Diploma Thesis

Outcome of patients with acute myeloid leukaemia after allogeneic haematopoietic stem cell transplantation

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Abstract

Introduction: Allogeneic HSCT is the most effective treatment for curing patients with AML. Accordingly, consensus exists across current guidelines to recommend allogeneic HSCT for eligible patients with intermediate- or high-risk AML in first complete remission as well as for all patients with AML relapse. However, despite advances in transplantation medicine as well as supportive care during the last two decades, relapse- and treatment-related mortality of patients with AML undergoing allogeneic HSCT remains a major concern negatively affecting long-term outcome.

Methods: We performed a retrospective analysis of AML patients treated with allogeneic HSCT at the Division of Haematology, Medical University of Graz to determine overall survival and to identify (novel) risk factors for adverse outcome. Descriptive statistical methods as well as Kaplan Meier estimates and Cox regression models were used for analyzing the collected data.

Results: In total, 204 patients (108 males and 96 females) were included in the study. After a median follow up of 35 months, median survival was 27 months with an estimated overall survival (OS) at 5 years of 45%. We observed similar 5-year OS across matched donor types (matched related donor, MRD: 56% and matched unrelated donor, MUD: 53%), but significant inferior survival for patients receiving mismatched umbilical cord blood (21%) or an allograft from a mismatched donor (25%). With an overall incidence of 29% at 5 years, relapse was the main cause of death in this cohort. In multivariate analysis, remission status at time of transplantation (not in first complete remission, p<0,001), hyperglycemia during the engraftment period (p=0,003) and HLA-mismatch (p=0,045) were identified as independent negative risk factors for OS.

Conclusion: Relevant survival parameters as well as risk factors for adverse outcome observed in our retrospective study compare well with published studies. In addition, we identified hyperglycemia during the engraftment period as a major

risk factor for OS after HSCT in AML patients. Prospective clinical trials are therefore warranted to validate this newly discovered risk factor but also to evaluate the impact of strict blood glucose management on outcome of AML patients undergoing HSCT.

Zusammenfassung

Einführung: Die Durchführung einer allogenen hämatopoetischen Stammzelltransplantation (HSCT) kann trotz schwerwiegender mitunter sogar tödlicher Nebenwirkungen in vielen Fällen zu einer vollständigen Heilung von Patienten mit akuter myeloischer Leukämie führen. Dank neuer Erkenntnisse prognosebestimmender molekularer und genetischer Eigenschaften der Erkrankung, wurden distinkte Subgruppen definiert, die am besten von dieser intensiven Therapie profitieren.

Methoden: Anhand einer retrospektiven Studie analysierten wir alle AML-Patienten, die zwischen 1996 und 2013 mit einer allogenen Stammzelltransplantation an der Abteilung für Hämatologie des LKH-Univ. Klinikum Graz behandelt wurden. Primäres Ziel war die Erfassung des Gesamtüberlebens und die Identifikation von Risikofaktoren, die Einfluss auf das Überleben haben. Deskriptive statistische Methoden, Kaplan-Meier Kurven sowie Cox Regressionmodelle wurden zur Datenanalyse herangezogen.

Ergebnisse: Wir konnten 204 AML-Patienten in die Studie einschließen. Nach einer medianen Nachbeobachtungszeit von 35 Monaten lag das mediane Gesamtüberleben bei 27 Monaten und die 5-Jahres-Überlebensrate bei 45%. Vergleichbare Überlebensraten wurden zwischen HLA-identen Familien- (56%) und Fremdspendern (53%) beobachtet. Hingegen zeigten Empfänger von Nabelschnurblut (21%) und nicht HLA-identen Stammzellen (25%) ein signifikant schlechteres Überleben. Ein Rezidiv der Erkrankung trat bei bis zu 29% der Patienten nach fünf Jahren auf und stellte somit die häufigste Todesursache in diesem Patientenkollektiv dar. In einer multivariaten Analyse konnten eine hyperglykämische Stoffwechsellage während der Engraftmentphase ($p=0,003$), HLA-Inkompatibilität ($p=0,045$) sowie der Remissionsstatus der Krankheit zum Zeitpunkt der Transplantation ($p<0,001$) als unabhängige Risikofaktoren für das Gesamtüberleben identifiziert werden.

Schlussfolgerung: In dem von uns untersuchten Patientenkollektiv zeigten sich vergleichbare Überlebensdaten wie in anderen publizierten Studien. Neben bekannten Risikofaktoren konnten wir eine hyperglykämische Stoffwechsellage während der Engraftmentphase als neuen Risikofaktor für ein vermindertes Gesamtüberleben für AML-Patienten nach Stammzelltransplantation identifizieren. Wir empfehlen daher die Durchführung prospektiver Studien in Bezug auf Blutzucker-Management in diesem Patientenkollektiv.

Table of contents

Abbreviations

aGvHD	Acute Graft-versus-Host disease
AML	Acute myeloid leukaemia
ARA-C	Cytarabine
BM	Bone marrow
BMT	Bone marrow transplant
CBF-AML	Core-binding factor acute myeloid leukaemia
CEBPA	CCAAT/enhancer-binding protein alpha
cGvHD	Chronic Graft-versus-Host disease
CMV	Cytomegalovirus
CN-AML	Cytogenetic normal acute myeloid leukaemia
CR	Complete remission
CR 1	First complete remission
CR 2	Second complete remission
del	Deletion
EFS	Event-free survival
e.g.	exempli gratia, for example
ELN	European Leukaemia Net
FLT-3	FMS-like tyrosine kinase-3
GvHD	Graft-versus-Host disease
GvL	Graft-versus-Leukaemia
inv	Inversion
ICU	Intensive care unit
ITD	Internal tandem duplication
HCT-CI	Hematopoietic cell transplantation comorbidity index
HiDACS	High-dose cytarabine
HLA	Human leukocyte antigen
HR	Hazard Ratio
HSCT	Haematopoietic stem cell transplantation
LDH	Lactate dehydrogenase
LFS	Leukaemia-free survival

MAC	Myeloablative conditioning
MDS	Myelodysplastic syndrome
MEDOCS	Medical Documentation and Communication System
MMUD	Mismatched unrelated donor
MPO	Myeloperoxidase
MRD	Matched related donor
MUD	Matched unrelated donor
NPM1	Nucleophosmin 1
NSE	Nonspecific esterase
OS	Overall survival
PBSC	Peripheral blood stem cells
PD	Progressive disease
PR	Partial remission
RFS	Relapse-free survival
RIC	Reduced intensity conditioning
s-AML	Secondary acute myeloid leukaemia
SBB	Sudan black B
t	Translocation
t-AML	Therapy-related acute myeloid leukaemia
T-cell	A type of lymphocyte
TRM	Treatment related mortality
UCB	Umbilical cord blood
URD	Unrelated donor
WBC	White blood cell

Register of illustration

List of tables

1. Introduction

“AML is a heterogeneous neoplastic disorder of the bone marrow characterized by an abnormal proliferation and differentiation of myelopoietic precursors.”(1), resulting in incomplete maturation and insufficient production of healthy haematopoietic cells. (2)

Acute myeloid leukaemia (AML) occurs in people of all age ranges, but it is more common in elderly with a median age of 72 years, as reported by the Swedish Acute Leukaemia Registry. (3) With a prevalence of 3,8 cases per 100000 adults aged up to 65 years and 17,9 cases per 100000 adults aged older than 65 years it is the most frequent acute leukaemia in adults. (4) The disease manifests in symptoms like fatigue, dyspnoe (caused by anaemia), infections (because of neutropenia) and haemorrhage (caused by thrombocytopenia). AML is a heterogeneous disease, beside morphological criteria it can be classified into cytogenetic and molecular genetic subtypes which show different prognosis and response to treatment. The etiology of AML is still not known in most cases, but there are known risk factors like exposure to ionizing radiation, previous chemotherapy, predisposing diseases like myelodysplastic syndromes (MDS) and hereditary factors like trisomy 21. (2,5)

If patients have a history of previous cytotoxic and/or radiation therapy, therapy-related AML (t-AML) can arise five to seven years after therapy with alkylating agents or irradiation or within two to three years after administration of agents targeting topoisomerase II, mostly associated with unfavorable cytogenetics. Compared with de novo AML, overall survival of t-AML patients is worse, most likely caused by persistence of primary malignant disease, harmed organs from prior therapy, damaged marrow stroma because of radiation therapy, and chronic immunosuppression. (6)

Secondary AML (sAML) develops from an underlying myeloid disorder like myeloproliferative disease or - more commonly - myelodysplastic syndromes, which are “[…] a heterogeneous group of clonal hematologic disorders characterized by clonal expansion of dysplastic myeloid cells in the bone marrow.

[…] Some of these patients have stable and indolent disease over years, whereas others show rapid progression into secondary acute myeloid leukemia (sAML).” (7)

1.1. Diagnostic procedures

The diagnosis of AML is based on a marrow or blood blast cell count (cells of incomplete maturation) of at least 20%. The classification of AML is based on morphology, cytochemistry, immunophenotype, genetics and clinical features. (8) Cytogenetic and molecular analyses at the time of diagnosis allow categorizing of the patients’ risk as favorable, intermediate, or adverse in terms of disease outcome. (9)

1.1.1. Morphology

Blood and marrow smears are part of the routine diagnostic work-up. To diagnose AML, a marrow or blood blast count of at least 20% of all nucleated cells is needed. The blast count involves myeloblasts, monoblasts and megakaryoblasts, in cases of AML with monocytic or myelomonocytic differentiation also monoblasts and promonocytes are counted. Cytochemistry using myeloperoxidase (MPO), Sudan black B (SBB) and nonspecific esterase (NSE) stains allow lineage determination of blasts. In addition, immunophenotyping using flow cytometry is used for diagnosis and for identification of lineage involvement by determining expression patterns of surface (e.g. CD34, CD33, CD13) and cytoplasmic antigens (e.g. MPO). If blasts express distinct markers of more than one lineage, a so called mixed phenotype acute leukaemia is identified. (6)

1.1.2. Cytogenetic

“The karyotoype of the leukemic cells is the strongest prognostic factor for response to induction therapy and for survival.”(6)

An obligatory component in the diagnostic work-up of AML is cytogenetic analysis. About 55% of adults with AML have chromosome abnormalities, which can be differentiated in balanced abnormalities (aberrations without loss of genetic material like translocations or inversions) and unbalanced abnormalities (loss or gain of (parts of) chromosomes like deletion 5q or 7q). Complex karyotype, defined as the presence of 3 or more chromosome abnormalities in the absence of t(8;21), inv(16) or t(16;16) and t(15;17), correlates with a very poor outcome. Monosomal karyotoype, defined as the presence of a single monosomy (most commonly monosomy 7 or 5) with an additional (structural) chromosome abnormality, confers an even worse prognosis. (6) In contrast, balanced abnormalities including t(8;21), inv(16) and t(15;17) are prognostically favorable (see also Table 1). (10)

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I*	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse†
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	−5 or del(5q); −7; abnl(17p); complex karyotype‡

Table 1: Cytogenetic and molecular data in AML categorized into groups (6)

1.1.3. Molecular genetics

Gene mutations and deregulated gene activity are very important prognostic factors and allow us to map the genetic variety within the different cytogenetic groups, particularly in the large group of patients with cytogenetic normal AML (CN-AML). A multistep mechanism is needed to develop AML that is determined by at least two genetic and/or chromosomal aberrations. (6)

"Leukaemogenesis needs, at a minimum, activating mutations in class I genes that stimulate signal transduction pathways and induce cellular proliferation, in conjunction with mutations in class II genes that affect transcription factors and compromise normal differentiation." (2)

Cytogenetic markers	Molecular markers	Clinical factors
Favourable prognostic factors		
t(8;21) inv(16)/t(16;16) t(15;17)	Mutated *CEBPA* (double) Mutated *NPM1* (without *FLT3*–ITD mutation)	MRD-negative
Adverse prognostic factors		
inv(3)/t(3;3) t(9;22) t(9;11) t(6;9) –5 or del(5q) –7 abn(17p) Complex karyotype Monosomal karyotype	Enhanced *Evi-1* expression *MLL* rearrangements *FLT3*–ITD mutation *DNMT3A* mutation *BAALC* expression *ERG* expression *MN1* expression *WT1* polymorphism *BCR–ABL*-positive	Increased age Elevated WBC count Extramedullary disease No early complete remission Persistent MRD $CD34^+$ blasts Treatment-related AML

Abbreviations: AML, acute myeloid leukaemia; *BAALC*, gene encoding brain and acute leukaemia cytoplasmic protein; *CEBPA*, gene encoding CCAAT/enhancer binding protein; *DNMT3A*, gene encoding DNA (cytosine-5)-methyltransferase 3A; *ERG*, gene encoding transcriptional regulator ERG; Evi-1, MDS1 and EVI1 complex locus protein EVI1 (also known as ecotropic viral integration site 1); *FLT3* fms-like tyrosine kinase receptor-3; ITD, internal tandem duplication; *MLL*, gene encoding histone-lysine *N*-methyltransferase MLL; MRD, minimal residual disease; *MN1*, gene encoding probable tumour suppressor protein MN1; *NPM1*, gene encoding nucleophosmin; WBC, white blood cell; *WT1*, gene encoding Wilms tumour protein.

Table 2: AML-related prognostic parameters (1)

Mutations in so called class I genes, such as RAS, FLT3 and c-KIT, activate signal transduction pathways resulting in cell proliferation, which leads to clonal expansion of affected haematopoietic progenitor cells. Class II mutations like RUNX1/ETO, CBFB/MYH11, and PML/RARα are fusion transcripts caused by chromosomal abnormalities t(8;21), inv(16), and t(15;17) and are able to stop myeloid differentiation. The frequent combined occurrence of these mutations has led to the statement, that only this conjunction causes leukaemia. (2,4,9)

Internal tandem duplication (ITD) of the FLT3 gene contributes to worse outcome of CN-AML patients in comparison to those without FLT3-ITD. The nucleophosmin gene, called NPM1, is the most commonly mutated gene in AML and affects about 48-64% of CN-AML patients. There is evidence that patients with NPM1 mutation but without FLT3-ITD have better overall and relapse-free survival. This is also seen in patients with biallelic CEPBA mutations. (4) Core-binding-factor AML (CBF-AML), involving t(8;21) or inv(16), belongs to the cytogenetically favorable risk group, but in the presence of a KIT mutation it is associated with inferior outcome. Using cytogenetic as well as the discussed genetic data of AML cells allows categorization of AML patients into four prognostic risk groups according to the current guidelines of the European Leukemia Net (ELN) (see Table 1). (6)

1.2. Treatment

Intensive chemotherapy is still the gold standard in the treatment of AML. It is categorized into three phases:

- Induction therapy
- Consolidation or postremission therapy after achievement of remission (includes conventional chemotherapy as well as haematopoietic stem cell transplantation (HSCT)
- Maintenance therapy after these intensive therapy phases. Nowadays maintenance therapy is not commonly used due to lack of clear beneficial evidence). (5)

“The aim of induction is to achieve complete remission (CR) whereas consolidation is designed to eliminate residual leukaemia cells that persist after induction.” (2) In general, AML requires immediate treatment and it has been shown that postponing chemotherapy beyond five days after diagnosis has an adverse effect on outcome. (6)

1.2.1. Induction therapy

The standard induction regimen is the administration of an anthracycline for three days (daunorubicin or idarubicin) combined with a continuous infusion of cytarabine for seven days (so called 3+7 regimen). Several studies attempted to improve the rate and quality of CR achieved with the 3+7 regimen by using additional cytotoxic agents. However, significant benefit has not yet been achieved. (2) In general, therapy response is assessed by bone marrow biopsy 28 days after start of induction. Table 3 shows all categories of remission status after induction therapy. About 70% of patients aged younger than 60 years achieve a complete remission (CR), those who just attain CR with incomplete recovery (CRi) commonly have inferior outcome. (11)

Category	Definition
Complete remission (CR)*	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0 × 10^9/L (1000/μL); platelet count > 100 × 10^9/L (100 000/μL); independence of red cell transfusions
CR with incomplete recovery (CRi)†	All CR criteria except for residual neutropenia (< 1.0 × 10^9/L [1000/μL]) or thrombocytopenia (< 100 × 10^9/L [100 000/μL])
Morphologic leukemia-free state‡	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Cytogenetic CR (CRc)§	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm)‖	No standard definition; depends on molecular target
Treatment failure	
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase 2/3 trials), or failure to achieve CR, CRi, or PR (phase 1 trials); only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or < 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse¶	Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease

Table 3: Response criteria in AML (6)

1.2.2. Postremission/consolidation therapy

Without further treatment after induction therapy most, if not all patients would relapse. To prevent (immediate) relapse, various postremission strategies have been explored, including intensive chemotherapy, autologous and allogeneic haematopoietic stem cell transplantation (HSCT). (6) “Allogeneic HSCT as a postremission strategy is associated with the lowest rates of relapse.” (6) This is probably due to the graft versus leukaemia effect, but the benefit can be outweighed by transplant-related mortalitiy (TRM) and morbidity. Therefore, the implementation of allogeneic HSCT needs to be evaluated on an individual basis. With the help of scores and identification of risk factors for TRM, relapse and overall survival the decision either to proceed to allogeneic HSCT or to applicate a non-transplant strategy should be balanced for every single patient. (1) (Details on this topic are discussed in chapter 1.3.)

