**Diploma thesis**

# Conventional post-remission therapy in adult acute myeloid leukemia – a systematic review / meta-analysis


Submitted by

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Graz, 22.05.2016……..

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# Zusammenfassung

**Einleitung**: Akute myeloische Leukämie (AML) ist eine heterogene maligne Erkrankung, die durch eine Veränderung der hämatopoetischen Stamm- und Vorläuferzellen ensteht. Zurzeit besteht die potentiell kurative Therapie aus einer Induktion mit Cytarabin und Anthrazyklinen, gefolgt von einer Postremissionstherapie. Bei zweiterem kann unter Miteinbeziehung von leukämie- und patientenspezifischen Faktoren entweder eine allogene Stammzelltransplantation oder eine konventionelle chemotherapeutische Konsolidierungstherapie durchgeführt werden. Jedoch bleiben die Fragen offen, was dabei die optimale Cytarabin-Dosis ist und wie viele Therapiezyklen optimalerweise appliziert werden. Dieser systematische Review inklusive Meta-Analyse untersucht den Vergleich der verschiedenen Cytarabin Dosen (hoch / mittel / niedrig), die optimale Anzahl an Konsolidierungszyklen, sowie den Vergleich zwischen Mono- und Kombinationstherapie in Bezug auf jüngere (≤65 Jahre) und ältere PatientInnen (>65 Jahre).

**Methodik:** Um diese Forschungsfrage zu klären, wurde ein systematischer Review, der eine Meta-Analyse inkludiert, laut PRISMA Statement durchgeführt. Die Datenbanken von Pubmed und Cochrane Library im Zeitraum von 1990 bis 2014, sowie die Archive der „American Society of Hematology“ und die „NCCN Guidelines for AML“ wurden durchsucht. Originalarbeiten, die randomisierte Studien mit mindestens 100 PatienInnen unter konventioneller Konsolidierungstherapie einschlossen, wurden ausgewählt. Die relevanten Daten wurden extrahiert, beschrieben und in die Meta-Analyse inkludiert.

**Ergebnisse**: Von 38,814 potentiell relevanten Arbeiten wurden schließlich 18 Studien, die unsere Einschlusskriterien erfüllten und insgesamt 14,572 PatientInnen umfassten, in diesen systematischen Review eingeschlossen. Fünfzehn der Studien konnten für die primäre Meta-Analyse von PatientInnen unter 65 Jahren analysiert werden. Der Vergleich von niedriger/mittlerer Dosis mit einer hohen Dosis von Cytarabin zeigte einen Hinweis darauf, dass das Disease Free Survival (DFS) durch eine Hochdosistherapie sich verbessern kann (HR 0.90 [0.80; 1.02]), aber es war deutlich weniger ausgeprägt für das Overall Survival (OS) (HR 0.96 [0.87; 1.06]). Unsere Ergebnisse ermittelten keine signifikanten Unterschiede zwischen Langzeit- und Kurzzeittherapie in Bezug auf OS (HR 1.06 [0.89; 1.25]), jedoch fand sich ein signifikantes Ergebnis, dass weniger Therapiezyklen zu einem schlechter DFS führen können (HR 1.16 [1.03; 1.29]). Auch zeigte sich kein signifikanter Unterschied, ob Mono- oder Kombinationstherapie besser ist, sowohl beim DFS (HR 1.02 [0.93; 1.11]) als auch beim OS (HR 1.08 [0.97; 1.20]).

**Schlussfolgerung**: Dieser systematische Review inklusive Meta-Analyse demonstriert, dass Hochdosis Cytarabin das DFS, jedoch nicht das OS, bei einer konventionellen Konsolidierungstherapie für PatientInnen mit AML verbessern kann. In Bezug auf die Therapiezyklen konnten wir zeigen, dass eine vermehrte Anzahl an Zyklen mit Chemotherapie das DFS im Gegensatz zum OS erhöhen kann. Es gab keinen Vorteil, wenn man weitere Chemotherapeutika zur Cytarabin Basistherapie hinzufügte. Eine weitere Auswertung wird diese Parameter in Anbetracht bestimmter AML Subgruppen, vor allem in Bezug auf zytogenetische Anomalien, untersuchen.

# Abstract

**Background:** Acute myeloid leukemia (AML) is a heterogenous malignancy caused by transformation of hematopoietic stem and precursor cells. Current therapies applied with curative intention consist of induction using cytarabine and anthracycline followed by post-remission treatment. The latter can be either allogeneic stem cell transplantation (HSCT) or conventional consolidation chemotherapy according to leukemia and patient-related factors. However, the question of the optimal cytarabine dose and number of treatment cycles remains open. This systematic review and meta-analysis aims to investigate the comparison of the different doses of cytarabine (high / intermediate / low), the optimal number of consolidation cycles and whether mono- or combination therapy is of advantage with respect to younger patients (≤65 years) and elderly patients (>65 years).

**Methods:** To investigate this research question, a systematic review including a meta-analysis was performed according to the PRISMA Statement. The databases of Pubmed and Cochrane Library from 1990 to 2014 as well as the archives of the American Society of Hematology and the NCCN guidelines for AML were screened. Original articles reporting randomized clinical trials that at least included 100 patients undergoing conventional consolidation therapy were identified. Relevant data were extracted, described and processed by a meta-analysis.

**Results**: Of 38,814 potentially relevant articles, 18 were finally included in this systematic review fulfilling our inclusion critera reporting on a total of 14,572 patients with AML. Fifteen matched to the primary goal of the systematic review and meta-analysis of patients aged 65 years and younger. The comparison of low/intermediate versus high dose cytarabine indicated that disease free survival (DFS) may be improved by high dose administration (HR 0.90 [0.80; 1.02]) but was less pronounced for overall survival (OS) (HR 0.96 [0.87; 1.06]). Our results did not show any significant differences between long term and short term therapy with respect to OS (HR 1.06 [0.89; 1.25]) either, but there was a significant result that less cycles may lead to worse DFS (HR 1.16 [1.03; 1.29]). Evaluating whether monotherapy or combination therapy is of advantage, no significant results could be found for both, DFS (HR 1.02 [0.93; 1.11]) and OS (HR 1.08 [0.97; 1.20]).

**Conclusion**: This systematic review and meta-analysis demonstrate that high dose cytarabine may improve DFS but not OS used as conventional consolidation treatment for patients with AML.. Concerning the number of cycles, we could show that an increased number of consolidation chemotherapy cycles enhance DFS but not OS. There was no benefit in adding other chemotherapy drugs to the cytarabine treatment schedule. Further work will focus on assessing these parameters in certain AML subgroups, most importantly those based on cytogenetic aberrations.

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# 1 Introduction

## *1.1 Acute Myeloid Leukemia*

### 1.1.1 Definition and basic information of AML

Acute myeloid leukemia, in short AML, is a hematological malignancy, which is characterized by infiltration of the bone marrow, blood, and other tissues by proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated blast cells of the hematopoietic system (Döhner, Weisdorf & Bloomfield 2015). The significant amount of those blast cells of the myeloid lineage that leads to the diagnosis of AML can be encountered by 20% and more, except for AML with t(15;17), t(8;21), inv(16) or t(16;16), and some cases of erythroleukemia (Döhner et al. 2010). As the European LeukemiaNet expert panel mentions, myeloblasts, monoblasts as well as megakaryocytic blasts are included in the blast count. They also state that in AML with monocytic or myelomonocytic differentiation, monoblasts and promonocytes, but not abnormal monocytes, can be counted as blast equivalents, whereas erythroblasts are not counted as blasts except in case of pure erythroid leukemia, which is very rare. Acute myeloid leukemia is typical of a rapid proliferation in neoplastic clones mainly in bone marrow or peripheral blood and a following arrest in their growth.

With an incidence of 3 – 4 per 100,000 men and women per year AML can be considered as the most common acute leukemia in adults (Schlenk 2014). About 18,300 new cases occur in Europe each year (Smith et al. 2004b) and around 12,000 new patients are diagnosed with acute myeloid leukemia in the United States annually (Juliusson et al. 2012). The generic patient population affected with this sort of cancer can be observed with a median age at presentation that has been steadily increasing over the past few decades and now is approaching around 70 years (Rowe 2009). Particular statistical analyses were carried out within the framework of the Surveillance, Epidemiology, and End Results (SEER) paper, which have pointed out the global differences among the incidence of AML: among males in the United States the incidence is higher than

in Sweden for all age groups and particularly higher in men older than 50 years and women than 75 years, whereas the United Kingdom incidence appears to be lower (Juliusson et al. 2012). The explanation for these differences is still unknown but has a big potential to be a subject of further study.

### 1.1.2 Symptoms

The clinical presentation of acute myeloid leukemia is determined by the increasing hematopoietic insufficiency due to the infiltration of the bone marrow. The normal bone marrow is replaced with malignant leukemic blast cells which leads to decrease of the normal cell lines, like platelets, red and white blood cells. In further consequence patients suffer from symptoms of anaemia like primarily paleness, fatigue and reduced physical performance, as well as symptoms of neutropenia, such as an increased number of opportunistic bacterial infections, especially in the pharynx, the skin or systemic mycosis. Besides, there can be thrombopenia symptoms, for example petechiae, easy bruising and bleeding or epistaxis (Smith et al. 2004b). The bleeding tendency is also caused by disseminated intravascular coagulation and hyper fibrinolysis. In case of exceeding leucocytosis, patients with hematopoietic failure can sustain a leukostasis, which causes hypoxia, pulmonary opacities, retinal bleedings and neurological symptoms. The state of a leukostasis constitutes a severe hematological emergency and can be treated with immediate decrease of leucocytes by chemotherapy or leukapharesis. The opportunistic infections mentioned before are still one of the most common reasons for death in patients with hematological malignancies. Besides hematological patients present general symptoms, like loss of appetite, bone and joint pain and unwanted weight loss. In addition, most common extramedullary infiltrations occur in skin infiltration, meningeosis leukaemica, gingival hyperplasia, as well as infiltration of the spleen and liver. Without any therapy this disease constitutes a life-threatening condition, leading to death within a few months.

### 1.1.3 Diagnosis

The first step of the diagnostic process, after performing standard physical examination and basic routine laboratory reports, is to examine the bone marrow aspirate and further trephine biopsy in patients with a dry tap (punctio sicca) (Döhner et al. 2010).

The principle method for the diagnosis of AML and its further sub classification consists of light microscopy supplemented by cytochemistry. Therefore the bone marrow and peripheral blood samples are stained with Wright Giemsa or May-Grünwald-Giemsa (MGG) to distinguish blast cells from normal blood cells. The pathological myeloblasts appear as large cells with a low nuclear-cytoplasmic ratio, a finely stippled nuclear chromatin pattern and frequently multiple prominent nucleoli. Approximately over one third of cases present so-called Auer rods in the cytoplasmic azurophilic granules, which are irregular cell organelles containing lysosomal enzymes and point out an abnormal maturation as well as proliferation of the cell.

Another mandatory diagnostic tool is immunophenotyping. This procedure is essential to identify the leukemic origin, whether the blast cells are myeloid or lymphoid, or when cytochemistry is uninformative or equivocal. Two possibilities to analyse immunophenotypically is by flow cytometry (commonly 3- to 4 colour) or by immunocytochemistry. Moreover, immunophenotyping assists to identify evidence of monocytic, erythroid, or megakaryocytic differentiation (Swerdlow et al. 2008). According to the European LeukemiaNet expert panel some AMLs with recurrent genetic abnormalities are associated with characteristic immunophenotyping. For instance, AMLs with t(8;21) frequently express the lymphoid markers CD19, CD7 or CD56; AMLs with inv(16) tend to express the T lineage-associated marker CD2, whereas AMLs with NPM1 mutation typically show high CD33 but low CD43 expression (Kita et al. 1992, Baer et al. 1997, Adriaansen et al. 1993).

Last, but not least, there is another mandatory diagnostic instrument, namely, cytogenetics: on the one hand, there is conventional cytogenetic analysis and on the other hand, there is molecular cytogenetics. Cytogenetics is definitely one of the most important criteria in diagnosis as it leads to the main classification of the different types of AML and in further consequence to the different risk groups of patients according to the chromosomal abnormalities and karyotypic changes. One of the most common numerical cytogenetic abnormalities that can be seen is trisomy 8, for example. Furthermore, typical structural chromosomal translocations are: t(8;21), t(15;17), inv(16), abnormalities of 11q23, t(6:9), inv(3) or t(8;16) (Smith et al. 2004b). According to the European LeukemiaNet expert panel a minimum of 20 metaphase cells analysed from bone marrow is considered mandatory to establish the diagnosis of a normal karyotype, and recommended to define an abnormal karyotype. This investigation can be diagnosed from blood specimens. Concerning molecular cytogenetics, cell pellets fixed in methanol/acetic acid should be stored. In case conventional cytogenetic analysis fails, fluorescence in situ hybridization (FISH) is an option to detect gene rearrangements, for instance RUNX1-RUNX1T1 or loss of chromosome 5q and 7q material (Fröhling et al. 2002).

Furthermore, molecular diagnosis should especially focus on analysis of DNA and RNA extraction by reverse transcriptase-polymerase chain reaction (RT-PCR) for the recurring gene fusions NPM1, CEBPA and FLT3 at least among patients with cytogenetically normal AML (CN-AML) who will receive treatment other than low-dose chemotherapy or best supportive care (Döhner et al. 2010). The most common recurrent gene mutations are NPM1 with a frequency of 25-35% of patients, CEBPA in 6-10% of patients, RUNX1 in 5-15% of patients and FLT3-ITD mutation in approximately 20% of patients. Further frequent mutations can be observed in KIT, TET2, and NRAS as well as in DNMT3A (Döhner, Weisdorf & Bloomfield 2015).

### 1.1.4 WHO Classification

Acute myeloid leukemia is classified according to the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues and this classification was last updated in 2008 (Swerdlow et al. 2008). Previously the main classification was constituted of the French-American-British (FAB) classification of Bennett et al in 1985 (*Table 1*), which divides AML into 8 subgroups, varying from M0 to M7 type according to appearance of cancer cells under the microscope and cytochemistry. Swerdlow et al. speak of major categories of the current WHO classification including AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related AML, and AML not otherwise specified. This categorization has been more established because it provides more reliable prognostic information than the FAB classification. As the number of acute leukemias categorized by cytogenetics or molecular genetic abnormalities is steadily increasing, the WHO was forced to reorganize their classification in different new subgroups. They define acute myeloid leukemia by a leukemic blast count of 20% or higher in the bone marrow and/or in the peripheral blood. Morphology and cytochemistry, but also immunological as well as cytogenetic analyses count as the mandatory diagnostic tools (Swerdlow et al. 2008).

The subgroup “AML with recurrent genetic abnormalities” includes a number of primary AML entities. In this subgroup the focus was on mutations in molecular genetic lesions, like for example RUNX1-RUNX1T1, MLLT3-MLL or DEK-NUP214. The mutation in RUNX1-RUNX1T1 and CBFB-MYH11 are considered as acute myeloid leukemia regardless of bone marrow blast counts. Two new provisional entities should be mentioned in this category as well, namely, NPM1 and CEBPA. The former subgroup termed “AML with multilineage dysplasia” is now renamed “AML with myelodysplasia-related changes”. Patients who belong to this entity are typical of prior myelodysplastic syndrome or myeloproliferative disease that developed into AML. “Therapy-related myeloid neoplasms” is still a distinct subgroup. But it is very difficult to reproduce which therapies patients had before, so a division according to the type of previous

therapy is often not feasible (Sill et al. 2011). Myeloid leukemia associated with Down syndrome is now listed as a distinct entity. The last main category of the WHO classification is “AML not otherwise specified”. A detailed list of all relevant AML subgroups is quoted in *Table 2*. (Döhner et al. 2010)

***Table 1. French-American-British (FAB) classification of AML***

| **Type** | **Name (% of cases)** | **Cytogenetics** |
|---|---|---|
| M0 | Acute myeloblastic leukemia with minimal differentiation (3%) | inv(3q26) and t(3;3) |
| M1 | Acute myeloblastic leukemia, without maturation (15-20%) | |
| M2 | Acute myeloblastic leukemia, with maturation (25-30%) | t(8;21), t(6;9) |
| M3 | Acute promyelocytic leukemia (5-10%) | t(15;17), t(11;17), t(5;17) |
| M4<br>M4eo | Acute myelomonocytic leukemia (20%)<br>Acute myelomonocytic leukemia with abnormal eosinophils (5-10%) | 11q23, inv(3q26) and t(3;3), t(6;9)<br>inv(16), t(16;16) |
| M5 | Acute monocytic leukemia (2-9%) | 11q23, t(8;16) |
| M6 | Erythroleukemia (3-5%) | |
| M7 | Acute megakaryocytic leukemia (3-12%) | t(1;22) |

***Table 2. WHO classification of AML 2008***

- **Acute myeloid leukemia with recurrent genetic abnormalities**
  - AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
  - APL with t(15;17)(q22;q12); PML-RARA
  - AML with t(9;11)(p22;q23); MLLT3-MLL
  - AML with t(6;9)(p23;q34); DEK-NUP214
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
  - AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
  - Provisional entity: AML with mutated *NPM1*
  - Provisional entity: AML with mutated *CEBPA*
- **Acute myeloid leukemia with myelodysplasia-related changes**
- **Therapy-related myeloid neoplasms**
- **Acute myeloid leukemia, not otherwise specified (NOS)**
  - Acute myeloid leukemia with minimal differentiation
  - Acute myeloid leukemia without maturation
  - Acute myeloid leukemia with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)
- **Myeloid sarcoma**
- **Myeloid proliferations related to Down syndrome**
  - Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder)
  - Myeloid leukemia associated with Down syndrome
- **Blastic plasmacytoid dendritic cell neoplasm**
- **Acute leukemias of ambiguous lineage**
  - Acute undifferentiated leukemia
  - Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1
  - Mixed phenotype acute leukemia with t(v;11q23); MLL rearranged
  - Mixed phenotype acute leukemia, B/myeloid, NOS
  - Mixed phenotype acute leukemia, T/myeloid, NOS
  - Provisional entity: Natural killer (NK)–cell lymphoblastic leukemia/lymphoma

### 1.1.5 Prognostic Index

The prognostic index results from mainly two sub categories: on the one hand, there are the patient-related factors and on the other hand, there are AML-related factors. These various prognostic factors can assist the clinicians to predict the risk of disease recurrence as well as the most probable progression of disease and they are important tools guiding treatment decisions.

Age is one of the most important patient-related prognostic factors within this leukemic disease leading to a major impact on the management and outcome for patients with AML (Appelbaum et al. 2006, Juliusson et al. 2009). According to the SEER Medicare database over half of patients diagnosed with acute myeloid leukemia are 65 years or older. Other relevant patient-related prognostic factors are comorbidities, which are mostly related to a higher age, and poor performance status (Appelbaum et al. 2006). Medeiros et al showed in a retrospective study with a patient population of 8336 patients, where 40% received chemotherapy within 3 months of diagnosis and 60% did not, that treated patients suffered 33% less from risk of death compared to untreated patients. In addition they observed increasing age, increasing comorbidity score (measured by the National Cancer Institute comorbidity index) and poor performance status were significantly associated with higher mortality risks (Medeiros et al. 2015). Appelbaum et al reported AML as less proliferative disease with lower white blood cell counts and peripheral blast percentages in the elderly, however, the range of cytogenetic abnormalities changed with a much higher incidence of abnormalities involving chromosomes 5, 7, and 17 and a lower incidence of the translocations related to favourable treatment outcomes. The reasons for the association of increasing age with these specific chromosomal abnormalities are still in the dark and could be further object of study (Appelbaum et al. 2006).

