC study by Krejci *et al.* have published these figures. They reported that the most frequent toxicities were grade III/IV infections, occurring in 84 % of all patients (207). This is similar to the findings of the Treo/Flu/AraC study, with 85 % of the patients experiencing a grade III/IV infectious complication. This suggests that although patients conditioned with Treo/Flu/AraC may experience the same number of infectious complications, there is a lower mortality rate from these infections.

In the two FLAMSA-RIC studies in which the RIC protocol contains treosulfan, thereby giving us the best toxicity comparison to our protocol, the reporting of side-effects is scanty at best. The small study by Chemnitz *et al.* (66) use the Bearman criteria to grade toxicities. The Bearman grade toxicities as follows; grade I: reversible without treatment, grade II: requires treatment but not life-threatening, grade III: life-support intervention required, grade IV: fatal toxicity (208). According to these criteria 29.4 % of patients in the Chemnitz study experienced grade III/IV regimen-related toxicities. There was no reported case of SOS. Hepatic, renal and CNS toxicities were also published. The spectrum of toxicities is similar to that of the Treo/Flu/AraC study, but due to the smaller size of the study and the different grading systems, a direct comparison is not possible. FLAMSA combined with TBI- or busulfan-based RIC has been far more widely studied and reported on. Schneidawind observed a very high incidence of mucositis, with 58 % of all patients experiencing grade III/IV symptoms. This is far higher than the rate of 23 % in the Treo/Flu/AraC study and may be attributable to the use of TBI. Hepatobiliary toxicities were comparable between the study by Schneidawind *et al.* and the

Treo/Flu/AraC study (16 % vs 22 %). Renal toxicity was slightly higher in the Schneidawind study (15 % vs. 1.3 % grade III/IV) perhaps due to the use of cyclophosphamide in the RIC protocol. The toxicities and adverse events recorded during the Treo/Flu/AraC study, compare well to those reported following FLAMSA-RIC. However, to make any conclusions about the incidence of toxicities and adverse events between the FLAMSA-RIC and Treo/Flu/AraC regimens, prospective randomised controlled trials need to be conducted.

Two patients developed a secondary malignancy following HSCT. The first developed a palliative gallbladder cancer three years following the mMUD transplantation for AML M5. The second developed a ductal carcinoma *in situ* (ER negative, PR positive, Ki67 30 %, pTis, pN0, R0, G3) just over two and a half years following MUD transplantation for CML. The development of secondary malignancies following allogeneic HSCT is a well-defined late complication (209). They are responsible for some 5-10 % of late deaths following transplantation. Patients who have undergone allogeneic HSCT develop solid tumours at double the rate of the general population (210). Major risks factors for their development are TBI as part of the conditioning regimen and the development of moderate to severe cGvHD. Neither of our patients had either of these risk factors. Whilst the increased risk of breast cancer has been well studied and defined with a twenty-five-year cumulative incidence of 11 % (211), the occurrence of gallbladder carcinoma seems to be extremely rare. In fact, the only reference to a biliary cancer in the literature is a single case report of a patient developing a tubular adenocarcinoma of the lower bile duct nine years after allogeneic HSCT (212). Astoundingly, the adenocarcinoma in this case contained cells of donor origin. There is still much to be learned about the development of secondary malignancies after allogeneic HSCT and regular screening is recommended during long-term follow-up of patients.

Duration of Neutropenia

The severity and duration of neutropenia is a major factor in predicting the outcomes for patients following treatment with chemotherapy (213). A study looking at 396 patients who experienced febrile neutropenia following cancer treatment with chemotherapy found that 18.4 % suffered an unfavourable outcome and 3.4 % died as a result (214). Outcomes considered unfavourable were as follows; respiratory failure, refractory hypotension, intensive care unit admission, decreased mental status and renal failure requiring dialysis. A prolonged neutropenia (four or more days of a neutrophil count < 500/µl) was independently associated with an unfavourable outcome.

Due to the increased risk of unfavourable outcomes dependent on the duration of neutropenia, this is an important factor to consider when designing a conditioning regimen. The duration of the pre-engraftment neutropenic phase following the conditioning regimen and transplantation is critical. It has been shown in a large prospective multicentre study of patients treated for

various haematological malignancies with autologous or allogeneic HSCT, that one of the risk factors for the development of gram-negative sepsis is the duration of pre-engraftment neutropenia (215). In the univariate analysis of allogeneic HSCT patients, the presence of pretransplant neutropenia was not a significant risk factor for death within four months following transplantation.

Indeed, the most recent trial phase III trial comparing the outcomes of patients undergoing Treo/Flu or Bu/Flu conditioning had to temporarily suspend patient accrual as a result of concerns over the duration of neutropenia and infectious complications in the Treo/Flu group (93). As a result, the dose of treosulfan used in the study was reduced from 14 g/m^2 to 10 g/m^2 and the first day of the infusion schedule was changed from -6 to -4.

Eighteen patients in the Treo/Flu/AraC study were neutropenic prior to the start of conditioning (day -7). These patients had a significantly worse OS compared to those patients who became neutropenic after day -7. Although there was a trend to higher rates of relapse and worse RFS and NRM amongst patients neutropenic prior to day -7, the differences did not reach significance. The reason for the poorer OS is manifold and not solely a result of the longer period of neutropenia that these patients experienced. This was a heavily pretreated patient group. Of the 18 patients, four had had one previous allogeneic HSCT, and one had already had two. All but one patient had undergone two or more previous lines of treatment, and four had undergone five. The trend to the higher cumulative incidence of relapse in those patients who were neutropenic prior to day -7 suggests that these patients had higher risk disease. A combination of this and poorer NRM likely led to the significantly worse OS.

The majority of patients in the Treo/Flu/AraC study were neutropenic before day 0 and some for quite some time before this. As discussed previously, 25 patients in this study were given treosulfan on days -4 to -2 instead of -6 to -4, in the theoretical hope of reducing the duration of the vulnerable pre-engraftment phase. It was found, however, that the difference in timing of the administration of treosulfan did not lead to a significant difference in the duration of neutropenia. A larger follow-up study would be required to reproduce or disprove this result and to observe the effect that the timing of administration of treosulfan has on the risk of infectious complications.

AML Subgroup Analysis

To aid comparison between the outcomes of patients treated with Treo/Flu/AraC and FLAMSA-RIC, a subgroup analysis of patients in the Treo/Flu/AraC study with a diagnosis of AML at the time of transplantation, was performed. Table 16 (Appendix I) summarises the outcomes of adult patients treated with FLAMSA-RIC for AML. Table 17 (Appendix I) summarises outcomes of studies comparing treatment with Treo/Flu and FLAMSA-RIC.

FLAMSA-RIC is primarily employed to condition patients with refractory or high-risk AML (see (167) for a definition). As discussed previously in the introduction the rationale behind the FLAMSA-RIC regimen is to reduce the leukaemic burden in high-risk AML and MDS patients by giving a short intensive course of chemotherapy and then RIC before transplantation. There are several iterations of the FLAMSA-RIC treatment protocol, but the one most closely resembling our Treo/Flu/AraC regimen consists of; fludarabine 30 mg/m^2, cytarabine 2000 mg/m^2 and amsacrine 100 mg/m^2 day -13 to -10, treosulfan 10 g/m^2 day -6 to day -4 and cyclophosphamide on day -3 to -2 (40 mg/kg/day for an MRD and 60 mg/kg/day for an unrelated donor). This protocol differs with respect to the Treo/Flu/AraC regimen in the timing of the administration of fludarabine, cytarabine and treosulfan and with the addition of amsacrine and cyclophosphamide. The patients in the Treo/Flu/AraC study also fit in the definition of high-risk or refractory AML. The results can be seen in Table 12.

Table 12: *Outcomes of the AML subgroup analysis of the Treo/Flu/AraC study*

Outcome Observed	**Result (%)**			
	100 days	One year	Two years	Three years
Relapse-free Survival	Not reported	43.3	38.1	33.5
Overall Survival	Not reported	56.5	48.9	43.5
Non-relapse Mortality	11.7	21.7	21.7	Not reported
Relapse Rate	Not reported	35.0	41.2	42.5

There have only been two FLAMSA-RIC studies where TBI has been replaced by treosulfan. Chemnitz *et al.* treated 17 patients with refractory or high-risk AML (median age 57), with only 29 % being in CR1 at the time of transplantation (66). Holtick *et al.* treated 58 patients with high risk or relapsed AML (median age 60), with 31 % of patients being in CR1 at the time of transplantation (167). The results of these two studies are summarised in Table 13 and Table 14.

Table 13: *Outcomes of the FLAMSA-RIC study with treosulfan replacing TBI from Chemnitz* et al. 2012

Outcome Observed	**Result (%)**			
Chemnitz *et al. (66)*	100 days	One year	Two years	Three years
Relapse-free Survival	Not reported	55.0	Not reported	Not reported
Overall Survival	Not reported	62.0	Not reported	Not reported
Non-relapse Mortality	Not reported	20.0	Not reported	Not reported
Relapse Rate	Not reported	25.0	Not reported	Not reported

Table 14: *Outcomes of the FLAMSA-RIC study with treosulfan replacing TBI from Holtick et al. 2017*

Outcome Observed	**Result (%)**			
Holtick *et al.* (167)	100 days	One year	Two years	**Four years**
Relapse-free Survival	Not reported	Not reported	Not reported	41.0
Overall Survival	Not reported	Not reported	Not reported	47.0
Non-relapse Mortality	Not reported	Not reported	Not reported	28.0
Relapse Rate	Not reported	Not reported	32.0	Not reported

As can be seen by these short summaries, as is often the problem with comparing publications, is that the endpoints are calculated at different times. However, the results seem to be comparable with the Treo/Flu/AraC study. Extrapolating the results further gives a four-year RFS rate of 45.5 % from patients in the Treo/Flu/AraC study, which again compares well to the FLAMSA-RIC study by Holtick *et al*. The relapse rate was higher in the Treo/Flu/AraC study compared with either of the FLAMSA-RIC studies. A prospective head to head comparison of both conditioning regimens would need to be performed to ascertain if this difference is reproducible and significant.

The FLAMSA-RIC protocols that use TBI have also had comparable results to our study with one exception (see Table 16, Appendix I). The 2016 study by Pfrepper *et al.* observed a three-year OS of 15 %, a three-year event-free survival of 12 % and an NRM and a relapse rate of 18 % and 69 % respectively. A total of 43.2 % of the 44 AML patients in the study by Pfrepper *et al.* had an adverse cytogenetic risk profile according to the ELN and over half of the patients had a blast count of ≥20 % prior to conditioning. The extremely poor risk factors of these patients compared with the patients in the Treo/Flu/AraC study and those of the other FLAMSA-RIC studies, could explain the very high risk of relapse and mortality of these patients.

Future Perspectives

Retrospective studies have several limitations including the risk of bias and confounding factors. However, they are useful to collect pilot data that may be helpful in the design of a prospective study. To more accurately assess the safety of Treo/Flu/AraC conditioning in the treatment of AML/MDS/MPN a prospective trial would need to be conducted. To assess efficacy against an existing conditioning regimen such as busulfan/fludarabine or treosulfan/fludarabine, a controlled prospective randomised trial would need to be conducted. Although Treo/Flu/AraC has been shown in this study to have an acceptable safety profile, the addition of cytarabine at a dose of 2 g/m^2 is not without the possibility of worsening side-effects over the established Treo/Flu regimen. It is therefore important to identify the patients who would benefit most from the use of this regimen. A slightly higher NRM rate may be acceptable if the total RFS of carefully selected patients is significantly higher. Patients who would

potentially benefit from the increased antileukaemic effect of this conditioning regimen include those with high risk AML and those with higher blast counts prior to HSCT.

During the patient accrual phase of the recently published phase III Treo/Flu versus Bu/Flu trial, there were concerns about prolonged neutropenia and related infectious complications (93). As a result, the first day of administration of treosulfan was moved from day -6 to day -4. To reduce the length of neutropenia produced by the Treo/Flu/AraC regimen, the first day of administration of cytarabine could be also moved from day -6 to day -4. As fludarabine is administered from day -6 to day -2, the synergistic antileukaemic effect that the two agents have together could still be exploited.

