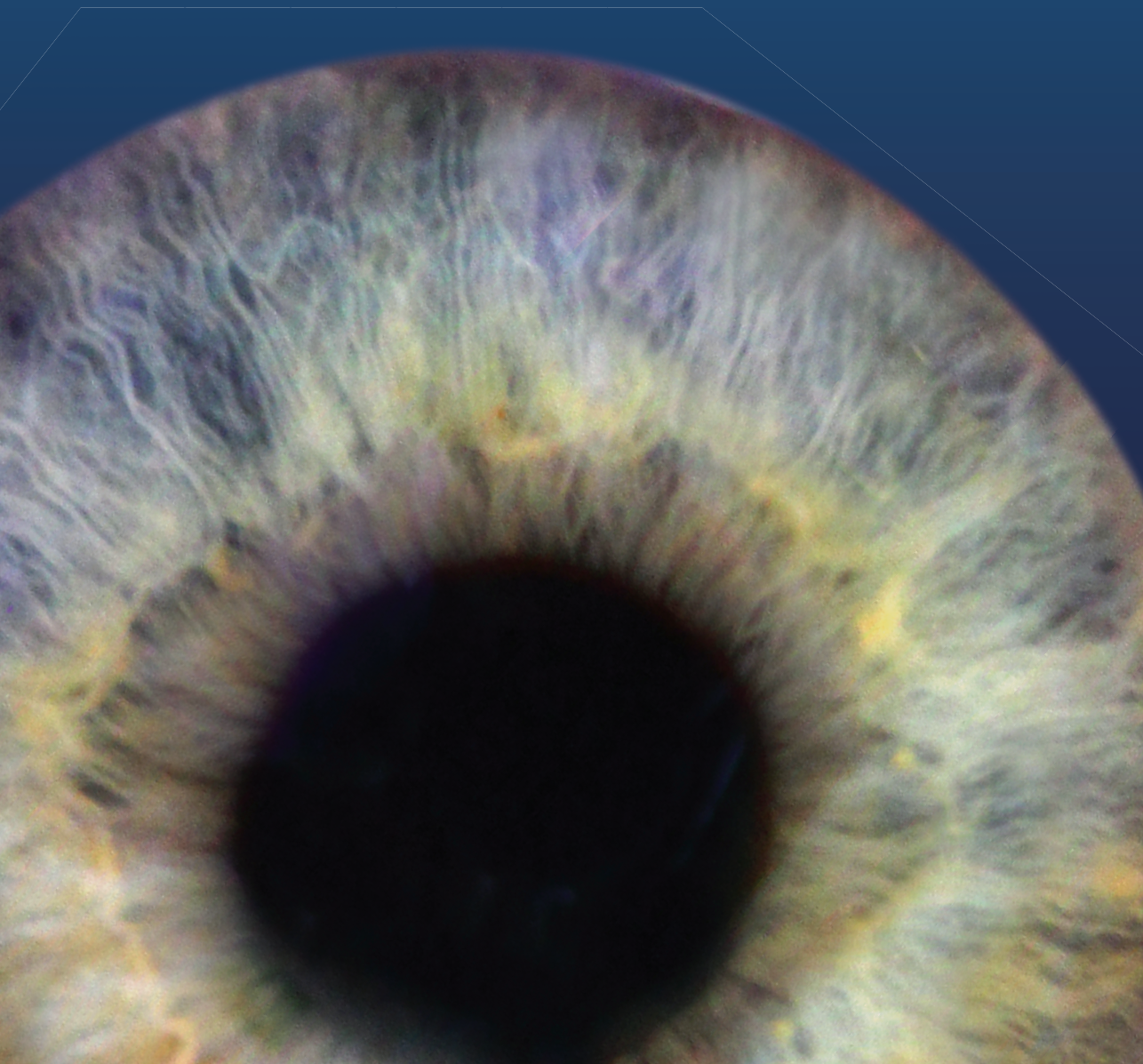
Allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia

a personalized approach

Jurjen Versluis



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Allogeneic Hematopoietic Stem Cell Transplantation in patients with Acute Myeloid Leukemia

a personalized approach

Allogene hematopoïetische stamcel transplantatie voor patiënten met acute myeloïde leukemie

een gepersonaliseerde benadering

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The eye sees all, but the mind shows us what we want te see.
William Shakespeare

CONTENTS

1

GENERAL INTRODUCTION

ACUTE LEUKEMIA

Acute myeloid leukemia (AML) is a malignant disorder of the bone marrow characterized by impaired maturation of myeloid progenitor cells and increased proliferation of these ineffective myeloid precursors.[1] AML patients have impaired normal hematopoiesis by replacement of immature cells resulting in cytopenias of the different peripheral blood cell types. The majority of patients present with signs of bone marrow failure including anemia, neutropenia, or thrombocytopenia. Fatigue, infections, and hemorrhage are the most common clinical manifestations at presentation of AML patients, whereas enlargement of the spleen and liver are frequent clinical signs of the rapid proliferation of the abnormal myeloid precursors.

Epidemiology

AML is the most common form of acute leukemia in adults, accounting for approximately 80% of the cases.[2] The overall incidence of AML ranges from 3 to 5 cases per 100.000 persons in large registries,[2-4] and estimated around 3 per 100.000 persons in the Netherlands.[5] The annual incidence of AML increases with age, estimating >20 cases per 100.000 in persons above the age of 70 years.[4,5] AML is a disease of the elderly population with a median age at diagnosis of 65 to 70 years. Relative survival of patients with AML decreases with age but may not be generalized as survival also greatly depends on the underlying characteristics of the leukemia.

Etiology and pathophysiology

AML can be secondary to other hematological disorders such as myelodysplastic syndrome or myeloproliferative neoplasms, and may also be related to previous therapy including treatment with topoisomerases II inhibitors (anthracyclines), alkylating agents or radiotherapy.[6] However, the majority of patients develop a de novo AML. The development of AML from normal myeloid stem cells has been suggested to occur along a gradual, "evolutionary" path, whereby myeloid precursor cells with a proliferative advantage ultimately prevail. Leukemogenesis consists of the stepwise acquisition of mutations affecting key cellular programs, including proliferation, differentiation, cell cycle physiology, and/or apoptosis. Earlier, a two-hit hypothesis was proposed, consisting of class I mutations (eg, *FLT3*-ITD or *K/N-RAS*) conferring a proliferative advantage resulting in clonal expansion of myeloid progenitors, and class II mutations resulting in impaired differentiation (eg, *NPM1* or *CEBPA*).[7,8] However, advances in DNA sequencing of leukemic cells of individual patients resulted in the discovery of many genetic events, which may have a complex interaction. Clonal hematopoiesis with somatic mutations may occur as early events in the development of hematological malignancies.[9,10] These mutations are gradually acquired and may ultimately result in coexisting malignant clones with usually one dominant clone and

multiple subclones harboring different genetic mutations.[11,12] Studies have shown that these clones may evolve during the development of leukemia, but also during or after treatment, with clones emerging or disappearing depending on their proliferative advantage and the supportive or counteracting effects exerted by their (micro)environment and therapeutic pressure.[12,13] Genetic mutations are identified in the vast majority (>95%) of patients with de novo AML, commonly without the presence of chromosomal abnormalities.[11,14-16] The most frequently observed genetic alterations are mutated *NPM1*, *FLT3*-ITD, mutated *DNMT3A*, or mutated *IDH*, and genetic mutations may frequently co-occur with different impact on outcome of the individual patient.[11,14-16] Coinciding with these genomic discoveries, molecular aberrations were demonstrated to identify subgroups of AML patients with distinct prognostic features, increasingly necessitating tailored treatment approaches. As a result, molecular analysis has become pivotal in the diagnosis and risk classification of AML patients. The applied techniques are rapidly evolving and were recently summarized.[16,17]

Diagnosis and risk classification

Morphology of peripheral blood and bone marrow smears is still the cornerstone of diagnosing AML.[18] A bone marrow or peripheral blood blast count of more than 20% is required for the diagnosis of AML according to the latest European LeukemiaNET (ELN) guideline.[18] In addition to morphology, the evaluation of patients with AML should also include immunophenotyping, cytogenetics and molecular analysis of the leukemic blasts as the WHO classification of myeloid neoplasm is increasingly based on molecular genetic diagnostics. The latest WHO 2016 classification includes AML with *BCR-ABL1* and mutated *RUNX1* as two new provisional entities.[18] In addition, a new category called "myeloid neoplasm with germ line predisposition" was added, recognizing myeloid malignancies being associated with inherited or de novo germ line mutations.[18] The increased and extensive use of genetic data for patients with AML has provided the opportunity to address the predictive capacity of specific genetic mutations.[14-16,19] Previously, conventional cytogenetics and mutations of *NPM1*, *FLT3*-ITD and *CEBPA* were included in the ELN 2010 risk classification of AML patients.[20] The current ELN 2017 AML risk classification has added mutations in three genes including *RUNX1*, *ASXL1*, and *TP53* (Table 1).[18] Similar to the previous risk classification, the ELN 2017 risk classification is advocated to be used for risk-stratifying AML and to a risk-adapted treatment approach of patients with AML.

Induction treatment

The first goal of treatment of patients with AML is to obtain a complete hematological remission (CR), which is defined as a blast count of less than 5% in the bone marrow and recovery of peripheral neutrophils and platelets. Since the early 1980s, the backbone of AML induction treatment has been the "7 + 3" regimen, which is a combination of cytarabine and an anthracycline.[21,22] Cytarabine is generally dosed as 100-200 mg/m^2 daily as a continuous

Table 1 European LeukemiaNET 2017 AML risk classification*

Risk category	Genetic or molecular abnormality
Favorable	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*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable 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)*
	–5 or del(5q); –7; –17/abn(17p)
	Complex karyotype,§ monosomal karyotype||
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*{
	Mutated *ASXL1*{
	Mutated *TP53*

* Table adapted from Dohner et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-47; Table 5.[17]

† Low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5); semi quantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "*FLT3*-ITD" divided by area under the curve "*FLT3*-ITD wild-type"

‡ The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

§ Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*

|| Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML)

{ These markers should not be used as an adverse prognostic marker if they co-occur with favorable risk AML subtypes

infusion for seven days for induction, whereas intensified cytarabine has been the cornerstone of consolidation chemotherapy. The HOVON approach was established as induction chemotherapy consisting of cytarabine 200 mg/m^2 daily for seven days and idarubicin 12 mg/m^2 for three days, followed by consolidation therapy with an anthracycline and high-dose cytarabine, which dosage has currently been established at 1000 mg/m^2.[23-35]

Higher dosing of cytarabine up to 3000 mg/m^2 has been associated similar CR rates, whereas increased toxicity has been reported.[32,36,37] The anthracycline in the induction regimens may be three days of either idarubicine 12 mg/m^2 daily or daunorubicine 60 mg/m^2 daily. Different doses of anthracyclines have been investigated, with daunorubicine 90 mg/m^2 being associated with higher response rates as compared with daunorubicine 45 mg/m^2,[29,38,39] whereas a recent randomized study found increased early mortality compared with a dosage of 60 mg/m^2.[40] The ELN 2017 guideline has recommended a dose of 60 mg/m^2 daunorubicine as the minimum dose.[18] Other groups have compared idarubicine 12 mg/m^2 with daunorubicine either dosed at 50 mg/m^2 or 80 mg/m^2 and found no significant differences in response rate or outcome parameters.[41,42] Thus the "7 + 3" regimen with cytarabine and an anthracycline (either daunorubicine or idarubicine) has been adopted as the backbone of induction chemotherapy. That regimen may result in an hematological CR in 60 to 90% of patients aged 60 years and younger, whereas CR rates are lower (40 to 60%) in elderly patients above the age of 60 years.[29,32,35-42] Decreased CR rates and shorter duration of CR in elderly patients may be related to the disease itself harboring more high-risk features resulting in a higher incidence of refractory disease, but also to increased mortality following induction treatment due to concurrent diseases.[43]

A number of drugs have been added to the backbone of AML treatment in prospective phase 3 trials of younger and elderly patients to improve the response rate and outcome of AML induction treatment.[44] These drugs include etoposide,[45,46] nucleoside analogues (ie, fludarabine, cladribine, or clofarabine),[28,35,37,47-50] or the anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin.[30,51-55] The latter drug was initially withdrawn because of negative results, but has regained interest after a meta-analysis was published, which reported lower relapse rates and improved survival in patients with favorable or intermediate risk AML.[56] A number of *FLT3* inhibitors (ie, sorafenib, midostaurin, lestaurtinib) have also been studied in randomized phase 3 trials as additive drug to the backbone induction treatment of AML patients.[57-60] Recently, midostaurin was associated with improved overall survival (OS) compared with placebo in patients with AML harboring a *FLT3* mutation, which resulted in approval for midostaurin in AML patients with *FLT3* mutations.[60] Currently, multiple new drugs are being investigated, including drugs targeting specific molecular mutations, hypomethylating agents, and new chemotherapeutic drug formulations.[61] These drugs are currently primarily used in the setting of relapsed or refractory AML patients, but may proceed to the induction treatment of AML within the next years.

Post-remission treatment

The majority of patients obtain a first hematological CR after induction treatment, but the risk of a relapse without further treatment is considerably high. Post-remission treatment may reduce the risk of relapse and may consist of continued chemotherapy, autologous hematopoietic stem cell transplantation (HSCT) and allogeneic HSCT (alloHSCT). The clinical

decision for post-remission treatment is primarily based on the risk of the leukemia, but also on other factors, including the potential to harvest stem cells, the availability of an allogeneic donor, and patients performance status or concurrent morbidity.

Continued chemotherapy

Post-remission chemotherapy has been associated with acceptable toxicity profiles and may be specifically applied in patients who appear chemotherapy sensitive.[62] The HOVON approach has been to continue chemotherapy in such patients with mitoxantrone and etoposide,[27,32] whereas other groups have used post-remission cytarabine. The optimal dose of cytarabine has been addressed by different study groups, although a dose of 1500 mg/m^2 has been associated with less toxicity and similar OS compared with higher doses of cytarabine.[37,63] Different other chemotherapeutic regimens did also not yield better outcome compared with high doses of cytarabine (ie, >2000 mg/m^2).[64-66] Although subgroups of AML patients may have profited from higher doses of cytarabine, no convincing evidence is currently available that higher doses of cytarabine are preferred over a dose of >1000 mg/m^2 as post-remission chemotherapy.[67]

Autologous hematopoietic stem cell transplantation

Autologous HSCT consists of high dose chemotherapy followed by the infusion of autologous hematopoietic progenitor cells to ensure hematopoietic recovery. Initially, autologous HSCT was performed using autologous bone marrow,[68,69] with randomized studies and their meta-analyses reporting better relapse-free survival (RFS) following autologous bone marrow transplantation, but similar OS compared with chemotherapeutic post-remission treatment or no further treatment.[70-73] Autologous HSCT with peripheral blood stem cells was increasingly being used as from the early 1990s, which transplants contain a significantly higher number of stem cells compared with bone marrow resulting in improved hematological recovery and less mortality from infectious or bleeding complications.[74-77] The comparative value of autologous peripheral blood HSCT and chemotherapeutic consolidation has not been studied extensively. The HOVON-SAKK group has performed a prospective randomized study of autologous peripheral blood HSCT compared with chemotherapeutic consolidation and found significantly less relapse, a trend towards better RFS, but similar OS compared with post-remission chemotherapy.[31] Retrospective studies have also compared autologous HSCT with alloHSCT following sibling donors. These studies showed less non-relapse mortality (NRM) following autologous HSCT, but an increased cumulative incidence of relapse which resulted in similar outcome in good and intermediate risk patients.[78-80] Although autoHSCT provides a stronger cytotoxic anti-leukemic effect than continued chemotherapy, the application of autoHSCT may be limited to subgroups of patients, including patients lacking an appropriate stem cell donor or being ineligible for alloHSCT, but having a sufficient autologous stem cell harvest.[81,82]

Allogeneic hematopoietic stem cell transplantation

AML has become the most dominant indication for alloHSCT worldwide.[83,84] AlloHSCT was originally developed with an intensive cytotoxic conditioning regimen for leukemic ablation, but also to provide an immunosuppressive effect preventing graft rejection of allogeneic bone marrow cells.[85] The anti-leukemic effect was initially attributed to high dose chemotherapy and radiotherapy, but was subsequently largely ascribed to the immunological graft-versus-leukemia (GVL)-effect, initially based on the observation of an inverse relation of relapse rates and grades of graft-versus-host disease (GVHD).[86] T-cells were found to be largely responsible for GVL, with alloreactivity resulting from recognition of disparate allogeneic antigens. These disparities were later found to involve minor and major human leucocyte antigens (HLA) as well as some specific tumor antigens.[87-91] Retrospective studies confirmed the observation of an increased relapse rate in patients who received an alloHSCT with either T-cell depletion of the donor graft or patients who received intensified immunosuppressive regimens for prevention of GVHD. These strategies resulted in less GVHD at the expense of increased relapse, indicating that the GVL-effect was abrogated upon elimination of donor T-cells.[92-94] The GVL-effect has been extensively investigated since these observations, and it was subsequently exploited with the development of donor lymphocyte infusions after transplantation.[95,96]

Although alloHSCT was shown to offer the most effective anti-leukemic therapy in AML, counterbalancing NRM compromised that favorable effect compared with chemotherapy or autologous HSCT.[72] As randomized studies proved to be difficult to perform, comparative studies evaluated alloHSCT by donor availability in so-called donor-versus-no-donor studies. The HOVON-SAKK group also performed such a donor-versus-no-donor analysis and found no benefit of myeloablative conditioning (MAC) alloHSCT in patients with a favorable risk AML, whereas improved OS and RFS were reported in patients with an intermediate or poor risk AML.[97] These results were confirmed by others and also documented in several meta-analysis of these studies.[97-99] However, the meta-analysis by HOVON-SAKK suggested that the survival benefit of MAC alloHSCT was limited to patients below the age of 40 years, due to increased NRM in older patients.[97] These and other studies highlighted the need to reduce NRM associated with alloHSCT.[97-102] As the beneficial effect of alloHSCT depended on GVL rather than on intensive chemotherapy and radiotherapy, attempts were made to reduce the toxicity of the fully myeloablative regimens. Reduced intensity conditioning (RIC) regimens were developed, which were associated with decreased toxicity and mortality.[103-109] The GVL-effect of alloHSCT following RIC regimens was still present and was found to be stronger in patients who experienced chronic GVHD, whereas patients who received a T-cell depleted graft had a higher incidence of relapse.[110-112] One of the least intensive nonmyeloablative conditioning regimen was developed by the Seattle group consisting of fludarabine and a low dose of total body irradiation, which was associated with effective engraftment and limited early mortality.[109] However, the anti-leukemic effects of these less intensive

conditioning regimens were questioned in patients with high risk leukemias in which patient groups higher relapse rates were observed.[113] Therefore, others added intermediate dose alkylating agents (ie, busulfan, treosulfan, melphalan) to the conditioning to enhance the anti-leukemic efficacy.[114-119] These RIC regimens were suggested to be associated with less relapse compared with nonmyeloablative regiments, but no randomized prospective studies have been conducted so far comparing these regimens. Nevertheless, these less intensive conditioning regimens broadened the application of alloHSCT, particularly for elderly patients or for patients with concurrent morbidity. Meanwhile, the increased availability of alternative donors has allowed to offer alloHSCT to the majority of AML patients for whom transplantation is indicated. Increased availability of stem cell donors resulted from an enormous increase of potential volunteer unrelated donors in the larger registries, including the American and several European and Asian registries.[83,84,120] In addition, unrelated cord blood was developed as an alternative stem cell source, whereas haplo-identical family donors have regained interest more recently.[121-126] Although the degree of antigen disparity is greater using alternative donors, most transplants with the use of these donors are currently performed following RIC and with more intensive immunosuppressive approaches.[124-126] Collectively, studies in AML with alloHSCT following RIC confirmed the potency of the immunotherapeutic GVL-effect of alloHSCT, but GVHD related toxicity and mortality of alloHSCT have remained a major challenge, especially following transplants with alternative donors.[103,126-130]

AIMS AND OUTLINE OF THE THESIS

This thesis addresses the value of post-remission therapy with alloHSCT and weighs the beneficial anti-leukemic effects of alloHSCT versus toxicity and non-relapse mortality.

The first part of the thesis addresses the comparative value of alloHSCT as post-remission treatment in subgroups of patients. In **Chapter 2**, post-remission treatment with chemotherapy, autologous HSCT, or alloHSCT following RIC of MAC regimens was compared in patients aged 40 to 60 years. The comparative value of these post-remission therapies were considered in three leukemia risk groups with different effects of alloHSCT on OS, RFS and the cumulative incidence of relapse. Outcome of elderly patients who receive intensive treatment for AML may be worse as compared with younger patients. **Chapter 3** addresses outcome following post-remission treatment in elderly patients above the age of 60 years, comparing no further post-remission treatment with chemotherapy, autologous HSCT or alloHSCT following RIC. The outcome of post-remission treatment was again compared in different AML risk groups. Post-remission treatment has been specifically debated in patients with an intermediate risk AML, especially taking molecular markers (eg, *NPM1* and *FLT3*-ITD) into account. We addressed the impact of *NPM1* and *FLT3*-ITD including the *FLT3*-

ITD allelic ratio on the outcome in patients with cytogenetically normal AML in **Chapter 4**. Patients were classified based on these molecular markers and outcome of post-remission treatment with alloHSCT vs autologous HSCT or chemotherapy was compared. A risk-adapted approach of AML may be further developed taking minimal residual disease (MRD) into account. **Chapter 5** addresses whether and to what extent the GVL-effect of alloHSCT was present in patients with or without MRD to provide further risk- and MRD-adapted recommendations for post-remission treatment with alloHSCT.

The second part of the thesis focuses on complications and toxicity of alloHSCT. In **Chapter 6**, the preferred type of donor for patients with poor risk AML in first CR was investigated in a large cohort of alloHSCT recipients. Outcome was compared for patients receiving alloHSCT with matched related donors, matched unrelated donors, mismatched unrelated donors, cord blood grafts, or haplo-identical donors. Secondly, outcome and mortality following alloHSCT may be predicted before transplantation using transplant risk scores. **Chapter 7** addresses the prediction of NRM in recipients of RIC alloHSCT. Previous established risk scores were evaluated and the variables of these scores were subsequently reassessed and combined into a new NRM risk score for AML patients proceeding to RIC alloHSCT. **Chapter 8** describes the incidence and sequelae of hepatitis E virus as an opportunistic pathogen in recipients of alloHSCT having clinical implications.

Finally, **Chapter 9** summarizes the most important findings of this thesis and provides a risk-adapted approach for a personalized post-remission treatment of AML patients taking into account the risk of relapse, but also the risk of mortality.

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2

COMPARATIVE THERAPEUTIC VALUE OF POST-REMISSION APPROACHES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AGED 40–60 YEARS

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ABSTRACT

The preferred type of post-remission therapy (PRT) in patients with acute myeloid leukemia (AML) in first complete remission (CR1) is a subject of continued debate, especially in patients at higher risk of non-relapse mortality (NRM), including patients >40 years of age. We report results of a time-dependent multivariable analysis of allogeneic hematopoietic stem cell transplantation (alloHSCT) (n = 337) versus chemotherapy (n = 271) or autologous HSCT (autoHSCT) (n = 152) in 760 patients aged 40-60 years with AML in CR1. Patients receiving alloHSCT showed improved overall survival (OS) as compared with chemotherapy (respectively 57 ± 3% vs 40 ± 3% at 5 years, P < 0.001). Comparable OS was observed following alloHSCT and autoHSCT in patients with intermediate risk AML (60 ± 4% vs 54 ± 5%). However, alloHSCT was associated with less relapse (hazard ratio [HR] 0.51, P < 0.001) and better relapse-free survival (RFS) (HR 0.74, P = 0.029) as compared with autoHSCT in intermediate risk AMLs. AlloHSCT was applied following myeloablative conditioning (n = 157) or reduced intensity conditioning (n = 180), resulting in less NRM, but comparable outcome with respect to OS, RFS and relapse. Collectively, these results show that alloHSCT is to be preferred over chemotherapy as PRT in patients with intermediate and poor risk AML aged 40-60 years, whereas autoHSCT remains a treatment option to be considered in patients with intermediate risk AML.

INTRODUCTION

Although hematological first complete remissions (CR1) may be achieved in ~80% of younger patients with newly diagnosed acute myeloid leukemia (AML), the relapse rate is still unacceptably high and varies according to age and the underlying cytogenetic and molecular profile of the leukemia.[1-5] Post-remission therapy (PRT) is applied for prevention of relapse and may include either consolidation chemotherapy or hematopoietic stem cell transplantation (HSCT) using either allogeneic (alloHSCT) or autologous (autoHSCT) stem cell grafts. Although alloHSCT offers the most effective antileukemic therapy, enhanced non-relapse mortality (NRM) may compromise that favorable effect. As a result, alloHSCT is no longer indicated for favorable risk AML,[6] and currently being discussed in patients with intermediate risk AML.[7-9]

NRM following myeloablative conditioning (MAC) alloHSCT increases with age and/or comorbidities,[10-12] as a result of which a net survival benefit of alloHSCT in AML patients beyond 40 years could not be demonstrated in a meta-analysis of the earlier studies by HOVON, MRC, EORTC and the French BGM group.[6,13-16] Following that observation, several HOVON centers introduced reduced intensity conditioning (RIC) alloHSCT in patients beyond 40 years of age to reduce NRM, while maintaining graft versus leukemia (GVL) effects.[17,18] Meanwhile, by virtue of the use of peripheral blood stem cells instead of bone marrow, results of autoHSCT gradually improved in AML.[19] These developments, as well as results of more recent retrospective and prospective studies,[20-22] urged us to readdress the question of preferred PRT in a more recent cohort of AML patients, aged 40-60 years. Particularly this age-cohort allowed us to compare PRT by alloHSCT, using either RIC or MAC, versus chemotherapy or autoHSCT. We evaluated these PRT modalities by time-dependent analysis, a method that has lately increasingly been applied for evaluation of alloHSCT, as the sibling donor versus no-donor methodology can no longer be applied with the increased use of unrelated donors.[7,23-26] The method allows for comparing patients actually transplanted versus non-transplanted patients without the bias caused by the time to transplant.[27]

PATIENTS AND METHODS

Patients

A total of 760 patients between 40 and 60 years of age with newly diagnosed AML receiving PRT in CR1, who participated in two consecutive, prospective HOVON-SAKK phase III trials (AML42/42A, and AML92), were included.[19,28,29] Patients were classified for leukemia risk, based on the cytogenetic and molecular profile of the underlying AML, according to the latest European LeukemiaNET (ELN) AML risk classification.[1] In the present analysis, the intermediate-I and intermediate-II risk groups of the ELN risk classification were combined because of similar outcome of these subgroups (Supplementary Figure 1). Both AML42/42A and AML92 had been approved by ethics committees of participating institutions and were conducted in accordance with the Declaration of Helsinki. All participants had given written informed consent. Detailed description of the inclusion and exclusion criteria of these studies are previous reported.[28,29]

Treatment protocols

Treatment in the AML42/42A and AML92 studies involved a maximum of two remission induction cycles, including anthracyclin with cytarabine chemotherapy, as previously described.[28,29] Three different types of PRT were applied in patients in CR1 according to a predefined strategy as outlined in the AML42 and AML 92 protocol, including a third cycle of chemotherapy with mitoxantrone and etoposide, high-dose chemotherapy with busulfan and cyclophosphamide followed by autoHSCT, or alloHSCT following either RIC or MAC. These different therapeutic modalities were applied according to a risk-adapted strategy.[19,28,29] (1) Patients with AML classified as favorable risk, according to cytogenetic and available molecular analysis, were planned for a third cycle of chemotherapy; (2) intermediate risk patients were preferentially treated by alloHSCT using a human leukocyte antigen (HLA) matched sibling donor or a fully HLA-matched unrelated donor if available; and (3) patients with poor risk AML proceeded to alloHSCT using either a sibling or unrelated donor, using 7/8 or 8/8 matched donors. Patients alternatively received an autoHSCT or a third cycle of chemotherapy if no suitable donor was available.[19]

Transplantation protocols

Patients received either a MAC or RIC regimen followed by the infusion of donor cells. RIC-alloHSCT was introduced in patients below 60 years as from 2001, whereby the indication for RIC or MAC was selectively determined by age and consistently adhered to by the individual center throughout the AML42/42A and AML92 studies. Whereas some centers maintained their policy of MAC-alloHSCT for all patients up to the age of 60, a number of centers changed their policy by setting the age limit for MAC at <40 and RIC for patients of 40 years and beyond. The degree of HLA-matching for unrelated donors was 8/8 allele

match for HLA-A, B, C, and DRB1 for intermediate risk patients and ≥ 7/8 allele match for poor risk patients. The MAC regimen contained high-dose cyclophosphamide with total body irradiation (TBI) in 110 (70%) patients, whereas the remainder received busulfan with cyclophosphamide. T-cell depletion was only performed in recipients of MAC-alloHSCT, whereby partial T-cell depletion was performed by CD34-selection and add-back of T-cells to the graft to ensure 1×10^5 T-cells/kg bodyweight of the recipient, as described earlier.[30] Although RIC regimens varied, the majority contained 2.0 Gy TBI preceded by fludarabine (n = 126, 70%), as described earlier.[17] A calcineurin inhibitor (either ciclosporin or tacrolimus) plus mycophenolate mofetil or methotrexate was given as prophylaxis for graft versus host disease (GVHD). Recipients of a T-cell depleted MAC-alloHSCT received a calcineurin inhibitor (either ciclosporin or tacrolimus) plus methotrexate as GVHD prophylaxis.

Endpoints

The primary endpoint of the study was overall survival (OS), according to the type of PRT received. OS and relapse-free survival (RFS) were measured from the date of start of PRT. The event for OS was death whatever the cause, and patients were censored at the date of last contact, if alive. The events for RFS were death in CR1, designated as NRM or hematological relapse. The cumulative risks of relapse and NRM over time were calculated as competing risks with actuarial methods where patients alive in continuing CR1 were censored at the date of last contact.

Statistical methods

A time-dependent analysis of PRT was performed as described previously,[24] by applying multivariable Cox regression with time-dependent covariates autoHSCT and alloHSCT. The multivariable analysis is conceptually similar to a Mantel-Byar analysis,[31] but more general as it allows for adjustment for other factors. Some patients received PRT with chemotherapy (n = 39) or autoHSCT (n = 3) first before they proceeded to alloHSCT. In both the multivariable analysis and the estimation of the survival curves, these patients were counted as at risk in the chemotherapy or autoHSCT group from the start of PRT until alloHSCT and after that as at risk in the alloHSCT group. Multivariable Cox regression analysis for OS, RFS, relapse and NRM was applied with stratification for leukemia risk and adjustment for late CR (after cycle II instead of I), time from CR to PRT, age, sex and year of treatment before or after 2006. Year of treatment before or after 2006 was included to adjust for a possible overall difference in outcomes between these two periods. Moreover, time from start induction to start post-remission treatment and T-cell depletion were added as factors to the model, but showed no significant effects on OS, RFS, relapse or NRM. A similar analysis restricted to

alloHSCT patients was done for a direct comparison of RIC-alloHSCT and MAC-alloHSCT, with stratification by leukemia risk and adjustment for late CR, time from CR to transplantation, age, sex, donor type and year of transplantation before or after 2006. In addition, time from start induction to transplantation, number of induction cycles, stem cell source, TBI, patient/donor gender mismatch and cytomegalovirus mismatch were not included in the model because of no significant effect on outcome. All P-values were based on log likelihood ratio tests, except when explicitly stated otherwise. Log likelihood ratio tests were also used to test for interactions. The proportional hazard assumption was tested on the basis of Schoenfeld residuals.[32] P-values have not been adjusted for multiple testing. All analyses were done with Stata Statistical Software: Release 13 (2013, College Station, TX, USA: Stata Corporation).

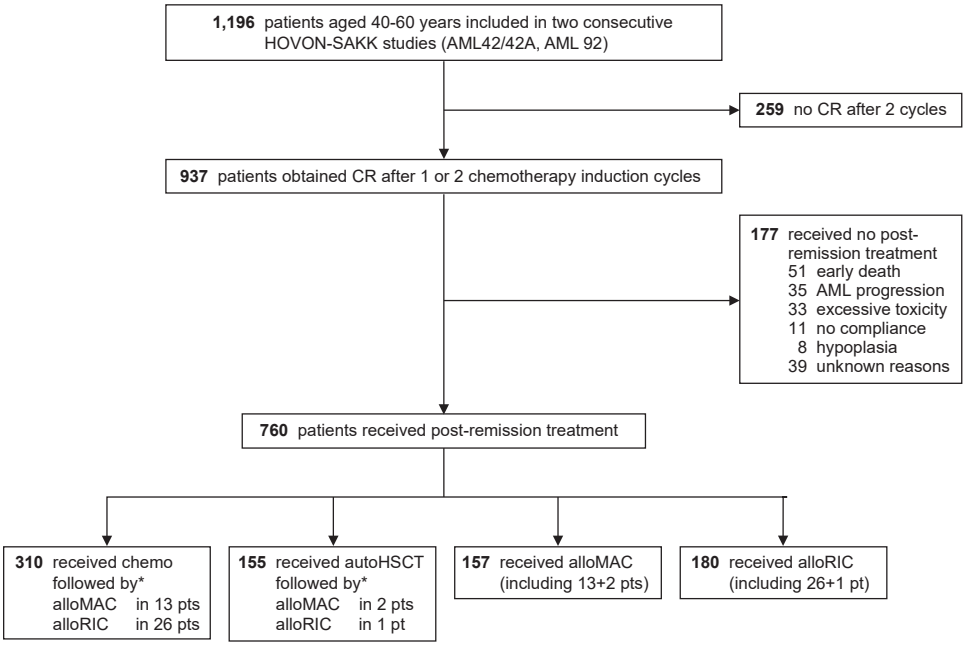

Figure 1 Consort diagram
*Counted as at risk in the transplantation group as from the day of transplant, according to time-dependent analysis

RESULTS

Characteristics of the patients

Between January 2001 and February 2010, induction chemotherapy was started in 1196 patients aged 40-60 years (Figure 1). CR after induction (2 cycles) was obtained in 937 (78%) patients, of whom 760 proceeded to PRT with either chemotherapy (n = 271), autoHSCT (n = 152), MAC-alloHSCT (n = 157) or RIC-alloHSCT (n = 180). One hundred and seventy seven patients in CR1 did not receive PRT, because of toxicity (n = 33), early death (n = 51), AML progression (n = 35) or other reasons (Figure 1). Patient characteristics are presented in Table 1. Owing to the preferred application in poor risk patients, more patients proceeding to alloHSCT exhibited adverse risk features. A higher percentage of alloHSCT recipients needed two cycles of chemotherapy instead of one cycle to obtain CR. AutoHSCT and alloHSCT were more frequently applied in recent years. The median follow-up of patients still alive was 79 months and differed between patients receiving chemotherapy (83 months), autoHSCT (71 months), MAC-alloHSCT (88 months) and RIC-alloHSCT (75 months). Table 2 presents transplantation characteristics of alloHSCT recipients, comparing the groups of RIC and MAC. Recipients of RIC-alloHSCT were significantly older and were transplanted more frequently in the recent years. Grafts were not manipulated in all RIC-alloHSCT patients, whereas 24% of MAC-alloHSCT patients received grafts, partially depleted of T-cells. Patients receiving RIC-alloHSCT and MAC-alloHSCT had similar donor source, stem cell source, CMV-serology status, female donors/male recipient's ratio and similar distributions of their leukemia risk profile[1] and EBMT-risk scores[10] (Tables 1 and 2).

Treatment outcome

OS appeared to be clearly different in the favorable, intermediate-I/intermediate-II, adverse leukemia risk groups as categorized by the ELN AML risk classification,[1] with OS at 5 years of 74 ± 4% in favorable risk, 51 ± 3% in intermediate-I risk, 47 ± 6% in intermediate-II risk and 33 ± 4% in adverse risk AMLs (Supplementary Figure 1). Because of similar survival in the ELN intermediate I and II risk subcategories, these patients were analyzed as one single intermediate risk group. Outcome estimates at 5 years for each type of PRT by ELN risk group can be found in Supplementary Table 1. Figure 2a and 2b show OS and RFS of all patients by type of PRT, stratified for leukemia risk. Improved OS was found for alloHSCT recipients as compared with patients receiving chemotherapy as PRT (57 ± 3% versus 40 ± 3% at 5 years, P<0.001, Figure 2a). In addition, OS was significantly improved in recipients of autoHSCT as compared with recipients of chemotherapy (54 ± 3% versus 40 ± 3% at 5 years, P = 0.02, Figure 2a).

Table 1 Patient characteristics

	Post-remission treatment			p-value (CT vs Auto)	p-value (CT vs Allo)
	CT (N=271)	Auto (N=152)	Allo (N=337)		
Gender					
Male	151 56%	83 55%	181 54%	.83	.62
Female	120 44%	69 45%	156 46%		
Age (years)					
Median	52	52	51	.72	.14
Range	40–60	40–60	40-60		
WBC at diagnosis (x10^9/l)					
Median	14	16	7	.55	.005
Range	1–400	1–220	0-300		
Karyotype classification					
t(8;21)	18 7%	4 3%	3 1%	.002	<.001
inv(16)	30 11%	3 2%	4 1%		
CN-X-Y	149 55%	91 60%	171 51%		
CA Rest	45 17%	33 22%	106 31%		
MK	15 6%	4 3%	40 12%		
Missing	14 5%	17 11%	13 4%		
Molecular classification (positive patients)*					
NPM1	64 24%	43 28%	61 18%	.078	.15
CEBPα dm	4 1%	7 5%	5 1%	.052	.91
FLT3-ITD ratio < 0.60	22 8%	21 14%	37 11%	.33	.23
FLT3-ITD ratio > 0.60	9 3%	3 2%	5 1%		
EVI1	8 3%	4 3%	15 4%	.97	.20
Risk AML†					
Favorable	86 32%	39 26%	29 9%	.28	<.001
Intermediate	150 55%	93 61%	161 48%		
Adverse	35 13%	20 13%	147 44%		
CR reached after					
Cycle 1 (early CR)	220 81%	130 86%	245 73%	.26	.014
Cycle 2 (late CR)	51 19%	22 14%	92 27%		
Time from start induction to start post-remission treatment (months)					
Median	3.3	3.6	3.8	.002	<.001
IQ range	3–4	3–4	3–4		
Time from CR to start post-remission treatment (months)					
Median	2.1	2.3	2.3	<.001	.039
IQ range	1–3	1–3	1–3		
Year of start treatment					
<2006	169 62%	73 48%	162 48%	.004	<.001
≥2006	102 38%	79 52%	175 52%		

Abbreviations: CT indicates chemotherapy; Auto, autologous hematopoietic stem cell transplantation; Allo, allogeneic hematopoietic stem cell transplantation; WBC, white blood cell count; CN-X-Y, cytogenetically normal or only loss of X or Y chromosome; CA, cytogenetically abnormal; MK, monosomal karyotype; *NPM1*, nucleophosmin 1; *CEBPα* dm, CCAAT/enhancer-binding protein alpha double mutations; *FLT3*-ITD, *F*ms-like tyrosine kinase 3 internal tandem duplication; *EVI1*, ecotropic virus integration site 1; AML, acute myeloid leukemia; CR, complete remission; and IQ, interquartile range; * Molecular analysis was available in 62% of the patients for *NPM1* and *Flt3*-ITD, and in 54% of the patients for *CEPBα* and *EVI1*; † According to the European LeukemiaNET AML risk classification

Table 2 Characteristics of alloHSCT recipients

	Conditioning				p-value
	AlloMAC (N=157)		AlloRIC (N=180)		
Age (years)					
Median	48		54		<.001
Range	40–59		40–60		
Risk AML*					
Favorable	16	10%	15	8%	
Intermediate	92	59%	110	61%	.81
Adverse	49	31%	55	31%	
CR reached after					
Cycle 1 (early CR)	108	69%	137	76%	.13
Cycle 2 (late CR)	49	31%	43	24%	
Year of start treatment					
<2006	92	59%	70	39%	<.001
≥2006	65	41%	110	61%	
Donor type					
HLA-identical sibling	117	75%	131	73%	
VUD	29	18%	41	23%	.42
Other	11	7%	8	4%	
T-cell depletion					
Yes	38	24%	0		<.001
No	119	76%	180	100%	
Stem cell source					
PB	141	90%	170	94%	.11
BM	16	10%	10	6%	
TBI given					
Yes	110	70%	131	73%	
No	41	26%	31	17%	.092
Unknown	6	4%	18	10%	
Female donor to male recipient					
Yes	35	22%	33	18%	.37
No	122	78%	147	82%	
CMV match patient/donor					
-/-	59	38%	57	32%	
-/+	13	8%	25	14%	.36
+/-	37	24%	44	24%	
+/+	48	31%	54	30%	
EBMT-score					
1 point	3	2%	2	1%	
2 points	90	57%	103	57%	.85
3 points	56	36%	68	38%	
4 points	8	5%	7	4%	

Abbreviations: AlloMAC indicates myeloablative conditioned allogeneic hematopoietic stem cell transplantation; alloRIC, reduced intensity conditioned allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; CR, complete remission; HLA, human leukocyte antigen; VUD, voluntary unrelated donor; PB, peripheral blood; BM, bone marrow; TBI, total body irradiation; CMV, cytomegalovirus; and EBMT, European Group for Blood and Marrow Transplantation; * According to the European LeukemiaNET AML risk classification

Intermediate risk AMLs

In intermediate risk patients, alloHSCT and autoHSCT significantly improved OS as compared with chemotherapy (60 ± 4% and 54 ± 5%, respectively, versus 36 ± 4% at 5 years, P<0.001, Figure 2c), while OS after alloHSCT versus autoHSCT was not significantly different. In contrast, improved RFS was found in patients with intermediate risk AML receiving PRT with alloHSCT as compared with autoHSCT (56 ± 4% versus 39 ± 5% at 5 years, respectively, P = 0.04, Figure 2d). Trends toward improved OS and RFS were found for alloHSCT and autoHSCT as compared with chemotherapy in the relatively small subgroups of favorable and unfavorable risk leukemia's (Supplementary Figure 2).

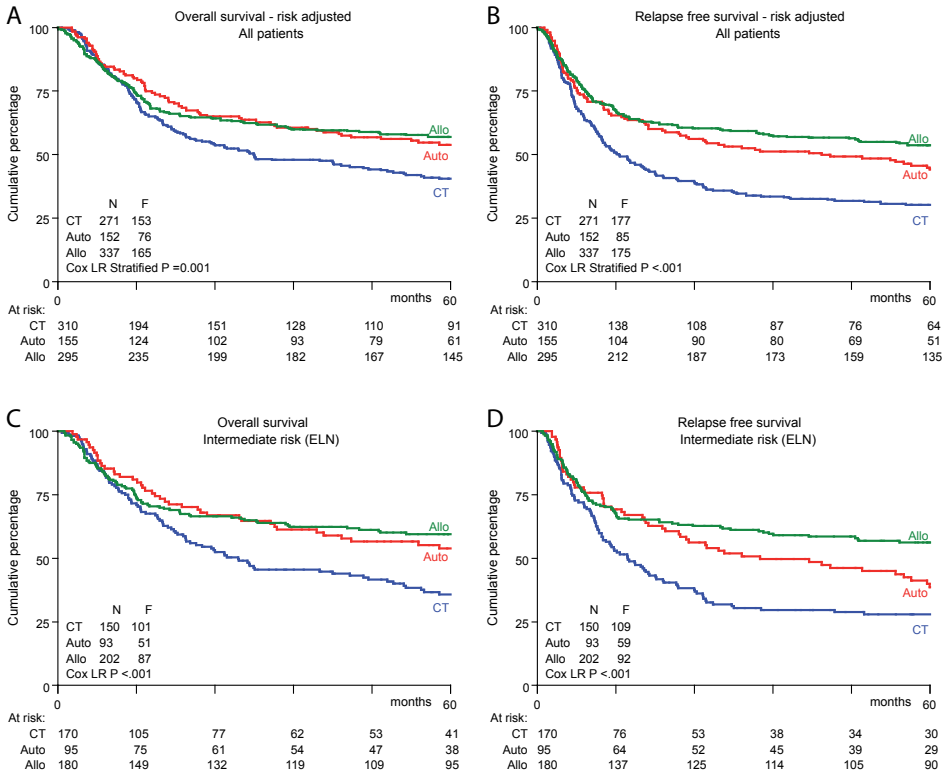

Figure 2 OS and RFS in all patients and intermediate-risk patients by post-remission treatment

Kaplan-Meier estimates of OS (**A**) and RFS (**B**) of patients with AML in first complete remission from start of post-remission treatment, according to post-remission treatment and with direct adjustment for differences in leukemia risk category among the treatment groups by the method of Gail and Byar.[55] Kaplan-Meier estimates of OS (C) and RFS (D) in intermediate risk patients. Of note, numbers of patients at risk (indicated below the x axis) differ from the patient numbers (indicated in Table 1 and within the figure) because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in CR1 to the transplantation curve. Abbreviations: CT, chemotherapy; Auto, autologous hematopoietic stem cell transplantation; Allo, allogeneic hematopoietic stem cell transplantation; F, number of failures (that is, death whatever the cause for OS, and death or relapse for RFS); N, number of patients; and Cox LR, Cox likelihood ratio

Second complete remission

A total of 358 patients developed a relapse after having received PRT. Two hundred and five patients proceeded to salvage chemotherapy and 125 (35%) entered a second CR. Ultimately, only 75 out of 358 relapsing patients proceeded to alloHSCT in second CR and 6 patients received an autoHSCT in second CR. Overall outcome of all relapsing patients was 12 ± 2% at 5 years from relapse.

Multivariable analysis

Table 3 shows the results of the multivariable analysis with stratification for leukemia risk and with adjustment for sex, age, late CR, time from CR to PRT, year of start of PRT before or after 2006 and PRT type. Both OS and RFS were significantly better after alloHSCT as compared with chemotherapy with HRs of 0.64 (P<0.001) and 0.51 (P<0.001), respectively (Supplementary Figure 2). Relapse was significantly reduced following alloHSCT as compared with chemotherapy (HR 0.33, P<0.001). AutoHSCT was also associated with significantly improved RFS (HR 0.69, P = 0.005) and a reduced risk of relapse (HR 0.66, P = 0.003) as compared with chemotherapy. OS was not significantly different comparing alloHSCT with autoHSCT (HR 0.83, P = 0.19), while RFS was significantly improved after alloHSCT as compared with autoHSCT (HR 0.74, P = 0.029).

Intermediate risk AMLs

With respect to OS and RFS in intermediate risk patients, the HRs comparing alloHSCT with chemotherapy were 0.54 (95% CI: 0.40-0.72, P<0.001) and 0.47 (95% CI: 0.35-0.63, P<0.001), respectively. The HRs comparing autoHSCT with chemotherapy in intermediate risk patients were 0.72 (95% CI: 0.51-1.02, P = 0.058) for OS and 0.73 (95% CI: 0.52-1.00, P = 0.048) for RFS. A trend was found toward improved OS comparing alloHSCT with autoHSCT in intermediate risk patients (HR 0.74, 95% CI: 0.52-1.06, P = 0.10), whereas RFS was significantly better for alloHSCT in intermediate risk patients (HR 0.65, 95% CI: 0.46-0.90, P = 0.011).

Tests for interaction

We tested for interactions between the type of PRT with age, time from CR to post-remission treatment, year of treatment (< or ≥ 2006), sex, late CR1 and leukemia risk. Only between age and PRT significant interactions were found, indicating that autoHSCT recipients experienced an increased event rate with age for all endpoints (details not shown). We have also tested for the interaction between the type of PRT and center size (above or below the median number of patients per center). No interaction was found between type of PRT and center size for all outcome parameters.