According to Döhner et al AML-related prognostic factors include white blood count (WBC), existence of prior MDS, previous cytotoxic therapy for another disease, and cytogenetic and molecular genetic changes in the leukemic cells at

diagnosis. Less common prognostic factors are splenomegaly and elevated serum lactate dehydrogenase (LDH), however, the significance of a prognostic factor always depends on the type of therapy given to a patient (Döhner et al. 2010). One of the strongest disease-related factors is the cytogenetic aspect (Mrozek, Heerema & Bloomfield 2004, Grimwade 2001). As per advice of the British Journal of Haematology guidelines cytogenetic examination at diagnosis allows patients to be divided into three groups with relapse risks varying from 35% to 76%. The standardized categorization results into the three main groups: favourable risk, intermediate risk (with respect to molecular genetics further division into intermediate-I and intermediate-II) and adverse risk (*Table 3*) (Grimwade et al. 1998, Byrd et al. 2002). These risk groups have a remarkable effect on deciding the therapy process. Even though a number of rarer cytogenetic abnormalities, for instance del(7q) or isolated trisomy 8, remain uncertain in categorization, a complex karyotype, which occurs in 10% to 12% of patients, has consistently been associated with a poor outcome or rather adverse risk (Mrozek 2008). Döhner et al define complex karyotype 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). AML with t(9;11), t(v;11), inv(3) or t(3;3), as well as t(6;9) should be excluded, because these groups build up separate entities according to the WHO classification (Vardiman et al. 2009). It should be noted that for elderly patients several other risk classifications have been proposed as the incidences of the different cytogenetic abnormalities vary from the incidences in younger patients (Leith et al. 1997, Bacher et al. 2005). The research group of Mrozek et al points out the differences in distribution of the genetic groups in younger and older adults, where in younger patients 41% belong to the favourable risk group, 18% intermediate-I, 19% intermediate-II and 22% adverse risk group, but in older patients only 20% allocated to the favourable risk group, 19% intermediate-I, 30% intermediate-II and 31% adverse risk group. Basically patients with core-binding factor AML (CBF-AML) with t(8;21)(q22;q22) or inv(16)(p13.1q22)/t(16;16)(p13.1;q22) can be classified in the favourable risk group, those with cytogenetically normal AML (CN-AML) in the intermediate risk group, and those with complex karyotype belong to the adverse risk group (Mrozek et al. 2012).

Last but not least molecular genetics figure prominently in AML-related prognostic factors. Molecular genetic lesions have been especially important for patients with CN-AML. The intermediate risk group mentioned before in aspect of cytogenetics can be separated into two subgroups with respect to the inclusion of molecular analysis, namely intermediate-I and intermediate-II (Mrozek et al. 2012) According to the European LeukemiaNet expert panel prognostic significance within CN-AML has been shown for mutations in the NPM1, CEBPA and FLT3 genes alone or in combination in younger adults (Schlenk 2014).

Even though remaining an active field of investigation, monitoring of minimal residual disease by RT-PCR detecting leukemia-specific targets or by flow cytometry is potentially useful to assess response to therapy according to risk stratification and guidance of postremission therapy (Freeman, Jovanovic & Grimwade 2008).

***Table 3. Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data***

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

### 1.1.6 Treatment

The treatment of acute myeloid leukemia consists of a therapy strategy which has not changed substantially for more than 30 years (Döhner, Weisdorf & Bloomfield 2015). The main cornerstones of treating AML are divided into three options: first of all there is the curative intention consisting of intensive chemotherapy with or without stem cell transplantation. This intensive chemotherapy involves induction therapy to potentially defeat the leukemic cells and postremission therapy, separated into consolidation therapy and maintenance therapy, to reduce the risk of relapse of acute myeloid leukemia. The second option for an AML patient is to get treated with demethylating low dose agents such as azacitidine or decitabine. On a final note, for patients with very bad prognostic factors, best supportive care appears to be the best treatment option in their given situation.

#### 1.1.6.1 Response criteria

To understand how patients with acute myeloid leukemia respond to their treatment, it is important to define the different states of their health status after the accomplishment of their therapy. Basically, a response assessment of each patient is carried out after the conventional induction therapy with three days of an anthracycline and seven days of cytarabine (“3+7”) or therapies of comparable intensity between day 21 and 28 after the start of treatment (Döhner et al. 2010).

The therapeutic target is to reach a complete remission so that the patient is amenable for further treatment to avoid relapse of the disease. ***Complete remission (CR)*** is defined as a state where the bone marrow blasts occur less than 5%, absence of blasts with Auer-rods, absence of extramedullary disease, absolute neutrophil count >1.0 x $10^9$/l, platelet count >100 x $10^9$/l and independence of red cell transfusions. A ***complete remission with incomplete recovery*** includes all CR criteria except for residual neutropenia (<1.0 x $10^9$/l) or thrombocytopenia (<100 x $10^9$/l). ***Partial remission (PR)*** means the patient has a bone marrow blast count between 5% and 25% or rather a bone marrow blast

decrease of at least 50%. Furthermore, there is a ***cytogenetic CR (CRc)*** defined as a reversion to a normal karyotype at the time of morphologic CR or CRi in cases with an abnormal karyotype at the time of diagnosis (this has to be based on the evaluation of at least 20 metaphase cells from bone marrow). Finally, patients can suffer from a ***resistant disease (RD)*** which means a failure to achieve CR, CRi or PR. (Cheson et al. 2003)

### 1.1.6.2 AML specific treatment options

#### *1.1.6.2.1 Cytarabine*

Cytarabine or rather chemically 1-β-D-arabinofuranosylcytosine is an antineoplastic analog of pyrimidine that is implemented into the DNA and in further consequence it leads to the replication of specific DNA segments. In addition, cytarabine inhibits the synthesis of glycoproteins and glycolipids, changes the membrane structure and finally, it induces the synthesis of ceramids and transcriptions factors. Though the exact mechanism of action of cytarabine has remained an open question. It is expected to be effective by its cytotoxicity and induction of apoptosis. This odorless, white to off-white crystalline powder which is freely soluble in water and slightly soluble in alcohol and in chloroform, can be administered intravenous, intrathecal or subcutaneous to the patient (the application depends very much on the given dose of cytarabine). (Bashir et al. 2015)

#### *1.1.6.2.2 Demethylating agents*

The demethylating agents azacitidine and decitabine appear to be two promising alternative options for AML patients who are not amenable for intensive therapy due to their poor performance status and / or high age. Azacitidine and decitabine are implemented as DNA-methyltransferase inhibitors into the DNA which results in a demethylation of the DNA, differentiation of the cell and finally, a p53-independent apoptosis takes place (Montalban-Bravo, Garcia-Manero 2015). These cytosine analogs have been approved for the treatment of high-risk MDS by

the Food and Drug Administration (FDA) and the European Medicine Agency (EMEA) (Döhner et al. 2010). According to a phase 3 randomized trial, azacitidine prolonged overall survival compared with conventional treatment in patients with intermediate-II or high-risk MDS, although one-third of these patients were classified to have AML according to the WHO criteria (blast counts between 20% and 30%). Therefore, based on these study results, azacitidine has been approved for AML in elderly patients with a blast count varying from 20% to 30% (Fenaux et al. 2009, Fenaux et al. 2010). The recommended dose of decitabine is 20mg/$m^2$ per day for 5 days every 4 weeks and for azacitidine, it is 75mg/$m^2$ for 7 days, also every 4 weeks (Al-Ali, Jaekel & Niederwieser 2014).

#### *1.1.6.2.3 Molecularly targeted therapy*

In the past few years, there was a rapidly increasing research activity about the molecular pathogenesis of acute myeloid leukemia. Genetic alterations, like for instance FLT3, KIT, RAS, NPM1 and many more, have been verifiably associated with AML. With this knowledge about the genetic modifications that are linked to this sort of cancer, latest research was able to develop some promising molecularly targeted therapy.

A quite recent area of induction therapy research concerns the evaluation of ***gemtuzumab ozogamicin (GO)*** given with conventional chemotherapy. This humanized anti-CD33 antibody is linked to the cytotoxic agent calicheamicin that leads to inhibition of DNA synthesis and finally induces apoptosis (Zein et al. 1988, Sievers et al. 2001, van Der Velden et al. 2001). However, gemtuzumab ozogamicin is only approved in the United States and Japan so far, but not in Europe, for patients with relapsed AML and not for standard induction therapy. Therefore, GO appears to be an interesting field for further study.

In addition, several ***FLT3-selective tyrosine kinase inhibitors*** (e.g. midostaurin (Weisberg et al. 2002) or lestaurtinib (Levis et al. 2002)) have been tested positively for in vitro cytotoxicity to leukemic blast cells. FLT3-inhibitors have demonstrated promising response rates in treatment of, especially relapsed,

AML with FLT3 mutations in pilot studies and in further consequence, randomized phase 3 trials are planned to evaluate the targeted therapy method as frontline therapy in younger adults (Stone et al. 2005, Smith et al. 2004a)

### 1.1.6.3 Induction therapy

The standard induction therapy remains continuous-infusion cytarabine with an anthracycline and its aim is to produce the maximal tumour destruction in order to restore normal haemopoiesis. This conventional induction therapy is given in the so-called “3+7 combination” – that means the patient is given 3 days of an anthracycline and 7 days of cytarabine (Schlenk 2014). Induction therapy is often put on a level with potentially curative AML therapy. It should be noted that doses vary from age group to age group. Younger patients (mostly defined as patients less than 65 years) receive a cytarabine dose of 100-200mg/$m^2$ continuously with daunorubicin in a dose of 60mg/$m^2$ in short infusion in the first cycle of induction (Burnett et al. 2013). If the first cycle does not lead to complete remission, an attempt of a second induction cycle with cytarabine 100-200mg/$m^2$ continuously with a dose of 45mg/$m^2$ of daunorubicin in short infusion can be given (Smith et al. 2004b). Smith et al state from analysing several international studies that daunorubicin turned out to be the best anthracycline for combination with cytarabine as it is less toxic than doxorubicin and idarubicin. Even though idarubicin is claimed to be superior to daunorubicin in patients less than 50 years by achieving more cases of complete remission after one course and the duration of this remission is longer, it is more myelosuppressive and hepatotoxic (Smith et al. 2004b). Furthermore, several other studies compared the efficacy of daunorubicin at a dose of 45-60 mg/m2 with other anthracyclines, such as idarubicin (Berman et al. 1991, Wiernik et al. 1992, Vogler et al. 1992) or aclarubicin (Hansen et al. 1991), with amsacrine (Berman et al. 1989), or with mitoxantrone (Arlin et al. 1990) and they have shown that none of those agents is superior to daunorubicin at equivalent doses.

However, treatment of acute myeloid leukemia in elderly is much more complicated due to their poor performance status and/or comorbidities, but also

genetic complications. Patients over 65 years can be given a “3+7” induction therapy of cytarabine continuously in a dose of 100-200mg/$m^2$ combined with daunorubicin 45mg/$m^2$ as short infusion (Löwenberg et al. 2009) or idarubicin 12mg/$m^2$ also as short infusion (Berman et al. 1991). According to Smith et al there has been interest in adding a third drug to an anthracycline and cytarabine combination, for example 6-thioguanine (6-TG) or etoposide, but these attempts failed to show significant improvements in remission induction, remission duration or overall survival. With the “3+7 conventional induction regimen” complete remission is achieved in 60% to 80% in younger adults, while older patients with good performance status and little comorbidity reach complete remission in about only maximum 50% (Sekeres et al. 2009). Therefore, for elderly patients with a poor performance status and / or many comorbidities a low dose cytarabine treatment can be targeted as an alternative induction therapy rather than best supportive care (Montalban-Bravo, Garcia-Manero 2015).

As mentioned before, it is mandatory to conduct a response assessment after conventional induction therapy with 3 days of an anthracycline and 7 days of cytarabine. This evaluation should be performed between day 21 and day 28 after start of therapy (Döhner et al. 2010). The European LeukemiaNet expert panel recommends an early response assessment in investigational studies made at 7 to 10 days after chemotherapy to assess the antileukemic efficacy of the chemotherapy. According to the National Cancer Institute criteria the relevant clinical definition of complete remission for the response assessment is a bone marrow blast amount under 5%, absence of blast cells with Auer rods and extramedullary infiltrations, granulocytes more than $1.5x10^9$/l, a platelet count of more than $100x10^9$/l and no need of red cell transfusions (Farag et al. 2005, Cheson et al. 2003).

### 1.1.6.4 Postremission therapy

Postremission therapy can be seen as continuation therapy for patients in complete remission and it has become standard practice as the median remission duration without postremission therapy lasts only 4 months (Smith et al. 2004b).

According to Rowe et al the need for any postremission therapy was established in the landmark study conducted by the ECOG in 1983 which prospectively included an observation arm as part of postremission strategy. But it should be noted that clearly, this study could not have been carried out according to the ethical policies nowadays (Rowe 2009). Various types of postremission therapy strategies are available including intensive conventional chemotherapy, prolonged maintenance therapy, and high-dose treatment followed by autologous or allogeneic haematopoietic stem cell transplantation (HSCT) (Estey, Döhner 2006, Löwenberg, Griffin & Tallman 2003).

It is important to mention the relevance of the cytogenetic and molecular genetic risk groups as two of the most influencing factors for the decision on continuation therapy.

#### *1.1.6.4.1 Consolidation therapy*

According to Döhner et al consolidation with intensive chemotherapy is the preferred postremission treatment in adults of 60 years and younger, but with respect to the individual risk group. The most exact appropriate dose and number of therapy cycles remains objective of further research and study. A landmark study performed by Cancer and Leukemia Group B (CALGB), published in 1994 and still valid, pointed out that 4 cycles of high-dose cytarabine with a dose of 3g/$m^2$ per 12 hours on days 1, 3 and 5 are superior to 4 courses of intermediate (400mg/$m^2$ continuous infusion on days 1 to 5) or standard dose (100mg/$m^2$ continuous infusion) of cytarabine (Mayer et al. 1994). Even though other study groups, for example the French ALFA-group, showed no beneficial effect on survival end points with the treatment schedule of CALGB, this kind of consolidation therapy has been established (Thomas et al. 2011). Patients of CALGB study also received 4 courses of monthly maintenance therapy, whereas the positive effect of the chemotherapy dose intensification was restricted to patients with CBF AML and, to a lesser extent, to patients with CN-AML (Bloomfield et al. 1998, Elonen et al. 1998).

Patients with a favourable genetic risk profile tend to benefit significantly from intensive chemotherapy as cure rates of 60% to 70% for this risk group are reported (Döhner, Weisdorf & Bloomfield 2015). However, randomized trials comparing single-agent cytarabine therapy with multi-agent chemotherapy in adults younger than 60 years have not shown a relevant difference in survival data (Schlenk 2014). A study by the German-Austrian AML Study Group (AMLSG) stated that favourable risk patients could even have a bigger disadvantage rather than a benefit from allogeneic HSCT, even though in rare individual cases, if the acute myeloid leukemia is refractory, high dose cytarabine in combination with stem cell transplantation could be an alternative treatment option (Schlenk et al. 2008).

In case a patient belongs to the intermediate-I or intermediate-II risk group, which represents the majority of AML patients, repetitive cycles of high dose cytarabine are a common therapy strategy, even though the outcome remains unsatisfactory (Cornelissen et al. 2007, Koreth et al. 2009, Meijer, Cornelissen 2008). For patients with a high risk of relapse, therefore, allogeneic HSCT is an attractive alternative with respect to the individual situation of each concerning patient.

Patients with an adverse genetic risk profile are the primary candidates for allogeneic HSCT as their outcome with conventional chemotherapy is dismal (Koreth et al. 2009, Bloomfield et al. 1998). The allogeneic haematopoietic stem cell transplantation from a matched related donor is currently considered the treatment of choice for patients with unfavourable cytogenetics in complete remission (Suciu et al. 2003, Basara et al. 2009). However, the outcome after allogeneic HSCT from fully matched unrelated donors seems to be not much varying from the outcome after allogeneic HSCT from matched related donors (Tallman et al. 2007). Basically, the combination of allogeneic HSCT with intensive postremission therapy is considered to have the strongest anti-leukemic effect in acute myeloid leukemia (Koreth et al. 2009). Unfortunately, the treatment option of HSCT is limited by non-relapse, treatment-related mortality (TRM). Thus, the decision whether a patient should receive stem cell transplantation is up to the risk of relapse after intensive chemotherapy versus allogeneic HSCT, TRM of

allogeneic HSCT and last but not least patient and transplant specific parameters such as comorbidity, donor type (HLA-matched related, unrelated and haploidentical donors, umbilical cord stem cell grafts), performance status and age (Cornelissen et al. 2015). At the end, it should be noted that TRM may vary between less than 15% and up to 50% (Harousseau et al. 1997, Cassileth et al. 1998, Burnett 2002).

Autologous haematopoietic stem cell transplantation is recommended as an alternative option for postremission treatment in patients with favourable or intermediate risk profiles, whereas it is not recommended in high-risk patients (Slovak et al. 2000, Schlenk et al. 2003, Breems, Löwenberg 2007). For the most part, autologous HSCT appears to be at least as good as conventional chemotherapy in specific subsets of AML (Breems, Löwenberg 2007).

#### *1.1.6.4.2 Maintenance Therapy*

Maintenance therapy in acute myeloid leukemia appears to be typically less aggressive or rather intensive and myelosuppressive than standard consolidation treatment (Smith et al. 2004b). The European LeukemiaNet is mentioning studies that show no benefit at all on the one hand, and on the other hand studies that show a superior outcome on disease free survival data (Buchner et al. 2003). Still maintenance chemotherapy has not been generally established as a routine strategy outside of clinical trials for patients with AML (Döhner et al. 2010). Smith et al speak of a maintenance treatment schedule which usually consists of short courses of mostly subcutaneously given cytarabine combined with oral agents such as thiopurine or etoposide given for 2 to 3 years. However, the opinion is deeply divided on this issue.

### 1.1.6.5 Best supportive care

In case a patient has many negative or high-risk factors and his or her medical condition is as severe as chemotherapy, demethylating agents or stem cell transplant can not be administered, best supportive care constitutes the

treatment of choice. Best supportive care is defined as assisting treatment which is not disease-related and which can not lead to remission of the cancer.

Patients with acute myeloid leukemia often suffer from neutropenia within their course of disease exposing them to a severe risk of getting an infection that they could not handle on their own due to lack of their immune system. This is why fungal and antibiotic prophylaxis as well as antiviral treatment is very important. Invasive fungal infections are a major reason for morbidity and mortality in patients with prolonged neutropenia (Enoch, Ludlam & Brown 2006). Regarding the antibiotic prophylaxis, it is recommended to be given after chemotherapy, because at that point bacterial infections are a common and dangerous cause of morbidity and mortality in neutropenic patients (Leibovici et al. 2006).

In individual cases growth factors, like GM-CSF or G-CSF, can be considered. Even though numerous studies have shown that myeloid growth factors have positive effects, for example acceleration of neutrophil recovery by 2 to 5 days, reduction of antibiotic use, duration of fever or numbers of days spent in hospital, it is not recommended in general as it did not lead to have a significant benefit on survival (Zittoun et al. 1996, Stone et al. 1995, Dombret et al. 1995, Rowe et al. 1995).