1.2.3. High-dose cytarabine

Conventional chemotherapy is a commonly used strategy for postremission therapy in AML. The Cancer and Leukemia Group B has shown that patients aged up to 60 years who received four cycles of high-dose cytarabine (HiDAC) (3g/m²

every 12 hours on days 1,3,5) have a survival advantage in contrast to four courses of intermediate- (400mg/m² continuous on days 1-5) or standard-dose (100mg/m² continuous on days 1-5) cytarabine. The greatest benefit of this strategy was found in patients with favorable cytogenetics like CBF AML and CN-AML. But it is still not clear, which is the optimum dose and what number of cycles are best for reduced toxicity without worsening the outcome. Currently, this postremission treatment strategy is the standard therapy in patients with favorable cytogenetic risk and in patients with intermediate risk lacking a suitable HLA-matched donor. (6)

1.2.3.1. Autologous HSCT

Another option for postremission therapy in patients with intermediate-risk cytogenetics lacking an HLA-matched donor is autologous haematopoietic stem cell transplantation (autologous HSCT). The outcome is comparable to HiDAC chemotherapy. (6)

1.2.4. Allogeneic HSCT

Allogeneic hematopoietic stem cell transplantation (allogeneic HSCT) is a potent treatment option for patients with AML. It is associated with lower relapse rates than autologous HSCT or chemotherapy (6), as a result of the graft-versus-leukaemia effect, which is evoked by alloreactive donor T cells. However, alloreactive T cells can also attack normal tissues of the recipient and thereby cause graft-versus-host disease (GvHD). (1) Severe GvHD has a major impact on treatment-related mortality (TRM), which can be as high as 10-25%. But also other factors like the patients' age, disease stage, time period from diagnosis to transplantation, donor type, donor-recipient sex combination, cytomegalovirus (CMV) serum status of recipient and donor have a substantial impact on overall survival (OS), TRM and leukaemia-free survival (LFS) after allogeneic HSCT. (2,6) "The value of allogeneic HSCT needs to be reassessed based on the identification of AML-related genetic changes that profoundly impact on prognosis, on the

availability of different transplant sources (bone marrow, blood) and donor types (matched related, unrelated and haploidentical donors, umbilical cord stem cell grafts) and in light of the use of reduced-intensity conditioning (RIC) regimens.[…] It is essential to assess whether the benefit of the reduced relapse rate outweighs TRM or will be offset by a high TRM. Comorbidity scores, such as the HCTCI, provide useful guidance in these decisions." (6)

According to the current guidelines of the European Leukemia Net (ELN) allogeneic HSCT is considered for patients of the intermediate risk group who do not display favorable mutations like biallelic CEBPA mutations or NPM1-mutations without FLT3-ITD, AML patients with adverse cytogenetic risk, s-AML as well as t-AML patients and for those who did not respond to induction chemotherapy. In addition, patients who experience relapse should undergo allogeneic HSCT irrespective of their remission status after reinduction chemotherapy. (12)
Before allogeneic haematopoietic stem cells can be applied, patients have to undergo conditioning therapy. While in earlier times only myeloablative conditioning (MAC) with high dose chemotherapy with or without total body irradiation was available, reduced intensity conditioning (RIC) regimens have been developed in recent years making allogeneic HSCT also suitable for patients with an age >50 years. Myeloablative conditioning was used to eradicate the disease and to suppress the recpient´s immune system to accept the allogeneic graft and typically consists of intravenous cyclophosphamide at 60mg/kg body weight for two days and fractionated total body irradiation (TBI, 12 Gy). TBI can also be substituted by the administration of busulfan. Because of high toxicity, these myeloablative regimens can only be applied to patients aged less than 50 years, who are in good physical condition and lack significant comorbidities. Reduced intensity conditioning (RIC) regimens have been developed for older (aged over 50 years) and less fit patients (due to comorbidities), who would not tolerate myeloablative conditioning. They contain various combinations of chemotherapeutics such as fludarabine, melphalan, cytarabine, amsacrin or busulfan and are sometimes combined with low dose TBI (2-4 Gy). (6) Although RIC is effective to assure engraftment and is associated with lower transplant

related mortality, relapse rates have been reported to be higher as compared to myeloablative regimens in some studies. (13) It can induce a potent GvL effect and is able to result in ongoing remission, but assures long-term disease free survival only in a fraction of patients. Moreover, the incidence of acute and chronic GvHD after T-cell repleted RIC is significantly higher and so optimizing the GvL effect with the help of donor lymphocyte infusion or early withdrawal of immunosuppression is limited. (7,12,14)

	RIC	MAC
Target group	Elderly patients, patients with comorbidities not eligible for myeloablative conditioning	Fit patients of younger age
Incidence of relapse	higher	lower
Incidence of TRM	lower	higher
Incidence of GvHD	higher	lower
Overall survival	similar between both groups	

Table 4: Comparison of RIC and MAC (RIC=reduced intensitiy conditioning, MAC=myeloablative conditioning)

Nevertheless, the availability of RIC allogeneic HSCT enables a curative approach in elderly patients with AML up to the age of 75 years. Anecdotal case presentations in the literature even report RIC allogeneic HSCT in patients aged 80 years or older. (15)

Only 30% of patients who are eligible for allogeneic HSCT have an HLA-identical sibling (matched related donor, MRD). (16) In the remaining cases, patients are reliant on unrelated donors, who is in the majority of cases HLA-identical to the recipient (matched unrelated donor, MUD) or displays one mismatched HLA allele (mismatched unrelated donor, MMUD). (17)

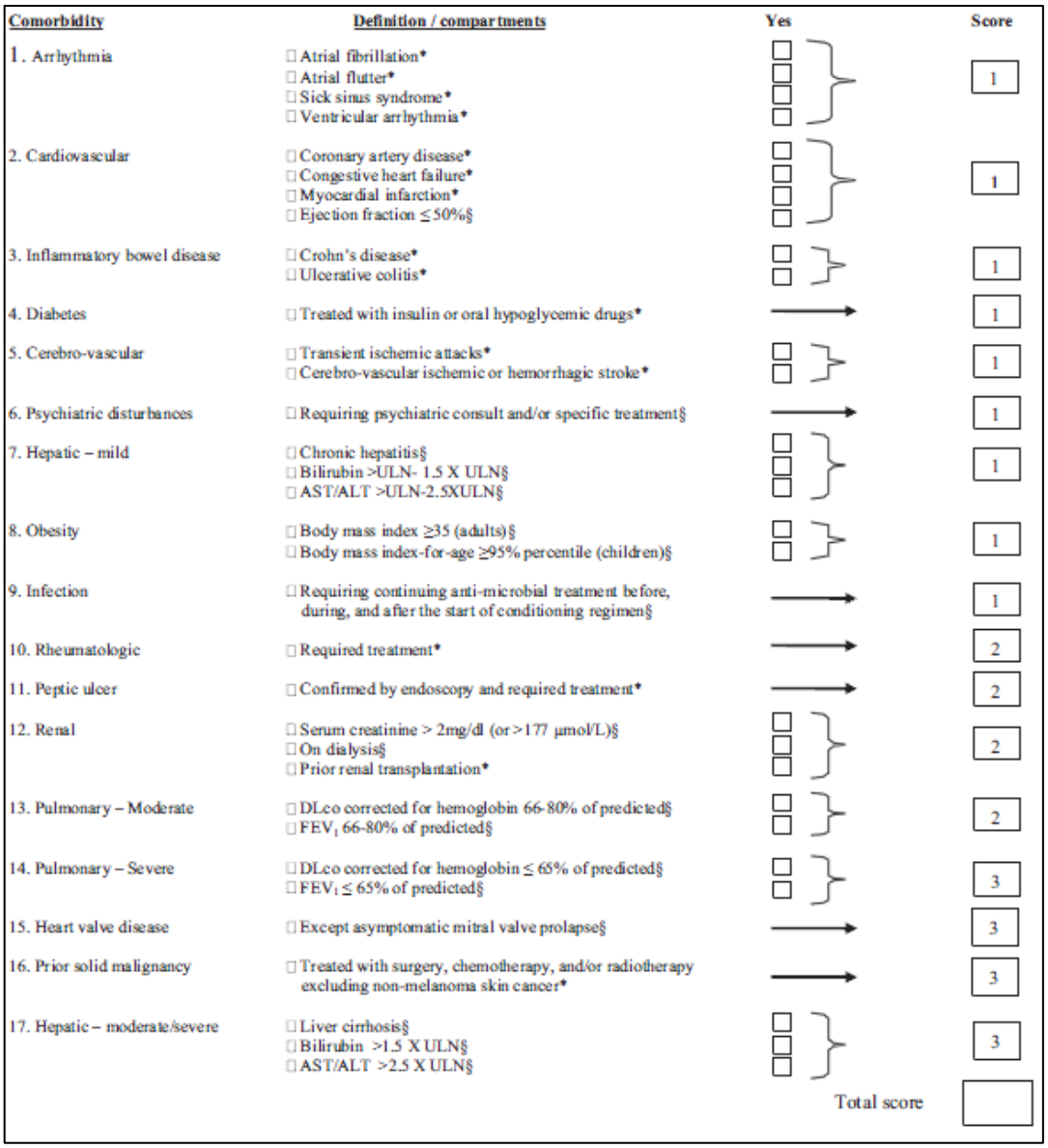
However, mismatch of HLA alleles increases treatment related mortality and thus results in decreased survival after allogeneic HSCT. With the help of high-resolution techniques for HLA-matching based on DNA sequencing and increasing the number of HLA-loci analyzed for matching outcome after MUD HSCT has been shown to be similar to that achieved with a HLA-identical sibling donor allograft. (16) (18) Due to a comprehensive, worldwide database of volunteers it is

nowadays possible to identify a suitable donor within 2 months in up to 80% of patients lacking an HLA-identical sibling. (16) If no suitable donor can be found, alternative stem cell sources like umbilical cord blood or haploidentical family donors can be used. These sources have the advantage of immediate availability, but are associated with higher TRM. (1)

1.3. Factors affecting outcome after allogeneic HSCT

“Prognostic factors may be subdivided into those related to patient characteristics and general health condition and those related to characteristics particular to the AML clone. The former subset usually predicts treatment-related mortality (TRM) and becomes more important as patient age increases while the latter predicts resistance to, at least, conventional therapy.” (6)

It is well established that increasing age as well as an increasing number of comorbidities affect outcome in (hematological) cancers. To estimate the impact of comorbidity on outcome after allogeneic HSCT, the hematopoietic cell transplantation comorbidity index (HCT-CI) has been developed. (19) This score has been proven very useful for prediction of early death and overall survival after HSCT. (6)



Comorbidity	Definition / compartments	Yes	Score
1. Arrhythmia	☐ Atrial fibrillation* ☐ Atrial flutter* ☐ Sick sinus syndrome* ☐ Ventricular arrhythmia*	☐☐☐☐	1
2. Cardiovascular	☐ Coronary artery disease* ☐ Congestive heart failure* ☐ Myocardial infarction* ☐ Ejection fraction ≤ 50%§	☐☐☐☐	1
3. Inflammatory bowel disease	☐ Crohn's disease* ☐ Ulcerative colitis*	☐☐	1
4. Diabetes	☐ Treated with insulin or oral hypoglycemic drugs*	→	1
5. Cerebro-vascular	☐ Transient ischemic attacks* ☐ Cerebro-vascular ischemic or hemorrhagic stroke*	☐☐	1
6. Psychiatric disturbances	☐ Requiring psychiatric consult and/or specific treatment§	→	1
7. Hepatic – mild	☐ Chronic hepatitis§ ☐ Bilirubin >ULN- 1.5 X ULN§ ☐ AST/ALT >ULN-2.5XULN§	☐☐☐	1
8. Obesity	☐ Body mass index ≥35 (adults)§ ☐ Body mass index-for-age ≥95% percentile (children)§	☐☐	1
9. Infection	☐ Requiring continuing anti-microbial treatment before, during, and after the start of conditioning regimen§	→	1
10. Rheumatologic	☐ Required treatment*	→	2
11. Peptic ulcer	☐ Confirmed by endoscopy and required treatment*	→	2
12. Renal	☐ Serum creatinine > 2mg/dl (or >177 µmol/L)§ ☐ On dialysis§ ☐ Prior renal transplantation*	☐☐☐	2
13. Pulmonary – Moderate	☐ DLco corrected for hemoglobin 66-80% of predicted§ ☐ FEV_1 66-80% of predicted§	☐☐	2
14. Pulmonary – Severe	☐ DLco corrected for hemoglobin ≤ 65% of predicted§ ☐ FEV_1 ≤ 65% of predicted§	☐☐	3
15. Heart valve disease	☐ Except asymptomatic mitral valve prolapse§	→	3
16. Prior solid malignancy	☐ Treated with surgery, chemotherapy, and/or radiotherapy excluding non-melanoma skin cancer*	→	3
17. Hepatic – moderate/severe	☐ Liver cirrhosis§ ☐ Bilirubin >1.5 X ULN§ ☐ AST/ALT >2.5 X ULN§	☐☐☐	3
		Total score	

Figure 1: The hematopoietic cell transplantation comorbidity index (20)

In addition to comorbidities, which are well covered by the HCT-CI, also cytomegalovirus serostatus, donor-recipient gender combination, graft source, conditioning regimen, stem cell source, and graft-versus-host-disease (GvHD) prophylaxis are factors that are known to affect TRM and therefore overall survival after HSCT. (6) Table 5 summarizes pre-, peri- and post-transplantion factors that influence outcome. For example, the survival of cytomegalovirus seropositive patients is 3-5% worse than that of seronegative patients. (1)

Pretransplantation	Peritransplantation	Post-transplantation
Favourable prognostic factors		
Sibling donor (HLA-matched) Shorter time from diagnosis to transplant* White ethnicity	Nonmyeloablative conditioning Stem-cell source (bone marrow or peripheral blood) T-cell depletion of the graft	Early immune recovery
Adverse prognostic factors		
Increased recipient age* Recipient and donor sex* Comorbidities (assessed using HCT–CI) Cytomegalovirus serostatus Cytokine polymorphism Unrelated donor HLA-mismatched Performance score Refractory leukaemia Therapy-related AML	Myeloablative conditioning regimen Alternative stem-cell source (umbilical cord blood)	Severe acute grade III–IV GVHD Persistent chronic extensive GVHD

*Incorporated into the EBMT risk score. Abbreviations: AML, acute myeloid leukaemia; EBMT, European Group for Blood and Marrow Transplantation; GVHD, graft-versus-host disease; HCT–CI, haematopoietic cell transplantation comorbidity index; HLA, human leukocyte antigen; HSCT, haematopoietic stem cell transplantation.