Table 3 Results of the multivariable analysis

	OS			RFS			Relapse			NRM		
	HR*	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**
Post-remission treatment												
Allo vs. CT	0.64	0.51-0.81	<.001	0.51	0.41-0.64	<.001	0.33	0.26-0.43	<.001	6.89	2.69-17.68	<.001
Auto vs. CT	0.77	0.58-1.02	.067	0.69	0.53-0.90	.006	0.66	0.50-0.87	.003	2.13	0.69-6.56	.18
Allo vs. Auto	0.83	0.63-1.10	.19	0.74	0.57-0.97	.029	0.51	0.38-0.69	<.001	3.24	1.53-6.88	<.001
Sex (female vs. male)	0.83	0.68-1.01	.066	0.82	0.67-0.99	.040	0.81	0.66-1.01	.058	0.84	0.53-1.33	.46
Age†	1.14	0.96-1.35	.14	1.07	0.90-1.26	.44	1.05	0.87-1.26	.61	1.11	0.75-1.66	.60
CR (late vs. early)	1.81	1.33-2.46	<.001	1.86	1.39-2.50	<.001	1.83	1.32-2.54	<.001	1.74	0.89-3.41	.11
Time CR to post-remission treatment‡	0.99	0.89-1.10	.84	0.99	0.89-1.09	.81	0.96	0.86-1.08	.54	1.05	0.85-1.30	.67
Year of start treatment (≥2006 vs. <2006)	1.08	0.88-1.33	.46	1.15	0.95-1.41	.16	1.06	0.85-1.32	.59	1.67	1.02-2.73	.041

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; Allo, allogeneic hematopoietic stem cell transplantation; vs., versus; CT, chemotherapy; and Auto, autologous hematopoietic stem cell transplantation;

* The HRs are the estimates of the effect of covariates for each outcome parameter, stratified by leukemia risk and adjusted for sex, age, CR (late vs. early), time from CR to post-remission treatment, year of treatment before or after 2006, and type of post-remission treatment;

† Linear with estimates of HRs for ten years difference;

‡ Linear with estimates of HRs for one month difference

AlloHSCT conditioning: RIC versus MAC

We additionally performed a direct comparison of patients receiving RIC-alloHSCT with MAC-alloHSCT. Figures 3a and 3b show OS and RFS, respectively, of alloHSCT recipients by conditioning type stratified for leukemia risk. NRM was significantly increased for recipients of MAC-alloHSCT as compared with recipients of RIC-alloHSCT (23 ± 3% versus 11 ± 2% at 5 years, P = 0.009, Figure 3d), whereas the cumulative incidence of relapse comparing RIC-alloHSCT with MAC-alloHSCT was not significantly different (37 ± 4% versus 29 ± 4% at 5 years, Figure 3c), resulting in no significant different OS (57 ± 4% versus 51 ± 4% at 5 years) and RFS (52 ± 4% versus 48 ± 4% at 5 years) for RIC-alloHSCT recipients compared with MAC-alloHSCT patients. Multivariable analysis with stratification for leukemia risk and adjustment for covariates including donor type (Table 4), showed decreased NRM following RIC-alloHSCT (HR 0.44, P = 0.004). Of note, within the group of patients receiving MAC-alloHSCT, NRM was increased in recipients of partially T-cell depleted MAC-alloHSCT (HR 4.00, 95% CI: 2.04-7.84, P<0.001), but relapse was not increased. No patients receiving RIC-alloHSCT received grafts that were depleted of T-cells. Relapse did not differ between MAC-alloHSCT and RIC-alloHSCT with an HR of 1.24 (P = 0.34). It resulted in similar OS (HR 0.78, P = 0.16) and RFS (HR 0.85, P = 0.34) between RIC-alloHSCT and MAC-alloHSCT. The similar outcome following either RIC or MAC was also observed in subgroups of patients, according to underlying leukemia risk. Specifically, the advantage of RIC- or MAC-alloHSCT versus chemotherapy in terms of RFS was observed in both intermediate and poor risk subgroups to a similar degree (Supplementary Figure 3).

GVHD

Incidences of grade II-IV acute GVHD after RIC-alloHSCT and MAC-alloHSCT were 9% and 26%, respectively. Incidences of chronic limited and chronic extensive GVHD were, respectively, 19% and 36% in RIC-alloHSCT and 32% and 29% in MAC-alloHSCT patients.

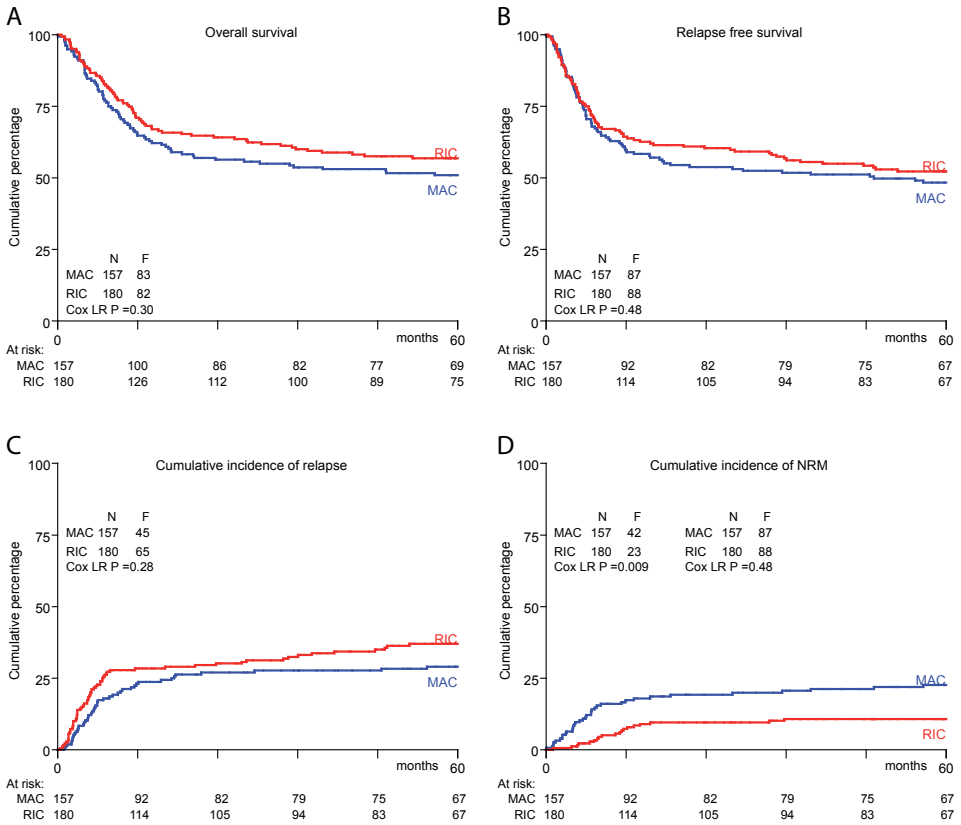

Figure 3 Outcome of allogeneic transplantation by conditioning type
Kaplan-Meier estimates of OS (**A**) and RFS (**B**) and cumulative incidence of relapse (**C**) and NRM (**D**) of patients with AML in first CR form start of transplantation, according to conditioning type and with direct adjustment for differences in leukemia risk category among the treatment groups by the method of Gail and Byar.[55] The cumulative incidences of relapse and NRM over time were calculated as competing risks with actuarial methods, where patients alive in continuing CR1 were censored at the date of last contact. Abbreviations: F, number of failures (that is, death whatever the cause); N, number of patients; and Cox LR, Cox likelihood ratio

Table 4 Results of the multivariable analysis

	OS			RFS			Relapse			NRM		
	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value
Transplantation type												
RIC vs. MAC	0.78	0.56-1.10	.16	0.85	0.60-1.19	.34	1.24	0.80-1.91	.34	0.44	0.25-0.79	.004
Sex (female vs. male)	1.07	0.78-1.48	.66	0.96	0.70-1.31	.80	0.94	0.63-1.39	.75	1.00	0.60-1.66	.99
Age†	1.27	0.94-1.71	.12	1.21	0.91-1.63	.19	1.19	0.82-1.72	.37	1.24	0.77-2.02	.38
CR (late vs. early)	2.00	1.33-3.02	.001	2.18	1.47-3.24	<.001	2.66	1.62-4.38	<.001	1.57	0.80-3.07	.20
Time CR to transplantation‡	1.02	0.89-1.17	.76	1.04	0.91-1.18	.59	1.03	0.87-1.21	.75	1.06	0.86-1.32	.58
Year of start transplantation												
≥2006 vs. <2006	1.27	0.91-1.76	.15	1.29	0.94-1.78	.12	1.18	0.80-1.75	.40	1.56	0.90-2.71	.11
Donor type												
VUD vs. Sib	1.20	0.78-1.86	.41	1.15	0.75-1.75	.52	1.11	0.66-1.88	.70	1.15	0.57-2.34	.70
Other vs. Sib	1.77	0.99-3.16	.069	1.45	0.82-2.59	.22	1.15	0.51-2.58	.74	2.01	0.87-4.63	.13

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; RIC, reduced intensity conditioning allogeneic hematopoietic stem cell transplantation; vs., versus; MAC, myeloablative conditioning allogeneic hematopoietic stem cell transplantation; VUD, voluntary unrelated donor; and Sib, sibling

* The HRs are the estimates of the effect of covariates for each outcome parameter, stratified by leukemia risk and adjusted for sex, age, CR (late vs. early), time from CR to transplantation, year of transplantation before or after 2006, and donor type

† Linear with estimates of HRs for ten years difference

‡ Linear with estimates of HRs for one month difference

DISCUSSION

The preferred type of PRT in younger patients with AML in CR1 is a subject of continued debate. While the GVL effect exerted by alloHSCT strongly reduces relapse irrespective of cytogenetic subcategory,[24] counterbalancing NRM may attenuate a favorable effect on OS, which is especially evident in good risk patients.[6,13-16,26] More recently, alloHSCT is also being discussed in intermediate risk patients,[7-9] especially in patients at higher risk of NRM. We and others previously observed increased NRM in alloHSCT recipients over the age of 40 years, which resulted in similar outcome for AML CR1 patients receiving alloHSCT as compared with conventional PRT using chemotherapy or autoHSCT.[6,13-16] Following the latter observations, several HOVON centers introduced RIC-alloHSCT for patients as from the age of 40 years, but adhered to alloHSCT as preferred PRT in intermediate risk patients. The latter approach signified the basis for the current study, addressing the value of alloHSCT versus conventional PRT and comparing recipients of a MAC-alloHSCT versus RIC-alloHSCT. Here, by time-dependent analysis, we observed improved OS by alloHSCT as compared with chemotherapeutic PRT in patients aged 40 to 60 years with AML in CR1. Of note, alloHSCT and autoHSCT did not significantly differ with respect to OS in intermediate risk patients, although RFS was better following alloHSCT. In addition, the intensity of the conditioning regimen did not significantly affect the rate of relapse after alloHSCT, thereby questioning the necessity of MAC in this category of patients.

Currently, it is generally accepted that patients with favorable risk AML do not qualify for alloHSCT as preferred PRT, because of a high probability of obtaining a second CR and subsequent favorable outcome upon proceeding to alloHSCT in second CR.[7,26,33] More recently, that policy was also advocated for intermediate risk patients,[7] although remission rates in relapsing intermediate risk patients are generally lower and also the percentage of patients actually proceeding to alloHSCT in second CR is compromised.[9,34] Younger patients with adverse risk AML are currently recommended for an alloHSCT in CR1 using sibling or alternative donors, provided the risk of NRM is not excessively high.[12] However, these recommendations are continuously evolving, because of a number of developments, including better results following autoHSCT,[19-22] less NRM following RIC-alloHSCT[17,18] and improved possibilities for risk-adapted therapy,[12] using at one hand better prognostic scores for leukemia risk,[1] better scores to predict NRM[10,11,35] and incorporation of quantified minimal residual disease (MRD) in decision making.[36-39] These developments urged us to readdress PRT, especially focusing on the place of alloHSCT in intermediate risk patients aged 40-60 years, for whom RIC-alloHSCT had been introduced in recent years. In contrast to our earlier observations,[13] the present study clearly showed an overall advantage for alloHSCT recipients as compared with patients proceeding to chemotherapeutic PRT. NRM following alloHSCT in the present study estimated 17 ± 2% at 5 years as compared with 25 ± 4% at 4 years in the earlier HOVON-SAKK study,[13] which thereby largely accounted for the observed

improvement. Improved outcome following alloHSCT as compared with chemotherapy in the present study was apparent in both MAC-alloHSCT and RIC-alloHSCT recipients, with fairly similar outcome. These results are in accordance with a recent German study, exploring PRT by prospective matched pair analysis.[40] That study showed significantly better OS for alloHSCT in non-favorable risk patients and especially in patients 45-59 years of age, which compares well with the present report. Also in their study, an increasing number of patients received an alloHSCT preceded by non-MAC or RIC, but recipients of RIC and MAC were not compared. In addition, the option of autoHSCT was not included as a PRT option in the AMLCG99 study.[40] Stelljes et al.[40] performed a prospective matched pair analysis, whereas alloHSCT was evaluated by time-dependent methodology in the present study. In the past, we and others evaluated the effect of transplantation by 'biological randomization' through so-called (sibling) donor versus no-donor studies.[6,13] These studies allow for an intention to treat analysis and thereby to approximate real randomized studies, although variable numbers of patients with a donor in those studies actually proceeded to transplantation. With the advent of MUDs and their increasing application in AML, sibling donor versus no-donor studies have become obsolete. Therefore, other statistical methods were introduced, including landmark analysis, matched pair analysis and multivariable models that include a particular type of PRT as a time-dependent covariate.[41] Although only a real randomization would more rigorously rule out selection, the methodology does allow for approximating a prospective comparison without the bias caused by the time to transplant and by including a multivariate analysis corrects for the most important, but not all, characteristics affecting relapse and NRM.[23,27]

Results with autografting have improved following the introduction of peripheral blood stem cells,[19-22] and a recent retrospective study by the Center for International Blood and Marrow Transplant Research suggested similar outcome for younger AML CR1 patients receiving either alloHSCT from an HLA-identical sibling or an autograft using peripheral blood stem cells.[21] Although recipients of alloHSCT exhibited more high risk features, had longer follow-up and experienced a lower risk of treatment failure, no significant difference in OS was noted. Better possibilities for salvage may have accounted for improved survival after autoHSCT. Salvage by MAC-alloHSCT after autologous bone marrow transplantation appeared associated with considerable NRM in the past,[42,43] but currently RIC-alloHSCT using either sibling or alternative donors may provide for better possibilities for PRT in second CR, as was suggested in the present study by a better RFS following alloHSCT but similar OS for autoHSCT and alloHSCT recipients.

RIC-alloHSCT is generally associated with reduced NRM as compared with MAC regimens, but concern has been raised that a reduction of NRM by RIC-alloHSCT is achieved at the expense of its antileukemic activity.[17,18] Although our study is not a prospective

randomized study by design and individual conditioning choice is poorly controllable, the methodology applied allowed to limit the bias associated with time to transplant, while including a multivariable analysis.[23,24,31,44] With a mature follow-up of >6 years (median) in recipients of RIC-alloHSCT, we found that the reduction of relapse by RIC-alloHSCT did not significantly differ from what was observed in recipients of MAC-alloHSCT, suggesting overall equivalent antileukemic efficacy. These results are in contrast with earlier observations of a possible higher relapse rate after RIC-alloHSCT.[45-51] A recent, prematurely closed prospective randomized study between RIC-alloHSCT and MAC-alloHSCT did not find major differences in outcome.[52] The RIC regimen in the latter study, however, involved a more intensive, near ablative conditioning with 8 Gy TBI.[52] The potent antileukemic effect of RIC-alloHSCT that we observed may be explained by a strong GVL-effect given the relatively high incidence of chronic extensive GVHD, which correlates with ongoing GVL.[53,54] Also, the strong antileukemic effects of dose-intensive remission-induction chemotherapy[28,29] may have obviated the need for further intensified chemoradiotherapy as part of the conditioning regimen. Therefore, our results suggest that reducing the intensity of the conditioning regimen before alloHSCT may result in less NRM without a significant increase of relapse in this group of intensively treated AML patients in CR1.

Collectively, our results suggest that alloHSCT is to be preferred over chemotherapy as PRT in patients with intermediate and poor risk AML aged 40-60 years, whereas autoHSCT remains a treatment option to be considered in patients with intermediate risk AML. Further refinement of decision making might result from taking into account at one hand evolving leukemia risk factors and at the other hand risk factors that predict for NRM.[12] In addition, risk factors that evolve during treatment such as MRD currently gain importance.[36-39] A number of recent studies have suggested that especially intermediate risk patients may be further subclassified on the basis of MRD, which might thereby allow for further optimization of personalized PRT in AML. Last, given the potent GVL activity and limited toxicity profile of RIC-alloHSCT, further evaluation of RIC alloHSCT in younger AML patients below the age of 40 years appears warranted.

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SUPPLEMENTARY APPENDIX

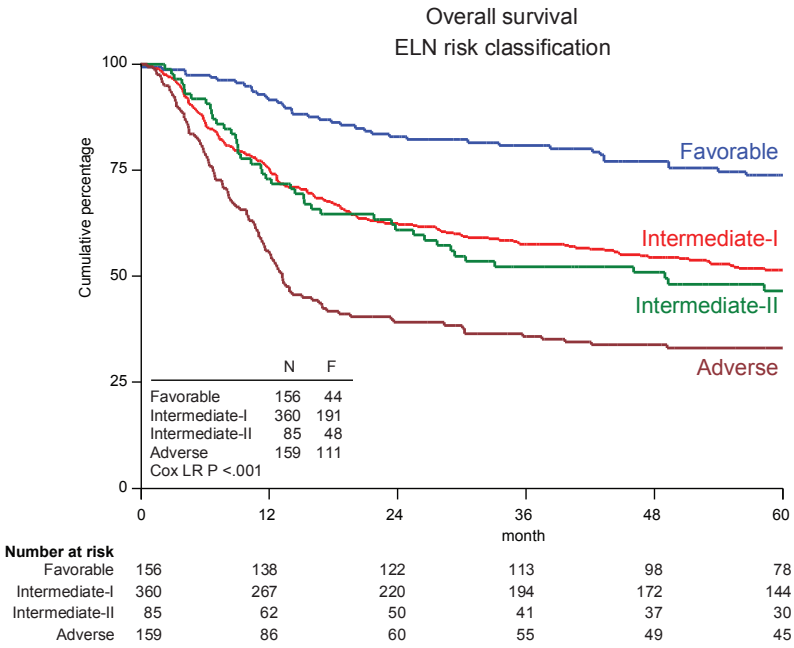
Supplementary Table 1 Outcome estimates at 5 years following post-remission treatment in each AML risk group

		Outcome after treatment (% at 5 years)			
Post-remission treatment	**No. of patients**	**OS**	**RFS**	**Relapse**	**NRM**
All patients*					
CT	271	40 ± 3	30 ± 3	60 ± 3	1 ± 1
Auto	152	54 ± 4	44 ± 4	47 ± 4	5 ± 2
Allo	337	57 ± 3	54 ± 3	36 ± 3	17 ± 2
Favorable-risk†					
CT	86	76 ± 5	56 ± 6	43 ± 6	1 ± 1
Auto	39	73 ± 7	73 ± 7	21 ± 6	6 ± 4
Allo	29	70 ± 8	70 ± 8	10 ± 5	20 ± 7
Intermediate-risk†					
CT	150	36 ± 4	28 ± 4	70 ± 4	2 ± 1
Auto	93	54 ± 5	39 ± 5	56 ± 5	5 ± 2
Allo	161	60 ± 4	56 ± 4	29 ± 3	15 ± 3
Adverse-risk†					
CT	35	19 ± 7	11 ± 6	89 ± 6	0
Auto	20	35 ± 11	31 ± 10	69 ± 10	0
Allo	147	37 ± 5	30 ± 5	51 ± 5	19 ± 4

Abbreviations: OS indicates overall survival; RFS, relapse-free survival; NRM, non-relapse mortality; CT, chemotherapy; Auto, autologous hematopoietic stem cell transplantation; and Allo, allogeneic hematopoietic stem cell transplantation

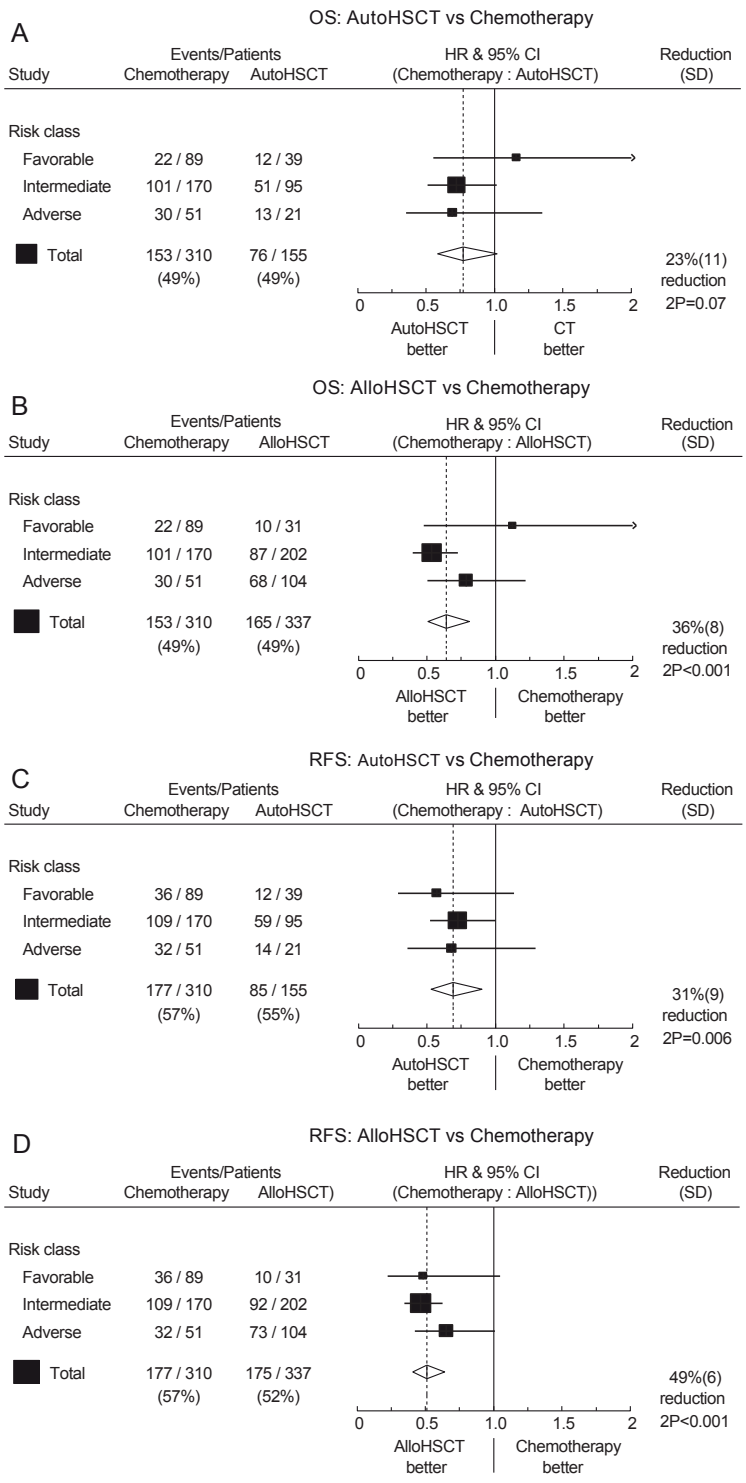
* Stratified for leukemia risk

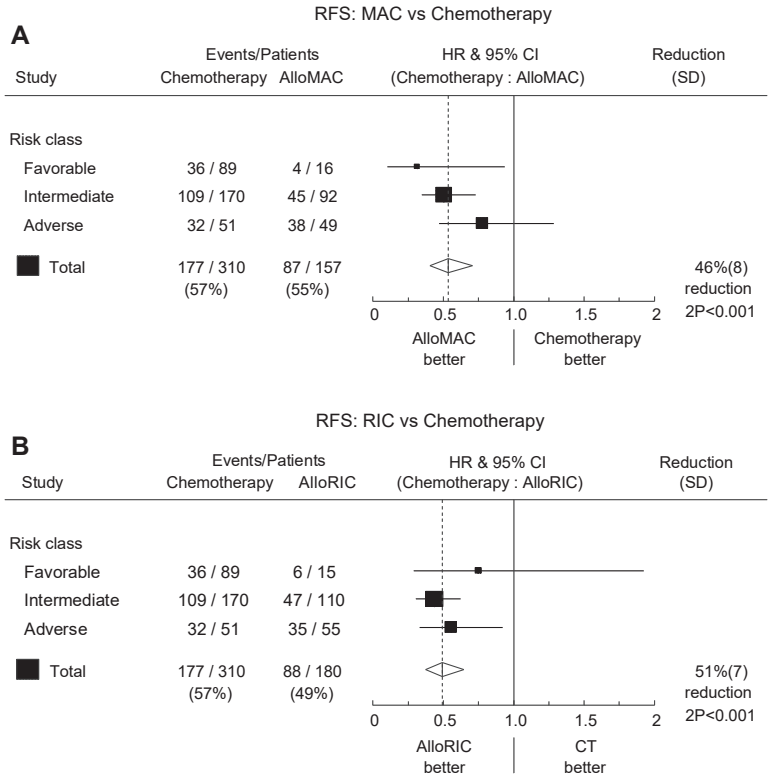
† According to the European LeukemiaNET AML risk classification



Supplementary Figure 1 Overall survival by European LeukemiaNET AML risk classification
Kaplan-Meier estimates of overall survival of patients with AML in first complete remission from start of post-remission therapy, according to European LeukemiaNET (ELN) AML risk classification.[1] Abbreviations: F, number of failures (i.e, death whatever the cause); N, number of patients; and Cox LR, cox likelihood ratio

Supplementary Figure 2 Forest plots of overall survival and relapse-free survival by post-remission therapy
Forest plots of pooled and individual estimates of the relative reduction (hazard ratio (HR) and 95% confidence interval (CI)) of overall survival (OS) and relapse-free survival (RFS) in each AML risk group, comparing autologous hematopoietic stem cell transplantation (Auto) versus chemotherapy (CT) (**A** and **C**), and allogeneic hematopoietic stem cell transplantation (Allo) versus CT (**B** and **D**). Multivariable cox regression analysis was applied with stratification for leukemia risk and adjustment for late CR (after cycle II instead of I), time from CR to PRT, age, sex, and year of treatment before or after 2006. Some patients received post-remission treatment with CT or Auto first before they proceeded to Allo. These patients were counted as at risk in the CT or Auto group from start of post-remission treatment until Allo and after that as at risk in the Allo group. Therefore the numbers in the Events/Patients table are different from the numbers reported in Table 1. Tests for heterogeneity were not significant for all comparisons (**A-D**).


A
OS: AutoHSCT vs Chemotherapy
Study
Events/Patients
Chemotherapy
AutoHSCT
HR & 95% CI
(Chemotherapy : AutoHSCT)
Reduction
(SD)
Risk class
Favorable 22 / 89 12 / 39
Intermediate 101 / 170 51 / 95
Adverse 30 / 51 13 / 21
Total 153 / 310 (49%) 76 / 155 (49%)
23%(11) reduction
2P=0.07
0 0.5 1.0 1.5 2
AutoHSCT better
CT better
B
OS: AlloHSCT vs Chemotherapy
Study
Events/Patients
Chemotherapy
AlloHSCT
HR & 95% CI
(Chemotherapy : AlloHSCT)
Reduction
(SD)
Risk class
Favorable 22 / 89 10 / 31
Intermediate 101 / 170 87 / 202
Adverse 30 / 51 68 / 104
Total 153 / 310 (49%) 165 / 337 (49%)
36%(8) reduction
2P<0.001
0 0.5 1.0 1.5 2
AlloHSCT better
Chemotherapy better
C
RFS: AutoHSCT vs Chemotherapy
Study
Events/Patients
Chemotherapy
AutoHSCT
HR & 95% CI
(Chemotherapy : AutoHSCT)
Reduction
(SD)
Risk class
Favorable 36 / 89 12 / 39
Intermediate 109 / 170 59 / 95
Adverse 32 / 51 14 / 21
Total 177 / 310 (57%) 85 / 155 (55%)
31%(9) reduction
2P=0.006
0 0.5 1.0 1.5 2
AutoHSCT better
Chemotherapy better
D
RFS: AlloHSCT vs Chemotherapy
Study
Events/Patients
Chemotherapy
AlloHSCT)
HR & 95% CI
(Chemotherapy : AlloHSCT))
Reduction
(SD)
Risk class
Favorable 36 / 89 10 / 31
Intermediate 109 / 170 92 / 202
Adverse 32 / 51 73 / 104
Total 177 / 310 (57%) 175 / 337 (52%)
49%(6) reduction
2P<0.001
0 0.5 1.0 1.5 2
AlloHSCT better
Chemotherapy better



Supplementary Figure 3 Forest plots of relapse-free survival by post-remission therapy

Forest plots of pooled and individual estimates of the relative reduction (hazard ratio (HR) and 95% confidence interval (CI)) of relapse-free survival (RFS) in each AML risk group, comparing chemotherapy (CT) versus myeloablative conditioned allogeneic hematopoietic stem cell transplantation (alloMAC) (**A**), and CT versus reduced intensity conditioned allogeneic hematopoietic stem cell transplantation (alloRIC) (**B**). Multivariable cox regression analysis was applied with stratification for leukemia risk and adjustment for late CR (after cycle II instead of I), time from CR to PRT, age, sex, and year of treatment before or after 2006. Some patients received post-remission treatment with CT first before they proceeded to Allo. These patients were counted as at risk in the CT group from start of post-remission treatment until Allo and after that as at risk in the Allo group. Therefore the numbers in the Events/Patients table are different from the numbers reported in Table 1. Tests for heterogeneity were not significant for both comparisons (**A-B**).

3

POST-REMISSION TREATMENT WITH ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS AGED 60 YEARS AND OLDER WITH ACUTE MYELOID LEUKAEMIA: A TIME-DEPENDENT ANALYSIS

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ABSTRACT

Background Acute myeloid leukaemia mainly affects elderly people, with a median age at diagnosis of around 70 years. Although about 50-60% of patients enter first complete remission upon intensive induction chemotherapy, relapse remains high and overall outcomes are disappointing. Therefore, effective post-remission therapy is urgently needed. Although often no post-remission therapy is given to elderly patients, it might include chemotherapy or allogeneic haemopoietic stem cell transplantation (HSCT) following reduced intensity conditioning reduced-intensity conditioning. We aimed to assess the comparative value of allogeneic HSCT with other approaches, including no post-remission therapy, in patients with acute myeloid leukaemia aged 60 years and older.

Methods For this time-dependent analysis, we used the results from four successive prospective HOVON-SAKK acute myeloid leukaemia trials. Between May 3, 2001, and Feb 5, 2010, a total of 1155 patients aged 60 years and older were entered into these trials, of whom 640 obtained a first complete remission after induction chemotherapy and were included in the analysis. Post-remission therapy consisted of allogeneic HSCT following reduced-intensity conditioning (n=97), gemtuzumab ozogamicin (n=110), chemotherapy (n=44), autologous HSCT (n=23), or no further treatment (n=366). Reduced-intensity conditioning regimens consisted of fludarabine combined with 2 Gy of total body irradiation (n=71), fludarabine with busulfan (n=10), or other regimens (n=16). A time-dependent analysis was done, in which allogeneic HSCT was compared with other types of post-remission therapy. The primary endpoint of the study was 5-year overall survival for all treatment groups, analysed by a time-dependent analysis.

Findings 5-year overall survival was 35% (95% CI 25-44) for patients who received an allogeneic HSCT, 21% (17-26) for those who received no additional post-remission therapy, and 26% (19-33) for patients who received either additional chemotherapy or autologous HSCT. Overall survival at 5 years was strongly affected by the European LeukemiaNET acute myeloid leukaemia risk score, with patients in the favourable risk group (n=65) having better 5-year overall survival (56% [95% CI 43-67]) than those with intermediate risk (n=131; 23% [19-27]) or adverse risk (n=444; 13% [8-20]) acute myeloid leukaemia. Multivariable analysis with allogeneic HSCT as a time-dependent variable showed that allogeneic HSCT was associated with better 5-year overall survival (HR 0.71 [95% CI 0.53-0.95], p=0.017) compared with non-allogeneic HSCT post-remission therapies or no post-remission therapy, especially in patients with intermediate risk (0.82 [0.58-1.15]) or adverse risk (0.39 [0.21-0.73) acute myeloid leukaemia.

Interpretation Collectively, the results from these four trials suggest that allogeneic HSCT might be the preferred treatment approach in patients of 60 years of age and older with intermediate risk and adverse risk acute myeloid leukaemia in first complete remission, but the comparative value should ideally be shown in a prospective randomised study.

INTRODUCTION

Acute myeloid leukaemia (AML) mainly affects older adults, with a median age at diagnosis of between 67 and 71 years.[1] Intensive remission induction chemotherapy can be initiated in patients with a favourable performance status and without substantial comorbidities. A first complete remission (CR1) is obtained in around 50-60% of patients.[2,3] Poor outcome of elderly patients can be explained by an increased incidence of relapse caused by a composite of poor risk AML characteristics. Thus, outcome is still disappointing in these patients, with 5-year overall survival (OS) of 15-30% after intensive induction chemotherapy.[2,3] Therefore, effective post-remission therapy (PRT) in elderly patients is urgently needed. PRT can include continued chemotherapy, autologous haemopoietic stem cell transplantation (HSCT), or allogeneic HSCT (alloHSCT), although maintenance treatment has never been proven to be effective. The issue of post-remission chemotherapy in older patients with AML has not been resolved, with equivalent outcomes reported for one cycle of chemotherapy compared with more than one cycle of post-remission chemotherapy.[4] An earlier study by the HOVON-SAKK consortia[5] did not show improved outcome following PRT with the monoclonal antibody gemtuzumab ozogamicin (GO), but the efficacy of this treatment has been the subject of ongoing debate.[5-8] AlloHSCT is the most effective PRT for the prevention of relapse in young patients with AML in CR1.[9-12] However, non-relapse mortality (NRM) can compromise these favourable effects on OS, especially in patients with advanced age, comorbidities, or both. Reduced intensity conditioning (RIC) regimens have been developed to reduce NRM, while maintaining graft-versus-leukaemia effects.[13] AlloHSCT following RIC has been shown to be feasible and is increasingly used in older patients with newly diagnosed AML.[14,15] Two comparative retrospective trials showed improved outcome by alloHSCT following RIC as compared with chemotherapy in elderly patients.[16,17] In the present study, we aimed to compare alloHSCT following RIC with other PRT approaches, including no further PRT in patients with AML aged 60 years or older, who were entered into four successive, prospective HOVON-SAKK AML trials.

METHODS

Study design and participants

Patients aged 60 years and older with newly diagnosed AML were included, who participated in consecutive, prospective HOVON-SAKK phase 3 trials (AML42/42A, AML43, AML81, and AML92) and who obtained CR1 after one or two induction cycles of chemotherapy and were alive at day 30 after the second cycle of treatment.[3,5,18] Detailed descriptions of the inclusion and exclusion criteria of these studies have been previously published.[3,5,18] Patients were classified by leukaemia risk, based on the cytogenetic and molecular profile of the underlying AML, according to the latest European LeukemiaNET (ELN) risk classification.[19] The intermediate-I and intermediate-II risk groups of the ELN classification were combined, on the basis of previous observations of similar outcome in patients 60 years of age and older.[20] All studies had been approved by the ethics committees of the participating institutions and were done in accordance with the Declaration of Helsinki. All participants had provided written informed consent. The results of the AML81 and AML92 trials have not yet been published, but trial information for both studies is available in the Netherlands Trial Register (NTR904 and NTR1446, respectively).

Procedures

Treatment in the AML42/42A, AML43, AML81, and AML92 studies involved a maximum of two remission induction cycles consisting of anthracycline with cytarabine chemotherapy, as previously described.[3,5,18] No further PRT was viewed as standard of care in patients aged 60 years or older (AML43 and AML81). Patients included in the AML43 study who had reached CR could be randomly allocated between GO 6 mg/m^2 every 4 weeks for three cycles or no PRT.[5] In the AML42/42A and AML92 studies, patients who were younger than 61 and 66 years, respectively, were eligible for PRT in a similar way as were younger patients. For patients treated with additional chemotherapy, they received a third cycle of chemotherapy with mitoxantrone 10 mg/m^2 per day intravenously for 5 days and etoposide 100 mg/m^2 per day intravenously for 5 days. For patients treated with autologous HSCT, they received high-dose chemotherapy with busulfan 1 mg/kg orally (or, alternatively busilvex 0.8 mg/kg intravenously) four times daily for 4 days and cyclophosphamide 60 mg/kg per day intravenously for 2 days followed by an autologous HSCT. Patients proceeded to alloHSCT after the identification of an human leukocyte antigen (HLA)-identical sibling or matched unrelated donor. Patients received a RIC regimen followed by the infusion of donor cells. The degree of HLA-matching for unrelated donors was eight out of eight alleles for HLA-A, HLA-B, HLA-C, and DRB1 for intermediate risk patients and at least seven out of eight alleles for adverse risk patients. RIC regimens mostly consisted of fludarabine 30 mg/m^2 per day intravenously for 3 days and 2 Gy of total body irradiation or fludarabine 30 mg/m^2 per day intravenously for 5 days combined with busulfan 1 mg/kg orally (or, alternatively busilvex

0.8 mg/kg intravenously) four times daily for 2 days. Rabbit antithymocyte globulin 2 mg/kg per day intravenously for 4 days was added to the RIC regimens in recipients of unrelated donors. Graft-versus-host disease (GVHD) prophylaxis was given with a calcineurin inhibitor (ie, ciclosporin or tacrolimus) based on serum trough levels (with a target of 250-350 µg/L for ciclosporin and 5-15 ng/mL for tacrolimus) combined with orally administered mycophenolate mofetil 15 mg/kg three times daily.

Outcomes

The primary endpoint of the study was 5-year OS for all treatment groups. OS and RFS were measured from day 30 after cycle 2, or from CR1 in cases where CR1 was obtained after day 30 of cycle 2. The event for OS was death whatever the cause, and patients were censored at the date of last contact if alive. Secondary endpoints were RFS, relapse and NRM. The events for RFS were death in CR1 (designated as NRM) or haematological relapse (recurrence of blasts in the marrow of ≥5% [excluding increased blasts in the context of regenerating marrow]). The cumulative risks of relapse and NRM over time were calculated as competing risks with actuarial methods, wherein patients alive in continuing CR1 were censored at the date of last contact.

Statistical analysis

We used the Kaplan-Meier method to estimate survival and the reverse Kaplan-Meier method to calculate the median time to follow-up. We did a time-dependent analysis of PRT as described previously,[12] taking into account the change in an individual's covariate status over time. We compared alloHSCT with non-alloHSCT PRT (ie, chemotherapy, autologous HSCT, or GO) and no PRT by applying multivariable Cox regression with the time-dependent covariate alloHSCT; we used this time-dependent covariate to avoid selectively favouring the alloHSCT group by attributing the favourable time period (since no relapse occurred) from CR to transplant to the alloHSCT group. The multivariable analysis is conceptually similar to a Mantel-Byar analysis,[21] but more general because it allows for adjustment for other factors. All patients initially received no PRT before proceeding to any type of PRT. Six patients received chemotherapy before alloHSCT. In both the multivariable analysis and the estimation of the survival curves, these patients were counted as at risk in the no PRT or non-alloHSCT group from start of the analysis until they received alloHSCT, and after that as at risk in the alloHSCT group. We applied a multivariable Cox regression analysis for OS, RFS, relapse, and NRM, with adjustment for the different types of non-alloHSCT treatment, leukaemia risk, white blood cell count (WBC) at diagnosis ($<20 \times 10^9$ cells per L *vs* $\geq 20 \times 10^9$ cells per L), late CR (after cycle 2 instead of cycle 1), age, sex, and year of treatment

(before 2006 *vs* 2006 or later). We included year of treatment (ie, before 2006 *vs* 2006 or later) to adjust for a possible overall difference in outcomes between these two periods. All p-values were based on log likelihood ratio tests, except when explicitly stated otherwise. We also used log likelihood ratio tests to test for interactions. The proportional hazards assumption was tested on the basis of Schoenfeld residuals.[21,22] The test of the assumption of proportionality indicated non-proportionality in the variable alloHSCT for all endpoints with increased HRs being limited to the early follow-up period, which would be expected because of the early toxicity of alloHSCT (Supplementary Table 1). We have not adjusted p values for multiple testing. All analyses were done with Stata version 13.1.

RESULTS

Between May 3, 2001, and Feb 5, 2010, induction chemotherapy was started in 1155 patients aged 60 years and older with AML in the four successive prospective HOVON-SAKK trials (Figure 1). 515 (45%) of 1155 patients were excluded from the analyses, because of refractory disease or death without achieving CR1 (427 [37%] of 1155 patients), death during cycle 2 of chemotherapy after an early CR (50 [4%] patients), or because they did not receive a second cycle of treatment due to toxicity (38 [3%] patients). CR after two cycles of induction therapy was obtained in 640 (55%) patients, of whom 274 (43%) proceeded to PRT with either GO (110 [17%] of 640), chemotherapy (44 [7%]), autologous HSCT (23 [4%]), or alloHSCT (97 [15%]). According to protocol, 366 (57%) of 640 patients who achieved a first complete remission did not receive further PRT after having received two cycles of chemotherapy, including 274 (75%) of 366 patients who obtained haematological remission after their first course of chemotherapy and 92 (25%) of 366 who did so after the second course of chemotherapy.

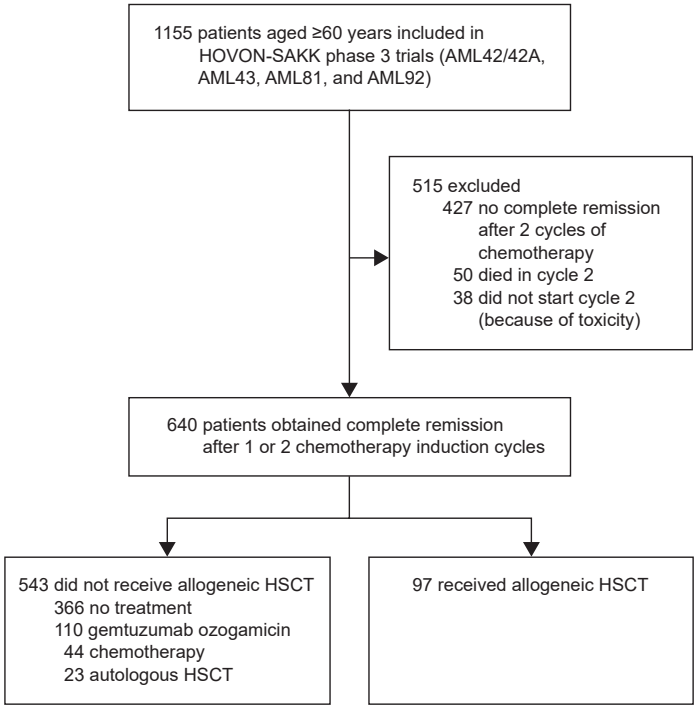

Figure 1 trial profile
Patients aged 60 years and older with acute myeloid leukaemia in the four consecutive HOVON-SAKK studies (AML42/42A, AML43, AML81, and AML92) included in this analysis. HSCT=haemopoietic stem cell transplantation.

Table 1 Patient characteristics

	Post-remission treatment			
	no PRT (N=366)	**Non-allo PRT (N=177)**	**alloHSCT (N=97)**	**p-value**
Gender				
Male	195 53%	99 56%	52 54%	.84
Female	171 47%	78 44%	45 46%	
Age (years)				
Median	68	65	64	<.0001
Range	60–82	60–78	60–74	
WBC at diagnosis (x10^9/l)				
Median	3.6	6.4	4.4	.18
Range	0.7-236	0.4-510	0.7-380	
Karyotype classification				
t(8;21)	15 4%	7 4%	0 0%	.40
inv(16)	9 2%	6 3%	1 1%	
CN-X-Y	188 51%	81 46%	53 54%	
CA Rest	92 25%	55 31%	30 31%	
MK	28 8%	15 8%	8 8%	
Missing	34 9%	13 7%	5 5%	
Risk AML*				
Favorable	33 9%	24 14%	8 8%	.15
Intermediate-I	213 58%	83 47%	51 53%	
Intermediate-II	51 14%	33 19%	13 13%	
Adverse	69 19%	37 21%	25 26%	
CR reached after				
Cycle 1 (early CR)	274 75%	120 68%	64 66%	.10
Cycle 2 (late CR)	92 25%	57 32%	33 34%	
Time from CR to start post-remission treatment (months)				
Median	NA	2.1	2.5	<.0001
IQ range	NA	1.3–3.0	1.6–3.7	
Year of start post-remission treatment				
<2006	224 61%	140 79%	43 44%	<.0001
≥2006	142 39%	37 21%	54 56%	

Abbreviations: AlloHSCT indicates allogeneic haematopoietic stem cell transplantation; WBC, white blood cell count; CN-X-Y, cytogenetically normal or only loss of X or Y chromosome; CA, cytogenetically abnormal; MK, monosomal karyotype; AML, acute myeloid leukaemia; CR, complete remission; IQ, interquartile range; and NA, not applicable
* According to the European LeukemiaNET AML risk classification

Table 1 shows the patient characteristics. Recipients of PRT were younger than patients who did not receive PRT. Most of the AMLs were classified as intermediate risk according to the ELN risk classification, and this did not differ significantly across the three groups. About a third of the patients in both PRT groups needed two cycles of chemotherapy to achieve CR. In patients who received PRT, time from CR to receiving alloHSCT was significantly longer than time from CR to receiving other types of PRT. Additionally, the number of patients receiving PRT in the form of alloHSCT has increased since 2006. The estimated median follow-up time was 69 months (interquartile range (IQR): 57-99) for patients receiving alloHSCT, 104 months (74-120) for patients receiving other types of PRT, and 82 months (54-100) for those who received no PRT.

Table 2 shows the transplantation characteristics of the patients who received alloHSCT. Grafts from HLA-identical siblings were used in 69 (71%) of 97 transplants. The majority of patients had a low European Group for Blood and Marrow Transplantation (EBMT) risk score.[23]

OS seemed to be strongly affected by the ELN AML risk classification. A small favourable risk group was identified, in which patients had better 5-year OS (56% [95% CI 43-67]), whereas the intermediate-I (23% [18-28]), intermediate-II (24% [16-34]), and adverse risk groups (13% [8-20]) had quite poor OS (Figure 2A). We subsequently pooled intermediate I and II patients together for further analyses (23% [19-27]). Figure 2B and 2C show OS and RFS of all patients by type of PRT. OS was better for alloHSCT recipients than patients receiving no PRT (5-year OS 35% [95% CI 25-44] *vs* 21% [17-26]; p=0.033), whereas the difference between alloHSCT and other types of PRT was not significant (26% [95% CI 19-33], p=0.43, Figure 2B). Similarly, RFS was significantly improved in patients receiving alloHSCT as compared with those who received no further treatment (5-year RFS 32% [95% CI 23-41] *vs* 14% [11-18], p=0.007, Figure 2C). 5-year RFS for patients receiving other types of PRT was 20% (95% CI 15-27). The cumulative incidence of relapse was 50% (95% CI 40-61) at 5 years in the patients who received alloHSCT, compared with 77% (72-81) in those who received no PRT, and 66% (57-74) in those who received non-alloHSCT PRT (Figure 3A). The 5-year cumulative incidence of NRM after alloHSCT was 18% (95% CI 12-27), compared with 14% (95% CI 8-25) in patients receiving no alloHSCT and 9% (6-12) in those patients receiving non-alloHSCT PRT (Figure 3B). Treatment outcomes for the different types of non-alloHSCT PRTs are shown in Supplementary Table 2. Both patients who received alloHSCT and those who did not, did not show significant improvement in OS over time (Supplementary Table 3). Extensive toxicity data for the different types of PRT were not available in detail for comparison between the different types of treatment, but Supplementary Table 4 provides information about the cause of death by type of PRT.

