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ABBREVIATIONS

ALL  acute lymphoblastic leukaemia
AML  acute myeloid leukaemia
ANC  absolute neutrophil count
ANLL  acute nonlymphocytic leukaemia
AraC  arabinofuranosyl cytidine (Cytarabine)
AUC  area under the curve
BMT  bone marrow transplantation
C<sub>max</sub>  maximum plasma concentration of the drug
CK-AML  complex karyotype AML
CLL  chronic lymphocytic leukaemia
CML  chronic myelogenous leukaemia
CNI  calcineurin inhibitor
CNS  central nervous system
CR  complete remission
d  days
DFS  disease free survival
DLI  donor lymphocyte infusion
DNR  daunorubicin
ECOG  Eastern Cooperative Oncology Group
EFS  event free survival
EORTC  European Organization for Research and Treatment of Cancer
FAB  French-American-British leukaemia classification
FLAG  fludarabine, AraC, G-CSF
FLAG-IDA  fludarabine, AraC, G-CSF, idarubicin
G-CSF  granulocyte colony-stimulating factor
GIMEMA  Gruppo Italiano Malattie EMatologiche dell’Adulto
GvL  graft versus leukaemia
Gy  Gray
HAM  high-dose AraC + mitoxantrone
HD-AraC  high-dose AraC
HiDAC  high-dose AraC
HMAC  high dose AraC and mitoxantrone
HOVON  Stichting Hemato-Oncologie voor Volwassenen Nederland (Dutch-Belgian Cooperative Trial Group for Haematology Oncology)
HR  hazard risk
IDAc  intermediate dose AraC
IMAc  intermediate dose AraC and mitoxantrone
iv    intravenous
LAME  Leucémie Aigue Myéloblastique Enfant
LFS   leukaemia free survival
LPI   labiles plasma iron
LVEF  left ventricular ejection fraction
MAMAc m-AMSA + AraC
MAv   mitoxantrone + AraC + VP-16
MRC   medical research council
MTD   maximal tolerated dose
MUGA scan multi gated acquisition scan
ns    non significant
NRM   non relapse mortality
NTBI  non-transferrin-bound iron
ORR   overall response rate
OS    overall survival
PMN   polymorphonuclear leukocytes
p.o.  per os
PR    partial response
pts   patients
RAEB  refractory anemia with excess blasts
RFS   relapse-free survival
sAML  secondary AML
tAML  therapy associated AML
SAL   Studien-Allianz Leukämie (study-alliance leukaemia)
SAKK  Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung
       (Swiss Group for Clinical Cancer Research)
SCT   stem cell transplantation
T½   half life
TAA  6-thioguanine + AraC + Amsacrine
TAD  6-thioguanine + AraC + daunorubicin
TBI   total body irradiation
TRM   therapy related mortality
VP16  etoposide
WBC   white blood cell count
FORWORD

ABOUT AMEKRIN®, AMSIDINE® AND AMSIDYL®

Amsacrine is an acridine derivative developed by Auckland Cancer Research Laboratory, New Zealand, and synthesised by Cain et al. in 1974. Amsacrine was developed to be a potent intercalating antineoplastic agent, and the first synthetic topoisomerase II inhibitor, effective in the treatment of acute myeloid leukaemia (AML) and acute lymphatic leukaemia (ALL). Auckland Cancer Research Laboratory entered into cooperation with Parke-Davis with whom Auckland Cancer Research Laboratory had close research collaborations. Amsacrine entered into clinical trials in 1976 and in 1982 it became available for the clinical treatment of leukaemia in adults in France, Belgium and the Netherlands.

Amsacrine was invented, produced and marketed by Parke-Davis. Parke-Davis was acquired by Warner-Lambert in the seventies, which in turn was bought by Pfizer in 2000. At that time Amsacrine was available in 28 countries.

In 2008, NordMedica A/S acquired all remaining MA’s including; Sweden, the Netherlands, Belgium, Luxembourg, Germany and Switzerland, all Pfizer’s inventory including; active pharmaceutical ingredient (API), finished manufactured products, packaging material, technical data, know-how and the Trade Marks Amekrin®, Amsidine® and Amsidyl®.

NordMedica A/S has established a new supply chain to secure a safe and continuous supply of Amsidyl®/Amsidine®/Amekrin® for all patients. Amsidyl®/Amsidine®/Amekrin® is sold as a registered product or on named patient basis in more than 25 countries worldwide.
1 INTRODUCTION

1.1 EPIDEMIOLOGY OF ACUTE LEUKAEMIAS

The annual incidence of acute myeloid leukaemia is around 2.5/100,000 and increases by age up to 12.13/100,000 in the age group above 65 years. The median age at diagnosis is 62 years.

Acute lymphatic leukaemia is the most common leukaemia in children, the annual incidence in adults is around 1/100,000 (ZENHÄUßERN 2003). Acute lymphoblastic leukaemia (ALL) in the elderly is a rare disease and has a poor prognosis. According to registry data, the incidence of ALL increases from 0.39/100,000 in adults aged 35–39 years to 2.1/100,000 in patients above 85 years. Moreover, approximately 30% of adult ALL cases arise in patients aged 60 years or older (ROBAK 2004).

1.2 ACUTE MYELOID LEUKAEMIA (AML)

Acute myeloid leukaemia (AML) is a malignant blood disorder characterized by blocked or impaired differentiation of hematopoietic stem cells, resulting in abnormal accumulation of immature precursors and suppression of growth and maturation of normal hematopoiesis (FERRARA 2004).

AML is the most common form of acute leukaemia among adults with an incidence that increases with age. Standard treatment of AML comprises one or two cycles of chemotherapy to induce complete remission (CR) followed by postremission treatment in order to prevent relapse of the disease (= consolidation therapy). This may include cycles of chemotherapy, autologous or allogeneic stem cell transplantation.

Induction therapy is in all FAB subtypes except M3 usually a combination with cytarabine (AraC) and an anthracycline (such as daunorubicin or idarubicin). This induction chemotherapy regimen is known as “7+3” (or “3+7”), because the cytarabine is given as a continuous IV infusion for seven consecutive days while the anthracycline is given for three consecutive days as an IV push.

Other alternative induction regimens, including high-dose cytarabine alone, investigational agents or combination regimens containing cytarabine, Amsacrine and other agents, may also be used. Because of the toxic effects of therapy, including myelosuppression and an increased risk of infection, induction chemotherapy may not be offered to the very elderly, and the options may include less intense chemotherapy or palliative care.

The great majority of patients with AML are older than 60 years. Median age is around 70 years, and physicians or patients are reluctant to undertake intensive chemotherapy. High age independently defines unfavorable disease outcome. Reduced anthracycline sensitivity has been reported in particular in older patients with AML. There is a prevailing opinion that intensive remission induction chemotherapy in the so-called fit elderly provides an outcome that overall is superior to a wait-and-watch approach or dose-attenuated cytoreductive treatment (BURNETT 2011).

Modern induction chemotherapy will result in complete remission in 50%–90% of patients with de novo disease, but between 10 and 25% of patients will have primary refractory disease and the majority of those who gain remission will relapse within 3 years of diagnosis (KELL 2006). After achieving first complete remission, favourable, intermediate, and poor-risk AML have probabilities of relapse of approximately 30%, 50% and 80%, respectively, and probabilities of overall survival of 60%, 40%, and 10%–20% (LODEWYCK 2008).
1.2.1 DEFINITION OF AML RELAPSE
Hematologic relapse is defined as the reappearance of blast cells to constitute more than 5% of the cells in the bone marrow and is generally considered as an absolute indication for treatment (FERRARA 2004).

1.2.2 PROGNOSTIC FACTORS FOR RELAPSED AML
Different clinical and prognostic parameters have a major role in predicting prognosis in newly diagnosed AML patients. In particular, age, cytogenetics and early blast clearance have been found to be significantly related to CR rate and survival, better results being achievable in young adult patients with favourable cytogenetics. As far as cytogenetic abnormalities are concerned, two substantially overlapping classification systems have been proposed by the United Kingdom Medical Research Council (MRC) Cooperative Group and the South West Oncology Group from the United States. Accordingly, three groups can be distinguished:

(i) a better prognosis group including those with t(8;21), inv(16) and t(16;16), accounting for 10%-15% of patients and more commonly occurring below the age of 60 years;

(ii) a group with an unfavourable prognosis, more frequently comprising elderly patients, with monosomies or long arm deletions of chromosomes 5 and 7 and abnormalities involving 3 or more chromosomes (complex karyotype);

(iii) an intermediate group accounting for 50%-60% of cases including normal karyotypes and all other aberrations.

While the prognostic relevance of karyotype in primary AML is well established and universally accepted, the value of cytogenetics at diagnosis for relapsed patients is not so well defined (FERRARA 2004). The probability of achieving a second remission is highly dependent on the duration of the first remission (CR1). Patients who relapse after a short duration of remission (in most studies the definition for short is a relapse within 12 months) have a grave prognosis (KIMBY 2001).

1.2.3 TREATMENT OF RELAPSED AML
Treatment of relapsed leukaemia is difficult and well-controlled trials in this group of patients are uncommon. Usually, patients are recruited to relatively small phase I and phase II trials examining the potential role for new drug approaches and many combination regimens based around high doses of cytarabine arabinoside, substitution of the anthracyclines mitoxantrone or idarubicin for daunorubicin or using Amsacrine have become established (KELL 2006). Also combinations of daunorubicin, cytarabine with fludarabine (DAF) or cladribine (DAC) were evaluated (HOLOWIECKI 2008). For patients with relapsed AML, the only proven potentially curative therapy is a haematopoietic stem cell transplant, if one has not already been performed.

1.2.4 PRIMARY REFRACTORY AML
AML patients who fail to achieve CR after first line chemotherapy have a very poor prognosis, and it is generally accepted that few cures can be achieved at this stage using chemotherapy alone. Failure generally occurred because of adverse biological determinants including multidrug resistance mechanisms. Obtaining long-term survival in these patients presents an important clinical challenge. Therapeutic options thus consist in dose escalation or total change of therapy. Classically, the only curative approach is haematopoietic stem cell transplantation (HSCT). Intensive reinduction therapy may therefore be justified in primary resistant AML patients. Indeed, the main goal in young adults with primary resistant AML is to achieve a complete remission (CR) so they can undergo HSCT (REVESZ 2003).
1.3 ACUTE LYMPHOID LEUKAEMIA
Acute lymphoblastic leukaemia (ALL) is a form of leukaemia characterized by excess lymphoblasts. Age at diagnosis is an independent poor-prognostic factor in ALL (ROBAK 2004).

1.3.1 TREATMENT OF ALL
The earlier acute lymphocytic leukaemia is detected, the more effective the treatment. The aim is to induce a lasting remission, defined as the absence of detectable cancer cells in the body (usually less than 5% blast cells on the bone marrow). Treatment for acute leukaemia can include chemotherapy, steroids, radiation therapy, intensive combined treatments (including bone marrow or stem cell transplants) and growth factors. Standard induction of adult ALL comprises at least a glucocorticoid, vincristine, and an anthracycline. Prednisone was replaced by dexamethasone in several trials based on paediatric results.

Intensive consolidation is standard in the treatment of ALL based on paediatric studies and historic comparisons. Several studies have also demonstrated that a modified induction (reinduction) improves outcome. The consolidation may contain cycles with new combination regimens (VP16, Amsacrine, mitoxantrone, idarubicine or other) (HOELZER 2009).

Maintenance to a total treatment duration of 2.5 years even after intensive induction and consolidation remains standard for adult ALL; all attempts to omit maintenance have led to inferior outcome.

Despite a great number of trials, the indications for SCT in first CR, scheduling, and conditioning regimens are still not defined satisfactorily. The parameters relevant for comparison of chemotherapy and HSCT are continuously changing. For example, there is a decreasing mortality with unrelated HSCT but also an improving survival with optimized chemotherapy. SCT offers an advantage in adult high-risk patients and has contributed to improved outcomes. However, procedures need to be optimized, including age limits for dose-reduced conditioning (GÖGBUGET 2009).

1.3.2 RELAPSED / REFRACTORY ALL
Although the overall prognosis for patients with adult acute lymphoblastic leukaemia (ALL) has improved to the point where 25%–35% may achieve prolonged disease-free survival (DFS), the majority of patients continue to relapse, and some are refractory to induction therapy. The strategy for these relapsed/refractory patients is reinduction chemotherapy followed by allogeneic stem cell transplantation, provided that the toxicity of the salvage treatment is acceptable. Most patients have already been exposed to intensive multiagent chemotherapy and most reinduction regimens in current use cause substantial toxicity (SPECCHIA 2005).

Currently available salvage regimens for adults with relapsed or refractory ALL produce CR in 20%–50% of cases, but these remissions last less than 4 months in most cases. Thus the best chance for these patients appears to be low-toxicity treatment-inducing CR, followed by intensification with allogeneic stem cell transplantation as soon as possible (SPECCHIA 2005).
2 AMSACRINE DEVELOPMENT

AMSIDINE®/AMSIDYL®/AMEKRIN® (AMSACRINE HYDROCHLORIDE)
Structural and molecular formulae and relative molecular mass

\[
\text{CH}_3
\]
\[
\text{O=S=O}
\]
\[
\text{NH}
\]
\[
\text{H}_2\text{CO}
\]
\[
\text{NH}
\]
\[
\text{N}
\]

C21H19N3O3S Relative molecular mass: 393.47
(Amsacrine – PubChem, Public Chemical Database, 2009)

2.1 COMPOUND SUMMARY
Amsacrine is also known as m-AMSA, meta-Amsacrine, Acridinylanisidide, Lamisine, Amecrin, and Amsine. Amsacrine [4-(9-acridinylamino)methanesulfon-m-anisidine] is an acridine derivative that was originally synthesized by Cain et al. in 1974 and that was found to have a potent antitumor activity against leukemic cells (TAVENIER 2003).

Amsacrine is a potent intercalating antineoplastic agent, effective in the treatment of acute leukaemia and malignant lymphoma. It is used frequently in combination therapy in various protocols. It produces predictable but acceptable myelosuppression and gives only cardiotoxic effects in 1%-2% of patients. (Material safety data sheet, 22 April 2009, NordMedica A/S).

2.2 INTRODUCTION AMSACRINE
Early data showed therapeutic activity in AML, blastic CML, and CLL; besides activity in leukaemia, the drug also induced phase I responses in Hodgkin’s disease, haepatoma and epidermoid carcinoma of the pesophagus. The recommended dosage in patients with solid tumours is 90-120 mg/m² intravenously every 3-4 weeks. Despite the encouraging reports from experimental models, m-AMSA has shown no real impact in the treatment of patients with a wide variety of solid tumours (HORNEDO 1985).

As single substance in previously treated acute leukaemia the best therapeutic results were observed when Amsacrine was used at a dose level of 75 to 90 mg/m²/d for 7 days (LEGHA 1980).

Patients with leukaemia appeared to tolerate doses of 90 to 150 mg/m²/d for five days. The MTDs found among paediatric patients (150 mg/m²/d) were similar to those observed in adults (LOUIE 1985). The recommended dose for phase II evaluation was 120 mg/m²/d for 5 days q 3 weeks, given as a 30 minute infusion.

Subsequent phase II studies in leukemic patients showed convincing activity in relapsed AML, with response rates up to 28% (23/82 patients); responses were also observed in ALL. Toxicity was predictable and manageable. The optimum dose of Amsacrine for remission induction in acute leukaemia was confirmed to be 120 mg/m²/d for 5 days q 3 weeks and to keep patients in complete response, a dose of 30 - 40 mg/m²/day for 5 days q 4 weeks was recommended.
So as single agent therapy, phase II data indicated that Amsacrine is a useful agent for the treatment of acute leukaemia and has activity comparable to that of other potent anti-leukemic drugs. Amsacrine is as effective as the most active drugs, cytarabine and daunorubicin and can produce complete remissions in patients refractory to these drugs (CASSILETH 1986). Amsacrine may be combined with AraC without significant negative effect to AraCTP (FREUND 1991). Several studies proved the interest of regimens using Amsacrine in combination with high-dose cytarabine (HD-AraC) in refractory AML.