Last but not least, transfusion support is a very important part of best supportive care. The introduction of platelet transfusions has led to a huge reduction of mortality from haemorrhage in AML (Freireich 2000). The American Society of Clinical Oncology guidelines recommend a threshold of $10 \times 10^9$/l for prophylactic platelet transfusions (Schiffer et al. 2001). According to the European LeukemiaNet expert panel, besides the platelet count, mucosal bleeding, infection, severe mucositis, and fever should be considered in the assessment of bleeding risk and should increase the transfusion threshold.

### 1.1.7 AML management in the elderly

Acute myeloid leukemia presents at all age groups, but it can be mainly seen as a disease of the elderly, especially as the median age increases steadily (varying from 66 to 70 years and older according to different studies) (Luger 2010). Over half of patients diagnosed with AML are 65 years or older, yet still the majority of 60% of elderly patients remain untreated in the United States (Medeiros et al. 2015). Older patients also tend to suffer more often from relapse of AML and mostly, have a lower survival rate (Tallman, Gilliland & Rowe 2005). The median survival of patients over 65 years with acute myeloid leukemia rests with 7.4 months and their five-year survival rate is only 10% (Montalban-Bravo, Garcia-Manero 2015).

Treatment management of acute myeloid leukemia has been established on clinical studies that are published based on data of younger patients. Thus, studies on older AML patients involve only few and highly selected patient populations. Most clinical trials elect older patients with higher performance status and less comorbidities which leads to a selection bias in the results. This is one of the main issues why there is no internationally agreed “standard treatment” for elderly patients.

The European LeukemiaNet expert panel basically divides older patients in two main age groups: patients 60 to 74 years and patients age 75 or older. Elderly are known to be more likely to suffer treatment related early death, to have more comorbidities, a worse performance status and a higher risk of relapse in disease. According to Döhner et al older age, per se, however, should not be a reason to withhold intensive therapy. Different studies have shown that remission induction chemotherapy provides better quality of life as well as longer overall survival than supportive care only (de Lima et al. 1997).

The same “3+7” induction therapy can be given to older adults (age group 60 to 74 years) as given to younger adults as long as their performance status is less than 2 and they have no severe comorbidities, wheras, patients 75 years and

older with performance status 2 or 3, suffering from comorbidities, or organ dysfunction should receive an alternative low dose induction (Burnett et al. 2007). The most suitable exact dose and cycles for induction as well as postremission treatment schedule remains despite several studies an open issue.

Wang et al presented an therapeutic approach for the consolidation treatment of older adults with AML. They state, in general, individuals without life-threatening organ failure or hyperleukocytosis may safely delay their therapy to allow an exact cytogenetic classification. This may lead to an overall better treatment outcome. Patients with good performance status and belonging to a favourable risk group should be offered intensive chemotherapy with cytarabine combined with anthracycline for 2 to 4 courses. Fit older patients with intermediate-risk AML may receive upfront intensive chemotherapy or hypomethylating therapy followed by the possibility of HSCT. Last but not least elderly with an adverse AML risk, who are unlikely to benefit from chemotherapy alone, should, as far as they reach CR, be referred as main candidates for allogeneic stem cell transplantation. It should be noted that any decision on treatment should be taken involving the older patients as for most of them AML will be a life-ending disease and so they should decide individually, with respect to quality of life and personal interest rather than a defined therapeutic algorithm, what kind of treatment schedule they want to go through.(Wang 2014)

Concerning the outcome data of elderly with acute myeloid leukemia, there are two main studies showing real world data. The first one is a data analysis of the Surveillance, Epidemiology and End Results (SEER) – Medicare database of the United States (Juliusson et al. 2009). Altogether, they analysed 8336 patients who met all study criteria, 40% of them received chemotherapy treatment within 3 months of diagnosis, the rest of them, 60%, did not receive any therapy. They state that increasing age, increasing comorbidity score and poor performance indicators were significantly associated with higher mortality risks. In their outcome data, with a median age of 66 years, it was clear that patients receiving intensive therapy were younger (mean age 73 vs. 78 and 81), were more likely male, were married and had less secondary AML as well as a lower comorbidity score compared to those receiving less intensive or no therapy. Patients treated with

intensive chemotherapy showed a 67% reduction in mortality. Among the treated patients there was only 8% of all patients undergoing HSCT therapy who also showed a 21% lower risk of death due to their therapy compared to elderly without any therapy. Medeiros et al believe these real-world data provide further support that age alone should not deter the use of guideline-recommended therapies particularly because of the high disparities in outcomes between treatment receipt and palliative care. However, it should be noted that the SEER Medicare data has several strengths on the one hand, as for instance the large patient population in their registry, but on the other hand, this analysis should be interpreted with caution due to the big amount of missing data like for example exact dose selection.(Medeiros et al. 2015)

The second important real world study that should be mentioned is the analysis of the Swedish Acute Leukemia Registry. Their investigations were based on 2767 adult patients over 16 years with non-APL AML, where the median age was 72 years. Juliusson et al compared patients receiving induction or palliation with or without chemotherapy. Patients with intensive chemotherapy showed higher CR rates (65% in patients with de Novo AML and 41% with secondary AML) as well as higher overall survival data compared to patients with less intensive treatment. (Juliusson et al. 2009)

## *1.2 Aim of the systematic review and meta-analysis*

As mentioned in the first part of the introduction acute myeloid leukemia is an aggressive malignancy of the hematopoietic system and a heterogeneous disorder with respect to clinical as well as genetic parameters. Current therapies applied with curative intention consist of induction therapy using cytarabine and an anthracycline and post-remission treatment. The latter is either allogeneic stem cell transplantation (HSCT) or conventional consolidation treatment in good-risk subtypes or patients not eligible for HSCT. However, the optimal number of conventional treatment cycles remains a matter of debate. The aim of this systematic review including a meta-analysis is to determine the optimal number of post-remission (consolidation) therapies in adult acute myeloid leukemia according to so far published randomised controlled trials. Here we propose a systematic review on this topic, which has not been published yet by other groups. The results may facilitate clinical patient management and give rise to further clinical trials. The outcome is equally important for male and female patients.

# 2 Material and methods

## *2.1 What is a systematic review?*

A systematic review can be defined as a quantitative or qualitative (or both involving) summary of evidence to a particular research question. Nowadays, there is an explosion in medical healthcare publishing so that it is difficult to keep up with primary research evidence for clinicians, therapists, healthcare managers and other people working in medical fields. Most of them have the problem of lack of time to go through all the mass of information provided online or in journals as well as they have other conflicts with their busy clinical or professional workload. At that point a systematic review helps to obtain up-to-date good quality information concerning the effectiveness, meaningfulness, feasibility and appropriateness of a specific healthcare matter. (Moher et al. 2009)

Systematic reviews are steadily more of importance and tend to replace the classic narrative reviews by their well-structured designing methods to package and transfer information that is understood and used in decision-making (Pearson et al. 2005). By comparison, a systematic review is much more detailed, based on a comprehensive plan and search strategy and it is trying to reduce bias by including all relevant studies on the research question, whereas a narrative review is mainly written in a descriptive style, has no systematic search of literature and last but not least it is often focused on the authors specific interest. This may cause selection and/or reporting bias and also lead to confusion of the reader as similar studies can have different results and conclusions that can not be replicated by the reader in most of the cases.

Signs of quality of a systematic review are a focused research question, a comprehensive literature search strategy, exactly defined inclusion and exclusion criteria, reproducible quality evaluation of the selected studies, examination of heterogeneity of the selected studies and last but not least the meta-analysis of the results, if possible.

### 2.1.1 The definition and role of meta-analysis

A meta-analysis is a statistical summary which synthesizes the data of the results of a systematic review into a single quantitative estimate or summary effect size and it depends on the quality or rather applicability of included studies (Petticrew, Roberts 2008). This statistical analysis, which is not mandatory for each systematic review, offers a certain security against bias and supports transparency as well as accuracy of the implemented structured review. It should be noted that a meta-analysis depends strongly on sensitivity analysis and the rigor of the carried out systematic review.

## 2.2 The PRISMA Statement

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Statement is a further development of the previously existing QUOROM (Quality of Reporting of Meta-analysis) guideline that includes a 27-item checklist and a four-phase flow diagram to support transparent reporting of a systematic review. (Moher et al. 2009)

### 2.2.1 Historical development

The QUOROM Statement was developed in 1996 and published in 1999 and its aim was to represent a reporting guideline for authors reporting a meta-analysis of randomized controlled trials. As the scientific world is steadily changing, this “Statement” had to be renewed and adapted. The “new version” of the QUOROM Statement, the PRISMA Statement, was designed by 29 people from different sections, such as review authors, medical editors, clinicians and consumers, in 2005. (Liberati et al. 2009)

### 2.2.2 Scope of Prisma

The PRISMA Statement itself focuses on details of the background and development of a systematic review and on methods for authors to ensure the transparent and complete reporting of systematic reviews and meta-analyses. Also from a viewpoint of a reader, this guideline leads to better understanding and replicating the results of the structured review (Atkins et al. 2005, Helfand, Balshem 2010).

### 2.2.3 The PRISMA checklist

The checklist of the PRISMA Statement consists of 27 items concerning the six main sections of an article: title, abstract, introduction, methods, results and discussion. The Prisma 2009 checklist, applied to the systematic review and meta-analysis performed here, is annexed in the appendix. (Moher et al. 2015).

The aim of the title is to state a clear description of the research question or topic with respect to the identification of the report as a systematic review, meta-analysis, or both. Liberati et al advise authors to use informative titles that make key information easily accessible to readers and they believe a title reflecting the PICOS approach (participants, interventions, comparators, outcomes, and study design) may help the readers to get an overview, too.

Providing a structured summary in the abstract should include background, objectives, data sources, study criteria, participants, interventions as well as results, limitations, conclusions, implications of key findings and funding for the systematic review.

The reader can enter the topic of the review easily by a good introduction that describes what is already known and again states in more detail the research topic according to the PICOS approach.

One of the most important parts of a systematic review is the methods section. It presents the whole process of how the review was conducted, starting from the study protocol over the eligibility criteria, information sources, search strategy details, study selection process up to the data collection process, risk of bias in selected studies, risk of bias across the studies, additional analyses as well as the evaluation of the quality of studies.

Study selection, study characteristics, risk of bias within studies, results of the individual studies, synthesis of results, and risk of bias across studies in further details come within the result's part. Also, additional analyses can be carried out.

For the discussion it is important to mention the summary of evidence and specific limitations of the systematic review. An outlook how to proceed on this topic in the future should also be included. Finally, sources of funding for the review and other support as for example supply of data should be noted, too.

## 2.3 Performing the systematic review

The systematic review and meta-analysis have been carried out and reported according to the PRISMA Statement and the quality of studies has been evaluated using the quality assessment tool of the Cochrane Collaboration.

### 2.3.1 Formulating the research question

Starting from landmark papers, such as the study by Mayer et al. or the recommendations of the European LeukemiaNet expert panel, we realized there was a lack of clarity concerning the question what dose of cytarabine and how many cycles of conventional chemotherapy in consolidation therapy should be given as the optimal solution for patients with acute myeloid leukemia. We decided to conduct a systematic review and a meta analysis on this topic. Therefore, we got on to this subject with the precise title: "Conventional consolidation therapy in adult acute myeloid leukemia – a systematic review / meta-analysis".

### 2.3.2 Literature search

To find all available relevant data we designed a literature search which included original papers, research letters and reviews in English or German that have been peer-reviewed within a time period from 1990 until the end of 2014. Furthermore, we checked the references of these publications for further articles.

At the first step we went through the databases Pubmed / MedLine and the Cochrane Library. Further literature search was done by hand search scouring the American Society of Hematology archive from 2005 to 2014 as well as all the references of the “NCCN guidelines for acute myeloid leukemia 2015”.

### 2.3.3 Search strategy

Our search strategy consisted of composing a determined list of search terms (*Table 4*) and according to the hits we went through titles and abstracts to find out whether the study would match the research question and our inclusion / exclusion criteria. After removing duplicates and excluding non-relevant studies we went through the full texts to further specify inclusion or exclusion of the material. One example for a search term combination is presented in *Figure 1*.

***Table 4. Search terms***

| | |
|---|---|
| **acut*** | **leukem*/leukaem*/leucem*/leucaem*** |
| **consolidation** | chemotherapy |
| **complete remission** | AML/aml |
| **non-lympho*/non lympho*** | myelo* |

***Figure 1. Search strategy***

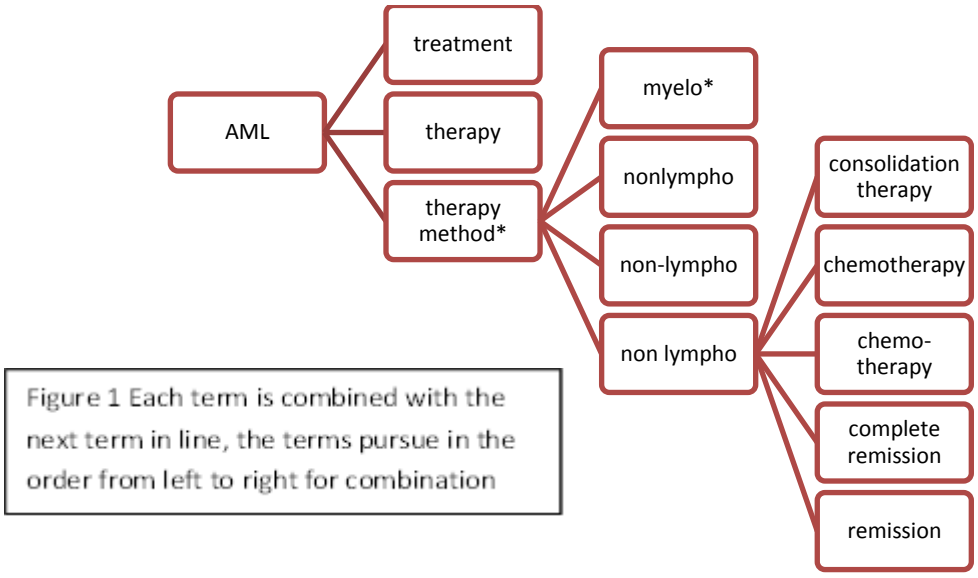


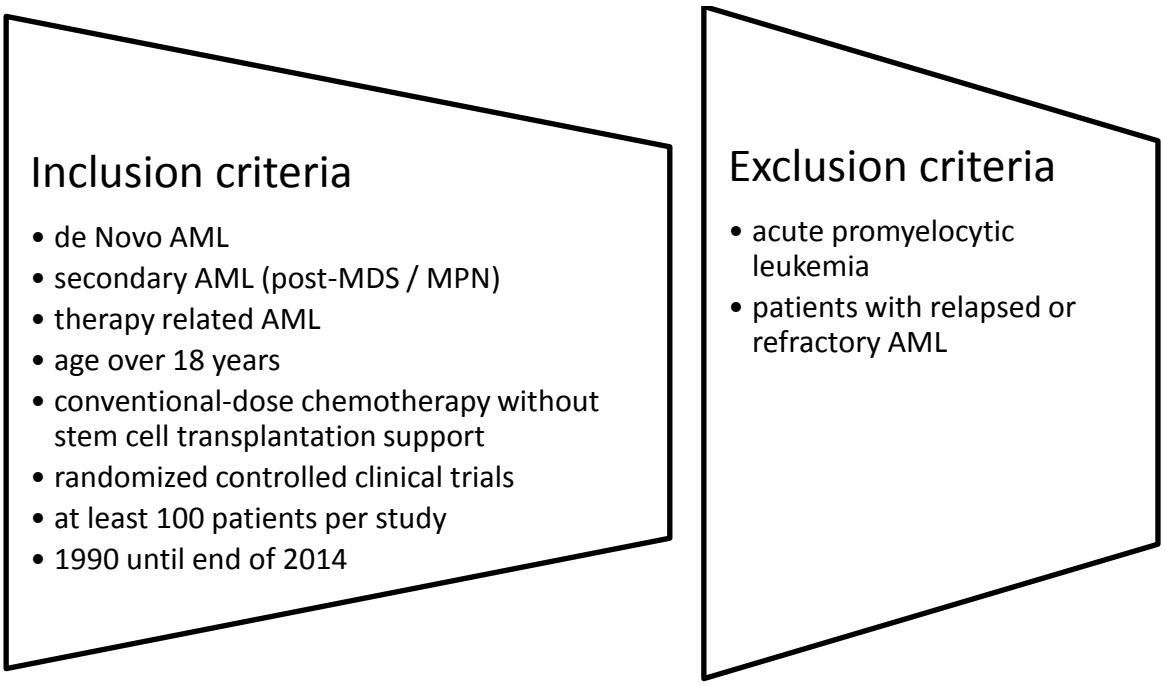

Figure 1 Each term is combined with the next term in line, the terms pursue in the order from left to right for combination

### 2.3.4 Trial characteristics

Each of our selected studies were screened in their titles, abstracts and fulltexts whether they fulfill our inclusion / exclusion criteria.

In the list below (*Figure 2*), there is a summary of all criteria that were considered:

***Figure 2. Inclusion and exclusion criteria for the selected studies***




### 2.3.5 Selection process

According to the PRISMA Statement, we created our own flow diagram (*Figure 3*) which presents a visualisation of our search process. It should also be noted that each step of our search strategy as well as our selecting process was undertaken by two reviewers (Kinkini Magina, Heinz Sill) independently and was well discussed afterwards. This should avoid any selection or reporting bias for our systematic review.

### 2.3.6 Appraisal of identified studies

After the final selection of studies for our work, we first extracted the relevant data according to the basic study information, like first author, publication year, period of enrollment, etc. and according to the specific study information due to our types of outcome measures, overall survival and disease free survival. In unclear cases, the authors were contacted via e-mail and asked for missing data.

Our target outcome measures in AML therapy for analysing our research questions were disease free survival (DFS) and overall survival (OS). We applied the definitions of the European LeukemiaNet expert panel for our systematic review and meta-analysis. “Disease free survival”, also designated “Relapse free survival” (RFS), is defined only for patients achieving complete remission, measured from the date of achievement of remission until the date of relapse or death from any cause and patients not known to have relapsed or died at last follow-up are censored on the date they were last examined, while overall survival is regarded the time frame for all patients of a trial, measured from the date of entry into a study to the date of death from any cause and patients not known to have died at last follow-up are censored on the date they were last known to be alive. (Döhner et al. 2010)

Furthermore, we evaluated the quality of a study with respect to the quality assessment tool of the Cochrane Collaboration. This assessment tool considers six aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and last but not least selective reporting.

### 2.3.7 Measures of treatment effect

Since we are dealing with survival data we used the hazard ratio (HR) with 95% confidence intervals as measure of treatment effect. If they are not available directly in the selected studies they are derived according to Parmar (Parmar, Torri

& Stewart 1998), using an Excel spreadsheet made available by Tierney et al (Tierney et al. 2007).

### 2.3.8 Assessment of heterogeneity

For the assessment of heterogeneity we used Higgins $I^2$ (Higgins, Thompson 2002, Higgins et al. 2003). In case of substantial heterogeneity ($I^2$ greater than 50%), we planned to perform subgroup and sensitivity analyses for the following items: age groups and study quality.

### 2.3.9 Data synthesis

Data are combined using the generic inverse-variance approach. We considered both fixed-effect and random-effects models for the meta-analyses. Fixed-effect models assume that all studies included in the systematic review estimate the same treatment effect; random-effects models assume a random distribution of treatment effects. Therefore, in a fixed-effect model all observed variation is assumed to be due to sampling variation but in a random-effects model variation is assumed to come from two sources, namely sampling variation and random variation owing to the fact that each study treatment effect is a sample from a distribution, Whereas fixed-effect models aim to provide an estimate of the 'true' underlying treatment effect, random-effects models aim to estimate the mean treatment effect and its standard deviation. Because of this subtle difference, fixed-effect models assign more weight to large studies than random-effects models (Borenstein et al. 2009). Unless there was good evidence for homogeneous effects across studies, we primarily summarised the data by means of a random-effects model. All analyses are depicted by forest plots. The analyses were performed using the R package 'meta' (R version 3.2.2).