Table 2 Transplantation characteristics

	alloHSCT (N=97)	
Donor type		
HLA-identical sibling	69	71%
Matched unrelated donor	20	21%
Other	8	8%
Stem cell source		
Peripheral blood	87	90%
Bone marrow	7	7%
Cord blood	3	3%
T-cell depletion		
In vitro	3	3%
Ex vivo	1	1%
None	93	96%
Conditioning		
Fludarabin / TBI	71	73%
Fludarabin / Busulfan	10	10%
Fludarabin / Cyclophosphamide / TBI	3	3%
Fludarabin / Cyclophosphamide	2	2%
Unknown	11	11%
Antithymocyte globulin used		
Yes	19	20%
No	55	57%
Unknown	23	24%
Female donor to male recipient		
Yes	5	5%
No	92	95%
Graft-versus-host disease prophylaxis		
Ciclosporin	6	6%
Ciclosporin / mycophenolate mofetil	70	72%
Tacrolimus / mycophenolate mofetil	2	2%
Unknown	19	20%
Cytomegalovirus status of patient/donor		
+/-	24	25%
+/+	36	37%
-/+	5	5%
-/-	18	19%
Unknown	14	14%
EBMT-score		
2 points	64	66%
3 points	30	31%
4 points	3	3%

Abbreviations: alloHSCT indicates allogeneic haematopoietic stem cell transplantation; HLA, human leukocyte antigen; TBI, total body irradiation; and EBMT, European Group for Blood and Marrow Transplantation

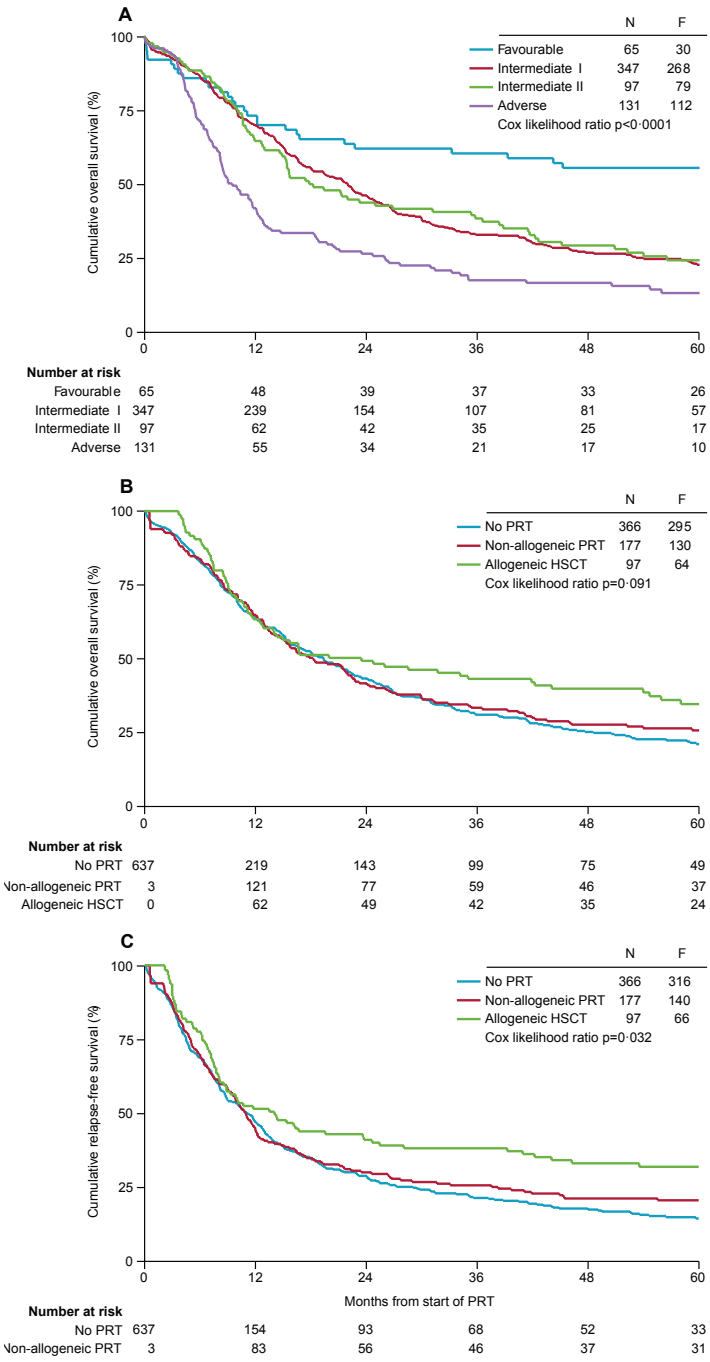

Figure 2 Overall survival and relapse-free survival

(**A**) Overall survival by acute myeloid leukaemia risk category. (**B**) Overall survival by post-remission therapy. (**C**) Relapse-free survival by post-remission therapy. All estimates are for patients aged 60 years and older with acute myeloid leukaemia in first complete remission, from start of post-remission treatment. Patients were classified by leukaemia risk, based on the cytogenetic and molecular profile of the underlying acute myeloid leukaemia, according to the latest European LeukemiaNET risk classification.[19] Non-allogeneic postremission therapy refers to chemotherapy, autologous HSCT, or gemtuzumab ozogamicin. Notably, numbers of patients at risk differ from the patient numbers in table 1 and within the figure because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in first complete remission.
N=number of patients; F=number of failures (ie, death, whatever the cause) PRT=post-remission therapy HSCT=haemopoietic stem cell transplantation

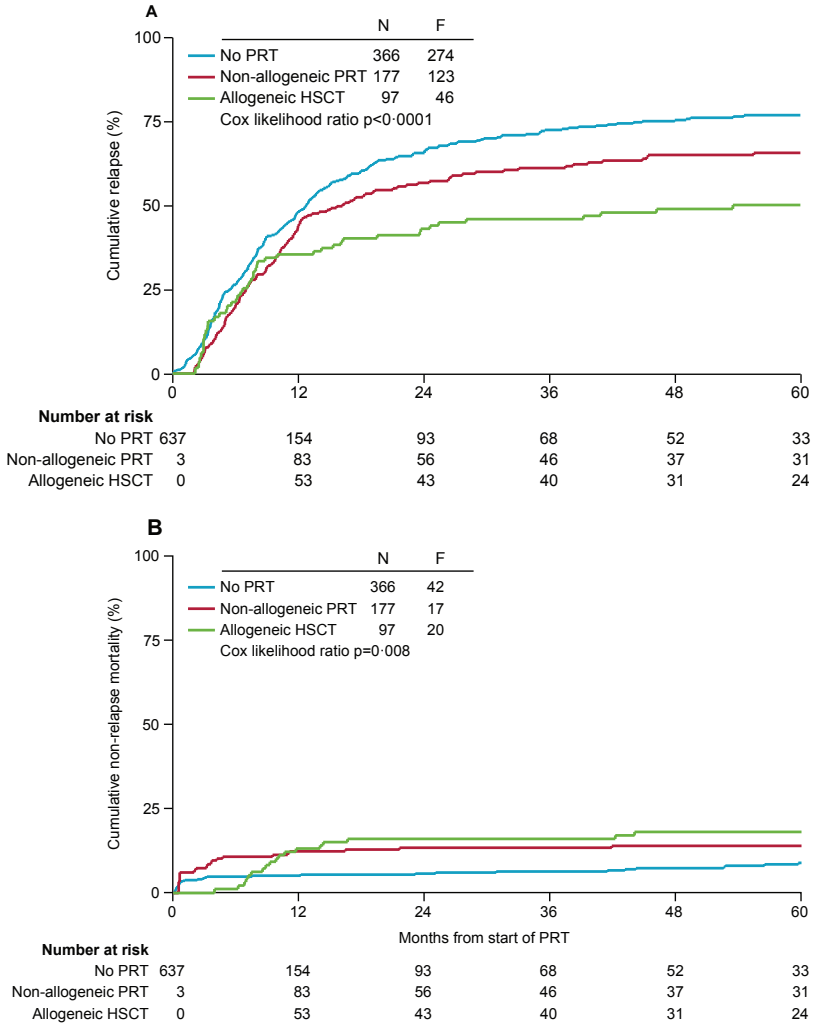

Figure 3 Cumulative incidence of relapse and non-relapse mortality
(**A**) Cumulative incidence of relapse by type of post-remission therapy. (**B**) Cumulative incidence of non-relapse mortality by type of post-remission therapy. All estimates are for patients aged 60 years and older with acute myeloid leukaemia in first complete remission, from start of post-remission treatment. Non-allogeneic postremission therapy refers to chemotherapy, autologous HSCT, or gemtuzumab ozogamicin. Notably, numbers of patients at risk differ from the patient numbers in table 1 and within the figure because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in first complete remission to the transplantation curve. N=number of patients. F=number of failures. PRT=postremission therapy. HSCT=haemopoietic stem cell transplantation.

Multivariable analysis

Table 3 shows the results of the multivariable analysis with adjustment for type of non-alloHSCT PRT, sex, age, leukaemia risk, WBC, late CR, and year of treatment. Both OS and RFS were significantly improved by alloHSCT compared with no PRT (Table 3). Relapse was significantly reduced by alloHSCT compared with no PRT; this effect was exerted similarly among the intermediate and adverse risk groups (Figure 4C). Women had a significantly better OS and RFS than did men (Table 3). Intermediate risk and adverse risk AMLs were associated with a highly significant HR for reduced OS and RFS, and increased rate of relapse compared with favourable AMLs (Table 3). Furthermore, patients with a WBC higher than 20×10^9 cells per L at diagnosis had a significant HR for worse OS and RFS, and significantly increased risk of relapse compared with those with a WBC of up to 20×10^9 cells per L. Minor differences in outcome were recorded between centres, but the multivariable analysis stratified by centre did not change OS (Supplementary Table 5). Figure 4 shows the forest plots of the HRs for OS, RFS, and relapse split by leukaemia risk group for a comparison of alloHSCT with non-alloHSCT (ie, patients who received other types of PRT and those who received no PRT). With respect to OS, the pooled estimates of the HR comparing alloHSCT versus non-alloHSCT was 0.71 (95% CI 0.53-0.95, p=0.014, Figure 4A), which seemed to be most prominent in adverse risk patients. With respect to RFS and relapse, the pooled estimates of the HR comparing alloHSCT with non-alloHSCT were 0.63 (95% CI 0.47-0.73; p=0.001) for RFS (figure 4B), and 0.46 (0.33-0.64; p<0.0001) for relapse (figure 4C), which were similarly improved by alloHSCT in patients with intermediate risk and adverse risk AMLs.

Table 3 Results of the multivariable analysis

	OS			RFS			Relapse			NRM		
	HR*	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**	**HR***	**95% CI**	**p-value**
Post-remission treatment												
AlloHSCT (n=97) vs. no PRT (n=366)	0.71	0.53-0.95	.017	0.63	0.47-0.73	.001	0.46	0.33-0.64	<.0001	3.10	1.59-6.02	.001
Non-allo PRT												
CT/auto (n=67) vs. no PRT (n=366)	1.02	0.73-1.43	.91	0.99	0.72-1.38	.98	0.86	0.60-1.24	.41	2.70	1.24-5.84	.018
GO (n=110) vs. no PRT (n=366)	0.80	0.62-1.03	.077	0.85	0.67-1.09	.20	0.83	0.65-1.08	.16	0.89	0.36-2.23	.80
Sex (female (n=294) vs. male (n=346))	0.79	0.66-0.95	.011	0.77	0.65-0.92	.004	0.78	0.64-0.94	.009	0.78	0.49-1.23	.27
Age†	1.15	0.93-1.42	.21	1.09	0.88-1.31	.43	1.08	0.86-1.35	.52	1.19	0.71-1.99	.51
Risk AML‡												
Intermediate (n=444) vs. Favorable (n=65)	2.40	1.63-3.53	<.0001	2.29	1.60-3.28	<.0001	3.24	2.06-5.09	<.0001	0.82	0.44-1.54	.54
Adverse (n=131) vs. Favorable (n=65)	4.26	2.78-6.51	<.0001	4.04	2.71-6.04	<.0001	6.48	3.97-10.56	<.0001	0.59	0.24-1.46	.25
WBC at diagnosis (>20 (n=187) vs. ≤20 (n=453))	1.33	1.09-1.63	.007	1.33	1.09-1.62	.006	1.42	1.15-1.76	.002	0.86	0.50-1.49	.59
CR (late (n=182) vs. early (n=458))	0.98	0.80-1.20	.83	1.03	0.85-1.25	.76	1.15	0.94-1.42	.18	0.41	0.21-0.80	.004
Year of treatment												
≥2006 (n=407) vs. <2006 (n=233)	0.99	0.82-1.21	.95	1.07	0.89-1.30	.46	1.00	0.81-1.23	.99	1.56	0.99-2.49	.059

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; Allo, allogeneic hematopoietic stem cell transplantation; vs., versus; PRT, post-remission treatment; CT, chemotherapy; auto, autologous HSCT; GO, gemtuzumab ozogamicin; AML, acute myeloid leukaemia; and WBC, white blood cell count

* The HRs are the estimates of the effect of covariates for each outcome parameter, adjusted for sex, age, AML risk, CR (late vs. early), WBC at diagnosis below or above 20, year of post-remission treatment before or after 2006, and type of post-remission treatment. The group after the term *"vs."* is regarded as the reference group.

† Linear with estimates of ten years difference

‡ According to the European LeukemiaNET AML risk classification[19]

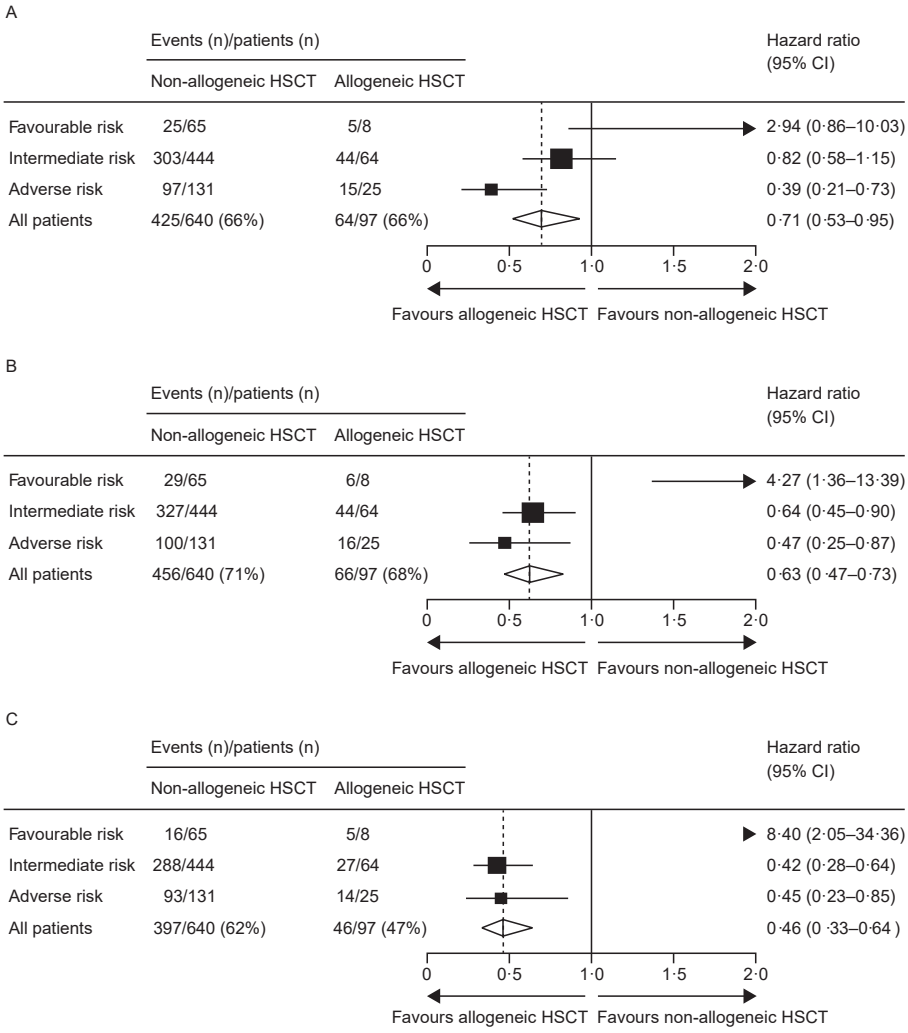

Figure 4 Forest plots of AlloHSCT versus non-alloHSCT

Forest plots of pooled and individual estimates of the relative reduction (HR) and 95% CI of overall survival (**A**), relapse-free survival (**B**), and relapse (**C**) in each acute myeloid leukaemia risk group, comparing allogeneic HSCT versus no allogeneic HSCT (non-allogeneic HSCT, which includes patients who received no post-remission therapy and those who received other types of post-remission therapy). Multivariable Cox regression analysis was used, with adjustment for the different types of non-allogeneic HSCT treatment, leukaemia risk, white blood cell count at diagnosis, late complete remission, age, sex, and year of treatment. To avoid bias, all patients started in the nonallogeneic HSCT group at the moment of first complete remission. In case patients proceeded to allogeneic HSCT, these patients were censored in the non-allogeneic HSCT group and switched to the allogeneic HSCT group at the very moment they received a transplant. Therefore, all patients were counted as at risk in the non-allogeneic HSCT group from start of follow-up until allogeneic HSCT and after that as at risk in the allogeneic HSCT group. Therefore the numbers in the events/patients table differ from those in table 1. HSCT=haemopoietic stem cell transplantation. HR=hazard ratio

DISCUSSION

At present, outcome in older patients with AML remains poor compared with that in younger patients because of an increased frequency of adverse cytogenetics,[24] more concurrent comorbidities,[25] and less fewer potentially curative treatment options.[26] In our analysis, we recorded improved RFS by alloHSCT compared with no further treatment and non-alloHSCT PRT in intermediate risk and adverse risk patients, and the latter subgroup also showed improved OS. PRT in the form of GO, chemotherapy and autologous HSCT, did not differ significantly from no further therapy beyond two cycles of chemotherapy for all patients.

The cytogenetic and molecular profile of the leukaemia is the most important factor affecting outcome and treatment decisions are largely based on the risk profile of the AML.[19] Younger patients with a favourable risk profile generally do not qualify for PRT by alloHSCT, but that issue remains unsolved in older patients with AML. Because the number of favourable risk patients was small in our study, we were not able to show a survival benefit for alloHSCT in that particular subgroup. In intermediate risk AMLs, alloHSCT significantly improved RFS compared with other types of PRT, although we did not observe a significant difference in OS. Salvage treatment by alloHSCT in second CR might account for that observation, since alloHSCT in second CR could be done in 25% of relapsing patients (data not shown). A clear survival benefit for alloHSCT was noted in patients with adverse risk AMLs. The latter observation may support the application of alloHSCT in that particular subgroup, if eligible. In addition to the risk profile of the AML, peripheral blood cell count recovery and especially minimal residual disease are often used for predicting the risk of relapse, but information of both cell count recovery and minimal residual disease after CR was not available in the present analysis.

NRM may compromise the favourable effects of alloHSCT on relapse, especially in elderly patients with comorbidities. Although NRM following alloHSCT was increased compared with other types of PRT in the present study, the cumulative incidence of NRM of 18% (95% CI 12-27) following alloHSCT might support the feasibility of this treatment following RIC in patients aged 60 years and older. However, this observation cannot easily be generalised, since our results are from a selected group of elderly patients, who were eligible for transplant and who had a well matched sibling or unrelated donor. The use of risk scores to estimate the risk of NRM pre-transplantation and the selection of only those patients with an acceptable NRM-score could further reduce NRM. Several risk scores are available with patient-related and transplantation-related parameters,[23] comorbidities,[27] or combination of parameters.[25] In particular, NRM risk assessment for patients with AML who qualify for RIC can be improved by a dedicated score, as was developed for AML in CR1 patients by the EBMT Acute Leukemia Working Party.[25] Information about specific comorbidities was not available in the present study, but the included patients had quite a low EBMT-score, suggesting that transplanted patients were thereby devoid of an excessive risk for NRM.

In our study, reduction of relapse and the acceptable toxicity of alloHSCT resulted in a survival benefit of alloHSCT compared with other types of PRT in adverse risk patients aged 60 years and older with AML in CR1. Although the present study is not a prospective randomised trial, the time-dependent nature of the analysis enables the comparison with no treatment, while avoiding the bias of time to transplantation by attributing that favourable time period to the no-transplant recipients (ie, control group).[21,28,29] Nevertheless, our study is hampered by selection of a relatively small group of 97 transplanted patients out of 640 patients in CR1, who met the eligibility criteria of the respective transplant centres, had a well matched donor, and actually underwent transplantation. Only a true randomised study will address those problems, and therefore results of a randomised study comparing alloHSCT with non-alloHSCT treatment, which is currently being done by cooperative groups in Europe (NCT00766779), are eagerly awaited.

In conclusion, our results show that alloHSCT following RIC is a feasible PRT in patients aged 60 years and older with AML in CR1. Survival was improved with alloHSCT compared with other types of PRT, especially in patients with adverse risk AML. Although transplant decisions should be based on a careful pre-transplant risk assessment of relapse and NRM,[30] these results suggest that the early search and identification of a compatible donor should also be pursued in elderly patients with an adverse AML risk profile.

Acknowledgements

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RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed before submission (final search on March 8, 2015) for original research articles published since 2001 about RIC alloHSCT versus chemotherapy in patients aged 60 years and older with AML in CR1, using the search terms “AML”, “allogeneic”, “reduced intensity”, and “elderly” or “60 years”. Although several studies showed the feasibility of alloHSCT following RIC in elderly patients with AML in CR1, no prospective randomised trials comparing alloHSCT versus chemotherapy have been reported in these elderly patients with AML. We identified two comparative retrospective trials that included patients with AML aged 60 years and older and compared alloHSCT with chemotherapeutic PRT. Both studies suggested improved outcome by alloHSCT compared with other PRTs, which was mostly present in intermediate risk and adverse risk AML subgroups. Similarly, in patients younger than 60 years of age with AML in CR1, we and others have reported improved survival with alloHSCT compared with chemotherapeutic PRT, especially in patients with intermediate risk and adverse risk AMLs.

Added value of this study

Our study included a large number of patients with long follow-up who all received induction treatment for AML in prospective phase 2 and 3 trials. Our results of a time-dependent analysis show that, in patients of 60 years and older with AML in CR1, alloHSCT might provide improved outcome compared with a non-alloHSCT PRT approach, especially in patients with intermediate risk and adverse risk AML.

Implications of all the available evidence

As outlined recently, tailoring of PRT in elderly patients depends not only on disease related risk factors, such as the underlying cytogenetic or molecular risk of the AML, but also on patient risk factors, including comorbidity status and performance status. Outcome of PRT in patients with AML, especially those aged 60 years and older, might benefit from a personalised treatment approach that takes into account both patient and disease factors, with the assessment of AML risk status, comorbidities, and performance status. Although alloHSCT might improve outcome in subgroups of elderly patients with AML, these results need to be confirmed in a prospective trial, which is currently ongoing (NCT00766779).

SUPPLEMENTARY APPENDIX

Supplementary Table 1 Tests of proportional hazards assumption

	Total cohort		<=12 months		>12 monhts			Schoenfeld Residuals	
	HR	95% CI	HR1	95% CI	HR2	95% CI	p-value*	rho	p-value
Overall survival									
AlloHSCT	0.71	0.53-0.95	1.02	0.68-1.53	0.55	0.36-0.84	.038	-0.083	.058
CT/auto	1.02	0.73-1.43	1.01	0.63-1.62	1.02	0.63-1.66	.98	-0.026	.54
GO	0.80	0.62-1.03	0.77	0.51-1.16	0.80	0.58-1.11	.87	-0.024	.60
WBC>20	1.33	1.09-1.63	1.71	1.29-2.26	1.10	0.81-1.48	.036	-0.104	.019
Relapse-free survival									
AlloHSCT	0.63	0.47-0.83	0.86	0.60-1.22	0.44	0.27-0.72	.031	-0.075	.075
CT/auto	0.99	0.72-1.38	1.17	0.80-1.72	0.61	0.32-1.17	.089	-0.044	.29
GO	0.85	0.67-1.09	0.94	0.69-1.28	0.71	0.48-1.05	.28	-0.011	.80
WBC>20	1.33	1.09-1.62	1.51	1.19-1.91	0.99	0.68-1.43	.058	-0.101	.018
Relapse									
AlloHSCT	0.46	0.33-0.64	0.60	0.40-0.90	0.35	0.20-0.61	.12	-0.090	.053
CT/auto	0.86	0.60-1.24	0.95	0.62-1.45	0.58	0.28-1.20	.25	-0.002	.97
GO	0.83	0.65-1.08	0.87	0.63-1.21	0.75	0.50-1.13	.57	0.014	.76
WBC>20	1.42	1.15-1.76	1.67	1.29-2.15	1.02	0.68-1.52	.041	-0.148	.0015
Non-relapse mortality									
AlloHSCT	3.10	1.59-6.02	7.38	3.08-17.7	1.20	0.42-3.42	.009	-0.139	.18
CT/auto	2.70	1.24-5.84	5.74	2.27-14.5	0.90	0.19-4.40	.048	-0.165	.096
GO	0.89	0.36-2.23	1.82	0.58-5.79	0.32	0.07-1.49	.077	-0.109	.33
WBC>20	0.86	0.50-1.49	0.81	0.43-1.55	0.84	0.30-2.37	.95	0.023	.83

Abbreviations: HR, hazard ratio; CI, confidence interval; alloHSCT, allogeneic haematopoietic stem cell transplantation; CT, chemotherapy; auto, autologous HSCT; and WBC, white blood cell count
* Indicating a test of equality of the HRs in period 1 and period 2 for each variable

Comments to Supplementary Table 1
The validity of the assumption of proportional hazards had been tested in two ways:
1. Using scaled Schoenfeld residuals (SSR) with the method of Grambs & Therneau[22]
2. By splitting the follow up period in two periods: <= 12 months and >12 months and performing separate Cox regression analysis (with the same variables as shown in Table 3) for each period

Supplementary Table 1 shows the variables with non-proportionality by outcome. No indication for non-proportionality was found in the other included variables in the multivariable analysis
The rho in the SSR section is the correlation coefficient between the scaled Schoenfeld residuals for each variable with the rank number of the event times. The P-values there are for a test whether that correlation coefficient is 0. The results indicate that non-proportionality was present for alloHSCT as compared with no further treatment, except for endpoint relapse. The endpoint non-relapse mortality showed a relatively high HR in the first year after alloHSCT, with almost no difference after the first year. That difference in non-relapse mortality impacted endpoints OS and RFS with HR closer to 1 and not significant in the first year, and smaller HR's around 0.5 in the period after 1 year. The results for WBC suggest an effect of WBC only during early follow up.

Supplementary Table 2 Outcome by non-alloHSCT PRT

		Estimates at 5 years (95% CI)			
	No. of patients	**OS**	**RFS**	**Relapse**	**NRM**
CT	44	26 (19-33)	20 (15-27)	66 (57-74)	14 (8-25)
Auto	23	26 (10-45)	18 (6-36)	62 (43-82)	20 (8-45)
GO	110	24 (16-33)	19 (12-27)	72 (60-83)	10 (3-31)

Abreviations: CI, confidence interval; OS, overall survival; RFS, relapse-free survival; NRM, non-relapse mortality; CT, chemotherapy; Auto, autologous haematopoietic stem cell transplantation; and GO, gemtuzumab ozogamicin

Supplementary Table 3 Overall survival by PRT categorized by year of PRT

	OS at 5 years (95% CI)			
	No. of patients	**CT/Auto/GO**	**No. of patients**	**AlloRIC**
Year of PRT - 2 categories				
<2006	136	24 (17-32)	42	30 (17-44)
≥2006	41	32 (18-47)	55	39 (26-52)
Year of PRT - 3 categories				
2001-2003	78	25 (16-36)	15	40 (16-63)
2004-2006	78	25 (16-35)	37	33 (19-48)
2007-2010	21	31 (12-52)	45	35 (21-49)

Abbreviations: CI, confidence interval; OS, overall survival; CT, chemotherapy; Auto, autologous haematopoietic stem cell transplantation; GO, gemtuzumab ozogamicin; alloRIC, allogeneic haematopoietic stem cell transplantation following reduced intensity conditioning; and PRT, post-remission treatment

Supplementary Table 4 Causes of Death by PRT

Cause of Death	**No PRT**		**No allo PRT**		**AlloRIC**		**Total**
AML	175	59%	89	68%	36	56%	300
Pneumonia	18	6%	6	5%	2	3%	26
Other Infection	29	10%	10	8%	7	11%	46
Hemorrhage	8	3%	5	4%	0	0%	13
GvHD	0	0%	0	0%	3	5%	3
2nd Cancer	5	2%	1	1%	1	2%	7
Other	34	11%	10	8%	11	17%	52
Unknown	27	9%	9	7%	5	8%	40
Total	296	100%	130	100%	64	100%	490

Abbreviations: PRT, post-remission treatment; allo, allogeneic haematopoietic stem cell transplantation; alloRIC, allogeneic haematopoietic stem cell transplantation following reduced intensity conditioning; AML, acute myeloid leukaemia; and GVHD, graft-versus-host disease

Supplementary Table 5 Results of the multivariable analysis stratified by center

		OS		
		HR*	95% CI	p-value
Post-remission treatment				
AlloHSCT (n=97) vs. no PRT (n=366)		0.58	0.41-0.80	<.001
Non-allo PRT				
CT/auto (n=67)	vs. no PRT (n=366)	1.02	0.65-1.36	.74
GO (n=110)	vs. no PRT (n=366)	0.81	0.62-1.07	.14
Sex (female (n=294) vs. male (n=346))		0.85	0.70-1.03	.097
Age†		1.17	0.92-1.49	.21
Risk AML‡				
Intermediate (n=444)	vs. Favourable (n=65)	2.44	1.63-3.67	<.001
Adverse (n=131)	vs. Favourable (n=65)	4.90	3.11-7.71	<.001
WBC at diagnosis (>20 (n=187) vs. ≤20 (n=453))		1.39	1.11-1.75	.005
CR (late (n=182) vs. early (n=458))		1.00	0.80-1.25	.99
Year of treatment				
≥2006 (n=407) vs. <2006 (n=233)		0.95	0.76-1.18	.63

Abbreviations: OS indicates overall survival (with event death whatever the cause); HR, hazard ratio; CI, confidence interval; Allo, allogeneic haematopoietic stem cell transplantation; vs., versus; PRT, post-remission treatment; CT, chemotherapy; auto, autologous HSCT; GO, gemtuzumab ozogamicin; AML, acute myeloid leukaemia; and WBC, white blood cell count

* The HRs are the estimates of the effect of covariates for each outcome parameter, stratified by centre and adjusted for sex, age, AML risk, CR (late vs. early), WBC at diagnosis below or above 20, year of post-remission treatment before or after 2006, and type of post-remission treatment. The group after the team "*vs.*" is regarded as the reference group; † Linear with estimates of ten years difference; ‡ According to the European LeukemiaNET AML risk classification[19]

Supplementary Table 6 Outcome by PRT in AML risk groups

	Outcome at 5 years (95% CI)	
	Non-allogeneic HSCT	Allogeneic HSCT
Overall survival		
Favourable risk	59 (45-71)	36 (8-66)
Intermediate risk	21 (17-26)	34 (23-46)
Adverse risk	8 (4-15)	34 (16-53)
Relapse-free survival		
Favourable risk	50 (36-62)	23 (3-53)
Intermediate risk	15 (11-18)	34 (23-46)
Adverse risk	6 (3-13)	28 (12-46)
Relapse		
Favourable risk	30 (19-44)	66 (35-93)
Intermediate risk	76 (71-80)	42 (31-56)
Adverse risk	89 (81-94)	64 (45-82)

Abbreviations: CI, confidence interval; and HSCT, haematopoietic stem cell transplantation

4

COMPARATIVE VALUE OF POST-REMISSION TREATMENT IN CYTOGENETICALLY NORMAL AML SUBCLASSIFIED BY *NPM1* AND *FLT3*-ITD ALLELIC RATIO

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ABSTRACT

Post-remission treatment (PRT) in patients with cytogenetically normal (CN) acute myeloid leukemia (AML) in first complete remission (CR1) is debated. We studied 521 patients with CN-AML in CR1, for whom mutational status of *NPM1* and *FLT3*-ITD was available, including the *FLT3*-ITD allelic ratio. PRT consisted of reduced intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (n = 68), myeloablative conditioning (MAC) alloHSCT (n = 137), autologous hematopoietic stem cell transplantation (autoHSCT) (n = 168), or chemotherapy (n = 148). Favorable overall survival (OS) was found for patients with mutated *NPM1* without *FLT3*-ITD (71 ± 4%). Outcome in patients with a high *FLT3*-ITD allelic ratio appeared to be very poor with OS and relapse-free survival (RFS) of 23 ± 8% and 12 ± 6%, respectively. Patients with wild-type *NPM1* without *FLT3*-ITD or with a low allelic burden of *FLT3*-ITD were considered as intermediate-risk group because of similar OS and RFS at 5 years, in which PRT by RIC alloHSCT resulted in better OS and RFS as compared with chemotherapy (hazard ratio (HR) 0.56, P = 0.022 and HR 0.50, P = 0.004, respectively) or autoHSCT (HR 0.60, P = 0.046 and HR 0.60, P = 0.043, respectively). The lowest cumulative incidence of relapse (23 ± 4%) was observed following MAC alloHSCT. These results suggest that alloHSCT may be preferred in patients with molecularly intermediate-risk CN-AML, while the choice of conditioning type may be personalized according to the risk for non-relapse mortality.

INTRODUCTION

Acute myeloid leukemia (AML) is a cytogenetically and molecularly heterogeneous disease. Cytogenetically normal AML (CN-AML) is the largest cytogenetic subgroup (40-50% of AML patients),[1] which currently can be further refined based on molecular markers. Mutations in *nucleophosmin 1* (*NPM1*) and *fms-like tyrosine kinase 3* internal tandem duplications (*FLT3*-ITD) are found in respectively 50% and 30% of patients with CN-AML.[2] Molecular diagnostic analyses provide additional prognostic information that may be used for a risk adapted treatment approach.[3-6] *FLT3*-ITD, particularly *FLT3*-ITD with a high mutant to wild-type ratio, is associated with an unfavorable prognosis, whereas *NPM1* mutations in the absence of *FLT3*-ITD are associated with a relatively favorable outcome.[2,3,7-11] Patients who obtain a first complete remission (CR1) are subsequently treated with post-remission treatment (PRT), including an additional cycle of chemotherapy, high dose chemotherapy followed by autologous hematopoietic stem cell transplantation (autoHSCT) or allogeneic HSCT (alloHSCT) following either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC). PRT in patients with CN-AML CR1 is a subject of continued debate, especially taking molecular markers into account.[12-19] AlloHSCT is generally not associated with better survival in patients with *NPM1* mutations without *FLT3*-ITD, whereas the role of autoHSCT and alloHSCT in patients with *FLT3*-ITD is not definitely settled.[3,9,10,12,19-21] In addition, large comparative studies of PRT including autoHSCT are lacking in molecularly defined subgroups. In the present study, we addressed the impact of *NPM1* and *FLT3*-ITD including the *FLT3*-ITD allelic ratio on the outcome in patients with CN-AML, treated upfront within four prospective, consecutive HOVON-SAKK and EORTC studies. Second, we compared outcome of PRT with alloHSCT and autoHSCT vs chemotherapy by time-dependent analysis in patients with AML in CR1, according to molecularly defined subgroups.

PATIENTS AND METHODS

Patients

A total number of 521 patients with newly diagnosed CN-AML were included, treated between 1995 and 2010 and who obtained CR1 after one or two induction cycles of chemotherapy. Patient data were derived from two cohorts including consecutive, prospective HOVON-SAKK phase III trials (AML29, AML42/42A, and AML92; n = 399),[22-24] and a prospective EORTC phase III trial (AML12; n = 122).[25] Patients were excluded if molecular information was not available or if *EVI1* overexpression was present. Figure 1 shows the total number of patients enrolled in different trials and reasons why patients were excluded in the present analysis. The ratio of *FLT3*-ITD mutant to wild-type, defined by *FLT3*-ITD divided by *FLT3*-ITD plus *FLT3*-wild-type, was available for 86% of the patients with *FLT3*-ITD AML. A predefined cutoff of >0.50 was applied to define subgroups with a low or high allelic ratio of *FLT3*-ITD. Patients were considered as having a low allelic ratio in case the ratio was not available in order to define a mere poor risk group. Details of the molecular analysis are provided in the Supplementary Appendix. All studies were approved by the ethics committees of participating institutions and were conducted in accordance with the Declaration of Helsinki. All participants had given written informed consent. A detailed description of the inclusion and exclusion criteria of the studies have been previously published[22-25].

Treatment protocols

Treatment in the HOVON-SAKK AML29, AML42/42A, and AML92 studies involved a maximum of two remission induction cycles consisting of an anthracycline with cytarabine chemotherapy, as previously described.[22-24] Induction chemotherapy was followed by three types of PRT in patients in CR1 according to a predefined strategy as outlined in the study protocols, including either a third cycle of chemotherapy with mitoxantrone and etoposide, high-dose chemotherapy with busulfan and cyclophosphamide followed by autoHSCT, or alloHSCT following either MAC or RIC. These different therapeutic modalities were applied according a risk-adapted strategy as previously described.[22-24,26,27] Induction treatment in the EORTC AML12 study consisted of a combination of anthracycline, etoposide, and cytarabine-based chemotherapy.[25] All patients in the EORTC AML12 study received PRT with at least one cycle of chemotherapy after obtaining CR1 followed by continued PRT with either autoHSCT or alloHSCT. The preferred type of PRT in patients below the age of 50 years with an available donor was alloHSCT, whereas in patients above the age of 50 years or patients lacking a donor autoHSCT was performed as the preferred PRT.[25] Conditioning with either RIC or MAC was performed based on center's choice.

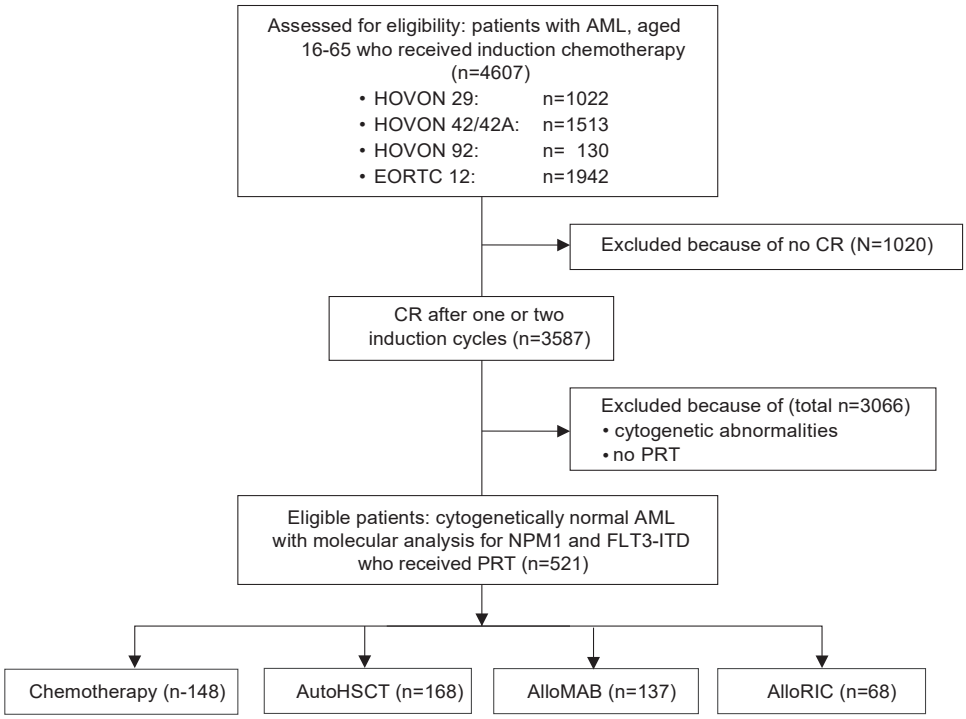

Figure 1 Patient flow chart
Patients with AML, included in EORTC and HOVON-SAKK trials, who were eligible for the present analysis with CN-AML in CR1 with available molecular analysis who received PRT

Transplantation protocols

Patients received either a MAC or a RIC regimen followed by the infusion of donor cells. RIC alloHSCT was introduced in patients below 60 years as from 2001, whereby the indication for RIC or MAC was selectively determined by age and consistently adhered to by the individual center throughout the HOVON AML42/42A and AML92 studies. While some centers maintained their policy of MAC alloHSCT for all patients up to the age of 60, a number of centers changed their policy by setting the age limit for MAC at <40 and RIC for patients of 40 years and beyond. The MAC regimen contained high-dose cyclophosphamide with total body irradiation (TBI) in 61 out of 81 (84%) HOVON patients, whereas the remainder received busulfan with cyclophosphamide. RIC regimens varied, but the vast majority consisted of 2.0 gray TBI preceded by fludarabine (n = 51, 93%). MAC alloHSCT in the EORTC study preferably consisted of high-dose cyclophosphamide with TBI and alternatively busulfan with high-dose cyclophosphamide. The most frequently used RIC regimen in the EORTC study was busulfan combined with fludarabin. A calcineurin inhibitor (either ciclosporin or tacrolimus) plus mycophenolate mofetil or methotrexate was given as prophylaxis for graft vs host disease.

End points

The primary end point of the study was overall survival (OS), according to the type of PRT received. OS and relapse-free survival (RFS) were measured from the date of starting the first PRT. OS was based on death from any cause, and patients were censored at the date of last contact if alive. The events for RFS were death in CR1, designated as non-relapse mortality (NRM) or hematological relapse. The cumulative risks of relapse and NRM over time were calculated as competing risks with actuarial methods, where patients alive in continuing CR1 were censored at the date of last contact.

Statistical Methods

A time-dependent analysis of PRT was performed as described previously,[27,28] by applying multivariable Cox regression with time-dependent covariates autoHSCT and alloHSCT following MAC or RIC. The multivariable analysis is conceptually similar to a Mantel-Byar analysis,[29] but more general as it allows for the adjustment of other factors. A number of patients received PRT with chemotherapy (n = 28) first before they proceeded to alloHSCT. In both the multivariable analysis and the estimation of survival curves, these patients were counted as at risk in the chemotherapy group from start of PRT until alloHSCT and after that as at risk in the MAC or RIC alloHSCT group. Multivariable Cox regression analysis for OS, RFS, relapse, and NRM was applied stratified by study cohort with adjustment for age, sex, white blood cell count at diagnosis, and late CR (after cycle II instead of I). Outcome estimates are at 5 years unless explicitly stated otherwise. All P-values were based on log likelihood ratio tests, except when explicitly stated otherwise. Log likelihood ratio tests were also used to test for interactions. The proportional hazard assumption was tested on the basis of Schoenfeld residuals.[29,30] P-values were not adjusted for multiple testing. All analyses were done with Stata Statistical Software: Release 13.1 (2013, Stata Corporation, College Station, TX, USA).

RESULTS

Patients

A total of 521 patients with CN-AML proceeded to PRT with either chemotherapy (n = 148), autoHSCT (n = 168) or alloHSCT following MAC (n = 137) or RIC (n = 68). Patient characteristics are presented in Table 1. Recipients of MAC alloHSCT were younger as compared with the other types of PRT. Patients with wild-type *NPM1* received RIC alloHSCT more frequently as compared with chemotherapy and autoHSCT. More allografted patients obtained a relatively late CR1 (achieved after two cycles of induction chemotherapy). In addition, time from remission to PRT was longer for recipients of autoHSCT, and RIC alloHSCT was performed more frequently in the recent years. The median follow-up of patients still alive was 77 months and differed between patients receiving chemotherapy (100 months), autoHSCT (70 months), MAC alloHSCT (79 months) and RIC alloHSCT (72 months). Patient's characteristics by the different study cohorts are presented in Supplementary Table 1. Due to different study protocols, time from CR1 to PRT was significantly longer for patients treated by the EORTC. All patients treated by the EORTC received PRT with chemotherapy followed by final PRT with either autoHSCT or alloHSCT with RIC or MAC.

Treatment outcome

OS and RFS of all patients were 53 ± 2% and 47 ± 2%, respectively, at 5 years from the start of PRT. Outcome by molecular subgroups demonstrated distinct favorable and poor risk subgroups (Figure 2). Outcome of patients with mutated *NPM1* was clearly determined by the absence or presence of *FLT3*-ITD with OS of 71 ± 4% and 39 ± 4%, respectively. In contrast, OS of patients with *FLT3*-ITD appeared to be not influenced by *NPM1* mutational status (*NPM1*^{mut} 39 ± 4%, *NPM1*^{wt} 39 ± 8%), but by the ratio of mutant to wild-type *FLT3*-ITD (low ratio 42 ± 3%, high ratio 23 ± 8%). Patients with mutated *NPM1* without *FLT3*-ITD had a favorable outcome with OS and RFS of 71 ± 4% and 65 ± 4%, respectively. In contrast, AML patients with a high *FLT3*-ITD mutant to wild-type ratio appeared to exhibit a very poor outcome with OS and RFS of 23 ± 8% and 12 ± 6%, respectively. A large group of AML patients, designated as molecular intermediate risk, with either a low *FLT3*-ITD ratio (mutant or wild-type *NPM1*) or wild-type *NPM1* without *FLT3*-ITD showed fairly similar OS and RFS estimating about 45% and 40%, respectively, allowing us to consider these three subgroups as one intermediate risk group.

Outcome by PRT in molecular subgroups

Favorable risk (NPM1 mutant without FLT3-ITD AML)

Patients with mutated *NPM1* without *FLT3*-ITD shared similar OS following chemotherapy, autoHSCT, MAC alloHSCT or RIC alloHSCT (68 ± 7% and 71 ± 6%, 74 ± 7% or 67 ± 14%, respectively, P = 0.94, Figure 3A, Table 2). Although autoHSCT or alloHSCT following either

MAC or RIC reduced relapse more strongly, RFS appeared not statistically significantly different as compared with chemotherapy (66 ± 6%, 71 ± 7% or 67 ± 14 vs 58 ± 7%, respectively, P = 0.78, Figure 3B, Table 2 and Supplementary Table 2). Limiting the analysis to strictly favorably risk patients with an early CR (after one cycle of induction chemotherapy) did not show any differences in OS or RFS.

Table 1 Patient characteristics

	Post-remission treatment			
	Chemotherapy (N=148)	**AutoHSCT (N=168)**	**AlloMAC (N=137)**	**AlloRIC (N=68)**
Sex				
Male	72 49%	87 52%	67 49%	36 53%
Female	76 51%	81 48%	70 51%	32 47%
Age (years)				
Median	50	48	44	54
Range	18–60	16–61	16–59	37-60
WBC at diagnosis				
Median	34	28	26	11
Range	0.8-400	0.8-278	0.6-291	0.9-182
NPM1				
Mutated	95 64%	96 57%	72 53%	30 44%
Wild-type	53 36%	72 43%	65 47%	38 56%
***FLT3*-ITD**				
Not present	94 64%	116 69%	92 67%	44 65%
Low ratio	39 26%	48 29%	37 27%	20 29%
High ratio	15 10%	4 2%	8 6%	4 6%
CR reached after				
Cycle 1 (early CR)	126 85%	155 92%	97 71%	49 72%
Cycle 2 (late CR)	22 15%	13 8%	40 29%	19 28%
Time from CR to PRT (months)				
Median	2.1	2.6	2.4	2.3
IQ range	1.4-2.7	2.0-2.9	1.0-2.9	1.2-2.8
Year of PRT				
<2005	104 70%	86 51%	76 55%	20 29%
≥2005	44 30%	82 49%	61 45%	48 71%

Abbreviations: AutoHSCT indicates autologous hematopoietic stem cell transplantation; AlloMAC, allogeneic hematopoietic stem cell transplantation following myeloablative conditioning, AlloRIC, alloHSCT following reduced intensity conditioning; WBC, white blood cell count; *NPM1*, nucleophosmin 1; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; IQ, interquartile range; CR, complete remission; and PRT, post-remission treatment

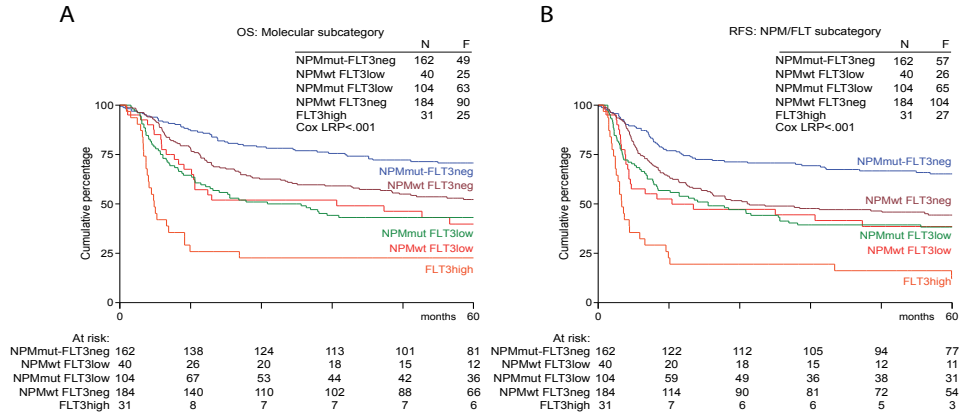

Figure 2 OS and RFS by molecular subcategory
Kaplan-Meier estimates of overall survival (OS, panel **A**) and relapse-free survival (RFS, panel **B**) by molecular subcategory of patients with CN-AML in first complete remission from start of post-remission treatment. Abbreviations: *NPM1*, nucleophosmin-1, *FLT3*neg, no *fms-like tyrosine kinase 3* internal tandem duplications; *FLT3*low, low allelic ratio of *FLT3*-ITD; *FLT3*high, high allelic ratio of *FLT3*-ITD. F, number of failures (ie, death whatever the cause); and N, number of patients

Intermediate risk (NPM1 wild-type without FLT3-ITD or low FLT3-ITD allelic ratio)

Recipients of RIC alloHSCT showed significantly better OS as compared with chemotherapy (63 ± 7% vs 39 ± 6%, respectively, P = 0.046). AutoHSCT and MAC alloHSCT had similar OS, which was not significantly different as compared with chemotherapy or RIC alloHSCT. RFS was improved by RIC alloHSCT as compared with chemotherapy (59 ± 7% vs 30 ± 5%, respectively, P = 0.008, Figure 3D). AutoHSCT and MAC alloHSCT reduced relapse more strongly as compared with chemotherapy, but RFS was not significantly different (40 ± 5%, 44 ± 5% vs 30 ± 5, respectively, P = 0.20, Figure 3D, Table 2 and Supplementary Table 2). These results remained similar in patients with an early CR with improved OS and RFS by RIC alloHSCT as compared with chemotherapy.