2.3 MECHANISM OF ACTION
Cytotoxicity of Amsacrine is greatest in cells in S and early G2 phases and a single in vivo dose is capable of killing 99% or more cycling cells. The cell cyclestage selectivity of Amsacrine is exactly complimentary to that of ionizing radiation or radiomimetic drugs (LINKESCH 1989).

Amsacrine induces DNA intercalation of the 3 coplanar rings and topo II (Type II Topoisomerase) inhibition with production of DNA double-strand breaks occurring at base sequences different from those of anthracyclines and etoposide. Intercalation is necessary but not sufficient for antitumor activity. Topoisomerase II is an enzyme with numerous and important functions for DNA function and cell survival. Despite the fact that they share the same target, Topoisomerase II inhibitors like anthracyclines or etoposide, Amsacrine have different mechanisms of action or specific sites of interaction. Type II DNA Topoisomerase catalyse the topological crossing of double-stranded DNA segments via a transient double-stranded DNA break. Topoisomerase II can break and re-join the DNA double helix by forming an equilibrium mixture of, at least, two types of complexes: non-cleavable and cleavable. The presence of two DNA segments at the interface of the two protein subunits presumably results in strand passing. Thus, Topoisomerases are known to be involved in many important DNA metabolism reactions including replication, recombination, transcription and chromosome segregation during mitosis (MALONNE 1997).

There are two highly homologous isoforms of human Topoisomerase II which have been designated Topoisomerase IIα and Topoisomerase IIβ. There is some evidence that these isoenzymes carry out different cellular functions and the role of each isoform in drug difference may differ. For Amsacrine and etoposide it was reported that the maximal cytotoxicity was achieved when cells were exposed during the S phase.

A synergy between Amsacrine and etoposide can be at least partially explained by the difference in structure of cleavable complexes, which may account for the retained activity of Amsacrine in etoposide-resistant cells and vice versa. Also, Amsacrine exerts its cytotoxicity by binding both Topoisomerase II isoforms α and β equally, whereas etoposide predominantly targets Topoisomerase IIβ. Importantly, hyperphosphorylation of Topoisomerase IIβ may compensate for hypophosphorylation of Topoisomerase IIβ to maintain normal Topoisomerase II function during proliferation making the Topoisomerase IIβ isoform an equally important drug target (HORSTMANN 2005).

Amsacrine was designed as a DNA binding agent, the ability to intercalate does not appear to be the sole determinant of drug activity. The non-intercalative m-AMSA head group enhanced enzyme-mediated DNA cleavage when it was detached from the acridine moiety, albeit with 100-fold lower affinity. It is suggested that much of the activity and specificity of m-AMSA as a Topoisomerase II poison is embodied in the head group, while DNA intercalation is used primarily to increase the affinity of m-AMSA for the Topoisomerase II-DNA cleavage complex (KETRON 2012).

2.4 PHARMACOLOGY
About 97% of a dose of Amsacrine is bound to protein bound in plasma in both cancer patients and
healthy volunteers. Studies of human plasma in vitro showed no change in protein binding across a concentration range of 1–100 µmol/L.

In cancer patients, Amsacrine undergoes biphasic elimination, with a distribution half-life of 0.25–1.6 h and an elimination half-life of 4.7–9 h. The total plasma clearance rate is 200–300 mL/min per m², and the apparent distribution volume is 70–110 L/m², suggesting concentration in tissues. During a 1-h infusion of Amsacrine at 90–200 mg/m², the peak plasma concentration was 10–15 µmol/L.

The drug is extensively metabolized in the liver, with cytotoxic metabolites excreted in urine (35% of dose) and bile (50% of dose). By liver impairment, T½ is prolonged (17 hours), with consequent increased stomatitis and myelotoxicity.

Amsacrine is taken up rapidly by nucleated blood cells in vivo, peak concentrations occurring shortly after the end of a 3-h infusion; the concentration was about five times greater than the peak plasma concentration. Over 24 hours, the mean integrated area under the timeconcentration curve (AUC) for cellular Amsacrine was eight times that of the AUC for plasma. The kinetics of elimination from peripheral blast cells was similar to elimination from plasma.

### 2.5 DRUG INTERACTIONS

Amsacrine is incompatible with chloride (Cl).

### 2.6 SAFETY

#### 2.6.1 HAEMATOLOGY

Myelosuppression is dose-limiting and the most important physiologic consequence of Amsacrine therapy. The degree of myelosuppression is dose dependent and was reversible in all trials. Leukopenia, thrombocytopenia, and, to a lesser extent, anaemia occur in virtually all patients. There is considerably less clinical experience in children, nevertheless at equivalent dosages, the myelosuppression produced in adults and children appears to be similar in frequency and severity (LOUIE 1985).

#### 2.6.2 LIVER FUNCTION

Transient liver function abnormalities are common when Amsacrine is given at high doses in multiple-dose schedules. The incidence may reach 35%. Hyperbilirubinemia is the most frequently reported
Abnormality; it appears to be dose-related and is usually reversible. Although transient liver function abnormalities are relatively common and cause little concern, the presence of liver function abnormalities before Amsacrine therapy requires careful monitoring of the patient. Since Amsacrine is eliminated by way of the liver, impaired liver function may lead to delayed clearance of the drug. In patients with leukaemia, however, serum bilirubin levels as great as 3 mg/dL (51 µmol/L) were occasionally associated with prolonged marrow hypoplasia, and a dosage reduction of 25% was recommended for patients with leukaemia whose bilirubin level increased beyond this level (LOUIE 1985).

2.6.3 RENAL FUNCTION
Abnormal renal function after Amsacrine treatment is rare.

2.6.4 INFECTIONS
Amsacrine is a potent myelosuppressive agent and is associated with increased risk of infection. The degree of risk appears to depend on the intensity of treatment with Amsacrine.

2.6.5 ADVERSE CARDIOVASCULAR EVENTS
Eighty-two cases of amsacrine-associated cardiotoxicity were observed among over 6000 patients in phase II studies who had received Amsacrine until 1986, indicating a total incidence of just above 1%. The more common effects were alterations in the electrocardiogram and arrhythmia, but cardiomyopathy and congestive heart failure also occurred. Amsacrine has been used safely in patients with pre-existing arrhythmia when a serum potassium concentration of >4 mmol/L was maintained (IARC 2000). There were reports on QT interval prolongation, ventricular arrhythmia and sudden death associated with Amsacrine administration, suggesting an effect of Amsacrine on cardiac repolarization (THOMAS 2004).

Amsacrine and hypokalemia may act together to decrease the rate of depolarization, and if sufficiently severe, may lead to ventricular arrhythmia through mechanisms already described for quinidine (LOUIE 1985).

The cardiac events appeared to be related to previous high-dose anthracycline therapy and to the dose and rate of administration of Amsacrine. No cardiac abnormalities were seen in children previously receiving <400 mg/m² of anthracycline or who were given <200 mg/m² Amsacrine within 48 hours (LOUIE 1985).

Amsacrine in high doses may play a contributing role, it is notable that there were no signs of cardiac toxicity in several large series of patients treated with Amsacrine in high doses using multiple-dose schedules. Amsacrine can be used to treat patients who already have cardiomyopathy from previous anthracycline treatment (KESLER 2008).

Many authors believe that Amsacrine may be given to patients with anthracycline cardiomyopathy without further impairment of left ventricular function. This finding suggests that the cardiac effects of anthracycline differs from, and are not necessarily worsened by subsequent Amsacrine therapy.

2.6.6 NEUROLOGIC ADVERSE EVENTS
Neurologic adverse events are uncommon and include rare reports of seizures and occasional reports of neuropathy, headache, dizziness, and CNS depression (LOUIE 1985).

2.6.7 GASTROINTESTINAL ADVERSE EVENTS
The principal gastrointestinal adverse events are nausea, vomiting and diarrhea. Nausea and vomiting are very common and to some extent occur in most trials, whether Amsacrine is administered at low or
high doses. The reported incidence of nausea and vomiting ranges from 0 to 30% (LOUIE 1985).

2.6.8 SKIN AND MUCOUS MEMBRANE ADVERSE EVENTS
Mucositis is common after both low and high doses of Amsacrine. It appears to be dose related and doses <120 mg/m² are usually associated with a low frequency of mucositis. It should be noted that the patients in these studies had refractory or relapsed acute leukaemias and many were at increased risk of mucositis and stomatitis regardless of the chemotherapeutic agent used simply because of the intensity of treatment required to achieve remission.

2.6.9 INJECTION SITE REACTIONS
Injection site reactions are not uncommon, but they are rarely of major importance.

2.6.10 AES IN CLINICAL TRIALS
Toxicity in 24 induction cycles as salvage regimen (adapted from FREUND 1991).

<table>
<thead>
<tr>
<th>Toxicity (WHO grade)*</th>
<th>0 (%)</th>
<th>1 (%)</th>
<th>2 (%)</th>
<th>3 (%)</th>
<th>4 (%)</th>
</tr>
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<tbody>
<tr>
<td>Granulopenia</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
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<td>–</td>
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<td>–</td>
<td>100</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>92</td>
<td>–</td>
<td>4</td>
<td>4</td>
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<td>Cardiac function</td>
<td>96</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Eye</td>
<td>92</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cerebellar</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*GOT/GPT, glutamic – oxaloacetic transaminase/glutamate – pyruvate transaminase; FUO, fever of unknown origin
Patients with AML received an Amsacrine based consolidation therapy after previous induction courses with Amsacrine as salvage regimen, the reported adverse events in this trial were (adopted from FREUND 1991):

Toxicity in 9 consolidation cycles (re-use)

<table>
<thead>
<tr>
<th>Toxicity (WHO grade)</th>
<th>0 (%)</th>
<th>1 (%)</th>
<th>2 (%)</th>
<th>3 (%)</th>
<th>4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulopenia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Thrombopenia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Bleeding</td>
<td>63</td>
<td>–</td>
<td>13</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>88</td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alk. Phosphatase</td>
<td>88</td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>GOT/GPT</td>
<td>50</td>
<td>13</td>
<td>25</td>
<td>–</td>
<td>13</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td>–</td>
<td>–</td>
<td>44</td>
<td>44</td>
<td>11</td>
</tr>
<tr>
<td>Mucositis</td>
<td>88</td>
<td>–</td>
<td>13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>–</td>
<td>63</td>
<td>25</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td>Creatinine</td>
<td>75</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>88</td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>38</td>
<td>38</td>
<td>25</td>
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<td>Alopecia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Local infection</td>
<td>78</td>
<td>11</td>
<td>11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sepsis</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FUO</td>
<td>22</td>
<td>33</td>
<td>44</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac rhythm</td>
<td>88</td>
<td>13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac function</td>
<td>75</td>
<td>–</td>
<td>–</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>Eye</td>
<td>89</td>
<td>–</td>
<td>11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cerebellar</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The conclusion of the authors was that the toxic side-effects are tolerable and the treatment is easy to handle (FREUND 1991).

The median hospitalization time for 91 AML patients receiving a combination of HD-AraC and Amsacrine as salvage regimen was 28 days (range, 5-77 days) (TAVERNIER 2003).

The median time to WBC recovery (>1 x 10⁹/l) was 21 days (range, 8-42 days). The median time to PMN recovery (>0.5 x 10⁹/l) was 21 days (range, 5-51 days) and the median time to platelet recovery (>100 x 10⁹/l) was 24 days (range, 9-53 days) (TAVERNIER 2003).

The main non-haematologic toxicity is infectious complications (TAVERNIER 2003, SUNG 2005). Nevertheless the salvage regimen consisting of Amsacrine plus intermediate dose AraC with or without etoposide seemed to be a safe and effective regimen for patients with refractory or relapsed acute leukaemia, and especially for SCT candidates (SUNG 2005).
3 CLINICAL RESULTS IN ACUTE LEUKAEMIA

3.1 CLINICAL RESULTS IN AML
Currently available chemotherapy schedules typically achieve CR in 75% to 80% of patients younger than 60 years, with approximately 40% to 45% of patients surviving 5 years, after which there are few events. The major challenge is reducing relapse. Both induction treatment (by delivering better quality remissions) and consolidation treatment may do this. However, intensification of induction can compromise delivery of consolidation (BURNETT 2010).

The choice of treatment approach and outcome in AML depends on the age of the patient. In younger patients, defined as being younger than 60 years, (BURNETT 2011) several anthracycline-based chemotherapy combinations are used. Complete remission rates in young adult patients with newly diagnosed AML range from 60% to 80% with long term survival in about 50% of cases (REVESZ 2003). In older patients the same anthracycline/cytarabine-based approach is deployed, the remission rate will be around 50%, but the risk of subsequent relapse is approximately 85% at 3 years (BURNETT 2011).

3.2 AMSA IN FIRST LINE TREATMENT
In AML Amsacrine is as effective as the two most active drugs, cytarabine and daunorubicine (CASSILETH 1986).

In a retrospective analysis of two studies with timed sequential therapy for adults with AML (ages 16 to 80) the Amsacrine based regimen resulted in a higher CR rate (76% for de novo AML and 54% for sAML). Each course consisted of daunorubicine at a dose of 45 mg/m²/d on days 1–3 and a total dose of 2 g/m² of AraC as a 72 hours continuous infusion beginning on days 1 and again on day 10. Because of toxicity in that study a less aggressive first course was used. On days 8, 9 and 10 instead of AraC, a daily bolus of Amsacrine at a dose of 200 mg/m²/d was given. Treatment with AraC-DNR-Amsa achieved remission in 63% of patients (GELLER 1989).

In four trials Amsacrine based regimens were compared with anthracycline based regimens (BERMAN 1989, LINKESCH 1989, SCHAICH 2002, KESSLER 2007). There are some prospective randomized trials comparing Amsacrine based first line regimens with anthracycline based regimens. In all these trials Amsacrine was not inferior to anthracyclines. In two prospective randomized trial combinations of Amsacrine with 6-thioguanine and AraC (TAA or AAT) were compared to DAT (Daunorubicin, AraC and 6-thioguanine). In both trials the response with TAA was in subgroups significant better, but the results for the whole group was not significant different.

For the subgroup of elderly patients an Austrian-German multicenter trial found a higher response rate in the Amsacrine arm (LINKESCH 1989).
A conclusion of this study was that especially for patients with preexisting congestive heart failure or prior anthracyline exposure AAT could be treatment of choice (LINKESCH 1989).

In patients with cardiac impairment who were unable to receive anthracyclines due to their high cardiac toxicity the group of Muenster use Amsacrine.

In a retrospective matched pair analysis they compared the efficacy of TAA (6-thioguanine, cytarabine and Amsacrine) in patients with contraindications against cardiotoxic substances) compared to the standard therapy of the German AML Cooperative Group 1999 protocol (TAD or HAM). Short- and long-term toxicities in both groups were tolerable and within the reported range for the AMLCG study group.

Figure 1 CR rates in elderly patients with ANLL with AAT or DAT

Figure 2 CR rates after one course of TAA or TAD/HAM in first line AML therapy, results are not significant.
The response rate was 56% vs. 31% with no significant difference, nevertheless we can conclude that the treatment with Amsacrine based chemotherapy was at least non-inferior to the standard regimen. The overall survival in the group receiving Amsacrine was 16 months compared to 8.5 months in the group receiving standard treatment, the difference was not significant.