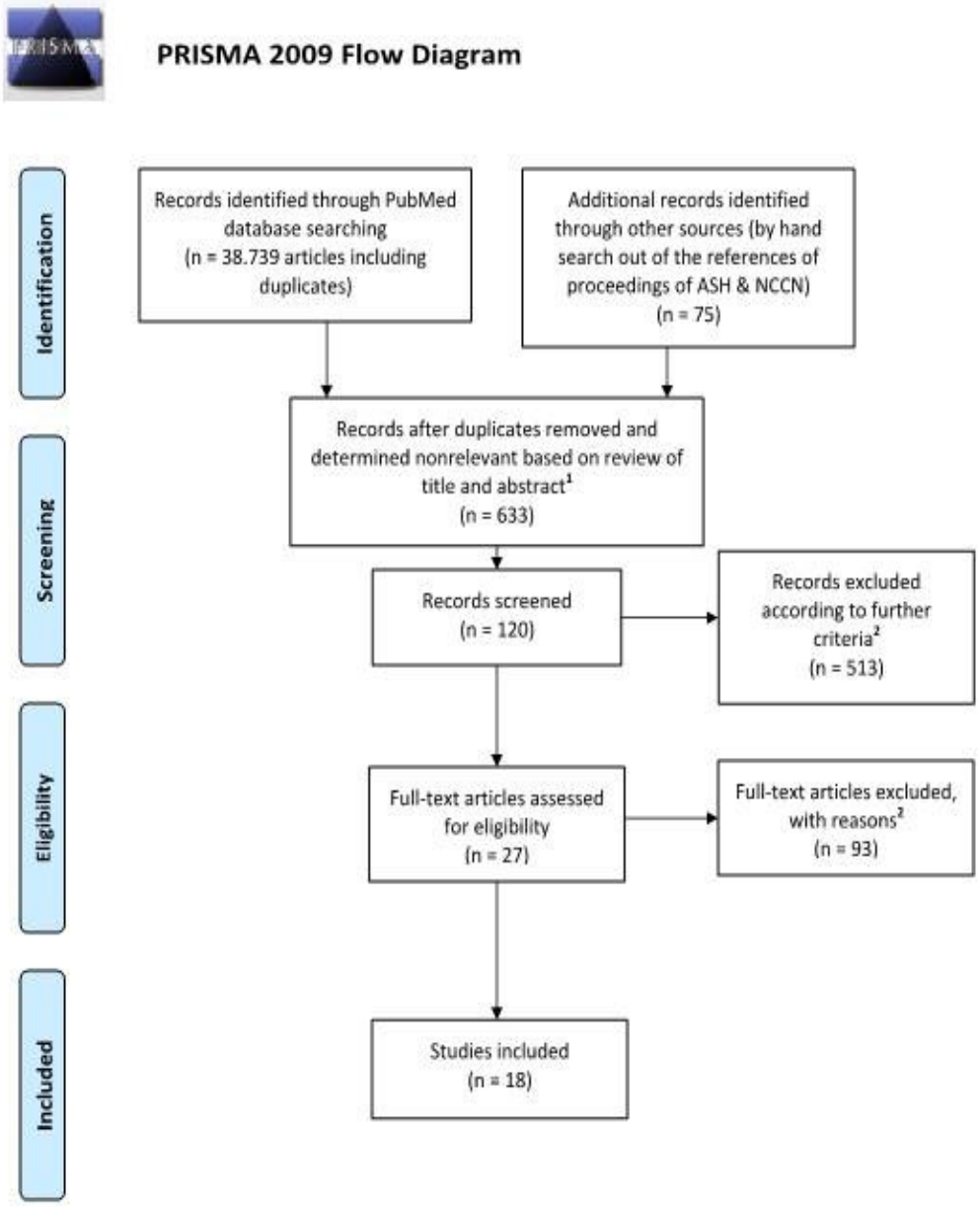
# 3 Results

After an intensive search of overall 38,739 potentially relevant articles identified via PubMed database and 75 potentially relevant articles identified by hand search of the reference list of “Proceedings of the American Society of Hematology (ASH)” and of the “National Comprehensive Cancer Network (NCCN)” the two investigators (Kinkini Magina, Heinz Sill) independently went through the articles by title and abstracts to remove duplicates and exclude non-relevant papers. After that step 633 articles were further evaluated for compliance with the inclusion criteria stated. In further consequence these records were screened for further inclusion criteria. Finally, 18 studies were included in this systematic review, 15 of which matched our primary research questions (13 studies with patients aged 65 years and younger and 2 studies with patients above 65 years). The remaining 3 studies are enclosed in a cytogenetic subanalysis that is not part of this diploma thesis. Our whole search process is presented in the flow diagram in *Figure 3* and the basic information of the selected studies is mentioned in *Table 5*.

*Table 5* shows the source (first author and year of publication), study acronym (as far as available), study period or rather period of enrollment, n total / n in CR (total amount of patients versus patients in complete remission), median age and age range in years, induction therapy (whether there was low dose cytarabine or high dose cytarabine or both administered), consolidation treatment, follow-up in years and last but not least whether the treatment schedule included concomitant cytotoxic medication and / or maintenance therapy.

Cytarabine was combined with several different concomitant (cytotoxic) drugs such as daunorubicin, amsacrine, azathioprine, aclarubicin, etoposide, vincristine, prednisolone, cyclophosphamide, diaziquone, mitoxantrone, idarubicin and vindesine.

***Figure 3. Flow diagram of our systematic review***




*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.* PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

**Used Filters in PubMed: Publication years: 01/01/1990 – 31/12/2014**

**1... addressing other issues, not human studies, not adult studies, under 20 patients investigated, case reports, not in English or German language available**

**2... under 100 patients investigated, Phase I/II studies, not randomised, retrospective**

*Table 5. Characteristics of included studies for consolidation therapy*

| *Source* | *Study ID* | *Study period* | *n total / n in CR* | *Median age [range] (in years)* | *Ind* | *Consolidation therapy* | *Follow Up (in years)* | *Mono /comb. Tx* | *Maint. Tx* |
|---|---|---|---|---|---|---|---|---|---|
| **Mayer RJ, 1994** | CALGB | 1985 - 1990 | 1088 / 693 | 52 (16 - 86) | LD | Ara-c 100mg/m$^2$x5 cont. 4 courses; Ara-c 400mg/m$^2$x4 cont. 4 courses; Ara-c 3000mg/m$^2$x6 short 4 courses | 4.3 | mono | yes |
| **Vogler WR, 1995** | na | 1981 - 1986 | 406 / 219 | na (15 - 50) | LD | Ara-c 200mg/m$^2$x7 cont. 3 courses + DNR; Ara-c 200mg/m$^2$x7 cont. 1 course + DNR/AMSA/AZA; Ara-c 100mg/m$^2$x10 short 3 courses + DNR | na | comb. | yes |
| **Weick JK, 1996** | SWOG | 1986 - 1991 | 723 / 361 | na (15 - 64) | HD | Ara-c 200mg/m$^2$x7 cont. 2 courses + DNR; Ara-c 2000mg/m$^2$x10 short 1 course + DNR | 4.3 | comb. | no |
| **Fopp M, 1997** | SAKK | 1985 - 1992 | 276 / 169 | 45 (16 - 64) | LD | Ara-c 100mg/m$^2$x7 cont. 1 course + DNR; Ara-c 3000mg/m$^2$x12 short 1 course + DNR | 6.0 | comb. | no |
| **Elonen E, 1998** | na | 1986 - 1992 | 248 / 192 | 46 (16 - 65) | LD | Ara-c 3000mg/m$^2$x4 short 1 course + AMSA, Ara-c 2000mg/m$^2$x10 short 1 course + DNR; Ara-c 3000mg/m$^2$x4 short 1 course + AMSA, Ara-c 2000mg/m$^2$x10 short 1 course + DNR/Acla/Eto/Vincr/Pred | 5.7 | comb. | no |
| **Bloomfield C, 1998** | CALGB | 1985 - 1990 | 826 / 321 | na (16 - 64) | LD | Ara-c 100mg/m$^2$x5 cont. 4 courses; Ara-c 400mg/m$^2$x4 cont. 4 courses; Ara-c 3000mg/m$^2$x6 short 4 courses | 7.0 | mono | yes |
| **Byrd JC, 1999** | CALGB 8461 | 1984 - 1995 | 50 / 50 | 35 (18 - 68) | LD | Ara-c 3000mg/m$^2$x6 short 4 courses; Ara-c 3000mg/m$^2$x6 short 3 courses; Ara-c 3000mg/m$^2$x6 short 1 course + Eto/Cyclo/AZQ/Mitox/GCSF | 5.0 | mono & comb. | yes |
| **Stone RM, 2001** | na | 1990 - 1993 | 388 / 205 | >60 | LD | Ara-c 100mg/m$^2$x5 cont. 4 courses; Ara-c 500mg/m$^2$x6 short 2 courses + Mitox | 7.7 | comb. | no |
| **Byrd JC, 2004** | CALGB 8461 | 1984 - 1995 | 48 / 48 | 36 (17 - 58) | LD | Ara-c 3000mg/m$^2$x6 short 4 courses; Ara-c 3000mg/m$^2$x6 short 3 courses; Ara-c 3000mg/m$^2$x6 short 1 course + Eto/Cyclo/AZQ/Mitox/GCSF | 7.8 | mono & comb. | yes |
| **Bradstock KF, 2005** | na | 1995 - 2000 | 292 / 234 | 43 (15 - 60) | HD | Ara-c 3000mg/m$^2$x8 short 1 course + IDR/Eto; Ara-c 100mg/m$^2$x5 cont. 2 courses + IDR/Eto | 3.8 | comb. | no |
| **Moore JO, 2005** | CALGB 9222 | 1992 - 1995 | 473 / 342 | 43 (17 - 59) | LD | Ara-c 3000mg/m$^2$x6 short 3 courses; Ara-c 3000mg/m$^2$x6 short 1 course + Eto/Cyclo/AZQ/Mitox | 8.3 | comb. | no |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Gardin C, 2007** | ALFA 9803 | 1999 - 2006 | 416 / 236 | 72 (65 - 85) | LD | Ara-c 60mg/m$^2$x5 cont. 6 courses + DNR or IDR; Ara-c 200mg/m$^2$x7 cont. 1 course + DNR or IDR | 2.8 | comb. | no |
| **Burnett AK, 2009** | MRC AML12 | 1994 - 2002 | 2934 / 2173 | 41 (0 - 68) | LD | Ara-c 200mg/m$^2$x5 cont. 1 course + AMSA/Eto, Ara-c 1000mg/m$^2$x6 short 1 course + Mitox; Ara-c 200mg/m$^2$x5 cont 1 course + AMSA/Eto, Ara-c 100mg/m$^2$x10 cont. 1 course + IDR/Eto, Ara-c 1000mg/m$^2$x6 short 1 course + Mitox | 8.4 | comb. | no |
| **Thomas X, 2011** | ALFA 9802 | 1999 - 2006 | 459 / 408 | na (15 - 50) | LD | Ara-c 3000mg/m$^2$x6 cont. 4 courses; Ara-c 500mg/m$^2$x3 cont. 1 course + Mitox, 500mg/m$^2$x3 short 1 course + Eto | 5.0 | mono & comb. | no |
| **Miyawaki S, 2011** | JALSG AML201 | 2001 - 2005 | 1057 / 823 | 47 (15 - 64) | LD | Ara-c 2000mg/m$^2$x10 short 3 courses; Ara-c 200mg/m$^2$x5 cont. 4 courses + Mitox/DNR/Aclarub/Eto/Vincr./Vind | 2.0 | comb. | no |
| **Hengeveld M, 2012** | AML 8B | 1986 - 1993 | 603 / 367 | 52 (13 - 60) | LD | Ara-c 500mg/m$^2$x12 cont. 1 course + AMSA, Ara-c 2000mg/m$^2$x8 1 course + DNR; Ara-c 200mg/m$^2$x7 cont. 1 course + DNR, Ara-c 100mg/m$^2$x10 cont. 6 courses + DNR | 7.5 | comb. | yes |
| **Schaich M, 2013** | AML2003 | 2003 - 2009 | 1179 / 696 | 48 (16 - 60) | LD | Ara-c 3000mg/m$^2$x6 short 3 courses; Ara-c 1000mg/m$^2$x12 short 2 courses + Mitox, Ara-c 1000mg/m$^2$x10 short 1 course + AMSA | 4.3 | mono & comb. | no |
| **Burnett AK, 2013** | AML15 | 2002 - 2009 | 3106 /1440 | 49 (0 -73) | LD /HD | Ara-c 200mg/m$^2$x5 cont. 1 course + AMSA/Eto, Ara-c 1000mg/m$^2$x6 short 1 course + Mitox; Ara-c 1500mg/m$^2$x6 short 2 courses; Ara-c 3000mg/m$^2$x6 short 2 courses | 5.6 | mono & comb. | no |

***Abbreviations***: *Ind, Induction therapy; LD, Low dose cytarabine; HD, high dose cytarabine; na, not available; Mono, Mono therapy; Comb., combination therapy; Tx, therapy; Maint., Maintenance therapy; Ara-c, Cytarabine; DNR, Daunorubicin; AMSA, Amsacrine; AZA, Azathioprine; Acla, Aclarubicin; Eto, Etoposide; Vincr., Vincristin; Pred, Prednisolon; Cyclo, Cyclophosphamide; AZQ, Diaziquone; Mitox, Mitoxantrone; GCSF, Granulocyte-colony stimulating factor; IDR, Idarubicin; Vind., Vindesine*

## 3.1 Application of the Cochrane Collaboration's tool for assessing risk of bias on our selected studies

For evaluating the risk of bias we used the Cochrane Collaboration's Tool for assessing risk of bias as the Prisma Statement requires a screening of the risk of bias within the selected studies. The quality of the included studies is judged according to 7 main categories: the random sequence generation (selection bias), the allocation concealment (selection bias), the blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias. Each category can be identified as "high risk of bias" (**-**), "low risk of bias" (**+**) or "unclear risk of bias". A brief explanation of each category including an example for low risk and high risk bias is given in *Table 6*. (Hartling L, Hamm M, Milne A, et al. 2012)

***Table 6. Explanation of the risk of bias categories***

| Category | Explanation |
|---|---|
| **Random sequence generation (selection bias)** | Procedure of adequate allocation generation<br>**+** choosing patients randomly by a computer<br>**-** choosing patients by date of birth |
| **Allocation concealment (selection bias)** | Procedure of adequate allocation concealment<br>**+** central allocation (web-based, by telephone)<br>**-** assignment envelopes were used without appropriate safeguards |
| **Blinding of participants and personnel (performance bias)**<br><br>**Blinding of outcome assessment (detection bias)** | Prevention of knowledge of the allocated interventions adequately during the study<br>**+** blinding ensured or no blinding, but has no effect on outcome<br>**-** incomplete blinding |
| **Incomplete outcome data (attrition bias)** | Clarification of incomplete data with reasons<br>**+** no missing outcome data<br>**-** potentially inappropriate application of simple imputation |

| | |
|---|---|
| **Selective reporting (reporting bias)** | Evaluation of selective reporting of outcome data<br>+ study protocol is available<br>- Not all of the study's pre-specified primary outcomes have been reported |
| **Other Bias** | Other bias that is not included in the categories mentioned before |

Based on our analysis we can generally speak of a very low risk of bias within our selected studies. The random sequence generation as well as the blinding of participants, personnel and outcome assessment was 100% low risk in overall for all ages summarized. This led to low selection bias, low performance bias and low detection bias for our systematic review and meta-analysis.

Allocation concealment was assessed with about 74% of low risk bias and 26% of unclear risk of bias. The concerning studies with unclear risk of bias were mostly old studies (Fopp et al. 1997, Gardin et al. 2007, Mayer et al. 1994, Vogler et al. 1992, Bradstock et al. 2005). It is safe to assume that the standard of performing a clinical trial in the 90s was not as precise as it is today. Surprisingly, the study of Gardin et al as well as the study by Bradstock et al did not report the procedure of allocation concealment although these were two quite new studies published in 2007 and 2005.

The aspect of incomplete data presented about 84% low risk of bias and 16% high risk of bias. The high risk of attrition bias of Hengeveld et al was at the basis of the fact that for the cytogenetic outcome data only 53% of the patients were mentioned. However, our standard for a low risk of attrition bias was defined by including 80% or more of the patients in outcome data in the trial. Besides, both studies by Byrd et al (1999, 2004) did not provide all necessary information.

Selective reporting was allocated 76% low risk of bias and 24% high risk of bias. Burnett et al (2013) had a high risk of reporting bias as some of the outcome was not comprehensible for our systematic review. Further studies that did not report correctly the full content of the trial were Bloomsfield et al and again, both studies by Byrd et al. Other bias could not be compiled in our selected studies. The assessment tool overview for comprising all age groups is reflected in *Figure 4* and *Figure 5*.