Poor risk (FLT3-ITD high mutant to wild-type ratio)

OS and RFS in patients with a *FLT3*-ITD mutant to wild-type ratio of >0.50 are very poor (Supplementary Figures 1A and 1B). Numbers of patients were low hampering a reliable comparison of the different types of PRT.

Table 2 Outcome by post-remission treatment in CN-AML patients subclassified by *NPM1* and *FLT3*-ITD mutational status

	Outcome at 5 years (%) by post-remission treatment											
	Chemotherapy			AutoHSCT			AlloMAC			AlloRIC		
Molecular subgroup	**No.**	**OS**	**RFS**	**No.**	**OS**	**RFS**	**No.**	**OS**	**RFS**	**No.**	**OS**	**RFS**
Favorable (*NPM1*^{mut} without *FLT3*-ITD) (n=162)	51	68±7	58±7	60	71±6	66±6	39	74±7	71±7	12	67±14	67±14
Intermediate (n=328)	82	39±6	30±5	104	47±5	40±5	90	47±5	44±5	52	63±7	59±7
NPM1^{wt} without *FLT3*-ITD (n=184)	43	43±8	27±7	56	48±7	40±7	53	54±7	52±7	32	65±9	59±9
NPM1^{mut} *FLT3*-ITD mut to wt ratio <0.50 (n=104)	30	44±9	42±9	33	45±9	35±8	27	41±9	37±9	14	42±13	42±13
NPM1^{wt} *FLT3*-ITD mut to wt ratio <0.50 (n=40)	9	0±11	0±0	15	49±14	50±14	10	30±14	20±13	6	100±0	100±0
Poor (*FLT3*-ITD mut to wt ratio >0.50) (n=31)	15	20±10	7±6	4	50±25	25±22	8	13±12	13±12	4	25±22	25±22

Abbreviations: OS indicates overall survival; RFS, relapse-free survival; AutoHSCT autologous hematopoietic stem cell transplantation; AlloMAC, allogeneic hematopoietic stem cell transplantation (alloHSCT) following myeloablative conditioning; AlloRIC, alloHSCT following reduced intensity conditioning; *NPM1*, nucleophosmin-1; mut, mutant; and *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication

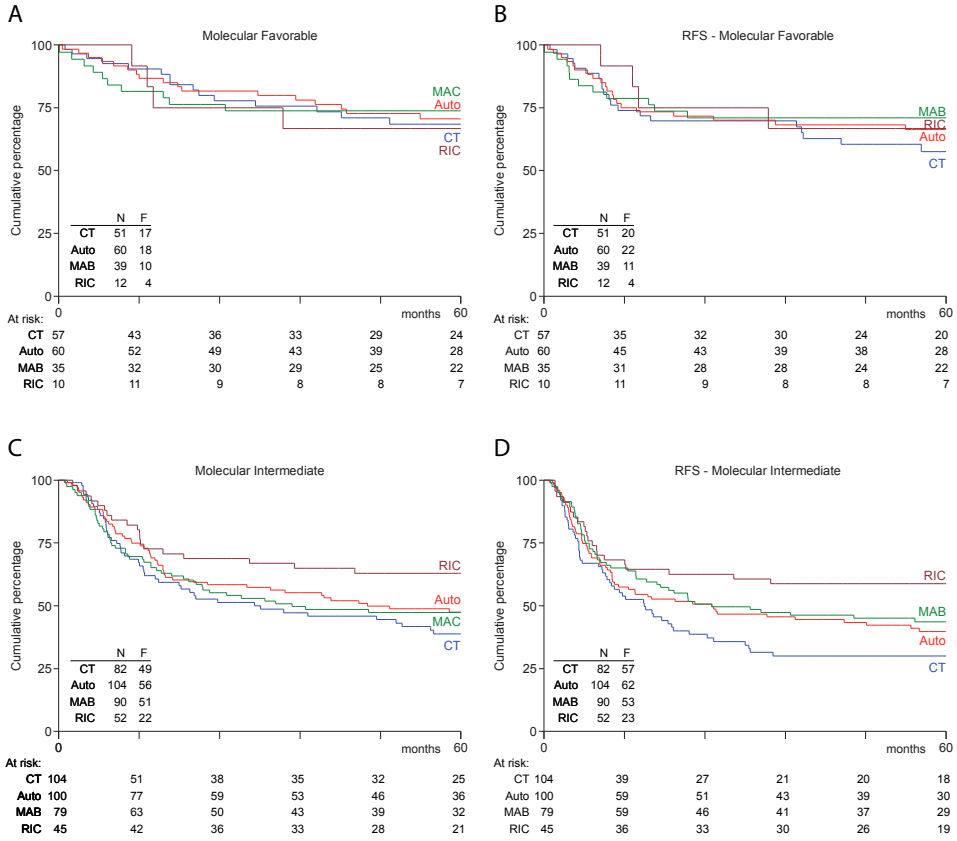

Figure 3 OS and RFS in molecular subcategories by post-remission treatment
Overall survival (OS) and relapse-free survival (RFS) in molecularly favorable risk (panels A and B) and molecularly intermediate risk (panels C and D) patients with CN-AML in first complete remission from start of post-remission treatment. Molecularly favorable includes patients with mutated *NPM1* without *FLT3*-ITD, and molecularly intermediate includes patients with wild-type *NPM1* without *FLT3*-ITD or patients with a low allelic ratio of *FLT3*-ITD. Of note, numbers of patients at risk (indicated below the x-axis) differ from the patient numbers (indicated in Table 1 and within the figure) because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in CR1 to the transplantation curve. Abbreviations: CT, chemotherapy; Auto, autologous hematopoietic stem cell transplantation (HSCT); MAC, myeloablative conditioned allogeneic HSCT (alloHSCT). RIC, reduced intensity conditioning alloHSCT, MAC; F, number of failures (ie, death whatever the cause); N, number of patients; and Cox LR, cox likelihood ratio

Multivariable analysis in molecularly intermediate risk patients

Table 3 shows the results of the multivariable analysis with adjustment for type of PRT, sex, age, white blood cell count below or above 100, and late CR. OS and RFS were better by RIC alloHSCT as compared with chemotherapy (hazard ratio (HR) 0.56, P = 0.022 and HR 0.50, P = 0.004, respectively) and autoHSCT (HR 0.60, P = 0.046 and HR 0.60, P = 0.043, respectively), whereas NRM was not significantly different comparing RIC alloHSCT with chemotherapy or autoHSCT (HR 2.54, P = 0.16 and HR 1.58, P = 0.42, respectively). Although no significant differences were found comparing autoHSCT and chemotherapy, the risk of relapse after autoHSCT was reduced with a HR of 0.71, P = 0.087. RFS was improved comparing MAC alloHSCT with chemotherapy (HR 0.67, P = 0.048), with a strongly decreased risk of relapse (HR 0.20, P<0.001) and counterbalancing increased risk of NRM following MAC alloHSCT (HR 9.14, P<0.001). OS and RFS following autoHSCT or MAC alloHSCT yielded similar results with a reduced risk of relapse following MAC alloHSCT as compared with autoHSCT (HR 0.29, P<0.001), but increased risk of NRM (HR 5.70, P<0.001). Furthermore, increasing age exhibited a significant HR for worse OS. In addition, late CR was associated with a significantly increased HR for OS, RFS and relapse as compared with CR after one cycle of induction chemotherapy. Of note, time from CR1 to start of PRT and year of treatment (before and after 2005) were added as factors to the model but showed no significant effects on OS, RFS, relapse or NRM. In addition, a sensitivity analysis of only patients receiving PRT after 2005 showed similar results for PRT on all outcome parameters.

Table 3 Multivariable analysis in molecularly intermediate-risk patients

	OS			RFS			Relapse			NRM		
	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value
Post-remission treatment												
Auto vs. CT	0.93	0.63-1.38	.72	0.83	0.57-1.20	.32	0.71	0.48-1.05	.087	1.60	0.40-6.48	.50
MAC alloHSCT vs. CT	0.86	0.57-1.30	.48	0.67	0.45-1.00	.048	0.20	0.12-0.35	<.001	9.14	2.74-30.42	<.001
RIC alloHSCT vs. CT	0.56	0.34-0.93	.022	0.50	0.31-0.82	.004	0.35	0.20-0.62	<.001	2.54	0.65-9.95	.16
MAC alloHSCT vs. Auto	0.93	0.62-1.38	.72	0.81	0.55-1.19	.29	0.29	0.16-0.50	<.001	5.70	2.33-13.89	<.001
RIC alloHSCT vs. Auto	0.60	0.36-1.00	.046	0.60	0.37-1.00	.043	0.49	0.27-0.89	.014	1.58	0.51-4.88	.42
Sex (female vs. male)	0.99	0.73-1.34	.94	0.96	0.72-1.29	.80	1.00	0.71-1.39	.99	0.81	0.44-1.48	.48
Age†	1.19	1.03-1.37	.014	1.08	0.95-1.23	.26	1.07	0.92-1.24	.37	1.17	0.86-1.60	.29
WBC at diagnosis (>100 vs. ≤100)	1.43	0.96-2.14	.086	1.34	0.90-1.99	.16	1.93	1.23-3.02	.006	0.49	0.19-1.25	.10
CR (late vs. early)	1.55	1.09-2.20	.019	1.51	1.07-2.12	.022	1.81	1.21-2.70	.006	1.21	0.63-2.33	.57

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; CT, chemotherapy; vs., versus; Allo, allogeneic hematopoietic stem cell transplantation; Auto, autologous hematopoietic stem cell transplantation; and WBC, white blood cell count. *The HRs are the estimates of the effect of covariates for each outcome parameter, adjusted for sex, age, CR (late vs. early), WBC at diagnosis below or above 100, and type of post-remission treatment. † Linear with estimates of ten years difference

DISCUSSION

The preferred type of PRT in patients with CN-AML in CR1 continues to be debated. Molecular diagnostics provide additional prognostic information to further stratify patients with CN-AML in CR1. Here, we demonstrate that type of PRT does not differentially affect outcome in the favorable group of patients with mutated *NPM1* without *FLT3*-ITD. Outcome in patients with a high allelic ratio of *FLT3*-ITD appeared very poor, with low patient numbers hampering a comparison by type of PRT. In contrast, outcome by type of PRT appeared to differ in a larger intermediate group, characterized by *FLT3*-ITD with a low allelic ratio and wild-type *NPM1* without *FLT3*-ITD AML. RIC alloHSCT appeared associated with significantly better OS and RFS as compared with chemotherapeutic PRT, whereas MAC alloHSCT and autoHSCT yielded similar OS, which did not significantly differ from PRT by chemotherapy.

The *FLT3*-ITD is an important molecular determinant of AML risk classification and outcome.[4,5,31] Here, not only *FLT3*-ITD itself, but especially the mutant to wild-type ratio strongly affected outcome with poor outcome for patients with a high allelic ratio. Based on these and previous results, the *FLT3*-ITD allelic ratio should be included in AML risk classifications and PRT decision making.[7-10,19,32] PRT has not extensively been studied in patients with AML, with a high allelic burden of *FLT3*-ITD, but improved outcome following alloHSCT has been suggested in patients with a *FLT3*-ITD allelic ratio of >0.50.[10,19,32] In our study, the few surviving patients with a high allelic burden of *FLT3*-ITD were recipients of an alloHSCT in either CR1 or CR2, which compares well with recent results by Ho et al,[19] suggesting improved outcome by alloHSCT.

Studies evaluating PRT by alloHSCT in patients with *FLT3*-ITD irrespective of the allelic ratio reported different results. While a study from the French GOELAMS study group reported improved outcome by alloHSCT,[33] a recent prospective matched pair study failed to show such a survival benefit.[34] The evaluation of all *FLT3*-ITD patients, including an unknown number of patients with a high allelic ratio, may have impacted on those results, questioning the comparability of those and other studies, focusing on *FLT3*-ITD. We combined patients with a low *FLT3*-ITD allelic ratio (irrespective of *NPM1* mutations) and patients with wild-type *NPM1* without *FLT3*-ITD into an intermediate risk group because of similar OS and RFS in these subgroups. In that molecularly intermediate risk group, OS and RFS were significantly better following RIC alloHSCT as compared with chemotherapy, which was confirmed by multivariable analysis stratified by study cohort and following adjustment for covariates. Of note, with a median follow-up of 72 months, NRM was low and a graft-vs-leukemia effect was preserved as evidenced by a HR of 0.35 for relapse as compared with chemotherapy. Although MAC alloHSCT showed an even stronger HR of 0.20, the anti-leukemic activity was counterbalanced by a significantly higher NRM (HR 9.14). Although a number of studies have shown a higher relapse rate following RIC alloHSCT as compared with MAC alloHSCT,[35-41] the net effect in terms of OS and RFS in well-defined and sufficiently sized subcategories of

AML CR1 patients is still underreported. Here, we show that the balance of a preserved graft-vs-leukemia and a low NRM eventually resulted in favorable outcome in molecularly intermediate risk AML CR1 recipients, who proceeded to RIC alloHSCT. MAC alloHSCT and autoHSCT yielded similar outcomes in that intermediate risk category of patients. Most comparative PRT studies in molecular subgroups compare alloHSCT with chemotherapy, but lack a group of autoHSCT recipients. Here, a large subgroup of recipients of an autograft was also included. Although autoHSCT was not significantly associated with improved outcome as compared with chemotherapy or MAC alloHSCT, autoHSCT may provide a valuable alternative PRT in these subgroups, especially in patients lacking a well matched donor or in patients at higher risk for NRM determined by risk scores.[42-44] In addition, the incorporation of minimal residual disease status assessed by flow cytometry[45,46] or molecular analysis[47] may add to that decision making, by the preferred application of autoHSCT in minimal residual disease negative, molecularly intermediate risk patients in CR1. Of note, while RFS following autografting estimated 40% in the intermediate risk group, OS was 47%, indicating that a considerable number of relapsing patients may be rescued by an allograft in CR2, as previously reported in AML patients.[48-50]

Combining results from two cooperative groups may implicate limitations. Although the induction chemotherapeutic regimens varied among the different study groups, all patients received cytarabine/anthracycline-based chemotherapy, obtained a hematological CR1 within two cycles of induction chemotherapy, and outcome was not significantly different among the different study groups. In addition, differences in PRT approach among the study groups may have resulted in selection bias, although that bias is presumably similar among the three molecularly defined groups in the analysis, which were not differentially approached by the study groups. The analysis presented did not prospectively compare RIC and MAC regimens prior alloHSCT, which withholds us from conclusions in that regard. Given the significant lower NRM associated with RIC, as shown in many studies, the presentation of RIC alloHSCT and MAC alloHSCT as two distinct categories is, however, warranted. The latter notion is supported by results of the prospective randomized US study, showing different outcomes following either conditioning type.[51] Although MAC alloHSCT is associated with a significantly stronger anti-leukemic effect, its counterbalancing effect on NRM need to be taken into account, especially in older patients with comorbidities. Therefore, as advocated before,[5] we prefer to apply either treatment modality in a personalized fashion, tailored by risk factors, predicting NRM.[52]

Collectively, these results suggest that RIC alloHSCT may provide better survival than chemotherapeutic PRT in patients with CN-AML with wild-type *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low allelic burden. AutoHSCT may be applied if not eligible, if no well-matched donor is available in CR1, or in case of absence of minimal residual disease. Although MAC

alloHSCT is associated with the strongest anti-leukemic effect, our results suggest that it might preferentially be applied in patients with an acceptable risk for complications and NRM.

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SUPPLEMENTARY APPENDIX

Supplementary methods - Molecular analysis

FLT3 ITD detection and FLT3 ITD/wild type ratio

Total RNA was extracted with phenol chloroform and reverse transcribed using Superscript II RT (Invitrogen, Breda, the Netherlands). The presence of *FLT3* ITD and *NPM1* mutations in the AML samples were determined as described previously.[1,2] AML samples harboring a *FLT3* ITD were subsequently analyzed by Roche 454 next generation sequencing (NGS). Amplicon fusion primers for bi-directional sequencing were designed according to the manufacturers protocols (Roche, Amplicon Library Preparation Method Manual-Lib-A, GS-Junior Titanium Series, May 2010 (Rev June 2010)) and consisted of an adapter sequence for the GS-Junior 454 system, a sample specific barcode sequence (MID – multiplex identifier) for both forward and reverse primers, and the template-specific sequence: *FLT3* exon 12: FLT3ex12: 5'-TAAACTCTCCAGGCCCCTTC-3 and *FLT3* exon 16: FLT3ex16: 5'-TGAGTGCCTCTCTTTCAGAGC-3. PCR and cycling conditions: 0.25mM dNTP, 0.4 µM primer, 2mM MgCl2, Taq polymerase and 1x buffer (Invitrogen, Breda, the Netherlands), cycling: 1 cycle 4' 94˚C, 35 cycles 1' 94˚C, 1' 60˚C, 1' 72˚C, and 1 cycle 10' 72˚C. Pooled PCR products were purified, diluted, clonally amplified by emulsion PCR and sequenced on the Roche 454 GS junior system and analyzed according to the manufacturer's protocols (Roche, Amplicon Library Preparation Method Manual-Lib-A, GS-Junior Titanium Series, May 2010, Rev June 2010). Samples with > 60% *FLT3* ITD as defined by (*FLT3* ITD reads/ (*FLT3* ITD reads + FLT3 wild type reads) were indicated as homozygous *FLT3* ITD. The threshold of 60% was chosen to ascertain that true *FLT3* ITD homozygous were included in the study. Samples lacking *FLT3*-ITDs were used as control.

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Supplementary Table 1 Patient characteristics by cohort

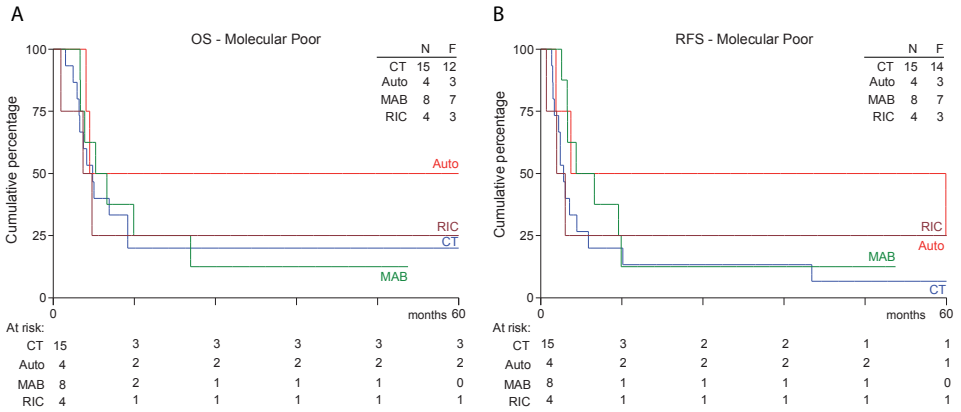
	EORTC (N=122)		HOVON (N=399)		p-value
Sex					
Male	54	44%	208	52%	.13
Female	68	56%	191	48%	
Age (years)					
Median	47		49		.038
Range	16-61		18-60		
NPM1					
Mutated	64	52%	229	57%	.34
Wild-type	58	48%	170	43%	
***FLT3*-ITD**					
Not present	87	71%	259	65%	.062
Low ratio	33	27%	111	28%	
High ratio	3	2%	29	7%	
CR reached after					
Cycle 1 (early CR)	114	93%	313	78%	<.001
Cycle 2 (late CR)	8	7%	86	22%	
Time from CR to start PRT (months)					
Median	3.0		2.3		<.001
IQ range	2-4		1-3		
PRT					
Chemotherapy	0	0%	148	37%	<.001
AutoHSCT	71	58%	97	24%	
MAB	47	39%	90	23%	
RIC	4	3%	64	16%	
Year of PRT					
<2005	71	58%	215	54%	.40
≥2005	51	42%	184	46%	

Abbreviations: AutoHSCT indicates autologous hematopoietic stem cell transplantation; AlloMAB, allogeneic hematopoietic stem cell transplantation following myeloablative conditioning, AlloRIC, alloHSCT following reduced intensity conditioning; WBC, white blood cell count; NPM1, nucleophosmin 1; FLT3-ITD, fms-like tyrosine kinase 3 internal tandem duplication; IQ, interquartile range; CR, complete remission; and PRT, post-remission treatment

Supplementary Table 2 Relapse and NRM by molecular subcategory

	Outcome at 5 years (%) by post-remission treatment											
	Chemotherapy			AutoHSCT			AlloMAC			AlloRIC		
Molecular subgroup	**No.**	**Relapse**	**NRM**	**No.**	**Relapse**	**NRM**	**No.**	**Relapse**	**NRM**	**No.**	**Relapse**	**NRM**
Favorable (*NPM1*^{mut} without *FLT3*-ITD) (n=162)	51	39±7	4±2	60	30±6	4±2	39	11±5	18±6	12	8±8	25±13
Intermediate (n=328)	82	68±5	2±2	104	54±5	6±2	90	23±4	33±5	52	32±7	9±4
NPM1^{wt} without *FLT3*-ITD (n=184)	43	71±7	0±0	56	53±7	7±3	53	18±5	30±6	32	32±8	9±5
NPM1^{mut} *FLT3*-ITD mut to wt ratio <0.50 (n=104)	30	58±9	9±9	33	62±9	3±3	27	22±8	41±9	14	44±13	14±9
NPM1^{wt} *FLT3*-ITD mut to wt ratio <0.50 (n=40)	9	91±9	0±0	15	40±13	10±9	10	51±16	30±14	6	0±0	0±0
Poor (*FLT3*-ITD mut to wt ratio >0.50) (n=31)	15	93±6	0±0	4	75±22	0±0	8	50±18	38±17	4	75±22	0±0

Abbreviations: OS indicates overall survival; RFS, relapse-free survival; AutoHSCT autologous hematopoietic stem cell transplantation; AlloMAC, allogeneic hematopoietic stem cell transplantation (alloHSCT) following myeloablative conditioning; AlloRIC, alloHSCT following reduced intensity conditioning; *NPM1*, nucleophosmin-1; mut, mutant; and *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication



Supplementary Figure 1 OS and RFS in molecularly poor subgroup

5

GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENEIC STEM CELL TRANSPLANTATION AND MINIMAL RESIDUAL DISEASE IN PATIENTS WITH AML IN FIRST CR

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ABSTRACT

Purpose

The detection of minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) may improve future risk-adapted treatment strategies. We assessed whether MRD positive and MRD negative AML patients benefit differently from the graft-versus-leukemia effect of allogeneic hematopoietic stem cell transplantation (alloHSCT).

Patients and Methods

A total of 1.511 patients were treated in subsequent HOVON-SAKK AML trials of whom 547 patients obtained a first complete remission, received post-remission treatment (PRT) and had available flowcytometric MRD prior to PRT. MRD-positivity was defined by more than 0.1% cells with a leukemia associated immunophenotype within the white blood cell compartment. PRT consisted of alloHSCT (n=282), or conventional PRT with a third cycle of chemotherapy (n=160) or autologous HSCT (autoHSCT, n=105).

Results

MRD was positive in 129 (24%) patients after induction chemotherapy before proceeding to PRT. OS and RFS were significantly better in patients without MRD prior to PRT compared with MRD-positive patients (65 ± 2% versus 50 ± 5% at 4 years, p=0.002, and 58 ± 3% versus 38 ± 4%, p<0.001, respectively), which was mainly because of a lower cumulative incidence of relapse (32 ± 2% compared to 54 ± 4 %, p<0.001, respectively). Multivariable analysis with adjustment for covariates showed that the incidence of relapse was significantly reduced following alloHSCT compared with chemotherapy or autoHSCT (HR 0.36, p<0.001), which was similarly exerted in both MRD-negative and MRD-positive patients (HR 0.38, p<0.001 and HR 0.35, p<0.001).

Conclusion

The graft-versus-leukemia effect of alloHSCT is equally present in MRD-positive and MRD-negative patients, which advocates a personalized application of alloHSCT taking the risk of relapse determined by AML risk group and MRD status as well as the counterbalancing risk of non-relapse mortality into account.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous malignancy, characterized by a variety of underlying cytogenetic and molecular aberrations, which are associated with distinct prognostic features.[1] Although current treatment approaches induce high percentages of hematological remission, relapse rates are high and vary according to the underlying risk profile.[2] Recently, the European LeukemiaNet (ELN) has developed an updated classification based on cytogenetic and molecular aberrancies distinguishing patients with a favorable, intermediate or adverse treatment response.[3] Post-remission treatment (PRT) decisions are currently tailored according to AML-risk groups, whereby allogeneic hematopoietic stem cell transplantation (alloHSCT) is generally not used in patients with favorable-risk AML, and on the other hand generally highly recommended in adverse-risk AML.[3-6] AML-risk classification may be further improved by introducing the assessment of minimal residual disease (MRD) early after induction chemotherapy, but also after PRT.[7-24] MRD after induction treatment being assessed by either multiparametric flow cytometry or quantitative PCR for specific markers, has firmly been shown to predict for relapse and overall outcome, irrespective of type of PRT.[10-24] Consequently, MRD negativity was introduced as clinical endpoint in patients with a hematological complete remission (CR).[3] Despite PRT with alloHSCT, a 2-5 fold increased incidence of relapse in MRD-positive recipients was observed compared with MRD-negative patients,[17-24] which observation questions whether and to what extent MRD-positive patients may benefit from the graft-versus-leukemia (GVL)-effect of alloHSCT. Conversely, the low relapse rate in MRD-negative alloHSCT recipients also evokes the question whether GVL is operational in that subgroup and to what extent it may be blunted by non-relapse mortality (NRM). Therefore, we set out to address whether and to what extent alloHSCT quantitatively reduces relapse compared with conventional PRT in upfront treated patients with MRD-positive or MRD-negative AML in first CR (CR1).

METHODS

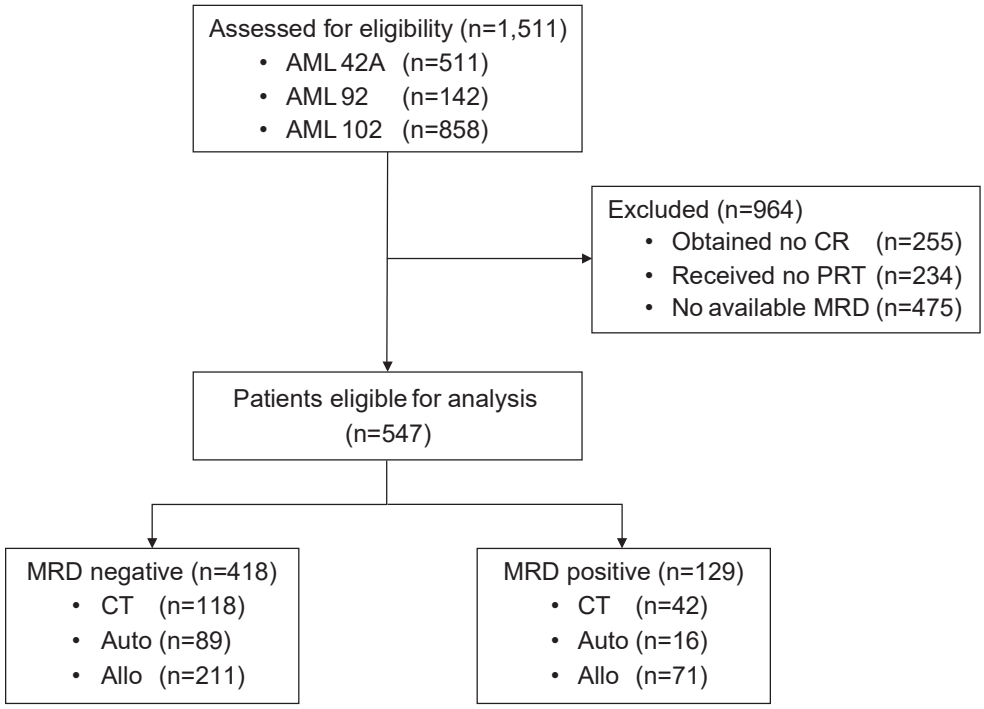
Patients

Patients participated in three prospective, consecutive HOVON-SAKK collaborative group trials (AML42, AML92 and AML102), for whom assessment of MRD after induction therapy and prior to PRT by either alloHSCT, chemotherapy or autologous HSCT (autoHSCT) was performed.[25,26] The results of the AML92 trial have not been published, but trial information is available in the Netherlands Trial Register (NTR1446). A total number of 1511 newly diagnosed AML patients were included for whom treatment was started between 2006 and 2014. Patients were excluded because of no CR1 after two induction cycles of chemotherapy (n=255, 17%), no application of PRT after obtaining CR1 (n=234, 15%). In addition, a total of 475 (31%) patients received a PRT in CR1 but had no available MRD status within a time window of four months before PRT. A total of 547 patients with available MRD status who received PRT in CR1 was available for analysis (Consort Diagram). Patients were classified by AML prognostic risk, based on the cytogenetic and molecular profile of the underlying AML, according to the ELN2017-risk classification.[3] Molecular analysis was available for the majority of patients, specifically for *NPM1* (93%), *FLT3*-ITD (91%), including the *FLT3*-ITD mutant to wild type ratio (86%), *EVI1* (79%), *ASXL1* (83%), *RUNX1* (47%), and *TP53* (47%). Patients for whom molecular analyses were not available were considered as not having the mutation in calculating the ELN2017-risk classification. All studies were approved by the ethics committees of participating institutions and were conducted in accordance with the Declaration of Helsinki. All participants had given written informed consent. A detailed description of the inclusion and exclusion criteria of the studies have been previously published.[25,26]

Treatment protocols

Treatment in the HOVON-SAKK AML42A, AML92, and AML102 trials involved a maximum of two remission induction cycles consisting of a first course of idarubicin with cytarabine and a second cycle of high dose cytarabine with amsacrine, as previously described.[25,26] Patients were randomized to G-CSF (AML42A), laromustine (AML92), and clofarabine (AML102). After obtaining CR1, patients subsequently received PRT to a predefined strategy as outlined in the study protocols, but without knowledge of the MRD status of the patients. PRT included either a third cycle of chemotherapy with mitoxantrone and etoposide, high-dose chemotherapy with busulfan and cyclophosphamide followed by autoHSCT, or alloHSCT following either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC). The MAC regimen contained high-dose cyclophosphamide with at least 8 Gy total body irradiation (TBI) in 83 (76%) patients, whereas the remainder received busulfan with cyclophosphamide. Although RIC regimens varied, the majority contained low dose (2 or 4 Gy) TBI preceded by fludarabine (n=126, 77%), whereas 23% of the patients received fludarabine with busulfan. These different PRT modalities were applied according a risk-

Consort Diagram



adapted strategy: (1) patients with AML classified as favorable-risk, according to cytogenetic and molecular analysis, were planned for a third cycle of chemotherapy; (2) intermediate-risk patients were preferentially treated by alloHSCT using a human leukocyte antigen (HLA) matched sibling donor or a fully HLA-matched unrelated donor if available; (3) patients with adverse-risk AML proceeded to alloHSCT using either a sibling donor, unrelated donor, or cord blood grafts; (4) patients alternatively received an autoHSCT or a third cycle of chemotherapy if no suitable donor was available.[25-28]

MRD detection and sample selection

MRD flow cytometric analysis was performed in a two-step procedure, as previously described.[18] In summary, the immunophenotype was determined on blasts defined by CD45 expression with a low sideward scatter. The leukemia associated immune phenotype (LAIP) at diagnosis was identified by detecting aberrantly expressed markers/marker combinations to distinguish leukemic blasts from normal hematopoietic progenitor cells. Bone marrow samples were collected at diagnosis to determine LAIP and follow-up after each chemotherapy cycle. The sensitivity of flow cytometry could lead to detection of one leukemic cell in 1,000 up to 100,000 white blood cells (WBC). MRD percentage was defined

as the percentage of LAIP cells within the WBC compartment multiplied by the correction factor (100% divided by the percentage of LAIP positive blasts at diagnosis). A percentage above 0.1% was considered as MRD-positive as validated in previous studies.[18] MRD samples obtained after cycle 2 in patients with AML in CR1 were used with a maximum time from the MRD sample to subsequent PRT of four months. The sample with the shortest time interval between PRT and the date of collection was selected for analysis.

Endpoints

The primary endpoint of the study was the cumulative incidence of relapse. Outcome estimates were measured from the date of starting the first PRT. Overall survival (OS) was based on death from any cause, and patients were censored at the date of last contact if alive. The events for relapse free survival (RFS) were death in CR1, designated as NRM, or hematological relapse. The cumulative risks of relapse and NRM over time were calculated as competing risks with actuarial methods, where patients alive in continuing CR1 were censored at the date of last contact.

Statistical methods

A time-dependent analysis of PRT was performed as described previously,[28,29] by applying multivariable Cox regression with alloHSCT as time-dependent covariate. The multivariable analysis is conceptually similar to a Mantel-Byar analysis,[30] but more general as it allows for adjustment of other factors. A number of patients received PRT with chemotherapy (n=44) first before they proceeded to alloHSCT in continuing CR1. In both the multivariable analysis and the estimation of the survival curves, these patients were counted as at risk in the chemotherapy group from start of PRT until alloHSCT and after that as at risk in the alloHSCT group. Forward selection with the variables significantly associated with relapse following univariable analysis was used for developing the multivariable model. Multivariable Cox regression analysis for relapse, OS, RFS, and NRM was applied stratified by the total number of induction courses. Stratification by the total number of induction courses (ie, I or II) was done in order to allow the baseline hazard to differ between these two patient groups. All p-values were based on log likelihood ratio tests, except when explicitly stated otherwise. The proportional hazard assumption was tested on the basis of Schoenfeld residuals.[30,31] P-values were not adjusted for multiple testing. All analyses were done with Stata Statistical Software: Release 13.1 (Stata Corporation 2013, College Station, TX, USA).

RESULTS

Patient characteristics

A total of 547 patients with AML in CR1 and available MRD-status proceeded to PRT with either alloHSCT (n=282), chemotherapy (n=160), or autoHSCT (n=105). A total of 129 (24%) patients were MRD-positive after induction chemotherapy before proceeding to first PRT. Patient characteristics are presented in Table 1. Patients with mutated *NPM1* were more frequently MRD-negative, whereas MRD-positive patients more frequently tended to obtain a late CR1 (ie, after induction cycle 2). The ELN2017-risk classification was similarly distributed among the MRD-negative and positive patients. Interestingly, the time-interval from CR1 to PRT for patients with a MRD-positive AML was shorter compared with their negative counterparts, which was mainly apparent in favorable-risk AML patients. The median follow-up of patients still alive was 50 months. Details of the characteristics of alloHSCT are shown in Table 2. No other differences as regards donor source, conditioning, CMV-serostatus, and EBMT-score were apparent between MRD-negative and MRD-positive patients. AlloHSCT recipients received a sibling donor in 50% of the transplants, whereas 42% patients were transplanted with a matched unrelated donor. AlloHSCT with RIC was predominantly performed, and conditioning mostly included total body irradiation. Patients with a high-risk for NRM according to the EBMT-score[32] (≥3 points) represented 45% of the transplanted patients.

Treatment outcome

OS and RFS were significantly better in patients without MRD before PRT compared with MRD-positive patients (65 ± 2% versus 50 ± 5% at 4 years, p=0.002, and 58 ± 3% versus 38 ± 4%, p<0.001, respectively, Figure 1A and 1B). Improved outcome was mainly caused by a lower cumulative incidence of relapse in MRD-negative patients compared with MRD-positive patients (32 ± 2% compared to 54 ± 4% at 4 years, p<0.001, respectively, Figure 1C), whereas NRM was not significantly different and estimated at 10 ± 1% (Figure 1D). More detailed outcome estimates according to MRD-status, type of PRT, and risk for NRM based on the EBMT-score are presented in Supplementary Table 1.

The cumulative incidence of relapse was significantly lower in patients without MRD receiving alloHSCT compared with chemotherapy or autoHSCT (26 ± 3% versus 38 ± 3% at 4 years, p=0.027, respectively, Figure 2A). The cumulative incidence of relapse in MRD-positive patients estimated 45 ± 6% compared to 66 ± 6% at 4 years (p=0.058), for recipients of alloHSCT compared with recipients of chemotherapy or autoHSCT, respectively (Figure 2B). RFS following alloHSCT proved similar compared with PRT with chemotherapy or autoHSCT in patients without MRD before PRT (58 ± 4% versus 58 ± 4% at 4 years, p=0.99, respectively, Figure 2C). RFS after alloHSCT in patients with positive MRD before PRT was 44 ± 6% compared to 31 ± 6% at 4 years (p=0.20) after chemotherapy or autoHSCT, respectively (Figure 2D).

Table 1 Patient characteristics

	MRD negative (N=418)		MRD positive (N=129)		P-value
Sex					.129
Male	207	50%	70	54%	
Female	211	50%	59	46%	
Age (years)					.099
Median	51		49		
Range	18-65		18-65		
WBC at diagnosis					.049
≤100	389	93%	113	88%	
>100	29	7%	16	12%	
Cytogenetics of AML					.008
t(8;21)	25	6%	7	5%	
inv(16)	15	4%	15	12%	
CN-X-Y	220	53%	56	43%	
Cytogenetic abnormalities	110	26%	36	28%	
Monosomal karyotype	30	7%	12	9%	
Missing	18	4%	3	2%	
***NPM1* mutation**					.001
No	238	57%	91	71%	
Yes	151	36%	27	21%	
Missing	29	7%	11	9%	
FLT3*-ITD					.87
Absent	289	69%	86	67%	
Low ratio	75	18%	24	19%	
High ratio	16	4%	6	5%	
Missing	38	9%	13	10%	
ELN2017 risk classification					.20
Favorable	163	39%	39	30%	
Intermediate	144	34%	51	40%	
Adverse	111	27%	39	30%	
CR reached after					.003
Cycle 1 (early CR)	373	89%	102	79%	
Cycle 2 (late CR)	45	11%	27	21%	
Post-remission treatment					.079
Chemotherapy	118	28%	42	33%	
Autologous HSCT	89	21%	16	12%	
Allogeneic HSCT	211	50%	71	55%	
Time from diagnosis to CR (days)					.084
Median	34		35		
IQ range	29-40		31-46		
Time from CR to PRT (days)					.009
Median	74		65		
IQ range	56-96		48-90		
Year of PRT					.11
Median	2011		2010		
Range	2006-2014		2006-2014		

Abbreviations: MRD, minimal residual disease; WBC, white blood cell count; AML, acute myeloid leukemia; NPM1, nucleophosmin-1; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; ELN, European LeukemiaNET; CR, complete remission; IQ, interquartile range; and PRT, post-remission treatment. *The cuf-off of the *FLT3*-ITD ratio is defined as 0.50

Table 2 Transplant characteristics

		MRD negative (N=211)		MRD positive (N=71)		P-value
Donor source						.29
HLA-identical sibling		107	51%	35	49%	
Matched unrelated donor		90	43%	29	41%	
Umbilical cord blood		7	3%	6	8%	
Other		7	3%	1	1%	
Conditioning						.85
Myeloablative	*Cyclophosphamide + TBI*	63	30%	20	28%	
	Cyclophospahmide + busulfan	20	9%	6	8%	
Reduced intensity	*Fludarabine + TBI*	89	42%	31	44%	
	Fludarabine + busulfan	24	11%	11	15%	
Unknown		15	7%	3	4%	
Stem cell source						.13
Bone Marrow		10	5%	4	6%	
Peripheral Blood		186	88%	59	83%	
Cordblood		6	3%	6	8%	
Missing		9	4%	2	3%	
CMV serostatus patient/donor						.059
Neg/Neg		25	12%	3	4%	
Other		108	51%	41	58%	
Missing		78	37%	27	38%	
Female donor to male recipient						.89
No		168	80%	56	79%	
Yes		43	20%	15	21%	
EBMT-score						.40
0		0		1	1%	
1		26	12%	9	13%	
2		93	44%	26	37%	
3		81	38%	31	44%	
4		11	5%	4	6%	

Abbreviations: MRD, minimal residual disease; HLA, human leukocyte antigen; TBI, total body irradiation; CMV, cytomegalovirus; and EBMT, European Group of Blood and Marrow Transplantation

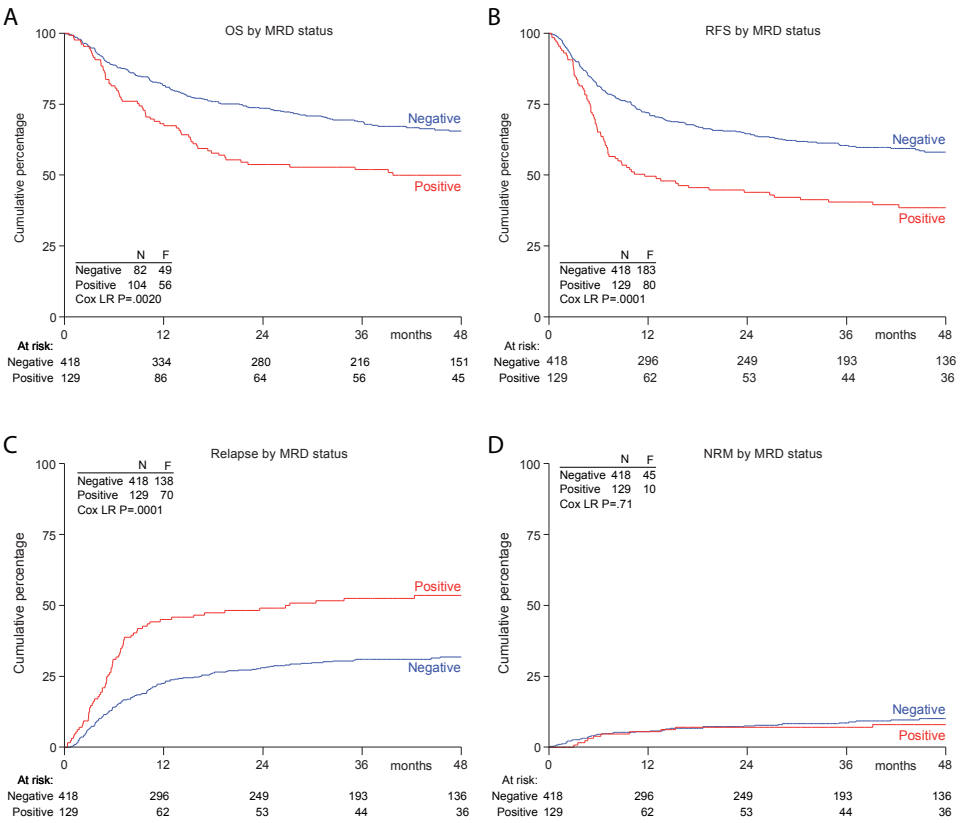


Figure 1 Outcome by minimal residual disease status
Kaplan-Meier estimates of overall survival (OS, panel **A**), relapse-free survival (RFS, panel **B**), cumulative incidence of relapse (panel **C**) and cumulative incidence of non-relapse mortality (NRM, panel **D**) by minimal residual disease (MRD) status in patients with AML in first complete remission from start of post-remission treatment. Abbreviations: F, number of failures (ie, death whatever the cause); and N, number of patients

The type of conditioning did not significantly impact on the incidence of relapse or RFS (Supplementary Figure 1). The cumulative incidence of NRM after alloHSCT was 15 ± 2% and was significantly affected by the EBMT-score (Supplementary Figure 2). NRM split by the EBMT-score showed less NRM in patients with a low EBMT-score compared with patients with a high EBMT-score (≤2 compared to >2, 10 ± 2% compared to 22 ± 4%, p=0.005, respectively).

A total of 208 patients developed a relapse after having received PRT, of whom 120 (57%) patients proceeded to salvage chemotherapy and 70 (59%) entered a second CR. Only 46 (22%) of relapsing patients proceeded to alloHSCT after obtaining second CR.

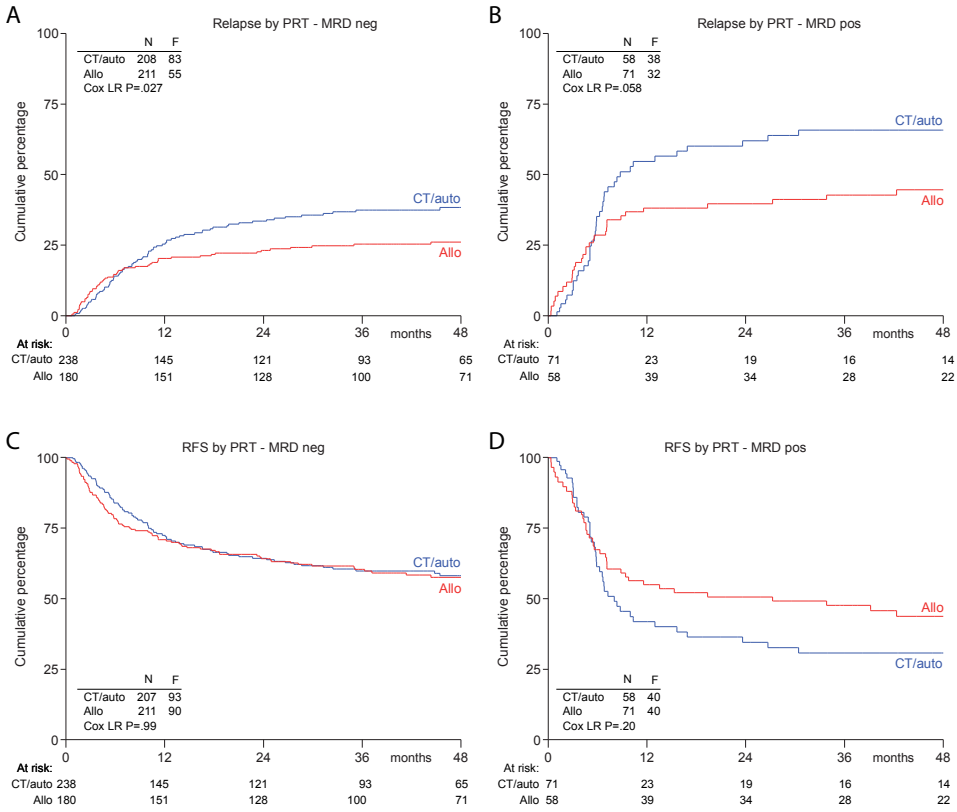


Figure 2 Outcome by post-remission therapy

Kaplan-Meier estimates of the cumulative incidence of relapse in minimal residual disease (MRD) negative patients (**A**), cumulative incidence of relapse in MRD positive patients (**B**), relapse-free survival (RFS) in MRD negative patients (**C**), and RFS in MRD positive patients (**D**) by type of post-remission treatment in patients with AML in first complete remission from start of PRT. Of note, numbers of patients at risk (indicated below the x-axis) differ from the patient numbers (indicated in Table 1 and within the figure) because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in CR1 to the transplantation curve. Abbreviations: CT/auto, chemotherapy or autologous hematopoietic stem cell transplantation; allo, allogeneic hematopoietic stem cell transplantation; F, number of failures (ie, death whatever the cause); and N, number of patients

Multivariable analysis

The following variables significantly predicted for relapse in the univariable analysis: MRD status, type of PRT, age, WBC category, *FLT3*-ITD category, year of PRT, time from diagnosis to CR, time from CR to PRT, cytogenetics, number of cycles to CR, ELN2017-risk classification, *NPM1* mutation, *EVI1* overexpression, and *CEBPA* double mutation. Following forward selection the multivariable analysis was performed, stratified by the total number of induction courses with adjustment for MRD status, type of PRT, age, WBC at diagnosis, *FLT3*-ITD, ELN2017-risk classification, number of cycles to CR, and year of PRT (Table 3). Relapse of AML was significantly reduced following alloHSCT compared with chemotherapy or autoHSCT (HR 0.36, p<0.001). That GVL-effect was similarly exerted in MRD-negative and MRD-positive patients (HR 0.38, p<0.001 and HR 0.35, p<0.001, Figure 3A), which was also similar comparing alloHSCT with either chemotherapy or alloHSCT with autoHSCT (Supplementary Figure 3). Despite significantly increased NRM (HR 2.94, p=0.003), RFS was better following alloHSCT compared with chemotherapy or autoHSCT (HR 0.53, p<0.001, Figure 3B, Supplementary Figure 4), whereas OS was not significantly different (Table 3). Different variables in the multivariable model were significantly associated, with relapse with the ELN2017-risk classification, *FLT3*-ITD mutant to wild-type ratio, number of cycles to reach CR, and type of PRT being the most important variables.