**Overall Survival**

![Overall Survival Graph]

Figure 3  Median OS time in months after one course of TAA or TAD/HAM in first line AML therapy. Results are not significant.

In this small but representative group replacing daunorubicine or mitoxantrone with amsacrine in the induction therapy of elderly AML patients did not significantly affect the CR rate, RFS or OS. Amsacrine may represent a suitable substitute for daunorubicine or mitoxantrone (KESSLER 2007).

Results suggest that AMSA may be substituted for anthracyclines without major loss of antileukemic effect, and that AMSA based combination therapy may be more effective than anthracycline-based combinations in selected groups of patients in whom analysis of prognostic factors indicates a low probability of achieving complete remission with conventional therapies (LOUIE 1985).

**Complete response rates from comparison in AML/ANLL**

![Complete Response Rates Graph]

Figure 4  CR rate in AML/ANLL reported from BERMAN (1989), LINKESCH (1989) and KESSLER (2007).
In three trials comparing AAT and DAA the results regarding remission rates were not significant in any whole study group (BERMAN 1989, LINKESCH 1989 and KESSLER 2007).

Interestingly the Amsacrine dose used in these three trials was high and nearly similar with 190 and 200 mg/m²/d for 3 days in 2 trials (BERMAN 1989, LINKESCH 1989) and 210 mg/m²/d for 3-5 days (KESSLER 2007). Linkesch reported a significant higher response rate in the elderly patients (see above) and BERMAN a significant (p=0.01) improved OS in the AAT group. Additional more patients reached CR after one course 48% compared to 28% (p=0.03) (BERMAN 1989).

3.3 DOSE INTENSIFICATION

Double induction is a strategy of very early intensification by starting a second induction course on day 21 of treatment irrespective of the presence or absence of residual leukemic blasts in the bone marrow after the first induction course (BÜCHNER 1999).

When remission induction had reached some standard dose levels, further increase in the remission rate appeared associated with progress in supportive care rather than intensification in antileukaemic treatment. In contrast with remission rates, chemotherapy dose effects have been found in the duration of remissions and relapse-free survival. Comparing an induction regimen containing HD-AraC with a regimen containing the drug at a standard dosage, both included in the second courses of double induction failed to show a significant difference regarding CR and early death rate or relapse free survival. A subgroup with poor prognosis was found in analysis where the TAD-HAM was superior over TAD-TAD regarding the CR rate, EFS and OS (BÜCHNER 1999). It should not be forgotten that increasing the intensity of front-line treatment to overcome primary resistance could even be detrimental if associated with greater drug toxicity and toxic death rates. This possibility can only be dealt with by improving supportive treatment at the same time (BASSAN 1995).
Double induction with an Amsacrine based regimen (MAV-MAMAC) in elderly patients (61-65 years) was used as an intensified regimen and compared to the outcome with the use of DAA regimen (2 cycles) in patients >65 years. Patients of both induction therapy groups who achieved a CR and were in good clinical condition were eligible to receive a cycle of MAMAC postremission chemotherapy with an interval of 6-8 weeks after double induction therapy. The dose intensification does not result in a higher response rate but to an increase of early death rate. On the other hand there was a trend to more long-term survivors showing the strong antileukaemic efficacy of the Amsacrine based regimen. The median DFS was longer for the MAV/MAMAC patients than DA/DA ones (18.6 vs. 11.6 months) resulting in a DFS rate of 27% and 8% after 51 months. Survival was better in patients who reached CR after intensified induction therapy than in the conventionally treated patients (SCHAICH 2002).

**MAV**: mitoxantrone 10 mg/m$^2$ days 4-8; AraC 100 mg/m$^2$ continuous infusion days 1-8; VP-16 100 mg/m$^2$ days 4-8.

**MAMAC**: AraC 1,000 mg/m$^2$ every 12h days 1-5 (total dose 10 g/m$^2$), Amsacrine 100 mg/m$^2$ days 1-5 (starting three weeks after the first course)

933 patients between the ages of 15 and 60 years who had untreated or de novo secondary AML were included in the AML96 study of the SAL. All randomly assigned patients uniformly received double induction therapy. One course of MAV was followed by one cycle of MAMAC starting three weeks after the first course. Of the 933 included patients, 619 (66%) achieved CR after double induction.

Patients who achieved CR were eligible for postremission therapy within an interval of 6 to 8 weeks after double induction therapy. This postremission therapy was stratified according to cytogenetic risk profile. A second consolidation therapy was administered to 276 patients, of whom 179 patients underwent autologous HSCT and 75 patients received chemo-consolidation with MAMAC. No significant differences in survival could be observed between the I-MAC (intermediate-dose of 12 g/m$^2$ cytarabine combined with mitoxantrone) and H-MAC (high-dose of 36 g/m$^2$ cytarabine combined with mitoxantrone) arms. The conclusion of the authors is that in young adults with AML receiving intermediate-dose cytarabine induction, intensification of the cytarabine dose beyond 12 g/m$^2$ within first consolidation did not improve treatment outcome (SCHAICH 2011).

The same findings were reported from a prospective randomized study from the HOVON and SAKK. They compared an intermediate-dose AraC with high-dose AraC. In both arms the induction cycle one was idarubicine and cytarabine and cycle two Amsacrine with a dose of 120 mg/m$^2$ on days 3, 5 and 7 in combination with cytarabine. The conclusions are that induction therapy with cytarabine at the lower dose already produced maximal antileukemic effects for all response end points, suggesting a plateau in the dose-response relationship above this level. High-dose cytarabine results in excessive toxic effects without therapeutic benefit (LÖWENBERG 2011).

For several years the HOVON-SAKK cooperative groups have employed a condensed treatment program in adults with AML that involves a backbone of two induction cycles with a first cycle of standard-dose cytarabine and a second cycle of intermediate-dose cytarabine and a third and final consolidation cycle without cytarabine (LÖWENBERG 2011). Like in the published AML 42 the backbone has not changed and also in the newer trials, which are running the induction 2 cycle includes AraC and Amsacrine.
THE ACTUAL 102 AML STUDY
Participating groups: HOVON, SAKK, Nordic group

Randomized study with a run-in feasibility phase to assess the added value of Clofarabine in combination with standard remission-induction chemotherapy in patients aged 18-65 years with previously untreated acute myeloid leukaemia (AML) or myelodysplasia (MDS/RAEB with IPSS ≥ 1.5).

Randomisation in Arm A or Arm B

<table>
<thead>
<tr>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induction cycle I</strong></td>
<td><strong>Induction cycle I</strong></td>
</tr>
<tr>
<td>Ida: 12 mg/m², days 1, 2, 3</td>
<td>Ida: 12 mg/m², days 1, 2, 3</td>
</tr>
<tr>
<td>AraC: 200 mg/m², days 1-7</td>
<td>AraC: 200 mg/m², days 1-7</td>
</tr>
<tr>
<td><strong>Induction cycle II</strong></td>
<td><strong>Induction cycle II</strong></td>
</tr>
<tr>
<td>Amsa: 120 mg/m², days 4, 5, 6</td>
<td>Amsa: 120 mg/m², days 4, 5, 6</td>
</tr>
<tr>
<td>AraC: 1000 mg/m², 3 hrs inf, q 12 hrs (x12), days 1-6</td>
<td>AraC: 1000 mg/m², 3 hrs inf, q 12 hrs (x12), days 1-6</td>
</tr>
<tr>
<td>Clofarabine: assigned dose</td>
<td>Clofarabine: assigned dose</td>
</tr>
<tr>
<td><strong>Further treatment</strong></td>
<td><strong>Further treatment</strong></td>
</tr>
</tbody>
</table>

Adapted to: http://www.hovon.nl/studies/studies-per-ziektebeeld/aml.html?action=showstudie&studie_id=72&categorie_id=4

The target number of patients is 870.

In the prospective randomized AML 2003 trial 807 patients were consolidated with cytarabine. Of these patients, 407 were randomized for AraC alone and 400 received cytarabine, mitoxantrone and Amsacrine (MAC/MAC/MAMAC). There was no difference in the five-year OS. The three-year event free survival showed a trend to superiority (30.5% vs. 35.6%; p=0.059) for patients receiving MAC/MAMAC (PARMENTIER 2011).

In a prospective multicenter trial a risk adapted approach was used. 305 patients received as first induction cycle idarubicine, etoposide and AraC. If they were good responders on day 15 to that treatment (n=208) they received a second cycle of this regimen. All other (n=93) received a second cycle consisting of Amsacrine 90 mg/m²/d, 3 doses and AraC 1 g/m², 8 doses. 51% (n=47) reached CR. All patients in CR after this second cycle received an early consolidation therapy with AraC 1 g/m²/d, 8 doses in combination with either m-AMSA (90 mg/m²/d, 3 doses) or daunorubicin (45 mg/m²/d, 3 doses) (HEIL 2004). The late consolidation treatment was risk adapted.
3.4 CONSOLIDATION WITH AMSACRINE

Amsacrine can not only be used for induction therapy, the use in consolidation is often reported. In the EORTC-AML-6 the post-remission treatment was six courses of intensive maintenance either repeated with daunorubicine, vincristine and AraC, or alternating treatment with Amsacrine combined with HD-AraC on cycle 1, 3, and 5 and with 5-azacytidine on cycle 2, 4, and 6. The dose of Amsacrine was only 150 mg/m² on day 1 of each course. The DFS appeared identical in the two randomized arms (ZITTOUN 1989). In a retrospective single center analyses an intensive consolidation chemotherapy involving two courses of intermediate/high-dose AraC and Amsacrine improved DFS and survival compared to less aggressive post-remission strategies from the EORTC-AML-6. A control group of patients who refused consolidation had significant less overall survival (JEHN 1994).

The Amsacrine dose is varying in consolidation regimens. The ECOG used 100 mg/m²/d for three days in combination with HD-AraC (3 g/m² every 12 hours for 12 doses) after an induction with 1-2 cycles TAD (CASSILETH 1992). In a prospective trial from the EORTC together with the GIMEMA an intensive course for consolidation therapy combining intermediate-dose AraC and Amsacrine was used. AraC with 1000 mg/m² every 12 hours for 12 doses and Amsacrine with 120 mg/m² on days 5, 6, and 7 were given. To reduce the incidence of lethal infections the AraC dose was decreased to 500 mg/m² after the first year of the study (ZITTOUN 1995).

The Danish Society of Haematology Study Group on AML observed no increased risk of solid tumours causally related to the intensive chemotherapy for de novo AML. 174 patients received in a prospective randomized trial a “7+3” regimen with either daunorubicine or aclorubicine, followed by early intensive consolidation with two alternating cycles of HD-Ara-C and two cycles of Amsacrine plus etoposide followed by 3 days of daunomycin plus 7 days of Ara-C. The 5 year overall survival rate was 23% (DE NULLY 1997). Of 174 evaluable patients 99 achieved CR and received the intensive consolidation regimen. Both the duration of remission and total survival decreased with increasing age (HANSEN 1991).

The use of multi-agent chemotherapy has generally been less effective for patients with myelodysplastic syndromes (MDS) in transformation to acute myeloid leukaemia (AML), with AML that has evolved from MDS, or AML after previous chemotherapy than for patients with de novo AML (GANSER 2000). Long-term benefit for individuals with high-risk (IPSS) myelodysplastic syndrome (MDS) or AML evolving either from MDS or after previous chemotherapy (secondary, sAML) can be achieved only by eradication of the abnormal clone and restoration of normal hematopoiesis. Studies giving intensive cytotoxic treatment to such patients have produced remission rates ranging from 22% to 79% (HOFMANN 2004).

In a prospective multi-center trial 110 of such patients received two induction courses with idarubicine, etoposide and AraC. For CR patients, this was followed by an identical early consolidation course. A late consolidation course contained Amsacrine, 60 mg/m², administered by i.v. bolus injection on days 1–5, and AraC at a dose of 600 mg/m² as a 2-h infusion every 12 hours on days 1–5. A total of 49 patients (45%) achieved a CR. The results of this trial suggest that improved treatment results can be obtained with intensive chemotherapy in patients with advanced myelodysplastic syndromes and high-risk leukaemia (GANSER 2000).

In a subgroup analysis of patients with secondary AML following a myelodysplastic syndrome or deriving as therapy-related AML included in the AML96 and in AML2003 and AML60+ the CR rate was 33%. Patients received double induction, in AML 96 MAV-MAMAC in AML2003 DA60 and DA60 and in the AML60+ trial DA45+DA45 and chemoconsolidation containing MAMAC or I-MAC (intermediate dose cytarabine and mitoxantrone) consolidation therapy consisted of MAMAC. With this subgroup analysis a new prognostic score could be obtained (STÖLZEL 2011).
Amsacrine can replace anthracyclines in patients with cardiac impairment, this is a problem often in elderly patients. Newly diagnosed AML patients aged >60 years receiving standard induction chemotherapy (“7+3”, with AraC 100 mg/m²/d and daunorubicin 60 mg/m²/d) with curative intent. Patients achieving CR were eligible to receive two consolidations, with “7+3” followed by mitoxantrone and etoposide. All patients underwent cardiac assessment prior to each cycle with MUGA scan. If the ejection fraction dropped to <50% or had fallen by >10%, Amsacrine (100 mg/m²/d for 5 days) was substituted for the anthracycline. 55 patients achieving CR proceeded to consolidation therapy, 39/55 patients went on to receive a second consolidation cycle.

Among patients proceeding to consolidation, 35/55 (63%) had a significant decline in LVEF during or at completion of, treatment: >10% drop in LVEF in 31 patients and LVEF <50% in 17 patients. 24/35 patients received a change in consolidation chemotherapy. There was no significant difference in RFS and OS between patients switched to Amsacrine versus those receiving standard protocol (WARD 2009).

Therefore Amsacrine can substitute anthracyclines in consolidation regimens without a loss in anti-leukaemic activity. Patients who run into cardiac problems with the use of anthracyclines can be switched to an Amsacrine based regimen, for induction therapy and also for consolidation.

3.5 SALVAGE IN AML (ADULTS)

At the moment there is no gold standard salvage chemotherapy and the therapeutic choice mostly depends on personal experience concerning efficacy and toxicity. Notwithstanding this, whatever the salvage therapy, best results are achievable when stem cell transplantation (SCT) is feasible after salvage therapy. On this basis, characteristics of an ideal salvage regimen should include either anti-leukaemic efficacy or acceptable extra-haematological toxicity. As a matter of fact, in clinical practice it is not rare that toxicity from previous therapy may preclude the feasibility of stem cell transplantation (FERRARA 2004).

Therapeutic failure remains a major concern. AML patients who fail to achieve CR after first-line chemotherapy have a very poor prognosis, and it is generally accepted that few cures can be achieved at this stage using chemotherapy alone (TAVERNIER 2002).

The activity of Amsacrine in combination with other anticancer agents in relapsed and refractory patients has been sufficient to inspire a number of trials of Amsacrine combinations for the primary therapy of acute leukaemia. Initial results suggest that AMSA may be substituted for anthracyclines without major loss of antileukaemic effect, and that Amsacrine based combination therapy may be more effective than anthracycline-based combinations in selected groups of patients in whom analysis of prognostic factors indicates a low probability of achieving complete remission with conventional therapies. Incorporation of AMSA into combination therapy has produced a number of active regimens (LOUIE 1985).

The combination of Amsacrine (120 mg/m² days 5-7) and ID-AraC (1 g/m² i.v. q 12h days 1-6) in 25 consecutive leukaemia patients with primary resistance (n=22) or resistant relapse (n=3) resulted in a CR rate of 48% (12/25 patients) in 10 after 1 cycle of induction and in 2 after 2 cycles. There was a significant survival advantage for responders compared to non-responders (10.7 vs. 3.2 month p=0.002) (JEHN 1993).