Table 5: Prognostic factors for outcome (1)

GvHD remains a significant cause of morbidity after HSCT and is responsible for the majority of patients succumbing to death due to TRM. It presents with a heterogeneous clinical picture involving various organs, such as the skin, the gastrointestinal tract, the liver and the lungs. The pathophysiology underlying GvHD is that "[…] immunocompetent donor cells recognize and attack host tissues in an immunocompromised recipient, […] leading to end organ fibrosis." (21) It is classified as acute GvHD (aGvHD) if appearing within 100 days of HSCT, and as chronic GvHD (cGvHD) if it is occurring beyond 100 days. Grading of aGvHD is based on the degree of organ involvement and commonly affects the skin, the liver and the gastrointestinal tract. The degree of HLA mismatch, age of recipient and previous aGvHD are risk factors for developing chronic GvHD. It classically affects skin, eyes, mouth, liver, gastrointestinal tract, lungs and genitalia and is graded as mild, moderate and severe, depending on the number of involved organs and pulmonary involvement. (21)

In addition to these patient- and procedure-based factors, AML-related factors like cytogenetic and molecular aberrations at the time of diagnosis, preceding haematological disease, elevated white blood counts and LDH levels at diagnosis have also a significant impact on outcome after HSCT. (6) Furthermore, several

clinical variables like time to achive complete remission (CR) as well as detection of minimal residual disease (MRD) after induction and/or consolidation therapy affect outcome of allogeneic HSCT. (1) Based on morphological criteria, CR is defined as less than 5% blasts in the bone marrow. “The persistence of blasts below the threshold of morphologic detection, identified by sensitive molecular and immunologic tests, is termed minimal residual disease (MRD).” (22) It has been well established that the presence of MRD confers a higher risk of relapse and adverse outcome in patients in their first CR (CR 1) undergoing allogeneic HSCT. (23) Not surprisingly, there is also good evidence that patients not being in CR1 before transplantation are more likely to relapse and show inferior survival. (24) Nevertheless, several studies showed a clear survival benefit of allogeneic HSCT in such high risk situations when compared to conventional postremission therapy, emphasizing the important role of allogeneic HSCT also in such adverse clinical situations. (25)

1.4. Aim of the study

In the last years much progress has been made in understanding leukaemia biology, which has allowed better risk stratification in AML patients with cytogenetic and molecular markers resulting in facilitating decision making whether to proceed to allogeneic HSCT or not. In addition reduced-intensity conditioning, better risk/benefit ratios, immediate availability of alternative donors, or improved transplant technologies provided more safety and access of allogeneic HSCT. Here, we performed a retrospective analysis of 204 patients with AML and MDS who underwent an allogeneic HSCT at our center. The primary endpoint was overall survival (OS) of AML patients after HSCT. We determined how OS is affected by patient-specific, disease-specific as well as transplant-related parameters in our cohort. With specific emphasis, we evaluated the impact of hyperglycemia during the first 30 days after allogeneic HSCT on outcome. Hyperglycemia was recently described as a risk factor in outcome of patients with severe underlying diseases.

2. Materials and methods

All patients diagnosed with AML who were treated with allogeneic HSCT between 1996 and 2013 at the Division of Haematology, Medical University of Graz, were included in this retrospective analysis. All data of in total 204 patients were retrieved with the help of the electronic documentation program MEDOCS (*Medical Documentation and Communication System)* and medical records. The following personal and medical data were collected: age at AML diagnosis, sex, blood counts and LDH levels at diagnosis, type of AML, cytogenetic and molecular aberrations at diagnosis, date of HSCT, disease remission status at HSCT, type of HSCT including stem cell source, HLA compatibility, CMV status of donor and recipient, comorbidities (HCT-CI, if available), (date of) relapse, date of death or last contact, overall survival. In addition, blood glucose levels during the first 28 days after transplantation were recorded. No minimum and maximum age was considered.

The main objective was to determine the overall survival (OS) of AML patients after allogeneic HSCT and how OS is affected by patient-specific and disease-specific parameters. OS was defined as the time from HSCT to death from any cause. Patients alive or lost to follow-up were censored at the time they were last seen alive. Event-free survival (EFS) was defined as the time from HSCT to disease relapse or death from any cause, whichever occurred first.

2.1. Statistical analysis

After anonymization the collected data were entered into a data collection sheet using Microsoft EXCEL© 2010. For statistical analysis SPSS® Statistics 22 and R Commander Version 2.1-2 was used. The probabilities of OS and EFS were estimated by using Kaplan-Meier curves and statistical significance was calculated by using the log-rank test. Risk factors for OS were examined in univariate and multivariate analysis by Cox regression analysis. In order to investigate the

incidence of TRM and relapse competing risk regressions were calculated. A p-value of 0.05 or less was considered to indicate statistical significance.

The study was approved by the Ethics committee of the Medical University Graz and was conducted in accordance with the Code of Ethics of the World Health Organization. The study character was non interventional hence no prior patient consent was needed.

3. Results

Between 1996 and 2013, a total of 204 adult patients (female n=96, male n=108) with AML underwent allogeneic HSCT at the Division of Haematology, Medical University of Graz and were therefore recruited for this retrospective study. Median follow-up of all patients was 10 months (8,28-11,64 months) and median follow-up of surviving patients was 34,8 months (24,20-45,39 months). Patient-, disease- and transplant-specific characteristics are shown in Table 6. Due to missing records not all data could have been collected for some patients.

3.1. Patient characteristics

At the time of AML diagnosis, 79,9% (n=163) of patients were aged between 18 and 59 years, 20,1% (n=41) were aged 60 years or older. The median age was 49,5 years, and the mean age was 47 (standard deviation ±12,7 years). The youngest patient at time of diagnosis was 18 years, the oldest 75 years old. Distribution of age of the entire cohort is shown in .

It is known that cytogenetic and molecular aberrations have a major impact on clinical outcome after allogeneic HSCT. (6) In 183 patients cytogenetic and molecular results were available and the patients were assigned to the proposed (cyto)genetic risk groups using the ELN (European Leukemia Net) recommendations. The largest proportion of patients (44,1%, n= 90) belonged to the intermediate I risk group, followed by intermediate II (21,6%, n=44) and the adverse risk group (20,1%, n=41). Only eight patients (3.9%) had favorable cytogenetic aberrations. Twenty-one patients (10,3%) could not be classified due to missing data.

The majority of patients (135 out of 204 patients, 66,2%) received RIC, in 69 patients (33,8%) MAC was performed.

Table 6: Patient characteristics

		Total 204
Age	Patients aged 18-59 years	163 (79,9%)
	Patients aged 60 years or older	41 (20,1%)
Sex	Male	108 (52,9%)
	Female	96 (47,1%)
Conditioning regimen	RIC	135 (66,2%)
	MAC	69 (33,8%)
Stem cell source	BM	10 (4,9%)
	PBSC	174 (85,3%)
	UCB	20 (9,8%)
HLA- Compatibility	Match	146 (71,6%)
	Mismatch	50 (24,5%)
	Unknown	8 (3,9%)
Donor	Related	69 (33,8%)
	Unrelated	115 (56,4%)
	UCB	20 (9,8%)
Risk score ELN guideline	Favorable	8 (3,9%)
	Intermediate I	90 (44,1%)
	Intermediate II	44 (21,6%)
	Adverse	41 (20,1%)
	Unknown	21 (10,3%)
Remission state at HSCT	CR 1	115 (56,7%)
	CR 2	15 (7,4%)
	PD	36 (17,7%)
	PR	37 (18,2%)
	Unknown	1 (0,5%)
Leukocytosis	>11,3 G/l	58 (28,4%)
	<11,3 G/l	90 (44,1%)
	Unknown	56 (27,5%)
Gender mismatch	Male to female	42 (20,6%)
	Female to male	42 (20,6%)
	Male and female	1 (0,5%)
	Unknown	24 (11,8%)
	Match	95 (46,6%)
CMV status	Positive	143 (70,1%)
	Negative	57 (27,9%)
	Unknown	4 (2%)
LDH	LDH <225 U/l	35 (17,2%)
	LDH >225 U/l	76 (37,3%)
	Unknown	93 (45,6%)

Concerning disease status at the time of HSCT more than half of 204 included patients (56,7%, n=115) were in first CR (CR 1), 7,4% (n=15) were in second CR

(CR 2), 18,2% (n=37) only in partial remission (PR) and 17,7% (n=36) of patients showed progressive disease (PD).

Bone marrow served as stem cell source in only 10 patients (4,9%), whereas the majority of patients (n=174, 85,3%) received peripheral blood stem cells. Twenty patients (9,8%) received umbilical cord blood. HLA-compatibility was given in 146 patients (71,6%), in 50 patients (24,5%) at least 1 HLA-allele was mismatched. In 8 cases, there were no data about HLA-matching available. Sixty-nine donors (33,8%) were related to the recipients and 115 (56,4%) were unrelated. Data on gender matching were available in 179 patients with 84 patients (41,2%) displaying a gender mismatch (for example male donor to a female recipient). 70,1% (n=143) of the patients were positive tested for CMV IgG antibodies, 27,9% (n=57) negative and in 2% (n=4) no data were available.

The number of leukocytes at AML diagnosis has been shown to be also a prognostic factor for overall survival after HSCT (6). In our cohort, these leukocyte counts were only available in 148 patients (72,5%). The median number of leukocyte counts was 5,62 G/l (range 0,46 to 445,2 G/l). When dividing patients according to their leukocyte counts into two groups 28,4% (n=58) showed leukocytosis (over 11,3 G/l), and 90 patients (44,1%) had normal or decreased leukocyte counts. Elevated LDH levels at diagnosis are also correlated with adverse prognosis. However, in our cohort LDH levels at diagnosis were only available for 111 patients (54,4%). Among these 76 (37,3%) of them had elevated LDH levels (above 225U/l), the rest (35, 17,2%) presented with normal LDH levels.

3.2. Overall survival

Allogeneic HSCT is a potentially curative treatment option for patients with AML. The main objective of this study was therefore to determine OS of AML patients undergoing allogeneic HSCT, which was defined as time from transplantation to death from any cause. Patients still alive or lost to follow up were censored at the time they were last seen alive. With a median follow up of 10 months (all patients) and 34,8 months (surviving patients), 96 of 204 (47,1%) patients died during the observation time and, 108 patients (52,9%) were still alive or were lost to follow-up. The median estimated survival time was 2,24 years. One-, 2- and 5-year OS survival rates were 51%, 46% and 45%, respectively (see Figure 2).

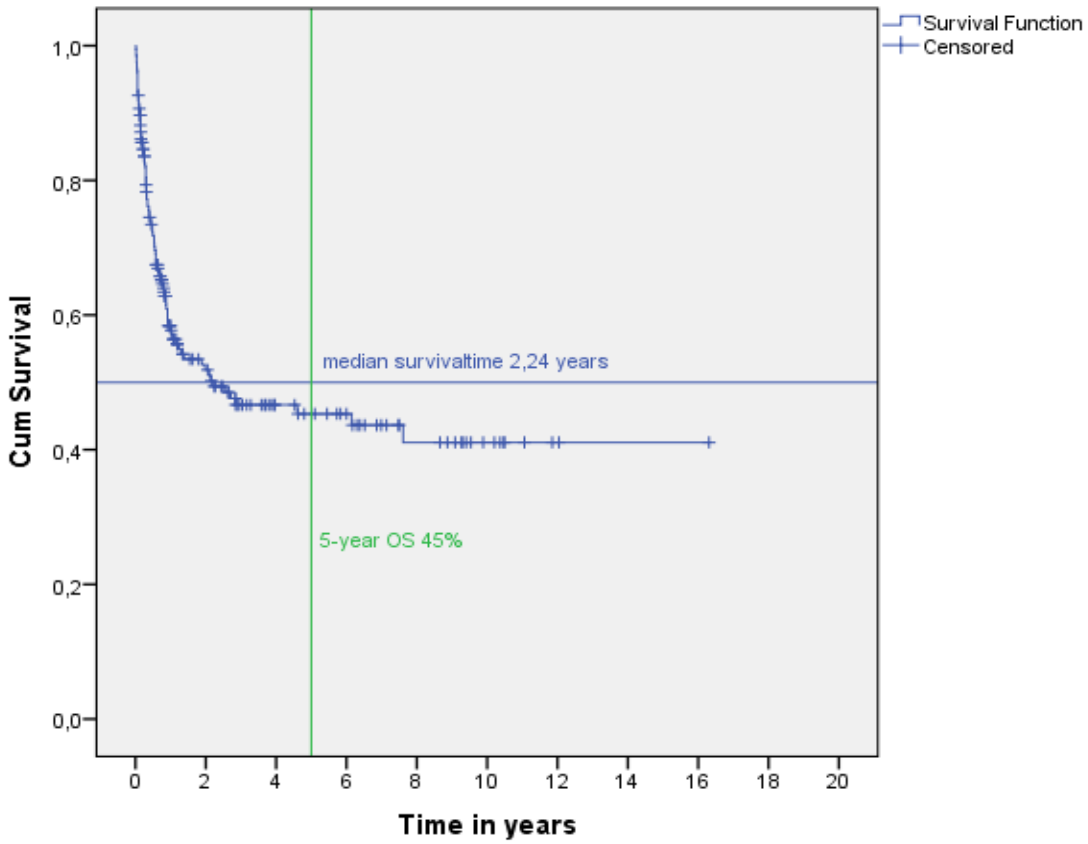
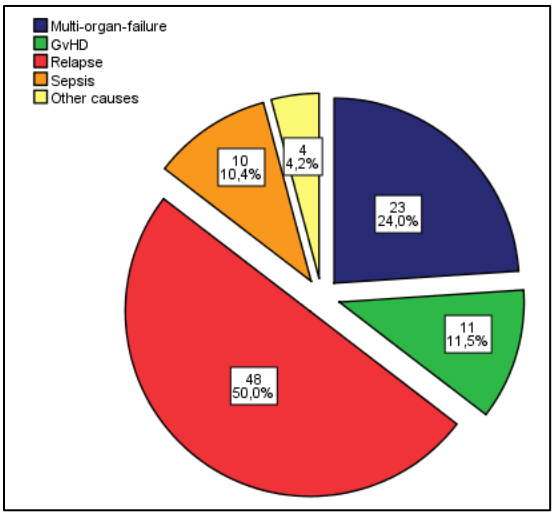


Figure 2: Overall survival of the entire cohort

Figure 3: Causes of death



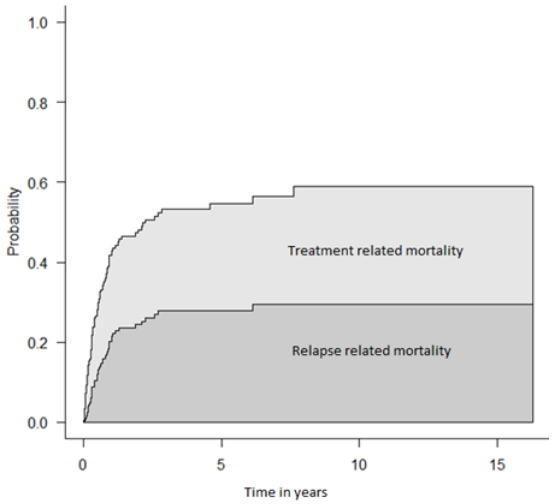


Figure 4: Treatment- and relapse-related mortality

The main reasons of death in patients undergoing allogeneic HSCT are either treatment-related mortality or disease relapse. In our cohort the main cause of death was relapse with an incidence of 50% (n=48), followed by multi-organ-failure (24%, n=23). Eleven patients (11,5%) died as a consequence of GvHD, and 10 (10,4%) due to sepsis. Four patients (4,2%) died of other causes (see Figures 3 and 4).

3.3. Event-free survival

Event-free survival (EFS) was defined as time from HSCT to relapse or death from any cause, whichever occurred first. The median EFS for patients in our cohort was 1,8 years, One-, 2- and 5-year EFS was 49%, 46% and 42%, respectively (see Figure 5). In total, 56 patients out of 204 relapsed and 48 of them died because of recurrence of their underlying disease and its complications. Eight relapsed patients were still alive or were lost to follow up. The median age of those who relapsed was 47,3 years, six of them were aged 60 years or older. Concerning risk factors, more patients with high risk disease as defined by cytogenetic or remission status at time of transplantation relapsed (see Figure 10 and Figure 12).