There have been several studies emerging looking at the combination of Treo/Flu conditioning with TBI. A preclinical study in a rat model of allogeneic HSCT found that treosulfan possesses some features of a radiosensitizer and that gastrointestinal toxicity was a limiting factor in this combination of treatments (216). A prospective phase II trial by Gyurkocza *et al*. found no increase in the incidence of NRM with the addition of 2 Gy TBI (217). They summarised that the regimen was effective in conditioning patients with AML/MDS prior to allogeneic HSCT and resulted in a low incidence of relapse. A further prospective randomised phase II 'pick the winner' trial in patients with AML or MDS conducted by Deeg *et al.,* found that the addition 2 Gy TBI to the Treo/Flu regimen improved the overall outcome for patients by reducing the risk of relapse (218). The effect of the addition of TBI was notably more marked in those with AML than with MDS. The authors report that even with the addition of TBI, the regimen was very well tolerated. Perhaps the addition of 2 Gy TBI to the Treo/Flu/AraC could reduce the risk of disease relapse even further. High-dose cytarabine (HDAC) combined with TBI and cyclophosphamide has already been used to condition patients with AML/MDS receiving cord blood transplants (219). The authors found an increased OS with the addition of HDAC and a reduction in tumour-related death without an increase in NRM. However, the same study design conducted in patient with AML/MDS who received a PBSC/BM transplant found no improvement in outcomes with the addition of HDAC to CY/TBI and an increase in NRM (220). It is unclear, then, from existing studies, if the addition of TBI to the Treo/Flu/AraC regimen would significantly improve outcomes. However, it is of potential interest to try this combination to reduce the risk of relapse of the underlying malignant disease.

There are several substances that have been shown to increase the antileukaemic effect of cytarabine. In one study in a retrovirus-mediated murine model of leukaemia, three leukaemia cell lines and seven primary AML samples, valproic acid enhanced the toxic effect of cytarabine on leukaemic cells by significantly upregulating the expression of the proapoptotic protein BAX (BCL-2 associated X protein) (221). The CD33 targeted antibody-drug conjugate, SGN-CD33A, has been shown in preclinical models of AML to act synergistically with cytarabine to

increase the antileukaemic activity of both substances (222). The potential to introduce a nonchemotherapeutic agent into a conditioning regimen to increase the antileukaemic activity of that regimen is an option that should be evaluated further in clinical trials.

A cytological complete remission of leukaemia is defined as <5 % blasts found within the BM. This could mean, though, that a large number of malignant cells remain within the patient. As a result, minimal residual disease monitoring is coming into more widespread use in the monitoring of the remission status of patients with myeloid malignancies. Measuring the level of expression of the Philadelphia chromosome has long been employed in the monitoring of minimal residual disease in CML (223). Due to the heterogeneity of the mutations present in MDS/AML, monitoring of these conditions using approaches to measure minimal residual disease has proven more difficult (224). However, thanks to improvement in DNA sequencing (next generation sequences), along with digital PCR and imaging techniques such as PET-CT, monitoring of these conditions has become easier and will continue to improve (225). It is of vital importance to quantify the level of minimal residual disease in the transplant setting. A large metanalysis has concluded that patients with AML transplanted in complete remission with minimal residual disease have a significantly increased risk of relapse compared to those in which minimal residual disease was not detected (226). Therefore, with improved minimal residual disease monitoring in AML, we may be able to identify further candidates that will benefit from a conditioning regimen with increased antileukaemic activity.

The use of existing chemotherapeutic agents and/or TBI to design disease-specific conditioning protocols is a direction that is being currently being explored and developed. The Treo/Flu/AraC protocol is one example of a conditioning protocol designed to maximise antileukaemic potential. The combination of fludarabine with melphalan has been used to condition patients prior to allogeneic HSCT for multiple myeloma (227, 228). Melphalan, an alkylating agent, which was first synthesised in the 1950s, has been used for many decades in the treatment of myeloma (229, 230). The Treo/Flu protocol has also been trialled in intensively pre-treated myeloma patients (84). Rituximab has been studied in RIC regimens for B-cell non-Hodgkin lymphoma and early data suggest it improves progression-free survival over non-Rituximab containing regimens (231). Treo/Flu has also proved effective as conditioning prior to allogeneic SCT in lymphoid malignancies (232). Perhaps using treosulfan alone or in combination with fludarabine as a backbone for more disease-specific conditioning protocols could reduce toxicity whilst improving relapse- or progression-free survival.

Conclusion

From the outcomes observed in this retrospective study, we conclude that the combination of treosulfan and fludarabine with cytarabine is a feasible and effective conditioning regimen with low non-haematological toxicities and low cumulative incidence of cGvHD, resulting in an acceptable NRM. Treo/Flu/AraC conditioning can be offered as an alternative to those not just with aggressive disease with a poor prognosis, but also for those of advanced age and patients with significant comorbidities. The RFS and OS observed in the Treo/Flu/AraC study compares well to the same figures reported in Treo/Flu and FLAMSA-RIC studies in similar high-risk populations.

The advantage of Treo/Flu/AraC over FLAMSA-RIC is the shorter duration of treatment with the potential of a reduced length of hospital stay and neutropenic phase. The results of the Treo/Flu/AraC study suggest that although the numbers of patients experiencing febrile neutropenia is similar between the two regimens, the mortality due to an infectious complication is lower following Treo/Flu/AraC conditioning that following FLAMSA-RIC. The rationale behind the use of amsacrine in the FLAMSA-RIC protocol is to target leukaemic blasts that previously did not respond to cytarabine-based therapy. However, cytarabine has been found to be effective in salvage therapies even after failure of standard cytarabine-based induction or consolidation regimens (98). The idea of disease-specific conditioning protocols, such as cytarabine for AML and melphalan for myeloma, using treosulfan as a backbone should be investigated in the era of targeted therapies.

The cumulative incidence of relapse reported here is, although relatively high, not unexpected in this high-risk patient population. The advantage of the Treo/Flu/AraC regimen over the original Treo/Flu conditioning is its increased antileukaemic effect. This is a result of the higher dose of treosulfan alongside the synergistic effect of the combination of fludarabine and cytarabine. The data indicate that treosulfan and fludarabine combined with cytarabine is an effective conditioning regimen for HCT in patients who have AML/MDS/MPN in any remission status at the time of HCT.

A comparison between studies with vastly different patient populations, as performed here, has many limitations. A large multicentre, multinational prospective randomised study incorporating this regimen is required to provide robust evidence with enough statistical power on the outcomes of patients conditioned with Treo/Flu/AraC. This regimen should be compared to the established Treo/Flu and FLAMSA-RIC protocols to establish the characteristics of patients who will benefit the most from this regimen.

References

1. Passweg JR, Baldomero H, Bader P, Basak GW, Bonini C, Duarte R, et al. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant. 2018;53(9):1139-48.
2. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124(3):344-53.
3. Kahl C, Storer BE, Sandmaier BM, Mielcarek M, Maris MB, Blume KG, et al. Relapse risk in patients with malignant diseases given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood. 2007;110(7):2744-8.
4. Corey SJ, Minden MD, Barber DL, Kantarjian H, Wang JC, Schimmer AD. Myelodysplastic syndromes: the complexity of stem-cell diseases. Nat Rev Cancer. 2007;7(2):118-29.
5. Van Etten RA, Shannon KM. Focus on myeloproliferative diseases and myelodysplastic syndromes. Cancer cell. 2004;6(6):547-52.
6. Malcovati L, Cazzola M. Myelodysplastic/myeloproliferative disorders. Haematologica. 2008;93(1):4-6.
7. Shetty V, Hussaini S, Broady-Robinson L, Allampallam K, Mundle S, Borok R, et al. Intramedullary apoptosis of hematopoietic cells in myelodysplastic syndrome patients can be massive: apoptotic cells recovered from high-density fraction of bone marrow aspirates. Blood. 2000;96(4):1388-92.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
9. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised International Prognostic Scoring System for Myelodysplastic Syndromes. Blood. 2012;120(12):2454-65.
10. Della Porta MG, Tuechler H, Malcovati L, Schanz J, Sanz G, Garcia-Manero G, et al. Validation of WHO classification-based Prognostic Scoring System (WPSS) for myelodysplastic syndromes and comparison with the revised International Prognostic Scoring System (IPSS-R). A study of the International Working Group for Prognosis in Myelodysplasia (IWG-PM). Leukemia. 2015;29(7):1502-13.
11. Steensma DP. Myelodysplastic syndromes current treatment algorithm 2018. Blood cancer journal. 2018;8(5):47.
12. Castelli R, Schiavon R, Rossi V, Deliliers GL. Management of anemia in low-risk myelodysplastic syndromes treated with erythropoiesis-stimulating agents newer and older agents. Med Oncol. 2018;35(5):76.
13. Steensma DP. Hematopoietic growth factors in myelodysplastic syndromes. Semin Oncol. 2011;38(5):635-47.
14. Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G. Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature. 1974;251(5474):437-8.
15. Boultwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, et al. Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. Blood. 2002;99(12):4638-41.
16. Magalhaes SMM, Velloso E, Buzzini R, Bernardo WM. Part 4: Myelodysplastic syndromes-Treatment of low-risk patients with the 5q deletion. Hematology, transfusion and cell therapy. 2018;40(3):274-7.
17. Stahl M, Zeidan AM. Lenalidomide use in myelodysplastic syndromes: Insights into the biologic mechanisms and clinical applications. Cancer. 2017;123(10):1703-13.
18. Gurion R, Vidal L, Gafter-Gvili A, Belnik Y, Yeshurun M, Raanani P, et al. 5-azacitidine prolongs overall survival in patients with myelodysplastic syndrome--a systematic review and meta-analysis. Haematologica. 2010;95(2):303-10.
19. Shapiro RM, Lazo-Langner A. Systematic review of azacitidine regimens in myelodysplastic syndrome and acute myeloid leukemia. BMC hematology. 2018;18:3.

20. Brierley CK, Steensma DP. Allogeneic stem cell transplantation in myelodysplastic syndromes: does pretransplant clonal burden matter? Curr Opin Hematol. 2016;23(2):167-74.
21. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med. 1999;341(14):1051-62.
22. Watts J, Nimer S. Recent advances in the understanding and treatment of acute myeloid leukemia. F1000Research. 2018;7.
23. Juliusson G, Antunovic P, Derolf A, Lehmann S, Mollgard L, Stockelberg D, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. Blood. 2009;113(18):4179-87.
24. Agrawal K, Miles L, Agrawal N, Khan A. Atypical Presentation of Acute Myeloid Leukemia. World J Oncol. 2018;9(1):29-34.
25. Maynor ML. Neurological manifestations as the initial presentation of acute myelogenous leukemia. Am J Emerg Med. 1989;7(5):481-4.
26. Potenza L, Luppi M, Morselli M, Tonelli S, D'Apollo N, Facchini L, et al. Leukaemic pulmonary infiltrates in adult acute myeloid leukaemia: a high-resolution computerized tomography study. Br J Haematol. 2003;120(6):1058-61.
27. Verhaak RG, Goudswaard CS, van Putten W, Bijl MA, Sanders MA, Hugens W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood. 2005;106(12):3747-54.
28. Dohner K, Schlenk RF, Habdank M, Scholl C, Rucker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005;106(12):3740-6.
29. Zajac M, Dolnik A, Stasiak G, Zaleska J, Kielbus M, Czapinski J, et al. Analysis of NPM1 splice variants reveals differential expression patterns of prognostic value in acute myeloid leukemia. Oncotarget. 2017;8(56):95163-75.
30. Othus M, Garcia-Manero G, Godwin J, Weick JK, Anderson JE, Stirewalt D, et al. Associations between Complete Remissions (CRs) with 7+3 Induction Chemotherapy for Acute Myeloid Leukemia and 2-3 Year Survival ("Potential Cure") over the Past Four Decades: Analysis of SWOG Trial Data. Blood. 2017;130(Suppl 1):1301-.
31. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 2017;377(5):454-64.
32. Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood cancer journal. 2018;8(2):15.
33. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2012 update on diagnosis, monitoring, and management. Am J Hematol. 2012;87(11):1037-45.
34. Muller LP, Muller-Tidow C. The indications for allogeneic stem cell transplantation in myeloid malignancies. Dtsch Arztebl Int. 2015;112(15):262-70.
35. Barrett AJ, Ito S. The role of stem cell transplantation for chronic myelogenous leukemia in the 21st century. Blood. 2015;125(21):3230-5.
36. Khoury HJ, Kukreja M, Goldman JM, Wang T, Halter J, Arora M, et al. Prognostic factors for outcomes in allogeneic transplantation for CML in the imatinib era: a CIBMTR analysis. Bone Marrow Transplant. 2012;47(6):810-6.
37. Helbig G. Classical Philadelphia-negative myeloproliferative neoplasms: focus on mutations and JAK2 inhibitors. Med Oncol. 2018;35(9):119.
38. Vannucchi AM, Kiladjian JJ, Griesshammer M, Masszi T, Durrant S, Passamonti F, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med. 2015;372(5):426-35.
39. Harrison CN, Mead AJ, Panchal A, Fox S, Yap C, Gbandi E, et al. Ruxolitinib vs best available therapy for ET intolerant or resistant to hydroxycarbamide. Blood. 2017;130(17):1889-97.