Studies have shown that combination of Amsacrine with HD-AraC is an effective therapy with CR rate at 44%–51% and seems to be one of the best salvage induction regimens in refractory AML. In a
French study, CR could be achieved in 49% of cases. Although 11 patients died during salvage therapy, toxicity was acceptable as compared to the literature. Therefore, it was confirmed that Amsacrine is a safe and effective therapy for patients with acute leukaemia (TAVERNIER 2003).

The efficacy of the salvage regimen was independent from the type of the first course of induction chemotherapy (TAVERNIER 2003).

Therapeutic results for relapsed AML derive from retrospective studies, phase II single-agent studies, phase II combination-agent studies and a few phase III randomized trials. Overall, CR2 rates range from 20% to 80%, with a median duration of CR2 ≤14 months and on overall median survival of ≤12 months. The probability of 3-year survival ranges from 8% to 30%. Best results in terms of CR2 rate and duration have been reported after combination therapies in patients with CR1 lasting for more than 12 months with favorable or intermediate cytogenetics. High or intermediate dose cytarabine (AraC)-based regimens still represent the most frequently adopted therapy (FERRARA 2004). HD-AraC combined with Amsacrine was a useful salvage regimen in AML non-responding to a first course of chemotherapy.

Amsacrine has been shown to be effective as salvage regimen either as single agent or in combination with AraC. It was combined with HD-AraC or with AraC and etoposide or for consolidation with mitoxantrone, Ara-C and etoposide (KIMBY 2001). Results with FLAG-Amsa were reported recently (FONG 2011). There is no prospective randomized trial published where an Amsacrine based salvage regimen was inferior to the comparative arm.

In a retrospective single center analysis no statistical difference between FLAG with or without idarubicine was found. 59% of the treated patients achieved CR, around 60% were treated in relapse, 40% with de novo disease or secondary MDS or AML (VIRCHIS 2004). It is comparable to other reports. 46 patients treated with FLAG-IDA (30 patients in relapse after conventional chemotherapy and 10 patients refractory. The CR rate was 52.1%. 3/46 patients died during re-induction therapy (PASTORE 2003).

It is also here possible to substitute the anthracycline with Amsacrine. With FLAG-Amsacrine the overall CR/CRI rate was 61% in AML patients in first relapse. Most of the patients received previous HD-AraC. The EFS between patients who had received FLAG-Amsacrine following prior HD-AraC based therapy compared with those who had not received HD-AraC (FONG 2011).

3.6 SMALL META ANALYSIS
For this monograph 10 studies with Amsacrine based salvage therapy for AML were analysed.

In total 399 patients were included in these trials. The response rate was lower in patients with primary refractory disease than in patients with relapsed AML with 29% and 60% respectively (ARLIN 87). Patient groups were different. 230 of 399 patients (58%) responded to the salvage regimen, which shows the strong antileukaemic effect of Amsacrine based regimens.
In a trial from the Leukaemia Intergroup the role of HD-AraC in the treatment of adults with AML in first relapse was evaluated. Additionally the hypothesis was tested, that selective use of Amsacrine would increase the CR rate when leukaemia cells remained in the bone marrow after 6 days of HD-AraC was tested.

Of 155 included patients 36 (23%) had inadequate cytoreduction after the 6 days HD-AraC. They were randomized to no further chemotherapy or to 3 days Amsacrine (100 mg/m²/d). The CR rates after one course were 14% and 53%, respectively (p=0.01), and the fractions with resistant disease were 76% and 40% (LARSON 1992). The use of Amsacrine in patients with relapsed AML with inadequate cytoreduction after HD-AraC therapy results in significant higher CR rates than no treatment.

In a single arm study of 46 patients the TAA regimen was used as salvage regimen in 17 patients with refractory AML and 22 relapsed patients having daunorubicine and cytarabine maintenance therapy, or receiving >500 mg daunorubicine/m² and also included 7 previously untreated patients where cardiac disease was contraindicated with anthracycline therapy. 46% reached CR, probability of survival was comparable to published results for first-line treatment with daurornubicine plus cytarabine regimens (WATSON 1994). It should be noted that in patients who were 60-76 years of age, there was no statistically significant difference in CR rate or probability of survival relative to patients <60 years.

In a single center study 48 patients with AML not eligible for anthracycline treatment, mostly due to cardiac contraindications, were given aggressive therapy using Amsacrine and conventional or HD-AraC for remission induction. For first line therapy TAA and for relapsed or refractory AML either TAA or HA-AMSA (HD-AraC and Amsacrine) or sequential (sHA-AMSA) administered HD-AraC and Amsacrine was used. Overall 60.4% responded to treatment. The results in patients with first relapse are comparable to those achieved with HD-AraC in combination with mitoxantrone. The responses with HA-AMSA in

Graph 6  CR rates reached with Amsacrine based salvage regimens in AML

Overall response rate in AML/ANLL with AMSA containing salvage therapy
patients treated for refractory AML indicate an antileukaemic efficacy also in extensively pretreated AML patients (MASCHMEYER 1992).

Amsacrine in combination with AraC is successful also as second-line salvage therapy e.g. after failure of FLAG + anthracycline. Over 70% reached CR after one course, one patient after two courses (TAURO 2003).

3.7 ALL (ADULTS)
In adults with acute lymphoblastic leukaemia (ALL) complete remission rates (CR) with induction chemotherapy programmes range from 60% to 90%. Among patients achieving CR the long-term disease-free survival (DFS) rates are only 20%–40%. Therefore, the majority of adult patients will require treatment in relapsed or refractory disease. Outcome after salvage therapy in adult ALL remains unsatisfactory and is worse than that of newly diagnosed patients (TEDESCHI 2007). In most studies the disease in 10%–25% of patients is resistant to a standard four or five drug regimen based on vincristine, prednisone, L-asparaginase and anthracycline. ALL of the elderly is a rare disease with poor prognosis. In a study of the PALG no differences in either biology or outcome between patients aged 60–69 years and those aged more than 70 years was observed. 40% showed primary resistance to chemotherapy (ROBAK 2004).

There are only very limited data to the first-line use of Amsacrine in ALL. 32 patients with untreated ALL (n=26) or lymphoblastic lymphoma (n=6) were treated with a short remission induction course with VP-16, Amsacrine, intermediate dose AraC for 6 days, prednisone and intrathecal methotrexate, followed by a consolidation course with vincristine, Amsacrine, HD-AraC for 4 days, prednisone and intrathecal methotrexate. No further maintenance was planned. 23/32 patients (72%) achieved a CR. Ten of 13 patients with T-ALL or lymphoma, six of eight patients with pre-B or common ALL and 7 of 11 patients with B-ALL or Burkitt’s lymphoma achieved CR. Overall survival of the whole group was 35% at 5 years. Long-term survival for patients with B or T-ALL was approximately 60%, compared with 15% for those with common or pre B-ALL. The results are comparable to those obtained with long-term maintenance regimens (WILLEMZE 1995).

3.8 CONSOLIDATION WITH AMSACRINE IN ALL
In a multicenter trial 130 adult patients with ALL were treated with standard induction therapy (vincristine, prednisone, daunorubicine and asparginase). Consolidation therapy consisted of three cycles, each consisting HD-AraC in combination with Amsacrine 120 mg/m²/d on day 1-5 in the first consolidation course, with mitoxantrone in the second consolidation course and etoposide in the third
consolidation course. No maintenance therapy after consolidation was used in this study. 86 patients (66%) achieved a CR after the induction phase and a cumulative number of 95 patients (73%) after the first course of postinduction regimen. The strongest predictor of CR was age: 82% of patients younger than 50 years achieved a CR versus 41% of those older than 50 years (p<0.001). The estimated 5-year OS was 22%. The outcome for adult patients with ALL receiving intensive consolidation not followed by maintenance therapy may be worse or even inferior compared with studies using long-term maintenance therapy (DEKKER 1997). The results with this consolidation regimen could perhaps be improved by using conventional maintenance.

3.9 SALVAGE THERAPY IN ALL
Among patients achieving CR the long-term disease-free survival (DFS) rates are only 20%–40%. Therefore, the majority of adult patients will require treatment in relapsed or refractory disease. Outcome after salvage therapy in adult ALL remains unsatisfactory and is worse than that of newly diagnosed patients with a median DFS of only 2–7.5 months. For these patients the only realistic chance of cure lies in achieving remission followed by a successful transplant strategy. In the salvage setting the use of chemotherapeutic agents similar to those administered during initial therapy may induce CR but the likelihood of a second CR is less than 50%. Additionally, this approach is limited particularly in patients with primary resistant disease. The main alternative to high-dose AraC as a single agent is to utilize HD-AraC combined with L-asparaginase, fludarabine, anthracyclines (idarubicine, doxorubicine) or non-anthracycline intercalators (mitoxantrone and m-AMSA) (TEDESCHI 2007).

Currently available salvage regimens for adults with relapsed or refractory ALL produce CR in 20–50% of cases, but these remissions last less than 4 months in most cases; the best chance for these patients appears to be low-toxicity treatment-inducing CR, followed by intensification with allogeneic stem cell transplantation as soon as possible (SPECCHIA 2005).

Amsacrine was used in the EORTC ALL-3 trial as salvage regimen. This study was conducted from November 1986 to November 1996 in 20 European centers. Patients older than 15 years of age and younger than 60 years of age with de novo ALL and lymphoblastic non-Hodgkin’s lymphoma (NHL) were included in the study. All patients received a first induction therapy. If complete remission was not achieved by day +28 salvage therapy was administered. This salvage therapy consisted of cytosine arabinoside (1 g/m² given as a 2-hour infusion every 12 hours, 12 times in 6 days) and Amsacrine (120 mg/m²/d in a one-hour infusion on days 5, 6 and 7 after the beginning of salvage therapy. 67% of patients reached CR after induction, a total of 74% of patients achieved CR after induction remission, with or without salvage therapy (LABAR 2004).

In five studies with the use of Amsacrine based salvage regimen the reported response rate was 60% (Response in 76 from 126 patients), this shows also for ALL that AMSA may be substituted for anthracyclines without loss of antileukemic effect (see graph on next page).

Arlin used a regimen that included Amsacrine 200 mg/m²/d i.v. for three days with AraC 3 g/m² for five days. The toxicity of this regimen was comparable to other reinduction regimens for ALL, but the side effects characteristic of HD-AraC therapy were absent (ARLIN 1988). It is suggested that this Amsacrine combination is non-cross-resistant with other agents. The CR rate in ALL makes this therapy among the best available for relapsed ALL (ARLIN 1987).
Sung used a lower dose of Amsacrine 150 mg/m²/day intravenously as a short infusion over 3 days (days 1–3) and AraC 2 g/m²/day intravenously as a short infusion over 5 days (days 1–5) (SUNG 2005).

There are no prospective randomized trials comparing Amsacrine based salvage therapy with other salvage regimens in ALL. For regimens like FLAG-IDA response rates around 40-50% are reported. Due to the lack of data it is not possible to make evidence based decisions as to which salvage regimen in ALL might be the better one.
4 CLINICAL PROTOCOLS IN ACUTE LEUKAEMIA

**MACE (MRC CONSOLIDATION)**
- m-Amsacrine 100 mg/m² daily by 1-hour i.v. infusion (in 5% dextrose) on days 1-5 inclusive (5 doses)
- Cytosine Arabinoside 200 mg/m² daily by i.v. infusion over 19 hours on days 1-5 inclusive (5 doses)
- Etoposide 100 mg/m² daily by 4-hour i.v. infusion on days 1-5 inclusive (5 doses)
  (BURNETT 2010)

**ACE** (same substances in combination but different dosages)
- Amsacrine 150 mg/m²/d on days 2 and 4
- AraC 3 g/m² x 2 days 1, 3, 5
- Etoposide 150 mg/m² days 1-3
  (HÖGLUND 2003)

**MAMAC**
- AraC 1,000 mg/m² every 12h days 1-5 (total dose 10 g/m²),
- Amsacrine 100 mg/m² days 1-5
  (SCHAICH 2002, SCHAICH 2011, STÖLZEL 2011)

**TAA / AAT**
- Amsacrine 210 mg/m² i.v. (2 h) days 3 through 5
- 6-thioguanine 200 mg/m² p.o. days 3 through 9
- AraC 100 mg/m² i.v. (24 h) days 1 and 2
- AraC 100 mg/m² q 12 h i.v. (30 min) days 3 through 8
  (LINKESCH 1989, MASCHMEYER 1992, KESSLER 2007)
- Amsacrine 190 mg/m² i.v. (2 h) days 1-3
- 6-thioguanine 100 mg/m² p.o. every 12 hours for 5 days
- Ara-C 25 mg/m² IV bolus
- Ara-C 160 mg/m² as continuous IV infusion daily for 5 days
  (BERMAN 1989)

**HA-AMSA (MASCHMEYER 1992)**
- Amsacrine 210 mg/m² i.v. (2 h) days 2 through 4
- AraC 3 g/m² q 12 h i.v. (3 h) days 1 through 4
- Only small dose adjustments regarding Amsacrine were made (TAURO 2003) with
  Amsacrine 200 mg/m² i.v. for 3 days and AraC 3 g/m² once daily for 5 days.
- It was used for HR-leukaemia, AML and ALL.

- Amsacrine 90 mg/m²/day days 5 to 7 and
  HD-AraC 3 g/m² q 12 days 1 through 4
  (TAVERNIER 2003).
4.1 STEM CELL TRANSPLANTATION

Autologous stem cell transplantation (ASCT) is an important treatment modality for AML. The role of ASCT in first remission patients remains controversial. Phase II and phase III studies demonstrate that patients with favorable-risk cytogenetics benefit from ASCT, with reduction in relapse and improvement in leukaemia-free survival. Patients with poor-risk cytogenetics do not appear to benefit significantly from ASCT and should be preferentially treated with allogeneic transplant. The role of ASCT for patients with intermediate risk disease is uncertain (LINKER 2003).

The EORTC and the GIMEMA conducted together a prospective trial of three postremission treatments to examine disease-free and overall survival. All patients received an intensive course of consolidation therapy combining intermediate-dose cytarabine and Amsacrine. Patients who had an HLA-identical sibling were allowed to undergo allogeneic BMT. The remaining patients were randomly assigned to receive either autologous BMT or a second course of intensified chemotherapy. The relapse rate was highest in the intensive-chemotherapy group and lowest in the allogeneic-transplantation group, whereas the mortality rate was highest after allogeneic transplantation and lowest after chemotherapy. The overall survival after complete remission was similar in the three groups (ZITTOUN 1995).

By intention to treat analysis of patients in the EORTC-LG/GIMEMA AML-10 trial in CR1 aged younger than 46 years assigned to allo-SCT has a significantly better outcome than for those who were planned to undergo an auto-SCT. This finding seems specifically true for patients with bad or very bad risk cytogenetics. This conclusion is justified because this AML-10 trial was the first large study in which prospectively only the two transplantation modalities were offered at an early time point of entering CR. In the patients with good risk the strategy to perform an allo-SCT led to a slightly lower DFS rate than the strategy to perform an auto-SCT. The lower relapse incidence in the allo-SCT group could not balance the higher TRM observed after transplantation (SUCIU 2003).

If an allo-SCT is planned the question about which donor is best is relevant. In a single center retrospective study with 92 consecutive patients with AML (n=87) or MDS (n=5) treated between 1996 and 2006 with a uniform preparative regimen of Bu and Cy and PBSCT from related donors (n=46) or unrelated donors (n=46) the outcome was analysed.