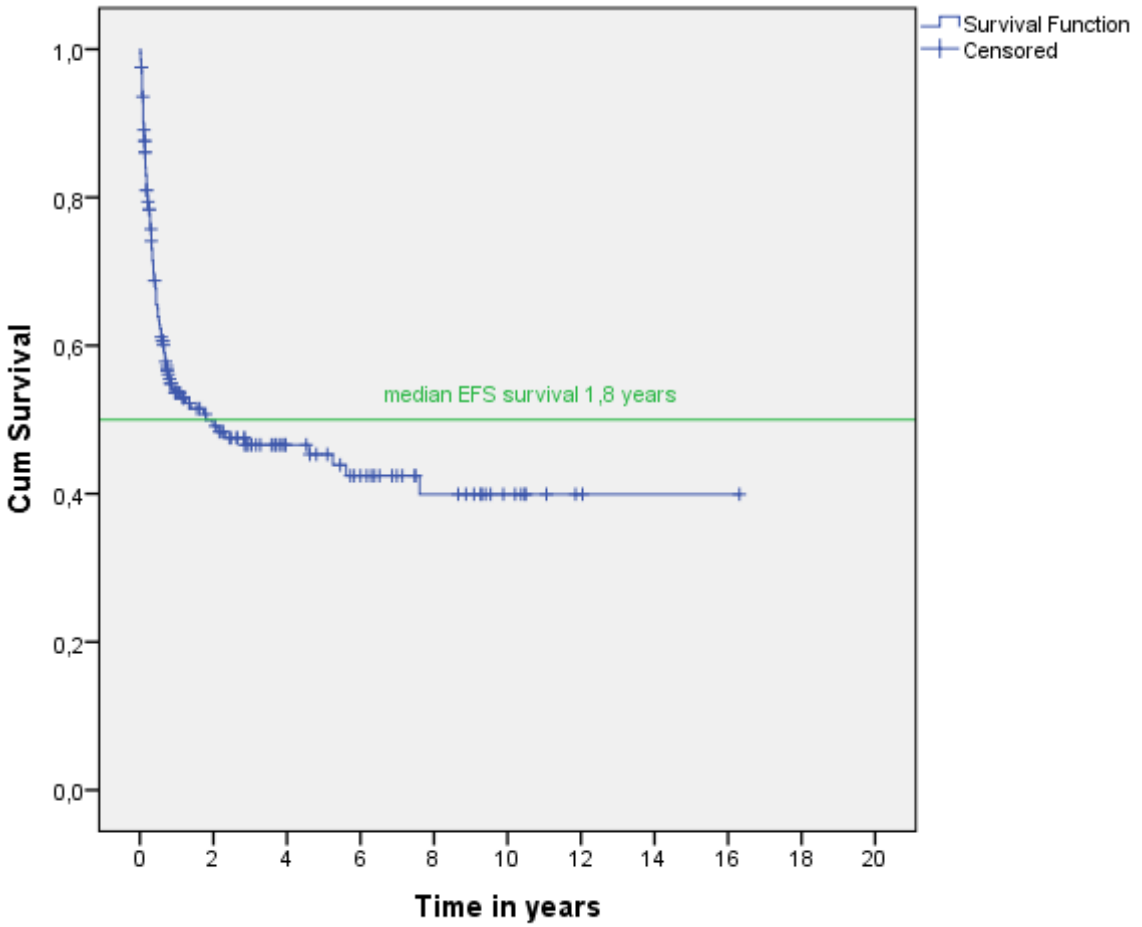


Figure 5: Event-free survival defined as time from transplantation to relapse or death from any cause

The overall incidence of relapse at 1 year was 26% (95%CI 20%-32,4%), at 2 years 28,2% (95%CI 21,8%-34,8%) and at 5 years 29% (95%CI 22,5%-35,8%), as seen in Figure 5. Relapse was more frequent for those who underwent transplantation without achieving CR 1 (Incidence of relapse at 1 year 32,3%, 95%CI 22,5%-42,5%, at 2 years 35,4%, 95%CI 25%-45,9%, at 5 years 37,2%, 95%CI 26,5%-47,9%) compared with patients in CR 1 during transplantation (incidence of relapse at 1 year 21,4%, 95%CI 14,2%-29,6%, at 2 years 22,6%, 95%CI 15,1%-31%, at 5 years 22,6%, 95%CI 15,1%-31%) (p=0,027).

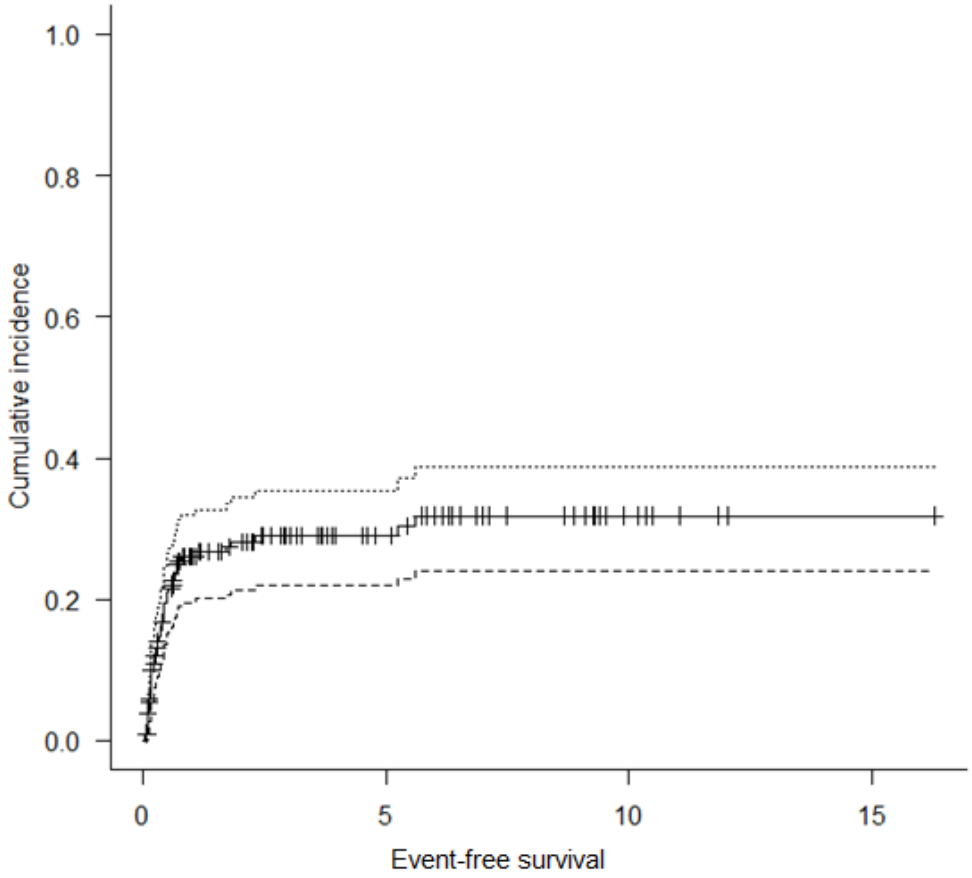


Figure 6: Incidence of relapse with 95%CI

3.4. Factor affecting overall and event-free survival in AML patients undergoing allogeneic HSCT

In the following paragraphs, the results on analyzing the effect of patient-, transplant- as well as disease-specific parameters on OS as well as the impact of some of these factors on relapse risk and TRM are presented. Additionally, a univariate and multivariate analysis determining the influence of these risk factors on OS was calculated.

3.4.1. Age

Increasing age is correlated with inferior outcome in AML patients. (1) In our cohort the median age was 49,5 years. Age distribution of the entire cohort is shown in Figure 8. When dividing the cohort into two age groups the median survival time of the younger age group (18-59 years) was 1,98 years (95%CI 0,511-3,449 years) and the estimated 1-, 2-, and 5-year OS rates were 52%, 48% and 43%, respectively. Surprisingly, estimated 1-, 2- and 5-year OS in the older age group (60-75 years) were 61%, 55% and 55%, respectively, and therefore higher than what was observed in the younger age group. However, no statistical significance was reached between these two groups concerning OS (log rank test p=0,361).

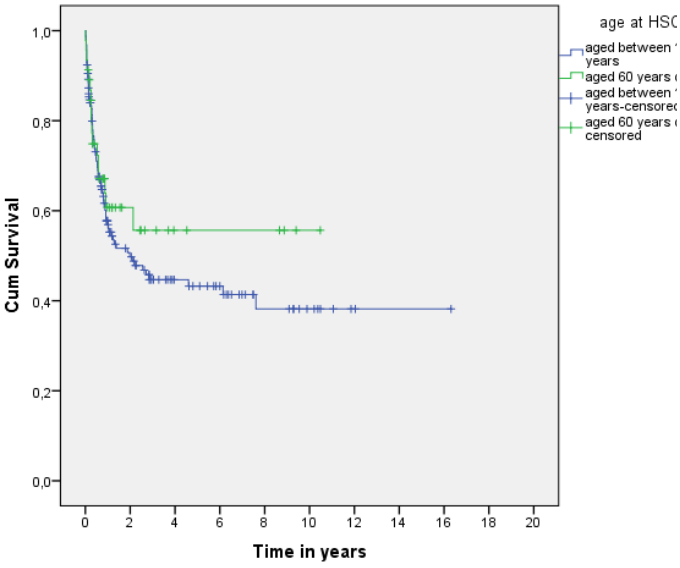


Figure 7: Overall survival according to age

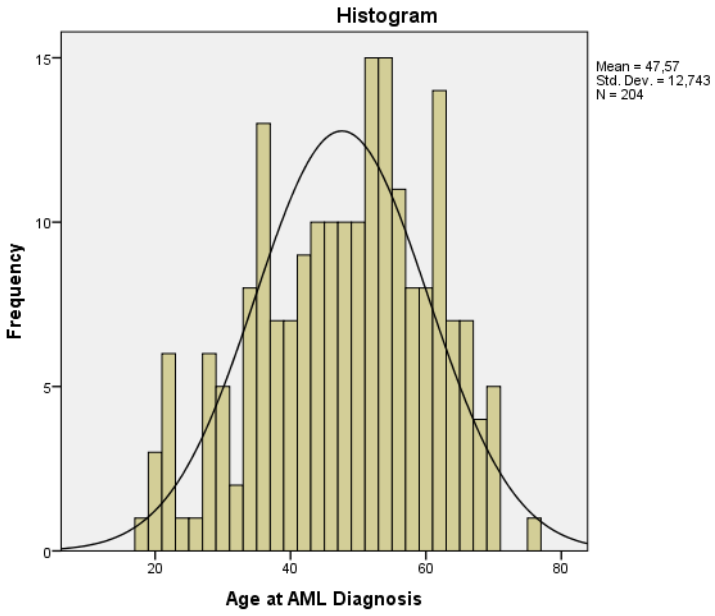


Figure 8: Age distribution of the entire cohort

3.4.2. Cytogenetic risk

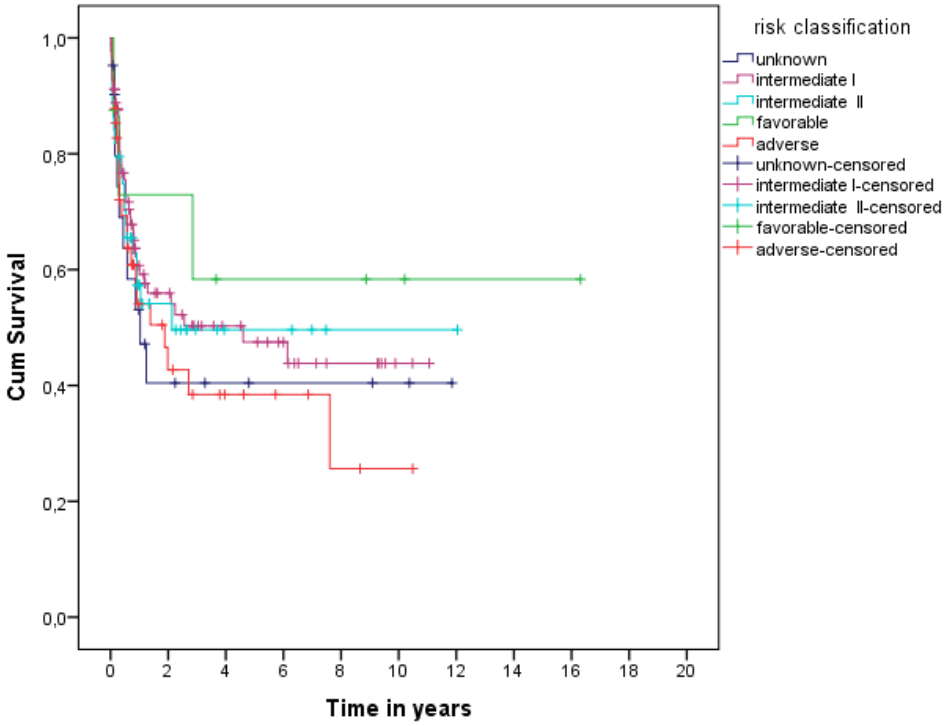


Figure 9: Overall survival according to risk classification

With the use of cytogenetic and molecular markers patients were assigned into the ELN-proposed risk groups (favorable, intermediate I, intermediate II and adverse risk). Although there was a trend for inferior OS in adverse risk patients as compared with prognostic more favorable groups like intermediate I (median survival time 1,8 for adverse risk versus 4,6 years in intermediate I patients), no statistical significance was reached (log rank test p=0,758, see Figure 9). Estimated overall survival rates for all cytogenetic risk groups are given in Table 7.

	1-year OS	2-year OS	5-year OS
Favorable (n=8)	73%	59%	59%
Intermediate I (n=90)	56%	50%	47%
Intermediate II (n=44)	52%	47%	47%
Adverse (n=41)	42%	38%	38%
Unknown (n=21)	41%	41%	41%

Table 7: Overall survival rates according to cytogenetic risk

Regarding recurrence of underlying disease, cytogenetically high risk patients were more likely to relapse as low risk patients. However, due to low patient numbers this difference was not statistically significant. Figure 10 gives all numbers of relapsed patients according to cytogenetic risk groups.

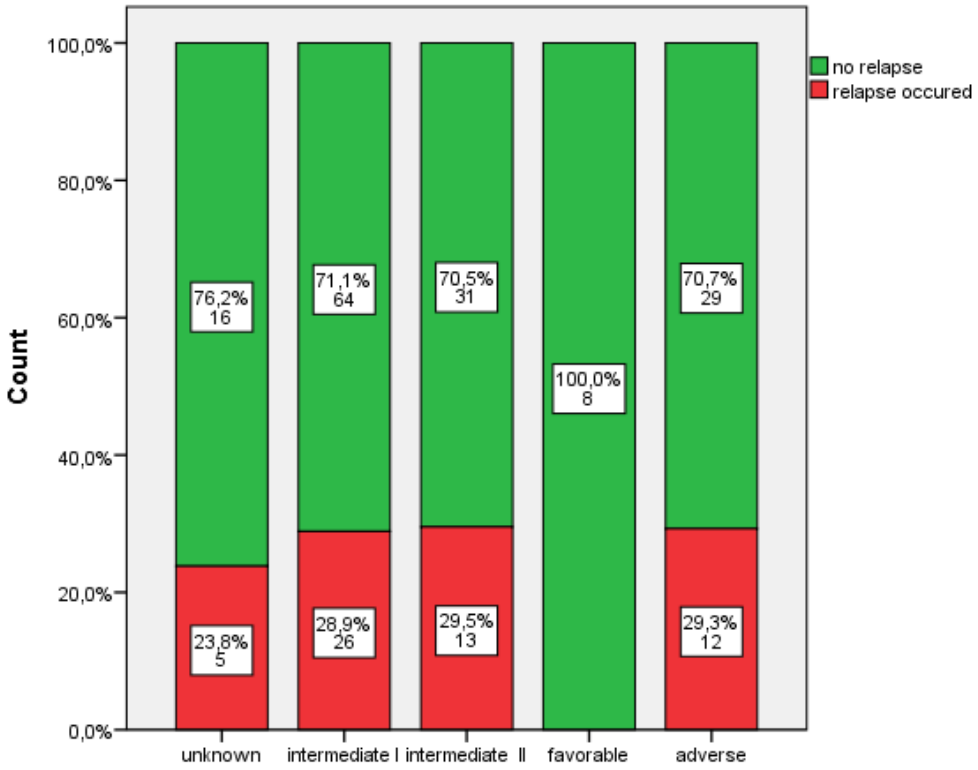


Figure 10: Percentage of patients experiencing relapse based on cytogenetic risk

3.4.3. Disease status at transplantation

Disease status at transplantation is a well-known risk factor affecting outcome parameters in AML after HSCT. In our cohort, the majority of patients was in complete remission (CR) after initial induction chemotherapy (CR 1, n=115, 56,7%).