***Figure 4. Risk of bias for all ages in summary***

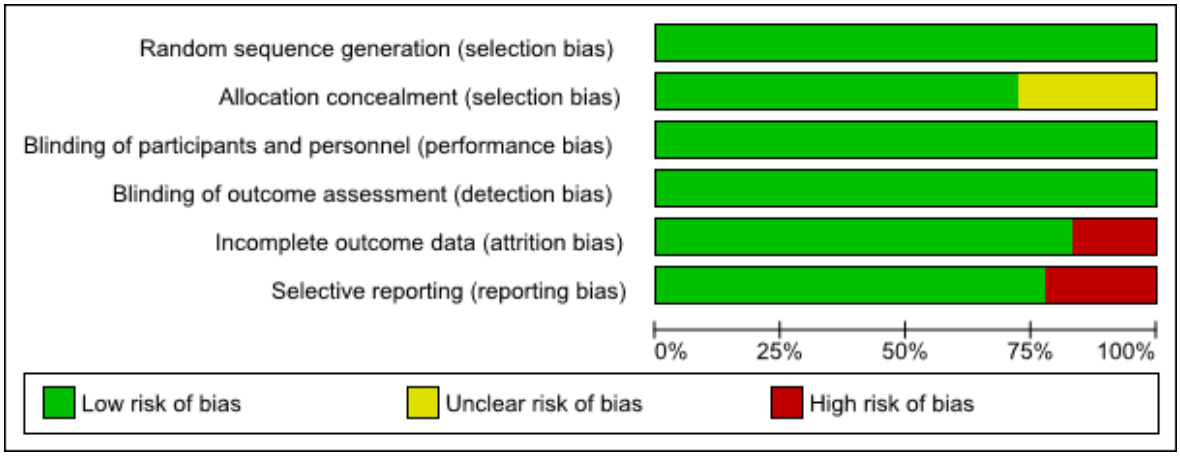
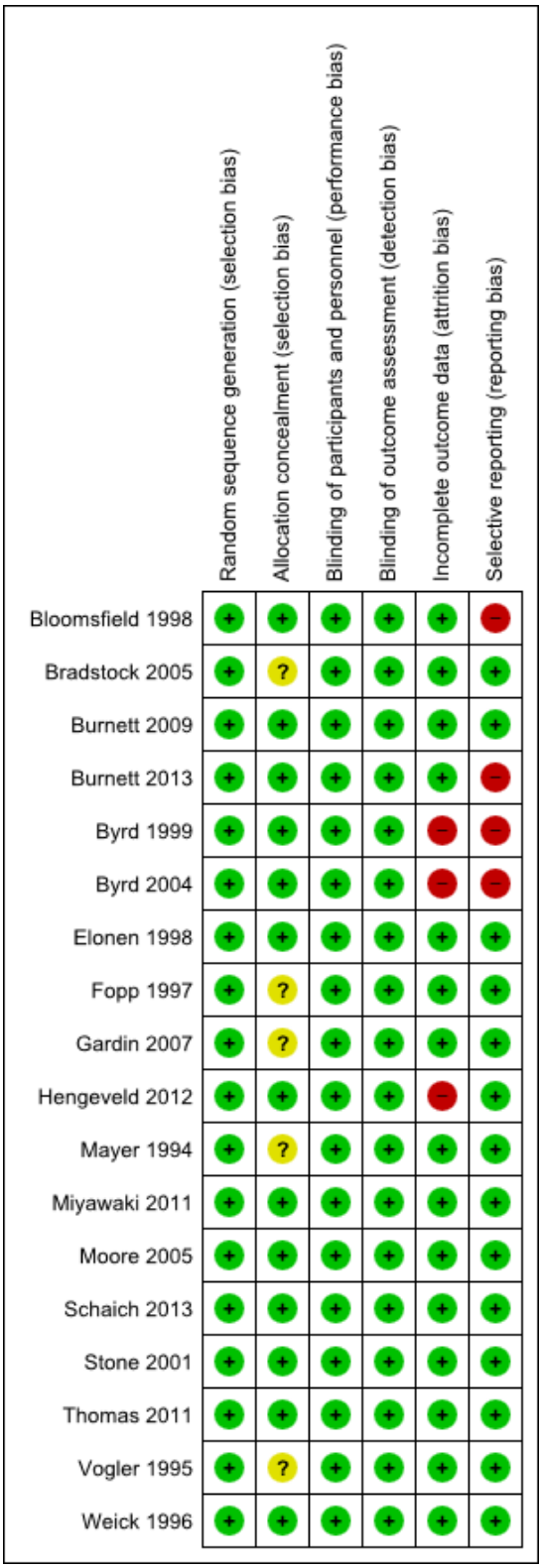


***Figure 5. Risk of bias for all ages in detail***



## 3.2 Main research questions

Our main research questions concerning this systematic review and meta-analysis were as following and they were done within two different patient groups, a group of younger patients (patients aged 65 years and younger) and elderly patients (patients above 65 years).

3.2.1 Intensity of the cytarabine dose in relation to the outcome of the patients

a. High dose versus intermediate dose cytarabine in consolidation treatment
b. High dose versus low dose cytarabine in consolidation treatment

3.2.2 Comparison of the number of consolidation cycles that were independently of the cytarabine dose administered

3.2.3 Comparison of monotherapy versus combination consolidation therapy

First of all, the definitions of the different doses of cytarabine should be explained as this has been the most important drug in consolidation treatment, there are three different levels of cytarabine that are used in hospitals based on international classification. Low dose cytarabine means a cytarabine dose between 100 $mg/m^2$ and 200 $mg/m^2$, intermediate dose cytarabine is a dose varying from 1.0 $g/m^2$ to 1.5 $g/m^2$, whereas high dose cytarabine is defined by a dose between 2.0 $g/m^2$ and 3.0 $g/m^2$. However, we extended our analysis and included an assessment of the cumulative dose of cytarabine administered during the whole consolidation treatment period.

We report on a population of a total of 14,572 patients with acute myeloid leukemia, of these 8977 patients reached a status of complete remission (equals 61.6% of all patients). All studies included minors under the age of 18 years, except two studies that only focused on elderly patients (Stone et al. 2001, Gardin et al. 2007). The oldest patient was 86 years old and the youngest patient was an infant under the age of one year.

The selected studies were published between 1994 (Mayer et al. 1994) and 2013 (Burnett et al. 2013). The median follow-up time varied between 2.0 years (Miyawaki et al. 2011) and 8.4 years (Burnett et al. 2009).

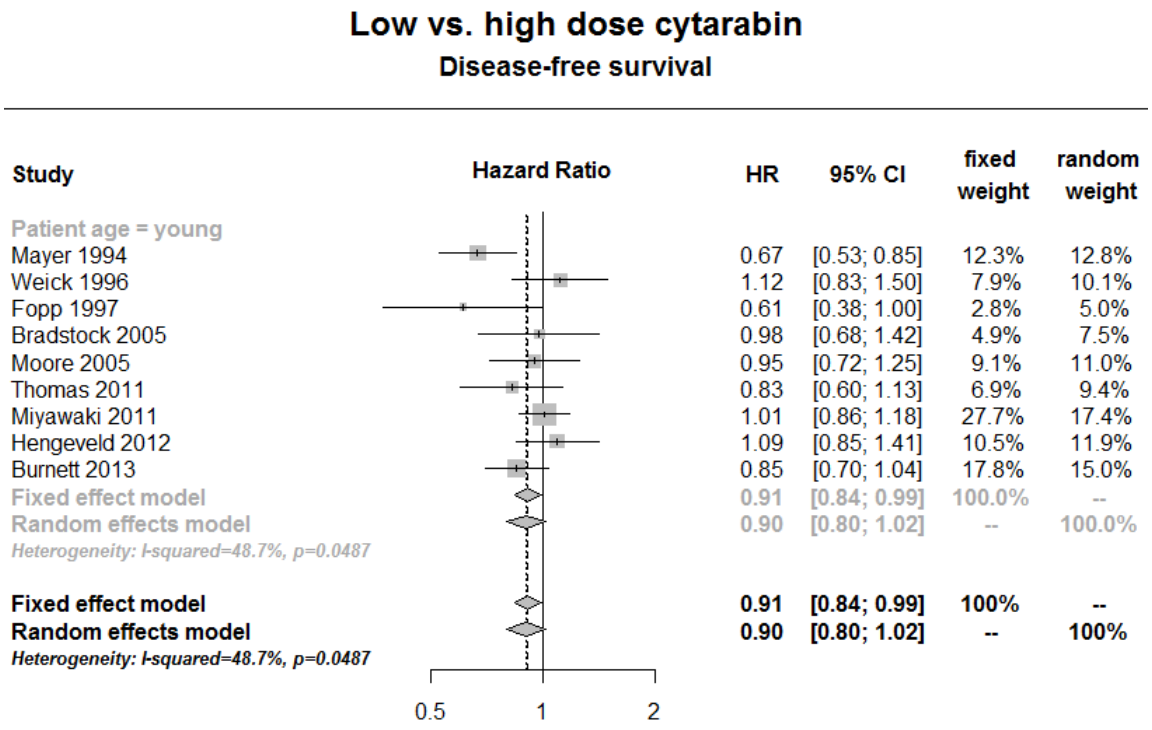
Most of the trials used a combination therapy, only two used monotherapy (Mayer et al. 1994, Bloomfield et al. 1998) and five studies administered mono- and combination therapies (Byrd et al. 1999, Byrd et al. 2004, Thomas et al. 2011, Burnett et al. 2013, Schaich et al. 2013). Maintenance therapy was described in six studies (Mayer et al. 1994, Volger et al. 1995, Byrd et al. 1999, Byrd et al. 2004, Bloomfield et al. 1998, Hengeveld et al. 2012). The majority of trials used low dose cytarabine in induction treatment except two studies that used high dose cytarabine (Bradstock et al. 2005, Weick et al. 1996) and one study administering both (Burnett et al. 2013).

### 3.2.1 Intensity of the cytarabine dose in relation to the outcome of the patients

Regarding the question about the intensity of cytarabine “high dose” versus “intermediate dose” a statistical calculation could not be performed as there was only one study directly addressing this particular comparison (Thomas et al. 2011). However, we performed a statistical analysis of the comparison “high dose” versus “low dose”, where we added the intermediate dose therapy to the low dose treatment to get an overall comparison of all studies available. Our cut-off level between high and low / intermediate dose cytarabine was defined by the cumulative dose of 20.000 mg/m$^2$ of cytarabine. This implies that all doses less than 20.000 mg/m$^2$ were classified as low / intermediate dose cytarabine, whereas greater or equal 20.000 mg/m$^2$ was assigned as high dose cytarabine. The lowest dose applied in all studies was a cumulative dose of 700 mg/m$^2$ (Fopp et al. 1997) and the highest cumulative dose administered was 72.000 mg/m$^2$ (Mayer et al. 1994, Thomas et al. 2011). We were able to determine 9 studies from the younger patient group (Mayer et al. 1994, Weick et al. 1996, Fopp et al. 1997, Thomas et al. 2011, Miyawaki et al. 2011, Hengeveld et al. 2012, Burnett et al. 2013, Moore et al. 2005, Bradstock et al. 2005) that compare high dose cytarabine with low / intermediate dose cytarabine in consolidation therapy. There was no study available in the elderly patient group addressing this question.

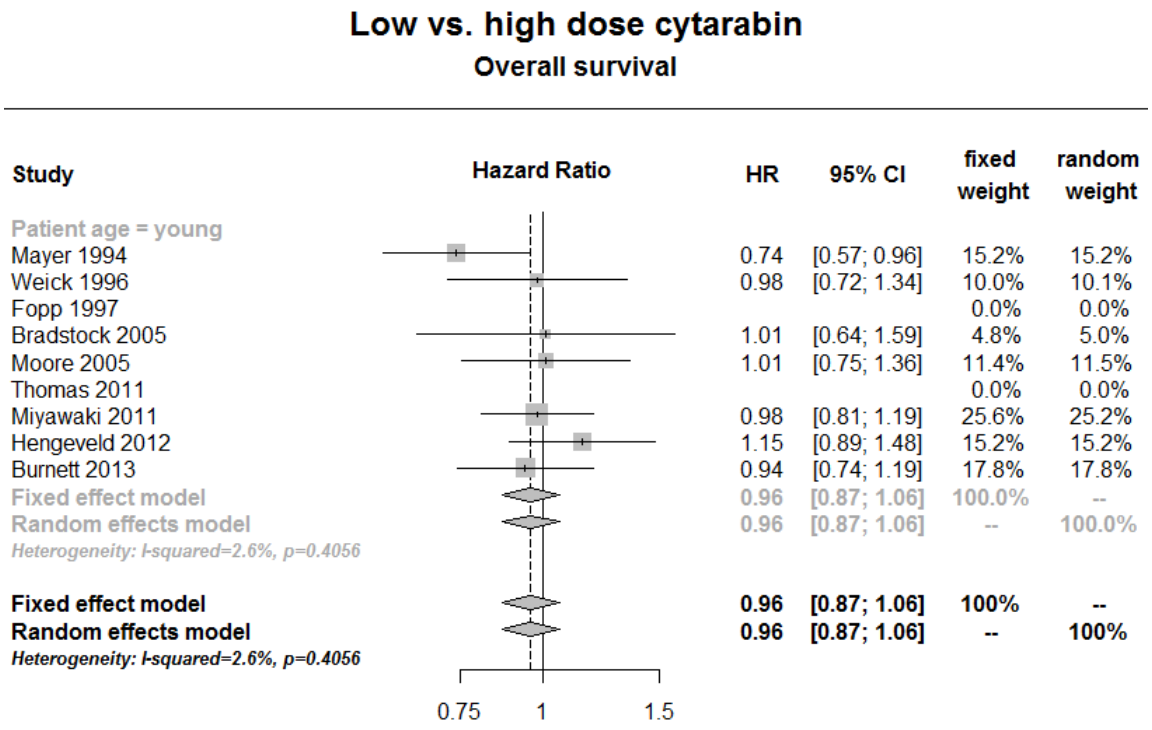
The comparison of low / intermediate dose versus high dose cytarabine with respect to disease free survival showed a hazard ratio (HR) of 0.90 [0.80; 1.02] using a random effects model. There is a trend that high dose cytarabine may improve disease free survival. Two rather old studies, Mayer et al (HR 0.67 [0.53; 0.85]) and Fopp et al (HR 0.61 [0.38; 1.00]), had significant results preferring high dose treatment with cytarabine. The test of heterogeneity gave an overall P value of 0.048 ($I^2$ = 48.7%) showing that there is heterogeneity among the selected studies. The relevant data are presented in *Figure 6*.

***Figure 6. Disease free survival benefit in the comparison of low / intermediate dose versus high dose cytarabine treatment***




We analysed the comparison of low / intermediate dose versus high dose cytarabine on overall survival. We did not get any significant difference between the administration of low / intermediate dose versus high dose treatment which means that there is no preference if low / intermediate dose or high dose consolidation should be favoured to improve overall survival. The overall random effects model gave an HR of 0.96 with a 95% confidence interval between 0.87 and 1.06. The only significant study tending to use high dose cytarabine was performed by Mayer et al (HR 0.74 [0.57; 0.96]). The studies by Thomas et al and Fopp et al had to be excluded from this analysis as we did not have all relevant data. The test of heterogeneity gave a P value of 0.4 ($I^2$ = 2.6%) showing almost no heterogeneity among the selected studies. The relevant data is shown in *Figure 7*.

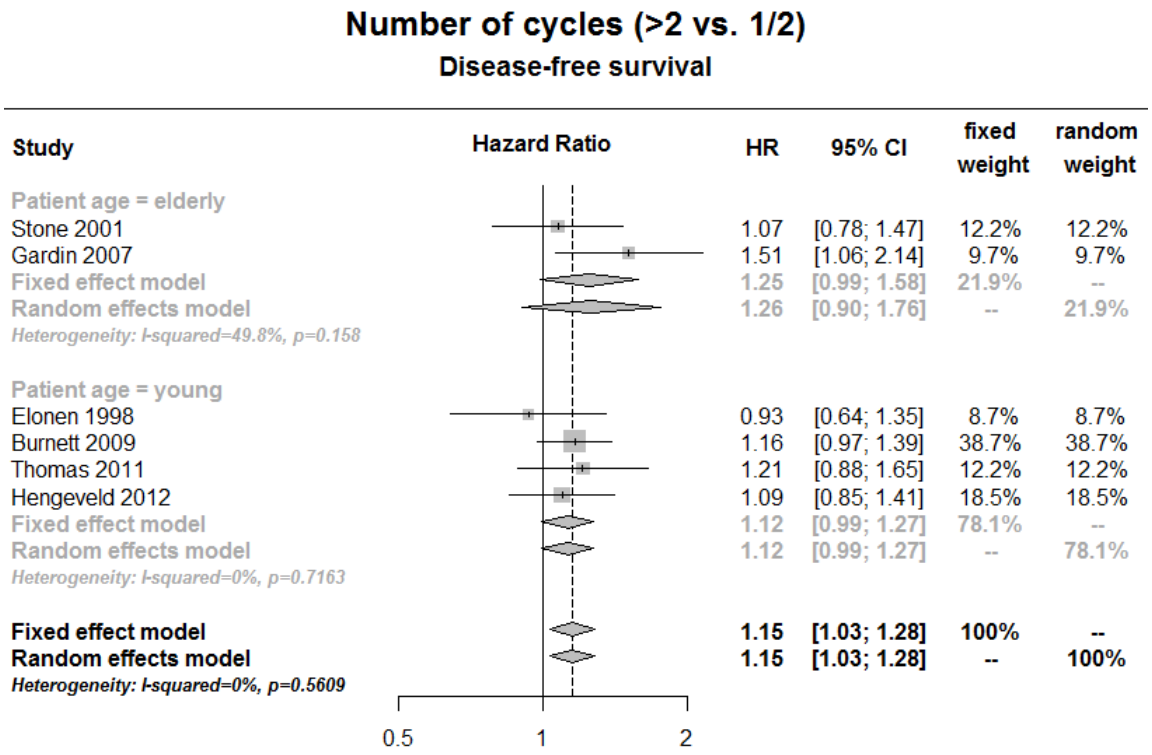
***Figure 7. Overall survival benefit in the comparison of low / intermediate dose versus high dose cytarabine treatment***



### 3.2.2 Comparison of the number of cycles administered independently of cytarabine dose

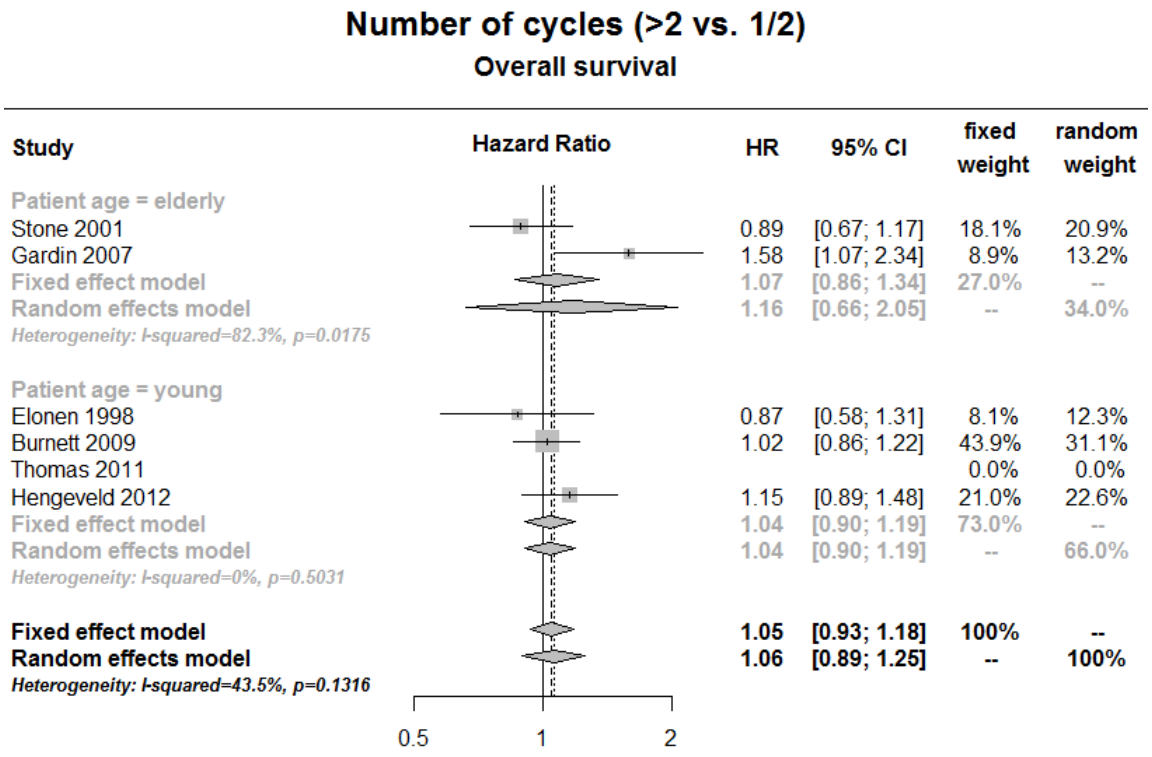
For the comparison of the number of cycles that were administered independently from dose of cytarabine we compared a long term consolidation therapy with more than 2 cycles against a short term consolidation therapy with two or less than 2 cycles of chemotherapy. Our results concerning disease free survival did not show significant differences between long term and short term therapy looking at both age groups separately. The analysis within our younger patients revealed that 3 of 4 analysed studies favoured the long term therapy concept. In our elderly patient group we were able to analyse two studies and both of them preferred a long term therapy. However, the study by Gardin et al was the only significant study (HR 1.51 [1.06; 2.14]). In the overall (both age groups combined) random effects model, a HR of 1.15 [1.03; 1.29]) was obtained which confirms the benefit long term therapy. The test of heterogeneity gave a P value of 0.56, and Higgins $I^2$ indicated overall no heterogeneity ($I^2$ = 0%). The corresponding data for this issue is pictured in *Figure 8*.