In unrelated donor HCTs performed in patients in CR1 or in a mismatch situation ATG-F (60 mg/kg b.w.) was used and in patients not in CR1 (40 mg/kg b.w.). The 5-year relapse-free survival rates and the OS were not significantly different. In a subgroup analysis it was shown that high-risk patients with an unfavourable remission status before HCT had a higher benefit from unrelated HCT than related HCT, showing a significantly better 5-year relapse free survival of 46% vs. 22% (p=0.04) (DENZ 2010).

The outcome after allogeneic transplantation improved over the decades, but also the supportive care. High-risk patients may benefit more from allogeneic transplantation as they have a higher risk of relapse. For standard-risk patients a postremission therapy with chemotherapy may be the better choice.

4.2 AUTOLOGOUS HSCT

Whether autografting should be performed during CR1 or whether it should be used as salvage treatment after the second complete remission remains a controversial issue. Autologous BMT is however a well accepted method of consolidating AML during CR2 (MAK 2000).

In a retrospective registry analysis of 94 patients receiving BAVC regimen with 432 patients receiving busulfan + cyclophosphamid (BuCy) as conditioning regimen for autologous transplantation in AML the relapse incidence was higher and transplant related mortality lower with BAVC. The study indicates that BAVC may offer a good alternative to BuCy.
BAVC and BuCy were compared in order to evaluate the long term antileukaemic effect of BAVC. Adult patients (>16 years old) with AML receiving either the BAVC regimen (BCNU 800 mg/m², Amsacrine 150 mg/m²/day for 3 days, VP-16 150 mg/m²/day for 3 days and AraC 300 mg/m²/day for 3 days) or the reduced BuCy regimen (busulfan 4 mg/kg/day for 4 days and cyclophosphamide 60 mg/kg/day for 2 days) and autologous stem cell transplantation (ASCT) in first complete remission (CR1). The comparison of 5-year outcomes in the patients treated with BAVC and BuCy showed a LFS of 37 ± 5% and 46 ± 2% (p = 0.23), a RI of 58 ± 11% and 44 ± 5% (p = 0.015), a TRM of 4 ± 4% and 11 ± 3% (p = 0.04) and an OS of 47 ± 5% and 50 ± 2% (p = 0.85), respectively (FOUILLARD 2004).

The incidence of relapse was significantly higher with BAVC and the TRM significantly lower. This study shows that patients with AML who received BAVC and ASCT had an identical survival to that of patients receiving BuCy. Although it cannot be concluded from this retrospective study that BAVC should replace BuCy, the low TRM observed with BAVC indicates that when BuCy or even TBI cannot be given, considering the patient’s age and physical conditions, BAVC may offer a good alternative (FOUILLARD 2004). Because of its reduced toxicity, the BAVC regimen is particularly suitable for older patients (THOMAS 2007) and/or patients in CR2 (MAK 2000).

BAVC can also successfully be used for ABMT in second remission. In a small number of patients (n=21) with AML in CR2 transplanted with BAVC the duration of CR2 has exceeded the duration of CR1 in all patients in continuous complete remission, the projected probability of disease-free survival was 52% at 63 months (MELONI 1990).

Leukemic relapse remains the most frequent reason for treatment failure in patients with AML treated with ASCT. The possible role of autologous bone marrow transplant (ABMT) in patients with AML who relapsed after ASCT was tested. 17 patients in untreated relapse underwent ABMT with the BAVC regimen. 76% achieved CR. The DFS probability at 3 years was 36%. BAVC conditioning followed by ABMT is associated with a low treatment-related toxicity (DE LA RUBIA 1996).

In a prospective trial the GOELAM compared to intensive consolidation regimens in young patients (15-50 years) with previously untreated primary AML. All patients received a first induction cycle of cytarabine and either idarubicine or rubidazone. The second cycle of induction treatment was high-dose cytarabine and the same anthracycline as in cycle one. Patients were randomly assigned to either receive a second course of intensive chemotherapy with Amsacrine (150 mg/m²/d day 1-5) and etoposide (100 mg/m²/d day 1-5) or a combination of busulfan and cyclophosphamide followed by an unpurged autologous BMT. The 4 year overall survival was similar in the two groups (54.5% vs. 50% p=0.72). The type of postremission therapy had no significant impact on the outcome (HAROUSSEAU 1997).

If an autologous transplant with PBSC shall be done enough CD34+ cells will be needed. There is a good correlation between the number of CD34+ cells infused and haematopoietic recovery. Successful mobilization is defined as the collection of ≥2.0 x 10⁶ CD34 cells/kg b.w. within a maximum of three leukaphereses. In AML, the optimal regimen for mobilizing PBSC has not been yet defined.

In a comparison of two consecutive cohorts with AML CR1 the efficacy of two PBSC mobilization regimens, mini–ICE + filgrastim (second consolidation) and HiDAC + AMSA + filgrastim (third consolidation) was analysed. 38 patients with de novo AML at six different hospitals were included in this study. All received remission induction chemotherapy with idarubicin and AraC. Patients not obtaining CR were given ACE (Amsacrine, AraC and etoposide). All patients who obtained CR1 received HiDAC as first consolidation.
The first 18 patients (12 of them obtained CR1 following only one induction cycle, whereas 6 had required a second induction with ACE) were mobilized with mini-ICE (idarubicine 8 mg/m², AraC 800 mg/m² and etoposide 150 mg/m² days 1-3). Only four patients reached >5 CD34+ cells/µl blood and were able to undergo leukaphereses. 20 patients (16 of them obtained CR1 following only one induction cycle, whereas 4 had required a second induction with ACE) received HiDAC + AMSA (AraC 3 g/m² b.i.d. days 1,3,5 + Amsacrine 150 mg/m² q.d. days 2, 4) followed by filgrastim. A total of 18 patients reached >5 CD34+ cells/µl blood and underwent PBSC harvesting starting on day 23 (14-29). Of 20 patients 17 (85%) reached the defined target of ≥2.0 x 10⁶ CD34 cells/kg after 1-3 leukaphereses.

It was shown that consolidation with HiDAC + Amsacrine + filgrastim – but not mini-ICE + filgrastim mobilises CD34+ cells efficiently in most patients within AML CR1 (HÖGLUND 2003). Amsacrine and cytarabine is an efficient combination with regards to PBSC mobilization.

4.3 ALLOGENEIC HSCT

Compared with non-allogeneic SCT therapies, allogeneic SCT has significant relapse free survival and overall survival benefits for intermediate- and poor-risk AML but not for good-risk AML in first complete remission (KORETH 2009).

The only curative treatment for high-risk or refractory AML is allogeneic stem cell transplant (allo SCT). Reduced intensity conditioning (RIC) lowers treatment related mortality (TRM), but at the expense of a higher relapse rate (WILHELM 2011).

Amsacrine is used broadly in concepts with aplasia conditioning. The FLAMSA-RIC protocol introduced 1999 by Kolb and Schmid for high-risk patients with AML and MDS has shown promising results in refractory disease as well as in first complete remission.

The concept of the FLAMSA-RIC protocol was to minimize the leukemic burden by intensive chemotherapy before reduced conditioning for allogeneic HSCT. For cytoreduction a fludarabine- and high-dose cytarabine based regimen was chosen. Similar protocols were effective in high-risk AML and advanced CML. Instead of anthracyclines, Amsacrine was introduced. This drug has shown to be similarly effective in poor-prognosis AML, and it also is less cardiotoxic. AML patients have been treated regularly with considerable doses of anthracyclines before transplantation. Therefore, the use of a different drug seemed reasonable to prevent chemotherapy resistance. The regimen was well tolerated and nevertheless ensured effective cytoreduction (SCHMID 2005).

An overall CR rate of 88% was achieved, thereby demonstrating a superior antileukemic efficacy of the protocol as compared with nonmyeloablative procedures, after which CR rates in the range of 45% have been observed. Interestingly, neither cytogenetics nor the stage of the disease at transplantation had a significant influence on outcome. No difference was seen in terms of survival between the intermediate and the poor prognostic cytogenetic subgroups.
In a prospective study of 75 consecutive patients high risk AML patients (median age, 52.3 years), high risk was defined by progressive or refractory disease (n=59), second remission after early relapse (n=8), or first remission with poor prognosis based on cytogenetics or delayed response to induction therapy (n=8). Unfavourable karyotypes were found in 49% of informative patients, and 68 patients had medical contraindications against standard conditioning. Fludarabine (30 mg/m²), cytarabine (2 g/m²), and Amsacrine (100 mg/m²/d for 4 days) were used for cytoreduction. After 3 days of rest, RIC consisted of 4 Gy total-body irradiation, antithymocyte globulin (ATG-Fresenius), and 80 to 120 mg/kg cyclophosphamide. 31 patients had an HLA-identical sibling donor; 44 patients had an unrelated and/or HLA-mismatched donor. pDLT was given from day +120 in patients who were not receiving immunosuppression and were free of graft-versus-host disease (GvHD). Complete remission was induced in 66 patients (88%). With a median follow-up of 35.1 months (range, 13.6 to 47.6 months), 2-year overall and leukaemia-free survival were 42% and 40%, respectively (SCHMID 2005).

Single center data retrospectively analysed showed that AML patients with the fast transplant approach (FLAMSA-RIC) yields encouraging long-term survival in high-risk AML patients, refractory to induction therapy, nearly approaching the results of patients transplanted in CR using classical conditioning (DÜNZINGER 2010). In another single center retrospective analysis the results of a high-risk cohort of 23 transplanted AML patients were compared to the outcome of 21 consecutive standard-risk patients <55 years, who had received standard, myeloablative sibling SCT in CR1 AML within the same center and time period. Survival and cumulative incidences of relapse and NRM were identical in both groups. In conclusion, the FLAMSA-RIC regimen produces long-term remission in a high proportion of patients with high-risk AML transplanted in CR1. In this cohort, FLAMSA-RIC showed equivalent antileukemic activity as compared to the standard protocols (Schmid 2008).

In a subsequent study in refractory AML, a lower number of chemotherapy courses given before FLAMSA–RIC was associated with superior outcome. On the basis of these observations, it was tested...
to improve the outcome of patients with CK-AML by employing the FLAMSA–RIC regimen for HSCT, as early as possible in the course of the disease (SCHMID 2011). CK-AML represent a rather small subgroup, constituting 10%–12% of all patients and 6%–8% of patients <60 years. Allogeneic HSCT is the recommended treatment for CK-AML and other subgroups with unfavourable cytogenetics, although data from prospective trials are scarce. The strategy was based on the poor results of CK-AML after conventional treatment, usually resulting in low CR rates, early relapse and short OS. The relatively intensive FLAMSA chemotherapy was intended to serve as a platform for a GvL reaction, established by RIC–HSCT following immediately.

OS and LFS at 4 years from SCT are 61% and 54%, respectively OS was 100% for patients transplanted in CR/CRi, supporting immediate HSCT, once control of the leukaemia has been achieved (SCHMID 2011).

Nowadays there are some adapted protocols published, like e.g. a protocol including 12 Gy TBI for younger ALL patients (ZOHREN 2009). On the other hand the FLAMSA-RIC protocol containing 4 Gy TBI / cyclophosphamide / ATG-F and implicates acute toxicity mainly due to TBI preventing its usage in elderly as well as in patients with severe concomitant diseases. To further reduce acute toxicity the Cologne group substituted 4 Gy TBI with treosulfan. This provides an alternative opportunity to treat elderly or patients with concomitant diseases when TBI appears not feasible due to possible toxicity (CHEMNITZ 2010).

In a small pilot study the Hamburg group generated first data regarding reducing toxicity of FLAMSA and to enhance the outcome. In patients undergoing allogeneic SCT non-transferrin-bound iron (NTBI) has been shown to appear quickly after onset of chemotherapy, while not being detectable at baseline. This indicates that cytotoxic therapy is associated with release of catalytically active iron. Pre-transplant iron overload has been associated with a higher risk of complications, e.g. infections. This could be due to released NTBI catalyzing the generation of reactive oxygen species and being an essential factor for pathogen growth. Labile plasma iron (LPI) represents the chelatable, redox-active and toxic fraction of NTBI.

Nine patients receiving FLAMSA-RIC, five consecutive patients received an iron chelator for the first 5 days of chemotherapy. On day 2 and 5 no LPI was detectable in patients receiving the iron chelator whereas in control group 3/4 patients had positive LPI values. The group with iron chelation therapy had a lower incidence of bacterial bloodstream infections with 2/5 vs. 4/4 in the control group (FRITSCH 2011).

Adaptions to the FLAMSA-RIC were made regarding incorporation of busulfan instead of TBI (DETRAIT 2011) or in modification of the GvHD prophylaxis, e.g. use of rapamune for a CNI free immunsuppression (SCHLEUNING 2009) or interventional treatment with everolimus due to severe toxicity induced by CNIs (SAYER 2010).

All these protocols are using aplasia conditioning, so patients receive a cytotherapeutic chemotherapy including Amscarine and other substances e.g. fludarabine. Patients are transplanted some days later in aplasia, without waiting for the recovery of blood cells upfront of transplantation.
of poor-risk patients in the aplasia conditioning group. There was no significant increase of NRM. For patients with refractory AML, survival was significantly better in the aplasia conditioning group than in the conventional conditioning group, suggesting that aplasia conditioning might be the preferable treatment option for these patients (JUNG 2011).

FLAMSA-RIC enables long-term disease free survival, even in primary refractory or relapsed AML patients. The sequential approach of this regimen seems to overcome the dismal prognosis of these patients. The moderate toxicity allows the application of this curative salvage therapy option even in elderly patient populations (SCHNEIDAWIND 2011).