	1-year OS	2-year OS	5-year OS
CR 1	63%	60%	56%
CR 2	33%	22%	22%
PD	38%	33%	33%
PR	44%	41%	33%

Table 8: Overall survival rates regarding disease status at transplantation (CR1=first complete remission, CR2=second complete remission, PD=progressive disease, PR=partial remission)

In regard to OS, disease status at time of transplantation significantly influenced survival (log rank test p=0,001) resulting in a superior OS for patients who were in CR 1. Table 8 gives details on the OS rates according to disease status at time of transplantation. When combining all patients who were not in CR 1 at transplantation, overall survival was inferior compared with patients in CR 1 (estimated 1-, 2- and 5-years OS. 40%, 35% and 31% versus 63%, 60% and 56%; see Figure 11). For patients in CR 1 follow-up was not long enough to observe 50% of failures as well as the Kaplan-Meier estimator was over 50% during the entire follow-up time. In contrast, median survival time for patients not being in CR 1 at transplantation was 9,4 months (95%CI 5,2 months-1,139 years). This difference between both groups was highly significant (log rank test p<0,001). We also analyzed the incidence of relapse based on remission status at time of transplantation. Recurrence of AML was significantly more often observed in patients not in CR 1 (Fisher's exact test 2-sided p=0,040), as seen in Figure 12.

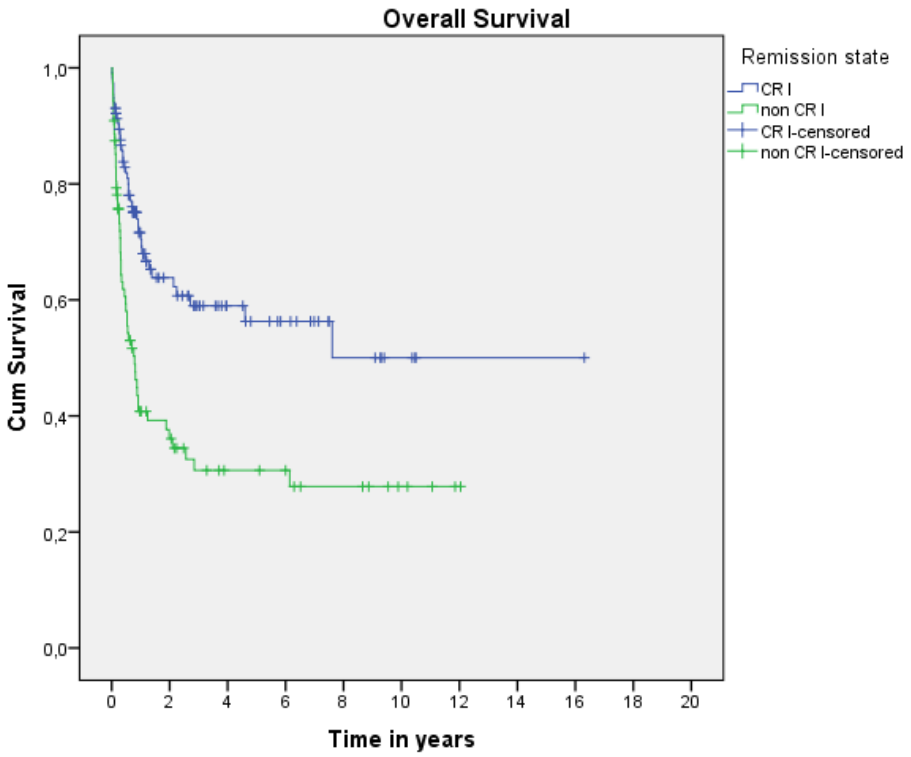


Figure 11: Overall survival according to disease status at transplantation (CR 1= first complete remission)

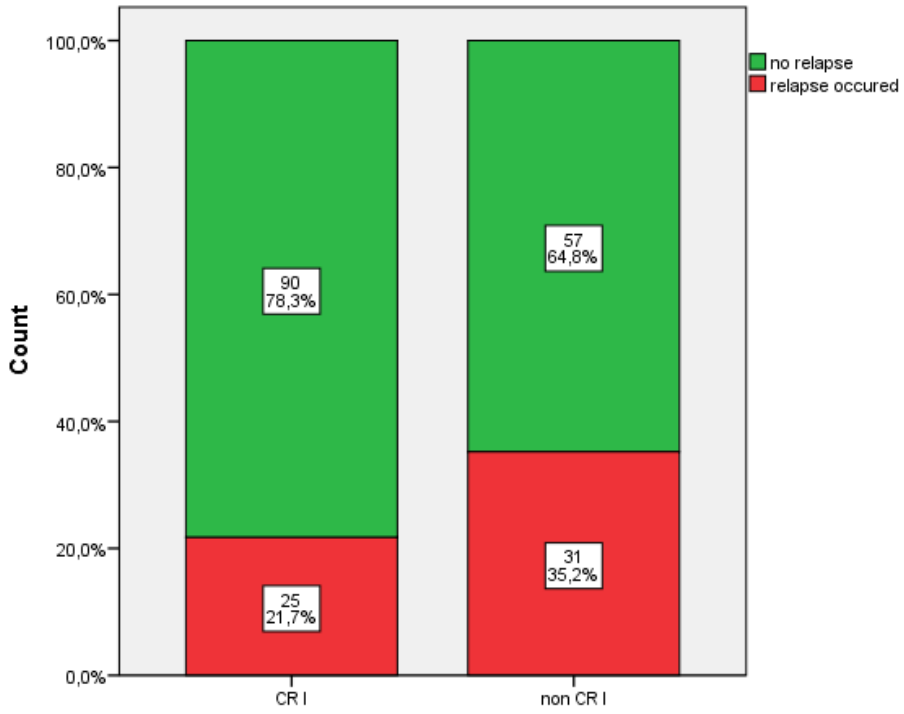


Figure 12: Observed frequency of relapse based on remission status at time of transplantation

3.4.4. HLA-matching

The immediate availability of a matched donor has prognostic value on OS for AML patients. (26) In our cohort 67 patients (32,8%) had an HLA-identical sibling donor. In the case of lacking an HLA-identical sibling, a matched unrelated donor (MUD) served as stem cell donor in 79 patients (38,7%), in 30 cases (14,7%, 29 unrelated, 1 related donor) at least one allele was mismatched and UCB transfusion was used in 20 patients (9,8%). As by definition all cord-blood grafts were HLA-mismatched, in total 50 patients (24,5%) received HLA-mismatched allografts. In seven unrelated (3,4%) and one related (0,5%) allografts records about HLA-Compatibility were missing.

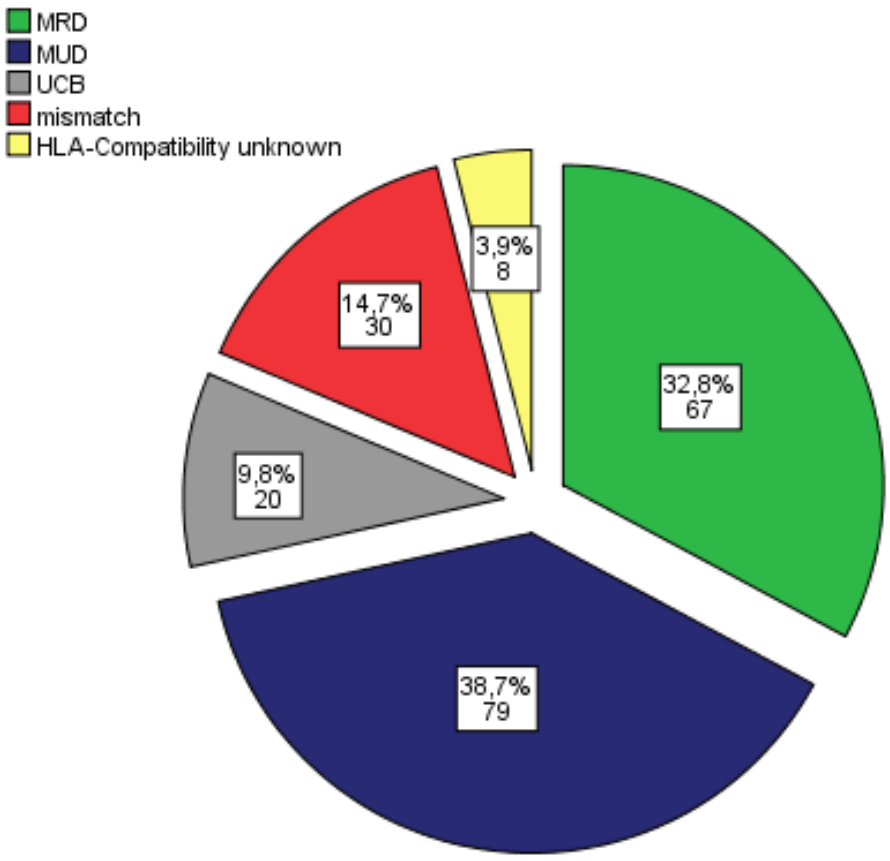


Figure 13: Donor types

Regarding OS we found no difference between patients receiving a MRD allograft or an allograft from a matched unrelated donor. In contrast, a significantly worse outcome was seen in cord blood recipients with a median survival time of 9,5 months (95%CI 4,4 months-1,211 years) (log rank test p=0,006) as well as in patients receiving an allograft from a mismatched donor (median survival time 9,1 months, 95%CI 0,4 months-1,483 years).

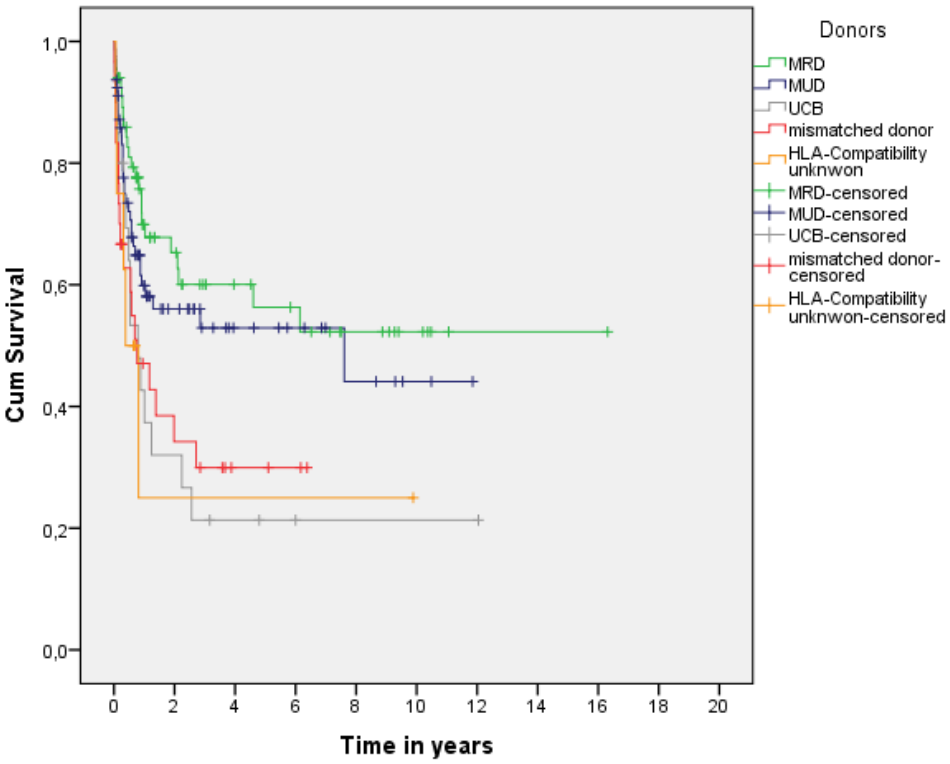


Figure 14: Overall survival according to donor type (MRD=matched related donor, MUD=matched unrelated donor, UCB=umbilical cord blood)

By combining the groups with a matched donor (MRD and MUD) and the groups with a mismatched donor (UCB and MMUD) we also analyzed influence of HLA-compatibility on OS. 34 patients out of 50 who received a mismatched donor graft died and the median survival time was 9 months (95%CI 4 months-1,182 years). In clear contrast, the median survival time was 7,61 years (estimation is limited because the largest observation is uncensored) for recipients of an HLA identical graft, as seen in Figure 15. In 8 cases no information about HLA-compatibility was

available. Analysis using the log rank test identified a highly significant influence of HLA-compatibility (p<0,001) on OS. Table 9 gives an overview of OS rates based on donor type and HLA-compatibility.

Donor type	1-year OS	2-year OS	5-year OS
MRD	68%	60%	56%
MUD	56%	56%	53%
UCB	32%	27%	21%
MMUD	35%	30%	30%
HLA-Compatibility	**1-year OS**	**2-year OS**	**5-year OS**
Mismatched graft	35%	30%	25%
Matched graft	61%	58%	54%

Table 9: Overall survival rates regarding donor type and HLA-Compatibility (MRD=matched related donor, MUD=matched unrelated donor, UCB=umbilical cord blood, MMUD=mismatched unrelated donor)

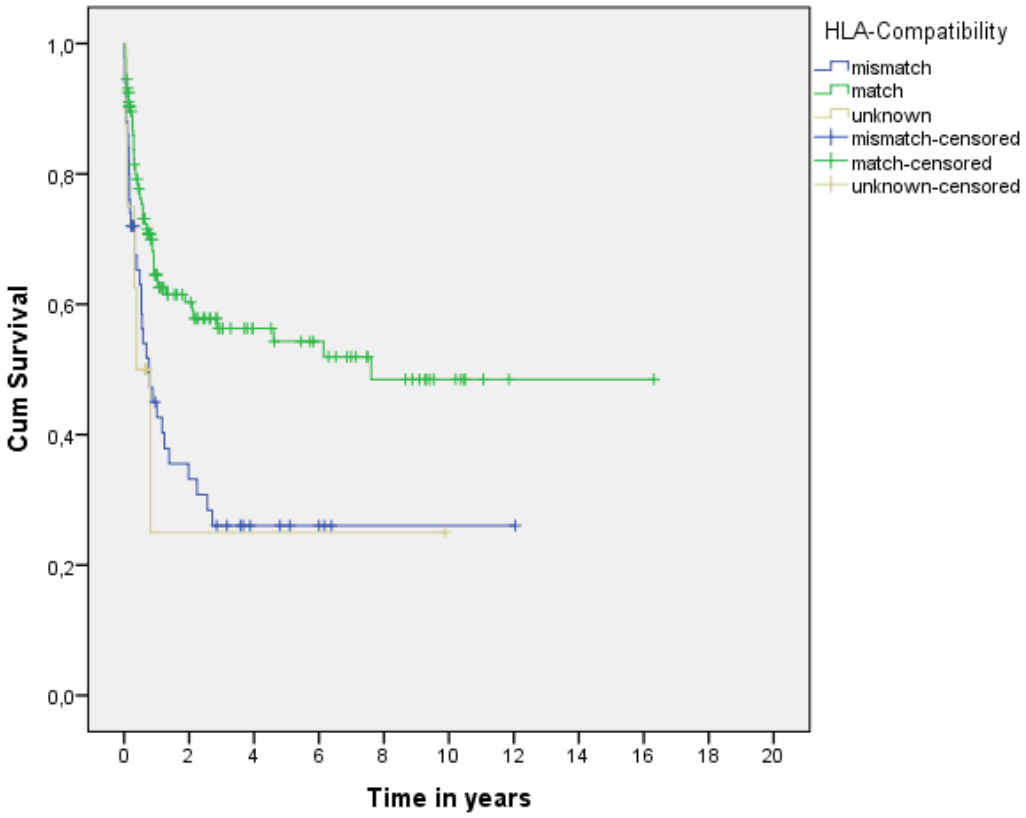


Figure 15: Influence of HLA-Compatibility on overall survival

3.4.5. Conditioning regimen

In the majority of our cohort (n=135, 66,2%) RIC therapy was used as conditioning regimen. 69 patients (33,8%) received myeloablative conditioning (MAC). The median survival time for patients receiving RIC was 6,14 years (95%CI 0,979-11,301 years) versus 1,3 years (95%CI 0,258-2,502 years) for those treated with MAC. However, using the log rank test displayed no statistically significant difference between both groups concerning OS (p=0,333). One-, 2- and 5-year OS rates for patients receiving RIC were 56%, 51% and 51%, for patients treated with MAC 45%, 39% and 37%, respectively.