Table 3 Multivariable analysis

	Relapse			OS			RFS			NRM		
	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value
PRT (Allo vs. CT/Auto)	0.36	0.26-0.50	<.001	0.97	0.71-1.32	.83	0.53	0.40-0.71	<.001	3.01	1.38-6.59	.003
MRD (Postive vs. Negative)	2.01	1.48-2.73	<.001	1.52	1.11-2.08	.011	1.70	1.28-2.25	<.001	0.74	0.34-1.64	.44
Age†	1.19	1.04-1.35	.008	1.37	1.20-1.58	<.001	1.25	1.11-1.41	<.001	1.59	1.18-2.13	.001
WBC at diagnosis (>100 vs ≤100)	2.36	1.53-3.63	<.001	1.66	1.04-2.65	.044	1.93	1.27-2.94	.004	0.64	0.15-2.82	.53
***FLT3*-ITD category**												
Low ratio vs. Negative	1.41	0.97-2.06	.082	1.54	1.07-2.21	.025	1.46	1.05-2.04	.030	1.48	0.71-3.11	.31
High ratio vs Negative	2.98	1.60-5.56	.002	2.99	1.66-5.38	<.001	2.76	1.56-4.87	.001	2.49	0.71-8.72	.20
ELN2017-risk classification												
Intermediate vs. Favorable	2.03	1.40-2.96	<.001	1.58	1.06-2.34	.024	1.75	1.24-2.47	.001	1.13	0.50-2.56	.77
Adverse vs. Favorable	3.92	2.65-5.81	<.001	3.23	2.18-4.79	<.001	3.40	2.38-4.84	<.001	2.20	0.99-4.89	.051
Late CR vs early CR (attained after cycle II vs. cycle I)	3.00	2.07-4.36	<.001	3.37	2.35-4.83	<.001	2.80	1.99-3.92	<.001	2.07	0.92-4.63	.098
Year of PRT	1.12	1.05-1.20	<.001	1.02	0.95-1.09	.60	1.07	1.00-1.13	.033	0.91	0.80-1.04	.17

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; PRT, post-remission treatment; Allo, allogeneic hematopoietic stem cell transplantation; CT, chemotherapy; Auto, autologous hematopoietic stem cell transplantation; MRD, minimal residual disease; WBC, white blood cell count; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; ELN2017, European LeukemiaNET 2017; and CR, complete remission

* The HRs are the estimates of the effect of covariates for each outcome parameter, stratified by the number of induction courses and adjusted for type of PRT, MRD status, age, WBC at diagnosis, *FLT3*-ITD category, ELN2017-risk, number of cycles to CR, and year of PRT

† Linear with estimates of ten years difference

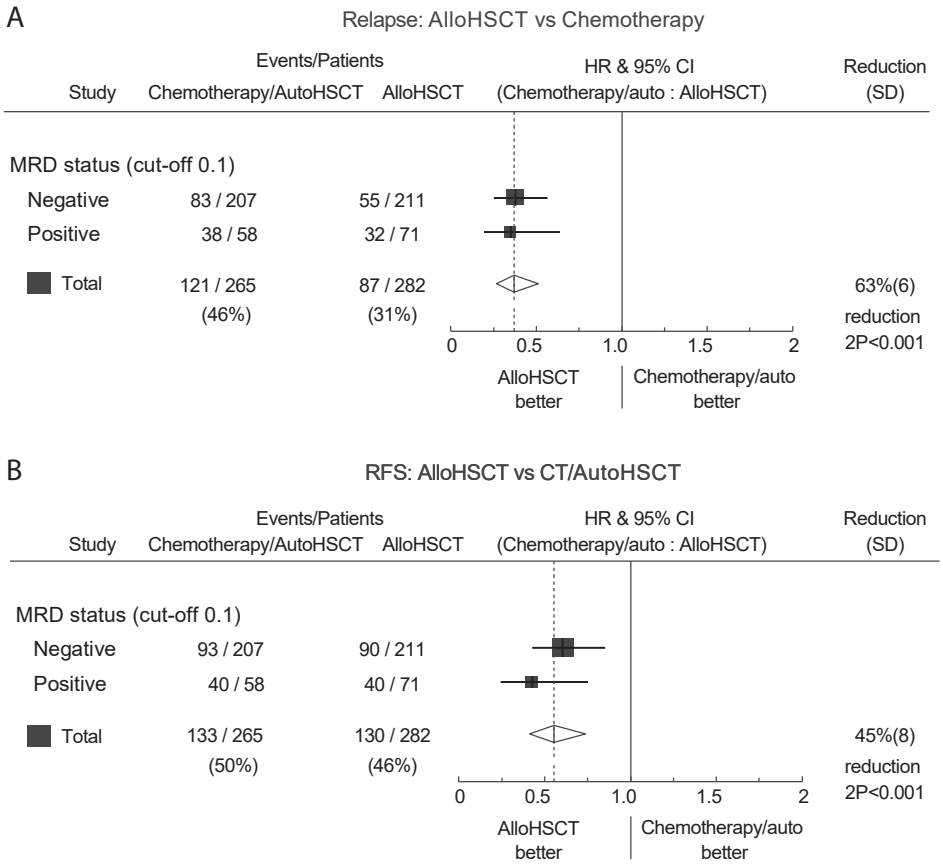


Figure 3 Forest plots of relapse comparing alloHSCT versus chemotherapy or autoHSCT
Forest plot of pooled estimates of the relative reduction (hazard ratio (HR) and 95% confidence interval (CI)) of relapse (**A**) and relapse-free survival (**B**) by MRD status comparing allogeneic hematopoietic stem cell transplantation (HSCT) and chemotherapy or autologous HSCT

DISCUSSION

The development of treatment approaches in AML patients is increasingly personalized by using genetic and molecular leukemia characteristics at diagnosis and individual treatment response.[3-6] Response and especially MRD, detected by either multiparametric flow cytometry or quantitative PCR, has become an important parameter in a more precise treatment approach of AML patients.[10-24] Currently it is unknown whether and how the presence or absence of MRD should guide the application of alloHSCT as PRT. Recently, the quantitative detection of mutated *NPM1* has been shown of high predictive value and recommendations to tailor the application of alloHSCT by MRD were done.[10-12] Balsat et al.[10] suggested to refrain from alloHSCT in *NPM1* MRD-negative patients and to selectively proceed to alloHSCT as PRT in CR1 in MRD-positive patients or adverse-risk patients based on karyotype or the presence of *FLT3*-ITD. In addition, Buccisano et al.[33] concluded in a recent analysis that MRD-positive patients as determined by flow cytometry could also benefit from alloHSCT. Although overall outcome was suggested to be improved by alloHSCT in MRD-positive patients, the question to what quantitative extent alloHSCT reduces relapse in MRD-positive patients and how that compares to MRD-negative patients is still open.

Here, we show that the allogeneic GVL-effect as estimated by the relative reduction of relapse is similar in MRD-positive and MRD-negative patients with a reduction of 63% by alloHSCT compared with chemotherapy or autoHSCT. These results compare well to earlier findings in cytogenetic subgroups, in which the GVL-effect appeared to be similar among patients with a monosomal karyotype, core binding factor AML, or patients with a normal karyotype.[34] These observations are most readily explained by the abundant expression of class I and II HLA-antigens on malignant myeloid precursor cells and their susceptibility to alloreactive T-cells, including T-cells recognizing minor or major HLA-antigens.[35-37] It suggests that T-cell alloreactivity might exert anti-leukemic effects irrespective of underlying subcategory of AML, although absolute estimates of relapse incidences do differ and may rather reflect differences in disease biology such as intrinsic resistance.

Although alloHSCT provides a strong GVL-effect, counterbalancing NRM may be of concern. As NRM critically depends from a number of different risk factors, it has become imperative to assess the NRM-risk profile in addition to leukemia characteristics and response to induction chemotherapy.[4] In the present study, the subset of patients with low EBMT-risk scores showed excellent outcome, whereas the GVL-effect of alloHSCT may be blunted by NRM in patients with a high-risk for NRM. Therefore, refined genetic leukemia-risk scores supplemented with MRD status may improve the latest risk score systems for NRM.[32,38-44] Transplant-risk scores have been developed and validated based on patient and transplant characteristics, including the EBMT-risk score[32] and the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI)[40], which is continuously being refined including age, disease status, or bio-markers.[39,41,42] The EBMT-acute leukemia working party (ALWP) has developed

an integrated score based on the EBMT-risk score and the HCT-CI with increased predictive power in the setting of RIC alloHSCT.[43] Alternatively, a more sophisticated, machine based learning model was developed by the EBMT-ALWP, which resulted in an alternating decision tree model with high predictive power for mortality at 100-days extending to 2-years.[44] As advocated before, by weighing both the risk for NRM and the risk for relapse, a more personalized treatment approach can be applied.[5] Although that approach suits the precision needed for individual patients, it might also impair the prospective, randomized evaluation of AML treatment approaches, because patient selection may occur at various time points during treatment. Nevertheless, the advantages of personalized treatment for the individual patient are obvious and continuously being refined by updated and better risk scores. Also new technologies to better define MRD, like quantification of leukemic stem cell content, standardized protocols and antibody panels, and novel software possibilities, are emerging.[45-47]

A personalized approach including MRD identifies patients with an high-risk of relapse, who qualify for alloHSCT, but who might benefit from attempts to induce MRD-negativity prior transplantation. Previously, a number of investigators reported that patients in CR1 with persistence of MRD before alloHSCT have worse survival compared with recipients of alloHSCT with a MRD-negative CR1.[17,19-24] Although alloHSCT is clearly indicated in MRD-positive patients, it is important to study the value of approaches intended to induce MRD-negativity before alloHSCT. A prospective inclusion of all MRD-positive patients subsequently analyzing such a strategy in a randomized fashion might answer this important question. It has been suggested that continued chemotherapy with one or two consolidation cycles may not be the preferred strategy to obtain a MRD-negative CR before alloHSCT,[48] but several new drugs are currently being developed and evaluated in AML.[49] Possible other strategies may include efforts to improve allogeneic immunotherapy by early tapering of immunosuppression and/or pre-emptive donor lymphocyte infusions, which also could be guided by MRD. In addition, the continued application of novel post-transplant strategies including epigenetic therapy to enhance the GVL-effect (ie, demethylating agents and histone deacetylase inhibitors[50,51]), new agents such as tyrosine kinase inhibitors for specific molecular mutations (ie, *FLT3*-ITD[52], *IDH*1/2[53,54]) or targeted immunotherapy with chimeric antigen receptor T-cells in MRD-positive patients may offer further therapeutic options minimizing relapse after alloHSCT.

Collectively, our study shows that the GVL-effect was strikingly similar in MRD-positive and MRD-negative patients. The personalized application of alloHSCT should take MRD-response into account and also risk scores for NRM as GVL is not invariably blunted by NRM. Further prospective studies are needed to evaluate whether the conversion of MRD-positivity into a MRD-negative remission prior alloHSCT further optimizes outcome and how the GVL-effect after alloHSCT can be optimized. Precision medicine for patients with AML is urgently needed, thus the decision to transplant or not in an individual patient might depend on weighing the risk of relapse versus the personalized risk of NRM.

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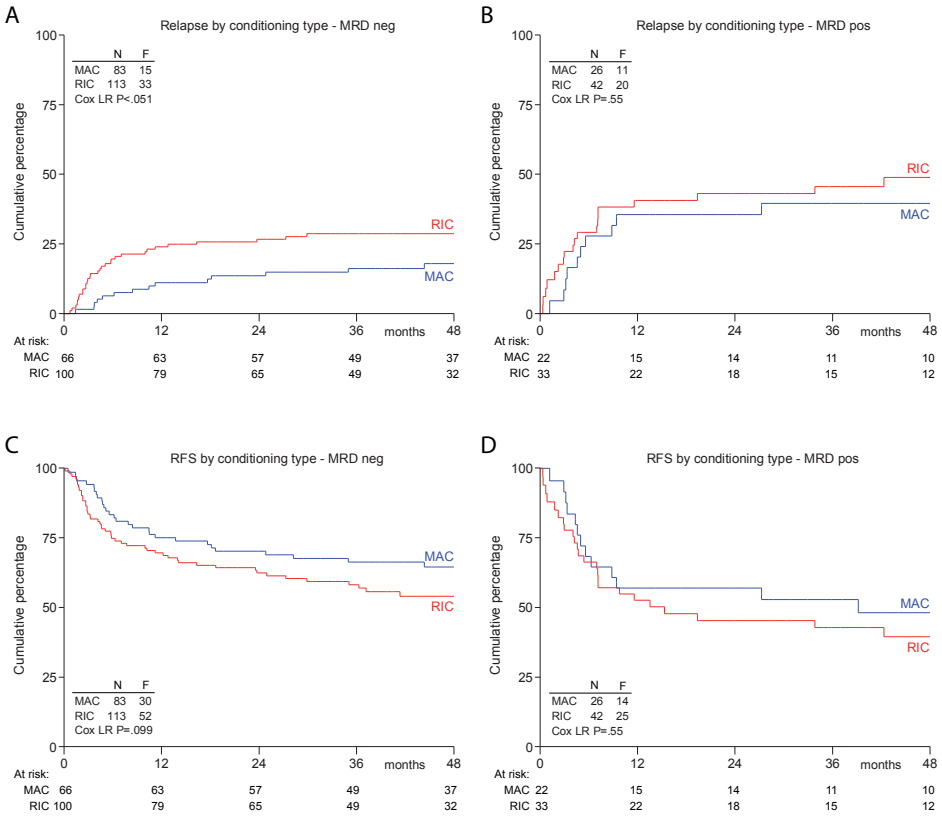
SUPPLEMENTARY APPENDIX

Supplementary Table 1 Treatment outcome at 4 years

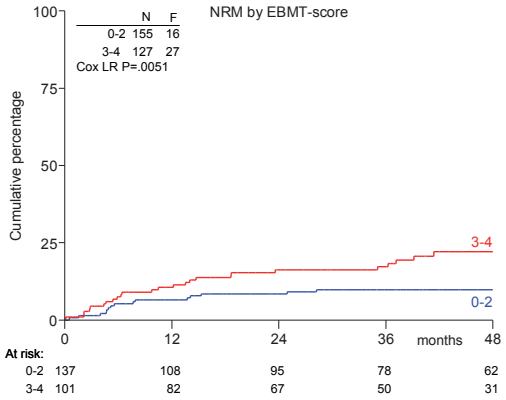
	MRD negative (N=418)	MRD positive (N=129)
All patients		
OS	65 ±2%	50 ±5%
RFS	58 ±3%	38 ±4%
Relapse	32 ±2%	54 ±4%
NRM	10 ±2%	8 ±2%
CT/auto		
OS	71 ±3%	53 ±7%
RFS	58 ±4%	31 ±6%
Relapse	38 ±3%	66 ±6%
NRM	4 ±1%	3 ±2%
Allo		
OS	60 ±4%	47 ±6%
RFS	58 ±4%	44 ±6%
Relapse	26 ±3%	45 ±6%
NRM	16 ±3%	12 ±4%

Abbreviations: MRD, minimal residual disease; OS, overall survival; RFS, relapse-free survival; NRM, non-relapse mortality; CT, chemotherapy; Auto, autologous hematopoietic stem cell transplantation; Allo, allogeneic hematopoietic stem cell transplantation; EBMT,Eeuropean group for Blood and Marrow Transplantation

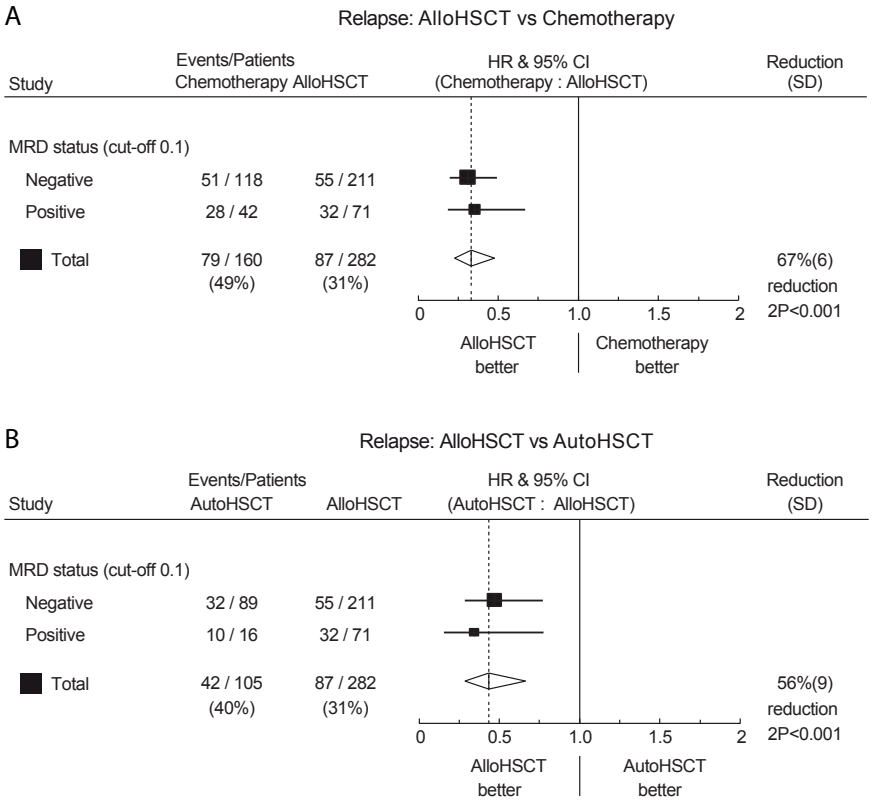


Supplementary Figure 1 Outcome by conditioning type

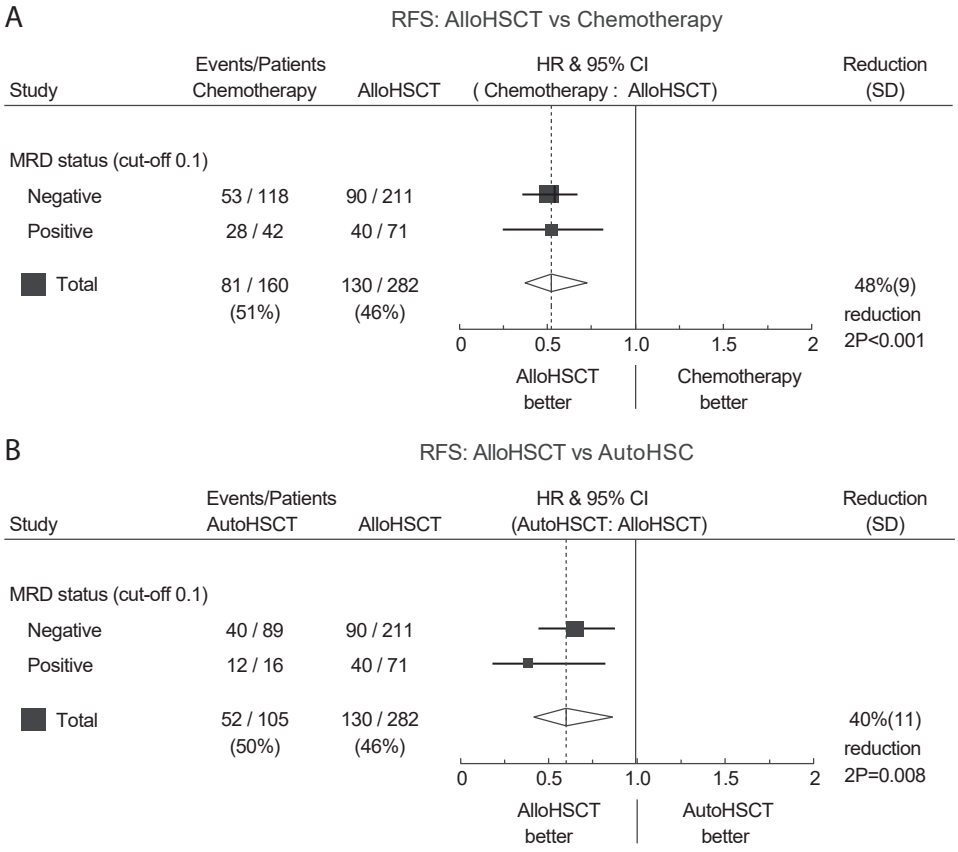
Kaplan-Meier estimates of the cumulative incidence of relapse in minimal residual disease (MRD) negative patients (**A**), cumulative incidence of relapse in MRD positive patients (**B**), relapse-free survival (RFS) in MRD negative patients (**C**), and RFS in MRD positive patients (**D**) by conditioning type in patients with AML in first complete remission from start of PRT. Abbreviations: MAC, myeloablative conditioning; RIC, reduced intensity conditioning; F, number of failures (ie, death whatever the cause); and N, number of patients



Supplementary Figure 2 NRM by EBMT-score
Kaplan-Meier estimates of non-relapse mortality (**A**) by EBMT-score, split into a low and a high risk group, from start of alloHSCT. Abbreviations: F, number of failures (ie, death whatever the cause); and N, number of patients



Supplementary Figure 3 Forest plots of relapse comparing alloHSCT to CT and autoHSCT
Forest plot of pooled estimates of the relative reduction (hazard ratio (HR) and 95% confidence interval (CI)) of relapse by MRD status comparing alloHSCT and chemotherapy (**A**) or alloHSCT and autoHSCT (**B**)



Supplementary Figure 4 Forest plots of RFS comparing alloHSCT to CT and autoHSCT
Forest plot of pooled estimates of the relative reduction (hazard ratio (HR) and 95% confidence interval (CI)) of RFS by MRD status comparing alloHSCT and chemotherapy (**A**) or alloHSCT and autoHSCT (**B**)

6

ALTERNATIVE DONORS FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN POOR RISK AML IN CR1

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Blood Advances. 2017;1:477-85.

ABSTRACT

Allogeneic hematopoietic stem cell transplantation (alloHSCT) remains the treatment of choice to consolidate remission in patients with poor-risk acute myeloid leukemia (AML). With increasing alternative donors available, the preferred donor or stem cell source is debated. We set out to study outcome in recipients of alloHSCT with poor-risk AML in first complete remission (CR1) by donor type. A total of 6545 adult patients with poor-risk AML in CR1 receiving an alloHSCT using matched related donor (MRD, n = 3511) or alternative donors, including 10/10 (n = 1959) or 9/10 matched unrelated donors (MUDs, n = 549), umbilical cord blood (UCB) grafts (n = 333), or haplo-identical (haplo) donors (n = 193) were compared. Overall survival (OS) at 2 years following MRD alloHSCT was an estimated 59 ± 1%, which did not differ from 10/10 MUD (57 ± 1%) and haplo alloHSCT (57 ± 4%). OS, however, was significantly lower for 9/10 MUD alloHSCT (49 ± 2%) and UCB grafts (44 ± 3%), respectively ($P < .001$). Non-relapse mortality (NRM) depended on donor type and was estimated at 26 ± 3% and 29 ± 3% after haplo alloHSCT and UCB grafts at 2 years vs 15 ± 1% following MRD alloHSCT. Multivariable analysis confirmed the impact of donor type with OS following MRD, 10/10 MUD, and haplo alloHSCT not being statistically significantly different. NRM was significantly higher for alternative donors as compared with MRD alloHSCT. Collectively, these results suggest that alloHSCT with MRDs and 10/10 MUDs may still be preferred in patients with poor-risk AML in CR1. If an MRD or 10/10 MUD is not available, then the repertoire of alternative donors includes 9/10 MUD, UCB grafts, and haplo-identical donors. The latter type of donor is increasingly applied and now approximates results with matched donors.

KEY POINTS

- The preferred donor for patients with poor-risk AML in CR1 proceeding to alloHSCT include MRD or 10/10 MUD.
- Alternative donors are 9/10 MUD, UCB grafts, and especially haplo, but sufficient numbers and follow-up to define a hierarchy are lacking.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the most effective post-remission treatment for prevention of relapse in poor-risk acute myeloid leukemia (AML) in first complete remission (CR1).[1,2] Although most patients lack an human leukocyte antigen (HLA) matched related donor (MRD), an alternative donor is available for almost every patient in need of an alloHSCT.[3] Although the probability of identifying an adult matched unrelated donor (MUD) can be as high as 60% to 80% for Caucasian patients, finding a suitable MUD for patients from ethnic minorities is less successful.[4-6] One allele mismatched unrelated donor (MMUD) may serve as a good alternative, but outcome has been associated with approximately 10% loss in overall survival (OS), which has predominantly been ascribed to increased non-relapse mortality (NRM).[7] Following the favorable results in pediatric patients, alloHSCT with umbilical cord blood (UCB) grafts was also developed in adult patients. Results of UCB alloHSCT in retrospective registry studies approximated those of MUD alloHSCT, although hematopoietic recovery is delayed compared with MUD alloHSCT and graft failure was more frequently observed.[8-10] More recently, a revived interest in the use of haplo-identical (haplo) donors has become apparent, because of improved transplantation techniques and pharmacological manipulation of host-versus-graft and graft-versus-host reactions.[11] Although each type of donor and/or stem cell source has its own advantages and drawbacks, comparative studies evaluating survival estimates in well-defined groups of patients are scarce. Here, we set out to compare outcome in patients with poor-risk AML in CR1 receiving alloHSCT between 2000 and 2014, using either MRD, 10/10 or 9/10 MUDs, haplo or UCB grafts.

METHODS

Patients

A total of 6545 adult patients with poor-risk AML in CR1 receiving an alloHSCT between 2000 and 2014 and reported to the European Society for Blood and Marrow Transplantation (EBMT) Acute Leukemia Working Party and Eurocord were eligible for the analysis. Patients were transplanted with an MRD (n = 3511) or alternative donors, including 10/10 (n = 1959) or 9/10 MUDs (n = 549), UCB grafts (n = 333), or haplo (n = 193). Poor-risk AML was defined as described previously,[12] with either white blood cell count (WBC) >100 x 10^9/L at diagnosis, secondary AML, cytogenetic abnormalities associated with adverse risk according to European LeukemiaNET classification,[13] presence of an fms-like tyrosine kinase 3 internal tandem duplication (*FLT3*-ITD), or no CR after 1 cycle of induction chemotherapy. Patients received conditioning therapy followed by infusion of donor cells with either MRD or alternative donors. In vivo T-cell depletion was defined as the use of either antithymocyte globulin (ATG) or alemtuzumab. No ex vivo T-cell depletion was performed on any of the patient grafts. The degree of HLA-matching was 9 or 10 of a 10 allele match for the MUDs, with mismatches allowed at HLA-A, HLA -B, HLA -C, HLA -DR, or HLA-DQ levels for the 9/10 MUDs (Supplementary Table 1). AlloHSCT with UCB grafts was performed with either single or double cords. HLA matching for UCB was done according to the standard criteria on antigen level for A and B and allele level for DRB1. Haplo was defined as ≥4/8 HLA match. This study was a retrospective multicenter analysis and was performed in accordance with the principles of the Declaration of Helsinki and approved by the Acute Leukemia Working Party of the EBMT group. The EBMT is a nonprofit, scientific society representing more than 600 transplant centers, mainly in Europe. The EBMT promotes all activity aiming to improve stem cell transplantation or cellular therapy, which includes registering all the activity relating to stem cell transplants. Data are entered, managed, and maintained in a central database with Internet access; each EBMT center is represented in this database. There are no restrictions on centers for reporting data, except for those required by the law on patient consent, data confidentiality, and accuracy. Quality control measures included several independent systems: confirmation of validity of the entered data by the reporting team, selective comparison of the survey data with minimum essential data A data sets in the EBMT registry database, cross-checking with the national registries, and regular in-house and external data audits. Since 1990, patients have provided informed consent authorizing the use of their personal information for research purposes.

End points

The primary end point of the study was OS at 2 years. Secondary end points included relapse-free survival (RFS), relapse, NRM, and acute and chronic graft-versus-host disease (GVHD) at 2 years. All outcome parameters were measured from the date of transplantation. The

event for OS was death, whatever the cause, and patients were censored at the date of last contact if alive. The events for RFS were death in CR1, designated as NRM, or hematological relapse of the leukemia. Cumulative incidences of chronic GVHD were estimated in patients without graft failure with death without chronic GVHD as competing risk.

Statistical methods

Patient, disease, and transplant characteristics were compared by using the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. Probabilities of OS and RFS were calculated using the Kaplan-Meier estimate.[14] Cumulative incidence curves were used to estimate NRM and relapse because NRM and relapse were competing events.[15] Outcome estimates are at 2 years unless explicitly stated otherwise. Multivariable Cox regression analysis for OS, RFS, relapse, and NRM was applied with adjustment for covariates, which were selected based on a *P* value < .05 by univariate analysis. All analyses were done with Stata Statistical Software: release 13.1 (Stata Corporation; 2013, College Station, TX, USA).

RESULTS

Patient characteristics

A total of 6545 patients with poor-risk AML in CR1 were included. Patients received an alloHSCT from either an MRD (n = 3511), 10/10 MUD (n = 1959), 9/10 MUD (n = 549), UCB graft (n = 333), or haplo (n = 193). Patient characteristics are shown in Table 1, including the different poor-risk features of the AML, which were differentially distributed among the donor types. Recipients of UCB grafts were significantly younger compared with the other donor types (median 48 vs 52 years, *P* < .001). Poor-risk cytogenetics and a *FLT3*-ITD were more frequently present in recipients of UCB grafts and haplo alloHSCT (*P* = .016 and *P* < .001). The median time from diagnosis to alloHSCT was 4.7 months, which was significantly shorter for recipients of an MRD compared with the alternative donors (median 5.8 months, *P* < .001). In addition, alternative donor transplantation has been performed more frequently in recent years as compared with MRD alloHSCT (*P* < .001). The median follow-up of patients still alive differed between the patient groups with a follow-up time of 43 months (range, 1-188) for recipients of MRD alloHSCT, whereas follow-up was shorter for 10/10 MUD (24 months; range, 1-159), 9/10 MUD (26 months; range, 1-139), UCB (24 months; range, 2-124), and haplo (22 months; range, 1-120).

Table 1 Patient characteristics

	Donor source										p-value
	MRD (N=3511)		MUD 10/10 (N=1959)		MUD 9/10 (N=549)		CB (N=333)		Haplo (N=193)		
Sex											.019
Male	1809	52%	1034	53%	275	50%	146	44%	110	57%	
Female	1701	48%	925	47%	273	50%	187	56%	83	43%	
Age (years)											<.001
Median	50		54		52		48		51		
Range	18-74		18-80		18-74		18-72		18-75		
WBC at diagnosis											.009
>100	1716	49%	897	46%	264	48%	177	53%	69	36%	
<=100	435	12%	176	9%	47	9%	36	11%	8	4%	
Missing	1360	39%	886	45%	238	43%	120	36%	116	60%	
Secondary AML											<.001
No	2207	63%	1068	55%	318	58%	208	62%	116	60%	
Yes	1304	37%	891	45%	231	42%	125	38%	77	40%	
Poor risk cytogenetics (-7/-5/complex/11q23)											.016
No	2028	58%	1109	57%	300	55%	163	49%	101	52%	
Yes	1483	42%	850	43%	249	45%	170	51%	92	48%	
***FLT3*-ITD**											<.001
No	366	10%	271	14%	83	15%	53	16%	50	26%	
Yes	605	17%	353	18%	101	18%	72	22%	42	22%	
Missing	2540	72%	1335	68%	365	66%	208	62%	101	52%	
CR reached after											<.001
Cycle 1 (early CR)	2536	72%	1529	78%	404	74%	253	76%	144	75%	
Cycle 2 (late CR)	975	28%	430	22%	145	26%	80	24%	49	25%	
Time from diagnosis to PRT (months)											<.001
Median	4.7		5.6		6.1		5.9		6.2		
IQ range	3.7-6.1		4.3-7.2		4.7-8.1		4.8-7.8		4.5-8.7		
Year of PRT											<.001
Median	2008		2011		2011		2010		2012		
Range	2000-2014		2000-2014		2000-2014		2000-2014		2000-2014		

Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haplo-identical; WBC, white blood cell count; AML, acute myeloid leukemia; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; IQ, interquartile range; CR, complete remission; and PRT, post-remission treatment

Table 2 Transplant characteristics

	Donor source										p-value
	MRD (N=3511)		MUD 10/10 (N=1959)		MUD 9/10 (N=549)		CB (N=333)		Haplo (N=193)		
Conditioning											<.001
MAC	1951	56%	888	45%	256	47%	145	44%	104	54%	
RIC	1518	43%	1056	54%	291	53%	183	55%	89	46%	
Missing	42	1%	15	1%	2	0%	5	2%	0		
Peripheral stem cells											<.001
No	712	20%	315	16%	76	14%	333	100%	100	52%	
Yes	2799	80%	1644	84%	473	86%	0		93	48%	
CMV patient/donor											<.001
neg/neg	717	20%	585	30%	129	23%	64	19%	32	17%	
pos/neg	522	15%	582	30%	166	30%	93	28%	35	18%	
neg/pos	324	9%	166	8%	74	13%	42	13%	12	6%	
pos/pos	1587	45%	582	30%	166	30%	78	23%	111	58%	
Missing	361	10%	44	2%	14	3%	56	17%	3	2%	
TBI given											<.001
No	2480	71%	1422	73%	424	77%	130	39%	141	73%	
Yes	1028	29%	535	27%	124	23%	203	69%	52	27%	
Female donor to male recipient											<.001
No	2710	77%	1739	89%	472	86%	273	82%	147	76%	
Yes	801	23%	220	11%	77	14%	60	18%	46	24%	
In vivo T cell depletion											<.001
No	2354	67%	535	27%	73	13%	198	59%	111	58%	
ATG	707	20%	1201	61%	393	72%	123	37%	78	40%	
Alemtuzumab	237	7%	216	11%	80	15%	0		3	2%	
Missing	213	6%	7	0%	3	1%	12	4%	1	1%	

Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haplo-identical; MAC, myeloablative condioning; RIC, reduced intensity condtioning; CMV, cytomegalovirus; TBI, total body irradiation

Transplant characteristics

Myeloablative conditioning (MAC) was applied in a higher proportion in recipients of haplo (54%) and MRD (56%) compared with patients receiving MUD (46%) or UCB grafts (45%) (Table 2). The vast majority of MRD and MUD received peripheral blood stem cells, whereas 52% of the haplo recipients received bone marrow cells. Both family donors had a cytomegalovirus (CMV) donor match in more than two-thirds of the transplants, whereas a CMV mismatch was present in MUDs and UCB grafts in about 40% of the transplants.

Conditioning with total body irradiation was performed mostly in recipients of UCB grafts (69%), particularly in patients who received a UCB graft following reduced intensity conditioning (RIC) (80%). In vivo T-cell depletion with either ATG or alemtuzumab was used in the majority of MUDs (75%). In the haplo group, 90 (47%) haplo recipients received post-transplant cyclophosphamide without ATG, whereas 62 (32%) patients received an ATG-based regimen as GVHD-prophylaxis. Fourteen (7%) patients received both ATG and post-transplant cyclophosphamide following a haplo donor.

Table 3 Transplant outcome

	Donor source					p-value
	MRD (N=3511)	**MUD 10/10 (N=1959)**	**MUD 9/10 (N=549)**	**CB (N=333)**	**Haplo (N=193)**	
						<.001
Graft failure	53 2%	29 1%	20 4%	30 9%	11 6%	
Time to engraftment (days)						<.001
Median	16	17	17	23	18	
IQ range	13-20	14-20	14-20	17-30	15-23	
Acute Graft-versus-Host Disease (maximum grade)						<.001
Grade 0-1	2623 75%	1369 70%	374 68%	220 66%	140 73%	
Grade 2-4	769 22%	518 26%	156 28%	99 30%	49 25%	
Unknown	119 3%	72 4%	19 3%	14 4%	4 2%	
Time transplant to acute GvHD (days)						.11
Median	28	27	25	28	29	
IQ range	18-47	17-43	16-42	19-38	18-48	
Chronic Graft-versus-Host Disease						<.001
Mild	533 15%	283 14%	81 15%	28 8%	39 20%	
Extensive	712 20%	310 16%	73 13%	37 11%	18 9%	
Time transplant to chronic GvHD (months)						<.001
Median	6.0	5.4	5.6	4.9	6.4	
IQ range	4.0-9.6	3.7-8.3	3.7-8.3	4.1-6.9	4.1-9.6	
Outcome at 2 years						
OS	59±1%	57±1%	49±2%	44±3%	57±4%	
RFS	53±1%	53±1%	44±2%	41±3%	52±4%	
Relapse	32±1%	27±1%	31±2%	30±3%	22±3%	
NRM	15±1%	20±1%	24±2%	29±3%	26±3%	

Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haplo-identical; GvHD, graft versus host disease; IQ, interquartile; OS, overall survival; RFS, relapse-free survival; NRM, non-relapse mortality.

Transplant outcome

The rate of graft failure at 100 days after transplant was significantly higher in recipients of UCB grafts and haplo compared with MRD and 10/10 MUD alloHSCT (9% and 6% vs 2% and 1%, $P < .001$, respectively; Table 3). Maximum grade of acute GVHD was slightly increased in recipients of 9/10 MUDs and UCB grafts ($P < .001$; Table 3; Supplemental Table 2). Limited chronic GVHD was less frequently present in recipients of UCB grafts, and the highest incidence of chronic extensive GVHD was observed in MRD recipients (Table 3). The cumulative incidence of chronic GVHD by MRD, 10/10 MUD, 9/10 MUD, UCB and haplo at 2 years is an estimated 38 ± 1%, 36 ± 1%, 33 ± 2%, 24 ± 2%, 37 ± 4%, respectively. With a median follow-up of 32 months, OS at 2 years was not significantly different between MRD alloHSCT, 10/10 MUD alloHSCT, and haplo alloHSCT (59 ± 1%, 57 ± 1%, and 57 ± 4%, respectively, $P = .19$; Figure 1A).

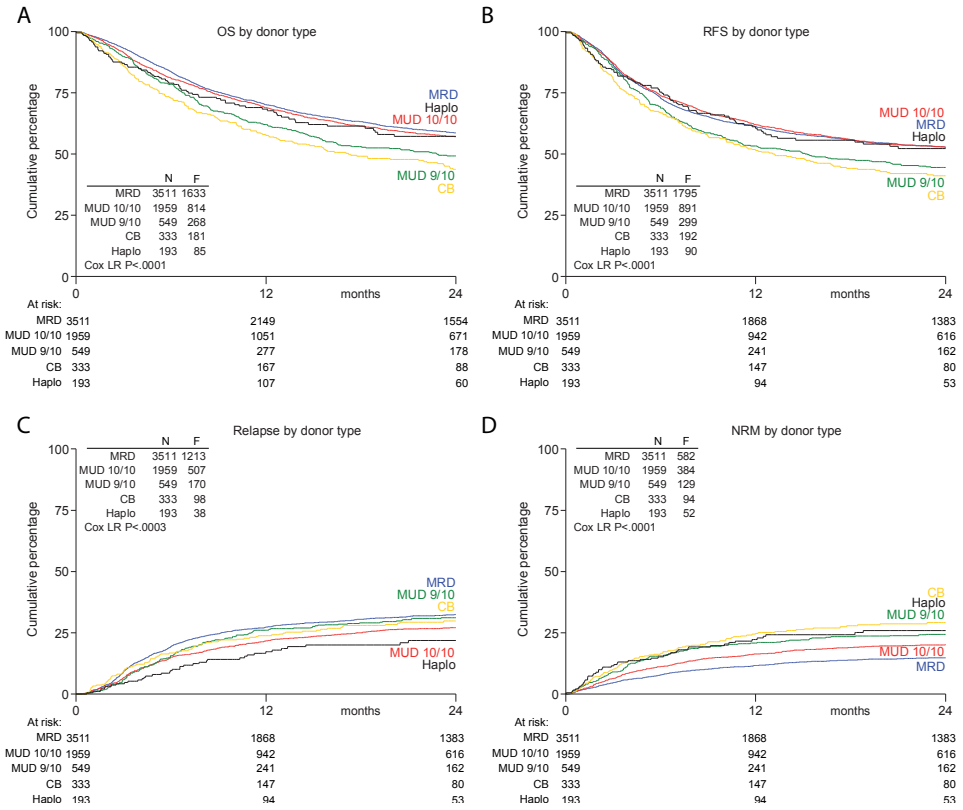

Figure 1 Outcome by different donor types
Kaplan-Meier estimates of OS (**A**), RFS (**B**), relapse (**C**), and NRM (**D**) by donor type of patients with poor-risk AML in CR1. Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haplo-identical donor; F, number of failures (ie, death whatever the cause); N, number of patients; and Cox LR, cox likelihood ratio

However, OS was significantly lower in recipients of 9/10 MUD alloHSCT and UCB grafts compared with MRD alloHSCT (49 ± 2%, 44 ± 3%, and 59 ± 1%, respectively, *P* < .001; Figure 1A). These results were similar in subgroups of poor-risk cytogenetics and secondary AML (Supplementary Figure 1). RFS was 53 ± 1%, 53 ± 1% and 52 ± 4% at 2 years following MRD, 10/10 MUD, and haplo alloHSCT, respectively, which was significantly (*P* < .001) better than following UCB grafts (41 ± 3%) or 9/10 MUD alloHSCT (44 ± 2%) (Figure 1B). The cumulative incidence of relapse at 2 years is an estimated 22 ± 3% for haplo alloHSCT, whereas other types of donor transplantation were associated with a relapse incidence of about 30% (Figure 1C). NRM depended on donor type and is an estimated 26 ± 3% and 29 ± 3% after haplo and UCB alloHSCT at 2 years, respectively, vs 15 ± 1% following MRD alloHSCT (Figure 1D). Causes of death by donor type are shown in Supplementary Table 3. Infections and GVHD were the most common causes of non-relapse death, which were increased in the alternative donor transplants.

Multivariable analysis

The multivariable analysis is shown in Table 4 and was performed with adjustment for donor type, age, cytogenetics, secondary AML, time interval from diagnosis to transplant, year of transplant, in vivo T-cell depletion, and conditioning type. OS was not significantly different comparing alloHSCT following MRD with 10/10 MUD, and haplo alloHSCT (hazard ratio [HR], 0.99 and 1.12, respectively). OS following both 9/10 MUD and UCB grafts was significantly worse compared with MRD (HR, 1.23; *P* = .001; and HR, 1.54; *P* < .001, respectively). A similar pattern was found for RFS with non-significant differences for MRD, 10/10 MUD, and haplo alloHSCT, whereas both 9/10 MUD alloHSCT and UCB grafts were associated with worse RFS. Relapse was decreased for 10/10 MUD (HR, 0.74; *P* < .001), and haplo (HR, 0.60; *P* = .001) compared with MRD alloHSCT. NRM was significantly higher for all alternative donors compared with MRD alloHSCT. Older age was associated with increased risk for all outcome parameters. Both poor-risk cytogenetics and secondary AML had an increased HR for OS, RFS and relapse, whereas a shorter time from diagnosis to transplant predicted for better OS, RFS, and relapse. A higher HR for relapse was found for RIC compared with MAC (HR, 1.23; *P* < .001), which was counterbalanced by a lower HR for NRM (0.78; *P* < .001), resulting in similar OS and RFS comparing RIC and MAC. A detailed analysis of the different alternative donors following either a RIC or MAC preparative regimen is presented in Supplementary Table 4. Higher NRM associated with 9/10 MUD, UCB, and following haplo donors was observed after both RIC and MAC, but appeared most pronounced after MAC. OS again showed similar survival following MRD and 10/10 MUD.

Table 4 Multivariable analysis

	OS			RFS			Relapse			NRM		
	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value
Donor source												
MUD 10/10 vs. MRD	0.99	0.90-1.09	.89	0.93	0.85-1.02	.11	0.74	0.66-0.83	<.001	1.39	1.19-1.62	<.001
MUD 9/10 vs. MRD	1.23	1.07-1.42	.005	1.17	1.02-1.34	.023	0.93	0.78-1.11	.44	1.77	1.43-2.20	<.001
CB vs. MRD	1.54	1.31-1.81	<.001	1.37	1.17-1.60	<.001	0.96	0.77-1.19	.69	2.41	1.92-3.04	<.001
Haplo vs. MRD	1.12	0.89-1.40	.34	1.00	0.81-1.25	.97	0.60	0.44-0.84	.001	1.98	1.47-2.68	<.001
Age†	1.17	1.13-1.21	<.001	1.13	1.09-1.16	<.001	1.07	1.02-1.12	.002	1.22	1.16-1.29	<.001
Poor risk cytogenetics (yes vs. no)	1.31	1.20-1.44	<.001	1.36	1.25-1.48	<.001	1.66	1.49-1.85	<.001	0.95	0.82-1.09	.47
Secondary AML (yes vs. no)	1.35	1.23-1.48	<.001	1.33	1.22-1.46	<.001	1.28	1.14-1.44	<.001	1.40	1.21-1.61	<.001
Time from diagnosis to transplant‡	0.99	0.98-1.00	.095	0.99	0.98-1.00	.039	0.97	0.96-0.99	.002	1.00	0.99-1.02	.69
Year of transplant†	0.96	0.85-1.09	.50	0.95	0.84-1.06	.36	1.03	0.89-1.19	.68	0.80	0.66-0.98	.029
Conditioning (RIC vs. MAC)	0.98	0.90-1.06	.59	1.03	0.95-1.12	.42	1.23	1.11-1.37	<.001	0.78	0.68-0.89	<.001
In vivo T-cell depletion (yes vs. no)	1.04	0.95-1.13	.37	1.06	0.98-1.15	.15	1.13	1.02-1.25	.023	0.94	0.82-1.07	.35

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; MUD, matched unrelated donor; MRD, matched related donor; CB, cord blood; Haplo, haplo-identical; *FLT3*-ITD, *fms-like tyrosine kinase 3* internal tandem duplication; AML acute myeloid leukemia; RIC, reduced intensity conditioning; and MAC, myeloablative conditioning

* The HRs are the estimates of the effect of covariates for each outcome parameter, adjusted for donor, age, poor risk cytogenetics, secondary AML, time from diagnosis to transplant, year of transplant, conditioning type, and in vivo T-cell depletion

† Linear with estimates of ten years difference

‡ Linear with estimates of one month difference

DISCUSSION

Post-remission therapy by alloHSCT remains the treatment of choice in poor-risk AML patients upon achieving CR1 and qualifying for intensive therapy.[1,2] Possible donor sources currently include MRDs or alternative donors such as MUDs with either a 10/10 or 9/10 HLA match, UCB grafts, or haplo. The present retrospective study from the EBMT Acute Leukemia Working Party demonstrates similar OS for patients with poor-risk AML in CR1 following alloHSCT with either MRD or 10/10 MUD. In contrast, recipients of 9/10 MUD and UCB grafts experienced worse outcome compared with MRD or 10/10 MUD, which was mainly the result of increased NRM. Recipients of T-cell replete haplo alloHSCT showed encouraging outcomes, which appeared not statistically different from MRD and 10/10 MUD, although a larger cohort and longer follow-up may be needed.