FLAMSA-based sequential conditioning therapy is effective for previously untreated patients with high-risk MDS or sAML (SAURE 2012).
## SECOND-LINE THERAPY TREATMENT RESULTS IN ACUTE LEUKAEMIA (METAANALYSIS)

### AML second line therapy results with Amsacrine

<table>
<thead>
<tr>
<th>Study</th>
<th>n (--)</th>
<th>Regimen</th>
<th>First-Line Regimen</th>
<th>Response Rate</th>
<th>LFS</th>
<th>OS</th>
<th>Treatment-related mortality (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Thomas (2007)</td>
<td>23 AML</td>
<td>Amsacrine 100 mg/m²/d x 3 days and AraC 3 g/m²/d on days 1, 3, 5, 7</td>
<td>Prospective study, total 299 patients randomized</td>
<td>70% CR, 25% PR</td>
<td>Even 50% CR in patients after fourth relapse</td>
<td>Median 6 months, patients entering CR 8 months</td>
<td>Median OS = 8.1 months</td>
<td>4%</td>
</tr>
<tr>
<td>JD Hines (1984)</td>
<td>40 ANLL, 10 refractory to DAT</td>
<td>HD-ArAC 3 g/m² every 12 hours for 6 days and m-AMSA at doses of 75 mg/m² (6 patients) and 100 mg/m² (34 patients) on days 7-9</td>
<td>All patients had received between 450 mg/m² and 640 mg/m² anthracycline before</td>
<td>70% CR, 65% PR</td>
<td>Median remission duration 9-10 months</td>
<td>Median OS = 8.1 months</td>
<td>27.5%</td>
<td>Heavily pretreated patients, 13 first relapse, 9 in second relapse, 8 in third relapse and 6 in fourth relapse</td>
</tr>
<tr>
<td>M Freund (1991)</td>
<td>22 AML</td>
<td>Amsacrine 100 mg/m²/d for 5 days and AraC 2 x 1000 mg/m² i.v. days 1-5</td>
<td>54% CR, 14% PR, 6% OR</td>
<td>Median OS = 8 months</td>
<td>Median OS = 12 months</td>
<td>15%</td>
<td>In most patients the AMLA based regimen was third-line therapy</td>
<td></td>
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<tr>
<td>ZA Adin (1987)</td>
<td>47 patients, 12 relapsed ALL, 20 relapsed AML, 7 primary refractory AML</td>
<td>Amsacrine 200 mg/m²/d x 3 and HIDAC 3 g/m²/d on days 1-5</td>
<td>47%, 63% CR in relapsed AML, 29% CR in primary refractory AML</td>
<td>Median 3 months</td>
<td>Median OS = 10.8 months</td>
<td>15%</td>
<td>In most patients the AMLA based regimen was third-line therapy</td>
<td></td>
</tr>
<tr>
<td>S Tsecco (2003)</td>
<td>20 ALL, 12 ALL, 2 advanced CML</td>
<td>Amsacrine 200 mg/m²/d for 3 days and AraC 3 g/m²/d for 5 days</td>
<td>56% CR, 63% PR, 62% OR, 60% CR in AML and 67% in ALL</td>
<td>Median OS = 8.1 months</td>
<td>Median OS = 12 months</td>
<td>15%</td>
<td>HR relapsing or refractory AML</td>
<td></td>
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<tr>
<td>E Tenevery (2003)</td>
<td>91 AML</td>
<td>Amsacrine 130 mg/m²/d on days 1-4 and AraC 3 g/m²/d on days 5-7</td>
<td>CYAM 85 (69 patients) or LAM 90 trial (22 patients)</td>
<td>45% CR</td>
<td>11.5 months</td>
<td>Median OS = 7.5 months long term survivors 13%</td>
<td>12%</td>
<td>HR leukemia</td>
</tr>
<tr>
<td>WJ Sung (2005)</td>
<td>29 AML, 12 ALL</td>
<td>Amsacrine 100 mg/m²/d for 3 days and IDAC and etoposide, Amsa 350 mg/m²/d for 3 days = IDAC</td>
<td>IDA 12 mg/m²/day x 3 and AraC 100 mg/m²/day x 7</td>
<td>45% CR, 63% OR</td>
<td>Median remission duration 1.5 months</td>
<td>Median OS = 5 months</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>W Heldemann (1984)</td>
<td>12 AML</td>
<td>Amsacrine 210 mg/m²/d for 3-4 days and etoposide 330 mg/m²/day 1 and 5</td>
<td>33% PR</td>
<td>57% CR</td>
<td>Median DFS 21 weeks</td>
<td>Median OS = 25 weeks</td>
<td>17%</td>
<td>Heavily pretreated patients</td>
</tr>
<tr>
<td>Williamse (1988)</td>
<td>79 AML, 56 first relapse, 15 prem. refractory</td>
<td>AraC 1 g/m²/every 12 h for 6 days and Amsacrine 120 mg/m² x 3 d</td>
<td>57% CR</td>
<td>57% CR</td>
<td>Median DFS 21 weeks</td>
<td>Median OS = 25 weeks</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Fang (2011)</td>
<td>56 AML, 12 first relapse</td>
<td>Amsacrine 200 mg/m²/d for 3-4 days and etoposide 330 mg/m²/day 1 and 5</td>
<td>33% PR</td>
<td>57% CR, 61% OR/CR</td>
<td>57% CR</td>
<td>Median DFS 21 weeks</td>
<td>Median OS = 25 weeks</td>
<td>17%</td>
</tr>
</tbody>
</table>

**Second-line therapy results with Amsacrine**

- **Regimen:** Amsacrine 100 mg/m²/d for 3-4 days and etoposide 330 mg/m²/day 1 and 5
- **Response Rate:** 57% CR, 61% OR/CR
- **Median DFS:** 21 weeks
- **Median OS:** 25 weeks
## ALL second line therapy results with Amsacrine

<table>
<thead>
<tr>
<th>Study</th>
<th>(n=)</th>
<th>Regimen</th>
<th>First-Line Regimen</th>
<th>Response Rate</th>
<th>LFS</th>
<th>OS</th>
<th>Treatment-related mortality (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WJ Sung (2005)</td>
<td>29 AML 22 ALL</td>
<td>Amsacrine 100 mg/m²/d for 3 days and IDAc and etoposide. Amsa 350 mg/m²/d for 3 days = IDAC</td>
<td>AML 22 ALL</td>
<td>IDA 1.2 mg/m²/day x 3 and Amsa 100 mg/m²/day x 7</td>
<td>45% CR</td>
<td>68% CR</td>
<td>Median remission duration = 1.5 months</td>
<td>Median OS = 5 months</td>
</tr>
<tr>
<td>S Taruo (2003)</td>
<td>20 AML 12 ALL 2 advanced CML</td>
<td>Amsacrine 200 mg/m²/d for 3 days and AraC 3 g/m²/d for 5 days</td>
<td>ALL 2 advanced CML</td>
<td>56% CR</td>
<td>60% CR</td>
<td>67% CR</td>
<td>Median OS = 10.8 months 3 years OS in pts receiving SCT = 40% without SCT = 6.5%</td>
<td>HR leukaemia</td>
</tr>
<tr>
<td>ZA Arlin (1987)</td>
<td>47 patients 12 relapsed ALL 20 refractory AML 7 primary refractory AML</td>
<td>Amsacrine 200 mg/m²/d x 3 and HDAC 3 g/m²/d x 5</td>
<td>ALL 12 relapsed ALL 20 refractory AML 7 primary refractory AML</td>
<td>47% CR</td>
<td>66% CR in relapsed ALL 60% CR in refractory ALL 29% CR in primary refractory AML</td>
<td>Median 3 months</td>
<td>15%</td>
<td>In most patients the AMLA-based regimen was third-line therapy</td>
</tr>
<tr>
<td>ZA Arlin (1988)</td>
<td>36 relapsed and 4 primary refractory ALL</td>
<td>Amsacrine 200 mg/m²/d for 3 days, AraC 3 g/m²/d for 5 days</td>
<td>ALL 36 relapsed and 4 primary refractory ALL</td>
<td>75% CR</td>
<td>90% CR</td>
<td>Median 4 months (no consolidation treatment)</td>
<td>7.5%</td>
<td>12 pts in 2nd relapse, one in 4th relapse</td>
</tr>
<tr>
<td>O Raman (2004)</td>
<td>40 relapsed ALL at least 3 months after first line</td>
<td>Amsacrine 200 mg/m²/d for 3 days, AraC 3 g/m²/d for 5 days</td>
<td>ALL 40 relapsed ALL at least 3 months after first line</td>
<td>40% CR</td>
<td></td>
<td>Median 3.2 months, 3 years DFS of 12%</td>
<td>Median OS = 5.4 months</td>
<td>4%</td>
</tr>
</tbody>
</table>

## Non Amsacrine Salvage Therapies in ALL (examples)

<table>
<thead>
<tr>
<th>Study</th>
<th>(n=)</th>
<th>Regimen</th>
<th>First-Line Regimen</th>
<th>Response Rate</th>
<th>LFS</th>
<th>OS</th>
<th>Treatment-related mortality (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Tedeschi (2007)</td>
<td>12 refractory ALL 13 refractory ALL</td>
<td>Idarubicine 45 mg/m² on day 3, AraC 3 g/m² days 1-5 and 6 CR from day 7</td>
<td>ALL 12 refractory ALL 13 refractory ALL</td>
<td>50% CR</td>
<td>38% CR</td>
<td>Median DFS for all pts. was 6 months</td>
<td>Median OS for all pts. was 98 months</td>
<td></td>
</tr>
<tr>
<td>G Specchia (2005)</td>
<td>n=2 5 refractory ALL 18 first relapse ALL</td>
<td>FLAG-IDA = Fludarabine 30 mg/m²/day for 5 days, AraC 2 g/m²/day for 5 days, IDA 10 mg/m²/day for 3 days, GCSE 5 µg/kg</td>
<td>ALL 2 5 refractory ALL 18 first relapse ALL</td>
<td>30% (8/28) 0% (0/0)</td>
<td>50% (15/29)</td>
<td>DFS of CR pts. 6 months</td>
<td>All pts. OS = 4.5 months</td>
<td>4%</td>
</tr>
</tbody>
</table>
### Comparisons: Amsacrine based regimens and other therapies in AML/ANLL

<table>
<thead>
<tr>
<th>Study</th>
<th>(n=)</th>
<th>Regimen</th>
<th>First-Line Regimen</th>
<th>Response Rate</th>
<th>LFS</th>
<th>OS</th>
<th>Treatment-related mortality (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Horstmann (2005)</td>
<td>33 AML age 61-65</td>
<td>MAM-NC = Amsacrine 100 mg/m² every 12h for 5 days, Aracytin 100 mg/m² for 5 days</td>
<td>MAV</td>
<td>33% CR</td>
<td>25% (5.1 months)</td>
<td>18% (6.4 months)</td>
<td>42% ED (p=0.04)</td>
<td>AML</td>
</tr>
<tr>
<td>M Horstmann (2005)</td>
<td>39 AML age &gt; 65 years</td>
<td>DA II (45 mg/m², Amsacrine 100 mg/m²)</td>
<td>DA II</td>
<td>39% CR</td>
<td>8% (p=0.22)</td>
<td>6% (64 months, p=0.78)</td>
<td>18% ED</td>
<td>AML</td>
</tr>
<tr>
<td>T Kessler (2007)</td>
<td>16 AML age 66 years</td>
<td>TAA = 6-thioguanine 200 mg/m², days 3-9, Amsacrine 100 mg/m²/24h for 1-2 days, Amsacrine 20 mg/m² for 3-2 days</td>
<td>56% CR</td>
<td>32 months</td>
<td>16 months</td>
<td>6.25% ED</td>
<td>First-line in patients with impaired cardiac function</td>
<td>T A E</td>
</tr>
<tr>
<td>T Kessler (2007)</td>
<td>16 AML age 66 years</td>
<td>AMLCG (10 HAM 6 TAD)</td>
<td>AMLCG (10 HAM 6 TAD)</td>
<td>32% CR</td>
<td>9.5 months</td>
<td>3.5% ED</td>
<td>Matched pair analysis with normal AMLCG patients</td>
<td>T A E</td>
</tr>
<tr>
<td>W Linkesch (1989)</td>
<td>34 ANLL</td>
<td>AML Amsacrine 200 mg/m² for 3 days, Aracytin 100 mg/m²/12h x 10</td>
<td>58% CR</td>
<td>9 months</td>
<td>3.5 months</td>
<td>60%</td>
<td>First-line prospective, randomized trial in adults</td>
<td>W L I n k e s c h (1989)</td>
</tr>
<tr>
<td>W Linkesch (1989)</td>
<td>35 ANLL</td>
<td>DAT = Daunorubicine 50 mg/m² for 3 days, Amsacrine 100 mg/m²/12h for 10 days</td>
<td>90% CR</td>
<td>11.5 months</td>
<td>8 months</td>
<td>9 months</td>
<td>3.5 months</td>
<td>3%</td>
</tr>
<tr>
<td>E Berman (1989)</td>
<td>46 ANLL</td>
<td>AAT = Amsacrine 190 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days</td>
<td>70% CR</td>
<td>5 months</td>
<td>3.5 months</td>
<td>60%</td>
<td>Median remission duration = 1.5 months</td>
<td>E B e r m a n (1989)</td>
</tr>
<tr>
<td>E Berman (1989)</td>
<td>46 ANLL</td>
<td>DAT = Daunorubicine 50 mg/m² for 3 days, Amsacrine 25 mg/m² IV bolus followed by 100 mg/m²/12h for 5 days, 6-thioguanine 100 mg/m²/12h for 10 days</td>
<td>54% CR</td>
<td>8.5 months</td>
<td>3.5 months</td>
<td>25%</td>
<td>First-line prospective, randomized trial in adults</td>
<td>E B e r m a n (1989)</td>
</tr>
</tbody>
</table>

### Results of Amsacrine containing salvage regimens in paediatric acute leukaemia

<table>
<thead>
<tr>
<th>Study</th>
<th>(n=)</th>
<th>Regimen</th>
<th>First-Line Regimen</th>
<th>Response Rate</th>
<th>LFS</th>
<th>OS</th>
<th>Treatment-related mortality (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Horstmann (2005)</td>
<td>24 ALL</td>
<td>AEP Amsacrine 100 mg/m² for 2 days, etoposide 500 mg/m² for 2 days, methylprednisolone 1 g/day for 3 days</td>
<td>AEP</td>
<td>42% CR</td>
<td>6% (p=0.01)</td>
<td>5%</td>
<td>0%</td>
<td>M H o r s t m a n n (2005)</td>
</tr>
<tr>
<td>CP Steuber (1996)</td>
<td>47 children with AML, 41 primary refractory AML, 12 relapsed AML</td>
<td>Amsacrine 100 mg/m² for 3 days, etoposide 200 mg/m² for 1-3 days, Amsacrine 20 mg/m² for 3-5 days (200 mg/m²)</td>
<td>Amsacrine 20 mg/m² for 3 days (200 mg/m²)</td>
<td>34% CR</td>
<td>21% (p=0.03)</td>
<td>10%</td>
<td>3%</td>
<td>5%</td>
</tr>
</tbody>
</table>

---

**Notes:**
- **LFS:** Long-term survival.
- **OS:** Overall survival.
- **ED:** Event-related death.
- **D Alt:** Daunorubicine.
- **MAV:** Amsacrine 100 mg/m² every 12h for 5 days, Aracytin 100 mg/m² for 5 days.
- **TAA:** 6-thioguanine 200 mg/m², days 3-9, Amsacrine 100 mg/m²/24h for 1-2 days, Amsacrine 20 mg/m² for 3-2 days.
- **AEP:** Amsacrine 100 mg/m² every 12h for 2 days, etoposide 500 mg/m² for 2 days, methylprednisolone 1 g/day for 3 days.
- **AMLCG:** Amsacrine 100 mg/m² every 12h for 3 days, Aracytin 100 mg/m²/12h for 10 days.
- **DAT:** Daunorubicine 50 mg/m² for 3 days, Amsacrine 100 mg/m²/12h for 10 days.
- **AAT:** Amsacrine 190 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
- **AEP:** Amsacrine 100 mg/m² for 2 days, etoposide 500 mg/m² for 2 days, methylprednisolone 1 g/day for 3 days.
- **HSA:** Daunorubicine 50 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
- **HSA:** Daunorubicine 50 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
- **HSA:** Daunorubicine 50 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
- **HSA:** Daunorubicine 50 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
- **HSA:** Daunorubicine 50 mg/m² for 3 days, Aracytin 25 mg/m² IV bolus followed by 100 mg/m²/12h for 10 days, 6-thioguanine 100 mg/m²/12h for 10 days.
The prognosis of relapsed and/or primary resistant acute leukaemia and secondary AML is quite poor, and an effective reinduction regimen for these types of leukaemias is quite rare. Allogeneic and autologous hematopoietic stem cell transplantation offers a chance for long-term survival in these childhood cases with poor-prognosis acute leukaemia (TAVIL 2010).

Therefore, the main purpose of a salvage therapy for acute leukaemia is not only the achievement of CR but also bridging patients into an SCT program without increasing organ toxicity. Amsacrine has been related to a lower incidence of cardiac events than anthracyline analogs, meaning that Amsacrine would appear to be safe with regards to toxicity in a salvage setting (SUNG 2005).

5.1 ALL (PAEDIATRIC)

In children with multiple relapsed and/or refractory ALL an institutional, non-randomized, single-arm protocol with 24 children comprising Amsacrine, etoposide, and high-dose methyl-prednisolone as a salvage treatment resulted in a response rate of 58%. Refractory disease could be overcome in 4 of 10 patients. Patients received Amsacrine 100 mg/m²/day iv for 2 days, etoposide 500 mg/m²/day iv for 2 days, and methylprednisolone 1 g/m²/day iv or p.o. for 3 days (AEP). There was no AEP-related mortality after a total of 34 treatment cycles in 24 patients.

The CoALL is using in their actual prospective study an Amsacrine based regimen for second line therapy.