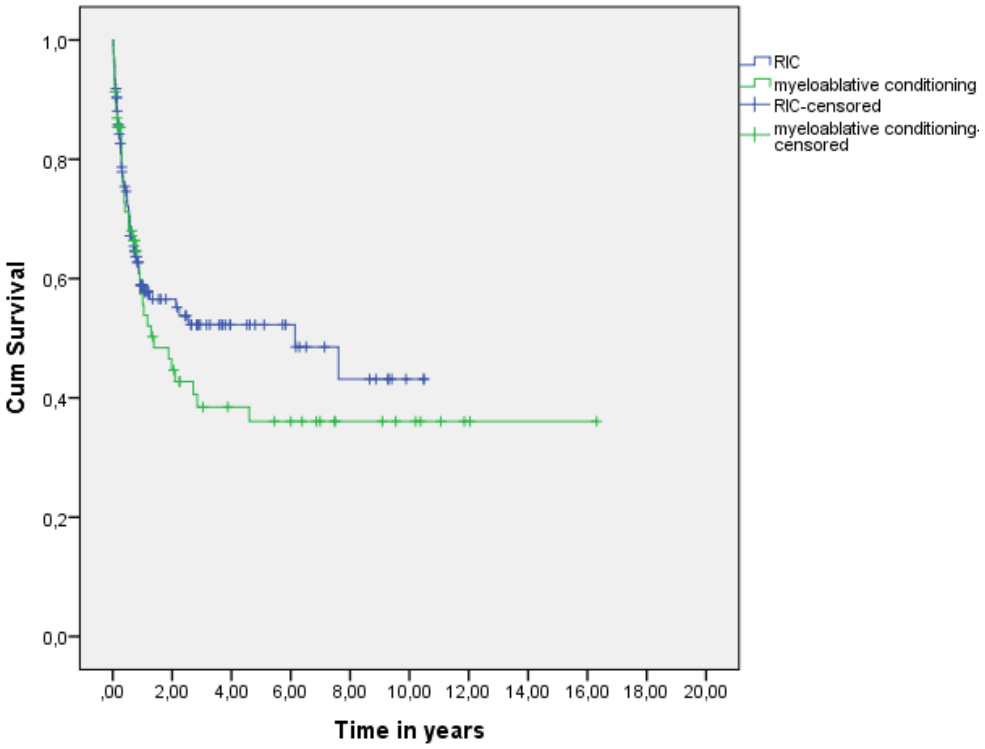


Figure 16: Overall survival regarding conditioning regimen (RIC= reduced intensity conditioning)

Treatment-related mortality was not different between both conditioning regimen groups (Figure 17). Full results on this topic are given in Table 10. Results did not differ significantly (p=0,745).

Incidence of TRM	RIC	95%CI		MAC	95% CI	
		lower	upper		lower	upper
1-year TRM	23%	16%	30,7%	17,9%	9,8%	28%
2-year TRM	24,2%	16,9%	32,1%	21,6%	12,4%	32,4%
5-year TRM	25,5%	17,9%	33,7%	26%	15,5%	37,8%
10-year TRM	30,7%	18,7%	43,4%	26%	15,5%	37,8%

Table 10: Incidences of Treatment-related mortality (TRM) according conditioning regimen (RIC=reduced intensity conditioning, MAC=myeloablative conditioning)

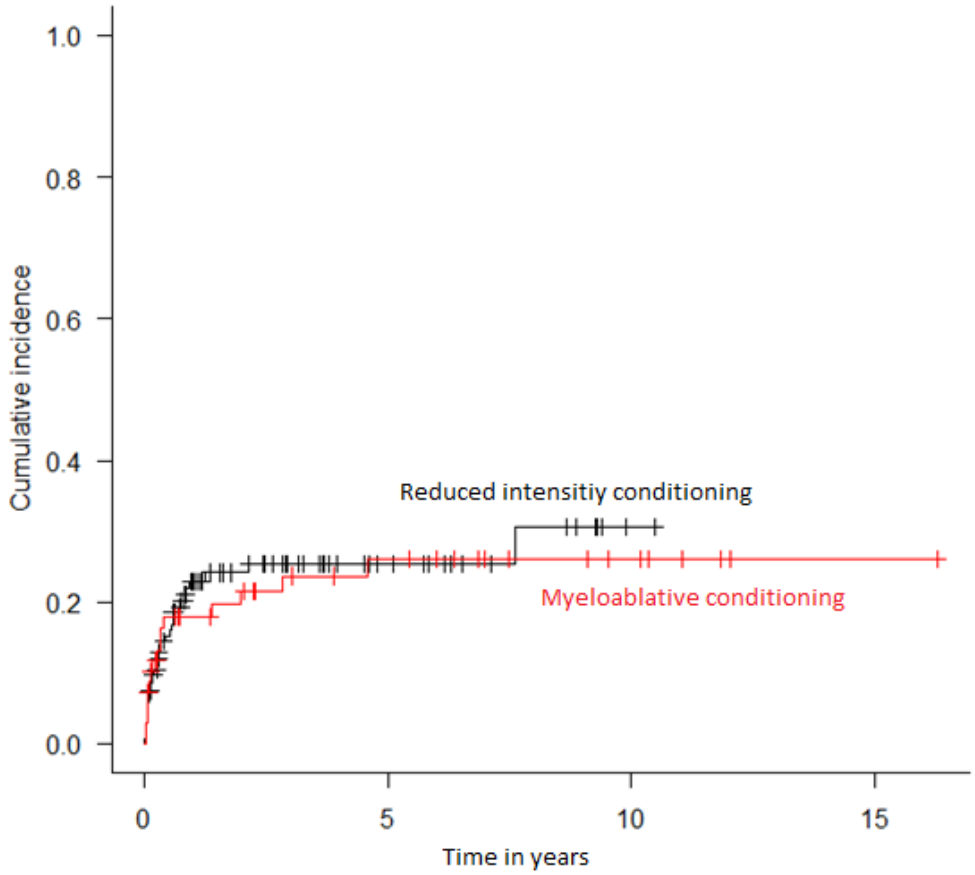


Figure 17: Incidence of Treatment-related mortality based on conditioning regimen

Next, we analyzed the influence of conditioning intensity on the incidence of relapse. As seen in Figure 18, cumulative incidence of relapse tended to be higher in the MAC group (incidence of relapse at 1 year 31%, 95%CI 20,1%-42,5%, at 2 years 36,4%, 95%CI 24,6%-48,3%, at 5 years 36,4%, 95%CI 24,6%-48,3%) compared with the RIC group (incidence of relapse at 1 year 23,4%, 95%CI

16,4%-31%, at 2 years 23,4%, 95%CI 16,4%-31%, at 5 years 24,7%, 95%CI 17,4%-32,7%). However, this difference was not statistically significant (p=0,201).

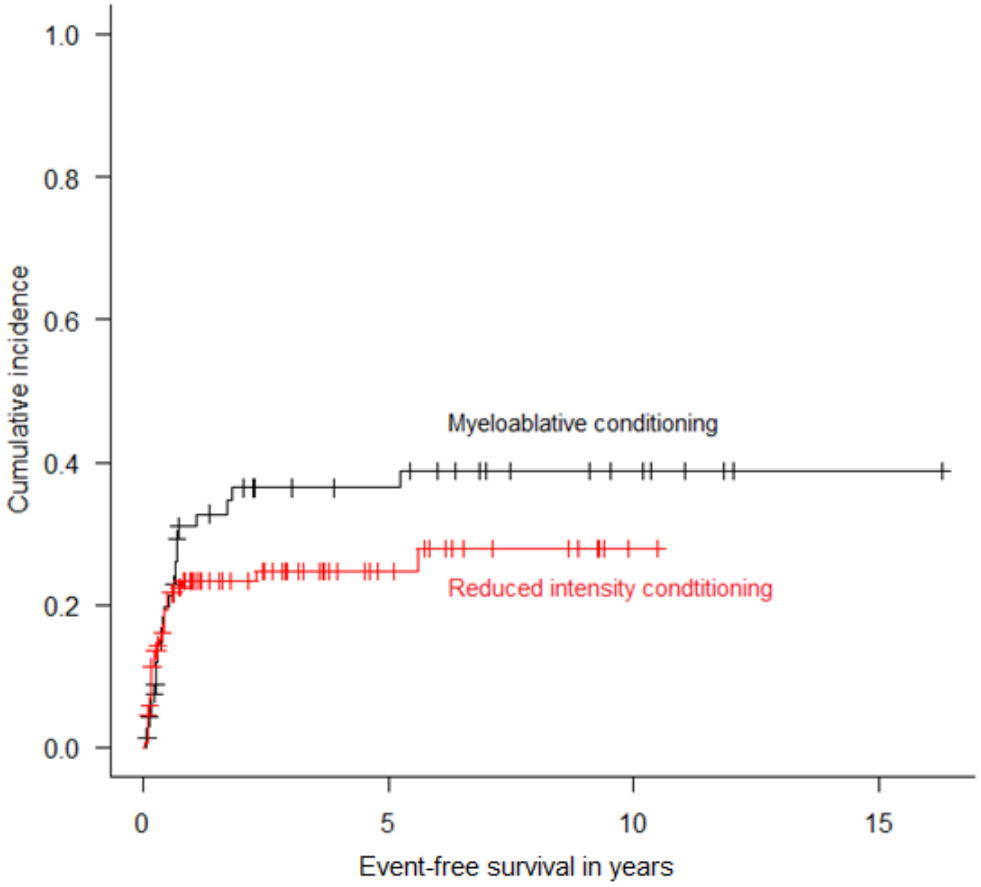


Figure 18: Incidence of relapse according to conditioning regimen

Searching for an explanation of our results, that OS tended to be lower in patients receiving MAC as compared to RIC, which is not in line with published literature (see Table 4), we analyzed disease burden prior to transplantation in both groups. Figure 19 shows that the majority of patients (56,5%, n=39) treated with MAC were not in CR 1 at transplantation. In comparison, 63,4% of patients (n=85) receiving RIC were in CR 1 (Fisher's exact test, 2-sided, p=0,007). This imbalance might explain the somewhat lower OS in patients receiving MAC.

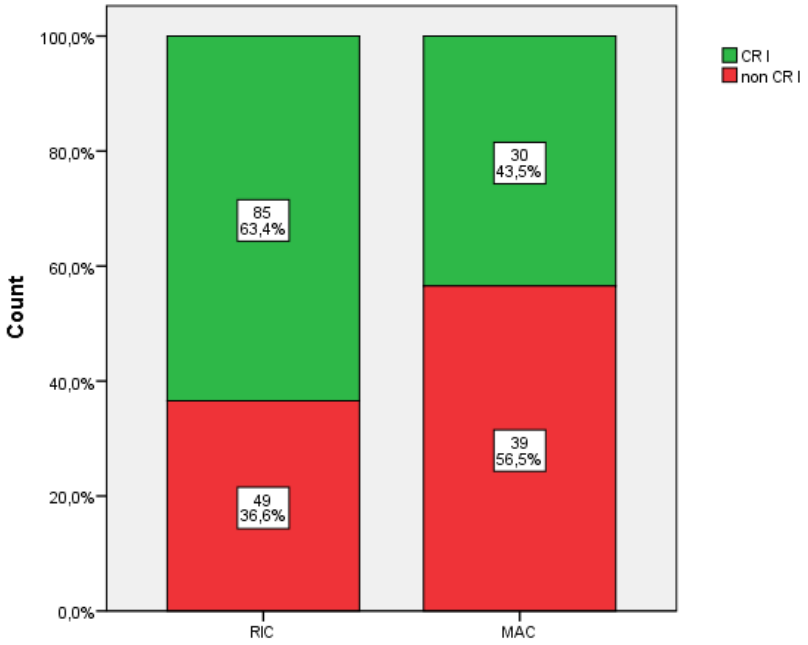


Figure 19: Remission status at time of transplantation in regard to conditioning regimen (RIC=reduced intensity conditioning, MAC=myeloablative conditioning, CR 1=first complete remission)

Thus, we calculated OS rates of both conditioning groups based on remission status at time of transplantation, as seen in Table 11. Inferior survival was observed in patients not in CR 1 regardless of which conditioning regimen was used. However, comparing RIC and MAC in patients in CR 1 at transplantation, we still observed somewhat higher OS rates in the RIC group, although this was not statistically significant (p=0,782).

CR 1	**1-year OS**	**2-year OS**	**5-year OS**
RIC	67%	61%	61%
MAC	55%	50%	45%
Non CR 1	**1-year OS**	**2-year OS**	**5-year OS**
RIC	36%	33%	33%
MAC	38%	30%	30%

Table 11: Overall survival rates according to entering CR 1 or Non CR 1 at time of transplantation and conditioning regimen (CR 1= first complete remission)

3.4.6. Acute and chronic GvHD

GvHD remains a significant cause of morbidity and mortality after allogeneic HSCT. Although GvHD prophylaxis was given to all patients, 84 patients (41,2%) developed acute GvHD (occurred within 100 days after HSCT). Biopsies were taken to confirm the diagnosis and dependent on severity topical or systemic therapy was given. Forty-six patients (22,5%) developed chronic GvHD (persistent or newly occurred GvHD beyond day 100). Among these 46 patients, 26 had already suffered from acute GvHD. Thus, previous acute GvHD had a significant impact for development of chronic GvHD (Fisher's exact test, 2-sided, p=0,018).

	Chronic GvHD		**Total**
	No chronic GvHD	Chronic GvHD	
Acute GvHD	58 (36,7%)	26 (56,5%)	84 (41,2%)
No acute GvHD	100 (63,3%)	20 (43,5%)	120 (58,8%)
Total	158 (100%)	46 (100%)	204 (100%)

Table 12: Occurrence of chronic GvHD in relation to previous acute GvHD

In addition, HLA-mismatch was associated with the development of GvHD. Recipients of a mismatched graft (MMUD and UCB) were more likely to develop acute GvHD (Fisher's exact test 2-sided, p=0,069). In contrast, HLA-compatibility had no impact on occurrence of chronic GvHD (Fisher's exact test 2-sided p=0,553).

	HLA-Compatibility (8 data missing)		**Total**
	match	mismatch	
Acute GvHD	54 (37,0%)	26 (52,0%)	80 (40,8%)
No acute GvHD	92 (63,0%)	24 (48,0%)	116 (59,2%)
Total	146 (100%)	50 (100%)	196 (100%)
Chronic GvHD	34 (23,3%)	9 (18,0%)	43 (21,9%)
No chronic GvHD	112 (76,7%)	41 (82,0%)	153 (78,1%)
Total	146 (100%)	50 (100%)	196 (100%)

Table 13: Occurrence of acute and chronic GvHD in relation to HLA-matching of the graft

Despite all negative clinical impact of GvHD, occurrence of chronic GvHD is associated with reduced risk of relapse. (13) In our study, there was also an inverse correlation between relapse and the occurrence of chronic GvHD (see Figure 20). Patients with chronic GvHD showed reduced recurrence of AML (Fisher's exact test 2-sided p=0,014).