Historically, alloHSCT with an MRD has been the preferred type of donor for patients with hematological diseases. However, 70% of the patients lack a suitable MRD and the use of older MRD in elderly AML patients has recently been questioned.[16,17] The present study confirms that an MRD should still be considered the preferred donor. AlloHSCT with a 10/10 MUD yielded similar survival in the present study, confirming 10/10 MUD as the preferred alternative if an MRD is not available. Several study groups have compared outcome of transplantation using either MRD or MUD in patients with AML and reported similar survival rates.[18-22] Some studies reported slightly higher NRM following MUD, whereas counterbalancing lower relapse resulted in similar outcome compared with MRD, which was also found in the present study.

Inferior survival was found for recipients of MMUDs (9/10 HLA-match) compared with MRD, which was primarily caused by increased incidence of (severe) GVHD and subsequent NRM. A recent meta-analysis of 7 retrospective studies comparing 10/10 MUD and 9/10 MUD alloHSCT showed a 27% increased risk of mortality for recipients of a 9/10 MUD.[23] Here, a similarly increased risk (25%) was found when comparing 9/10 MUDs with 10/10 MUDs in patients with poor-risk AML in CR1. These results suggest that transplants using 9/10 MUD may be followed by more stringent prevention of GVHD to limit NRM. Studies addressing the value of intensified GVHD-prophylaxis, such as being applied in a haplo alloHSCT setting, may possibly direct how to improve transplants with MMUDs.[11,24]

Following the initial favorable results in pediatric patients, alloHSCT with UCB grafts was also developed in adults with acute leukemia. Although a higher incidence of graft failure and delayed hematopoietic recovery are associated with UCB grafts, largely similar outcomes compared with MRD, MUD, or MMUD alloHSCT were reported.[9,10,25,26] However, these studies included different groups of patients, which hampered a precise comparison. The present study in a homogenous group of patients shows that alloHSCT with UCB grafts is still associated with higher NRM compared with MRD, which resulted in significantly lower OS. The incidence of graft failure following alloHSCT with UCB grafts in our study is an estimated

9% and the majority of causes of death were infections, to which graft failure and delayed recovery contributed. No significant differences in outcome were found between single vs double UCB grafts, and no difference between UCB with low vs high total nucleated cells at infusion of the UCB grafts were found, although information was not available for all UCB grafts (data not shown). These results suggest that improving hematopoietic engraftment and hematopoietic recovery remains a major challenge in UCB graft alloHSCT in adult patients, which is currently addressed and studied by several groups exploring expansion of UCB hematopoietic stem cells.[27,28]

Allogeneic transplantation with a haplo-identical family donor was extensively studied by the Perugia group.[29] Although that approach consisting of transplantation with high numbers of $CD34^+$ cells, intensified conditioning and stringent GVHD-prophylaxis appeared to result in favorable engraftment in the majority of patients, a relatively high NRM precluded application on a broader scale. More recently, both the approach by the Baltimore group based on post-transplant cyclophosphamide and the Chinese approach based on in vivo T-cell depletion, were demonstrated to result in favorable engraftment, limited GVHD, and limited NRM.[30,31] A recent biologically randomized study from China suggested similar outcomes using matched related or haplo-identical family donors.[30] Updated results from the Baltimore group also suggested similar survival following haplo alloHSCT and matched donor alloHSCT.[31] A recent retrospective study from the Acute Leukemia Working Party of the EBMT showed a similar relapse incidence in patients receiving T-cell replete and T-cell depleted haplo alloHSCT as compared with MRD, which suggested a similar graft-versus-leukemia effect.[32] The present study included T-cell replete haplo alloHSCT which appeared associated with a stronger graft-versus-leukemia effect compared with MRD (HR 0.60), whereas severe grades of acute and chronic GVHD were relatively low as compared with the other donor types. A large retrospective study of the Center for International Blood an Marrow Transplant Research comparing MRD with haplo alloHSCT using post-transplant cyclophosphamide also found less GVHD following haplo alloHSCT and an overall similar outcome.[33] Our study showed a relatively low incidence of relapse following haplo alloHSCT, but a higher incidence of NRM, resulting in similar outcome as compared with MRD alloHSCT. Both the relatively short follow-up and unknown patient selection preclude more definite conclusions as regards the comparison with sibling donors. However, haplo alloHSCT was also suggested to result in better outcome as compared with UCB alloHSCT. In a less homogenous group of AML patients, UCB and haplo alloHSCT were previously suggested to result in similar overall outcome.[34] The latter results are in line with the 2 parallel phase 2 trials of Brunstein et al,[35] which addressed UCB grafts and unmanipulated haplo alloHSCT including post-transplant cyclophospamide. Although haplo was associated with less NRM, a higher relapse rate counterbalanced that favorable effect, resulting in similar

RFS.[35] Currently, a prospective randomized phase 3 trial is being conducted comparing UCB and haplo alloHSCT (BMT CTN 1101), which will address the question how UCB and haplo alloHSCT compare in patients with hematological malignancies.

Our study may have several limitations. First, a center's preference regarding preferred alternative donors may result in selection bias.[36] Reasons for the choice of an alternative donor transplant are not registered in the EBMT database and therefore not known. We focused on a homogenous group of poor-risk AML patients with no important differences in baseline characteristics, but time intervals from diagnosis to transplant did differ, which may be associated with selection resulting from exclusion of early relapses in the group of alloHSCT recipients with the longest timeframe. Second, an increasingly important parameter is the presence or absence of minimal residual disease, which was unknown in the present study, but has recently been shown to strongly predict for subsequent relapse and overall outcome.[37-40] Although not recorded, residual disease is not routinely assessed in most centers and also not uniformly used for risk-adapted treatment; as a result, it is unlikely to have resulted in a strong selection bias. Last, the retrospective multicenter nature of our study implies that the physician/centers intention and/or preference is not taken into account, which can only be addressed in a prospective randomized study. However, prospective studies with more than 2 or 3 donor types will be extremely difficult, necessitating larger registry studies. To our knowledge, our study is the largest comparative study of MRD and alternative donors in the homogenous subgroup of patients with poor-risk ALM in CR1 in urgent need of an alloHSCT. Our results compare well with a recent study by Raiola et al, who performed a single-center retrospective study of 459 patients that received alloHSCT using different donors, including unmanipulated haplo, MRD, MUD or UCB grafts. Although the recipients suffered from various hematological malignancies and haplo has been performed more frequently in recent years, their results also suggested higher NRM in MMUD alloHSCT and following alloHSCT with UCB grafts.[41]

In conclusion, our study suggests that well-matched donors including MRD and 10/10 MUD are to be preferred over UCB and MMUD patients with poor-risk AML in CR1. Nine of 10 MUD, UCB grafts, and haplo-identical donors could be used as alternatives in case a fully matched donor is not available or an urgent transplant is required. Haplo-identical donors are increasingly used and results are encouraging. However, comparative prospective studies of haplo alloHSCT with other donor types are warranted and longer follow-up after haplo alloHSCT may be needed to definitely establish its place in the hierarchy of alternative donors.

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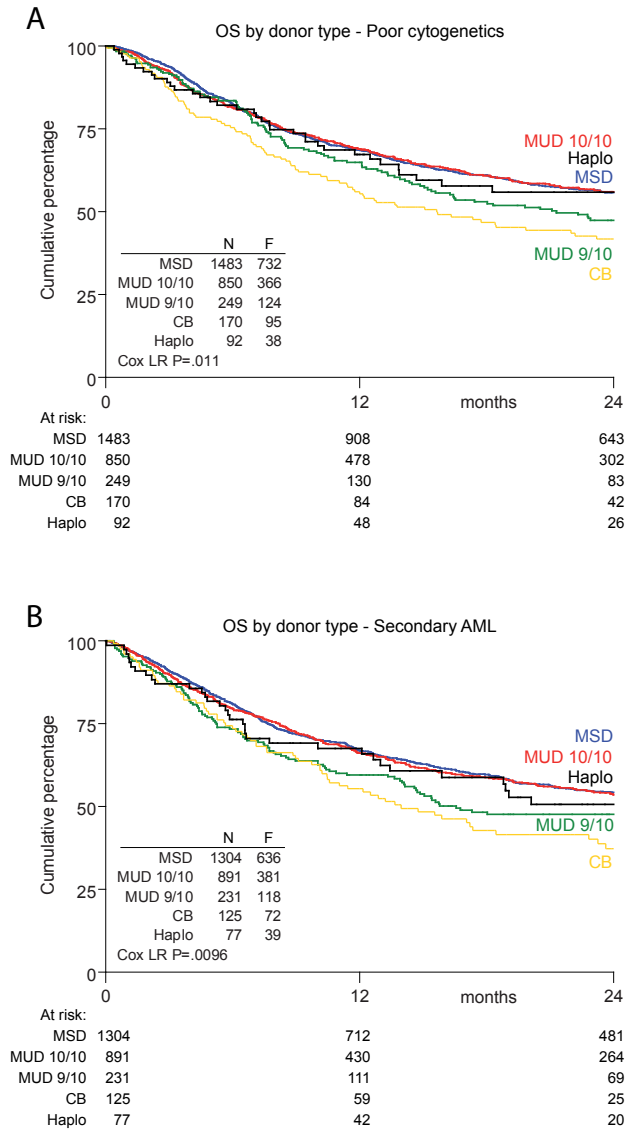
SUPPLEMENTARY APPENDIX



Supplementary Figure 1 OS by donor type in AML poor risk subcategories
Kaplan-Meier estimates of OS by donor type in patients with AML with poor risk cytogenetics (A) or secondary AML (B). Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haplo-identical donor; F, number of failures (ie, death whatever the cause); N, number of patients; and Cox LR, cox likelihood ratio

Supplementary Table 1 Mismatch loci of 9/10 matched unrelated donors

	MUD 9/10 (N=549)	
Loci		
A	150	27%
B	80	15%
C	167	30%
DR	49	9%
DQ	103	19%

Supplementary Table 2 Grades of acute graft-versus-host disease by donor

	Donor source									
	MRD (N=3511)		MUD 10/10 (N=1959)		MUD 9/10 (N=549)		CB (N=333)		Haplo (N=193)	
Acute Graft-versus-Host Disease (maximum grade)										**p<0.001**
Grade 0	2049	58%	966	49%	255	46%	160	48%	108	56%
Grade 1	574	16%	403	21%	119	22%	60	18%	32	17%
Grade 2	478	14%	351	18%	96	17%	62	19%	35	18%
Grade 3	196	6%	113	6%	37	7%	23	7%	8	4%
Grade 4	95	3%	54	3%	23	4%	14	4%	6	3%
Unknown	119	3%	72	4%	19	3%	14	4%	4	2%

Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; and haplo, haplo-identical

Supplementary Table 3 Causes of Death

	Donor source									
	MRD (N=3511)		MUD 10/10 (N=1959)		MUD 9/10 (N=549)		CB (N=333)		Haplo (N=193)	
Cause of Death										
Relapse of AML	912	56%	384	47%	118	44%	80	44%	28	33%
GvHD	247	15%	134	17%	59	22%	30	17%	10	12%
Infection	198	12%	144	18%	49	18%	43	24%	26	31%
Second malignancy	30	2%	15	2%	4	1%	4	2%	2	2%
Interstitial pneumonitis	24	1%	19	2%	4	1%	3	2%	3	4%
Veno occlusive disease	23	1%	10	1%	5	2%	6	3%	3	4%
Haemorrhage	21	1%	5	1%	2	1%	2	1%	1	1%
Cardiac toxicity	8	0%	8	1%	1	0%	0		0	
Failure/Rejection	2	0%	5	1%	0		0		1	1%
Other transplant related	74	5%	35	4%	16	6%	9	5%	7	8%
Missing	87	5%	51	6%	9	3%	4	2%	3	4%

Abbreviations: MRD, matched related donor; MUD, matched unrelated donor; CB, cord blood; haplo, haploidentical; AML, acute myeloid leukemia; and GvHD, graft versus host disease

Supplementary Table 4 Multivariable analysis by conditioning type

	OS			RFS			Relapse			NRM		
	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value	HR*	95% CI	p-value
Donor source (RIC)												
MUD 10/10 vs. MRD	0.98	0.87-1.11	.74	0.91	0.81-1.03	.14	0.70	0.60-0.81	<.001	1.50	1.22-1.85	<.001
MUD 9/10 vs. MRD	1.18	0.98-1.43	.089	1.14	0.95-1.36	.16	0.93	0.74-1.16	.51	1.74	1.29-2.35	<.001
CB vs. MRD	1.28	1.02-1.60	.038	1.21	0.98-1.51	.086	1.04	0.79-1.36	.79	1.72	1.20-2.48	.005
Haplo vs. MRD	0.93	0.67-1.31	.68	0.82	0.59-1.14	.22	0.51	0.31-0.83	.003	1.65	1.04-2.61	.045
Donor source (MAC)												
MUD 10/10 vs. MRD	0.98	0.84-1.14	.76	0.92	0.79-1.07	.26	0.76	0.63-0.92	.005	1.23	0.98-1.56	.082
MUD 9/10 vs. MRD	1.25	1.01-1.56	.045	1.18	0.96-1.46	.12	0.89	0.67-1.17	.40	1.81	1.32-2.48	<.001
CB vs. MRD	1.80	1.40-2.30	<.001	1.50	1.18-1.91	.002	0.73	0.49-1.08	.10	3.23	2.35-4.44	<.001
Haplo vs. MRD	1.26	0.93-1.72	.15	1.15	0.86-1.54	.36	0.67	0.43-1.04	.060	2.22	1.49-3.30	<.001

Abbreviations: OS indicates overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete remission (CR) or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); NRM, non-relapse mortality (with event death in first CR and censored at relapse); HR, hazard ratio; CI, confidence interval; MUD, matched unrelated donor; MRD, matched related donor; CB, cord blood; Haplo, haplo-identical; RIC, reduced intensity conditioning; and MAC, myeloablative conditioning

* The HRs are the estimates of the effect of covariates for each outcome parameter, adjusted for donor, age, poor risk cytogenetics, secondary AML, time from diagnosis to transplant, year of transplant, conditioning type, and in vivo T-cell depletion

7

PREDICTION OF NON-RELAPSE MORTALITY IN RECIPIENTS OF REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION WITH AML IN FIRST COMPLETE REMISSION

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ABSTRACT

Non-relapse mortality (NRM) after allogeneic hematopoietic stem cell transplantation (alloHSCT) can be predicted by the hematopoietic cell transplantation comorbidity index (HCT-CI) and the European Group for Blood and Marrow Transplantation (EBMT) score, which are composed of different parameters. We set out to integrate the parameters of both scores in patients with acute myeloid leukemia (AML) in first complete remission (CR1) receiving reduced intensity conditioning (RIC) alloHSCT. All parameters from the HCT-CI and the EBMT-score with the addition of patient and donor cytomegalovirus serology were evaluated in 812 patients by multivariable analysis with end-point NRM at 2 years. Subsequently, 16 parameters were selected based on hazard ratio >1.2 and were incorporated into a novel score, which was further internally validated by bootstrapping. Both the HCT-CI and the EBMT-score showed relatively weak predictive value, whereas the integrated score allowed to identify three clearly distinct risk groups with 2-year NRM estimates of 8 ± 2% (low-risk), 17 ± 2% (intermediate-risk) and 38 ± 4% (high-risk), which also translated in prediction of overall survival. Collectively, integration of the most dominant parameters from the HCT-CI and the EBMT-score allowed to develop a simple and robust, integrated score with improved prediction of NRM for AML patients proceeding to RIC alloHSCT in CR1.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the most effective post-remission treatment for prevention of relapse in patients with acute myeloid leukemia (AML) in first complete remission (CR1) as compared with non-alloHSCT approaches.[1-3] However, non-relapse mortality (NRM) may compromise the favorable effects of alloHSCT on overall survival (OS). Currently, an increasing number of patients receive their allograft after reduced intensity conditioning (RIC), because of age and/or comorbidities. AML has become the predominant indication for RIC alloHSCT and with a median age of 67-71 years in newly diagnosed AML patients,[4,5] the application of RIC alloHSCT is steadily increasing.[6] Apart from age, other important risk factors for NRM include pre-existing co-morbidities and transplantation-related risk factors such as donor source and patient/donor histocompatibility.[7-9]

In order to quantify the risk of NRM, composite risk scores have been established, which allowed to predict NRM and survival before transplantation. The most widely accepted and clinically applied predictive models are the hematopoietic cell transplantation comorbidity index (HCT-CI)[10] and the European Group for Blood and Marrow Transplantation (EBMT) score.[9] The HCT-CI originated from the Charlson Comorbidity Index[7] and was further developed by the Seattle group consisting of 17 comorbidities which contribute to a cumulative score based on each parameters' own hazard ratio (HR) as regards NRM.[10] Subsequently, three risk groups were defined with 2-year cumulative incidences of NRM of 14%, 21% and 41% in the low-, intermediate- and high-risk groups, respectively. The HCT-CI was developed and validated in a cohort of alloHSCT patients with different diseases (that is, leukemia, lymphoma, myeloma and non-malignant diseases), but has been confirmed in other disease categories, including AML.[11,12] The EBMT-score was originally developed to predict overall outcome in 3142 chronic myeloid leukemia patients by Gratwohl et al.,[9] with two patient parameters (that is, age and disease stage) and three transplantation-related parameters (that is, donor source, gender mismatch and time from diagnosis to transplantation). The score predicted for overall outcome and NRM and the score was externally validated in another chronic myeloid leukemia cohort,[13] and also in other patient categories, including AML patients.[14]

The ability of both scores to predict NRM was also confirmed in patients receiving RIC alloHSCT.[15-17] However, some reports did not confirm the predictive ability of the HCT-CI in RIC recipients with much higher baseline NRM risk (that is, older patients with a higher prevalence of comorbidities).[18-20] The EBMT-score also performed less well in older patients receiving RIC alloHSCT.[14,20-22] Considering that RIC alloHSCT is currently applied more frequently in older, medically less fit AML patients and that the EBMT-score and HCT-CI take different parameters into account, we set out to integrate the predominant parameters of each score into a novel scoring system in AML CR1 patients receiving RIC alloHSCT.

PATIENTS AND METHODS

Patients

A total of 812 adults with de novo or secondary AML in CR1 who received consolidation therapy with RIC alloHSCT between 2000 and 2011 were included in this study. All patients were documented in the EBMT database. RIC regimens consisted of either fludarabine with total body irradiation (n = 191, 23%) or chemotherapy (that is, fludarabine with busulfan or melphalan; n = 621, 77%). The dose ranges of melphalan, busulfan and fludarabine were 110-150 mg/m^2, 3.2-8.6 mg/kg and 90-300 mg/m^2 respectively. Anti-thymocyte globulin supplemented the conditioning regimen in 340 (42%) patients. Post-transplantation graft versus host disease (GVHD) prophylaxis consisted of a calcineurin inhibitor with mycopenolate mofetil or methotrexate. Patients received standard infection prophylaxis with acyclovir and trimethoprim-sulfamethoxazole post-transplant. Cytomegalovirus (CMV) DNA was monitored regularly and early treatment with ganciclovir was started in case of reactivation. In addition to baseline characteristics, pre-transplantation data were available for all patients in order to assess both the HCT-CI and the EBMT-score. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Acute Leukemia Working Party of the EBMT group.

End points

The primary end point of the study was 2-year NRM. Secondary end points included 2-year OS, relapse-free survival, relapse, 5-year NRM and acute and chronic GVHD. All outcome parameters were measured from the date of transplantation. The event for OS was death whatever the cause and patients were censored at the date of last contact if alive. The events for relapse-free survival were death in CR1, designated as NRM, or hematological relapse of the leukemia.

Statistical methods

Cumulative incidence curves were used to estimate NRM and relapse, as NRM and relapse were competing events, as well as probability of acute and chronic GVHD, with NRM without GVHD being treated as a competing event.[23] Probabilities of OS and relapse-free survival were calculated using the Kaplan-Meier estimate.

Variable selection and model development

Multivariable analysis of all parameters of both the HCT-CI and the EBMT-score was performed. We also added patient and donor CMV serology status before transplantation to the analysis, in accordance with previous observations that positive patient and/or donor CMV serology is associated with an increased risk of NRM.[14,24-26] The 17 comorbidities of the HCT-CI were assessed as previously defined.[7,10,27] In addition, donor type, donor/recipient

gender combination, time from diagnosis to transplant and age were included, as applied in the EBMT-score.[9] Of note, different cutoff points for the time interval from diagnosis to transplantation and patient age were applied, as the median time to transplant was close to 6 months and the median patient age was close to 60 years in these RIC alloHSCT recipients. The EBMT-score parameter disease stage was omitted, because all patients were transplanted in CR1. All covariates were included in both the Cox proportional hazards model and the Fine-Gray model[28,29] as follows: patient age (< versus ≥ 60 years), interval from diagnosis to transplant (< versus ≥ 6 months), type of donor (unrelated versus HLA identical sibling), female donor to male recipient (versus other gender combinations), patient CMV serology (positive versus negative), donor CMV serology (positive versus negative) and all comorbidities (present versus not present). The Cox and the Fine-Gray models were stratified on the type of RIC regimens (with or without total body irradiation) in order to allow the underlying hazard function to vary across these two conditioning modalities. Integer weights were derived from HRs estimated by the Cox model. The integrated score was the sum of integer weights obtained by rounding HRs of all covariates associated with HR > 1.2, similar to variable selection as was applied in the original development of the HCT-CI.[10]

Model validation

The model's ability to discriminate between patients with or without NRM at 2 years post-transplant was quantified by using *c* statistics.[30] For a binary outcome (survival without relapse larger or smaller than 2-years post-transplant), *c* statistics reflect the area under a receiver operating characteristic curve. A model with a *c* statistic of 1.0 indicates no false-positives, which means that no patients who did not die of NRM were missed by the model. The internal validity of the regression model was assessed using the bootstrapping technique ('rms' R package), which includes taking out random samples with replacement 300 times. For the analysis including all variables, a predictive model was developed at each step including variable selection using a P-value of 0.15.[31] The same procedure, without variable selection, was used for the Cox model including the score in three classes. The bootstrapping procedure leads to estimates of the optimism-corrected performance, which is calculated as apparent performance minus optimism. Time-dependent receiver operating characteristic curves and the area under curves (AUCs) were also estimated non-parametrically using the inverse probability of a censored weighting approach[32] with competing risks.[33] The 'timerROC' R package was used to derive confidence intervals and for comparison of the AUC obtained with the different scores. All statistical tests were performed with R package (R 3.0.1 by R development Core Team).

Table 1 Patient characteristics: transplantation related parameters

Parameter	No.	(%)
No. of patients	812	
Age (years)		
Median	58	
Range	20-76	
Time from diagnosis to alloHSCT (days)		
Median	167	
Range	50-959	
Year of transplantation		
Median	2008	
Range	2000-2011	
Female donor to male recipient		
No	658	(81)
Yes	154	(19)
Donor type		
Sib	432	(53)
MUD	380	(47)
Cytogenetic risk of the AML[34]		
Good	22	(3)
Intermediate	446	(55)
Poor	86	(11)
Unknown	258	(32)
Patient CMV serology		
Negative	250	(31)
Positive	562	(69)
Donor CMV serology		
Negative	362	(45)
Positive	450	(55)
Conditioning regimen		
Fludarabine + busulfan	372	(45)
Fludarabine + melphalan	162	(20)
Fludarabine + 2 Gy TBI	173	(21)
Fludarabine + 4 Gy TBI	18	(2)
Other	87	(11)

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplantation; Sib, sibling; MUD, matched unrelated donor; AML, acute myeloid leukemia; CMV, cytomegalovirus; Gy, Gray; TBI, total body irradiation

RESULTS

Patient characteristics

Characteristics of the 812 patients are shown in Table 1. The median age at transplantation was 58 (range: 20-76) years. Fifty percent of patients were transplanted within 167 days after diagnosis of AML. Grafts from female donors for male recipients were used in 19% and patients received an unrelated graft in 47% of the transplants. The majority of underlying leukemia's exhibited an intermediate-risk karyotype.[34] CMV serology was positive in 55% of the donors and 69% of the patients before transplantation. The median follow up time in patients alive was 34 (range: 3-138) months. The comorbidities of all patients are listed in Table 2, with active infection, cardiac disease and moderate pulmonary disease being the most frequently observed comorbidities with a prevalence of more than 15%.

Table 2 Patient characteristics: comorbidities

Parameter	No.	(%)
No. of patients	812	
Comorbidity*		
Arrhythmia	38	(5)
Cardiac	128	(16)
Cerebrovascular disease	20	(2)
Diabetes	69	(8)
Heart valve disease	45	(6)
Hepatic (mild)	80	(10)
Hepatic (severe)	21	(3)
Inflammatory bowel disease	17	(2)
Infection	192	(24)
Obesity	40	(5)
Peptic ulcer	18	(2)
Psychiatric disturbance	49	(6)
Pulmonary (moderate)	170	(21)
Pulmonary (severe)	73	(9)
Renal (moderate/severe)	27	(3)
Rheumatologic disease	21	(3)
Solid tumor	83	(10)

* Comorbidities are defined as described previously[7,10,27]

Predictive value of the EBMT-score and the HCT-CI

All patients scored at least one point on the EBMT-score but the vast majority (90%) were captured with scores of two (n = 331, 41%) or three points (n = 401, 49%). Figure 1A depicts the cumulative incidence of NRM of the 812 patients, according to the EBMT-score. The 2-year cumulative incidence of NRM by the EBMT-score estimated 14 ± 2%, 20 ± 2% and 24 ± 5% for patients with scores 1-2, 3 and ≥ 4, respectively. The *c* statistic was 0.546, indicating a weak predictive value. Non parametric estimation of the AUC was 0.575 (95% confidence interval: 0.523-0.627).

With respect to comorbidities, almost 80% of the patients scored at least one point on the HCT-CI. The 2-year cumulative incidence of NRM by the subgroups as defined by the HCT-CI estimated 11 ± 2%, 21 ± 2% and 18 ± 2%, for patients with scores 0, 1-2 and ≥ 3, respectively (Figure 1B). Also, the HCT-CI showed relatively weak predictive power with a *c* statistic of 0.580. Non parametric estimation of AUC was 0.584 (95% confidence interval: 0.527-0.640).

Model development, validation and predictive value

The HRs of all individual parameters for 2-year NRM as predicted by the integrated, multivariable Cox regression analysis including all covariates are shown in Table 3. Based on this analysis, variable selection and model development was performed. As a result, seven parameters were dismissed from the integrated model because of HRs < 1.2 (Table 3), including six comorbidities and one transplantation-related parameter: cerebrovascular disease, moderate pulmonary disease, prior solid tumors, mild hepatic disease, cardiac disease, diabetes mellitus and female donor to male recipient. Subsequently, 11 comorbidities, age, donor type, time interval from diagnosis to transplantation and patient and donor CMV serology were selected for the integrated model based on HRs of > 1.2 (Table3).

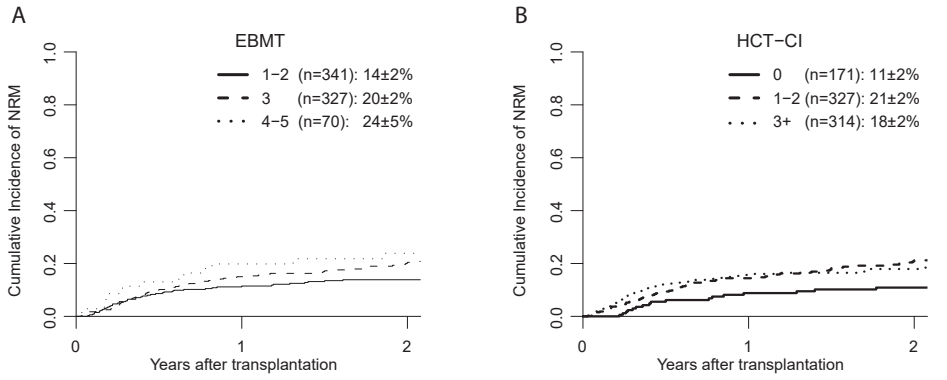

Figure 1 Non-relapse mortality by EBMT-score and the HCT-CI

Cumulative incidences of non-relapse mortality by (**A**) the EBMT-score and (**B**) the HCT-CI. Performance of the EBMT-score and the HCT-CI in predicting non-relapse mortality in these patients was tested using *c* statistics (0.546 versus 0.580, respectively), likelihood ratios (4.88 versus 3.68) and non-parametric area under the curve (0.575 versus 0.584). The event for NRM was death in CR1 with relapse as a competing event.

The selected parameters were then attributed points, as determined by their quantified, rounded HR (Table 4). As a result, six parameters were given one point, including active infection, severe pulmonary disease, unrelated donor, positive donor CMV serology, severe hepatic disease and interval from diagnosis to transplantation ≥ 6 months. Furthermore, 10 parameters were attributed two points including peptic ulcer, inflammatory bowel disease, heart valve disease, psychiatric disturbance, age at transplantation ≥ 60 years, arrhythmia, positive patient CMV serology, obesity, rheumatologic disease and moderate/severe renal disease (Table 4). As a result, the integrated score was completed to 16 parameters and *c* statistic using this model was estimated at 0.691. After bootstrapping including variable selection, the corrected *c* statistic was 0.641. Subsequently, the score was collapsed into three groups according to the number of patients and 2-year NRM in each category: 0-3 points for low-risk patients, 4-6 points for intermediate-risk patients and 7 or more for high-risk patients.

Table 3 Hazard ratios for 2-year NRM by Cox regression

Variable	HR
Cerebrovascular disease	0.76
Female donor to male recipient	0.80
Pulmonary (moderate)	0.83
Solid tumor	0.93
Hepatic (mild)	1.01
Cardiac	1.07
Diabetes	1.08
Infection	1.22
Pulmonary (severe)	1.22
Unrelated donor	1.31
Donor CMV serology positive	1.31
Hepatic (severe)	1.34
Interval diagnosis to alloHSCT	1.35
Peptic ulcer	1.51
Inflammatory bowel disease	1.52
Heart valve disease	1.53
Psychiatric disturbance	1.68
Age at alloHSCT	1.79
Arrhythmia	1.84
Patient CMV serology positive	1.87
Obesity	2.20
Rheumatologic disease	2.40
Renal (moderate/severe)	2.42

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplantation; and CMV, cytomegalovirus

Table 4 Definitions and weights of parameters included in the integrated score

Parameter	Definition*	Attributed score
Infection	Requiring continuation of antimicrobial treatment after alloHSCT	1
Pulmonary disease	DLco and/or FEV_1 ≤ 65% or dyspnea at rest or requiring oxygen	1
Unrelated donor	Matched unrelated donor	1
Donor CMV	Donor CMV IgG serology positive	1
Hepatic disease	Liver cirrhosis, bilirubin > 1.5 ULN, or AST/ALT > 2.5 x ULN	1
Time interval to alloHSCT	Time interval from diagnosis to alloHSCT ≥ 6 months	1
Peptic ulcer	Requiring treatment	2
Inflammatory bowel disease	Crohn disease or ulcerative colitis	2
Heart valve disease	Any valve disorders, except prolapsed mitral valve	2
Psychiatric disturbance	Depression or anxiety requiring psychiatric consultation or treatment	2
Age at alloHSCT	≥ 60 years	2
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	2
Patient CMV	Patient CMV IgG serology positive	2
Obesity	Patients with a body mass index > 35 kg/m^2	2
Rheumatologic disease	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Renal disease	Serum creatinine > 2mg/dL or > 177 micromol/L, on dialysis, or prior renal transplantation	2

Abbreviations: alloHSCT, allogeneic hematopoietic stem cell transplantation; DLco, diffusing capacity of carbon dioxide; FEV_1, forced expiratory volume in 1 second; CMV, cytomegalovirus; IgG, immunoglobuline G, ULN, upper limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; and CTD, connective tissue disease

* Parameters are defined as described previously[7,9,10,27]

The cumulative incidence of 2-year NRM in the integrated score estimated 8 ± 2%, 17 ± 2% and 38 ± 4% for low-, intermediate- and high-risk patients, respectively (Table 5 and Figure 2A). The non-parametric estimation of AUC 0.680 (95% confidence interval: 0.630-0.730) for the integrated score with three groups indicated relatively strong predictive power for NRM at 2-years as compared with the EBMT-score and the HCT-CI (P = 0.0009 and P = 0.0018, respectively). In addition, higher scores in the integrated score were more strongly associated with increased NRM as compared with the HCT-CI and the EBMT-score based on the likelihood ratio (50.5 versus 3.7 and 4.9, respectively). The integrated model with 3 groups was internally validated after bootstrapping with a corrected *c* statistic of 0.66. Moreover, cumulative incidences of NRM at 5 years from transplant increased to 12 ± 2%, 21 ± 2% and 44 ± 5% in the low-, intermediate- and high-risk subgroups, respectively. The cumulative incidences of 2-year NRM were similar among the different RIC regimens (data not shown). The integrated score was also significantly associated with OS, as depicted in Figure 2B and shown in Table 5. A non-significant trend was observed for the association of

the integrated score with cumulative incidence of acute GVHD grade 2 to 4 with estimates at 2 years of 18 ± 2%, 20 ± 2%, 26 ± 4% (P = 0.10) for low-, intermediate- and high-risk patients, respectively. Cumulative incidences for chronic GVHD were not different among the three risk groups, estimating 50 ± 3%, 53 ± 3%, 50 ± 5% (P = 0.49) at 2 years for low-, intermediate- and high-risk patients, respectively. Furthermore, the integrated score did not predict for relapse, with similar relapse rates among all three groups (data not shown).

Table 5 Integrated score: cumulative incidence of NRM and OS

			Probability at 2 years	
Score		**(%)**	**NRM**	**OS**
0 – 3 points	272	(33)	8 ± 2	69 ± 3
4 – 6 points	392	(48)	17 ± 2	60 ± 3
≥ 7 points	148	(18)	38 ± 4	43 ± 4
corrected c statistics			0.665	0.578
non-parametric AUC			0.680	0.598
likelihood ratio			50.5	20.7

Abbreviations: NRM, non-relapse mortality; OS, overall survival; and AUC area under the curve

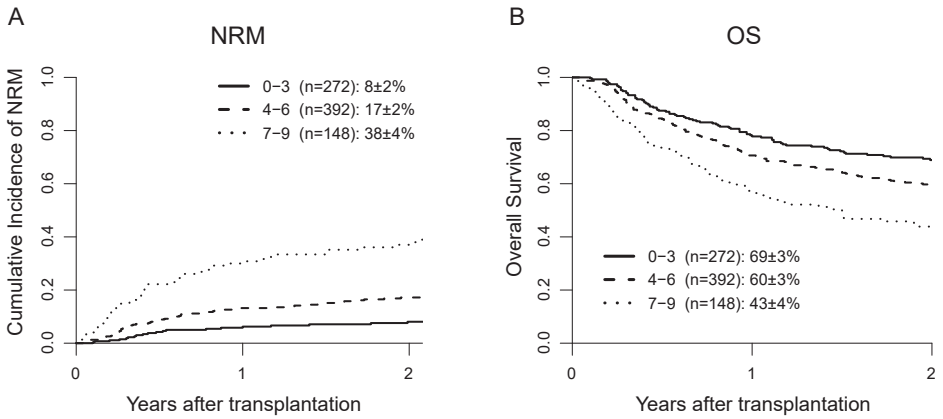

Figure 2. Overall survival and non-relapse mortality by the integrated score. (**A**) Cumulative incidence of non-relapse mortality (NRM) and (**B**) Kaplan-Meier estimates of overall survival (OS) by the integrated score. Performance of the integrated score in predicting NRM and OS in these patients is described in Table 5. The event for NRM was death in CR1 with relapse as a competing event. The event for OS was death whatever the cause and patients were censored at the date of last contact if alive.

DISCUSSION

Currently, RIC alloHSCT is increasingly applied as post-remission treatment in patients with AML in CR1,[6] who do not qualify for myeloablative alloHSCT because of age or pre-existing comorbidities. Transplant decisions in older or medically less fit patients increasingly depend on risk assessment of both the underlying leukemia and the risk of NRM.[21] In the past, composite risk scores were developed in order to optimize NRM risk assessment. The most widely applied and validated risk scores include the HCT-CI and the EMBT-score, which each use different parameters.[9,10] Of these, the EBMT-score appeared to discriminate risk groups less clear in older recipients of RIC alloHSCT.[14,21] Here, we set out to integrate these scores by reassessing the individual parameters in recipients of RIC alloHSCT with AML in CR1. Multivariable analysis of all parameters from both scores plus patient and donor CMV serology status resulted in the identification of 16 predominant parameters, including 11 comorbidities, 3 EBMT-score parameters and patient and donor CMV serology status. Subsequently, three risk groups were defined in an integrated score based on these parameters and their respective weights. The integrated score yielded a strong NRM risk categorization, particularly for low- and high-risk patients and was significantly associated with NRM and OS at 2 years after RIC alloHSCT. Moreover, NRM was found to increase at 5 years post-transplantation with continued discriminative power of the integrated score. Thus, the integrated score may contribute to NRM risk assessment in patients with AML in CR1, for whom RIC alloHSCT is considered as post-remission treatment for prevention of AML relapse.

The EBMT-score, which was initially developed to assess OS after myeloablative conditioning alloHSCT in chronic myeloid leukemia patients, demonstrated relatively weak predictive power in the present study. Several explanations may be considered. First, the EBMT-score was originally not selectively developed for predicting NRM. The score was designed to predict overall outcome by incorporating both disease parameters and transplant parameters.[9] Here, we selectively focused on NRM after RIC alloHSCT. In addition, only AML CR1 patients were studied, which patient group exhibits different characteristics as compared with the original and confirmatory reports of the EBMT-score.[9,14] Reassessment of the individual parameters of the EMBT-score in an integrated model resulted in different HRs as compared with the original report.[9] The HRs of the parameters age and time from diagnosis to transplantation were largely similar in the present study, although these parameters were defined differently as compared with the original report of the EBMT-score.[9] Strikingly, male patients receiving grafts from female donors were not associated with an increased risk for NRM at 2 years. The dismissal of the latter parameter may be explained by differences in patient characteristics; especially type of conditioning regimen, which itself is a risk factor for GVHD and NRM. Of note, in the original reports of the EBMT-score female donor to male recipient was also the parameter with the lowest relative risk and non-significant in several disease subgroups (for example, acute lymphoblastic leukemia

and myelodysplastic syndrome).[9,14] In the present study, the parameter donor type was associated with an increased HR for 2-year NRM, similar to the EBMT-score.[9,14] Furthermore, positive patient and donor CMV serology affected outcome in a confirmatory analysis of the EBMT-score, but CMV serostatus did not alter the EBMT-score and was therefore not included in the score.[14] However, in the present study, patient and donor CMV serology status independently predicted for 2-year NRM and were therefore both included in the model. Last, differences in HRs may be partly explained by the statistical method used in the original EBMT-score,[9] because NRM and relapse were evaluated separately by cause-specific hazards at that time, while ignoring NRM and relapse as competing risks. In contrast, the present score was based on a competing risk regression model (that is, Fine-Gray model[29]) which allows for a more accurate estimation of these end points.

The HCT-CI appeared to be associated with a relatively low predictive value in the present study, which may be partly explained by strong differences in patient characteristics as compared with the original report.[10] The present study included a homogeneous cohort of AML patients with an increased frequency of comorbidities as compared with the original description of the HCT-CI.[10] In particular, cardiovascular-related comorbidities were found more frequently (that is, cardiac disease, heart valve disease and diabetes), which would be expected given the relative advanced median age of this cohort as compared with the Seattle cohort (median 58 years versus 44 years, respectively).[10] Furthermore, we found a relative high prevalence of infections (24%, versus 4% in the Seattle cohort), which may be explained by the intensive pre-transplant chemotherapy of AML, rendering patients susceptible for invasive infections requiring antimicrobial treatment at the time of transplant. Following integrated reassessment of all individual parameters, mild and moderate comorbidities (that is, pulmonary disease and hepatic disease) showed weak associations with 2-year NRM, which may be explained by decreased toxicity of RIC. It resulted in the omission of mild hepatic disease and moderate pulmonary disease from the integrated score. Previously, the Seattle group reported similar findings that recipients of RIC alloHSCT experienced less pulmonary toxicity, despite having worse lung function pre-transplantation.[35] The parameter prior solid tumor was also dismissed despite a considerable prevalence (10%) in our cohort. Collectively, our integrated reassessment in AML CR1 patients receiving RIC alloHSCT yielded HRs that differed from the original report by Sorror et al.[10] Overall, it suggests that prediction of NRM may merit from a more tailored model, here as developed for older patients with AML in CR1 proceeding to RIC alloHSCT, which is currently the predominant indication for allografting.

Apart from the established EBMT-score and HCT-CI, other groups developed predictive models for NRM.[18,22,36-38] Some groups modified the weights of the EBMT-score and the HCT-CI,[18,37,38] whereas others combined transplant-related parameters and patient characteristics.[22,36] Recently, Barba et al.[22] showed the combined ability of the EMBT-score

and the HCT-CI to predict NRM, whereas leaving each individual score unchanged. Improved discrimination of patients at high risk for NRM was demonstrated. Of note, parameters of both scores were not reassessed individually while the original scores showed relatively weak predictive value. Most alternative models performed reasonably well in the original reports, but external validation in other cohorts of patients and/or diseases is often lacking. [18,22,36-38] In general, the performance of a predictive model within a population the model was derived from may be too optimistic, which may also apply to our integrated score. In the present study, the internal validity of the regression model was assessed using a bootstrapping technique in order to avoid a possible increase in false positive and false negative error rates that would occur if we would have split the data into a training and test set. However, despite bootstrapping, external validation of a predictive score remains mandatory and should be performed in an independent cohort of RIC alloHSCT recipients with AML, but also in other disease groups. Besides validity, the consistency of parameter analysis and coding is another important concern in constructing scores, as recently stressed by Sorror.[27] He proposed a brief training program in order to have consistent guidelines in assessing comorbidities and he found marked improvement in interevaluator agreement after using those guidelines. The data used in the present study have been gathered by the EBMT Acute Leukemia Working Party among more than 100 centers from 23 countries. Although 30 centers included more than two-thirds of the patients, the accuracy in the assessment of comorbidities may be of concern, which could lead to an over- or underestimation of the prevalence of comorbidities and may compromise the performance of the score, as well as the individual HRs upon reassessment. Therefore, the integrated score proposed in the present study would need external validation preferable in centers with standardized scoring systems and guidelines.

In conclusion, we present an integrated score for the assessment of NRM in patients with AML in CR1 eligible for RIC alloHSCT, based on a selection of parameters of the original EBMT-score and HCT-CI. The integrated score yielded relatively strong prediction of 2-year NRM that allowed for a clear discrimination of both low-risk and high-risk patients. Optimizing composite risk scores is of importance in order to support individual risk assessment in transplant decisions, as recently stressed by the European LeukemiaNET AML Working Party.[21] The working party advocated that individual transplant decisions should be made on both leukemia-related and NRM-related parameters, necessitating the use of scores with a high predictive value. Although the integrated score in the present study showed improved predictive power, future research is needed, in order to validate this score in an independent cohort of RIC alloHSCT recipients.

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8

HEPATITIS E VIRUS: AN UNDERESTIMATED OPPORTUNISTIC PATHOGEN IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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ABSTRACT

Hepatitis E virus (HEV) is increasingly acknowledged as a cause of hepatitis in healthy individuals as well as immunocompromised patients. Little is known of HEV infection in recipients of allogeneic hematopoietic stem cell transplantation (alloHSCT). Therefore, we set out to study the incidence and sequelae of HEV as a cause of hepatitis in a recent cohort of 328 alloHSCT recipients. HEV-RNA was tested in episodes of liver enzyme abnormalities. In addition, HEV-RNA and HEV serology were assessed pre- and post-alloHSCT. We found 8 cases (2.4%) of HEV infection, of which 5 had developed chronic HEV infection. Seroprevalence pre-alloHSCT was 13%. Four patients died with HEV viremia, with signs of ongoing hepatitis, having a median time of infection of 4.1 months. The 4 surviving patients cleared HEV after a median period of 6.3 months. One patient was diagnosed with HEV reactivation after a preceding infection prior to alloHSCT. Although the incidence of developing acute HEV post-alloHSCT is relatively low, the probability of developing chronic hepatitis in severely immunocompromised patients is high. Therefore, alloHSCT recipients should be screened pre-transplantation by HEV serology and RNA. Furthermore, a differential diagnosis including hepatitis E is mandatory in all alloHSCT patients with severe liver enzyme abnormalities.

KEY POINTS

- The incidence of acute HEV infection after alloHSCT is relatively low, in contrast to a high probability of developing chronic hepatitis.
- HEV infection or reactivation should be included in the differential diagnosis of liver enzyme abnormalities in alloHSCT recipients.

INTRODUCTION

In 1983, a new waterborne hepatitis agent was found after an outbreak of unexplained hepatitis at a military camp, later identified as hepatitis E virus (HEV). HEV is endemic in resource-limited countries and an emerging health issue in industrialized countries.[1,2] It is a causative agent of acute and chronic hepatitis, transmitted via fecal-oral route, with a mostly self-limiting course in healthy individuals. In human HEV infection, there are 4 known genotypes prevalent, with genotypes 1 and 2 responsible for large waterborne HEV outbreaks in developing countries (Africa and Asia), and genotypes 3 and 4 generally seen in sporadic cases as a zoönotic infection in industrialized countries.[1,3] Since the first evidence of chronic hepatitis due to HEV in recipients of solid organ transplants, an increasing awareness for HEV has become apparent.[4,5]

Persistent chronic infection and cirrhosis have been reported in immunocompromised patients, with most cases in solid organ transplant recipients.[5] However, HEV was recently also reported in recipients of allogeneic hematopoietic stem cell transplantation (alloHSCT).[6-9] A prevalence of 1% to 3% of hepatitis E viremia in recipients of solid organ transplants has been reported, with 47% to 83% of the patients developing chronic hepatitis.[10-13] So far, the incidence and sequelae of hepatitis due to HEV in recipients of alloHSCT is largely unknown.

After 2 recent cases of HEV infection in our clinic, we set out to retrospectively evaluate the point prevalence and clinical sequelae of HEV infection in a cohort of alloHSCT recipients in our clinic, and we studied the role of HEV in transplant recipients presenting with liver enzyme abnormalities.

METHODS

Sample collection

We conducted a retrospective cross-sectional analysis of all adult alloHSCT recipients transplanted in the period January 2006 to July 2011, whose serum or EDTA-plasma samples were available in the biobank of Erasmus Medical Center (ErasmusMC; Rotterdam, The Netherlands). These samples, stored at -20°C or -80°C, had been collected during routine visits to our outpatient clinic for clinical assessment of cytomegalovirus (CMV) and EpsteinBarr virus (EBV) reactivation. To select samples, we performed a Laboratory Information Management System database search for last pre-transplantation and most recent post-transplantation sample availability. In addition to the cross-sectional analysis, samples were selected from patients experiencing episodes with alanine transaminase (ALT) abnormalities grade 2 to 4, according to Common Terminology Criteria for Adverse Events version 3.0. Common Terminology Criteria for Adverse Events grades 2 to 4 ALT abnormalities are defined as at least 2.5 times the upper limit of normal. This study was approved by the medical ethical committee (MEC) of ErasmusMC (MEC approval: 2012-522). This study was conducted in accordance with the Declaration of Helsinki.

Virological parameters

For detection of both HEV-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) in serum or plasma samples, the commercially available HEV enzyme-linked immunosorbent assays (Wantai, Beijing, People's Republic of China) were used. Available peripheral blood, feces and cerebrospinal fluid (CSF) samples of HEV RNA positive patients were retrospectively analyzed during the course of infection to study the kinetics of serum antibody responses (IgM and IgG) and viremia in different body compartments.

All samples were screened for HEV RNA by an internally controlled quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR), described previously.[13] The RT-PCR had a lower limit of detection (95% hit rate) of 143 (2.16 log) IU/mL as determined by the first World Health Organization standard for HEV RNA nucleic amplification testing-based assays (6329/10; Paul Ehrlich Institute, Langen, Germany). Phylogenetic analysis was performed to determine genotype, to exclude a common source of infection, and to examine potential HEV reactivation. Statistical analysis and data collection were performed using Microsoft Office Excel 2007 and SPSS (version 20).

Case definition

A case of HEV infection was defined as a patient with an HEV RNA positive serum or plasma sample and was confirmed either by showing HEV-specific serum IgM or IgG antibody response, or by showing the presence of HEV RNA in sequential samples. Chronic infection was diagnosed by retrospective testing of stored samples of identified cases and was defined as having HEV viremia of more than 6 months.