40. Verstovsek S, Passamonti F, Rambaldi A, Barosi G, Rumi E, Gattoni E, et al. Ruxolitinib for essential thrombocythemia refractory to or intolerant of hydroxyurea: long-term phase 2 study results. Blood. 2017;130(15):1768-71.
41. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9):799-807.
42. Fedratinib Becomes New Option in Myelofibrosis. Cancer Discov. 2019;9(10):1332.
43. Tiribelli M, Palandri F, Sant'Antonio E, Breccia M, Bonifacio M. The role of allogeneic stem-cell transplant in myelofibrosis in the era of JAK inhibitors: a case-based review. Bone Marrow Transplant. 2019.
44. Thomas ED, Lochte HL, Jr., Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med. 1957;257(11):491-6.
45. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968;2(7583):1366-9.
46. Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet. 1968;2(7583):1364-6.
47. Thomas ED, Collins JA, Herman EC, Jr., Ferrebee JW. Marrow transplants in lethally irradiated dogs given methotrexate. Blood. 1962;19:217-28.
48. Graw RG, Jr., Brown JA, Yankee RA, Leventhal BG, Whang-Peng J, Rogentine GN, et al. Transplantation of HL-A identical allogeneic bone marrow to a patient with acute lymphocytic leukemia. Blood. 1970;36(6):736-47.
49. Thomas ED, Storb R, Fefer A, Slichter SJ, Bryant JI, Buckner CD, et al. Aplastic anaemia treated by marrow transplantation. Lancet. 1972;1(7745):284-9.
50. Graw RG, Jr., Yankee RA, Rogentine GN, Leventhal BG, Herzig GP, Halterman RH, et al. Bone marrow transplantation from HL-A matched donors to patients with acute leukemia. Toxicity and antileukemic effect. Transplantation. 1972;14(1):79-80.
51. Santos GW, Tutschka PJ. Marrow transplantation in the busulfan-treated rat: preclinical model of aplastic anemia. J Natl Cancer Inst. 1974;53(6):1781-5.
52. Tutschka PJ, Santon GW. Bone marrow transplantation in the busulfin-treated rat. III. Relationship between myelosuppression and immunosuppression for conditioning bone marrow recipients. Transplantation. 1977;24(1):52-62.
53. Kapoor N, Kirkpatrick D, Blaese RM, Oleske J, Hilgartner MH, Chaganti RS, et al. Reconstitution of normal megakaryocytopoiesis and immunologic functions in Wiskott-Aldrich syndrome by marrow transplantation following myeloablation and immunosuppression with busulfan and cyclophosphamide. Blood. 1981;57(4):692-6.
54. Parkman R, Rappeport JM, Hellman S, Lipton J, Smith B, Geha R, et al. Busulfan and total body irradiation as antihematopoietic stem cell agents in the preparation of patients with congenital bone marrow disorders for allogenic bone marrow transplantation. Blood. 1984;64(4):852-7.
55. Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. Blood. 1987;70(5):1382-8.
56. Clift RA, Buckner CD, Appelbaum FR, Sullivan KM, Storb R, Thomas ED. Long-term follow-Up of a randomized trial of two irradiation regimens for patients receiving allogeneic marrow transplants during first remission of acute myeloid leukemia. Blood. 1998;92(4):1455-6.
57. Marks DI, Forman SJ, Blume KG, Perez WS, Weisdorf DJ, Keating A, et al. A comparison of cyclophosphamide and total body irradiation with etoposide and total body irradiation as conditioning regimens for patients undergoing sibling allografting for acute lymphoblastic leukemia in first or second complete remission. Biol Blood Marrow Transplant. 2006;12(4):438-53.
58. Rosales F, Peylan-Ramu N, Cividalli G, Varadi G, Or R, Naparstek E, et al. The role of thiotepa in allogeneic bone marrow transplantation for genetic diseases. Bone Marrow Transplant. 1999;23(9):861-5.
59. Helenglass G, Powles RL, McElwain TJ, Lakhani A, Milan S, Gore M, et al. Melphalan and total body irradiation (TBI) versus cyclophosphamide and TBI as conditioning for allogeneic matched

sibling bone marrow transplants for acute myeloblastic leukaemia in first remission. Bone Marrow Transplant. 1988;3(1):21-9.
60. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-33.
61. Terenzi A, Aristei C, Aversa F, Perruccio K, Chionne F, Raymondi C, et al. Efficacy of fludarabine as an immunosuppressor for bone marrow transplantation conditioning: preliminary results. Transplant Proc. 1996;28(6):3101.
62. Cain BF, Atwell GJ. The experimental antitumour properties of three congeners of the acridylmethanesulphonanilide (AMSA) series. Eur J Cancer. 1974;10(8):539-49.
63. Ketron AC, Denny WA, Graves DE, Osheroff N. Amsacrine as a topoisomerase II poison: importance of drug-DNA interactions. Biochemistry. 2012;51(8):1730-9.
64. Cassileth PA, Gale RP. Amsacrine: a review. Leuk Res. 1986;10(11):1257-65.
65. Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol. 2005;23(24):5675-87.
66. Chemnitz JM, von Lilienfeld-Toal M, Holtick U, Theurich S, Shimabukuro-Vornhagen A, Krause A, et al. Intermediate intensity conditioning regimen containing FLAMSA, treosulfan, cyclophosphamide, and ATG for allogeneic stem cell transplantation in elderly patients with relapsed or high-risk acute myeloid leukemia. Ann Hematol. 2012;91(1):47-55.
67. Malard F, Labopin M, Stuhler G, Bittenbring J, Ganser A, Tischer J, et al. Sequential Intensified Conditioning Regimen Allogeneic Hematopoietic Stem Cell Transplantation in Adult Patients with Intermediate- or High-Risk Acute Myeloid Leukemia in Complete Remission: A Study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2017;23(2):278-84.
68. Schneidawind D, Federmann B, Faul C, Vogel W, Kanz L, Bethge WA. Allogeneic hematopoietic cell transplantation with reduced-intensity conditioning following FLAMSA for primary refractory or relapsed acute myeloid leukemia. Ann Hematol. 2013;92(10):1389-95.
69. Shimoni A, Nagler A. Optimizing the conditioning regimen for allogeneic stem-cell transplantation in acute myeloid leukemia; dose intensity is still in need. Best Pract Res Clin Haematol. 2011;24(3):369-79.
70. Dupont B. Immunology of hematopoietic stem cell transplantation: a brief review of its history. Immunological reviews. 1997;157:5-12.
71. Henig I, Zuckerman T. Hematopoietic stem cell transplantation-50 years of evolution and future perspectives. Rambam Maimonides medical journal. 2014;5(4):e0028.
72. Gropp M, Meier W, Hepp H. Treosulfan as an effective second-line therapy in ovarian cancer. Gynecol Oncol. 1998;71(1):94-8.
73. Chekerov R, Kaltenecker G, Reichert D, Gohler T, Klare P, Oskay-Ozcelik G, et al. Treosulfan in the Treatment of Advanced Ovarian Cancer - Results of a German Multicenter Non-interventional Study. Anticancer Res. 2015;35(12):6869-75.
74. Harstrick A, Wilke H, Eberhardt W, Klaassen U, Strumberg D, Korn M, et al. A Phase I Dose Escalation Trial of Intravenous Treosulfan in Refractory Cancer. Oncology Research and Treatment. 1996;19(2):153-6.
75. Litzow MR, Perez WS, Klein JP, Bolwell BJ, Camitta B, Copelan EA, et al. Comparison of outcome following allogeneic bone marrow transplantation with cyclophosphamide-total body irradiation versus busulphan-cyclophosphamide conditioning regimens for acute myelogenous leukaemia in first remission. Br J Haematol. 2002;119(4):1115-24.
76. Scheulen ME, Hilger RA, Oberhoff C, Casper J, Freund M, Josten KM, et al. Clinical phase I dose escalation and pharmacokinetic study of high-dose chemotherapy with treosulfan and autologous peripheral blood stem cell transplantation in patients with advanced malignancies. Clin Cancer Res. 2000;6(11):4209-16.

77. Beelen DW, Trenschel R, Casper J, Freund M, Hilger RA, Scheulen ME, et al. Dose-escalated treosulphan in combination with cyclophosphamide as a new preparative regimen for allogeneic haematopoietic stem cell transplantation in patients with an increased risk for regimen-related complications. Bone Marrow Transplant. 2005;35(3):233-41.
78. Danylesko I, Shimoni A, Nagler A. Treosulfan-based conditioning before hematopoietic SCT: more than a BU look-alike. Bone Marrow Transplant. 2012;47(1):5-14.
79. Munkelt D, Koehl U, Kloess S, Zimmermann SY, Kalaaoui RE, Wehner S, et al. Cytotoxic effects of treosulfan and busulfan against leukemic cells of pediatric patients. Cancer Chemother Pharmacol. 2008;62(5):821-30.
80. Casper J, Knauf W, Kiefer T, Wolff D, Steiner B, Hammer U, et al. Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. Blood. 2004;103(2):725-31.
81. Fan CQ, Crawford JM. Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). J Clin Exp Hepatol. 2014;4(4):332-46.
82. Hilgendorf I, Wolff D, Gromke T, Trenschel R, Elmaagacli AH, Pichlmeier U, et al. Retrospective analysis of treosulfan-based conditioning in comparison with standard conditioning in patients with myelodysplastic syndrome. Bone Marrow Transplant. 2011;46(4):502-9.
83. Casper J, Wolff D, Knauf W, Blau IW, Ruutu T, Volin L, et al. Allogeneic hematopoietic stem-cell transplantation in patients with hematologic malignancies after dose-escalated treosulfan/fludarabine conditioning. J Clin Oncol. 2010;28(20):3344-51.
84. Schmidt-Hieber M, Blau IW, Trenschel R, Andreesen R, Stuhler G, Einsele H, et al. Reduced-toxicity conditioning with fludarabine and treosulfan prior to allogeneic stem cell transplantation in multiple myeloma. Bone Marrow Transplant. 2007;39(7):389-96.
85. Kroger N, Shimoni A, Zabelina T, Schieder H, Panse J, Ayuk F, et al. Reduced-toxicity conditioning with treosulfan, fludarabine and ATG as preparative regimen for allogeneic stem cell transplantation (alloSCT) in elderly patients with secondary acute myeloid leukemia (sAML) or myelodysplastic syndrome (MDS). Bone Marrow Transplant. 2006;37(4):339-44.
86. Shimoni A, Rand A, Shem-Tov N, Hardan I, Volchek Y, Yerushalmi R, et al. Fludarabine and Treosulfan Conditioning for Allogeneic Stem-Cell Transplantation; a Dose- Intense Regimen with Limited Toxicity. Blood. 2010;116(21):3473-.
87. Boztug H, Zecca M, Sykora KW, Veys P, Lankester A, Slatter M, et al. Treosulfan-based conditioning regimens for allogeneic HSCT in children with acute lymphoblastic leukaemia. Ann Hematol. 2015;94(2):297-306.
88. Burroughs LM, Nemecek ER, Torgerson TR, Storer BE, Talano JA, Domm J, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. Biol Blood Marrow Transplant. 2014;20(12):1996-2003.
89. Bernardo ME, Piras E, Vacca A, Giorgiani G, Zecca M, Bertaina A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood. 2012;120(2):473-6.
90. Shimoni A, Labopin M, Savani B, Hamladji RM, Beelen D, Mufti G, et al. Intravenous Busulfan Compared with Treosulfan-Based Conditioning for Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia: A Study on Behalf of the Acute Leukemia Working Party of European Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2017.
91. Sheth V, Labopin M, Canaani J, Volin L, Brecht A, Ganser A, et al. Comparison of FLAMSA-based reduced intensity conditioning with treosulfan/fludarabine conditioning for patients with acute myeloid leukemia: an ALWP/EBMT analysis. Bone Marrow Transplant. 2018.
92. Mauri L, D'Agostino RB. Challenges in the Design and Interpretation of Noninferiority Trials. New England Journal of Medicine. 2017;377(14):1357-67.
93. Beelen DW, Trenschel R, Stelljes M, Groth C, Masszi T, Remenyi P, et al. Treosulfan or busulfan plus fludarabine as conditioning treatment before allogeneic haemopoietic stem cell transplantation for older patients with acute myeloid leukaemia or myelodysplastic syndrome (MC-FludT.14/L): a randomised, non-inferiority, phase 3 trial. The Lancet Haematology. 2019.