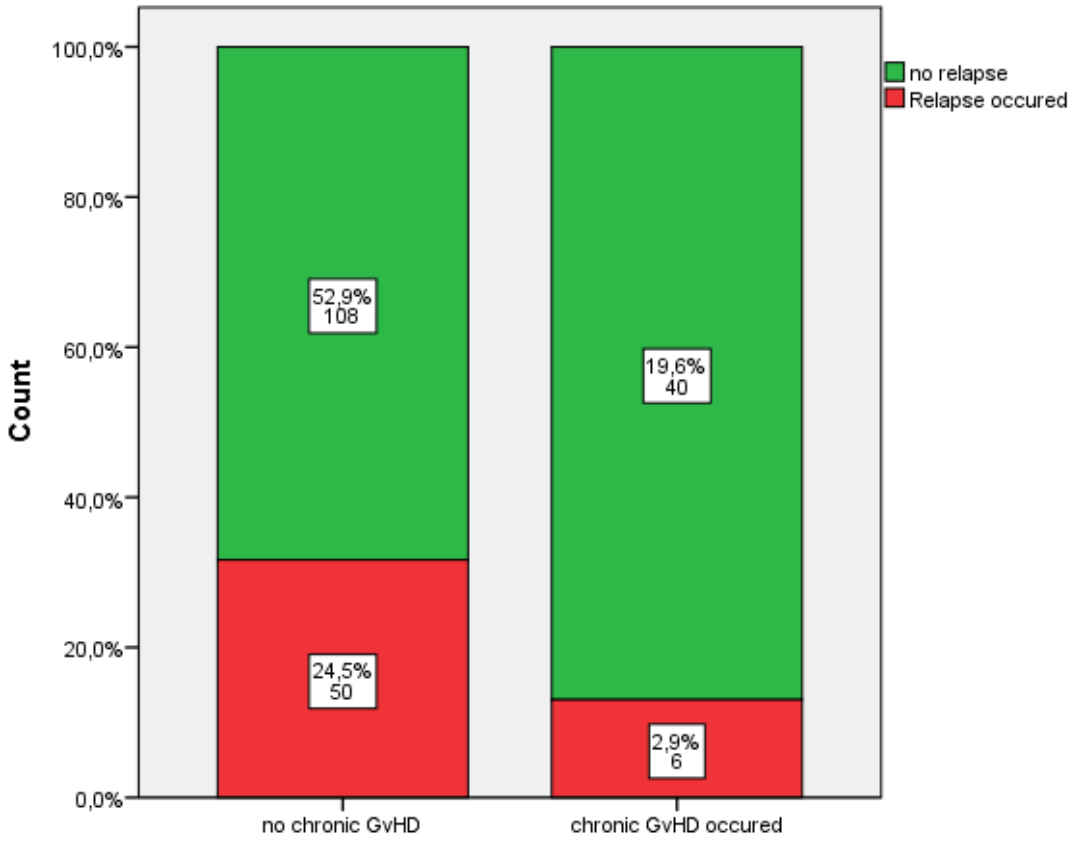


Figure 20: Observed frequency of relapse regarding occurrence of chronic GvHD

Regarding OS, developement of acute GvHD significantly influenced survival (log rank test p=0,035, see Figure 21). One-, 2- and 5-year OS rates for patients developing acute GvHD were 38%, 34% and 34%, patients without occurrence of acute GvHD had One-, 2- and 5-year OS rates of 61%, 54% and 52%, respectively. The occurrence of cGvHD had no impact on overall survival in our cohort.

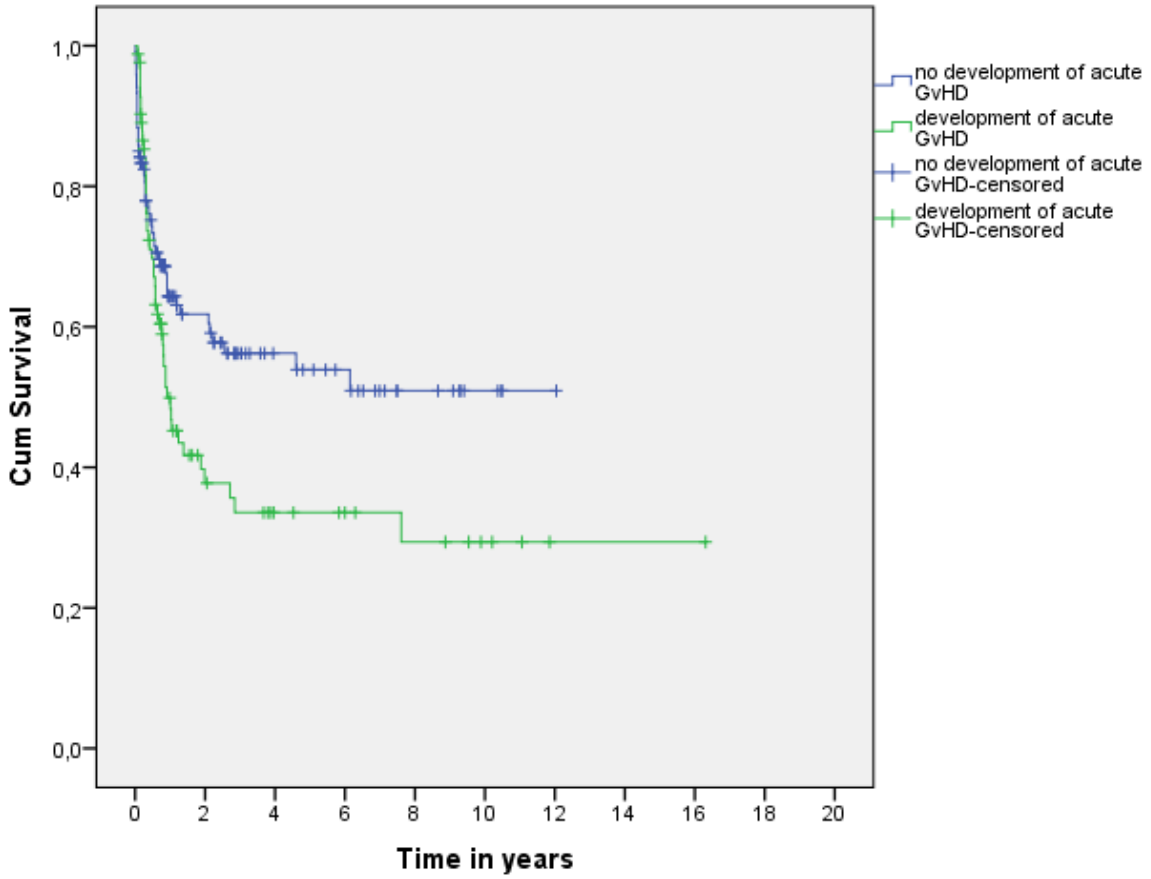


Figure 21: Overall survival based on development of acute GvHD

3.4.7. Hyperglycemia

A specific aim of this retrospective study was to evaluate the impact of hyperglycemia during the first 30 days after allogeneic HSCT on outcome. In 160 patients blood glucose levels were regularly determined within the first 30 days after allogeneic HSCT starting on the day of transplantation. A median of 24 blood glucose measurements were available for every allogeneic HSCT recipient resulting in 3646 analyzed blood glucose measurements in total. Hyperglycemia was defined as 20% of blood glucose measurements higher than 125mg/dl during the observation period. We observed an increased rate of death in patients with hyperglycemia. While 35 out of 57 hyperglycemic patients (61,4%) died, only 41 out of 103 patients (39,8%) with normal glucose levels succumbed to death (Fisher's exact test 2-sided p=0,013, see Figure 22). Median overall survival for patients with hyperglycemia was significantly shorter as compared to patients with normal glucose levels (6,8 months vs. 34,2 months, log rank test p=0,001, see Figure 23). Accordingly, estimated OS rates were lower in patients with hyperglycemia, as seen in Table 14.

	1-year OS	2-year OS	5-year OS
Glucose <125mg/dl	60%	49%	49%
Glucose >125mg/dl	38%	38%	29%

Table 14: Overall survival rates for patients with normal blood glucose levels and hyperglycemia

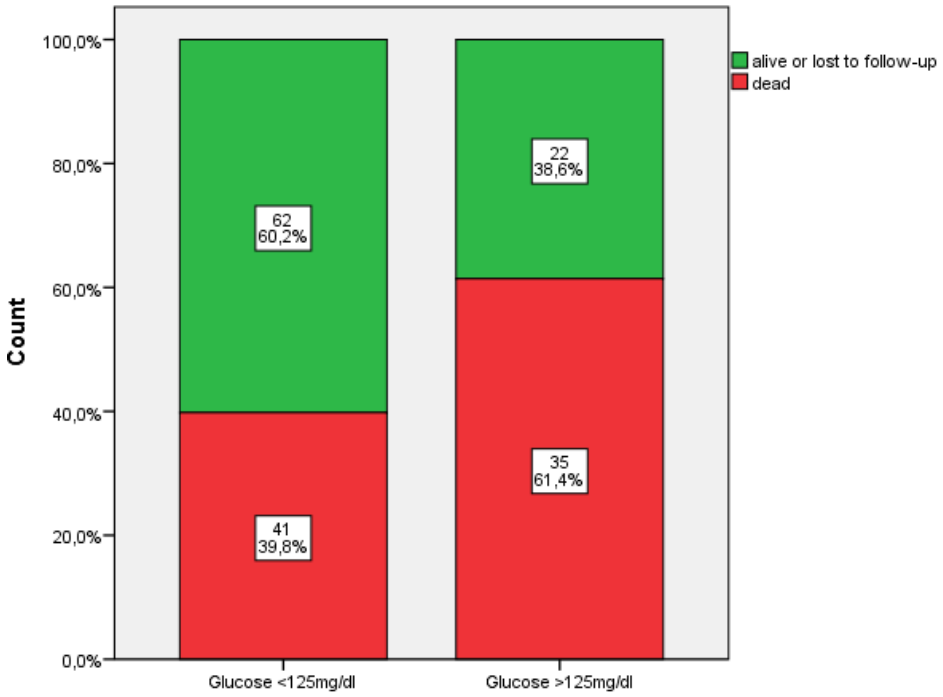


Figure 22: Percentage of patients alive, lost to follow-up or dead based on blood glucose levels

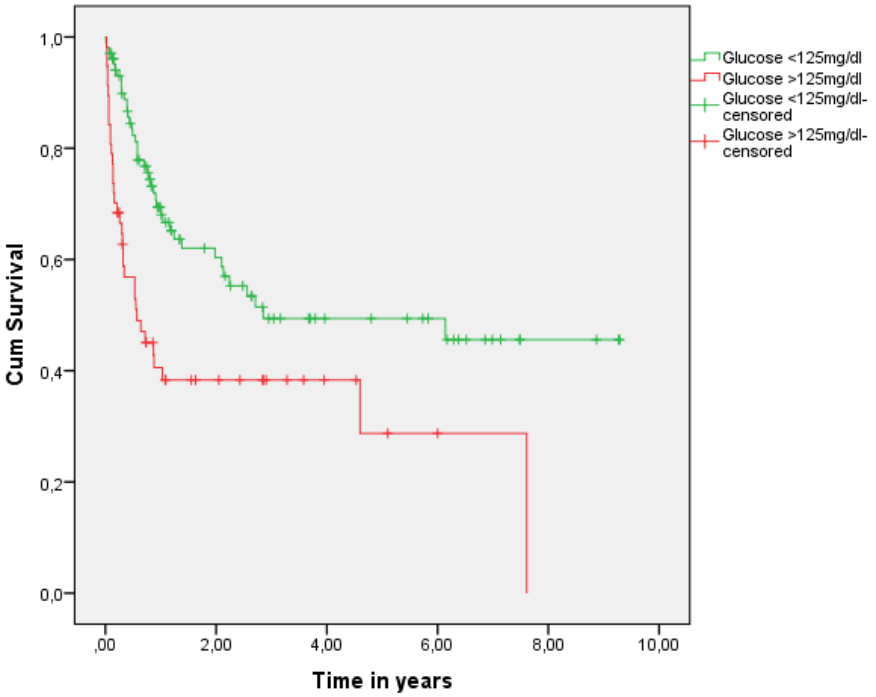


Figure 23: Overall survival according to blood glucose levels

3.4.8. Univariate analysis of risk factors affecting survival

Known risk factors influencing overall survival like donor/recipient gender mismatch, CMV status, occurrence of acute GvHD, HLA-compatibility, age, conditioning regimen, cytogenetic risk group, remission status at time of transplantation, LDH values, leukocytosis, as well as hyperglycemia were analyzed in a univariate manner using Cox regression analysis. The Log rank test was used to compare survival of the samples. Due to missing records, not all patients could be included in all analyses.

	Coefficient	Significance	Hazard Rate Exp (B)	95% CI	
				lower	upper
Donor/Recipient Gendermismatch	-0,065	p=0,773	0,937	0,601	1,459
LDH	-0,069	p=0,808	0,933	0,535	1,630
CMV status	0,253	p=0,291	1,289	0,805	2,063
Leukocytosis	0,098	p=0,676	1,103	0,698	1,743
Acute GvHD	0,427	p=0,037	1,533	1,026	2,291
Hyperglycemia	0,782	p=0,001	2,187	1,389	3,443
HLA-Compatibility (mismatch)	0,739	p=0,001	2,093	1,367	3,205
Age (<60 years)	-2,56	p=0,363	0,774	0,446	1,344
RIC	-0,201	p=0,336	0,818	0,543	1,232
Remission status (non CR 1)	0,811	p=0,000	2,251	1,500	3,377
Intermediate I		p=0,764	1,000		
Intermediate II	0,071	p=0,795	1,074	0,627	1,838
Favorable	-0,335	p=0,577	0,715	0,221	2,320
Adverse	0,272	p=0,305	1,313	0,780	2,209
Unknown	0,241	p=0,479	1,273	0,653	2,482

Table 15: Univariate analysis of prognostic risk factors (RIC=reduced intensity conditioning, CR 1=first complete remission)

OS was not affected by gender mismatch (graft from male donor to female recipient and reversed, log rank test p=0,772, Cox regression p=0,773) and CMV

status of the recipient (log rank test p=0,289, Cox regression p=0,291). Laboratory findings like elevated LDH levels (log rank test p=0,808, Cox regression p=0,808) and white blood counts (WBC, log rank test p=0,675, Cox regression p=0,676) had also no influence on OS in our cohort. The majority of patients were aged less than 60 years. Regarding OS, there was no statistical significance reached comparing the two age ranges and their impact on survival (log rank test p=0,361, Cox regression p=0,363). Comparing the two conditioning modalities RIC and MAC on OS, also no significance was reached (log rank test p=0,333, Cox regression p=0,363). Interestingly, the different risk groups based on cytogenetics, had no impact on overall survival in relation to the intermediate I risk group (Cox regression for intermediate II p=0,795, favorable p=0,577, adverse p=0,305).

In contrast to the above mentioned factors, HLA-compatibility (p=0,001), remission status at transplantation (p<0,001), acute GvHD (p=0,037) and hyperglycemia (p=0,001) were identified as risk factors predictive for overall survival.

For patients not in CR 1 at time of allogeneic HSCT, the risk of death was more than twice as high as for patients in CR 1 (HR 2,251, 95%CI 1,500-3,377).

Comparably, recipients of mismatched donor grafts had a more than doubled risk of death as compared to recipients of matched allografts (HR 2,093, 95%CI 1,367-3,205). AML patients who met the criteria for hyperglycemia also had an increased risk of death, which was more than twice as high as for patients with normal glucose levels after transplantation (HR 2,187, 95%CI 1,389-3,443). Furthermore, occurrence of acute GvHD correlated with inferior outcome (p=0,037, Hazard Rate 1,533, 95%CI 1,026-2,291).

3.4.9. Multivariate Analysis of risk factors affecting survival

We also performed a multivariate analysis using Cox-proportional-hazard-model taking into account age at time of diagnosis, remission status at time of HSCT, risk classification based on cytogenetics, blood glucose levels, HLA-compatibility and conditioning regimen. Remission status, hyperglycemia and HLA-compatibility independently remained risk factors for OS, as seen in Table 16. Patients not in CR 1 before transplantation were at a more than twice as high risk to die (HR 2,505, 95%CI 1,504-4,172, p<0,001) as well as patients with elevated blood glucose levels after transplantation (HR 2,121, 95%CI 1,291-3,485, p=0,003). For recipients of mismatched allografts risk to experience death was 72,9% higher than for those receiving an HLA-identical allograft (HR 1,729, 95%CI 1,013-2,952, p=0,045). Patients assigned to the intermediate II cytogenetic risk group tended to have inferior survival (HR 1,902, 95%CI 0,996-3,632, p=0,051).

	Coefficient	Significance	Hazard Rate Exp (B)	95% CI	
				lower	upper
Conditioning regimen	0,379	p=0,155	1,461	0,866	2,466
Remission status	0,918	p=0,000	2,505	1,504	4,172
Age at time of diagnosis	-0,136	p=0,703	0,873	0,434	1,755
Hyperglycemia	0,752	p=0,003	2,121	1,291	3,485
HLA-Compatibility	0,548	p=0,045	1,729	1,013	2,952
Intermediate I		p=0,232	1,000		
Intermediate II	0,643	p=0,051	1,902	0,996	3,632
Favorable	-0,266	p=0,728	0,767	0,172	3,419
Adverse	0,522	p=0,105	1,686	0,897	3,168
Unknown cytogenetic	0,469	p=0,293	1,599	0,667	3,831

Table 16: Multivariate analysis

4. Discussion

Allogeneic HSCT is still the most effective treatment for curing patients with AML. Accordingly, consensus exists across current guidelines to recommend allogeneic HSCT for patients with intermediate- or high-risk AML in first complete remission as well as for all patients with AML relapse, who are eligible for HSCT and who have an HLA-matched (either related or unrelated) donor. However, despite advances in transplantation medicine as well as supportive care during the last two decades, relapse- and treatment-related mortality of patients with AML undergoing allogeneic HSCT remains a major concern negatively affecting long-term outcome. In this diploma thesis we retrospectively analyzed the outcome of AML patients undergoing allogeneic HSCT at the Division of Hematology, Medical University of Graz. The analyzed cohort encompassed 204 patients having undergone allogeneic HSCT between 1996 and 2013. The primary endpoint was overall survival.