***Figure 8. Disease free survival benefit according to the number of cycles of cytarabine with respect to the age group***




Considering overall survival with reference to the number of cycles of consolidation chemotherapy, our results did not show any significant differences in the comparison of long term and short term treatment. Thomas et al had to be excluded from this analysis as there was not all relevant data provided. However, Gardin et al (HR 1.58 [1.07; 2.34]) was the only significant study preferring a long term therapy. The overall random effects model amounted to a HR of 1.06 [0.89; 1.25] (*Figure 9*). The test of overall heterogeneity gave a P value of 0.13 ($I^2$ = 43.5%).

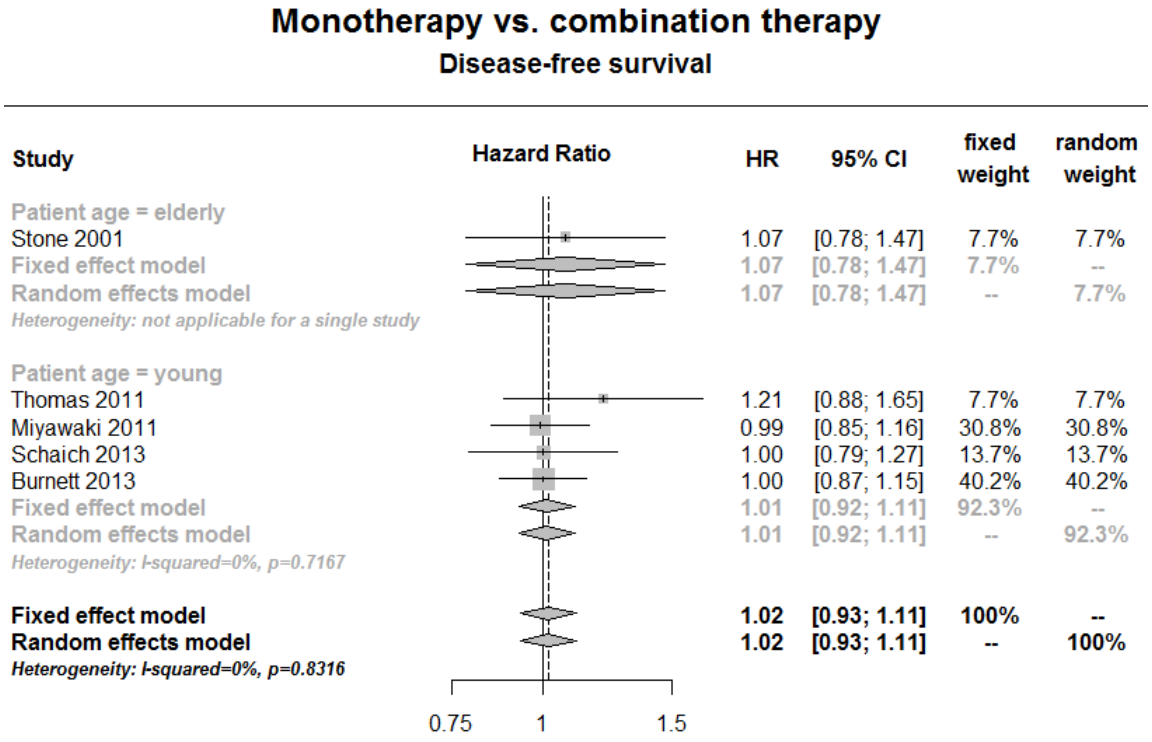
***Figure 9. Overall survival benefit according to the number of cycles of cytarabine with respect to the age group***



### 3.2.3 Monotherapy versus combination consolidation therapy

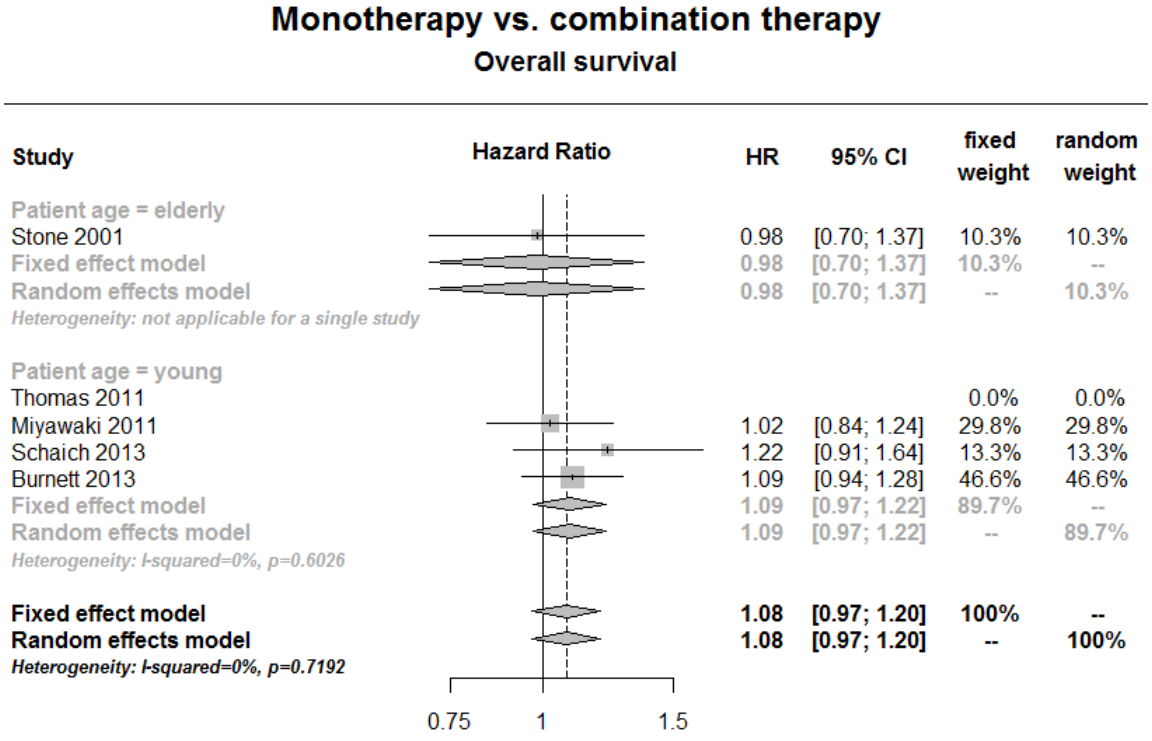
Our results of the evaluation whether monotherapy or combination consolidation chemotherapy was of advantage with respect to disease free survival showed no significant differences in both age groups with an overall HR of 1.02 [0.93; 1.11] for the random effects model. All enclosed trials with younger patients (Miyawaki et al. 2011, Schaich et al. 2013, Burnett et al. 2013, Thomas et al. 2011) were illustrating that it did not matter for disease free survival if they had used mono- or combination consolidation treatment. For analysing the elderly patients regarding this research question we could only include one study (Stone et al. 2001). The test of heterogeneity gave a P value of 0.83 ($I^2$ = 0%). *Figure 10* refers to the relevant data for the disease free survival with respect to the comparison of monotherapy and combination therapy.

***Figure 10. Disease free survival benefit according to comparison of mono- and combination consolidation therapy with respect to the age group***



We assessed the comparison of monotherapy versus combination therapy with respect to the overall survival. As a consequence we were not able to observe any significant differences between the two treatment options either which is based on an overall HR of 1.08 [0.97; 1.20] for the random effects model for both age groups combined. In our younger patient group the study of Thomas et al had to be excluded once again due to lack of data. For the elderly patient group, there was just one study available, Stone et al showed a HR of 0.98 [0.70; 1.37]. The test of overall heterogeneity gave a P value of 0.71 ($I^2$ = 0%). The corresponding data is presented in *Figure 11*.

***Figure 11. Overall survival benefit according to comparison of mono- and combination consolidation therapy with respect to the age group***



# 4 Discussion

Acute myeloid leukemia can be considered as the most common acute leukemia in adults with an incidence of 3 – 4 per 100,000 men and women per year (Schlenk 2014). The generic affected patient population concerns mostly elderly people, with a median age approaching almost 70 years (Rowe 2009). In light of the fact that age represents a significant prognostic parameter, this systematic review and meta-analysis analysed all patients in the selected studies according to two age groups: younger patients (aged 65 years and younger) and elderly patients (patients above 65 years).

Treatment with curative intention of this malignancy is based on an induction therapy followed by a post-remission treatment. The current major drug used for the therapy of AML has been cytarabine for the past three decades (Döhner, Weisdorf & Bloomfield 2015), which is in the center of this systematic review. Further best supportive care is the alternative therapy for patients, albeit with dismal outcome prediction.

Cytarabine combined with an anthracycline in the “3+7 combination” (3 days of anthracycline and 7 days of cytarabine) represents the standard induction therapy (Schlenk 2014). Younger patients mostly receive low dose cytarabine for induction, varying from 100 - 200 mg/m$^2$ continuously with mostly daunorubicin in a dose of 60 mg/m$^2$ in short infusion (Smith et al. 2004b). As elderly patients often have a poor performance status and/or comorbidities the induction dose of cytarabine is adapted to 100 – 200 mg/m$^2$ continuously in combination with a short infusion of daunorubicin 45 mg/m$^2$ (Löwenberg et al. 2009) or idarubicin 12 mg/m$^2$ (Berman et al. 1991). Alternatively, in case an elderly patient is not amenable for a chemotherapy due to poor performance status or too many comorbidities best supportive care can be carried out. Nevertheless, in our selected studies we observed the administration of high dose cytarabine in induction in three studies (Weick et al. 1996, Bradstock et al. 2005, Burnett et al. 2013).

Post-remission treatment of AML as continuation therapy for patients in complete remission shows mainly two different options. On the one hand, there is

intensive conventional chemotherapy and on the other hand, high dose treatment followed by haematopoietic stem cell transplantation can be performed. Beyond that, maintenance therapy is a further strategy which is not regarded an evidence-based approach. This systematic review and meta-analysis focused on conventional chemotherapy without any stem cell support. As AML can be seen as a disease of the elderly, the treatment of patients aged above 65 years is a very important aspect of consolidation therapy. We also tried to explore statistically the optimal treatment schedule for elderly patients.

In order to compare the different dose levels of cytarabine, we extended our analysis and included an assessment of the cumulative dose of cytarabine administered during the entire consolidation treatment period instead of using at single-dose approach of this drug. Our cut-off level between high and low/intermediate dose therapy was defined by a total cumulative dose of 20.000 $mg/m^2$ of cytarabine. All doses less than 20.000 $mg/m^2$ were classified as low/intermediate dose cytarabine, whereas greater or equal 20.000 $mg/m^2$ was assigned as high dose cytarabine.

## *4.1 Discussion of the results*

We performed a statistical analysis of the comparion “high dose” versus “low dose cytarabine”, where we added the intermediate dose therapy to the low dose treatment according to the definitions outlined to get an overall comparison of all studies available. It was possible to determine 9 studies from the younger patient group for this research question, but no study with elderly patients was available. Our results showed that regarding the outcome “disease free survival” patients may benefit from high dose cytarabine administration, whereas overall survival was not improved by any specific dose of cytarabine. The landmark study of Mayer et al was one of two significant studies in our analysis preferring a high dose schedule. They showed that 4 courses of high dose cytarabine (3000 mg/m$^2$ short infusion x 6, cumulative dose 72.000 mg/m$^2$) are superior to 4 courses of intermediate or low dose of cytarabine (400 mg/m$^2$ continuously x 4, cumulative dose 8.000 mg/m$^2$; 100 mg/m$^2$ continuously x 5, cumulative dose 2.000 mg/m$^2$) (Mayer et al. 1994). The other significant study showing a benefit with high dose cytarabine was carried out by Fopp et al. They used one cycle of low dose cytarabine (100 mg/m$^2$ continuously x 7, cumulative dose 700 mg/m$^2$) against one cycle of high dose cytarabine (3000 mg/m$^2$ short infusion x 12, cumulative dose 36.000 mg/m$^2$) and they suggested that cytarabine intensity may be more important than total dosage. On a final note, it should be mentioned that we observed the issue of high toxicity of cytarabine in all our selected studies that used a high dose regimen. Therefore, the side effects of cytarabine should be considered, too, when it comes to a treatment decision. Toxic reactions can vary from severe neutropenia, thrombocytopenia over infections, fever up to involvement of other organ systems such as for instance cerebral, cardiac, gastrointestinal or renal toxicities which, in a substantial number are severe or life-threatening (Bradstock et al. 2005).

Concerning the comparison of the number of cycles that were administered independently from the dose of cytarabine we compared a long term consolidation therapy with more than 2 cycles against a short term consolidation therapy with two or less cycles of chemotherapy. No significant difference with respect to overall survival was found if long term or short term therapy was used in both age

groups. Though, it can be stated that there is a trend that less cycles of consolidation therapy may lead to worse disease free survival. The only study that significantly revealed a preference of long term treatment for disease free survival as well as for overall survival was performed by Gardin et al. Their administration of very low dosed cytarabine in long term schedule (60 mg/m2 continuously x 5 for 6 courses) was superior to short term schedule (200 mg/m2 continuously x 7 for 1 course), both therapy arms were combined with either daunorubicin or idarubicin (Gardin et al. 2007). As this study was performed on elderly patients only, it is not possible to generalize this result for the whole patient population which includes also younger patients. Furthermore, the applied dose of cytarabine is a very low dose that would not have been normally administered on patients younger than 65 years since the recommended dose of cytarabine is substantially higher for them (Döhner et al. 2010).

We further compared monotherapy versus combination therapy with respect to disease free survival and overall survival. In both cases it did not matter if they had used mono- or combination consolidation treatment. Therefore, it can be stated that it does not have any benefit to add other drugs to the basic cytarabine therapy in the setting of consolidation therapy for AML. On the contrary, several studies reported on increased toxic side effects (Volger et al. 1995, Weick et al. 1996, Stone et al. 2001, Moore et al. 2005, Thomas et al. 2011, Miyawaki et al. 2011, Schaich et al. 2013).

Even though the cytogenetic subanalysis has not been carried out yet a trend of a benefit for certain cytogenetic risk groups is predictable according our selected studies. Patients with core-binding factor AMLs showed a superior outcome compared with AML patients with normal karyotype or other abnormality karyotype (Bloomfield et al. 1998). Furthermore, patients with t(8; 21)(q22;q22) as well as patients with inv(16) / t(16;16) seemed to benefit from a sequential high-dose cytarabine regimen with a decreased likelihood of relapse (Byrd et al. 1999, Byrd et al. 2004). The results of our selected studies mostly confirm the recommendation of the European LeukemiaNet expert panel stating that intensive conventional chemotherapy may have a beneficial effect on favourable risk group patients (Bloomfield et al. 1998, Byrd et al. 1999, Byrd et al. 2004, Miyawaki et al.

2011, Burnett et al. 2013). However, we also observed studies showing no evidence of benefit for any specific cytogenetic risk group by using intensive conventional chemotherapy (Bradstock et al. 2005, Moore et al. 2005). One study revealed a trend that was in favour of high dose cytarabine among patients with intermediate cytogenetic risk (Thomas et al. 2011). There was no data regarding outcome parameters available for the risk group stratified analysis of elderly patients. On a final note, it should be mentioned that all our selected studies agree with the recommendation of the European LeukemiaNet expert panel by reporting that an allogeneic stem cell transplantation from a matched related donor is currently considered the treatment of choice for patients with adverse / unfavourable cytogenetics (Döhner et al. 2010, Koreth et al. 2009)

In a recent meta-analysis the role of cytarabine in acute myeloid leukemia treatment has also been assessed regarding the optimal dose (Li et al. 2014). In contrast to our systematic review and meta-analysis, Li et al investigated not only conventional consolidation treatment, but also induction therapy and they included bone marrow transplantation in their treatment options. Furthermore, their selection of studies was undertaken with other inclusion criteria. Our systematic review includes only randomized controlled clinical trials with adult patients and our time period of literature search gathered papers published from 1990 until the end of 2014. Besides, our analysis included elderly patients over 65 years which was not a subject of the systematic review of Li et al. Another important difference is that Li et al investigated only the comparison of “high dose” cytarabine versus “low dose” cytarabine. However, we analysed not only the question of the intensity of cytarabine dose, but also the comparison of consolidation cycles as well as the effect of mono- versus combination therapy. In contrast to Li et al, our meta-analysis is based on the cumulative dose of cytarabine which seemed to be more precise to us for a comparison. Though, altogether we had 7 studies in common. As far as it concerns the results of both systematic reviews, we were able to agree on the fact that there was a benefit for disease free survival by administering high dose cytarabine, but no significant difference in overall survival. Considering the stratified cytogenetic subanalysis they observed a significant benefit in disease free survival in the favourable risk group. This particular subanalysis remains open in our meta-analysis as the statistical evaluation has not been performed yet.

## *4.2 Limitations*

One limitation we had to face while performing this systematic review was that a majority of our selected studies included minors under the age of 18 years (Mayer et al. 1994, Volger et al. 1995, Weick et al. 1996, Fopp et al. 1997, Elonen et al. 1998, Bradstock et al. 2005, Moore et al. 2005, Thomas et al. 2011, Miyawaki et al. 2011, Schaich et al. 2013, Burnett et al. 2013). Though, these studies were all included in our meta-analysis because the proportion of patients younger than 18 years was very low and did not have a crucial impact on our evaluation. Our literature search was restricted from a number of 38,814 potential hits to finally 18 relevant studies as there were many duplicates and just a few studies directly addressing our research topic as randomized controlled trials. Furthermore, we found a minority of studies with elderly patients due to the fact that most clinical trials are based on data of younger patients or only few and highly selected elderly patients which cause a selection bias. Therefore, it is difficult to make a general statement regarding conventional consolidation therapy in elderly patients. Two studies had to be excluded from the cytogenetic subanalysis (Byrd et al. 1999, Byrd et al. 2004), which still is being evaluated. However, we did not want to exclude them from our systematic review as a whole as they demonstrated important results for specific cytogenetic subgroups. One of the most serious limitations we had to deal with was the fact that we were not able to gather all relevant statistic information for our meta-analysis. Thomas et al did not provide the required outcome data concerning the overall survival as well as Burnett et al did not supply the relevant outcome data either since we were not able to contact the lead authors. On a final note, it should be mentioned that on grounds of several different treatment strategies used in the selected studies we received a considerably inhomogenous variety of treatment regimens. This led us to the decision that we should compare the trials according to the cumulative dose rather than the single dose of cytarabine.

## *4.3 Outlook*

This systematic review and meta-analysis emphasizes how difficult it is to determine the optimal dose of cytarabine in conventional consolidation treatment as well as the most appropriate number of courses that should be applied. In addition, we observed how challenging the therapy can especially be in elderly patients aged above 65 years. However, our results showed that there is a certain benefit of high dose cytarabine for the improvement of disease free survival, even if the overall survival is not affected. Also, it can be stated that there is a trend that less cycles of consolidation therapy may lead to worse disease free survival, wheras overall survival is not touched. We found out that it does not have any benefit to add other drugs to the basic cytarabine therapy. For elderly patients we could not clarify an exact therapy recommendation either, but it was obvious that lower doses of cytarabine in an prolonged treatment schedule could lead to a positive effect on the outcome. Though, conventional chemotherapy should only be offered to elderly patients with a good performance status and less comorbidities. With respect to cytogenetic risk groups we noted that conventional consolidation treatment may be beneficial to patients with favourable cytogenetics and in particular cases patients with intermediate risk cytogenetics can also benefit from this therapy regimen, whereas the treatment of choice for patients with adverse risk cytogenetics remains the allogeneic haematopoietic stem cell transplantation. However, caution should be taken when high dose cytarabine is administered due to its high toxicity as mentioned before. In conclusion, individualized consolidation approaches for patients with acute myeloid leukemia considering the patient-related and AML-related prognostic factors appear to be the most reasonable strategy to find an optimal treatment. In the future, it will be an aim of further studies to evaluate more precisely the clinical use of cytarabine in larger patient populations with acute myeloid leukemia. Especially more studies focusing on elderly patients are essential to establish a proper satisfying therapy.