94. Gandhi V, Plunkett W. Cellular and clinical pharmacology of fludarabine. Clin Pharmacokinet. 2002;41(2):93-103.
95. Ricci F, Tedeschi A, Morra E, Montillo M. Fludarabine in the treatment of chronic lymphocytic leukemia: a review. Ther Clin Risk Manag. 2009;5(1):187-207.
96. Ben-Barouch S, Cohen O, Vidal L, Avivi I, Ram R. Busulfan fludarabine vs busulfan cyclophosphamide as a preparative regimen before allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. Bone Marrow Transplant. 2016;51(2):232-40.
97. Capizzi RL. Curative chemotherapy for acute myeloid leukemia: the development of high-dose ara-C from the laboratory to bedside. Invest New Drugs. 1996;14(3):249-56.
98. McLaughlin B, Im A, Raptis A, Agha M, Hou JZ, Redner R, et al. Fludarabine and cytarabine in patients with relapsed acute myeloid leukemia refractory to initial salvage therapy. Int J Hematol. 2012;96(6):743-7.
99. Lamba JK. Genetic factors influencing cytarabine therapy. Pharmacogenomics. 2009;10(10):1657-74.
100. Gandhi V, Estey E, Keating MJ, Plunkett W. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol. 1993;11(1):116-24.
101. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. Leukemia. 2007;21(7):1387-94.
102. Arai Y, Jo T, Matsui H, Kondo T, Takaori-Kondo A. Efficacy of antithymocyte globulin for allogeneic hematopoietic cell transplantation: a systematic review and meta-analysis. Leuk Lymphoma. 2017;58(8):1840-8.
103. Baron F, Mohty M, Blaise D, Socie G, Labopin M, Esteve J, et al. Anti-thymocyte globulin as graft-versus-host disease prevention in the setting of allogeneic peripheral blood stem cell transplantation: a review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Haematologica. 2017;102(2):224-34.
104. Bacigalupo A. Antilymphocyte/thymocyte globulin for graft versus host disease prophylaxis: efficacy and side effects. Bone Marrow Transplant. 2005;35(3):225-31.
105. Theurich S, Fischmann H, Shimabukuro-Vornhagen A, Chemnitz JM, Holtick U, Scheid C, et al. Polyclonal anti-thymocyte globulins for the prophylaxis of graft-versus-host disease after allogeneic stem cell or bone marrow transplantation in adults. Cochrane Database Syst Rev. 2012(9):Cd009159.
106. Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin RE, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood. 2011;117(25):6963-70.
107. Sellar RS, Peggs KS. Recent progress in managing graft-versus-host disease and viral infections following allogeneic stem cell transplantation. Future Oncol. 2012;8(12):1549-65.
108. Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemary M, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. Lancet Oncol. 2016;17(2):164-73.
109. Weiden PL, Doney K, Storb R, Thomas ED. Antihuman thymocyte globulin for prophylaxis of graft-versus-host disease. A randomized trial in patients with leukemia treated with HLA-identical sibling marrow grafts. Transplantation. 1979;27(4):227-30.
110. Kroger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. N Engl J Med. 2016;374(1):43-53.
111. Bacigalupo A. ATG in allogeneic stem cell transplantation: standard of care in 2017? Point. Blood advances. 2017;1(9):569-72.
112. Bacigalupo A, Lamparelli T, Barisione G, Bruzzi P, Guidi S, Alessandrino PE, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-

related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006;12(5):560-5.
113. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10(9):855-64.
114. Kekre N, Antin JH. ATG in allogeneic stem cell transplantation: standard of care in 2017? Counterpoint. Blood advances. 2017;1(9):573-6.
115. Soiffer RJ, Kim HT, McGuirk J, Horwitz ME, Johnston L, Patnaik MM, et al. Prospective, Randomized, Double-Blind, Phase III Clinical Trial of Anti-T-Lymphocyte Globulin to Assess Impact on Chronic Graft-Versus-Host Disease-Free Survival in Patients Undergoing HLA-Matched Unrelated Myeloablative Hematopoietic Cell Transplantation. J Clin Oncol. 2017;35(36):4003-11.
116. Research CfIBaMT. Response criteria [Available from: https://www.cibmtr.org/manuals/fim/1/en/topic/aml-response-criteria.
117. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-9.
118. Sorror ML. How I assess comorbidities before hematopoietic cell transplantation. Blood. 2013;121(15):2854-63.
119. Birninger N, Bornhauser M, Schaich M, Ehninger G, Schetelig J. The hematopoietic cell transplantation-specific comorbidity index fails to predict outcomes in high-risk AML patients undergoing allogeneic transplantation--investigation of potential limitations of the index. Biol Blood Marrow Transplant. 2011;17(12):1822-32.
120. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-47.
121. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110(13):4576-83.
122. Olsson RF, Logan BR, Chaudhury S, Zhu X, Akpek G, Bolwell BJ, et al. Primary graft failure after myeloablative allogeneic hematopoietic cell transplantation for hematologic malignancies. Leukemia. 2015;29(8):1754-62.
123. Olsson R, Remberger M, Schaffer M, Berggren DM, Svahn BM, Mattsson J, et al. Graft failure in the modern era of allogeneic hematopoietic SCT. Bone Marrow Transplant. 2013;48(4):537-43.
124. Larocca A, Piaggio G, Podesta M, Pitto A, Bruno B, Di Grazia C, et al. Boost of CD34+-selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation. Haematologica. 2006;91(7):935-40.
125. Lee SJ. Classification systems for chronic graft-versus-host disease. Blood. 2017;129(1):30-7.
126. Nassereddine S, Rafei H, Elbahesh E, Tabbara I. Acute Graft Versus Host Disease: A Comprehensive Review. Anticancer Res. 2017;37(4):1547-55.
127. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transplant. 2007;40(4):381-7.
128. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. Bone Marrow Transplant. 2010;45(9):1388-95.
129. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Controlled clinical trials. 1996;17(4):343-6.
130. Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48-56.
131. Shimoni A, Labopin M, Savani B, Volin L, Ehninger G, Kuball J, et al. Long-term survival and late events after allogeneic stem cell transplantation from HLA-matched siblings for acute myeloid

leukemia with myeloablative compared to reduced-intensity conditioning: a report on behalf of the acute leukemia working party of European group for blood and marrow transplantation. Journal of Hematology & Oncology. 2016;9(1):118.
132. Socie G, Stone JV, Wingard JR, Weisdorf D, Henslee-Downey PJ, Bredeson C, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N Engl J Med. 1999;341(1):14-21.
133. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.e1.
134. Kanda J. Effect of HLA mismatch on acute graft-versus-host disease. Int J Hematol. 2013;98(3):300-8.
135. Beelen D, Markiewicz M, Stelljes M, Remenyi P, Wagner-Drouet E-M, 3rd, Dreger P, et al. Final Evaluation of a Clinical Phase III Trial Comparing Treosulfan to Busulfan-Based Conditioning Therapy Prior to Allogeneic Hematopoietic Stem Cell Transplantation of Adult Acute Myeloid Leukemia and Myelodysplastic Syndrome Patients Ineligible to Standard Myeloablative Regimens. Biology of Blood and Marrow Transplantation. 2019;25(3):S3.
136. Ruutu T, Volin L, Beelen DW, Trenschel R, Finke J, Schnitzler M, et al. Reduced-toxicity conditioning with treosulfan and fludarabine in allogeneic hematopoietic stem cell transplantation for myelodysplastic syndromes: final results of an international prospective phase II trial. Haematologica. 2011;96(9):1344-50.
137. Baronciani D, Rambaldi A, Iori AP, Di Bartolomeo P, Pilo F, Pettinau M, et al. Treosulfan/fludarabine as an allogeneic hematopoietic stem cell transplant conditioning regimen for high-risk patients. Am J Hematol. 2008;83(9):717-20.
138. Holowiecki J, Giebel S, Wojnar J, Krawczyk-Kulis M, Markiewicz M, Holowiecka-Goral A, et al. Treosulfan and fludarabine low-toxicity conditioning for allogeneic haematopoietic stem cell transplantation in chronic myeloid leukaemia. Br J Haematol. 2008;142(2):284-92.
139. Carreras E, Dufour C, Mohty M, Kröger N. EBMT Handbook 2019 Hematopoietic Stem Cell Transplantation and Cellular Therapies2019.
140. Weltermann A, Fonatsch C, Haas OA, Greinix HT, Kahls P, Mitterbauer G, et al. Impact of cytogenetics on the prognosis of adults with de novo AML in first relapse. Leukemia. 2004;18(2):293-302.
141. Weisdorf DJ, Millard HR, Horowitz MM, Hyare PS, Champlin R, Ho V, et al. Allogeneic transplantation for advanced acute myeloid leukemia: The value of complete remission. Cancer. 2017;123(11):2025-34.
142. Biggs JC, Horowitz MM, Gale RP, Ash RC, Atkinson K, Helbig W, et al. Bone marrow transplants may cure patients with acute leukemia never achieving remission with chemotherapy. Blood. 1992;80(4):1090-3.
143. Fung HC, Stein A, Slovak M, O'Donnell M R, Snyder DS, Cohen S, et al. A long-term follow-up report on allogeneic stem cell transplantation for patients with primary refractory acute myelogenous leukemia: impact of cytogenetic characteristics on transplantation outcome. Biol Blood Marrow Transplant. 2003;9(12):766-71.
144. Todisco E, Ciceri F, Boschini C, Giglio F, Bacigalupo A, Patriarca F, et al. Factors predicting outcome after allogeneic transplant in refractory acute myeloid leukemia: a retrospective analysis of Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Bone Marrow Transplant. 2017;52(7):955-61.
145. Kassim AA, Savani BN. Hematopoietic stem cell transplantation for acute myeloid leukemia: A review. Hematol Oncol Stem Cell Ther. 2017;10(4):245-51.
146. Nagler A, Savani BN, Labopin M, Polge E, Passweg J, Finke J, et al. Outcomes after use of two standard ablative regimens in patients with refractory acute myeloid leukaemia: a retrospective, multicentre, registry analysis. The Lancet Haematology. 2015;2(9):e384-e92.