4.1. Overall survival and relapse risk in general

The observed median overall survival in our cohort was 2,24 years. One-, 2- and 5-year OS survival rates were 51%, 46% and 45%, respectively. These results compare well with other studies analyzing overall survival in AML patients after allogeneic HSCT in general (Shimoni et al. (27), Saber et al. (28), Hegenbart et al. (29) and Warlick et al. (30)). Summarized data on overall survival in these studies as compared to our results are given in Table 17.

	Shimoni et al.	Saber et al.	Warlick et al.	Hegenbart et al.	Our cohort
Patients	**112**	**2223**	**414**	**122**	**204**
OS (1 year)	NA	**52%**	NA	NA	**51%**
OS (2 years)	**48%**	**42%**	NA	**48%**	**46%**
OS (3 years)	NA	**37%**	NA	NA	NA
OS (5 years)	NA	NA	NA	NA	**45%**
OS (6 years)	NA	NA	**44%**	NA	NA

Table 17: Comparison of overall survival after allogeneic HSCT

In total, 96 out of 204 patients died during follow-up. Forty-eight patients died due to recurrence of AML making relapse the major cause of death in our cohort. This correlates well with published literature, in which disease relapse has been identified to be the major cause of death after allogeneic HSCT in AML (for review see Vyas et al.). Patient-specific parameters (age, comorbidities, performance status), disease-specific factors like cytogenetic and molecular characteristics and failure to achieve CR after induction therapy as well as transplant-specific factors, like the intensity of the conditioning regimen affect the risk of relapse. (13) Concerning disease-specific parameters for example, patients within the cytogenetically adverse risk group or patient not in first complete remission after induction chemotherapy have the highest risk of relapse after allogeneic HSCT. (13,31) Accordingly, in our study frequently more patients with high risk disease as defined by cytogenetics or not being in CR 1 at time of transplantation relapsed as compared to other patients. In a study of Warlick et al including 414 AML patients the overall incidence of relapse at 2 years was 29% and higher relapse rates were observed in patients undergoing transplantation not in CR 1 ($p<0,04$). (30) Hegenbert et al. reported higher relapse rates for patients in advanced disease status at time of transplantation as well. (29) Again, these published results compare well with the data from our cohort. Table 18 summarizes all data.

	Warlick et al	Hegenbart et al.	Our cohort
Overall incidence of relapse at 2 years	29% (95%CI, 24%-34%)	39% (95%CI 0,7-1,8)	28,2% (95%CI 21,8%-34,8%)
Incidence of relapse in CR 1	26% (95%CI 21%-31%)	16% (95%CI NA)	22,6% (95%CI 15,1%-31%)
Incidence of relapse not in CR 1	36% (95%CI, 27%-45%)	42% (95%CI NA)	35,4% (95%CI 25%-45,9%)

Table 18: Comparison of incidence of relapse

In the literature an inverse correlation between either the development of chronic GvHD as well as the intensity of post-transplantation immunosuppression and the risk of relapse has been reported. Lower intensity and shorter duration of immunosuppression after transplant can reduce risk of relapse, probably resulting from a potent GvL effect, but is linked with the risk of developing GvHD. (13) Accordingly, in our cohort we found a reduced risk of AML relapse in patients with chronic GvHD.

An increased risk of relapse has also been reported in patients receiving RIC before allografting. (14) In a retrospective analysis including 878 AML and MDS patients the European Group for Blood and Marrow Transplantation reported an increased relapse rate in patients receiving RIC as compared to MAC (relapse risk 23% in MAC versus 39% in RIC transplantations). (32) On the contrary, Bornhauser et al. reported similar incidences of relapse at 36 months (28% for RIC and 26% for MAC) in patients with AML. (33) Interestingly, we observed an increased incidence of relapse in the MAC group, but this increase was not statistically significant (p=0,201, incidence of relapse at 2-year 36,4% versus 23,4% in the RIC group). A possible explanation for these discrepant results is the finding, that patients receiving MAC frequently had a more advanced disease status at time of transplantation as patients receiving RIC in our cohort. As discussed above, the remission status at transplantation has a strong impact on the incidence of relapse.

Using uni- and multivariate analysis we identified several risk factors affecting overall survival in our cohort of AML patients undergoing allogeneic HSCT

including remission status at transplantation, HLA compatibility and development of acute GvHD. These risk factors as well as factors not associated with outcome in our cohort, but described in the literature to affect survival after allogeneic HSCT are discussed in detail in the following subchapters.

4.2. Remission status at transplantation

The risk factor, which we identified to have the biggest impact on survival in our cohort of AML patients, was the remission status at transplantation. Patients not in CR 1 had a 2,5-fold increased risk of death as compared to patients in CR 1. These results are in line with published studies from Shimoni et al. (27), Michallet et al. (34) and Hegenbart et al. (29), comparing patients in CR 1 with patients not in CR 1 at time of HSCT (see Table 19).

	Shimoni et al [a]	Michalet et al.	Hegenbart et al..[b]	Our cohort[a]
Patients	**112**	**379**	**122**	**204**
OS (1 year)	NA	NA	NA	**63% / 40%**
OS (2 years)	**69% / 31%**	NA	**51%/28%**	**60% / 35%**
OS (5 years)	NA	**35% / 13%**	NA	**56% / 31%**

Table 19: Comparison of overall survival after allogeneic HSCT for AML patients in CR 1 versus non CR 1 (CR 1=first complete remission)

The impact of advanced disease status at time of transplantation was also described by Warlick et al. In a retrospective study analyzing 414 AML patients undergoing MAC or RIC allogeneic transplantation, patients aged 18-39 undergoing MAC had a superior survival compared with a same age patient group undergoing RIC. This adverse outcome was due to a poorer performance status and an advanced remission status at transplantation (not in CR 1) in RIC patients. (30) Conversely, in our cohort we observed a somewhat worse outcome in patients with MAC, which was also most likely due to the fact that the majority of patients receiving MAC was not in CR 1 (56,5%) at time of transplantation. In

comparison, almost two thirds of our patients treated with RIC were in CR 1 (63,4%). In a prospective German multicenter trial including 195 young AML patients all in CR 1 and comparing RIC versus MAC, the 3-year OS and EFS as well as non-relapse mortality were identical. (33) Combining all these results indicates that remission status at time of transplantation has a major impact on OS as well as relapse risk and that this adverse risk cannot be (fully) overcome by treatment with MAC.

4.3. Influence of donor type

In our cohort we observed no significant difference in OS in patients receiving HSC from a matched related (MRD) or matched unrelated (MUD) donor (5-year OS 56% versus 53%). In contrast, 5-year OS of cord blood recipients (21%) and recipients of a mismatched unrelated allograft (30%, p=0,006) was significantly lower. By combining the groups with a matched donor (MRD and MUD) and the groups with a mismatched donor (UCB and MMUD), we observed a clinically important and statistically significant worse outcome for patients receiving HLA-mismatched allografts (5-year OS 25% versus 54% for HLA identical allografts, p<0,001).

Thirty percent of patients eligible for allogeneic HSCT have an HLA-identical sibling (MRD). In the remaining cases patients are reliant on unrelated donors or alternative stem cell sources like UCB. (16) In earlier times, outcome of MRD transplants was suggested to be superior when compared to MUD allografts and therefore preferred. However, more recent data report similar results on survival when comparing these two donor types. (25)

A large CIBMTR study of AML patients treated between 2002 and 2006 using either matched URD or sibling donors observed similar survival rates between these donor types. (28) Our results are in line with these reports. The availability of high-resolution techniques for HLA-matching based on DNA sequencing and increasing the number of HLA-loci analyzed for matching are the two main factors being responsible for the improved outcome after MUD HSCT within the last two decades. (16)

In case of lacking a matched donor, UCB can be used as an alternative source of HSC for transplantation in AML patients. (35) UBC grafts are mismatched in the vast majority of cases, but display a reduced risk of GvHD. (36) However, significant variations in clinical outcome are reported in studies comparing UCB HSCT with grafting using adult donors. Laughlin et al. reported that survival after HSCT UCB was comparable to that of one mismatched HLA-antigen URD BMT and inferior to HLA-identical URD BMT. (37) In contrast, Rocha et al. concluded that outcome after MUD and UCB allografts was identical. (38) Takahashi et al. even reported superior results after UCB as compared to MUD transplantation. (39) A retrospective study from Eapen et al. observed similar leukaemia-free survival, but higher transplant-related mortality after UCB transplantation when compared with 8/8 and 7/8 allele-matched peripheral blood progenitor cells or bone-marrow transplantation. (35) In our cohort, survival of patients receiving UCB was comparable to patients receiving MMUD transplantation but was significantly lower when compared to matched donors.

4.4. Impact of age on transplantation outcome

Although AML occurs in people of all age ranges, it is more common in elderly with a median age of 71 years at time of diagnosis. (1) As expected our cohort of transplanted AML patients was dominantly younger and just 20,1% were aged 60 years or older. The underuse of HSCT in elderly patients is well reported in the scientific literature. (25) Barriers to apply allogeneic HSCT in elderly people include high TRM related to the presence of comorbidities, increased incidence of adverse cytogenetics or preceded, often unrecognized blood disorders like MDS in older AML patients. With the advent of RIC, TRM rates could have been reduced and the number of potential recipients of allogeneic HSCT with higher age broadened. (2,26) In a multicenter study from the Seattle consortium including 274 AML patients, thereof 135 aged 60 years or older, outcomes after treatment with non-myeloablative conditioning were not influenced by age. (26) Similar results were seen in a recent report from the European Group for Blood and Marrow Transplantation including 719 patients with a median age of 58 years. In a

multivariate analysis no impact of patient age was found on survival (40). Also in our cohort, increased age did not negatively affect OS.

For the decision whether to proceed to allogeneic HSCT or not in older patients, a careful patient selection and risk calculation is important. (41) An analysis from Mawad et al. revealed that not older age, but poor performance status affected outcome of allogeneic HSCT. (42) With the help of scores like the HCT-CI Score, a thorough assessment of comorbidities can identify older patients who are able to tolerate intensive therapy and who will benefit from allogeneic HSCT. (20) However, informative prospective studies are still scarce, because most transplant studies exclude patients aged 65 years or older. In a Medicare population based study including 5480 AML patients aged >65 years only 46 patients underwent allogeneic HSCT (0,8%). Within this group, OS of patients aged 65-69 years and 70-74 years was similar, but strongly affected by high comorbidity scores (29 versus 8 months median survival for patients with low versus high comorbidity scores). (43) The HCT-CI Score has been used at the Division of Hematology in Graz since 2010. Therefore, this information was missing for the majority of patients included in this study and no analysis could have been made. Recent reports are encouraging and in absence of comorbidities, allogeneic HSCT may provide a potential curative therapy in a selected cohort of older AML patients.

4.5. Impact of conditioning regimens: RIC versus MAC

In our retrospective analysis about two thirds of patients (n=135, 66,2%) received RIC therapy and 69 patients (33,8%) myeloablative conditioning (MAC) therapy reflecting that the use of RIC has increased in recent years. (44)

With the exception of patients aged 55 years or older, the decision whether to use MAC or RIC is currently not accurately defined, since comprehensive studies, comparing both regimens in younger patients are still lacking. The only available prospective study comparing a RIC and MAC protocol is a German multicenter trial including 195 AML patients all being in CR 1 and younger than 60 years. In this study the 3-year OS and EFS as well as non-relapse mortality was identical in both groups. However, this study was stopped early because of slow patient

accrual. (33) In general, the intensity of the different conditioning regimens often varies from center to center and the difference between truly MAC and RIC is not exactly defined. Additionally, “the dose-response curves are not so steep that every study has found a difference, particularly when the intervals in dose intensity are small or when the numbers of patients are limited.” (13) In a recent review Craddock et al. stated that MAC regimen should be considered as gold standard in fit patients for allogeneic HSCT. (14) This statement is based on the fact that some retrospective analyses showed similar OS in AML patients undergoing MRD or MUD transplantation with RIC or MAC, but also observed higher relapse rates after RIC. Indeed, increasing the intensity of conditioning therapy reduces the incidence of relapse, but is associated with increased TRM. (32,45) In terms of selection of a convenient preparative regimen, the risk of TRM has to be outweighed individually for every single patient against the burden, behavior and risk of relapse of the underlying disease. (13)

4.6. Hyperglycemia during the engraftment period is an adverse risk factor after allogeneic HSCT

By performing a univariate analysis we confirmed well described risk factors on OS after allogeneic HSCT in AML patients, like the occurrence of acute GvHD (p= 0,037), HLA-mismatch (p=0,001), and not being in CR 1 at time of transplantation (p<0,001). The remission status (p<0,001) and HLA compatibility (p=0,045) were also shown to significantly affect OS in a multivariate analysis. However, in conflict to several studies (1,41), age (p 0,703) and cytogenetics had no statistically significant impact on OS in the present study. Since we observed a trend towards an increased risk in patients with adverse cytogenetics, a possible explanation could be the fact that the number of patients in our adverse cytogenetic risk group was just too low to reach statistical significance.

So far, hyperglycemia during the engraftment period was not known to be a risk factor in allogeneic HSCT, but well described to affect outcome in patients with other severe diseases. (46) Accordingly, van den Berghe et al. showed that strict control of hyperglycemia with insulin therapy in intensive care unit (ICU) patients

reduces morbidity and incidence of infections. (46) In a vicious cycle hyperglycemia facilitates infections and exacerbates underlying diseases resulting in increased organ dysfunction and mortality. (46) By evaluating glucose levels during the first four weeks after transplantation we found a significant inferior OS in patients meeting the criteria of hyperglycemia. Both, in multivariate and univariate analysis, hyperglycemia was an independent strong risk factor for overall survival in AML patients undergoing allogeneic HSCT. In a retrospective analysis of 112 patients with various hematological malignancies undergoing myeloablative allogeneic HSCT, Fuji et al. reported a significant impact of hyperglycemia on organ dysfunction, NRM and risk of aGvHD. (47) The importance of glucose metabolism became also evident from a study of Chen et al. They reported in a metabolomics study with 400 AML patients and 446 healthy controls that AML patients with increased glycolysis in leukemic cells showed decreased response and sensitivity to Ara-C, a conventional chemotherapeutic substance used for induction therapy. (48)

Although these findings on hyperglycemia during the engraftment phase after HSCT may have an important impact, our study has several limitations due to its retrospective nature. A consistent protocol for blood glucose measurements and nutritional support was not applied. In addition, data on the use of corticosteroids and preexisting diabetes mellitus were not available for our analysis. But even with these limitations, we think that our findings are important considering the challenging issue of decreasing transplant-associated mortality after allogeneic HSCT. We propose that prospective studies testing the impact of hyperglycemia as well as a strict blood glucose management will help to improve the outcome in this clinical setting.

4.7. Conclusion

In summary, we observed a 5-year OS rate of 45% in our retrospective analysis of 204 AML patients treated with allogeneic HSCT at the Division of Hematology of the Medical University of Graz. Relapse and transplant-related morbidity such as severe infections as well as GvHD were the main causes of death. Remission status at time of HSCT and HLA-compatibility turned out to have a significant impact on OS. All these data are in good agreement with published reports in the literature. In addition, our data support the use of MRD and MUD as graft source, but alternative sources like UCB as well as mismatched donors should be handled with care due to a significantly inferior survival in our study. A careful assessment of disease risk in comparison to transplantation risk is crucial in such situations, when a matched donor is not available. Finally, we identified hyperglycemia during the engraftment period as a major risk factor for OS after allogeneic HSCT. Prospective clinical trials are therefore warranted to validate this newly discovered risk factor but also to evaluate the impact of strict blood glucose management on outcome of AML patients after HSCT.

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