# 5 References


Adriaansen, H.J., te Boekhorst, P.A., Hagemeijer, A.M., van der Schoot, C.E., Delwel, H.R. & van Dongen, J.J. 1993, "Acute myeloid leukemia M4 with bone marrow eosinophilia (M4Eo) and inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression", *Blood,* vol. 81, no. 11, pp. 3043-3051.

Al-Ali, H.K., Jaekel, N. & Niederwieser, D. 2014, "The role of hypomethylating agents in the treatment of elderly patients with AML", *Journal of geriatric oncology,* vol. 5, no. 1, pp. 89-105.

Appelbaum, F.R., Gundacker, H., Head, D.R., Slovak, M.L., Willman, C.L., Godwin, J.E., Anderson, J.E. & Petersdorf, S.H. 2006, "Age and acute myeloid leukemia", *Blood,* vol. 107, no. 9, pp. 3481-3485.

Arlin, Z., Case, D.C.,Jr, Moore, J., Wiernik, P., Feldman, E., Saletan, S., Desai, P., Sia, L. & Cartwright, K. 1990, "Randomized multicenter trial of cytosine arabinoside with mitoxantrone or daunorubicin in previously untreated adult patients with acute nonlymphocytic leukemia (ANLL). Lederle Cooperative Group", *Leukemia,* vol. 4, no. 3, pp. 177-183.

Atkins, D., Fink, K., Slutsky, J., Agency for Healthcare Research and Quality & North American Evidence-based Practice Centers 2005, "Better information for better health care: the Evidence-based Practice Center program and the Agency for Healthcare Research and Quality", *Annals of Internal Medicine,* vol. 142, no. 12 Pt 2, pp. 1035-1041.

Bacher, U., Kern, W., Schnittger, S., Hiddemann, W., Haferlach, T. & Schoch, C. 2005, "Population-based age-specific incidences of cytogenetic subgroups of acute myeloid leukemia", *Haematologica,* vol. 90, no. 11, pp. 1502-1510.

Baer, M.R., Stewart, C.C., Lawrence, D., Arthur, D.C., Byrd, J.C., Davey, F.R., Schiffer, C.A. & Bloomfield, C.D. 1997, "Expression of the neural cell adhesion molecule CD56 is associated with short remission duration and survival in acute myeloid leukemia with t(8;21)(q22;q22)", *Blood,* vol. 90, no. 4, pp. 1643-1648.

Basara, N., Schulze, A., Wedding, U., Mohren, M., Gerhardt, A., Junghanss, C., Peter, N., Dolken, G., Becker, C., Heyn, S., Kliem, C., Lange, T., Krahl, R., Ponisch, W., Fricke, H.J., Sayer, H.G., Al-Ali, H., Kamprad, F., Niederwieser, D. & East German Study Group Hematology and Oncology (OSHO) 2009, "Early related or unrelated haematopoietic cell transplantation results in higher overall survival and leukaemia-free survival compared with conventional chemotherapy in high-risk acute myeloid leukaemia patients in first complete remission", *Leukemia,* vol. 23, no. 4, pp. 635-640.

Bashir, Y., Geelani, S., Bashir, N., Mir, S.A., Mushtaq, M., Jan, M.A. & Rasool, J. 2015, "Role of low dose cytarabine in elderly patients with acute myeloid leukemia: An experience", *South Asian journal of cancer,* vol. 4, no. 1, pp. 4-6.

Berman, E., Arlin, Z.A., Gaynor, J., Miller, W., Gee, T., Kempin, S.J., Mertelsmann, R., Andreeff, M., Reich, L. & Nahmias, N. 1989, "Comparative trial of cytarabine and thioguanine in combination with amsacrine or daunorubicin in patients with untreated acute nonlymphocytic leukemia: results of the L-16M protocol", *Leukemia,* vol. 3, no. 2, pp. 115-121.

Berman, E., Heller, G., Santorsa, J., McKenzie, S., Gee, T., Kempin, S., Gulati, S., Andreeff, M., Kolitz, J. & Gabrilove, J. 1991, "Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia", *Blood,* vol. 77, no. 8, pp. 1666-1674.

Bloomfield, C.D., Lawrence, D., Byrd, J.C., Carroll, A., Pettenati, M.J., Tantravahi, R., Patil, S.R., Davey, F.R., Berg, D.T., Schiffer, C.A., Arthur, D.C. & Mayer, R.J. 1998, "Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype", *Cancer research,* vol. 58, no. 18, pp. 4173-4179.

Borenstein, M., Hedges, L.V., Higgins, J.P. & Rothstein, H.R. 2009, "A basic introduction to fixed-effect and random-effects models for meta-analysis", *Research synthesis methods,* vol. 1, no. 2, pp. 97-111.

Bradstock, K.F., Matthews, J.P., Lowenthal, R.M., Baxter, H., Catalano, J., Brighton, T., Gill, D., Eliadis, P., Joshua, D., Cannell, P., Schwarer, A.P., Durrant, S., Gillett, A., Koutts, J., Taylor, K., Bashford, J., Arthur, C., Enno, A., Dunlop, L., Szer, J., Leahy, M., Juneja, S., Young, G.A. & Australasian Leukaemia and Lymphoma Group 2005, "A randomized trial of high-versus conventional-dose cytarabine in consolidation chemotherapy for adult de novo acute myeloid leukemia in first remission after induction therapy containing high-dose cytarabine", *Blood,* vol. 105, no. 2, pp. 481-488.

Breems, D.A. & Löwenberg, B. 2007, "Acute myeloid leukemia and the position of autologous stem cell transplantation", *Seminars in hematology,* vol. 44, no. 4, pp. 259-266.

Buchner, T., Hiddemann, W., Berdel, W.E., Wormann, B., Schoch, C., Fonatsch, C., Loffler, H., Haferlach, T., Ludwig, W.D., Maschmeyer, G., Staib, P., Aul, C., Gruneisen, A., Lengfelder, E., Frickhofen, N., Kern, W., Serve, H.L., Mesters, R.M., Sauerland, M.C., Heinecke, A. & German AML Cooperative Group 2003, "6-Thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): a randomized trial of the German AML Cooperative Group", *Journal of clinical oncology : official*

*journal of the American Society of Clinical Oncology,* vol. 21, no. 24, pp. 4496-4504.

Burnett, A.K. 2002, "Acute myeloid leukemia: treatment of adults under 60 years", *Reviews in clinical and experimental hematology,* vol. 6, no. 1, pp. 26-45; discussion 86-7.

Burnett, A.K., Hills, R.K., Milligan, D.W., Goldstone, A.H., Prentice, A.G., McMullin, M.F., Duncombe, A., Gibson, B. & Wheatley, K. 2009, "Attempts to optimize induction and consolidation treatment in acute myeloid leukemia: results of the MRC AML12 trial", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 28, no. 4, pp. 586-595.

Burnett, A.K., Milligan, D., Prentice, A.G., Goldstone, A.H., McMullin, M.F., Hills, R.K. & Wheatley, K. 2007, "A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment", *Cancer,* vol. 109, no. 6, pp. 1114-1124.

Burnett, A.K., Russell, N.H., Hills, R.K., Hunter, A.E., Kjeldsen, L., Yin, J., Gibson, B.E., Wheatley, K. & Milligan, D. 2013, "Optimization of chemotherapy for younger patients with acute myeloid leukemia: results of the medical research council AML15 trial", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 31, no. 27, pp. 3360-3368.

Byrd, J.C., Dodge, R.K., Carroll, A., Baer, M.R., Edwards, C., Stamberg, J., Qumsiyeh, M., Moore, J.O., Mayer, R.J., Davey, F., Schiffer, C.A. & Bloomfield, C.D. 1999, "Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 17, no. 12, pp. 3767-3775.

Byrd, J.C., Mrozek, K., Dodge, R.K., Carroll, A.J., Edwards, C.G., Arthur, D.C., Pettenati, M.J., Patil, S.R., Rao, K.W., Watson, M.S., Koduru, P.R., Moore, J.O., Stone, R.M., Mayer, R.J., Feldman, E.J., Davey, F.R., Schiffer, C.A., Larson, R.A., Bloomfield, C.D. & Cancer and Leukemia Group B (CALGB 8461) 2002, "Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461)", *Blood,* vol. 100, no. 13, pp. 4325-4336.

Byrd, J.C., Ruppert, A.S., Mrozek, K., Carroll, A.J., Edwards, C.G., Arthur, D.C., Pettenati, M.J., Stamberg, J., Koduru, P.R., Moore, J.O., Mayer, R.J., Davey, F.R., Larson, R.A. & Bloomfield, C.D. 2004, "Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): results from CALGB 8461", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 22, no. 6, pp. 1087-1094.

Cassileth, P.A., Harrington, D.P., Appelbaum, F.R., Lazarus, H.M., Rowe, J.M., Paietta, E., Willman, C., Hurd, D.D., Bennett, J.M., Blume, K.G., Head, D.R. & Wiernik, P.H. 1998, "Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission", *The New England journal of medicine,* vol. 339, no. 23, pp. 1649-1656.

Cheson, B.D., Bennett, J.M., Kopecky, K.J., Buchner, T., Willman, C.L., Estey, E.H., Schiffer, C.A., Doehner, H., Tallman, M.S., Lister, T.A., Lo-Coco, F., Willemze, R., Biondi, A., Hiddemann, W., Larson, R.A., Lowenberg, B., Sanz, M.A., Head, D.R., Ohno, R., Bloomfield, C.D. & International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia 2003, "Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 21, no. 24, pp. 4642-4649.

Cornelissen, J.J., van Putten, W.L., Verdonck, L.F., Theobald, M., Jacky, E., Daenen, S.M., van Marwijk Kooy, M., Wijermans, P., Schouten, H., Huijgens, P.C., van der Lelie, H., Fey, M., Ferrant, A., Maertens, J., Gratwohl, A. & Lowenberg, B. 2007, "Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom?", *Blood,* vol. 109, no. 9, pp. 3658-3666.

Cornelissen, J.J., Versluis, J., Passweg, J.R., van Putten, W.L., Manz, M.G., Maertens, J., Beverloo, H.B., Valk, P.J., van Marwijk Kooy, M., Wijermans, P.W., Schaafsma, M.R., Biemond, B.J., Vekemans, M.C., Breems, D.A., Verdonck, L.F., Fey, M.F., Jongen-Lavrencic, M., Janssen, J.J., Huls, G., Kuball, J., Pabst, T., Graux, C., Schouten, H.C., Gratwohl, A., Vellenga, E., Ossenkoppele, G., Lowenberg, B., HOVON & SAKK Leukemia Groups 2015, "Comparative therapeutic value of post-remission approaches in patients with acute myeloid leukemia aged 40-60 years", *Leukemia,* vol. 29, no. 5, pp. 1041-1050.

de Lima, M., Strom, S.S., Keating, M., Kantarjian, H., Pierce, S., O'Brien, S., Freireich, E. & Estey, E. 1997, "Implications of potential cure in acute myelogenous leukemia: development of subsequent cancer and return to work", *Blood,* vol. 90, no. 12, pp. 4719-4724.

Döhner, H., Estey, E.H., Amadori, S., Appelbaum, F.R., Buchner, T., Burnett, A.K., Dombret, H., Fenaux, P., Grimwade, D., Larson, R.A., Lo-Coco, F., Naoe, T., Niederwieser, D., Ossenkoppele, G.J., Sanz, M.A., Sierra, J., Tallman, M.S., Lowenberg, B., Bloomfield, C.D. & European LeukemiaNet 2010, "Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet", *Blood,* vol. 115, no. 3, pp. 453-474.

Döhner, H., Weisdorf, D.J. & Bloomfield, C.D. 2015, "Acute Myeloid Leukemia", *The New England journal of medicine,* vol. 373, no. 12, pp. 1136-1152.

Dombret, H., Chastang, C., Fenaux, P., Reiffers, J., Bordessoule, D., Bouabdallah, R., Mandelli, F., Ferrant, A., Auzanneau, G. & Tilly, H. 1995, "A controlled study of recombinant human granulocyte colony-stimulating factor in elderly patients after treatment for acute myelogenous leukemia. AML Cooperative Study Group", *The New England journal of medicine,* vol. 332, no. 25, pp. 1678-1683.

Elonen, E., Almqvist, A., Hanninen, A., Jansson, S.E., Jarventie, G., Koistinen, P., Koivunen, E., Lahtinen, R., Lehtinen, M., Nousiainen, T., Pelliniemi, T.T., Rajamaki, A., Remes, K., Timonen, T., Vilpo, J., Volin, L. & Ruutu, T. 1998, "Comparison between four and eight cycles of intensive chemotherapy in adult acute myeloid leukemia: a randomized trial of the Finnish Leukemia Group", *Leukemia,* vol. 12, no. 7, pp. 1041-1048.

Enoch, D.A., Ludlam, H.A. & Brown, N.M. 2006, "Invasive fungal infections: a review of epidemiology and management options", *Journal of medical microbiology,* vol. 55, no. Pt 7, pp. 809-818.

Estey, E. & Döhner, H. 2006, "Acute myeloid leukaemia", *Lancet (London, England),* vol. 368, no. 9550, pp. 1894-1907.

Farag, S.S., Ruppert, A.S., Mrozek, K., Mayer, R.J., Stone, R.M., Carroll, A.J., Powell, B.L., Moore, J.O., Pettenati, M.J., Koduru, P.R., Stamberg, J., Baer, M.R., Block, A.W., Vardiman, J.W., Kolitz, J.E., Schiffer, C.A., Larson, R.A. & Bloomfield, C.D. 2005, "Outcome of induction and postremission therapy in younger adults with acute myeloid leukemia with normal karyotype: a cancer and leukemia group B study", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 23, no. 3, pp. 482-493.

Fenaux, P., Mufti, G.J., Hellstrom-Lindberg, E., Santini, V., Finelli, C., Giagounidis, A., Schoch, R., Gattermann, N., Sanz, G., List, A., Gore, S.D., Seymour, J.F., Bennett, J.M., Byrd, J., Backstrom, J., Zimmerman, L., McKenzie, D., Beach, C., Silverman, L.R. & International Vidaza High-Risk MDS Survival Study Group 2009, "Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study", *The Lancet.Oncology,* vol. 10, no. 3, pp. 223-232.

Fenaux, P., Mufti, G.J., Hellstrom-Lindberg, E., Santini, V., Gattermann, N., Germing, U., Sanz, G., List, A.F., Gore, S., Seymour, J.F., Dombret, H., Backstrom, J., Zimmerman, L., McKenzie, D., Beach, C.L. & Silverman, L.R. 2010, "Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 28, no. 4, pp. 562-569.

Fopp, M., Fey, M.F., Bacchi, M., Cavalli, F., Gmuer, J., Jacky, E., Schmid, L., Tichelli, A., Tobler, A., Tschopp, L., Von Fliedner, V. & Gratwohl, A. 1997, "Post-remission therapy of adult acute myeloid leukaemia: one cycle of high-dose versus standard-dose cytarabine. Leukaemia Project Group of the Swiss Group for Clinical Cancer Research (SAKK)", *Annals of Oncology : Official Journal of the European Society for Medical Oncology / ESMO,* vol. 8, no. 3, pp. 251-257.

Freeman, S.D., Jovanovic, J.V. & Grimwade, D. 2008, "Development of minimal residual disease-directed therapy in acute myeloid leukemia", *Seminars in oncology,* vol. 35, no. 4, pp. 388-400.

Freireich, E.J. 2000, "Supportive care for patients with blood disorders", *British journal of haematology,* vol. 111, no. 1, pp. 68-77.

Fröhling, S., Skelin, S., Liebisch, C., Scholl, C., Schlenk, R.F., Dohner, H., Dohner, K. & Acute Myeloid Leukemia Study Group, Ulm 2002, "Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 20, no. 10, pp. 2480-2485.

Gardin, C., Turlure, P., Fagot, T., Thomas, X., Terre, C., Contentin, N., Raffoux, E., de Botton, S., Pautas, C., Reman, O., Bourhis, J.H., Fenaux, P., Castaigne, S., Michallet, M., Preudhomme, C., de Revel, T., Bordessoule, D. & Dombret, H. 2007, "Postremission treatment of elderly patients with acute myeloid leukemia in first complete remission after intensive induction chemotherapy: results of the multicenter randomized Acute Leukemia French Association (ALFA) 9803 trial", *Blood,* vol. 109, no. 12, pp. 5129-5135.

Grimwade, D. 2001, "The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia", *Best practice & research.Clinical haematology,* vol. 14, no. 3, pp. 497-529.

Grimwade, D., Walker, H., Oliver, F., Wheatley, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A. & Goldstone, A. 1998, "The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties", *Blood,* vol. 92, no. 7, pp. 2322-2333.

Hansen, O.P., Pedersen-Bjergaard, J., Ellegaard, J., Brincker, H., Boesen, A.M., Christensen, B.E., Drivsholm, A., Hippe, E., Jans, H. & Jensen, K.B. 1991, "Aclarubicin plus cytosine arabinoside versus daunorubicin plus cytosine arabinoside in previously untreated patients with acute myeloid leukemia: a Danish national phase III trial. The Danish Society of Hematology Study Group on AML, Denmark", *Leukemia,* vol. 5, no. 6, pp. 510-516.

Harousseau, J.L., Cahn, J.Y., Pignon, B., Witz, F., Milpied, N., Delain, M., Lioure, B., Lamy, T., Desablens, B., Guilhot, F., Caillot, D., Abgrall, J.F., Francois, S.,

Briere, J., Guyotat, D., Casassus, P., Audhuy, B., Tellier, Z., Hurteloup, P. & Herve, P. 1997, "Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM)", *Blood,* vol. 90, no. 8, pp. 2978-2986.

Hartling L, Hamm M, Milne A, et al. 2012, "Validity and Inter-Rater Reliability Testing of Quality Assessment Instruments [Internet]. Appendix B, Guidelines for Risk of Bias Assessments.", *Rockville (MD): Agency for Healthcare Research and Quality (US),* .

Helfand, M. & Balshem, H. 2010, "AHRQ series paper 2: principles for developing guidance: AHRQ and the effective health-care program", *Journal of clinical epidemiology,* vol. 63, no. 5, pp. 484-490.

Hengeveld, M., Suciu, S., Karrasch, M., Specchia, G., Marie, J.P., Muus, P., Petti, M.C., Rotoli, B., Amadori, S., Fioritoni, G., Leoni, P., Morra, E., Thaler, J., Resegotti, L., Fazi, P., Vignetti, M., Mandelli, F., Zittoun, R. & de Witte, T. 2012, "Intensive consolidation therapy compared with standard consolidation and maintenance therapy for adults with acute myeloid leukaemia aged between 46 and 60 years: final results of the randomized phase III study (AML 8B) of the European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups", *Annals of Hematology,* vol. 91, no. 6, pp. 825-835.