147. Oki Y, Kantarjian HM, Zhou X, Cortes J, Faderl S, Verstovsek S, et al. Adult acute megakaryocytic leukemia: an analysis of 37 patients treated at M.D. Anderson Cancer Center. Blood. 2006;107(3):880-4.
148. Garderet L, Labopin M, Gorin N-C, Polge E, Baruchel A, Meloni G, et al. Hematopoietic stem cell transplantation for de novo acute megakaryocytic leukemia in first complete remission: a retrospective study of the European Group for Blood and Marrow Transplantation (EBMT). Blood. 2005;105(1):405-9.
149. Casper J, Holowiecki J, Trenschel R, Wandt H, Schaefer-Eckart K, Ruutu T, et al. Allogeneic hematopoietic SCT in patients with AML following treosulfan/fludarabine conditioning. Bone Marrow Transplant. 2012;47(9):1171-7.
150. Michallet M, Sobh M, Milpied N, Bay JO, Furst S, Harousseau JL, et al. Phase II prospective study of treosulfan-based reduced-intensity conditioning in allogeneic HSCT for hematological malignancies from 10/10 HLA-identical unrelated donor. Ann Hematol. 2012;91(8):1289-97.
151. Sakellari I, Mallouri D, Gavriilaki E, Batsis I, Kaliou M, Constantinou V, et al. Survival Advantage and Comparable Toxicity in Reduced-Toxicity Treosulfan-Based versus Reduced-Intensity Busulfan-Based Conditioning Regimen in Myelodysplastic Syndrome and Acute Myeloid Leukemia Patients after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2017;23(3):445-51.
152. Schlenk RF, Dohner K, Mack S, Stoppel M, Kiraly F, Gotze K, et al. Prospective evaluation of allogeneic hematopoietic stem-cell transplantation from matched related and matched unrelated donors in younger adults with high-risk acute myeloid leukemia: German-Austrian trial AMLHD98A. J Clin Oncol. 2010;28(30):4642-8.
153. Yakoub-Agha I, Mesnil F, Kuentz M, Boiron JM, Ifrah N, Milpied N, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. J Clin Oncol. 2006;24(36):5695-702.
154. Saber W, Opie S, Rizzo JD, Zhang MJ, Horowitz MM, Schriber J. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. Blood. 2012;119(17):3908-16.
155. Gupta V, Tallman MS, He W, Logan BR, Copelan E, Gale RP, et al. Comparable survival after HLA-well-matched unrelated or matched sibling donor transplantation for acute myeloid leukemia in first remission with unfavorable cytogenetics at diagnosis. Blood. 2010;116(11):1839-48.
156. Woolfrey A, Lee SJ, Gooley TA, Malkki M, Martin PJ, Pagel JM, et al. HLA-allele matched unrelated donors compared to HLA-matched sibling donors: role of cell source and disease risk category. Biol Blood Marrow Transplant. 2010;16(10):1382-7.
157. Ringden O, Pavletic SZ, Anasetti C, Barrett AJ, Wang T, Wang D, et al. The graft-versus-leukemia effect using matched unrelated donors is not superior to HLA-identical siblings for hematopoietic stem cell transplantation. Blood. 2009;113(13):3110-8.
158. Pidala J, Lee SJ, Ahn KW, Spellman S, Wang HL, Aljurf M, et al. Nonpermissive HLA-DPB1 mismatch increases mortality after myeloablative unrelated allogeneic hematopoietic cell transplantation. Blood. 2014;124(16):2596-606.
159. Petersdorf EW. Mismatched unrelated donor transplantation. Seminars in hematology. 2016;53(4):230-6.
160. Armand P, Gibson CJ, Cutler C, Ho VT, Koreth J, Alyea EP, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. Blood. 2012;120(4):905-13.
161. Nagler A, Labopin M, Beelen D, Ciceri F, Volin L, Shimoni A, et al. Long-term outcome after a treosulfan-based conditioning regimen for patients with acute myeloid leukemia: A report from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Cancer. 2017;123(14):2671-9.

162. Sorror ML, Storb RF, Sandmaier BM, Maziarz RT, Pulsipher MA, Maris MB, et al. Comorbidity-Age Index: A Clinical Measure of Biologic Age Before Allogeneic Hematopoietic Cell Transplantation. Journal of Clinical Oncology. 2014;32(29):3249-56.
163. Claudiani S, Marktel S, Piemontese S, Assanelli A, Lupo-Stanghellini MT, Carrabba M, et al. Treosulfan based reduced toxicity conditioning followed by allogeneic stem cell transplantation in patients with myelofibrosis. Hematol Oncol. 2016;34(3):154-60.
164. Marks DI, Cullis JO, Ward KN, Lacey S, Syzdlo R, Hughes TP, et al. Allogeneic bone marrow transplantation for chronic myeloid leukemia using sibling and volunteer unrelated donors. A comparison of complications in the first 2 years. Ann Intern Med. 1993;119(3):207-14.
165. Mielcarek M, Storer BE, Sandmaier BM, Sorror ML, Maloney DG, Petersdorf E, et al. Comparable Outcomes after Nonmyeloablative Hematopoietic Cell Transplantation with Unrelated and Related Donors. Biology of Blood and Marrow Transplantation. 2007;13(12):1499-507.
166. Singhal S, Powles R, Henslee-Downey PJ, Chiang KY, Treleaven J, Godder K, et al. Allogeneic transplantation from HLA-matched sibling or partially HLA-mismatched related donors for primary refractory acute leukemia. Bone Marrow Transplant. 2002;29(4):291-5.
167. Holtick U, Herling M, Pflug N, Chakupurakal G, Leitzke S, Wolf D, et al. Similar outcome after allogeneic stem cell transplantation with a modified FLAMSA conditioning protocol substituting 4 Gy TBI with treosulfan in an elderly population with high-risk AML. Ann Hematol. 2017;96(3):479-87.
168. Lim Z, Brand R, Martino R, van Biezen A, Finke J, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation for patients 50 years or older with myelodysplastic syndromes or secondary acute myeloid leukemia. J Clin Oncol. 2010;28(3):405-11.
169. Bokhari SW, Watson L, Nagra S, Cook M, Byrne JL, Craddock C, et al. Role of HCT-comorbidity index, age and disease status at transplantation in predicting survival and non-relapse mortality in patients with myelodysplasia and leukemia undergoing reduced-intensity-conditioning hemopoeitic progenitor cell transplantation. Bone Marrow Transplant. 2012;47(4):528-34.
170. Patel H, Molina A, Nikanjam M, Schiller GJ. Risk Factors for Relapse Following Allogeneic Transplant for Acute Myeloid Leukemia in the UCLA Patient Population. Blood. 2016;128(22):5855-.
171. Stern M, de Wreede LC, Brand R, van Biezen A, Dreger P, Mohty M, et al. Impact of Graft-Versus-Host Disease On Relapse After Allogeneic Hematopoietic Stem Cell Transplantation, an EBMT Megafile Study. Blood. 2012;120(21):469-.
172. Stern M, de Wreede LC, Brand R, van Biezen A, Dreger P, Mohty M, et al. Sensitivity of hematological malignancies to graft-versus-host effects: an EBMT megafile analysis. Leukemia. 2014;28(11):2235-40.
173. Solomon SR, Sizemore CA, Zhang X, Brown S, Holland HK, Morris LE, et al. Impact of Donor Type on Outcome after Allogeneic Hematopoietic Cell Transplantation for Acute Leukemia. Biol Blood Marrow Transplant. 2016;22(10):1816-22.
174. Holtick U, Shimabukuro-Vornhagen A, Chakupurakal G, Theurich S, Leitzke S, Burst A, et al. FLAMSA reduced-intensity conditioning is equally effective in AML patients with primary induction failure as well as in first or second complete remission. Eur J Haematol. 2016;96(5):475-82.
175. Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood. 2006;107(9):3481-5.
176. Champlin RE, Schmitz N, Horowitz MM, Chapuis B, Chopra R, Cornelissen JJ, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood. 2000;95(12):3702-9.
177. Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol. 2010;11(7):653-60.

178. Bitan M, Or R, Shapira MY, Resnick IB, Gesundheit B, Ackerstein A, et al. Time to engraftment following allogeneic stem cell transplantation is significantly longer in patients with myelodysplastic syndrome than with acute myeloid leukemia. Bone Marrow Transplant. 2008;41(1):69-78.
179. Keever-Taylor CA, Klein JP, Eastwood D, Bredeson C, Margolis DA, Burns WH, et al. Factors affecting neutrophil and platelet reconstitution following T cell-depleted bone marrow transplantation: differential effects of growth factor type and role of CD34(+) cell dose. Bone Marrow Transplant. 2001;27(8):791-800.
180. Heimfeld S. HLA-identical stem cell transplantation: is there an optimal CD34 cell dose? Bone Marrow Transplant. 2003;31(10):839-45.
181. Kim DH, Won DI, Lee NY, Sohn SK, Suh JS, Lee KB. Non-CD34+ cells, especially CD8+ cytotoxic T cells and CD56+ natural killer cells, rather than CD34 cells, predict early engraftment and better transplantation outcomes in patients with hematologic malignancies after allogeneic peripheral stem cell transplantation. Biol Blood Marrow Transplant. 2006;12(7):719-28.
182. Shimoni A, Hardan I, Shem-Tov N, Yeshurun M, Yerushalmi R, Avigdor A, et al. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. Leukemia. 2006;20(2):322-8.
183. Luger SM, Ringden O, Zhang MJ, Perez WS, Bishop MR, Bornhauser M, et al. Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. Bone Marrow Transplant. 2012;47(2):203-11.
184. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative Versus Reduced-Intensity Hematopoietic Cell Transplantation for Acute Myeloid Leukemia and Myelodysplastic Syndromes. J Clin Oncol. 2017;35(11):1154-61.
185. Liesveld J, Pawlowski J, Chen R, Hyrien O, Debolt J, Becker M, et al. Clinical factors affecting engraftment and transfusion needs in SCT: a single-center retrospective analysis. Bone Marrow Transplant. 2013;48(5):691-7.
186. Safdar A, Armstrong D. Infections in Patients With Hematologic Neoplasms and Hematopoietic Stem Cell Transplantation: Neutropenia, Humoral, and Splenic Defects. Clinical Infectious Diseases. 2011;53(8):798-806.
187. Stanworth SJ, Hudson CL, Estcourt LJ, Johnson RJ, Wood EM. Risk of bleeding and use of platelet transfusions in patients with hematologic malignancies: recurrent event analysis. Haematologica. 2015;100(6):740-7.
188. Trivedi M, Martinez S, Corringham S, Medley K, Ball ED. Optimal use of G-CSF administration after hematopoietic SCT. Bone Marrow Transplantation. 2009;43(12):895-908.
189. Blau IW, Schmidt-Hieber M, Leschinger N, Goldner H, Knauf W, Hopfenmuller W, et al. Engraftment kinetics and hematopoietic chimerism after reduced-intensity conditioning with fludarabine and treosulfan before allogeneic stem cell transplantation. Ann Hematol. 2007;86(8):583-9.
190. Remberger M, Torlen J, Serafi IE, Garming-Legert K, Bjorklund A, Ljungman P, et al. Toxicological effects of fludarabine and treosulfan conditioning before allogeneic stem-cell transplantation. Int J Hematol. 2017;106(4):471-5.
191. Kollman C, Howe CW, Anasetti C, Antin JH, Davies SM, Filipovich AH, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. Blood. 2001;98(7):2043-51.
192. Nakasone H, Fukuda T, Kanda J, Mori T, Yano S, Kobayashi T, et al. Impact of conditioning intensity and TBI on acute GVHD after hematopoietic cell transplantation. Bone Marrow Transplant. 2015;50(4):559-65.
193. Jagasia M, Arora M, Flowers ME, Chao NJ, McCarthy PL, Cutler CS, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. Blood. 2012;119(1):296-307.
194. Hagglund H, Bostrom L, Remberger M, Ljungman P, Nilsson B, Ringden O. Risk factors for acute graft-versus-host disease in 291 consecutive HLA-identical bone marrow transplant recipients. Bone Marrow Transplant. 1995;16(6):747-53.