RESULTS

Patient characteristics

A total of 207 episodes of acute ALT abnormalities, occurring in 138 out of 328 alloHSCT recipients, were evaluated, in addition to a cross-sectional RT-PCR analysis of all 328 patients (Figure 1). As delineated in Table 1, the cohort included 178 (54%) male and 150 (46%) female patients with a median age at transplantation of 50 (range: 17-66) years. Stem cell sources included sibling donors (n = 145, 44%), adult matched unrelated donors (MUD) (n = 137, 42%), and umbilical cord blood (UCB) grafts (n = 46, 14%). Acute myeloid leukemia was the most frequent diagnosis for transplantation (n = 142, 43%), followed by acute lymphoblastic leukemia (n = 49, 15%), and non-Hodgkin's lymphoma (n = 31, 9%). All patients received graft versus host disease (GVHD) prophylaxis with a combination of a calcineurin inhibitor (cyclosporine A) and mycophenolate according to local policy. Acute GVHD grades II to IV occurred in 130 (40%) patients, and chronic extensive GVHD was present in 122 (37%) patients. At the time of analysis (December 2012), 180 (55%) patients were still alive, with a median follow-up of 40.9 (range, 10-77) months from alloHSCT.

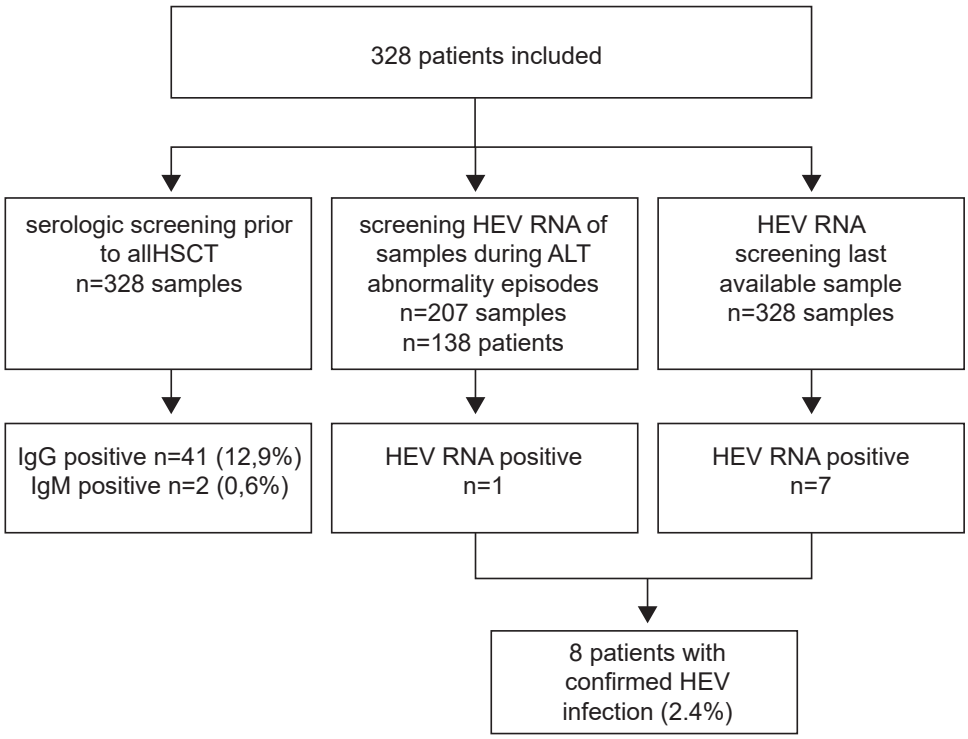


Figure 1 Overview of sample selection and study results

Table 1 Patient characteristics of the cohort (n = 328)

Characteristic	Number	
Age of Transplantation, years		
median (range)	50.4	(17-66)
Sexe, number (%)		
Male	178	(54%)
Female	150	(46%)
Diagnosis, number (%)		
AML	142	(43%)
ALL	49	(15%)
NHL	31	(9%)
CLL	24	(7%)
MM	18	(5%)
MDS	16	(5%)
Other	48	(15%)
Type of allogeneic transplantation, number (%)		
UCB	46	(14%)
MUD	137	(42%)
SIB	145	(44%)
GVHD, number (%)		
Acute grade I	42	(13%)
Acute grade II - IV	130	(40%)
Chronic limited	32	(10%)
Chronic extensive	122	(37%)
Patients alive		
number (%)	180	(55%)
Time to follow-up (months)		
median (range)	40.9	(10-77)

Abbreviations: AML indicaties acute myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome; UCB, umbilical cord blood; MUD, matched unrelated donor; SIB, sibling; and GVHD, graft versus host disease

Virological parameters

In total, 8 (2.4%) cases of confirmed HEV infection were found in 328 patients, of which seven (88%) were identified by cross-sectional analysis, and 1 (13%) by screening the episodes of acute ALT abnormalities.

HEV-specific IgG prior to alloHSCT was detected in 41 (13%) patients. Two (0.6%) patients were IgM positive, though HEV viremia could not be confirmed by RT-PCR. Presence or absence of HEV specific antibodies (both IgM and IgG) prior to alloHSCT was not predictive for HEV infection after alloHSCT, tested by Pearson's chi square- test of independency (P = .313).

The courses of HEV infection of all 8 cases are presented in Figure 2. Clinical and virological features are delineated in Table 2. Patients will be annotated according to their assigned letter: 'patients A–H'. Within the 8 cases, complete HEV-IgM and -IgG seroconversion occurred in 5 patients, of whom 4 eventually cleared the virus and 1 deceased with a HEV viremia (patient A–C, F, H). Median time from first HEV RNA detection to HEV-IgM and HEV-IgG conversion of these patients was 65 (range, 0-245) days, and 126 (range, -594-351) days, respectively. Three patients, who all died with HEV viremia, had aberrant serodynamics: 1 patient did not have detectable HEV-IgG, with only 1 serum sample testing HEV-IgM positive (patient G). Two patients did not have detectable HEV-IgM levels (patients D, E). One of them had detectable HEV-IgG in only 1 sample (patient D), and 1 had detectable HEV-IgG levels at time of alloHSCT, though declining to undetectable at the time of death 7 months later (patient E).

HEV-open reading frame 1b (ORF1b) sequences were generated for all 8 cases and deposited in Genbank under the accession numbers JQ015439, JQ015407, KC171439-KC1714444, KC171447, KC171450 and KC171451. Phylogenetic analysis did not identify a common or nosocomial source of HEV transmission. All HEV isolates were classified within genotype 3, as shown in the phylogenetic tree (Figure 3). Interestingly, confirmed HEV reactivation occurred in 1 patient, as described below (patient H).

Characteristics of HEV RNA positive patients

The median age of 8 HEV infected patients was 56 (range 39-66) years at transplantation, including 5 (63%) males and 3 (37%) females (Table 2). All patients were screened for hepatitis B virus, hepatitis C virus, EBV, adenovirus, varicella zoster virus, herpes simplex virus type 1 and 2, and CMV by PCR to exclude the role of other potential hepatrophic viruses. All tested samples were undetectable by PCR, except for 1 patient experiencing CMV reactivation at the time of HEV infection (patient E). In this patient, HEV viremia persisted after successful treatment with ganciclovir, excluding the role of CMV in hepatitis in this patient. All 8 patients received a graft from an alternative donor, including peripheral blood grafts from an adult MUD in 5 patients (63%) and UCB grafts in 3 (37%) patients. Plasma of the adult MUD grafts was HEV RNA negative. No samples of the UCB grafts were available for HEV RNA screening, yet 2 of 3 UCB recipients were HEV viremic at the time of alloHSCT (patients G,H). Six patients received multiple blood transfusions within 3 months prior to HEV infection, including platelet and red blood cell transfusions. None of the blood products were available for testing for HEV serology or RNA at the time of submission.

The median time from alloHSCT to infection was 4.6 (range, -2 to 18) months. The median peak ALT during HEV infection was 289 (range, 138-1507) U/L. At the time of infection, 6 (75%) patients were receiving intensive immunosuppressive therapy (>2 agents), prescribed for GVHD prevention (n = 2, 33%) or GVHD treatment (n = 4, 66%). In the HEV-infected patients, liver enzyme abnormalities were thought to be related to hepatic GVHD in 5 (63%) patients, and drug induced liver injury in 3 (38%) patients.

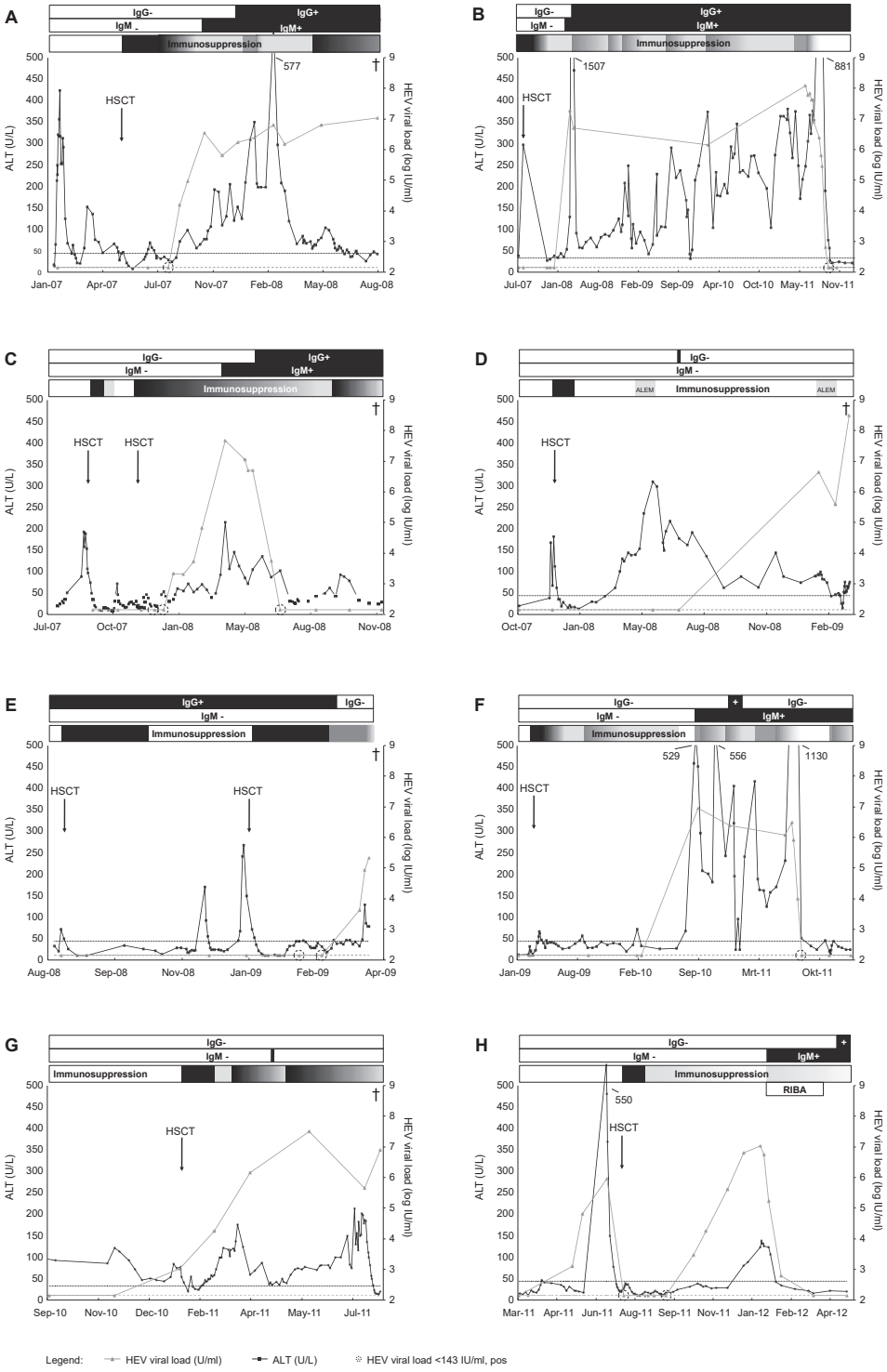
Table 2 Patient characteristics of Hepatitis E confirmed patients (n = 8)

Patient	Sexe	Age at allo-HSCT, years	Diagnosis	Stem cell source	Alive at EOF	GVHD	Initial diagnosis	At time of alloHSCT		HEV gt	Time from alloHSCT to infection, months	Duration of infection, months	Median (range) ALT levels during infection, U/l†	Immune-suppression at time of infection	Hepatic fibrosis in liver histology	No. of blood products received within 3 mos to infection
								HEV RNA	IgG status							
A	M	44	AML	UCB	no	Acute grade II-IV Chronic extensive	GVHD	-	-	3	2.7	12.5*	73 (24-577)	ciclosporin, prednisone, mycophenolate	N/A	21
B	F	54	NHL	MUD	yes	Acute grade II-IV Chronic limited	GVHD	-	-	3	8.4	42.4	207 (31-1507)	ciclosporin	F3	0
C	F	59	MDS	MUD	yes	Acute grade II-IV Chronic extensive	GVHD	-	-	3	3.4	6.3	72 (36-215)	ciclosporin, mycophenolate	N/A	48
D	M	43	CLL	MUD	no	None	DILI	-	+	3	14.0	1.6*	66 (15-309)	alemtuzumab	N/A	3
E	M	66	AML	MUD	no	Acute grade II-IV	DILI	-	+	3	5.8	1.7*	39 (19-268)	ciclosporin, prednisone, mycophenolate	N/A	35
F	M	58	NHL	MUD	yes	Acute grade II-IV Chronic extensive	GVHD	-	+	3	18.3	11.3	208 (25-1130)	sirolimus, prednisone	F1	0
G	F	39	SAA	UCB	no	Acute grade II-IV	DILI	+	-	3	0	6.5*	70 (12-213)	ciclosporin, mycophenol acid	F0	21
H	M	59	AML	UCB	yes	None	GVHD	+	-	3	-2.0	2.1 and 4.9	27 (10-550)	none	N/A	50

Abbreviations: alloHSCT indicates allogeneic hematopoietic stem cell transplantation; EOF, end of follow-up; GVHD, graft versus host disease; HEV, Hepatitis E virus; gt, genotype; ALT, alanine transaminase; AML, acute myeloid leukemia; UCB, umbilical cord blood; N/A, not available; NHL, non-Hodgkin's lymphoma; MUD, matched unrelated donor; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; DILI, drug induced liver injury; and SAA, severe aplastic anemia

* Patient died having a HEV viremia

† ALT Upper limited of normal, male = 44 U/l or female = 33 U/l


A
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Immunosuppression
HSCT
577
†
ALT (U/L)
HEV viral load (log IU/ml)
Jan-07 Apr-07 Jul-07 Nov-07 Feb-08 May-08 Aug-08
B
IgG-
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Immunosuppression
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ALT (U/L)
HEV viral load (log IU/ml)
Jul-07 Jan-08 Aug-08 Feb-09 Sep-09 Apr-10 Oct-10 May-11 Nov-11
C
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HEV viral load (log IU/ml)
Oct-07 Jan-08 May-08 Aug-08 Nov-08 Feb-09
E
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Immunosuppression
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ALT (U/L)
HEV viral load (log IU/ml)
Aug-08 Sep-08 Nov-08 Jan-09 Feb-09 Apr-09
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IgM+
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HSCT
ALT (U/L)
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Jan-09 Aug-09 Feb-10 Sep-10 Mrt-11 Okt-11
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ALT (U/L)
HEV viral load (log IU/ml)
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Immunosuppression
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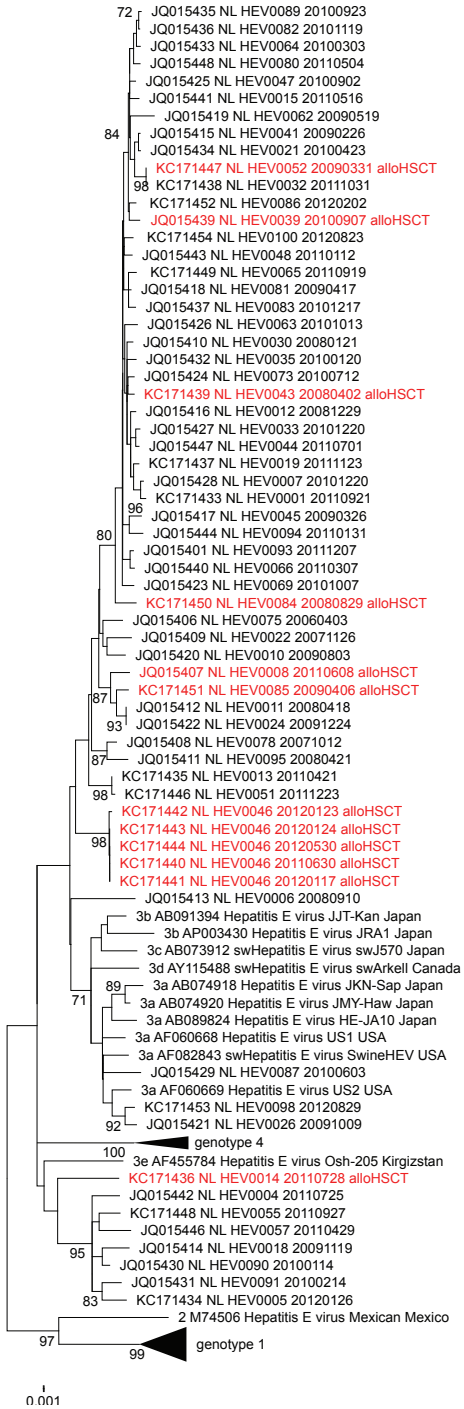
ALT (U/L)
HEV viral load (log IU/ml)
Mar-11 Apr-11 Jun-11 Aug-11 Sep-11 Nov-11 Jan-12 Feb-12 Apr-12
Legend:
HEV viral load (U/ml)
ALT (U/L)
HEV viral load <143 IU/ml, pos

Figure 2. Courses of Hepatitis E infection in all eight individual patients.
(**A**) HEV RNA persisted, although HEV-IgM and HEV-IgG seroconversion occurred under immunosuppressive therapy. This patient deceased of therapy refractory progressive gastro-intestinal GVHD with concurrent chronic HEV infection; (**B**) Acute ALT abnormalities arised during HEV infection. This patient was mistakenly diagnosed as hepatic GVHD, and immunosuppression was intensified multiple times because of persisting liver enzyme abnormalities. This patient cleared HEV with stopping all immunosuppression, after the diagnosis of HEV infection in this study; (**C**) This patient developed primary graft failure of a 8/8 HLA-matched unrelated donor graft after reduced intensity condition with rabbit antithymocyte globulin, fludarabine, and a single donor fraction of 2 gray total body irradiation. HEV RNA was present after second alloHSCT. This patient cleared infection after HEV-IgM and HEV-IgG seroconversion, supported by reduction of immunosuppressive therapy; (**D**) This patient developed graft failure of a 7/8 HLA-matched unrelated donor graft after reduced intensity conditioning with rabbit antithymocyte globulin, fludarabine, and a single fraction of 2 gray total body irradiation. Patient's disease relapsed three months after graft failure. Reinduction therapy was started with alemtuzumab (ALEM) and a second alloHSCT was prepared. However, due to recurrent infections, this patient was not able to complete treatment. Patient died shortly after his second cycle of alemtuzumab because of complications of a meningitis and secondary sepsis with *Escherichia coli*. Of note, patient's CSF samples tested positive for HEV; (**E**) Secondary graft failure occurred three months after the first alloHSCT. The second alloHSCT was complicated by multiple respiratory viral and bacterial infections, which eventually led to respiratory failure and death; (**F**) This patient was diagnosed as hepatic GVHD and immunosuppression was introduced in August 2010. Patient cleared HEV after cessation of all immunosuppression, following the diagnosis of HEV infection; (**G**) HEV RNA was detectable at time of alloHSCT and viral load increased under immunosuppressive therapy until patient deceased due to a respiratory viral infection. This patient showed HEV-IgM in one sample (black bar) and no HEV-IgG seroconversion; (**H**) HEV reactivation occurred after initial undetectable HEV RNA without seroconversion. After reduction of immunosuppressive therapy and addition of ribavirin (RIBA) the patient seroconverted and cleared HEV. For explanation of symbols and abbreviations see legend attached to Figure 2. The ALT upper limit of normal is 33 U/L and 44 U/L for females and males, respectively. The HEV RNA lower limit of detection is 143 (2.16 log) IU/ml.

Four (50%) patients died with persistent HEV viremia and signs of ongoing hepatitis (patients A, D, E, G). Median duration of HEV infection in deceased patients was 4.1 (range, 2-13) months, with acute HEV infection in 3 patients and chronic HEV infection in 1 patient. The cause of death was respiratory failure due to infection (fungal, bacterial, and viral) in 3 patients (patients D, E, G), and 1 patient died of therapy refractory progressive gastro-intestinal GVHD (patient A). Of note, one of the deceased patients appeared to have HEV RNA positive CSF with retrospective testing of CSF samples (patient D). These samples were obtained during an episode of meningitis and secondary sepsis with positive CSF and blood cultures for *Escherichia coli*. Radiological evaluation (computed tomography scan) revealed cerebral ischemia due to infection. This patient eventually died of respiratory failure due to fluid aspiration with a low level of consciousness since the meningitis.

The 4 (50%) living patients cleared HEV infection within a median period of 6.3 (range, 2-42) months (patients B, C, F, H). One patient received ribavirin treatment twice daily with 400 mg for 3 months after a starting dose of three times 600 mg daily for 10 days because of a concurrent respiratory syncytial virus infection (patient H). Three patients cleared HEV during cessation of immunosuppressive therapy (patients B, F, H). The cessation rate depended on the presence or occurrence of GVHD. Among living patients, chronic HEV occurred in 3 patients (patients B, C, F), whereas 1 patient was able to clear HEV infection within 6 months (patient H).

Figure 3 Phylogenetic tree of ORF1b HEV sequences in eight HEV infected alloHSCT recipients



Phylogenetic relation of 321 bp ORF1b region was calculated using Maximum likelihood, K2P analysis with bootstrapping (n = 1000). Branch lengths are proportional to the evolutionary relationship between the sequences and internodal confidence of >70% is depicted in the tree. Genbank accession numbers, country of origin (eg, NL), HEV study number (eg, HEV001) and date of drawal (yyyymmdd) and alloHSCT recipients are indicated in the taxa (red text). No indication for a common origin or for nosocomial HEV transmission was found.

After HEV diagnosis was confirmed, a liver biopsy was taken from 2 patients (patients B, F), showing hepatitis, severe fibrosis, and portal inflammation (Figure 4). Liver histology was available in 1 patient by autopsy, showing no abnormalities (patient G).

Remarkably, 1 patient initially cleared the virus and showed reactivation after a period of 53 days of undetectable HEV RNA (patient H). At the time of alloHSCT, HEV RNA was detectable, though viral load was low (<143 IU/mL). The second viremic period was characterized as viral reactivation after alloHSCT, based on identical HEV-ORF1b sequences (Figure 3). This patient finally cleared the reactivated HEV infection within 2 months after diagnosis, supported by ribavirin treatment (as described above) and reduction of immunosuppressive therapy.

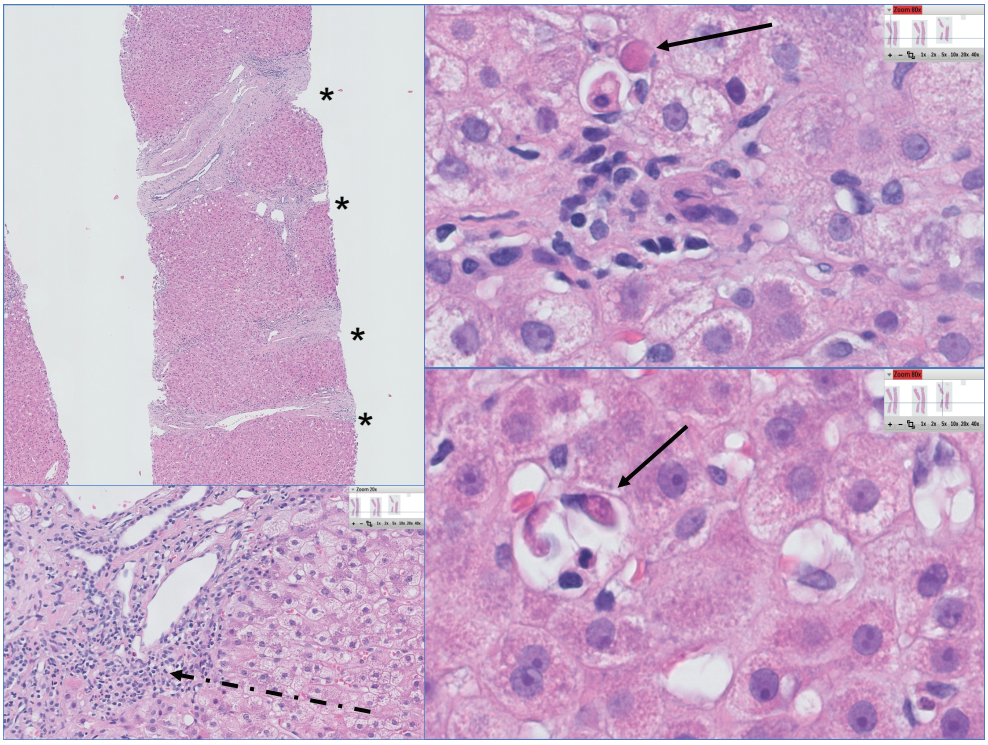


Figure 4 Liver histology of a patient with chronic HEV
The histopathology of chronic HEV infection in this patient is characterized by a dense lympho-plasmocellular infiltrate (dashed arrow) in the portal tracts, combined with severe fibrosis (F3) and porto-portal septation (*). Multiple foci of apoptotic bodies are seen in the lobuli surrounded by few inflammatory cells, indicating individual hepatocyte necrosis (Councilman-bodies: arrow) and probably caused by viral replication.

DISCUSSION

Recipients of allogeneic stem cell grafts, and especially those receiving alternative donor grafts, are at increased risk of opportunistic bacterial, fungal and viral infections. Here we describe the first retrospective cross-sectional study of HEV infection in a large cohort of alloHSCT patients. We report a relatively low incidence of 2.4%, in comparison with other opportunistic infections in alloHSCT recipients. Nevertheless, we found a high probability of 63% of developing chronic HEV infection.

Previously, 2 cohorts of 72 and 52 alloHSCT patients were screened for HEV by Abravanel et al.[6] and Koenecke et al.[8] respectively, without positive cases for HEV infection or reactivation, concluding that alloHSCT patients are at low risk for HEV infection and reactivation. However, these 2 cohorts of alloHSCT recipients comprised a more limited number of patients. In our study we identified 8 HEV cases in a larger cohort (n = 328), confirming the HEV prevalence of 2.4% in immunocompromised patients.[4,5,11,13] Second, the study of Abravanel et al.[6] included a restricted follow-up period of 6 months after alloHSCT, whereas our study had a median follow-up time of 41 months. Additionally, misdiagnosing HEV as drug induced liver injury has been reported previously by Dalton et al,[14] whereas this patient group was excluded in the study of Koenecke et al.[8] To reduce the risk of missing HEV infections, we screened all patients for HEV RNA at episodes of liver enzyme abnormalities in addition to last available samples. Of the confirmed HEV cases, 5 were misdiagnosed as GVHD, and 3 cases were mistakenly diagnosed as drug induced liver injury. Diagnosis of HEV in these patients is hampered by relatively low peak aminotransferase levels in comparison with non-immunocompromised patients,[15] which may be explained by intensive immunosuppressive therapy suppressing inflammation.

In our cohort, chronic hepatitis occurred in 5 out of 8 acute HEV cases. However, only 6 patients had sufficient follow-up for a potential diagnosis of chronic hepatitis, because 2 patients died within 2 months after acquiring HEV infection. Progression to chronic HEV in alloHSCT patients may be explained by an impaired immune reconstitution, including insufficient lymphocyte recovery, which are well known risk factors for post-transplantation infections.[16-18] In particular, impaired reconstitution of $CD4^+$ and $CD8^+$ T-cells predispose for infectious morbidity,[19] which is confirmed in studies with CMV and EBV viremia, with patients having low specific CMV and EBV $CD4^+$ and $CD8^+$ T-cell counts predisposing for CMV and EBV reactivation, respectively.[20,21]

Phylogenetic analysis of patient-derived HEV sequences before and after alloHSCT established HEV reactivation in 1 patient. This is the second case of HEV reactivation after alloHSCT described so far in the literature.[7] We could not unequivocally demonstrate a reinfection or reactivation in 3 viremic patients having detectable IgG prior to alloHSCT, because no HEV RNA was detected in available samples prior to alloHSCT. Four other patients were seronegative prior to transplantation, suggesting that transmission had occurred after alloHSCT.

In industrialized countries, HEV genotype 3 predominantly infects pigs, wild boars, and

deer but also humans and is recognized as a zoönotic agent. However the main modes of transmission of genotype 3 and 4 viruses remain to be determined.[2,3] The source of HEV infection is unclear, but HEV transmission may be enterically (food borne: porcine livers, shellfish), via blood or blood products, mother-to-child, and although rare, human-to-human.[1,22] Donors and donated blood are not routinely tested for HEV RNA worldwide, although reports of several cohorts in different countries of healthy blood donors reported HEV RNA and HEV IgM reactivity, suggesting active infection.[23-25] In our cohort, transmission of HEV by blood products cannot be excluded because 6 out of 8 viremic patients received multiple blood transfusions. Unfortunately none of these blood products were available for testing at the time of submission.

The high probability of developing chronic HEV found in this study was consistent with other studies in recipients of solid organ transplants.[4,5,11,13] HEV-infected patients are at risk of progression to fibrosis (67%) in 1 year from infection,[11] and also cirrhosis (10%).[10] Therefore immunocompromised patients should be screened prior to transplantation, and during episodes of liver enzyme abnormalities, post-transplantation. In our study, patients showed aberrant serology, which may be explained because of their impaired immune reconstitution. Thus, HEV RT-PCR testing is the preferred diagnostic method in these immunocompromised patients.

Treatment of HEV infection after transplantation includes reduction of immunosuppressive therapy, and there is no registered drug therapy. Anecdotal evidence supports the use of oral ribavirin in immunocompromised patients. In our study, 3 patients cleared HEV with a dose reduction of immunosuppressive agents (ie, cyclosporine A or prednisone) alone. Treatment with ribavirin should be considered in patients, for whom immunosuppression cannot be reduced, such as, for example, patients with active GVHD. The optimal daily dose of ribavirin is unknown; in case-reports sustained viral response has been described with daily dosages between 200 mg and 1200 mg.[11,26] If HEV infection is confirmed prior to alloHSCT, it can be considered as a contraindication to transplantation. Clearance of HEV viremia is therefore of high importance. AlloHSCT candidates are usually pretreated with chemotherapy, resulting in impaired or delayed immune reconstitution. Therefore, early ribavirin treatment can be initiated to support rapid HEV clearance in these future alloHSCT recipients.

In conclusion, this study shows that recipients of alloHSCT are at risk for HEV infection, albeit with a relatively low risk. However, the probability of developing severe chronic hepatitis in immunocompromised patients is high. Therefore, patients should be screened for HEV antibodies and HEV RNA prior to alloHSCT, and patients with acute liver enzyme abnormalities after alloHSCT should be analyzed for HEV reactivation or infection. Moreover, HEV should be included in the differential diagnosis of liver GVHD and drug-induced liver injury, because of the largely overlapping picture with respect to liver enzyme abnormalities.

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9

GENERAL DISCUSSION

INTRODUCTION

The majority of patients with newly diagnosed acute myeloid leukemia (AML) obtain complete hematological remission (CR) after induction chemotherapy, but the incidence of relapse is considerable despite chemotherapeutic consolidation therapy. The risk of relapse is predominantly determined by the genetic characteristics of the leukemia, which can be assayed by cytogenetic or molecular analysis.[1-6] In addition, assessment of minimal residual disease (MRD) upon achievement of hematological CR significantly adds to estimating the risk of disease recurrence.[7-9] Currently, post-remission treatment (PRT) for the prevention of relapse may include continued chemotherapy, autologous hematopoietic stem cell transplantation (HSCT), or allogeneic HSCT (alloHSCT). Although alloHSCT is associated with the lowest incidence of relapse, counterbalancing non-relapse mortality (NRM) may compromise overall outcome. The decision to perform an alloHSCT for patients with AML in first CR depends on the assessment of risks and benefits (ie, mortality and relapse risk reduction), which is based on disease features, but also factors related to patient characteristics, transplantation procedures and type of donor. Such a risk versus benefit evaluation of alloHSCT has evolved into a personalized approach for patients with AML in first CR.[10,11] The studies described in the first part of this thesis address the benefits of alloHSCT identifying different AML patient subgroups with improved outcome following alloHSCT. Secondly, the studies in this thesis also addressed morbidity and mortality following alloHSCT. In order to improve a risk-benefit evaluation, we developed a new NRM risk score. We have also described a new opportunistic infection in alloHSCT recipients. In the current chapter, we discuss the value of alloHSCT as PRT in specific AML subgroups, potential challenges with respect to alloHSCT-related NRM, and statistical considerations analyzing PRT for AML. Lastly, we present a personalized transplant decision approach for patients with AML in first CR, which may be applied in daily clinical practice.

The indication of alloHSCT as PRT in patients with AML in first CR

Risk classification

AML risk classifications define risk groups with distinct prognostic features that are continuously being refined. Recently, the European LeukemiaNet (ELN) described an updated classification based on cytogenetic and molecular features, classifying patients in three risk groups with a favorable, intermediate or adverse prognosis.[1] Applying that classification retrospectively to a cohort of 2.899 AML patients for whom induction treatment was started within four prospective HOVON-SAKK trials, the three risk groups were clearly identified (overall survival [OS] at 5 years: favorable risk 64 ± 2%, intermediate risk 41 ± 1%, and adverse risk 23 ± 1%, $p<0.001$, Figure 1).

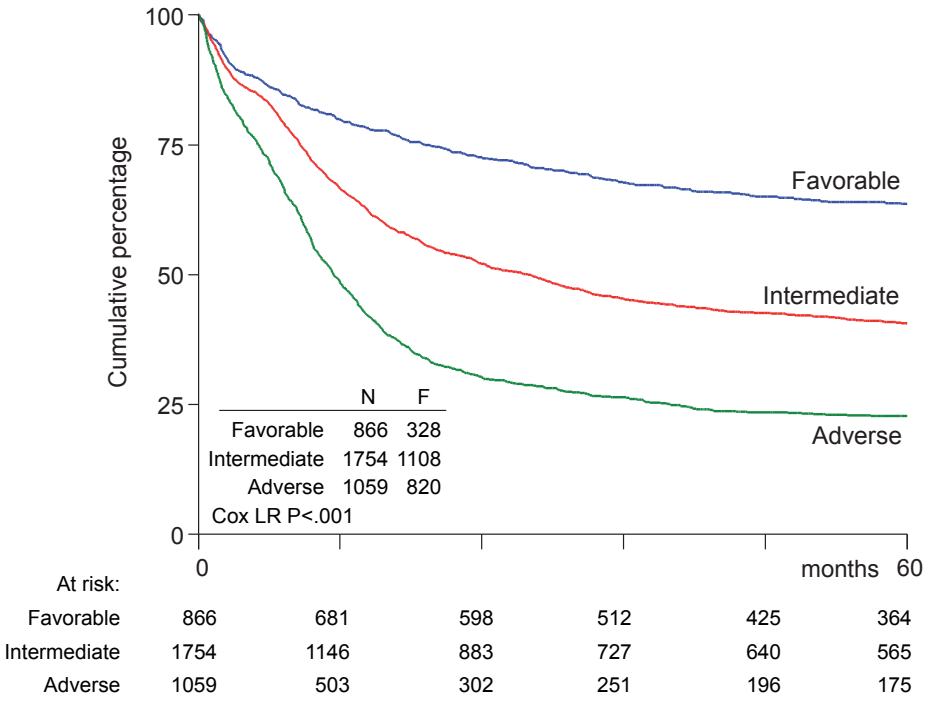

Figure 1. Overall survival from AML diagnosis by ELN risk classification.[1]
Patient data derived from AML29, AML42, AML92 and AML102 HOVON-SAKK studies.[142-144]

Risk classifications are used for optimizing induction and consolidation approaches, and for tailoring PRT decisions. AlloHSCT is generally not being indicated in patients with a favorable risk profile, but highly recommended for patients with adverse risk AML in first CR.[1,10-14] We have addressed outcome of AML patients in first CR by PRT in different age groups including 40-60 years, and beyond 60 years of age in **Chapter 2** and **Chapter 3**, respectively. Similar to previous studies and their meta-analyses,[15-20] outcome following alloHSCT as compared with chemotherapy or autologous HSCT was not significantly different in patients with a favorable risk profile. Patients with intermediate risk or adverse risk AML had improved OS and relapse-free survival (RFS) following alloHSCT as compared with chemotherapy, although the number of elderly alloHSCT recipients with intermediate risk AML was relatively small. Autologous HSCT was associated with improved RFS as compared with chemotherapy in patients aged 40-60 years with favorable, intermediate or adverse risk AML. However, OS appeared not significantly different because more recipients of chemotherapy could be rescued by an allograft in second CR.

Results of alloHSCT as compared with chemotherapy have previously yielded contradicting results in intermediate risk patients, especially taking molecular markers into account.[6,15-24] In **Chapter 4**, we have addressed the comparative value of alloHSCT in a large cohort of intermediate risk patients, who had cytogenetically normal AML in first CR with available molecular status of *NPM1* and *FLT3*-ITD including the allelic ratio. Similar to previous studies,[24-30] we confirmed the prognostic impact of the *FLT3*-ITD mutant to wild-type ratio, which is currently included in the latest ELN risk classification.[1] Importantly, the ELN classification has used a different calculation of the *FLT3*-ITD allelic ratio as compared with our analysis (**Chapter 4**) and previous studies.[26,30] The ELN has classified AML patients with mutated *NPM1* with a low ratio as favorable risk, whereas patients without *NPM1* and a low ratio of *FLT3*-ITD are classified as having an intermediate risk profile. Patients with mutated *NPM1* with a high allelic ratio of *FLT3*-ITD are currently also considered as intermediate risk, whereas a high ratio of *FLT3*-ITD without *NPM1* is categorized as adverse risk. However, the additive value of *NPM1* mutations in patients with *FLT3*-ITD remains questionable. Gale et al.[26] were able to show better outcome in patients with mutated *NPM1* with *FLT3*-ITD as compared with *NPM1* wild-type patients, but did not address the *FLT3*-ITD allelic ratio in that comparison. Other studies showed poor outcome of patients with a high allelic ratio of *FLT3*-ITD, and no differential impact of mutated *NPM1* on outcome,[24,28-30] questioning whether these patients may be classified as intermediate risk. We were also not able to identify differences in outcome of the subgroup with a high allelic ratio of *FLT3*-ITD with or without mutated *NPM1* (**Chapter 4**). In addition, similar outcome of patients with mutated *NPM1* with a low ratio of *FLT3*-ITD or mutated *NPM1* without *FLT3*-ITD was found in the study of Linch et al.,[30] whereas others were not able to confirm these results.[24,27,28] Our study showed inferior survival of patients with mutated NPM1 with a low ratio of *FLT3*-ITD as compared with patients having *NPM1* without *FLT3*-ITD (**Chapter 4**), again questioning whether these patients may be classified as favorable risk. These observations urge further studies to delineate the impact of *NPM1* mutations in the context of *FLT3*-ITD, irrespective of the allelic ratio. Nevertheless, the *NPM1* mutational status and *FLT3*-ITD should both be included in PRT decision making. We found that alloHSCT improves OS as compared with PRT with chemotherapy and autologous HSCT in patients with wild-type *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low allelic burden. Patients with favorable molecular characteristics in our cohort (ie, mutated *NPM1* without *FLT3*-ITD) were associated with a favorable prognosis, which was not further improved by alloHSCT. A small subset of patients with a high *FLT3*-ITD mutant to wildtype ratio was characterized by poor outcome, irrespective of type of PRT. Other research groups have suggested improved outcome following alloHSCT in AML patients with a high mutant to wild-type ratio of *FLT3*-ITD.[24,28,29] Collectively, alloHSCT is not indicated in patients with cytogenetically normal AML with mutated *NPM1* without *FLT3*-ITD, but alloHSCT may be considered in patients with cytogenetically normal AML with wild-type *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low or with a high allelic ratio.

Minimal residual disease

AML risk classification may be further improved by the assessment of MRD, which can be measured by either multiparametric flow cytometry or quantitative PCR for specific molecular markers.[7-9] MRD may be detected at time points early after induction treatment to assess the remission status of the AML, but also after PRT to detect imminent relapse. Consequently, MRD negativity was introduced as an endpoint in patients with a hematological CR.[1] MRD has been shown to predict for relapse and overall outcome early after induction treatment, but also after PRT.[31-45] Despite PRT with alloHSCT, a 2-5 fold increased incidence of relapse was observed in MRD positive AML patients as compared with MRD negative recipients of an allograft.[38-45] In **Chapter 5** we addressed whether and to what extent alloHSCT quantitatively reduces relapse as compared with conventional PRT in patients with or without MRD in first CR. We were able to demonstrate that the relative reduction of relapse by alloHSCT is similar in MRD positive and MRD negative patients as compared with chemotherapy or autologous HSCT. Of note, the relative reduction of relapse by alloHSCT as determined by a hazard ratio of 0.3-0.4 suggests a strong graft-versus-leukemia (GVL)-effect in our studies (**Chapters 2, 3, 4 and 5**). These results compare well to earlier findings in cytogenetic subgroups, in which the GVL-effect appeared to be similar among patients with a monosomal karyotype, core binding factor AML, or patients with a normal karyotype.[46] These observations are most readily explained by the abundant expression of class I and II HLA-antigens on malignant myeloid precursor cells and their susceptibility to alloreactive T-cells, including T-cells recognizing minor or major HLA-antigens.[47-49] Thus, T-cell alloreactivity may exert anti-leukemic effects irrespective of underlying subcategory of AML and MRD status. Collectively, the quantitative detection MRD should be used together with AML risk classifications to tailor the application of alloHSCT as PRT in patients with AML in first CR, which will be discussed in the last part of this chapter.

Although alloHSCT provides a strong GVL-effect, counterbalancing NRM may be of concern. As NRM critically depends from a number of different risk factors, it has become imperative to assess the NRM risk profile in addition to leukemia characteristics and response to induction chemotherapy.[10,11]

Morbidity and mortality in alloHSCT

AlloHSCT is associated with substantial NRM, which may be attributed to graft-versus-host disease (GVHD), infectious complications, organ toxicity and other causes.[50] A number of parameters may relate to alloHSCT-related NRM, including the procedure (eg, conditioning regimen, application of T-cell depletion), donor characteristics (eg, human leukocyte antigen [HLA]-matching) and recipient features (eg, age and comorbidity).

Conditioning

The infusion of allogeneic stem cells is preceded by a preparative regimen which permits engraftment. Myeloablative conditioning (MAC), containing high-dose chemotherapy and radiotherapy, has been developed to allow engraftment, to exert an anti-leukemic effect and to ensure a subsequent allogeneic GVL-effect.[51] However, such intensive conditioning resulted in significant toxicity and mortality prohibiting the use of alloHSCT in elderly patients or patients with comorbidities.[52] It prompted the development of reduced intensity conditioning (RIC) strategies limiting NRM and maintaining sufficient GVL-effect.[53,54] Which type of conditioning is preferred in patients with AML in first CR? In the past, several studies have compared conventional consolidation with MAC alloHSCT with sibling donors and found improved survival in younger recipients of MAC alloHSCT.[15-20] However, because of increased NRM in patients over the age of 40 years, the advantage appeared to be restricted to patients <40 years.[15-20] In **Chapter 2**, improved survival was noted in AML patients aged 40-60 years receiving RIC alloHSCT as compared with chemotherapeutic PRT. Although MAC alloHSCT also strongly reduced relapse, overall outcome was not significantly different comparing MAC alloHSCT with chemotherapy because of increased NRM following MAC alloHSCT. The prospective comparison of RIC vs MAC alloHSCT was done in a prematurely closed randomized study with no major differences in outcome, although the RIC regimen involved relatively intensive conditioning with 8 Gy of total body irradiation.[55] Recently, Scott et al.[56] reported a prospectively randomized study, including 272 patients with AML or myelodysplastic syndromes (MDS) receiving either RIC or MAC. The median age of included patients was 55 years with similar distribution of patient and disease characteristics. With a follow-up of only 18 months, RFS was significantly better following MAC alloHSCT, whereas OS was not significantly different as compared with RIC alloHSCT. As expected, RIC resulted in decreased NRM but higher relapse rates as compared with MAC. The cumulative incidence of NRM was remarkably low following MAC alloHSCT estimating 16% (95% CI 10–23%) at 18 months,[56] stressing the improvement of NRM as underlined by the longitudinal results of the Seattle group as reported by Gooley et al.[50] The European Group of Blood and Marrow Transplantation (EBMT) also conducted a prospective randomized study comparing RIC and MAC alloHSCT in 129 patients with MDS or secondary AML.[57] Similar OS and RFS at 2 years were reported, although a trend towards improved OS following RIC alloHSCT was observed (OS at 2 years: 76% (95% CI 66–87%) after RIC versus 63% (95% CI 51–75%) after MAC).[57] Based on the results of these trials, both RIC and MAC alloHSCT continue to be used in patients with MDS or AML. Obviously, longer follow-up from these studies is needed. Somewhat disappointingly, these prospective randomized studies failed to provide a definite answer but rather emphasize the use of a more personalized approach taking into account the risk for NRM, instead of applying a strict age cut-off to decide for RIC of MAC. Meanwhile, the risk of NRM continues to decline, most recently because of the introduction of post-transplantation cyclophosphamide for GVHD prevention.[58-63] Therefore, new prospective studies are needed re-evaluating established risk scores for NRM.

What is the optimal type of donor or graft source?

HLA-identical sibling donors and adult matched unrelated donors (MUD) have become the most common type of donor for alloHSCT.[64] The probability of identifying an adult MUD for patients who lack a HLA-identical sibling can be as high as 60-80% for Caucasian patients, whereas finding a suitable MUD for patients from ethnic minorities is less successful.[65-68] Alternative stem cell sources include umbilical cord blood (UCB) and stem cells from haplo-identical donors. UCB grafts are usually available within a few weeks and HLA-matching is less stringent for UCB as compared with adult donor grafts, HLA matching does impact on outcome following alloHSCT with UCB.[69] The main issue has been the relatively low number of hematopoietic progenitor cells in UCB grafts, which is associated with a higher rate of graft failure and delayed hematopoietic recovery.[70-74] Recently, the use of haplo-identical donors for alloHSCT has gained attention and application because of improved transplantation techniques and pharmacological manipulation of host-versus-graft and graft-versus-host reactions.[75]

Each type of donor and/or stem cell source has its own advantages and drawbacks, but which donor type has to be preferred? **Chapter 6** compared outcome of alloHSCT with HLA-identical siblings, MUD, mismatched unrelated donors (MMUD), UCB grafts and haplo-identical donors. Similar to previous studies,[76-80] we found slightly higher NRM following MUD, whereas counterbalancing lower relapse resulted in nearly equivalent outcomes as compared with HLA-identical siblings.

One or two allele MMUD may serve as a good alternative in case a HLA-identical sibling or MUD is not available, but HLA-incompatibility results in increased incidence of GVHD and subsequent increased NRM.[81] Although HLA-incompatibility may augment a GVL-effect resulting in slightly less relapse, MMUDs were associated with reduced OS because of higher NRM (**Chapter 6**). A recent meta-analysis compared outcomes of patients who were allografted with either 10/10 HLA-MUD or 9/10 HLA-MUD and found an estimated 27% increased risk of NRM for patients following 9/10 HLA-MUD.[82] These data strongly suggest that alloHSCT with a MMUD may benefit from intensified GVHD prophylaxis, such as being applied in the setting of haplo-identical donors.