The efficacy of AEP in the HSCT-setting appears to be similar to that of the recently introduced TVTG-regimen (topotecan, vinorelbine, thiopeta, dexamethasone and gemcitabine) for relapsed or refractory acute leukaemia. Compared to the latter regimen AEP resulted in fewer adverse events with faster hematopoietic recovery and fewer infectious complications without any treatment related deaths. AEP may be considered as one element of a rescue strategy in refractory or multiply relapsed ALL of childhood (HORSTMANN 2005).
5.2 CARDIOTOXICITY
Paediatric ALL patients are heavily pretreated before they will receive a salvage treatment or will be transplanted, often they received a high cumulative dosage of anthracyclines. In addition to clinical cardiotoxicity of anthracyclines, manifesting itself as clinical heart failure, studies have also reported subclinical cardiotoxicity, i.e. abnormalities measured by diagnostic techniques, in previously healthy survivors of childhood cancer (KREMER 2002).

Asymptomatic anthracycline-induced cardiac damage is a serious problem among young childhood cancer survivors. The frequency of asymptomatic anthracycline-induced cardiac damage has been reported to be as high as 57%. The risk of developing heart failure remains a life-long threat (VAN DALEN 2005).

It can manifest in patients as either clinical heart failure (i.e. with symptoms) or as asymptomatic heart damage (i.e. without symptoms, but on, for example, an ultrasound of the heart, abnormalities in heart function can be seen). Heart damage caused by anthracyclines does not only occur during treatment, but also years after the end of treatment. The consequences of heart damage caused by anthracyclines are extensive. First, it can cause a reduction in the amount of anthracyclines that a patient was supposed to receive and as a result, the chance of survival of that patient can be reduced. Also, cardiotoxicity can lead to long-term side effects, causing severe morbidity and reduced quality of life (VAN DALEN 2007).

There were no significant episodes of cardiac toxicity reported with the administration of more than 1.800 doses of Amsacrine to patients with a wide variety of prior anthracycline exposure. Despite the consideration that many of the patients in this trial had been heavily pretreated with anthracyclines, no significant cardiotoxicity was reported with the use of Amsacrine. Others have reported similar experience (STEUBER 1996). Amsacrine is as effective as the most active drugs, cytarabine and daunorubicine and can produce complete remission in patients refractory to these drugs (CASSILETH 1986). The relatively low toxicity of Amsacrine gives the ability to consolidate patients with SCT following the salvage regimen (TAURO 2003).

5.3 ALLOGENEIC HSCT IN PAEDIATRIC ALL
The cure rate in paediatric ALL increased over the last decades. Allogeneic HSCT is used in high-risk patients and is often performed in CR2 or even later.

One of the used transplant protocols is an Amsacrine-based regimen. FLAMSA-RIC, a less toxic, intermediate intensity regimen, which consists of a short sequence of one block of anti-leukemic chemotherapy with fludarbine, high-dose AraC, and Amsacrine, and a conditioning block with 4 Gy total body irradiation (TBI) plus cyclophosphamide, has been shown to be remarkably effective even in patients with high-risk disease with poor prognostic features (WILHELM 2011).
5.4 AML (PAEDIATRIC)

The response to therapy in childhood acute myeloid leukaemia (AML) is much worse than in acute lymphoblastic leukaemia (ALL). In contrast to ALL, optimal risk-group stratification so far has not been achieved in childhood AML (STYCZYNSKI 2008). Relapse remains the major cause of treatment failure in paediatric AML (SANDER 2010).

Success is limited in treating children with acute myeloid leukaemia (AML) who relapse or who fail the initial induction therapy. The outcome is somewhat dependent on the initial treatment and the duration of the first remission. Several active relapse regimens exist for adults and children with AML. Second remission rates of 50%–60% have been reported after high dose cytarabine (cytosine arabinoside, AraC) alone or in combination with anthracyclines, L-asparaginase, or Amsacrine (OZKAYNAK 1998).

The survival of patients with AML who relapse or fail to achieve an initial remission has been very poor (OZKAYNAK 1998).

5.5 FIRST LINE

A total of 868 children (477 boys, 391 girls) were registered at diagnosis on AML10 and AML12. In the AML10 trial, all children were scheduled to receive four courses of intensive chemotherapy. The two induction courses were based on the daunorubicin and cytarabine. The two consolidation courses were Amsacrine, cytarabine and etoposide (MACE) and mitoxantrone and cytarabine (MidAC). Drug doses were reduced by 25% for children aged <1 year or under 10 kg body weight (RAO 2005).
MAcE

CONSOLIDATION CHEMOTHERAPY FOR AML

Drugs/Dosage/Administration:

<table>
<thead>
<tr>
<th>Day</th>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 (5 doses) Give first each day due to short expiry</td>
<td>Amsacrine</td>
<td>100 mg/m² in 500 ml Glucose 5%</td>
<td>IV infusion over 1 hour</td>
<td>Once daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Then flush with glucose 5% before proceeding with etoposide (amsacrine will precipitate in presence of sodium chloride 0.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 (5 doses)</td>
<td>Etoposide</td>
<td>100 mg/m² in 1000 ml 0.9% Sodium Chloride</td>
<td>IV infusion over 1 hour</td>
<td>Once daily</td>
</tr>
<tr>
<td>1-5 (5 doses)</td>
<td>Cytarabine</td>
<td>200 mg/m² in 500 ml 0.9% Sodium Chloride</td>
<td>IV infusion over 22 hours</td>
<td>Once daily</td>
</tr>
</tbody>
</table>

In the AML 12 trial, children were allocated between three risk groups based on the results of cytogenetics and response to the first induction course. All children received consolidation therapy with MACE and MidAC (RAO 2005).

Overall, there were no significant differences in OS between Down Syndrome (DS) and non DS children with 5-year survival rates of 74% vs. 62%. However, model checking of the proportional hazard assumption showed evidence of a difference in HR over time: early on, DS patients had an increased risk of death, whereas later, these positions were reversed (RAO 2005).

The French LAME trials 89/91 included an induction phase (mitoxantrone plus cytarabine) and two consolidation courses, one containing timed-sequential high-dose cytarabine, asparginase and Amsacrine. 90% of patients achieved a CR. Total cumulative dose of intercalating agents was 460 mg/m² (given as daunorubicin dose with the conversion factors daunorubicin/doxorubicin: mitoxantrone, 1:5). Additionally a 450 mg/m² Amsacrine cumulative dose was delivered.

The optimal number of post-remission cycles has still not been determined. To be noted is that the MRC AML and LAME protocols are the only ones to include, according to various modalities, three different DNA intercalators, daunorubicin, mitoxantrone and Amsacrine (PEREL 2005).

5.6 SALVAGE REGIMENS

From a small phase I study a dose recommendation was given from the authors. They recommend for induction and consolidation/intensification treatment etoposide, 125 mg/m² and Amsacrine, 125 mg/m², intravenously daily for 5 days. A reduction by at least 50% in the dose of each drug is recommended for maintenance of complete remissions (LETENDRE 1985).

A randomized study compared the combination of Amsacrine (100 mg/m²/d on days 1 to 5) and etoposide (200 mg/m²/d on days 1 to 3) with the same two agents plus azacitidine (250 mg/m²/d on days 4 to 5) for the therapy of induction-resistant or relapsed childhood acute myeloid leukaemia (AML) after a successful pilot study (STEUBER 1991).
167 assessable children with AML who either had failed to respond to primary induction therapy (n=41) or had relapsed (n=126) were randomized.

Overall, there were 56 complete responses (34%). Among primary refractory patients, the complete response rate was higher with the three drug regimens (18% vs. 53%, p=0.03). In the relapsed patients, there was no difference in complete response rates related to treatment (31% vs. 35%, p=0.3). The major toxicities for both regimens were myelosuppression and infection.

The overall complete response rate of 34% in this patient population is indicative of effective anti-leukemic activity. A maximum of two courses of therapy was allowed for induction. A maximum of 10 courses of maintenance therapy was given for patients who achieved remission (STEUBER 1996).

Of the relapsed patients which responded to the therapy slightly over half (55%) of patients who responded did so after one course. So it is always worth to give a second course, because it gives nearly the same chance for response as the first course. For patients who entered remission and continued chemotherapy, dosage reduction for toxicity (primarily myelosuppression) was indicated after 35 of 116 post-induction courses (30%±4%) on treatment I and 43 of 98 post-induction courses (44%±5%) on treatment II (p=0.02) (STEUBER 1996).
5.7 AUTOLOGOUS HSCT

In a single center retrospective study with de novo AML comparing allo-BMT vs. auto-BMT after 1-2 induction courses and two consolidations (the second one contained AraC 2.4 g/m² for 4 days and Amsacrine 100 mg/m² on 3 days). Patients with a HLA-identical sibling received an allo-BMT all others an autologous-BMT. Hematologic reconstitution was significantly slower in auto-BMT recipients. Post-transplant 8-year EFS was 74.5% in allo-BMT and 74.2% in auto-BMT. Improved results in children with high-risk AML can be obtained by either allo- or auto-BMT performed after two courses of intensive consolidation therapy (ORTEGA 2003).

5.8 ALLOGENEIC HSCT IN PAEDIATRIC AML

Overall, one-third of children with relapsed AML can be cured today. SCT in CR2 is recommended for most patients (SANDER 2010).

The BMF group has started a prospective trial - the AML SCT-BFM 2007- with, the title:

“Allogeneic stem cell transplantation for children, adolescents and young adults with relapsed or refractory Acute Myeloblastic Leukaemia”

One of the study objectives is to evaluate whether “FLAMSA” increases survival as compared to a survival rate estimated from historical data (studies AML-BFM and Relapsed AML 2001/1) in children suffering from refractory AML or relapsed AML responding poorly to reinduction therapy.

5.9 CLINICAL BENEFIT OF AMSACRINE

Whatever the salvage therapy, best results are achievable when stem cell transplantation (SCT) is feasible after salvage therapy. On this basis, characteristics of an ideal salvage regimen should include either antileukaemic efficacy or acceptable extra-hematologic toxicity. As a matter of fact, in clinical practice it is not rare that toxicity from previous therapy may preclude the feasibility of stem cell transplantation (FERRARA 2004).
5.9.1 ANTI LEUKAEMIC EFFECT

Amsacrine has a strong antileukaemic effect. It is as effective as the most active drugs, cytarabine and daunorubicin and can produce complete remissions in patients refractory to these drugs (CASSILETH 1986). Amsacrine is not cross-resistant to anthracyclines (JEHN 1991). Amsacrine may be combined with AraC without significant negative effect to AraCTP (FREUND 1991). Several studies proved the interest of regimens using Amsacrine in combination with high-dose cytarabine (HD-AraC) in refractory AML and ALL.

5.9.2 LESS CARDIAC TOXICITY

Cytostatics of the anthracycline class are the best known of the chemotherapeutic agents that cause cardiotoxicity and this may occur >20% of patients treated with doxorubicin or daunorubicin. One of the risk factors is the cumulative dose of anthracyclines.

Cardiotoxicity can be prevented by modifying risk factors, aggressively monitoring for signs and symptoms as chemotherapy is administered and continuing follow-up after completion of a course. Modification of chemotherapy or use of appropriate drug therapy should be initiated on the basis of changes in monitoring parameters before the patient exhibits signs and symptoms of cardiotoxicity (PAI 2000).

In addition to clinical cardiotoxicity of anthracyclines, manifesting itself as clinical heart failure, studies have also reported subclinical cardiotoxicity, i.e. abnormalities measured by diagnostic techniques, in previously healthy survivors of childhood cancer (KREMER 2002). Asymptomatic anthracycline-induced cardiac damage is a serious problem among young childhood cancer survivors. The frequency of asymptomatic anthracycline-induced cardiac damage has been reported to be as high as 57%. The risk of developing heart failure remains a life-long threat (VAN DALEN 2005).

Anthracycline-induced cardiotoxicity after treatment for childhood cancer is a considerable and serious problem. An important side effect of anthracyclines is heart damage (cardiotoxicity). It can manifest in patients as either clinical heart failure (i.e. with symptoms) or as asymptomatic heart damage (i.e. without symptoms, but on, for example, an ultrasound of the heart, abnormalities in heart function can be seen). Heart damage caused by anthracyclines cannot only occur during treatment, but also years after the end of treatment. The consequences of heart damage caused by anthracyclines are extensive. First, it can cause a reduction in the amount of anthracyclines that a patient was supposed to receive and as a result, the chance of survival of that patient can be reduced. Also, cardiotoxicity can lead to long-term side effects, causing severe morbidity and reduced quality of life (VAN DALEN 2007).

The incidence and outcomes of cardiac complications in 278 recipients of RIC-HSCT was analysed. All patients received conditioning with BU, fludarabine and TBI. Patients were evaluated from conditioning therapy until 100 days after HSCT. Median age was 56 years. Cardiac events were defined as either one or more of the following: arrhythmias, myocardial infarction or congestive heart failure. 25 patients developed arrhythmias at a median of 3 days post-transplant. All patients converted to a normal rhythm by medical therapy at a median of 2 days. Day 100 mortality was 40% in this group. A history of high-dose anthracycline treatment and a low ejection fraction were risk factors for the development of cardiac complications. Patients were categorized as high risk according to either prior total anthracycline dose ≥400 mg/m² or an ejection fraction ≤45%. Day 100 mortality was high in the patients with cardiac event at 10/25 (40%) compared to 28/278 (10%) in patients who did not develop a cardiac event. All patients who developed cardiac complications had a preexisting cardiac condition. The majority of the patients having a cardiac complication had received an anthracycline dose of >400 mg/m². A pre-transplant echocardiogram with an LVEF ≤45% was also strongly associated with the
development of a cardiac toxicity. There may be a potential for prevention in patients for whom allo-
geneic HSCT is recognized as part of the treatment plan from the time of diagnosis. For example when
medically appropriate alternatives may exist, such as avoidance of high-dose anthracyclines during
induction, chemotherapy might reduce the risk of later cardiac complications (PERES 2010).

Cardiotoxicity of the anthracycline congestive type has not been observed with Amsacrine (JEHN
1991). Amsacrine has lower cardiotoxicity than anthracyclines. Anthracyclines have a cumulative
cardiotoxicity, which may have an impact on long term survivors. Many authors come to the conclusion
that high dose of anthracyclines should be avoided. Amsacrine is a good alternative for this (TAURO

Patients with relapsed AML or ALL and who had reduced left ventricular ejection fraction received
Amsacrine without incident if their serum potassium level was higher than 4.0 mEq/L at the time of
drug administration. Amsacrine is a safe and effective therapy for patients with acute leukaemia and
cardiac disease (ARLIN 1991).

A recently published trial compared two different dosages for daunorubicin in a prospective randomized
way. For the induction regimen the higher dose with 90 mg/m²/day for 3 days resulted in a significant
better CR rate 70.6% vs. 57.3% (p<0.001) and improved overall survival (median 23.7 vs. 15.7 months;
p=0.003) as the arm with 45 mg/m²/day (FERNANDEZ 2009).

Taking into account that many clinicians in Europe are using daunorubicine with 60 mg/m² for 3 days
in the “7+3” regimen and this often in induction regimen I + II, the dose at the end which the patient
received even without consolidation therapy is cumulative 360 mg/m² anthracyclines reaching CR1.

Often consolidation regimens also include anthracyclines, so that many patients being in CR1 had
received more than 400 mg/m².

Patients not reaching CR1 or patients who relapse need a salvage regimen. If this again includes
anthracyclines – which is often the case - they would have received a cumulative dose above 400 mg/m²
and may be on high risk for complications at transplantation.