195. Sjoo F, Hassan Z, Abedi-Valugerdi M, Griskevicius L, Nilsson C, Remberger M, et al. Myeloablative and immunosuppressive properties of treosulfan in mice. Exp Hematol. 2006;34(1):115-21.
196. Mohty M, Malard F, Abecassis M, Aerts E, Alaskar AS, Aljurf M, et al. Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives-a position statement from the European Society for Blood and Marrow Transplantation (EBMT). Bone marrow transplantation. 2015;50(6):781-9.
197. Corbacioglu S, Jabbour EJ, Mohty M. Risk Factors for Development of and Progression of Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome. Biol Blood Marrow Transplant. 2019.
198. Coppell JA, Richardson PG, Soiffer R, Martin PL, Kernan NA, Chen A, et al. Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. Biol Blood Marrow Transplant. 2010;16(2):157-68.
199. Nemecek ER, Guthrie KA, Sorror ML, Wood BL, Doney KC, Hilger RA, et al. Conditioning with treosulfan and fludarabine followed by allogeneic hematopoietic cell transplantation for high-risk hematologic malignancies. Biol Blood Marrow Transplant. 2011;17(3):341-50.
200. Martell RW, Sher C, Jacobs P, Monteagudo F. High-dose busulfan and myoclonic epilepsy. Ann Intern Med. 1987;106(1):173.
201. Marcus RE, Goldman JM. Convulsions due to high-dose busulphan. Lancet. 1984;2(8417-8418):1463.
202. Romanski M, Wachowiak J, Glowka FK. Treosulfan Pharmacokinetics and its Variability in Pediatric and Adult Patients Undergoing Conditioning Prior to Hematopoietic Stem Cell Transplantation: Current State of the Art, In-Depth Analysis, and Perspectives. Clin Pharmacokinet. 2018;57(10):1255-65.
203. Slatter MA, Rao K, Abd Hamid IJ, Nademi Z, Chiesa R, Elfeky R, et al. Treosulfan and Fludarabine Conditioning for Hematopoietic Stem Cell Transplantation in Children with Primary Immunodeficiency: UK Experience. Biol Blood Marrow Transplant. 2018;24(3):529-36.
204. Chaudhry HM, Bruce AJ, Wolf RC, Litzow MR, Hogan WJ, Patnaik MS, et al. The Incidence and Severity of Oral Mucositis among Allogeneic Hematopoietic Stem Cell Transplantation Patients: A Systematic Review. Biol Blood Marrow Transplant. 2016;22(4):605-16.
205. Naidu MUR, Ramana GV, Rani PU, Mohan IK, Suman A, Roy P. Chemotherapy-induced and/or radiation therapy-induced oral mucositis--complicating the treatment of cancer. Neoplasia. 2004;6(5):423-31.
206. Lowenberg B, Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, et al. Cytarabine dose for acute myeloid leukemia. N Engl J Med. 2011;364(11):1027-36.
207. Krejci M, Doubek M, Dusek J, Brychtova Y, Racil Z, Navratil M, et al. Combination of fludarabine, amsacrine, and cytarabine followed by reduced-intensity conditioning and allogeneic hematopoietic stem cell transplantation in patients with high-risk acute myeloid leukemia. Ann Hematol. 2013;92(10):1397-403.
208. Bearman SI, Appelbaum FR, Buckner CD, Petersen FB, Fisher LD, Clift RA, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol. 1988;6(10):1562-8.
209. Danylesko I, Shimoni A. Second Malignancies after Hematopoietic Stem Cell Transplantation. Curr Treat Options Oncol. 2018;19(2):9.
210. Rizzo JD, Curtis RE, Socie G, Sobocinski KA, Gilbert E, Landgren O, et al. Solid cancers after allogeneic hematopoietic cell transplantation. Blood. 2009;113(5):1175-83.
211. Friedman DL, Rovo A, Leisenring W, Locasciulli A, Flowers ME, Tichelli A, et al. Increased risk of breast cancer among survivors of allogeneic hematopoietic cell transplantation: a report from the FHCRC and the EBMT-Late Effect Working Party. Blood. 2008;111(2):939-44.
212. Haruki K, Shiba H, Futagawa Y, Wakiyama S, Misawa T, Yanaga K. Successfully-treated advanced bile duct cancer of donor origin after hematopoietic stem cell transplantation by pancreaticoduodenectomy: a case report. Anticancer Res. 2014;34(7):3789-92.
213. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med. 1966;64(2):328-40.

214. Ahn S, Lee YS, Chun YH, Kwon IH, Kim W, Lim KS, et al. Predictive factors of poor prognosis in cancer patients with chemotherapy-induced febrile neutropenia. Support Care Cancer. 2011;19(8):1151-8.
215. Girmenia C, Bertaina A, Piciocchi A, Perruccio K, Algarotti A, Busca A, et al. Incidence, Risk Factors and Outcome of Pre-engraftment Gram-Negative Bacteremia After Allogeneic and Autologous Hematopoietic Stem Cell Transplantation: An Italian Prospective Multicenter Survey. Clin Infect Dis. 2017;65(11):1884-96.
216. Sender V, Hofmeister-Mielke N, Sievert K, Teifke JP, Vogel H, Baumgart J, et al. Preclinical analysis of treosulfan in combination with total body irradiation as conditioning regimen prior to bone marrow transplantation in rats. Immunopharmacol Immunotoxicol. 2009;31(4):595-600.
217. Gyurkocza B, Gutman J, Nemecek ER, Bar M, Milano F, Ramakrishnan A, et al. Treosulfan, fludarabine, and 2-Gy total body irradiation followed by allogeneic hematopoietic cell transplantation in patients with myelodysplastic syndrome and acute myeloid leukemia. Biol Blood Marrow Transplant. 2014;20(4):549-55.
218. Deeg HJ, Stevens EA, Salit RB, Ermoian RP, Fang M, Gyurkocza B, et al. Transplant Conditioning with Treosulfan/Fludarabine with or without Total Body Irradiation: A Randomized Phase II Trial in Patients with Myelodysplastic Syndrome and Acute Myeloid Leukemia. Biol Blood Marrow Transplant. 2018;24(5):956-63.
219. Arai Y, Takeda J, Aoki K, Kondo T, Takahashi S, Onishi Y, et al. Efficiency of high-dose cytarabine added to CY/TBI in cord blood transplantation for myeloid malignancy. Blood. 2015;126(3):415-22.
220. Arai Y, Aoki K, Takeda J, Kondo T, Eto T, Ota S, et al. Clinical significance of high-dose cytarabine added to cyclophosphamide/total-body irradiation in bone marrow or peripheral blood stem cell transplantation for myeloid malignancy. Journal of Hematology & Oncology. 2015;8(1):102.
221. Liu N, Wang C, Wang L, Gao L, Cheng H, Tang G, et al. Valproic acid enhances the antileukemic effect of cytarabine by triggering cell apoptosis. International journal of molecular medicine. 2016;37(6):1686-96.
222. Sutherland MSK, Yu C, Anderson M, Emmerton K, Zeng W, O'Meara MM, et al. SGN-CD33A in Combination with Cytarabine or Hypomethylating Agents Demonstrates Enhanced Anti-Leukemic Activity in Preclinical Models of AML. Blood. 2014;124(21):3739-.
223. Radich JP. How I monitor residual disease in chronic myeloid leukemia. Blood. 2009;114(16):3376-81.
224. Grimwade D, Freeman SD. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? Blood. 2014;124(23):3345-55.
225. Roloff GW, Lai C, Hourigan CS, Dillon LW. Technical Advances in the Measurement of Residual Disease in Acute Myeloid Leukemia. J Clin Med. 2017;6(9):87.
226. Buckley SA, Wood BL, Othus M, Hourigan CS, Ustun C, Linden MA, et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. Haematologica. 2017;102(5):865-73.
227. Giralt S, Aleman A, Anagnostopoulos A, Weber D, Khouri I, Anderlini P, et al. Fludarabine/melphalan conditioning for allogeneic transplantation in patients with multiple myeloma. Bone Marrow Transplant. 2002;30(6):367-73.
228. Maymani H, Lin P, Saliba RM, Popat U, Bashir Q, Shah N, et al. Comparison of Outcomes of Allogeneic Hematopoietic Cell Transplantation for Multiple Myeloma Using Three Different Conditioning Regimens. Biol Blood Marrow Transplant. 2019;25(5):1039-44.
229. Samuels BL, Bitran JD. High-dose intravenous melphalan: a review. Journal of Clinical Oncology. 1995;13(7):1786-99.
230. Alexanian R, Haut A, Khan AU, Lane M, McKelvey EM, Migliore PJ, et al. Treatment for Multiple Myeloma: Combination Chemotherapy With Different Melphalan Dose Regimens. JAMA. 1969;208(9):1680-5.

231. Epperla N, Ahn KW, Ahmed S, Jagasia M, DiGilio A, Devine SM, et al. Rituximab-containing reduced-intensity conditioning improves progression-free survival following allogeneic transplantation in B cell non-Hodgkin lymphoma. Journal of hematology & oncology. 2017;10(1):117-
232. Yerushalmi R, Shem-Tov N, Danylesko I, Avigdor A, Nagler A, Shimoni A. Fludarabine and treosulfan compared with other reduced-intensity conditioning regimens for allogeneic stem cell transplantation in patients with lymphoid malignancies. Bone Marrow Transplant. 2015;50(12):1526-35.
233. Pfrepper C, Klink A, Behre G, Schenk T, Franke GN, Jentzsch M, et al. Risk factors for outcome in refractory acute myeloid leukemia patients treated with a combination of fludarabine, cytarabine, and amsacrine followed by a reduced-intensity conditioning and allogeneic stem cell transplantation. J Cancer Res Clin Oncol. 2016;142(1):317-24.
234. Saraceni F, Labopin M, Brecht A, Kroger N, Eder M, Tischer J, et al. Fludarabine-treosulfan compared to thiotepa-busulfan-fludarabine or FLAMSA as conditioning regimen for patients with primary refractory or relapsed acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT). J Hematol Oncol. 2019;12(1):44.

Appendix I

Table 15: *Summary of the results of Treo/Flu studies in patients with AML, MDS and/or MPN*

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Casper *et al.*, 2004 phase 1/2 (80)	30 patients with AML, MDS, CML, multiple myeloma, NHL or CLL. Median age 49	Treosulfan 10 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	Event-free survival 49 % at 22 months OS 73 % at 22 months	NRM 20 % at 22 months Relapse rate not reported	97 % PMN engraftment median day 11.5, plts 18 days	Day 14: 70 % Day 28: 81 %	aGvHD grade III/IV 14 %, cGvHD 39 %	ALT/AST rise grade 3/4 in 33 %, nausea and vomiting grade 3 in one patient, mucositis ≤ grade 2
Kroger *et al.,* 2006, prospective study (85)	26 patients with MDS (advanced disease) or sAML (7/15 in CR1) ineligible for standard conditioning. Median age 60	Treosulfan 10-14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 34 % at two years OS 36 % at two years	Treatment related mortality (TRM) 28 % at 100 days Two-year cumulative incidence (CI) of relapse 21 % at two years	Leucocyte engraftment 16 days, plts 17 days. No PGF, one SGF	13/16 achieved complete chimaerism at a median of 34 days	aGvHD II-III 23 %, no grade IV cGvHD 36 %, extensive in 18 %	Mucositis grade I-II 65 % Grade III toxicity: cardiac (n=2), pulmonary (n=1), hepatic (n=1), CNS (n=2), lethal grade IV toxicity in 3 cases
Baronciani *et al.,* 2008 Phase 2 (137)	46 patients (AML, ALL, MDS, MPN, MM, lymphoma), heavily pre-treated, age >50 years or presence of comorbidities. Median age 48.	Treosulfan 12-14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 38 % at 30 months OS 51 % at 30 months	TRM 9 % at 100 days, 15 % at 7 months CI of relapse not reported	96 % PMN engraftment median 15 days, plts 14 days, one PGF	97.5 % presented a complete chimaerism	aGvHD: 27 %, 8 grade I, 3 grade II, 1 grade III cGvHD: 24 %, limited in all but one case	GI grade I (1) Hepatotoxicity grade I (1), grade II (5) Nephrotoxicity grade I (2), grade II (1) No grade IV, one grade III constipation

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Casper *et al.* 2010 Prospective dose escalation study (83)	56 patients with various haematological malignancies incl.; AML 19, CML 6, MDS 6. 42 % CR, 58 % non-CR (incl. untreated MDS pts) Median age 50	Treosulfan 10-14 g/m^2 day -6 to -4 (20x10 g/m^2, 18x12g/m^2, 17x14 g/m^2), fludarabine 30 mg/m^2 day -6 to -2	RFS 49 % at two years OS 64 % at two years	NRM 13 % at day 100 18 % at one year 20 % at two years CI of relapse 31 % at two years	Day 28 cumulative incidence of engraftment: 98 % PMN, 80 % plts Median time to engraftment 14-14.5 days	77 % on day 28, 94 % on day 100	aGvHD at day 100 Grade II-IV: 42 % Grade III/IV: 5 % cGvHD limited and extensive 57 %	Grade III/IV: ALT rise 29 % AST rise 16 % Febrile neutropenia 29 % Hyperglycaemia 11 % Renal failure 11 % Two seizures
Shimoni *et al.* 2010 (86)	105 patients with AML/MDS/ CML/lymphoid malignancies/ myelofibrosis. High risk patient group, 23 % in CR (1st or later) Median age 57	Treosulfan 10-12 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 37 % at three years OS 45 % at three years	NRM 25 % at three years CI of relapse 30 % at three years	95 patients engrafted, 3 early PGF or SGF. PMN engraftment median 12 days, plts 16 days	Not reported	aGvHD overall 26 %, grade III-IV 11 % cGvHD 53 %	Not reported
Nemecek *et al.* 2011 Prospective study (199)	60 patients, AML, ALL, MDS High risk of relapse or NRM 26/44 AML patients in CR1, 18 % with relapsed or refractory disease Median age 46	Treosulfan 12-14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2 No ATG	RFS 58 % for all patients at two years, 88 % for those without high risk cytogenetics OS 65 % at two years	NRM 5 % at 100 days, 8 % at 2 years CI of relapse 33 % at two years	PMN 18 days Plts 16 days	CD3+: 82 % by day 28, 85 % by day 100 CD33+: 97 % by day 28, 97 % by day 100	aGvHD II-III 55.2 % at day 90 cGvHD 65 %	No SOS, mucositis grade I-II in 52 %, grade I-II AST/ALT rise in 60 %
Ruutu *et al.* 2011 International prospective non-randomised phase II trial (136)	45 patients with 1º MDS. Median age 50 78 % no prior treatment or chemotherapy, 18 % high-risk according to IPSS	Treosulfan 14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 67 % at two years OS 71 % at two years	NRM 17 % at two years CI of relapse 16 % at two years	Day 28: 96 % PMN engraftment, 87 % for plts. Median time to PMN recovery 17 days, platelets 16 days. One PGF, one SGF	78 % on day 28, 93 % on day 56 and 100	aGvHD at day 100: grade I-IV 56 %, II-IV 24 %, III-IV 16 % cGvHD at 2 years 59 %, extensive 28 %	87 % grade III-IV adverse event, infection (80 %), GI events (22 %). 2 % grade IV mucositis. Two patients grade II SOS, resolved