Results of alloHSCT with UCB grafts approximated those of MUD alloHSCT in retrospective registry studies, although hematopoietic recovery is delayed as compared with MUD alloHSCT and graft failure was more frequently observed.[83-85] In addition to a significant higher incidence of graft failure, we also observed that alloHSCT with UCB grafts is associated with higher NRM as compared with HLA-identical siblings and MUD, which resulted in significantly lower OS (**Chapter 6**). Thus, improving hematopoietic engraftment and hematopoietic recovery remains a major challenge in UCB graft transplantation. Currently, a number of groups are developing several techniques including ex-vivo expansion of hematopoietic cells,[86-89] priming of UCB progenitors,[90,91] or intra bone marrow injection.[92,93]

The use of haplo-identical donors was previously associated with extensive NRM precluding a broad application.[94] The development of a transplant strategy in which high-dose cyclophosphamide is infused after alloHSCT to selectively deplete proliferating alloreactive T-cells, resulted in favorable engraftment, limited GVHD, limited NRM and overall survival, approximating that following matched donor alloHSCT.[58-63] A recent biologically randomized study from China suggested similar outcome using HLA-identical donors or haplo-identical family donors with ATG as part of the conditioning regimen.[95] As shown in **Chapter 6**, we found encouraging outcome of haplo-identical donors, which approximated results of HLA-identical siblings and MUDs. However, conditioning and post-transplant regimens varied and follow-up was relatively short in the cohort of haplo-identical donors.

What is the optimal type of donor or graft source? As recently shown in a large cohort of patients from the EBMT, outcomes of alloHSCT were systematically improved with decreasing phenotypic and genotypic antigen disparity.[96] Thus, if a HLA-identical donor or MUD is available, the best match should be the donor of choice. Alternative donors including MMUD, UCB grafts and haplo-identical donors could be used when a fully matched donor is not available and an urgent transplant is required. Results following haplo-identical donors are encouraging, which donor type may step up in the donor hierarchy. However, comparative prospective studies including haplo-identical donors are needed to definitely establish its new place in the donor hierarchy.

Improving infectious complications

Apart from GVHD, infectious complications are a common cause of NRM following alloHSCT.[50,97] Several risk factors for infectious complications have been identified including conditioning regimen, prolonged neutropenia after alloHSCT, prolonged T-cell and B-cell immune reconstitution, the use of immunosuppressive drugs, the presence GVHD, the use of a central venous catheter and previous exposure to infectious agents during pre-alloHSCT treatment.[98-103] Recipients of an alloHSCT are at risk for different infectious complications, which occurrence is generally divided in three phases as summarized here.[103,104] Firstly, the early pre-engraftment phase is characterized by neutropenia and mucosal damage. The most common infectious threats are bacterial (ie, gram-negative bacteria related to mucositis, gram-positive bacteria related to central venous catheters and *Clostridium difficile*) and fungal pathogens (ie, *Candida* species and molds). Herpes simplex virus (HSV) may reactivate during neutropenia in most HSV-seropositive patients, which can be prevented by aciclovir. The second time-interval starts after engraftment and early hematopoietic recovery including neutrophil and NK-cell mediated immunity. The development of GVHD in the early post-engraftment phase poses an additional risk for bacterial infections, particularly in patients with intestinal GVHD whom are at risk for life-threatening bacteremia. Patients who require high-dose steroids may develop infections with *Aspergillus* species and other mold infections,

but also *Pneumocystis* pneumonia. The intensified immunosuppression for patients with GVHD further adds to the risk for opportunistic infections, including cytomegalovirus (CMV) viremia, which is still associated with transplant outcome (**Chapter 7**).[105,106] CMV viremia may precede pneumonia or enterocolitis, which may result in substantial morbidity and mortality.[105,106] In addition, adenovirus, and BK virus are encountered during that phase. The third phase usually starts as from three months after alloHSCT with gradual reconstitution of humoral and cellular immunity. Infectious complications may include similar pathogens as described in the second phase, as these are primarily related to GVHD and the prolonged use of immunosuppression. Late infections after alloHSCT may also include encapsulated bacteria (eg, *Streptococcus pneumoniae*) because of impaired opsonization, but also varicella zoster virus (VZV) infections. Of note, patients who have received a T-cell depleted graft or conditioning with ATG are at increased risk for Epstein-Barr virus reactivations and Epstein-Barr virus lymphoproliferative disease.[107-109] Other opportunistic infections may include parasites including Protozoa (eg, *Toxoplasma gondii, Leishmania* species, *Giardia Lamblia*) and Helminths (ie, *Strongyloides stercoralis*).[110] The risk of community-acquired respiratory viruses is considerable during all three phases after transplant, but predominantly related to seasonal outbreaks.[104,105] Recipients of alloHSCT are at increased risk of viral reactivations, but also de novo infections, with for example fulminant hepatitis following infection with hepatitis A virus, hepatitis B virus and hepatitis C virus.[111] In **Chapter 8**, we identified hepatitis E virus (HEV) as a novel opportunistic pathogen in alloHSCT recipients. We discovered a relatively high probability of developing chronic HEV infection in patients who were mistakenly diagnosed with drug induced liver injury or hepatic GVHD. The incidence of HEV infection may be up to 2.4%, which should be included in the differential diagnosis of liver enzyme abnormalities post-alloHSCT.[112] As serum immunoglobulins against HEV appeared not reliable for the diagnosis of HEV infection, we recommend the use of a HEV PCR in patients for whom impaired immune reconstitution is expected (eg, alloHSCT recipients).

Supportive care post-alloHSCT is intended to prevent many of the above infectious pathogens and also depends on the phase after transplant. Generally, alloHSCT recipients are screened for viral reactivations and prophylactically treated for *Pneumocystis* pneumonia, HSV and VZV. In case complications may develop, intensive screening for viral infections or reactivations is recommended, preferably not only by serology, but also by PCR, as immunoglobulin responses fail to occur in most alloHSCT recipients.

Predictive models

With GVHD and infections being the most common causes of alloHSCT-related mortality, the risk of mortality needs to be quantified. Composite risk scores have been established, which allowed to predict for NRM and overall outcome. Two generally approved transplant-risk scores have been developed and validated, including the EBMT-risk score[113] and the

Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI).[114] The EBMT-risk score is based on patient and transplantation characteristics, which was developed in CML patients and subsequently validated in other patient groups including AML.[115,116] The HCT-CI originated from the Charlson Comorbidity Index[117] and consists of 17 comorbidities which contribute to a cumulative score.[114] The HCT-CI was extensively validated and has been continuously being refined including age, disease status, or bio-markers.[118-120] Other groups have also developed predictive models for NRM, with some groups modifying the weights of the EBMT-risk score and the HCT-CI,[121-123] whereas others combined transplant-related parameters and patient characteristics.[124-126] A very interesting model was developed by the EBMT acute leukemia working party which is based on a machine learning algorithm.[127] The model was constructed and validated in acute leukemia patients and based on 10 variables which strongly predicted for NRM at 100 days and at 2 years.[126,128] With the introduction of RIC, alloHSCT has become a treatment option for older and medically less fit patients. However, both the EBMT-risk score and the HCT-CI performed less well in older patients receiving RIC alloHSCT.[115,121,124,129,130] In **Chapter 8**, we developed a dedicated score integrating the EBMT-risk score and HCT-CI by reassessing the individual parameters in recipients of RIC alloHSCT with AML in first CR. Three risk groups were identified with increased predictive power in this subgroup of elderly patients, although external validation is needed. The lack of predictive power of the established risk scores and the development of a refined and dedicated model emphasizes that prediction of NRM requires a continued reassessment of risk scores. Predictive scores may also merit from studying specific subgroups, such as older patients who receive RIC alloHSCT, which is currently the majority of alloHSCT recipients. In addition, recent results of post-transplantation cyclophosphamide suggest a further decline of NRM towards 10-15%.[58-63] Therefore, new developments in post-transplant care urges the need to continuously re-evaluate established risk scores for NRM, preferably in prospective studies.

How to deal with alloHSCT in statistical analysis of AML patients?

Analyzing alloHSCT as PRT may yield statistical challenges and a number of methods have been developed and used over the years. Table 1 summarizes the different statistical methods with each their own advantages and disadvantages, which will be discussed here.

Randomized studies

Obviously, the golden standard to compare treatment approaches continues to be a prospective randomized study to minimize selection bias and to allow for an intention to treat analysis. However, these studies have been extremely difficult to perform in the field of PRT in AML patients because of the divergent intensity of the treatment arms. Recently, patient numbers to be included in the randomized study from the EBMT (NCT00766779) comparing alloHSCT with no alloHSCT in elderly patients with AML had to be adapted due to slow accrual. Alternative comparative methods were developed and applied as randomized studies are scarce, but the need for comparative data is high, with ongoing improvement of both conventional treatment and transplantation approaches in AML.

Donor vs no-donor analysis

First, alloHSCT has been evaluated by comparing patients with a donor vs those without, which analysis is based on a biological treatment assignment with the availability of a sibling donor.[18-20,131] These studies allow for a prospective intention to treat analysis and thereby may approximate real randomized studies. However, concern has been raised that such a methodology may underestimate the donor group, because the percentage of patients actually receiving a transplant may be relatively low due to a number of reasons. First, the availability of a donor should not be mistaken for an intention to transplant, as indications and preferences may vary among countries, cooperative groups, centers, and individual physicians.[132] Second, the availability of an HLA-identical sibling does not take critical eligibility criteria of both donor and recipient into account. Third, the actual percentage of patients receiving a transplant may also decline in patients with high risk disease, failing to achieve remission or encountering early relapse. These patients are less likely to receive an allograft in first CR, despite an identified donor and being accounted for in the donor group of the analysis.[133,134] With the advent of MUDs and their increasing application in AML, sibling donor versus no-donor studies have become obsolete. Therefore, alternative statistical methods were applied to compare alloHSCT with other types of PRT, including retrospective analysis, landmark analysis, or a time-dependent analysis.[133,135,136]

Retrospective analysis

Retrospective registry studies have been performed on a broad scale since the establishment of large registries, including the EBMT and the International Blood and Marrow Transplant Research (IBMTR). Retrospective studies compare treatment groups that include only those

patients, who actually completed the originally allocated treatment. The analysis provides an estimate of the true efficacy of an intervention, although selection bias may result in an exaggerated treatment effect, because of more fit patients in the transplant group, and unfit patients ineligible for alloHSCT in the reference group. That selection bias can only be minimized by excluding those patients in a retrospective comparison, but still that bias may compromise the comparison. Another type of selection bias that occurs in retrospective analyses is a time-interval bias, also called the guarantee time, which is the time from CR to transplant.[133,136] Time to transplant may depend on different factors including donor search, hematological recovery and clinical recovery from previous chemotherapy. During the time needed to prepare for an allograft, patients may receive an additional course of chemotherapy, but also may experience disease relapse or even NRM. However, that does not apply to transplanted patients, especially those with a long guarantee time during which time period no early relapse occurred. The bias of guarantee time may be reduced when including that time-interval as a covariate in the multivariable analysis of a retrospective analysis. However, including a variable in a multivariable comparison does not solve the problem of its inherent bias.

Landmark analysis

The landmark analysis is a statistical method that may account for the time-interval bias by attributing predefined guarantee time to all patients. However, determining a clinically relevant landmark time is very difficult in transplant studies as time from first CR to transplant may vary from a few weeks to a year. In addition, the type of risk differs between treatment groups and evolves in time. Landmark analysis may be used when a clinically relevant fixed time-point is present before the start of PRT, such as the 30-day period after induction chemotherapy for recipients not receiving PRT, as described in **Chapter 3**. The same selection bias applies to a landmark approach as compared with retrospective studies. In addition, a number of patients are excluded from the analysis because of early events, which subsequently may favor the alloHSCT group as early transplant-related deaths are more frequently excluded than relapse-related deaths in the reference group.

Time-dependent analysis

The time-dependent methodology is a statistical method, which completely excludes the bias introduced by the time to transplant. That method was developed by Mantel and Byar attributing the favorable time period of guarantee time to the reference group.[137] In a time-dependent analysis, patients are initially counted as at risk in the control group (right-censoring) until alloHSCT and thereafter as at risk in the alloHSCT group (left-censoring). The time-dependent methodology has a number of disadvantages similar to the landmark analysis, including the selection bias of patients who actually received a transplant. Both analyses might favor alloHSCT recipients as patients who are not transplanted because of

ineligibility are included in the control group.[136] However, the weight of events for relapse or NRM is less in the reference group in the early time period as the denominator of that group is larger and it lacks the events of the (right-censored) patients waiting for their allograft. The time-dependent methodology may also correct for the most important characteristics affecting relapse and NRM by multivariable analysis, as previously described by Simon and Makuch.[138] We have used such an analysis with time-dependent covariates autologous HSCT and alloHSCT in **Chapters 2, 3, 4 and 5**.

Which type of statistical method has to be preferred for analyzing PRT in patients with AML? Previously, Hospital et al.[133] performed both a donor versus no-donor analysis and a time-dependent analysis of PRT with alloHSCT in a cohort of patients with adverse karyotype AML. The effect of alloHSCT was found to be stronger as compared with no alloHSCT in a time-dependent analysis, which may largely be explained by the percentage of patients not being transplanted in the donor group of the donor versus no-donor analysis. Collectively, in view of the advantages and disadvantages of the different statistical methods and the lack of randomized studies, a time-dependent method may currently be the preferred method for the comparative analysis of alloHSCT in AML, as retrospective and landmark studies may overestimate the effect of alloHSCT.

Table 1 Types of comparative studies evaluating alloHSCT

Type of study	Principle	Advantages	Disadvantages
Prospective randomized	Randomized allocation of treatment	• Intention to treat • No selection bias, which creates comparable groups • Observer bias is minimized	• Exclusion of non-eligible patients • Randomizing treatments with divergent intensity may not be ethical and feasible • Patient refusal • AlloHSCT not applied in all allocated patients
Donor vs no-donor	Treatment allocation based on the availability of a sibling donor	• Biological randomization based on sibling donor availability approximating a randomized study • Intention to treat • Observer bias is minimized • Can be incorporated in well-designed prospective AML studies	• Donor availability does not imply intention to transplant or actual receipt of alloHSCT • The use of unrelated donors overestimates the no-donor group
Retrospective	Comparison of patients who actually completed the treatment	• True effect of alloHSCT • Easily applicable by most international transplant registries	• Selection bias of patients who actually received transplant • Time-interval bias • Incomplete picture of preceding treatment and medical history • No or incomplete data monitoring
Landmark	Analysis from a fixed time point after the initiation of therapy	• Less time-interval bias • To be incorporated in prospective studies	• Clinical relevant landmark time may be difficult to determine • Selection bias of patients who actually received transplant • Loss of patients • Time interval bias caused by landmark (exclusion of more early events in the alloHSCT group than the reference group)
Time-dependent	Analysis from the start of post-remission treatment (or achievement of CR) allowing for patients to switch from treatment groups	• No time-interval bias • True effect of alloHSCT • Allows for evaluating a number of time-varying covariates	• Selection bias of patients who actually received transplant • Interpretation of non-proportionality of time-dependent covariates may be difficult

Future perspectives: personalized PRT approach for AML patients

Given the many variables associated with outcome and their possible opposing effects, it seems no longer acceptable to propose a 'one size fits all' model in decision making for PRT. That approach would imply an application of alloHSCT only based on AML risk groups, although neglecting a number of important parameters impacting on overall outcome after alloHSCT, autologous HSCT or continued chemotherapy. The following characteristics would need to be incorporated in a personalized approach: leukemia risk, MRD status, and the risk for NRM. Integrating all these parameters, I would propose a personalized model for the application of alloHSCT in patients with AML in first CR as summarized in Figure 2. Leukemia risk is based on the ELN risk classification,[1] and MRD status may be determined by multiparametric flow cytometry or quantitative PCR. NRM risk may be estimated by using risk scores which are preferably dedicated for a specific patient subgroup and validated in different studies.

Favorable risk AML patients without MRD may not be transplanted in first CR, which procedure may be reserved for salvage therapy in second CR. However, the risk of relapse in favorable risk patients who harbor MRD after induction chemotherapy may still be significant, evoking the question which type of PRT should be preferred. For those patients with a very low risk for NRM and a well matched donor, one might consider alloHSCT as PRT, whereas autologous HSCT or chemotherapy can be used in patients without a well matched donor and/or higher risk for NRM. AlloHSCT in patients with intermediate risk AML increasingly depends on the presence or absence of MRD. The possibility to apply alloHSCT in second CR provides an additional argument to refrain from allografting in intermediate risk patients without MRD, similar to the policy in favorable risk patients without MRD. Intermediate risk patients however with MRD qualify for alloHSCT unless a predicted NRM risk of 30% is exceeded. Adverse risk AML patients with MRD harbor the highest risk of relapse and are preferably transplanted as early as possible after obtaining CR. Patients with an adverse risk AML without MRD are also preferably transplanted, although patients with a high risk for NRM (>30%) may alternatively receive autologous HSCT or a third cycle of chemotherapy.

Donor availability should not affect the indication for an allograft primarily, although it may be important when considering alloHSCT in patients with favorable risk AML with MRD or intermediate risk AML without MRD, for whom a well-matched donor may be preferred.

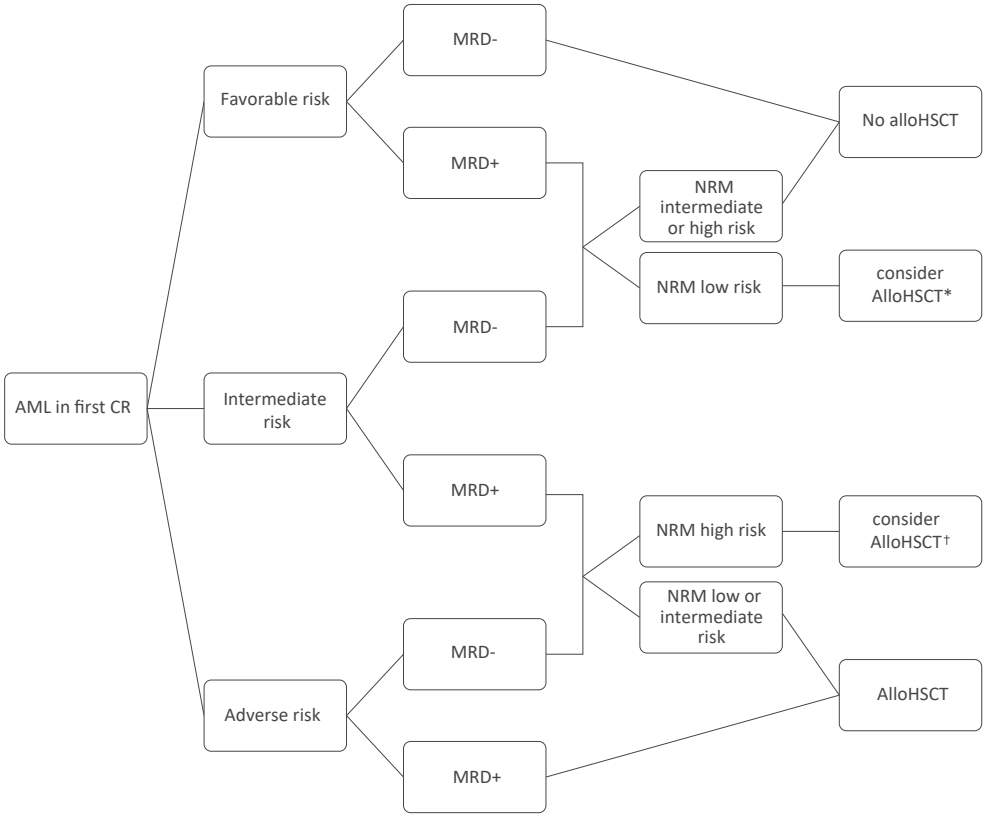

Figure 2 Personalized transplant decision model for patients with AML in first CR

- Leukemia risk is based on the ELN risk classification.[1]
- Minimal residual disease (MRD) may be measured by either multiparametric flow cytometry or quantitative PCR for specific molecular markers
- Non-relapse mortality (NRM) could be assessed by preferably dedicated predictive models. NRM low risk is considered as <15%, intermediate risk as 15-30%, high risk as >30%.

* In case a well-matched donor is available (ie, HLA-identical sibling or MUD)

† Consider an alloHSCT based on donor availability and alternatively proceed to PRT with autologous HSCT or a third cycle of chemotherapy.

The optimal conditioning strategy for patients with AML in first CR is still not clear, as recent randomized studies show no significant differences in overall outcome between MAC and RIC irrespective of age and comorbidity.[56,57] Some studies suggested that specific patient subgroups, especially with high risk disease, may benefit from a MAC alloHSCT. The open question of preferred conditioning type and the continued decline of NRM necessitate further prospective studies evaluating both conditioning type and the risk of NRM, including other variables associated with NRM.

Post-transplant strategies for alloHSCT are continuously evolving in order to improve outcome. GVHD prophylaxis with cyclophosphamide has been established as an excellent post-transplant platform with low toxicity with NRM rates of only 10-15%, but maintaining GVL-effect. These promising results may impact on the indication of alloHSCT, but also urge the need to validate predictive scores for NRM in the setting of post-transplant cyclophosphamide. In addition, allogeneic immunotherapy may be improved by early tapering of immunosuppression and/or pre-emptive DLI for optimal leukemic control. In addition, the continued application of novel post-transplant strategies including epigenetic therapy to enhance the GVL-effect (ie, demethylating agents and histone deacetylase inhibitors[63]), new agents such as tyrosine kinase inhibitors for specific molecular mutations (ie, *FLT3*-ITD[139], *IDH*1/2[140,141]) or targeted immunotherapy with chimeric antigen receptor T-cells may offer further therapeutic options minimizing relapse after alloHSCT.

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A

ADDENDUM

SUMMARY

Acute myeloid leukemia (AML) is a malignant disorder of the bone marrow characterized by impaired maturation and increased proliferation of myeloid progenitor cells. Although still based on morphological examination, genetic assays have become indispensable for diagnosis, risk classification, and treatment decision making. This thesis deals with the place of allogeneic hematopoietic stem cell transplantation (alloHSCT) as post-remission treatment in patients with newly diagnosed AML. The choice for alloHSCT increasingly depends on genetic characteristics of the leukemia, treatment response, and risk factors associated with the transplant procedure. The majority of patients obtain a first hematological complete remission (CR) after chemotherapeutic induction treatment, but the risk of relapse without further treatment constitutes the major obstacle. Post-remission treatment reduces the risk of relapse and may consist of continued chemotherapy, autologous HSCT and alloHSCT. The beneficial effect of alloHSCT is mainly based on an immunological graft-versus-leukemia (GVL)-effect of alloreactive T-cells resulting from allogeneic antigen disparity. The GVL-effect of alloHSCT was shown to provide the most effective anti-leukemic therapy in AML, but counterbalancing non-relapse mortality may compromise that favorable effect, especially in older patients or patients with comorbidities. Non-relapse mortality has been reduced with the development of reduced intensity conditioning regimens, whereas the alloreactive GVL-effect was still present. Reduced intensity conditioning regimens broadened the application of alloHSCT, particularly for elderly patients or for patients with concurrent morbidity. The clinical decision for post-remission treatment is primarily based on the genetic risk profile of the leukemia, but also on other factors, including the possibility to harvest autologous stem cells, the availability of an allogeneic donor, and patients' performance status with concurrent comorbidity (**Chapter 1**). The integration of all these parameters has become complex and asks for a personalized approach of patients with AML. The studies described in this thesis have addressed a number of these parameters, finally resulting in a personalized approach as regards transplant decision making.

Previous studies suggested that the favorable effect of alloHSCT was limited to patients below the age of 40 years as a result of increased non-relapse mortality following myeloablative conditioning alloHSCT. In **Chapter 2**, the comparative value of post-remission treatment with alloHSCT, autologous HSCT and chemotherapy was addressed in patients with AML aged 40 to 60 years. Improved overall survival was observed by alloHSCT compared with chemotherapeutic post-remission treatment in patients with intermediate risk and adverse risk AML. In addition, the intensity of the conditioning regimen did not significantly affect the rate of relapse after alloHSCT, thereby questioning the necessity of myeloablative conditioning in patients aged 40 to 60 years. Of note, alloHSCT and autologous HSCT did not significantly differ with respect to overall survival in intermediate risk patients, although relapse-free survival was better following alloHSCT, suggesting that autologous HSCT remains a treatment option to be considered in patients with intermediate risk AML.

Chapter 3 describes a comparative analysis of post-remission treatment in patients above the age of 60 years. Post-remission treatment in these elderly patients was performed with alloHSCT following reduced intensity conditioning, which was compared with other types of post-remission treatment including, chemotherapy, gemtuzumab ozogamicin or no further treatment. The analysis showed that alloHSCT was associated with better overall survival compared with other post-remission therapies or no post-remission therapy, especially in patients with intermediate risk or adverse risk AML. Non-relapse mortality in these elderly recipients of reduced intensity conditioning alloHSCT was relatively low.

Patients with a cytogenetically normal intermediate risk AML may be further subclassified based on molecular markers including *NPM1* and *FLT3*-ITD with its mutant to wild-type ratio. In **Chapter 4**, post-remission treatment with alloHSCT, autologous HSCT and chemotherapy was compared in a cohort of cytogenetically normal intermediate risk AML patients with available *NPM1* and *FLT3*-ITD mutational status. Post-remission treatment did not differentially affect outcome in a favorable group of patients with mutated *NPM1* without *FLT3*-ITD. Outcome in patients with a high allelic ratio of *FLT3*-ITD appeared very poor, with low patient numbers hampering a comparison by type of post-remission treatment. In contrast, overall survival by alloHSCT following reduced intensity conditioning compared with chemotherapy was improved in a larger intermediate group, characterized by *FLT3*-ITD with a low allelic ratio and wild-type *NPM1* without *FLT3*-ITD AML. Recipients of myeloablative conditioning alloHSCT and autologous HSCT yielded similar overall survival in that intermediate risk group, which did not significantly differ from post-remission treatment with chemotherapy. Thus, alloHSCT may be the preferred type of post-remission treatment in these patients with molecularly intermediate risk AML.

Chapter 5 addresses the GVL-effect of alloHSCT in patients with AML in first CR with or without minimal residual disease (MRD) as determined by flow cytometry. The GVL-effect was considered as the relative reduction of relapse of alloHSCT compared with post-remission treatment with chemotherapy or autologous HSCT. The GVL-effect appeared to be similar in MRD positive and MRD negative patients, which suggests that the alloreactive effect of T-cells rather depends on immunological characteristics and differences than on characteristics of the underlying AML. It further suggests that alloHSCT may be applied in both MRD negative and positive patients and that decision making should take other characteristics into account as well, such as risk scores for non-relapse mortality.

Although alloHSCT may be the preferred type of post-remission treatment for patients with adverse risk AML, the preferred type of donor may be debated. Alternative stem cell sources are increasingly being used for alloHSCT, including matched or mismatched unrelated donors, cord blood grafts or haplo-identical donors. In **Chapter 6**, these different type of donors were compared with HLA-identical siblings in patients with adverse risk AML in first CR. The comparative analysis suggests that well-matched donors including HLA-identical siblings and MUDs are to be preferred over cord blood grafts and mismatched

unrelated donors. Results of alloHSCT with haplo-identical donors are encouraging and approximate those of matched related or matched unrelated donors. However, comparative prospective studies of haplo-identical alloHSCT with other donor types are warranted and longer follow-up after haplo-identical alloHSCT may be needed to definitely establish its place in the hierarchy of alternative donors.

Non-relapse mortality after alloHSCT can be predicted by the hematopoietic cell transplantation comorbidity index (HCT-CI) and the European Group for Blood and Marrow Transplantation (EBMT) score, which are composed of different parameters. In **Chapter 7**, these composite risk scores were reassessed in a cohort of patients with AML in first CR who received an alloHSCT as post-remission treatment following reduced intensity conditioning. Both established risk scores showed relative weak predictive power. Subsequently, all parameters were reevaluated in a multivariable model resulting in the selection of a total of 16 parameters, which were subsequently integrated into a new score. That integrated score yielded increased predictive power for patients with AML in first CR. The lack of predictive power of the established risk scores and the development of a refined and dedicated model emphasizes that prediction of non-relapse mortality requires a continued reassessment of risk scores.

Infectious complications are a common cause of non-relapse mortality following alloHSCT. In **Chapter 8**, we have been the first to study the incidence and sequelae of hepatitis E virus infection in recipients of alloHSCT. The incidence of hepatitis E virus was 2.4% in our study, whereas a number of patients developed a chronic hepatitis E virus infection. In our cohort, patients with a hepatitis E virus infection were previously diagnosed with hepatic graft-versus-host disease or drug induced liver injury as a cause of liver enzyme abnormalities. Thus, hepatitis E virus infection should be included in the differential diagnosis in all recipients of alloHSCT with severe liver enzyme abnormalities.

Finally, a 'one size fits all' model seems no longer acceptable in decision making for post-remission treatment with many variables associated with outcome and their possible opposing effects. Precision medicine for patients with AML is urgently needed. Thus, the decision to transplant or not in an individual patient might depend on weighing the risk of relapse versus the personalized risk of non-relapse mortality. A personalized approach of post-remission treatment for patients with AML in first CR is proposed in **Chapter 9**. Such a personalized approach should be based on the risk of AML, MRD status and the risk for non-relapse mortality. However, prospective studies continue to be important to evaluate new risk parameters and to study their individual and integrated impact on personalized decision making.

NEDERLANDSE SAMENVATTING

Acute myeloïde leukemie (AML) is een kwaadaardige ziekte van het beenmerg waarbij er sprake is van een verminderde uitrijping en een toegenomen proliferatie van myeloïde voorlopercellen. Hoewel de diagnose van AML nog steeds gebaseerd is op morfologie wordt genetische analyse steeds belangrijker voor de diagnostiek, risico classificatie, en de keuze voor behandeling van AML patiënten. Dit proefschrift bespreekt de plaats van allogene (donor) hematopoïetische stamceltransplantatie (alloHSCT) als behandeling voor patiënten met een nieuw gediagnosticeerde AML, die een remissie bereikt hebben na 1 of 2 kuren chemotherapie. De keuze voor alloHSCT als post-remissie behandeling hangt steeds meer af van genetische kenmerken van de leukemie, de respons op de voorgaande behandeling, maar ook van risicofactoren die geassocieerd zijn met de transplantatie. De meerderheid van de patiënten behaalt weliswaar een eerste hematologische complete remissie (CR) na inductie behandeling met chemotherapie, maar het risico op een recidief van de leukemie zonder verdere behandeling blijft een groot struikelpunt. Post-remissie behandeling reduceert het risico op een recidief en kan uiteindelijk tot genezing leiden. Deze kan bestaan uit het continueren van chemotherapie, een autologe HSCT, of een alloHSCT. Het gunstige effect van alloHSCT is voornamelijk gebaseerd op een immunologisch anti-leukemie effect van alloreactieve T-cellen, die reageren op antigeen verschillen tussen donor en patiënt. Het is aangetoond dat dit anti-leukemie effect van alloHSCT de meest effectieve behandeling is, hoewel sterfte gerelateerd aan de transplantatieprocedure dat gunstige effect teniet kan doen. AlloHSCT behandelingssterfte speelt voornamelijk een rol bij oudere patiënten of patiënten met comorbiditeit. Deze sterfte is afgenomen sinds de ontwikkeling van een minder intensieve voorbehandeling of conditionering voorafgaande aan de transplantatie, terwijl het anti-leukemie effect nog steeds aanwezig bleek. De ontwikkeling van minder intensieve conditioneringsschema's hebben de toepassing van alloHSCT doen toenemen, in het bijzonder bij oudere patiënten of patiënten met relevante comorbiditeit. De klinische besluitvorming voor post-remissie behandeling is hoofdzakelijk gebaseerd op het genetische risico profiel van de leukemie (gunstig, intermediair of ongunstig), maar ook andere factoren spelen een rol zoals de mogelijkheid tot het verzamelen van autologe stamcellen, de beschikbaarheid van een allogene donor en de conditie van de patiënt inclusief comorbiditeit (**Hoofdstuk 1**). Het samenvoegen van al deze parameters voor de benadering van patiënten met AML is complex en vraagt om een individuele aanpak. De studies die worden beschreven in dit proefschrift hebben een aantal van deze parameters als onderwerp. Dit heeft geresulteerd in een voorstel tot een sterk geïndividualiseerde benadering van de besluitvorming om een alloHSCT al dan niet toe te passen.

Eerdere studies hebben laten zien dat het gunstige effect van alloHSCT zich beperkte tot patiënten met een leeftijd van 40 jaar of jonger als gevolg van een toegenomen behandelingssterfte na een myeloablatieve conditionering bij de oudere patiënten.

In **Hoofdstuk 2** wordt post-remissie behandeling met alloHSCT, autologe HSCT en chemotherapie vergeleken bij patiënten met AML in de leeftijdscategorie van 40 tot 60 jaar. De overleving van patiënten met een intermediair of ongunstig AML risicoprofiel bleek beter te zijn na een alloHSCT vergeleken met post-remissie chemotherapie. Tevens werd gevonden dat de intensiteit van de conditionering van alloHSCT geen significante invloed had op het optreden van een recidief, waardoor de noodzaak van een myeloablatieve conditionering voor patiënten in de leeftijdscategorie van 40 tot 60 jaar ter discussie gesteld kan worden. Overigens werd geen verschil gezien in overleving tussen alloHSCT en autologe HSCT in patiënten met een AML met een intermediair risicoprofiel, hoewel de ziektevrije overleving wel beter was na alloHSCT. Deze resultaten suggereren dat autologe HSCT een behandelingsoptie blijft voor patiënten met een intermediair risico AML.

Hoofstuk 3 beschrijft een vergelijkende analyse van post-remissie behandeling voor patiënten boven de leeftijd van 60 jaar. Post-remissie behandeling in deze oudere patiëntengroep bestond uit alloHSCT na minder intensieve voorbehandeling en werd vergeleken met andere vormen van post-remissie behandeling inclusief chemotherapie, gemtuzumab ozogamicin of geen verdere behandeling. De analyse laat zien dat patiënten die behandeld werden met een alloHSCT een betere overleving hebben vergeleken met andere post-remissie behandelingen of geen verdere post-remissie behandeling. Dit overlevingsvoordeel was vooral zichtbaar bij patiënten met een intermediair of ongunstig risicoprofiel. In deze oudere groep patiënten bleek de behandelingssterfte relatief laag door de toepassing van de minder intensieve voorbehandeling voorafgaande aan de transplantatie.

Patiënten met een intermediair risico AML met normale cytogenetica kunnen verder onderverdeeld worden op basis van moleculaire kenmerken zoals *NPM1* en *FLT3*-ITD, waarbij ook de ratio van gemuteerd versus wild-type *FLT3*-ITD in acht wordt genomen. In **Hoofdstuk 4** wordt post-remissie behandeling met alloHSCT, autologe HSCT en chemotherapie vergeleken in een patiëntengroep met een intermediair risico AML met normale cytogenetica, waarvan de *NPM1* en *FLT3*-ITD mutatie status beschikbaar was. Het type post-remissie behandeling bleek geen invloed te hebben op de reeds gunstige overleving van een groep AML patiënten met gemuteerd *NPM1* zonder *FLT3*-ITD. Patiënten met een hoge ratio van *FLT3*-ITD bleken een zeer slechte overleving te hebben, terwijl de patiënten aantallen te klein waren voor een vergelijking van de verschillende typen van post-remissie behandeling. Patiënten in een grote intermediaire groep bleken een betere overleving te hebben na alloHSCT met minder intensieve conditionering dan met post-remissie chemotherapie. Die intermediaire groep bestond uit patiënten met AML met een lage ratio van *FLT3*-ITD en uit patiënten met een wild-type *NPM1* zonder *FLT3*-ITD. Patiënten die behandeld werden met alloHSCT na een intensieve of myeloablatieve conditionering of een autologe HSCT hadden dezelfde overleving in deze intermediaire groep, hetgeen niet significant verschilde van post-remissie behandeling met chemotherapie. Derhalve kan alloHSCT worden beschouwd als de post-

remissie behandeling van keuze voor patiënten met een moleculair intermediair risico AML.

Hoofdstuk 5 gaat in op het anti-leukemie effect van alloHSCT bij patiënten met AML in eerste CR met of zonder restziekte ofwel "minimale residuale ziekte" (MRD) wat bepaald werd met flowcytometrisch onderzoek van beenmerg of perifeer bloedmonsters na het bereiken van die eerste CR. Het anti-leukemie effect werd berekend als de relatieve vermindering van de recidiefkans door alloHSCT te vergelijken met post-remissie behandeling met chemotherapie of autologe HSCT. Het anti-leukemie effect bleek exact hetzelfde te zijn in MRD positieve en MRD negatieve patiënten, wat suggereert dat donor T-cellen met name reageren op de immunologische eigenschappen van de leukemiecellen. Het anti-leukemie effect is blijkbaar minder afhankelijk van de genetische eigenschappen van de leukemiecellen. Deze observatie geeft ook aan dat alloHSCT zowel bij MRD positieve als MRD negatieve patiënten toegepast kan worden en dat de besluitvorming tot alloHSCT niet zozeer hoeft te berusten op de MRD status, maar meer op andere kenmerken zoals genetisch profiel van de leukemie en specifieke risico scores voor behandelingssterfte.

Hoewel alloHSCT de post-remissie behandeling van keuze is voor patiënten met een ongunstig risicoprofiel van de AML, zijn er verschillende typen van donoren mogelijk om de transplantatie mee uit te voeren. Stamcellen van een broer of zus, die wat betreft de witte bloedgroep, ofwel de "humane leucocyten antigenen" (HLA), identiek is aan de ontvanger, genieten nog steeds de voorkeur. Daarnaast zijn er verschillende andere mogelijkheden als een HLA-identieke broer of zus ontbreekt. Alternatieve stamcelbronnen worden steeds vaker gebruikt en bestaan uit goed passende vrijwillige onverwante donoren, navelstrengbloed transplantatie of haplo-identieke familiedonoren. Bij haplo-identieke donoren worden meerdere HLA verschillen tussen patiënt en familielid geaccepteerd. **Hoofstuk 6** vergelijkt de overleving na transplantatie middels deze verschillende typen alternatieve donoren met HLA-identieke broers of zussen bij patiënten met een ongunstig AML-risicoprofiel in eerste CR. De uitkomsten suggereren dat goed passende donoren bestaande uit HLA-identieke familie donoren en goed passende vrijwillige onverwante donoren de voorkeur genieten boven navelstrengbloed transplantatie of onverwante donoren met 1 of meer HLA verschillen. De resultaten met haplo-identieke donoren zijn veelbelovend en benaderen de resultaten van HLA-passende familiedonoren of onverwante donoren. Desalniettemin zijn prospectieve studies nodig die alloHSCT met haplo-identieke donoren vergelijken met andere type donoren. Pas dan kan alloHSCT met haplo-identieke donoren zijn definitieve plaats krijgen in de hiërarchie van de alternatieve donoren voor patiënten met AML.

Sterfte geassocieerd met de transplantatieprocedure ofwel behandelingssterfte kan worden voorspeld aan de hand van de 'hematopoietic cell transplantation comorbidity index (HCT-CI)' en de 'European Group for Blood and Marrow Transplantation (EBMT) score', welke scores bestaan uit verschillende parameters. In **Hoofdstuk 7** worden parameters van deze samengestelde scores opnieuw geëvalueerd bij patiënten met AML in eerste CR die behandeld werden met alloHSCT na een minder intensieve voorbehandeling.

Beide reeds gevestigde scores bleken een relatief zwak voorspellende waarde te hebben in deze patiëntengroep. Vervolgens werden alle parameters opnieuw opgenomen in een statistisch model, wat uiteindelijk resulteerde in een selectie van slechts 16 parameters, die behandelingssterfte goed bleken te voorspellen. Deze parameters werden geïntegreerd in een nieuwe score die een sterkere voorspellende waarde bleek te hebben dan de HCT-CI en de EBMT-score. Het gebrek aan voorspellende waarde van de gevestigde scores voor behandelingssterfte en deze ontwikkeling van een verfijnde en meer toegewijde score benadrukt dat een herevaluatie van reeds gevestigde risico scores noodzakelijk blijft.

Infectieuze complicaties zijn een belangrijke oorzaak van sterfte na alloHSCT. Wij hebben als eerste groep de incidentie en verschijnselen van hepatitis E virus infecties beschreven in patiënten die een alloHSCT kregen (**Hoofstuk 8**). De incidentie van hepatitis E virus infectie was 2.4% in onze studie, waarvan een deel van de patiënten een chronische hepatitis E virus infectie had ontwikkeld. De leverenzym afwijkingen bij deze patiënten werden initieel beschouwd als passende bij graft-versus-host ziekte of medicatie geïnduceerde leverschade. Hepatitis E virus infectie moet derhalve worden opgenomen in de differentiaal diagnose van alle patiënten die zich presenteren met ernstige leverenzymstoornissen na een alloHSCT.

Ten slotte kan worden geconcludeerd dat een algemeen geldend ‘one size fits all’ model niet langer acceptabel is voor de besluitvorming aangaande post-remissie behandeling van patiënten met AML vanwege de vele variabelen die invloed hebben op de uiteindelijke overleving. Een meer toegespitste benadering voor de individuele patiënt met AML is dringend nodig. Derhalve wordt voorgesteld om bij de besluitvorming tot wel of geen transplantatie zowel het risico van leukemie-recidief als het risico op behandelingssterfte mee te wegen. Een dergelijke individuele benadering van post-remissie behandeling voor patiënten met AML in eerste CR wordt voorgesteld in **Hoofdstuk 9**. Deze individuele benadering dient tenminste het genetische risicoprofiel van de leukemie, de hoeveelheid restziekte, en het individuele risico op behandelingssterfte te omvatten. Daarnaast blijven prospectieve studies nodig om nieuwe parameters te evalueren, maar ook om oude parameters in een nieuwe context opnieuw tegen het licht te houden.

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CURRICULUM VITAE

Jurjen Versluis was born on July 12th 1987 in Asperen, the Netherlands. In 2005, he graduated from the Gymnasium Camphusianum in Gorinchem and started his medical training at the VU University Medical Center in Amsterdam. He conducted his research internship at the department of medical oncology at the VU University Medical Center under the supervision of prof. dr. W.R. Gerritsen and focused on the treatment of patients with castrate resistant prostate cancer. He did a clinical internship at the Steve Biko Academic Hospital in Pretoria, South Africa, and he subsequently obtained his Medical Doctor degree in 2011. He then moved to Rotterdam and started to work as a resident at the department of hematology in the Erasmus Medical Center Cancer Institute in 2011 under the supervision of prof. dr. J.J. Cornelissen. During his clinical work at the Erasmus Medical Center Cancer Institute he initiated his clinical research with prof. dr. J.J. Cornelissen. In 2014, he started working as a resident of internal medicine at the Amphia Hospital in Breda supervised by dr. C. van Guldener and dr. J.W.J. van Esser as part of his medical specialization at the Erasmus Medical Center in Rotterdam supervised by prof. dr. J.L.C.M. van Saase and dr. S.C.E. Klein Nagelvoort-Schuit. Concurrently with his training in internal medicine, he proceeded with his research of allogeneic HSCT and AML which resulted in this PhD thesis. He returned to the Erasmus Medical Center in 2017 for his last year of training for internal medicine. In 2018, he will start working at the department of hematology for his specialization in hematology under the supervision of dr. P.A.W. te Boekhorst.

LIST OF PUBLICATIONS

This thesis

1. Versluis J, Kalin B, Zeijlemaker W, et al. Graft versus leukemia effect of allogeneic stem cell transplantation and minimal residual disease in patient with AML in first CR ICO Precision Oncology. **2017**; in press.

2. Versluis J, Labopin M, Ruggeri A, et al. Alternative donors for allogeneic hematopoietic stem cell transplantation in poor-risk AML in CR1. *Blood advances*. **2017**; 1: 477-85.

3. Versluis J, in 't Hout FEM, Devillier R, et al. Comparative value of post-remission treatment in cytogenetically normal AML subclassified by *NPM1* and *FLT3*-ITD allelic ratio. *Leukemia.* **2017**; 31: 26-33.

4. Versluis J, Hazenberg CL, Passweg JR, et al. Post-remission treatment with allogeneic stem cell transplantation in patients aged 60 years and older with acute myeloid leukaemia: a time-dependent analysis. *Lancet Haematol.* **2015**; 2: e427-36.

5. Cornelissen JJ, Versluis J, Passweg JR, et al. Comparative therapeutic value of post remission approaches in patients with Acute Myeloid Leukemia aged 40-60 years. *Leukemia*. **2015**; 29: 1041-50.

6. Versluis J, Labopin M, Niederwieser D, et al. Prediction of Non-Relapse Mortality in recipients of Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation with AML in first complete remission. *Leukemia.* **2015**; 29: 51-7

7. Versluis J, Pas SD, Agteresch HJ, et al. Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation. *Blood*. **2013**; 122: 1079-86.

Other publications

8. Beije N, Versluis J, Kraan J, et al. Circulating endothelial cell enumeration demonstrates prolonged endothelial damage in recipients of myeloablative allogeneic stem cell transplantation. *Haematologica*. **2015**; 100: e246-9.

9. van Dodewaard-de Jong JM, Santegoets SJ, van de Ven PM, Versluis J, et al. Improved efficacy of mitoxantrone in patients with castration-resistant prostate cancer after vaccination with GM-CSF transduced allogeneic prostate cancer cells. *Oncoimmunology*. **2016**; 5: e1105431 1-8

10. van den Eertwegh AJM, Versluis J, van den Berg HP, et al. A phase I trial of combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells (GVAX) and Ipilimumab in patients with metastatic castration-resistant prostate cancer. *Lancet Oncol*. **2012**; 13: 509-17.

11. Versluis J, and Suliman HM; Appendicitis in a patient wih Situs Inversus Totalis. *JBR-BTR*. **2014**; 97: 182-3.

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PhD PORTFOLIO

A summary of PhD training and teaching activities

PhD candidate:	Jurjen Versluis
Erasmus MC Department:	Hematology
Research school:	Molecular Medicine
Period:	2013 – 2017
Promotor:	Prof. Dr. J.J. Cornelissen

COURSES **YEAR**

Training

- Erasmus Summer Programme: Survival analysis. NIHES Institute Rotterdam 2013
- European Society for Blood and Marrow Transplantation / European Society of Hematology Statistics Course 2013

Workshops

- Erasmus Hematology Lectures 2013
- Regionale Nascholing Hematologie 2012-2013

Scientific meetings

- AIO/post-doc meetings at the Department of Hematology, Erasmus MC 2013
- Journal club at the Department of Hematology, Erasmus MC 2013

CONFERENCES AND PRESENTATIONS

Oral presentations

Prediction of Non-Relapse Mortality in recipients of Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation with AML in first complete remission.

- 54th Annual Meeting of the American Society of Hematology, Atlanta, GA, USA 2012
- 7th Dutch Hematology Congress, Arnhem, The Netherlands 2013
- 40th Annual Meeting of the European Society for Blood and Marrow Transplantation, Milan, Italy 2014

Post-remission treatment with allogeneic stem cell transplantation in patients aged 60 years and older with acute myeloid leukemia: a time-dependent analysis.

- 56th Annual Meeting of the American Society of Hematology, San Francisco, CA, USA 2014
- 9th Dutch Hematology Congress, Arnhem, The Netherlands 2015

Comparative value of post-remission treatment in cytogenetically normal AML subclassified by NPM1 and FLT3-ITD allelic ratio.

- 56th Annual Meeting of the American Society of Hematology, San Francisco, CA, USA 2014
- 9th Dutch Hematology Congress, Arnhem, The Netherlands 2015

Alternative donors for allogeneic hematopoietic stem cell transplantation in poor-risk AML in CR1.

- 56th Annual Meeting of the American Society of Hematology, San Francisco, CA, USA 2014
- 42nd Annual Meeting of the European Society for Blood and Marrow Transplantation, Valencia, Spain 2015

Graft versus leukemia effect of allogeneic stem cell transplantation and minimal residual disease in patient with AML in first CR.

- 22nd Congress of the European Hematology Association, Madrid, Spain 2017

Poster presentations

Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation

- 54th Annual Meeting of the American Society of Hematology, Atlanta, GA, USA 2012

Comparative value of post-remission treatment in cytogenetically normal AML subclassified by NPM1 and FLT3-ITD allelic ratio.

- Society of Hematologic Oncology Annual Meeting, Houston, Tx, USA 2016

Awards

- American Society of Hematology Abstract Achievement Award. 2012
- American Society of Hematology Outstanding Abstract Achievement Award. 2014

Teaching activities

- Supervising 2 master of science students: respiratory virus in immunocompromised patients. 2014