Higgins, J.P. & Thompson, S.G. 2002, "Quantifying heterogeneity in a meta-analysis", *Statistics in medicine,* vol. 21, no. 11, pp. 1539-1558.

Higgins, J.P., Thompson, S.G., Deeks, J.J. & Altman, D.G. 2003, "Measuring inconsistency in meta-analyses", *BMJ (Clinical research ed.),* vol. 327, no. 7414, pp. 557-560.

Juliusson, G., Antunovic, P., Derolf, A., Lehmann, S., Mollgard, L., Stockelberg, D., Tidefelt, U., Wahlin, A. & Hoglund, M. 2009, "Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry", *Blood,* vol. 113, no. 18, pp. 4179-4187.

Juliusson, G., Lazarevic, V., Horstedt, A.S., Hagberg, O., Hoglund, M. & Swedish Acute Leukemia Registry Group 2012, "Acute myeloid leukemia in the real world: why population-based registries are needed", *Blood,* vol. 119, no. 17, pp. 3890-3899.

Kita, K., Nakase, K., Miwa, H., Masuya, M., Nishii, K., Morita, N., Takakura, N., Otsuji, A., Shirakawa, S. & Ueda, T. 1992, "Phenotypical characteristics of acute myelocytic leukemia associated with the t(8;21)(q22;q22) chromosomal abnormality: frequent expression of immature B-cell antigen CD19 together with stem cell antigen CD34", *Blood,* vol. 80, no. 2, pp. 470-477.

Koreth, J., Schlenk, R., Kopecky, K.J., Honda, S., Sierra, J., Djulbegovic, B.J., Wadleigh, M., DeAngelo, D.J., Stone, R.M., Sakamaki, H., Appelbaum, F.R., Dohner, H., Antin, J.H., Soiffer, R.J. & Cutler, C. 2009, "Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials", *Jama,* vol. 301, no. 22, pp. 2349-2361.

Leibovici, L., Paul, M., Cullen, M., Bucaneve, G., Gafter-Gvili, A., Fraser, A. & Kern, W.V. 2006, "Antibiotic prophylaxis in neutropenic patients: new evidence, practical decisions", *Cancer,* vol. 107, no. 8, pp. 1743-1751.

Leith, C.P., Kopecky, K.J., Godwin, J., McConnell, T., Slovak, M.L., Chen, I.M., Head, D.R., Appelbaum, F.R. & Willman, C.L. 1997, "Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study", *Blood,* vol. 89, no. 9, pp. 3323-3329.

Levis, M., Allebach, J., Tse, K.F., Zheng, R., Baldwin, B.R., Smith, B.D., Jones-Bolin, S., Ruggeri, B., Dionne, C. & Small, D. 2002, "A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo", *Blood,* vol. 99, no. 11, pp. 3885-3891.

Li, W., Gong, X., Sun, M., Zhao, X., Gong, B., Wei, H., Mi, Y. & Wang, J. 2014, "High-dose cytarabine in acute myeloid leukemia treatment: a systematic review and meta-analysis", *PloS one,* vol. 9, no. 10, pp. e110153.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P., Clarke, M., Devereaux, P.J., Kleijnen, J. & Moher, D. 2009, "The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration", *Journal of clinical epidemiology,* vol. 62, no. 10, pp. e1-34.

Löwenberg, B. 2013, "Sense and nonsense of high-dose cytarabine for acute myeloid leukemia", *Blood,* vol. 121, no. 1, pp. 26-28.

Löwenberg, B., Griffin, J.D. & Tallman, M.S. 2003, "Acute myeloid leukemia and acute promyelocytic leukemia", *Hematology / the Education Program of the American Society of Hematology.American Society of Hematology.Education Program,* , pp. 82-101.

Löwenberg, B., Ossenkoppele, G.J., van Putten, W., Schouten, H.C., Graux, C., Ferrant, A., Sonneveld, P., Maertens, J., Jongen-Lavrencic, M., von Lilienfeld-Toal, M., Biemond, B.J., Vellenga, E., van Marwijk Kooy, M., Verdonck, L.F., Beck, J., Dohner, H., Gratwohl, A., Pabst, T., Verhoef, G., Dutch-Belgian Cooperative Trial Group for Hemato-Oncology (HOVON), German AML Study Group (AMLSG) & Swiss Group for Clinical Cancer Research (SAKK) Collaborative Group 2009, "High-dose daunorubicin in older patients with acute myeloid leukemia", *The New England journal of medicine,* vol. 361, no. 13, pp. 1235-1248.

Luger, S.M. 2010, "Treating the elderly patient with acute myelogenous leukemia", *Hematology / the Education Program of the American Society of Hematology.American Society of Hematology.Education Program,* vol. 2010, pp. 62-69.

Mayer, R.J., Davis, R.B., Schiffer, C.A., Berg, D.T., Powell, B.L., Schulman, P., Omura, G.A., Moore, J.O., McIntyre, O.R. & Frei, E.,3rd 1994, "Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B", *The New England journal of medicine,* vol. 331, no. 14, pp. 896-903.

Medeiros, B.C., Satram-Hoang, S., Hurst, D., Hoang, K.Q., Momin, F. & Reyes, C. 2015, "Big data analysis of treatment patterns and outcomes among elderly acute myeloid leukemia patients in the United States", *Annals of Hematology,* vol. 94, no. 7, pp. 1127-1138.

Meijer, E. & Cornelissen, J.J. 2008, "Allogeneic stem cell transplantation in acute myeloid leukemia in first or subsequent remission: weighing prognostic markers predicting relapse and risk factors for non-relapse mortality", *Seminars in oncology,* vol. 35, no. 4, pp. 449-457.

Miyawaki, S., Ohtake, S., Fujisawa, S., Kiyoi, H., Shinagawa, K., Usui, N., Sakura, T., Miyamura, K., Nakaseko, C., Miyazaki, Y., Fujieda, A., Nagai, T., Yamane, T., Taniwaki, M., Takahashi, M., Yagasaki, F., Kimura, Y., Asou, N., Sakamaki, H., Handa, H., Honda, S., Ohnishi, K., Naoe, T. & Ohno, R. 2011, "A randomized comparison of 4 courses of standard-dose multiagent chemotherapy versus 3 courses of high-dose cytarabine alone in postremission therapy for acute myeloid leukemia in adults: the JALSG AML201 Study", *Blood,* vol. 117, no. 8, pp. 2366-2372.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. & PRISMA Group 2009, "Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement", *Journal of clinical epidemiology,* vol. 62, no. 10, pp. 1006-1012.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A. & PRISMA-P Group 2015, "Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement", *Systematic reviews,* vol. 4, pp. 1-4053-4-1.

Montalban-Bravo, G. & Garcia-Manero, G. 2015, "Novel drugs for older patients with acute myeloid leukemia", *Leukemia,* vol. 29, no. 4, pp. 760-769.

Moore, J.O., George, S.L., Dodge, R.K., Amrein, P.C., Powell, B.L., Kolitz, J.E., Baer, M.R., Davey, F.R., Bloomfield, C.D., Larson, R.A. & Schiffer, C.A. 2005, "Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B Study 9222", *Blood,* vol. 105, no. 9, pp. 3420-3427.

Mrozek, K. 2008, "Cytogenetic, molecular genetic, and clinical characteristics of acute myeloid leukemia with a complex karyotype", *Seminars in oncology,* vol. 35, no. 4, pp. 365-377.

Mrozek, K., Heerema, N.A. & Bloomfield, C.D. 2004, "Cytogenetics in acute leukemia", *Blood reviews,* vol. 18, no. 2, pp. 115-136.

Mrozek, K., Marcucci, G., Nicolet, D., Maharry, K.S., Becker, H., Whitman, S.P., Metzeler, K.H., Schwind, S., Wu, Y.Z., Kohlschmidt, J., Pettenati, M.J., Heerema, N.A., Block, A.W., Patil, S.R., Baer, M.R., Kolitz, J.E., Moore, J.O., Carroll, A.J., Stone, R.M., Larson, R.A. & Bloomfield, C.D. 2012, "Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 30, no. 36, pp. 4515-4523.

Parmar, M.K., Torri, V. & Stewart, L. 1998, "Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints", *Statistics in medicine,* vol. 17, no. 24, pp. 2815-2834.

Pearson, A., Wiechula, R., Court, A. & Lockwood, C. 2005, "The JBI model of evidence-based healthcare", *International journal of evidence-based healthcare,* vol. 3, no. 8, pp. 207-215.

Petticrew, M. & Roberts, H. 2008, "Systematic reviews--do they 'work' in informing decision-making around health inequalities?", *Health economics, policy, and law,* vol. 3, no. Pt 2, pp. 197-211.

Rowe, J.M. 2009, "Optimal induction and post-remission therapy for AML in first remission", *Hematology / the Education Program of the American Society of Hematology.American Society of Hematology.Education Program,* , pp. 396-405.

Rowe, J.M., Andersen, J.W., Mazza, J.J., Bennett, J.M., Paietta, E., Hayes, F.A., Oette, D., Cassileth, P.A., Stadtmauer, E.A. & Wiernik, P.H. 1995, "A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (> 55 to 70 years of age) with acute myelogenous leukemia: a study of the Eastern Cooperative Oncology Group (E1490)", *Blood,* vol. 86, no. 2, pp. 457-462.

Schaich, M., Parmentier, S., Kramer, M., Illmer, T., Stolzel, F., Rollig, C., Thiede, C., Hanel, M., Schafer-Eckart, K., Aulitzky, W., Einsele, H., Ho, A.D., Serve, H., Berdel, W.E., Mayer, J., Schmitz, N., Krause, S.W., Neubauer, A., Baldus, C.D., Schetelig, J., Bornhauser, M. & Ehninger, G. 2013, "High-dose cytarabine consolidation with or without additional amsacrine and mitoxantrone in acute myeloid leukemia: results of the prospective randomized AML2003 trial", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 31, no. 17, pp. 2094-2102.

Schiffer, C.A., Anderson, K.C., Bennett, C.L., Bernstein, S., Elting, L.S., Goldsmith, M., Goldstein, M., Hume, H., McCullough, J.J., McIntyre, R.E., Powell, B.L., Rainey, J.M., Rowley, S.D., Rebulla, P., Troner, M.B., Wagnon, A.H. & American Society of Clinical Oncology 2001, "Platelet transfusion for patients with cancer: clinical practice guidelines of the American Society of Clinical Oncology", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 19, no. 5, pp. 1519-1538.

Schlenk, R.F. 2014, "Post-remission therapy for acute myeloid leukemia", *Haematologica,* vol. 99, no. 11, pp. 1663-1670.

Schlenk, R.F., Benner, A., Hartmann, F., del Valle, F., Weber, C., Pralle, H., Fischer, J.T., Gunzer, U., Pezzutto, A., Weber, W., Grimminger, W., Preiss, J., Hensel, M., Frohling, S., Dohner, K., Haas, R., Dohner, H. & AML Study Group Ulm (AMLSG ULM) 2003, "Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial", *Leukemia,* vol. 17, no. 8, pp. 1521-1528.

Schlenk, R.F., Dohner, K., Krauter, J., Frohling, S., Corbacioglu, A., Bullinger, L., Habdank, M., Spath, D., Morgan, M., Benner, A., Schlegelberger, B., Heil, G., Ganser, A., Dohner, H. & German-Austrian Acute Myeloid Leukemia Study Group 2008, "Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia", *The New England journal of medicine,* vol. 358, no. 18, pp. 1909-1918.

Sekeres, M.A., Elson, P., Kalaycio, M.E., Advani, A.S., Copelan, E.A., Faderl, S., Kantarjian, H.M. & Estey, E. 2009, "Time from diagnosis to treatment initiation predicts survival in younger, but not older, acute myeloid leukemia patients", *Blood,* vol. 113, no. 1, pp. 28-36.

Sill, H., Olipitz, W., Zebisch, A., Schulz, E. & Wolfler, A. 2011, "Therapy-related myeloid neoplasms: pathobiology and clinical characteristics", *British journal of pharmacology,* vol. 162, no. 4, pp. 792-805.

Slovak, M.L., Kopecky, K.J., Cassileth, P.A., Harrington, D.H., Theil, K.S., Mohamed, A., Paietta, E., Willman, C.L., Head, D.R., Rowe, J.M., Forman, S.J. & Appelbaum, F.R. 2000, "Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study", *Blood,* vol. 96, no. 13, pp. 4075-4083.

Smith, B.D., Levis, M., Beran, M., Giles, F., Kantarjian, H., Berg, K., Murphy, K.M., Dauses, T., Allebach, J. & Small, D. 2004a, "Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia", *Blood,* vol. 103, no. 10, pp. 3669-3676.

Smith, M., Barnett, M., Bassan, R., Gatta, G., Tondini, C. & Kern, W. 2004b, "Adult acute myeloid leukaemia", *Critical reviews in oncology/hematology,* vol. 50, no. 3, pp. 197-222.

Stone, R.M., Berg, D.T., George, S.L., Dodge, R.K., Paciucci, P.A., Schulman, P., Lee, E.J., Moore, J.O., Powell, B.L. & Schiffer, C.A. 1995, "Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. Cancer and Leukemia Group B", *The New England journal of medicine,* vol. 332, no. 25, pp. 1671-1677.

Stone, R.M., Berg, D.T., George, S.L., Dodge, R.K., Paciucci, P.A., Schulman, P.P., Lee, E.J., Moore, J.O., Powell, B.L., Baer, M.R., Bloomfield, C.D. & Schiffer, C.A. 2001, "Postremission therapy in older patients with de novo acute myeloid leukemia: a randomized trial comparing mitoxantrone and intermediate-dose cytarabine with standard-dose cytarabine", *Blood,* vol. 98, no. 3, pp. 548-553.

Stone, R.M., DeAngelo, D.J., Klimek, V., Galinsky, I., Estey, E., Nimer, S.D., Grandin, W., Lebwohl, D., Wang, Y., Cohen, P., Fox, E.A., Neuberg, D., Clark, J., Gilliland, D.G. & Griffin, J.D. 2005, "Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412", *Blood,* vol. 105, no. 1, pp. 54-60.

Suciu, S., Mandelli, F., de Witte, T., Zittoun, R., Gallo, E., Labar, B., De Rosa, G., Belhabri, A., Giustolisi, R., Delarue, R., Liso, V., Mirto, S., Leone, G., Bourhis, J.H., Fioritoni, G., Jehn, U., Amadori, S., Fazi, P., Hagemeijer, A., Willemze, R. & EORTC and GIMEMA Leukemia Groups 2003, "Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial", *Blood,* vol. 102, no. 4, pp. 1232-1240.

Swerdlow, S., Campo, E., Harris, N. & et al. 2008, "WHO Classification of tumours of haematopoietic and lymphoid tissues", *IARC Press,* .

Tallman, M.S., Dewald, G.W., Gandham, S., Logan, B.R., Keating, A., Lazarus, H.M., Litzow, M.R., Mehta, J., Pedersen, T., Perez, W.S., Rowe, J.M., Wetzler, M. & Weisdorf, D.J. 2007, "Impact of cytogenetics on outcome of matched unrelated donor hematopoietic stem cell transplantation for acute myeloid leukemia in first or second complete remission", *Blood,* vol. 110, no. 1, pp. 409-417.

Tallman, M.S., Gilliland, D.G. & Rowe, J.M. 2005, "Drug therapy for acute myeloid leukemia", *Blood,* vol. 106, no. 4, pp. 1154-1163.

Thomas, X., Elhamri, M., Raffoux, E., Renneville, A., Pautas, C., de Botton, S., de Revel, T., Reman, O., Terre, C., Gardin, C., Chelghoum, Y., Boissel, N., Quesnel, B., Hicheri, Y., Bourhis, J.H., Fenaux, P., Preudhomme, C., Michallet, M., Castaigne, S. & Dombret, H. 2011, "Comparison of high-dose cytarabine and timed-sequential chemotherapy as consolidation for younger adults with AML in first remission: the ALFA-9802 study", *Blood,* vol. 118, no. 7, pp. 1754-1762.

Tierney, J.F., Stewart, L.A., Ghersi, D., Burdett, S. & Sydes, M.R. 2007, "Practical methods for incorporating summary time-to-event data into meta-analysis", *Trials,* vol. 8, pp. 16.

Vardiman, J.W., Thiele, J., Arber, D.A., Brunning, R.D., Borowitz, M.J., Porwit, A., Harris, N.L., Le Beau, M.M., Hellstrom-Lindberg, E., Tefferi, A. & Bloomfield, C.D. 2009, "The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes", *Blood,* vol. 114, no. 5, pp. 937-951.

Vogler, W.R., Velez-Garcia, E., Weiner, R.S., Flaum, M.A., Bartolucci, A.A., Omura, G.A., Gerber, M.C. & Banks, P.L. 1992, "A phase III trial comparing idarubicin and daunorubicin in combination with cytarabine in acute myelogenous leukemia: a Southeastern Cancer Study Group Study", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 10, no. 7, pp. 1103-1111.

Volger, W.R., Weiner, R.S., Moore, J.O., Omura, G.A., Bartolucci, A.A. & Stagg, M. 1995, "Long-term follow-up of a randomized post-induction therapy trial in acute myelogenous leukemia (a Southeastern Cancer Study Group trial)", *Leukemia,* vol. 9, no. 9, pp. 1456-1460.

Wang, E.S. 2014, "Treating acute myeloid leukemia in older adults", *Hematology / the Education Program of the American Society of Hematology.American Society of Hematology.Education Program,* vol. 2014, no. 1, pp. 14-20.

Weick, J.K., Kopecky, K.J., Appelbaum, F.R., Head, D.R., Kingsbury, L.L., Balcerzak, S.P., Bickers, J.N., Hynes, H.E., Welborn, J.L., Simon, S.R. & Grever, M. 1996, "A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study", *Blood,* vol. 88, no. 8, pp. 2841-2851.

Weisberg, E., Boulton, C., Kelly, L.M., Manley, P., Fabbro, D., Meyer, T., Gilliland, D.G. & Griffin, J.D. 2002, "Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412", *Cancer cell,* vol. 1, no. 5, pp. 433-443.

Wiernik, P.H., Banks, P.L., Case, D.C.,Jr, Arlin, Z.A., Periman, P.O., Todd, M.B., Ritch, P.S., Enck, R.E. & Weitberg, A.B. 1992, "Cytarabine plus idarubicin or daunorubicin as induction and consolidation therapy for previously untreated adult patients with acute myeloid leukemia", *Blood,* vol. 79, no. 2, pp. 313-319.

Zittoun, R., Suciu, S., Mandelli, F., de Witte, T., Thaler, J., Stryckmans, P., Hayat, M., Peetermans, M., Cadiou, M., Solbu, G., Petti, M.C. & Willemze, R. 1996, "Granulocyte-macrophage colony-stimulating factor associated with induction treatment of acute myelogenous leukemia: a randomized trial by the European Organization for Research and Treatment of Cancer Leukemia Cooperative Group", *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* vol. 14, no. 7, pp. 2150-2159.

# 6 Appendix

### The Prisma 2009 Checklist applied on our systematic review and meta-analysis

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | **cover page** |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | **IV** |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | **1 ff.** |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | **23** |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | **-** |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | **28** |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | **28** |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | **29** |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | **30** |

| | | | |
|---|---|---|---|
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | **31** |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | **30** |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | **37 ff.** |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | **31** |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | **32** |
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | **37 ff.** |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | **-** |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | **33** |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | **35 – 36** |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | **37 ff.** |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | **43 ff.** |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | **43 ff.** |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | **37 ff.** |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | **-** |
| **DISCUSSION** | | | |

| | | | |
|---|---|---|---|
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | **51 ff.** |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | **56** |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | **57** |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | **-** |

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