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Hilgendorf *et al.* 2011 Retrospective analysis (82)	19 patients with MDS, primary or therapy related. HCT-CI > 2 37 % Median age 48.3	Treo/Flu (84 %) or Treo/Cyclophosphamide (16 %)	RFS 74 % at one year, 57 % at three years OS 74 % at one year, 57 % at three years	NRM 5 % at 100 days, 26 % at one year, 38 % at three years CI of relapse 5 % at three years	Not reported	Not reported	aGvHD by day 100 grade I/II 36.9 %, 21.1 % cGvHD 72 %, extensive 50 %	No SOS Mucositis grade I/II 57.9 %, grade III 10.5 %, no grade IV
Casper *et al.*, 2012 Prospective nonrandomised phase II trial (149)	75 patients (AML in CR), median age 45.	Treosulfan 14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	DFS 55 % at two years OS 61 % at two years	NRM 11 % at two years CI of relapse 34 % at two years	PMN: 93 % at day 28, MTE: 20 days Plts: 93 % at day 28, MTE: 14 days. No PGF, one SGF	72 % day 28 88 % day 56 92 % day 100	aGvHD II-IV 21 %, extensive cGvHD 16 %	Grade III-IV – infections 59 %, gastrointestinal 7 %
Michallet *et al.* 2012 Phase II prospective study (150)	56 patients, AML (CR1/CR2), MDS, CML, MM, CLL and ALL. Median age 57	Treosulfan 12 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 47 % event-free survival at three years OS 52 % at three years	NRM 20 % at 12 months, 23 at 24 months CI of relapse 25 % at three years	96 % engraftment, PMN recovery median 16 days, plts 11 days	90 % at one month, 95 % at four months, 100 % at 6 months	aGvHD ≥ grade II 31 % at three months, cGvHD limited 32 % at 12 months, 6 % extensive	Infection most frequent, 62 % ≥ grade II, sepsis in 27 % Three EBV-induced lymphomas
Claudiani *et al*. 2016 Retrospective study (163)	14 patients with myelofibrosis Median age 57.	Treosulfan 14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	RFS 46 % at three years OS 54 % at three years	NRM 39 % at two years CI of relapse not reported	All patients engrafted by day +60 60-day CI of plt engraftment 78 %	12/13 achieved full donor chimaerism by day +28	aGvHD grade II-IV 50 %, III-IV 36 % Moderate/ severe cGvHD 48 %	Mucositis grade I/II 4/13 No VOD Sepsis 5/13 FUO 3/13

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Remberger *et al.,* 2017, retrospective study looking at early toxicity of Treo/Flu (190)	118 patients, 93 with a haematological malignancy (AML/ALL, CML, lymphoma, MM). Median age 50	Treosulfan 12-14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	N/A	NRM 7.5 % at 100 days, 11.9 % at one year CI of relapse not reported	100 %	83 % day 28 76 % day 84	aGvHD grade II-IV 31 %, grade III-IV 6.8 %	AST/ALT rise grade 3 two cases Bilirubin rise grade 4 one case Creatinine rise grade 2 five cases
Nagler *et al.* 2017, retrospective multicentre analysis (161)	520 patients with AML, CR1 43 %, ≥CR2 21 %, active/advanced disease 36 %. Median age 57	Treosulfan based regimens, 94 % Treo/Flu	LFS 33 % at five years OS 38 % at five years	NRM five-year cumulative incidence 25 % CI of relapse 42 % at five years	96 %, one graft rejection	Not reported	aGvHD grade II-IV 24 %, grade III/IV 11 % cGvHD 5-year cumulative incidence 38 %	VOD 2.2 %, 2 deaths Cardiac toxicity 3 % of deaths Haemorrhage 5 % of deaths Infection 26 % of deaths Interstitial pneumonitis 3 % of deaths
Sakellari *et al.* 2017 Treo/Flu compared to historical Bu/Flu group (151)	31 patients, HCT >2 (medically infirm), AML or MDS without active disease. Median age 55	Treosulfan 14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	6 % relapse, one at three months, one at nine months post-transplant, 79 % at one year OS 76 % at one year	NRM 20.2 % at one year CI of relapse mortality 7.4 % at one year	100 % engraftment, PMN engraftment median day 10, plts median day 12	100 % CC by day 30	aGvHD grade II-IV 19.4 % at one year cGvHD 48.1 % at one year	No grade III/IV toxicity observed. Mucositis grade I/II in 27/31

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Shimoni *et al.* 2018 Retrospective multicentre analysis, Bu/Flu (FB4 or FB2) vs Treo/Flu (FT14 or FT12) (90)	3293 patients with *de novo* (2588) or secondary AML (705) FT14 median age 57, CR1 56 %, CR2/3 21 %, active disease 23 %, secondary AML 25 %	Treosulfan 12-14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2	LFS at two years: FT12 44%, FT14 46% OS at two years: FT12 51%, FT14 53%	NRM at two years: FT12 16%, FT14 21% CI of relapse at two years FT12 40%, FT14 34%	Overall 98.3 % PMN engraftment median day 16 (FT14 97.5 % 17 days)	Not reported	aGvHD grade II-IV 22 %, FT associated with lower risk of aGvHD cGvHD 37 %	Major causes of death: disease recurrence, GvHD, infection (17 %), organ toxicities (3 %). FT14 VOD no cases, FT12 VOD 2 cases
Deeg *et al.* 2018, prospective randomised phase II, Treo/Flu +/- TBI (218)	100 patients with MDS or AML (CR1/CR2, refractory 3 %) Median age 57	Treosulfan 14 g/m^2 day -6 to -4, fludarabine 30 mg/m^2 day -6 to -2 No ATG	Progression free survival 54 % at one year OS 69 % at one year	NRM 8 % at day 100, 9 % at one year CI of relapse 34 % at one year	PMN engraftment 15 days, plts 11 days	All patients who did not relapse achieved complete donor chimaerism by day 28. No graft rejection	aGvHD grade II 49 %, grade III-IV 20 % at a median of 32 days. cGvHD 44 % at 2 years	Grade 3 mucositis and skin rashes.

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Beelen *et al.* 2019, Multicentre prospective randomised phase III trial, Treo/Flu vs. Bu/Flu (93)	476 patients AML in CR or MDS, increased risk for standard regimens ≥50 yrs and/or HCT-CI >2 Median age 60	Treosulfan 10 g/m^2 day -4 to -2, fludarabine 30 mg/m^2 day -6 to -2	Event-free survival (event relapse, graft failure or death) 64 % at two years OS 71.3 % at two years	NRM 11.4 % at two years CI of relapse or progression 24.6 % at two years	28-day PMN engraftment 96.8 % Plts 96.8 %	93.5 % at day 28 86.4 % at day 100	aGvHD grade II-IV 52.1 % at 100 days, grade III/IV 6.4 % cGvHD 60.1 % at 2 years, extensive 18.4 %	Mucositis ≥3 grade 4.5 % Renal and urinary disorders grade 1/2 8 %, grade 4 1 % Hepatobiliary disorders grade 1/2 1 %, grade 3 <1 %

Abbreviations: PGF – primary graft failure, SGF – secondary graft failure, MTE – median time to engraftment, TBF – thiotepa/busulfan/fludarabine, FLAMSA – fludarabine, intermediate dose Ara-C, amsacrine, sAML – secondary acute myeloid leukaemia, plts – platelets

Table 16: *Results from a selection of FLAMSA-RIC studies observing outcomes of patients with AML*

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Chemnitz *et al*. 2012 Prospective study (66)	17 patients with refractory or high-risk AML. 29 % 1st CR, 29 % with primary induction failure, 42 % relapse after 1st CR. Median age 57.4	FLAMSA day -13 to -10. RIC: Treosulfan 10 g/m^2 day -6 to day -4, cyclophosphamide 40mg/kg/day MRD, 60mg/kg/day UD day -3 to -2	RFS 55 % at one year OS 62 % at one year	NRM 20 % at one year CI of relapse 25 % at one year	Leucocyte median time to engraftment 18, platelets 21 All patients engrafted One SGF	13/16 patients developed complete chimaerism by day 30. 13/14 patients developed complete chimaerism by day 100	aGvHD grade I in 6 patients, grade II in 3 patients. cGvHD limited: 6 patients, extensive: 2 patients	29.4 % of patients developed grade III/IV regimen related toxicities
Krejci *et al*. 2013 Retrospective analysis (207)	60 patients with high-risk AML. CR 57 %, active disease 43 % Median age 52	FLAMSA day -12 to -9. RIC: TBI 4Gy day -5, cyclophosphamide 40mg/kg/day MRD, 60mg/kg/day UD day -4 to -3	RFS 38 % at one year, 33 % at three years OS 45 % at one year, 42 % at three years	NRM 25 % at one year, 28 % at three years 38 % of patients experienced relapse after 37 months median follow-up	PMN median to engraftment 17 days, platelets 18 days 85 % achieved engraftment	Complete chimaerism in 71 % after a median of 30 days aGvHD	aGvHD 47 %, I/II 29 %, III/IV 18 % cGvHD 55 %, limited 39 %, extensive 16 %	Grade III/IV infections 84 %, Gastrointestinal toxicity grade III in 29 %
Schneidawind *et al*. 2013 Retrospective single centre analysis (68)	62 patients with primary refractory or relapsed AML. 68 % blast count >20 % prior to conditioning Median age 55	FLAMSA day -12 to -9. RIC either FLU/BU, TBI/CY or BU/CY	Event-free survival 26 % at two years OS 39 % at two years	NRM 22 % at two years CI of relapse 52 % at two years	Neutrophil engraftment at a median of 17 days, platelet engraftment at 22 days	38 % complete chimaerism at day 20, 65 % at day 60, 71 % at day 100	aGvHD ≥ grade II 21 % cGvHD 26 % (limited n=12, extensive n=4)	Mucositis grade III/IV 58 % Hepatobiliary system toxicity grade III/IV 16 % Renal toxicity grade III/IV 15 %

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Pfrepper *et al.* 2016 (233)	44 patients with primary refractory or relapsed AML. Median age 52	FLAMSA day -12 to -9. RIC: 4Gy TBI on day -5, cyclophosphamide 120mg/m^2 day -4 to -3	Event-free survival 12 % at three years OS 15 % at three years	NRM 18 % at three years CI of relapse 69 % at three years	Not reported	30/41 between day +12 and day +40	aGvHD in 55 %, grade I in 32 %, grade II-IV in 23 %. cGvHD in 8 patients (26 %). Six had limited, two had extensive	Not reported
Holtick *et al.* 2017 Retrospective analysis FLAMSA-RIC TBI vs. treosulfan (167)	130 patients with high risk or relapsed AML, 58 FLAMSA/treosulfan (median age 60), 72 FLAMSA/TBI (median age 46) 77 patients in CR, 53 with refractory disease or blast persistence	FLAMSA day -13 to -10 RIC: Treosulfan 10 g/m^2 day -6 to day -4 or 4Gy TBI, cyclophosphamide 40mg/kg/day MRD, 60mg/kg/day UD day -3 to -2	RFS 41 % at four years (FLAMSA/treo), 40 % at four years (FLAMSA/TBI) OS 47 % at four years (FLAMSA/treo), 43 % at four years (FLAMSA/TBI)	NRM 28 % at four years (FLAMSA/treo), 13 % at four years (FLAMSA/TBI) CI of relapse 46 % at two years for FLAMSA/TBI, 32 % for FLAMSA/Treo	FLAMSA/TBI PMN engraftment 69/72, median 16 days FLAMSA/Treo 58/59 median 15 days	FLAMSA/TBI 93 % CC at day 30, 87 % at day 100 FLAMSA/Treo 96 % CC on day 30, 90 % on day 100	Not reported Death from GvHD in seven patients (12 %) in treosulfan group	Not reported Death from infection in eight patients (13.8 %) in the treosulfan group.

Study	Population	Intervention	RFS and OS	NRM and CI of Relapse	Engraftment	Chimaerism	aGvHD and cGvHD	Toxicity
Malard *et al.* 2017 Retrospective multicentre analysis (67)	265 patients with intermediate- or high-risk AML in 1st (81.5 %) or 2nd (18.5 %) complete remission. Median age 55	FLAMSA day -12 to -9 RIC: Cyclophosphamide 40mg/kg/day MRD, 60mg/kg/day UD day -4 to -3 TBI 4Gy or Busulfan	LFS 52.8 % at two years OS 56.1 % at two years	NRM at two years 24 % CI of relapse 22.8 % at two years	96.2 % engrafted in TBI group, 95.3 % in the Bu group PMN TBI 17 days, Bu 14 days	Not reported	aGvHD grade II to IV day 100: 28.5 % cGvHD at 2 years: 31.8 %	2 deaths related to cardiac toxicity 2 cases of SOS 31 deaths related to infection

Regimens:
FLU/BU: fludarabine 30 mg/m2 on days −5 to −4, busulfan 0.8 mg/kg twice on day −6, and 0.8 mg/kg four times daily on days −5 to −4 (n=12)
TBI/CY: 4 Gy TBI on day −5, cyclophosphamide 60 mg/kg on days −4 to −3 (n=31)
BU/CY: busulfan 0.8 mg/kg once on day −6, 0.8 mg/kg four times daily on day −5, and 0.8 mg/kg three times on day −4, cyclophosphamide 60 mg/kg for mismatched and unrelated donors or 40 mg/kg for MRD on days −3 to −2 (n=19).

***Table 17**: Summary of the outcomes of two trials comparing FLAMSA-RIC with Treo/Flu conditioning*

Study	Population	Conclusions
Sheth *et al.* 2018 (91) Retrospective analysis comparing Treo/Flu, FLAMSA/TBI and FLAMSA/Busulfan	629 patients with AML, 281 Treo/Flu, 203 FLAMSA/TBI, 145 FLAMSA/Busulfan	Multivariate analysis: FLAMSA/TBI decreased risk of relapse and superior leukaemia-free survival compared to Treo/Flu. Acute GvHD rates significantly higher in FLAMSA/TBI group compared to Treo/Flu. OS, NRM and cGvHD were not significantly impacted by conditioning regimen used.
Saraceni *et al.* 2019, (234) retrospective analysis comparing Treo/Flu, thiotepa/busulfan/fludarabine (TBF), and FLAMSA/TBI	856 patients with AML, transplanted in active disease–primary refractory, first and second relapse, 113 patients received Treo/Flu (median age 58), 112 TBF (median age 52.1) and 631 FLAMSA/TBI (median age 51.5)	Results similar across protocols, OS determined by Karnofsky performance score (<80 %) and CMV serology. Age was not a determinant of OS. Global survival of 34 % at 2 years

Appendix II

CARL VON OSSIETZKY universität OLDENBURG
FAKULTÄT VI
MEDIZIN UND GESUNDHEITSWISSENSCHAFTEN

CARL VON OSSIETZKY UNIVERSITÄT OLDENBURG · 26111 OLDENBURG

Klinikum Oldenburg
Prof. Jochen Casper
Rahel-Straus-Str.10
26133 Oldenburg

Medizinische
Ethikkommission
-Geschäftsstelle-

TELEFONDURCHWAHL
+49 (0)441 798 31 09
FAX
+49 (0)441 798 4745
E-MAIL
med.ethikkommission@uni-oldenburg.de

VORSITZENDER
Prof. Dr. Frank Griesinger

JURISTIN
Ass. jur. Carola Alvarez Castillo

OLDENBURG, 05.11.2018

Unser Zeichen:	2018-106 (bitte stets angeben)
Antragsteller:	Prof. Jochen Casper
Titel:	Cytarabine in combination with treosulfan and flufarabine as a conditioning regimen for allogeneic haematopoietic stem cell transplantation patients with acute myeloid leukaemia, myelodysplastic syndrome and myeloproliferative disorders
Antrag vom:	02.10.2018

ems european medical school oldenburg-groningen

POSTANSCHRIFT
D-26111 Oldenburg

PAKETANSCHRIFT
Ammerländer Heerstraße 114 - 118
D-26129 Oldenburg

BESUCHERANSCHRIFT
Ammerländer Heerstr.140
V04-1-136/137
D-26129 Oldenburg

INTERNET
www.uni-oldenburg.de

BANKVERBINDUNG
Landessparkasse zu Oldenburg
IBAN DE46 2805 0100 0001 9881 12
BIC SLZODE22

Beratung gem. § 15 BO

Sehr geehrter Herr Prof. Casper,

Ihr Antrag hat der medizinischen Ethikkommission in ihrer Sitzung am 24.10.2018 zur Beratung vorgelegen.

Die medizinische Ethikkommission hat keine Bedenken gegen die Durchführung der o.g. Studie.

Hinweise/Empfehlungen:

Die medizinische Ethikkommission empfiehlt,

- das Einholen einer Patienteneinwilligung
- das Versenden des Anschreibens in einem neutralen Umschlag

Die medizinische Ethikkommission weist daraufhin, dass nach einer gewissen Anzahl von individuellen Heilversuchen eine Beratung durch eine medizinische Ethikkommission empfohlen wird, da es sich dann um systematische Heilversuche und damit um Forschung handelt.

1

Bitte beachten Sie noch folgende Punkte:

- Die Ethikkommission erwartet, dass ihr ohne Aufforderung ein Abschlussbericht mit dem beigelegten **Formular B** übermittelt wird.
- Unabhängig davon ist die Ethikkommission unaufgefordert und zeitnah über alle Änderungen am Prüfplan, sowie den in diesem Antrag vorgelegten Dokumenten unaufgefordert und unverzüglich zu unterrichten. Ihr sind unaufgefordert alle schweren unerwünschten Ereignisse mitzuteilen, soweit sie im Zuständigkeitsbereich der Ethikkommission aufgetreten sind.
- Die ethische medizinische und juristische Verantwortung des Studienleiters und des an der Studie beteiligten medizinischen und wissenschaftlichen Personal bleibt entsprechend der Beratungsfunktion der medizinischen Ethikkommission durch diese Stellungnahme unberührt.
- Die Ethikkommission kann dieses Votum jederzeit zurückziehen oder ändern. Dies wird dem Antragsteller mitgeteilt.
- Bitte machen Sie dieses Votum und die der Begutachtung zugrundeliegenden Dokumente allen beteiligten Ärztinnen und Ärzten und Wissenschaftlerinnen und Wissenschaftlern zugänglich.

An der Beratung und Beschlussfassung haben keine Kommissionsmitglieder teilgenommen, die selbst an dem Forschungsvorhaben mitwirken oder deren Interessen davon berührt werden.

Wir bitten um Mitteilung der teilnehmenden Ärztinnen und Ärzte im Zuständigkeitsbereich der Universität Oldenburg, sobald diese bekannt sind bzw. sofern im Verlauf weitere Ärztinnen und Ärzte hinzukommen.

Wir möchten darauf hinweisen, dass die Stellungnahme der medizinischen Ethikkommission und die studienrelevante Korrespondenz an alle teilnehmenden Ärztinnen und Ärzte weiterzuleiten ist.

Bitte informieren Sie die Ethikkommission unter Nutzung des beigefügten Formulars A über den Beginn der Rekrutierung an Ihrem Studienzentrum.

Wir wünschen Ihnen bei der Durchführung Ihrer Studie viel Erfolg.

Mit freundlichen Grüßen

Dr. jur. Hans Oehlers
stellvertretender Vorsitzender der medizinischen Ethikkommission

UNIVERSITÄTS KLINIKUM jena | Ethik-Kommission

Universitätsklinikum Jena · Ethik-Kommission · 07740 Jena

Universitätsklinikum Jena
Klinik für Innere Medizin II, - Abteilung für Hämatologie und Internistische Onkologie - Sektion für Stammzelltransplantation
PD Dr. med. Inken Hilgendorf
Am Klinikum 1
07747 Jena

Vorsitzender: Prof. Dr. med. Ulrich Brandl
Geschäftsstelle: Dr. phil. Ulrike Skorsetz

Bachstraße 18
07743 Jena

Telefon 03641 9 39 11 91
Telefax 03641 9 39 11 92

E-Mail: ethikkommission@med.uni-jena.de

18.02.2019

Reg.-Nr.: 2019-1316-BO Zweitvotum

Cytarabine in combination with treosulfan and fludarabine as a conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with acute myeloid leukaemia, myelodysplastic syndrome and myeloproliferative disorders

Sehr geehrte Frau Dr. Hilgendorf,

zum Antrag auf Teilnahme an der o. g. klinischen Studie teile ich Ihnen mit, dass sich die Ethik-Kommission der Friedrich – Schiller - Universität Jena der positiven Stellungnahme Medizinischen Ethikkommission der Carl von Ossietzky Universität Oldenburg vom 06.11.2018 anschließt.

Wir wünschen Ihnen viel Erfolg!

Mit freundlichen Grüßen
(i. A. Dr. Ulrike Skorsetz)

Prof. Dr. med. Ulrich Brandl
Vorsitzender der Ethik-Kommission

Appendix III

List of Tables

List of Figures

Acknowledgements

I would like to thank Professor Jochen Casper and Professor Claus-Henning Köhne for their assistance in the writing of this thesis. Furthermore, I would like to thank PD Dr. med. Inken Hilgendorf for her help and support collecting patient data from the University Hospital Jena.

I would also like to thank my mother Kay O'Hagan and my father Professor Brian Henderson who have always supported me throughout my academic career.

Lebenslauf

Name **Samantha O'Hagan Henderson**
geboren in Epsom, Großbritannien
verheiratet, zwei Kinder

Ausbildung

2009 – 2013 Studium der Humanmedizin
Abschluss: Bachelor of Medicine, Bachelor of Surgery (BM BCh)
University of Oxford, St Hugh's College, Großbritannien

2005 – 2009 Studium der Biochemie
Abschluss: Master of Biochemistry (MBiochem) Note: First class
Masterarbeit: The role of NALP2 in the molecular pathogenesis of myelodysplastic syndrome
University of Oxford, Lady Margaret Hall, Großbritannien

Berufstätigkeit

Seit 2015 Assistenzärztin in der Abteilung für Innere Medizin II (Professor C.H. Köhne), Onkologie und Hämatologie, Klinikum Oldenburg

2013 – 2015 Assistenzärztin in der West Midlands Deanery, Großbritannien

Approbation

Juni 2015 Approbation als Ärztin in der Bundesrepublik Deutschland
Niedersächsischer Zweckverband zur Approbationserteilung

August 2013 Approbation als Ärztin in Großbritannien

Publikationen

Dyson A, Ekbal N, Stotz M, Barnes S, Carré J, Tully S, **Henderson S**, Barrett L, Singer M. Component reductions in oxygen delivery generate variable haemodynamic and stress hormone responses. Br J Anaesth. 2014; 113: 708-716

Henderson B, **Henderson S.** Unfolding the relationship between secreted molecular chaperones and macrophage activation states. Cell Stress Chaperones. 2009; 14:329-341

Kongressbeiträge

Trifonas Papettas, **Samantha Henderson**, Julian Dickmann, Joel Lambert, Adam Lunt
Time to contrast enema and ileostomy closure rates following low anterior resection. Does laparoscopic surgery make a difference? Tripartite Colorectal Meeting, United Kingdom 2014

T. Papettas, **S. O. Henderson**, J. R. M. Dickmann, S. Freshney, V. Menon
Comparison of outcomes between left transthoracic and Ivor-Lewis oesophagectomies for the treatment of oesophageal cancer in one centre. Association of Surgeons of Great Britain and Ireland (ASGBI) Conference, United Kingdom 2014

S.O. Henderson, C. Arbuthnot, A. G. Borg
Palliative chemotherapy using prednisolone, etoposide, procarbazine and cyclophosphamide (PEP-C) is effective and tolerable in frail patients with aggressive lymphoma. Controversies in Hematology Conference, Istanbul 2014