Amsacrine can replace anthracyclines, without cumulative increase of the cardiotoxicity and with at
least the same antileukaemic effect. Thus it is a good substance to bridge to transplant.
6 BRIDGING TO TRANSPLANT

Allogeneic SCT can provide a chance to cure the disease. Therefore, the main purpose of a salvage therapy for acute leukaemia is not only the achievement of CR but also bridging patients into an SCT programme without increasing organ toxicity. In conclusion, a salvage regimen consisting of Amsacrine plus IDAC with or without etoposide seemed to be a safe and effective regimen for patients with refractory or relapsed acute leukaemia, and especially for SCT candidates, and the regimen may be valuable with regards to organ dysfunction and TRM (SUNG 2005).

Not only in AML patients can Amsacrine be used to bridge to transplant, this is also reported for ALL patients (SUNG 2005, TAURO 2003).
7 HANDLING INSTRUCTIONS

The Amsacrine ampoules and diluents are stored at room temperature, protected against light.

Amsacrine is presented as two sterile liquids which are combined immediately prior to use. The drug ampoule contains 1.5 ml of Amsacrine (dissolved in 1.3595 g N,N-dimethylacetamide), forming a bright orange-red liquid at a concentration of 50 mg/ml. The diluent vial contains 13.5 ml of 0.0353 M (42.93 mg) L-lactic acid. When 1.5 ml (75 mg) of concentrated Amsacrine is added to 13.5 ml of lactic acid diluent, the resulting solution contains 5 mg/ml (i.e. 75 mg in 15 ml).

It is recommended that the preparation of the drug should be carried out using a glass syringe due to possible extraction of components of rubber or certain plastic material, an effect having been reported due to the N,N-dimethylacetamide solvent. However, the Canadian provider (Erfa) claims that plastic syringes can be used, providing that Amsacrine remains in the syringes for no longer than 15 minutes. The solution should be added to 500 ml of 5% dextrose (in adults) and infused over a period of 60 – 90 minutes. Amsacrine is incompatible with sodium chloride 0.9%. Catheters flushed with heparin/saline solutions should be rinsed with sterile water before administering Amsacrine. The ready-to-use solution can be placed in plastic bags when diluted for IV infusion, and an intravenous cannula (Venflon) can be used. Observe that the infusion is not mixed in the proximal catheter with other fluids containing NaCl.

An infusion solution of maximal 400 mg amsacrine in 500 cc 5% glucose, stored in PVC or polythene infusion bags and prepared conform above instructions will be stable for 48 hours at room temperature and protected from light.

When handling Amsacrine, wear two layers of disposable gloves and safety glasses. Protective coveralls should be worn, where gloves are pulled over the sleeves.

By accidental release of Amsacrine, the spill is collected with absorbent material, and the area is cleaned thoroughly with water and soap. In case of skin contact, 5% isopropanol solution is used to clean the area.

By extravasation, the infusion is discontinued immediately. The infusion lead is replaced with a 5 ml syringe and slow aspiration without pressure is initiated: Aspirate as much as possible of the extravasated solution, and remove the IV access while aspirating. The affected extremity is kept elevated, and a cold pack is applied for at least an hour. Of more specific measures, it is recommended to topically apply dimethyl sulfoxide (DMSO, 99%) every 8 hours (with a sterile gauze pad without pressure), and to let the area air dry. The treatment is continued for a minimum of 7 days. Although Amsacrine is classified as a vesicant, very few cases of tissue necrosis have been reported in the literature, so non-invasive interventions are generally recommended (NordMedica A/S Product Brochure 2010).
8 SUMMARY

- **AMSIDYL®/AMSIDINE®/AMEKRIN®** is used for induction and consolidation therapy in acute myeloid leukaemia and in acute lymphatic leukaemia for adults who failed to conventional therapies.

- It can replace anthracyclines without a loss of the antileukemic efficacy.

- It is the Golden Standard in second-line therapy of acute leukaemia (LABAR 2004, BURNETT 2011)

  - up to 75% CR in ALL (ARLIN 1988)
  - up to 80% CR in AML (THOMAS 2008)

- Less cardiotoxicity compared to anthracyclines (SCHMID 2005, KESSLER 2008)

- Successful use over decades in regimens like MACE, MAMAC, BAVC, TAA, FLAMSA

- "Bridging to transplant" (SUNG 2005)
9 LITERATURE


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10 SUMMARY OF PRODUCT CHARACTERISTICS

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10.1 NAME OF THE MEDICINAL PRODUCT
Amsidine, concentrate for solution for intravenous infusion 75 mg/1.5 ml

10.2 QUALITATIVE AND QUANTITATIVE COMPOSITION
75 mg Amsacrine per 1.5 ml concentrate for solution for intravenous infusion.
For excipients, see 6.1.

10.3 PHARMACEUTICAL FORM
Concentrate for solution for intravenous infusion.
Description: Active ingredient in glass ampoule with orange-red liquid. Solvent in glass vial with colourless liquid.

10.4 CLINICAL PARTICULARS

10.4.1 THERAPEUTIC INDICATIONS
Acute non-lymphatic leukaemia refractory to other treatments.

10.4.2 POSOLOGY AND METHOD OF ADMINISTRATION

Dosage and administration
The usual dosage of amsacrine in the induction phase is 90 mg/m² during 5 successive days at an infusion rate/day of 30-90 minutes. The total dose per course is 450 mg/m². If bone marrow biopsy performed on day six displays over 50% cellularity and the blast count is over 30%, the treatment may be extended, bringing the total dose per course of treatment to 720 mg/m². More than one course of treatment may be required to achieve induction of a remission. Depending on the effectiveness of the first course the subsequent course can be started after a two week (if not effective) to four weeks (if effective) interval. In cases where no hypocellular marrow has been achieved after the first course of treatment, the daily dose of amsacrine may be increased to 120 mg/m² for the subsequent courses, unless this is contraindicated for reasons other than bone-marrow toxicity.

Dosage with hepatic or renal insufficiency
For patients with impaired liver function or impaired renal function, the dose of amsacrine should be decreased by 20-30%.

Dosage in paediatrics
Experience with amsacrine in children is limited.

10.4.3 CONTRAINDICATIONS
- hypersensitivity to amsacrine or acridine derivates;
- hypersensitivity to one of the other ingredients of the product;
- clear bone-marrow-suppression as a result of treatment with cytostatics or radiotherapy;
- lactation.

10.4.4 SPECIAL WARNINGS AND PRECAUTIONS FOR USE
Amsacrine should only be used under strict control of a specialised oncologist, with preference in institutions with experience with this kind of therapies.
Bone marrow suppression
Amsacrine can cause severe bone-marrow-depression, thus frequent blood control is necessary. Infections and haemorrhages can be fatal. With an already existing bone-marrow-depression caused by drugs, amsacrine should be administered cautiously and with extra controls. Also if a too strong decrease in white blood cells or blood platelets occurs, interruption of the amsacrine treatment or decrease of dosage can be necessary. Red blood cells and platelets should be available for transfusion as well as other facilities for the treatment of bone-marrow-depression.

Hyperuricemia
Amsacrine can induce hyperuricemia secondary to rapid lysis of neoplastic cells. Careful monitoring of blood uric acid levels is recommended. Consideration may be given to reducing uric acid levels prophylactically, prior to or concurrent with amsacrine treatment.

Patients with hepatic or renal impairment
Toxicity at recommended doses is enhanced by hepatic or renal impairment. Laboratory evaluation of hepatic and renal function is necessary prior to and during administration (see section 4.2 Posology and method of administration).

Adverse reactions (see section 4.8 Undesirable effects)
The physician should be aware of allergic reactions (anaphylaxia, oedema and skin reactions), GI problems, epileptic insults and should consider cardiotoxicity, renal insufficiency and hepatic impairment. Local necrosis can occur with extravasation of amsacrine.

Cardiac function
Careful monitoring of cardiac rhythm is recommended for detection of cardiotoxicity. Patients with hypokalemia are at increased risk of ventricular fibrillation. The risk of developing arrhythmias can be minimized by ensuring a normal serum potassium level immediately prior to and during amsacrine administration. Hypokalaemia should be corrected prior to amsacrine administration.

Laboratory Tests
Complete blood counts, liver and renal function tests, and electrolytes should be performed regularly. Electrolytes should be re-evaluated before each day’s treatment.

Conception
During treatment of man or female, 3 months after treatment of amsacrine for female and 6 months for man, conception should be prevented. Reversible azospermia in humans has been described (see section 4.6 pregnancy and lactation).

Use in children
Safety and activity in children have not yet been established.

10.4.5  INTERACTION WITH OTHER MEDICINAL PRODUCTS AND OTHER FORMS OF INTERACTION

Vaccines
Concomitant influenza or pneumococcal vaccination and immunosuppressive therapy have been associated with impaired immune response to the vaccine.

Other protein-bound drugs
Amsacrine may be displaced from serum albumin, with consequential increase in free drug and toxicity if used with other protein bound drugs.
Other cytotoxic drugs
Adverse events may be potentiated by use with other cytotoxic drugs.

10.4.6 PREGNANCY AND LACTATION
Pregnancy
Data on the usage of this compound during pregnancy in patients are not available to judge possible harmfulness. However based on its pharmacological activity harmfulness of treatment during pregnancy is possible. In animal studies teratogenicity and other reproductivity toxicity has been observed (see section 5.3) Based on animal studies and the mechanism of action of the substance, use during pregnancy is discouraged, especially during the first trimester. In every individual case the advantages of the treatment should be evaluated towards the possible risks to the foetus.

Lactation
As it is not clear whether amsacrine is excreted in the mother milk, lactation is contraindicated.

10.4.7 EFFECTS ON ABILITY TO DRIVE AND USE MACHINES
No data about this influence is known. In view of reported adverse effects profile patients are advised after administration of amsacrine to be cautious when driving or using machines.

10.4.8 UNDESIRABLE EFFECTS
The estimated frequencies of adverse events are categorized as follows: very common (³1/10); common (³1/100, <1/10); uncommon (³1/1.000, <1/100); rare (³1/10.000, <1/1.000); very rare (<1/10.000). The most common adverse events are nausea and/or vomiting, anaemia, fever and infection. Pain or phlebitis on infusion site has been reported.

Infections and infestations
Common: Infection, fever.

Blood and lymphatic system
All patients treated with a therapeutic dosage of amsacrine show bone marrow depression. Main complications are infections and haemorrhages. Minimal white blood cells occur on day 5-12, usually followed with complete recovery on day 25. The pattern of inhibition of blood platelets is similar to that of leucocytes. Common: thrombocytopenia, pancytopenia, haemorrhage.
Rare: anaemia, granulocytopenia, leukopenia, fever apparently not related to sepsis.

Immune system disorders
Rare: Allergic reactions amongst them anaphylaxia and edema.

Metabolism and nutrition disorders
Common: Hypokalaemia.
Rare: Weight decrease, weight increase.

Psychiatric disorders
Common: Emotional lability.
Rare: Lethargy, confusion

Nervous system disorders
Common: Grand mal seizures, sometimes paired with hypokalaemia. The seizures can be treated in the usual way, f.i. with phenytoin. Rare: headache, hypoesthesia, dizziness and peripheral neuropathy.
Eye disorders  
Rare: Visual disturbances.

Cardiac disorders  
Common: Cardiotoxic symptoms, cardiac arrhythmia, congestive heart failure (especially in paediatric patients, pretreated with antracyclines). Rare: atrial fibrillation, sinus tachycardia, fatal or life-threatening ventricular fibrillation (usually in patients with hypokalaemia), ventricular arrhythmias, cardiomyopathy, bradycardia, ECG changes, ejection fraction decrease.

Vascular disorders  
Very common: Hypotension  
Common: Haemorrhages.

Respiratory, thoracic and mediastinal disorders  
Common: Dyspnoea.

Gastrointestinal disorders  
Very common: Nausea and vomiting (mild to moderate), diarrhoea, abdominal pain. Mucosa of mouth and tractus digestivus are frequently effected ranging in severity from mild to life-threatening. Total oral mucosa can be affected; recovery takes several weeks.

Hepato-biliary disorders  
Common: Hepatitis, jaundice, hepatic insufficiency (see also section 4.2 Dosage with hepatic and renal insufficiency).

Skin and subcutaneous tissue disorders  
Very common: Purpura.  
Common: Alopecia, urticaria and rash.

Renal and urinary tract disorders  
Common: Haematuria.  
Rare: Anuria, proteinuria and acute renal insufficiency.

General disorders and administration site disorders  
Very common: Phlebitis on infusion.  
Common: Local tissue irritation, necrosis, cutaneous inflammatory reaction. This problem is related to the concentration of amsacrine infused. This can be prevented by diluting amsacrine in a greater volume 5 % glucose and infusion is spread over a larger period of time (minimal 1 hour). With extravasation necrosis can occur.

Investigations  
Common: Liver function tests show in 20-40% of the cases transient elevations of hepatic enzymes. If a serious increase is seen, dosage decrease is necessary.  
Rare: Increased laboratory values of bilirubin, BUN, alkaline phosphatase, creatinine and ASAT.

10.4.9 OVERDOSE  
No specific antidote is known in case of over dosage. Treatment should be symptomatic and supportive. Haemorrhage and infection, resulting from bone marrow hypoplasia or aplasia, may require intensive supportive treatment with red cell, granulocyte or platelet transfusions and appropriate antibiotics.
Vigourous symptomatic treatment may be necessary for severe mucositis, vomiting or diarrhoea.

10.5 PHARMACOLOGICAL PROPERTIES

10.5.1 PHARMACODYNAMIC PROPERTIES
Pharmacotherapeutic category: antineoplastic agent (ATC-code: L01X X01).

Amsacrine is a synthetic acridine-derivative. Though the mode of action is not fully understood, it is accepted that amsacrine binds to DNA by intercalation and external electrostatic binding. The synthesis of DNA is inhibited. DNA-fragmentation and chromosomal changes occur. RNA-synthesis is not changed. Clinically no cross resistance has been found with antracycline antibiotics such as doxorubicine and daunorubicine. Amsacrine gave in refractory patients a remission of short duration in 20%-30% of patients.

10.5.2 PHARMACOKINETIC PROPERTIES

Distribution
Amsacrine is extensively bound to tissue, probably especially to membrane structures. Also there exists a strong binding to plasma proteins which is concentration dependant. In animal experiments amsacrine penetrates the CNS system. The partition volume of amsacrine is about 1.5-2 l/kg body-weight.

Metabolism
Amsacrine is metabolised mainly in the liver.
Plasma-elimination curve shows firstly a fast decline (distribution phase) followed by an elimination phase with a half-life of 6-9 hours. The half-life of the slow phase is considerably longer in patients with hepatic insufficiency (more than 17 hours). Mild to moderate kidney insufficiency has hardly an effect on pharmacokinetics of amsacrine.

Excretion
Renal clearance of unchaged amsacrine is about 4% of total body clearance which is 200-500 ml/min. Inactive metabolites are excreted with the bile.

10.5.3 PRECLINICAL SAFETY DATA
Amsacrine caused embryotoxicity and teratogenicity in rats and mice. In view of its mechanism of action, amsacrine should be considered a potential carcinogen and mutagen in man.

10.6 PHARMACEUTICAL PARTICULARS

10.6.1 LIST OF EXCIPIENTS
An ampoule contains as solvents dimethylacetamide (DMA). A vial contains solvents L-lactic acid and water for injection.

10.6.2 INCOMPATIBILITIES
The amsacrine solution must only be diluted with the given lactic acid and with 5% glucose. Amsacrine is dissolved in dimethylacetamide (DMA). As DMA can interact with plastic and rubber, glass syringes should be used in preparing an intravenous solution (see section 6.6. Instructions for use and handling).
N.B.:  
1. Do not use other diluents  
2. Amsacrine is incompatible with saline  
3. Amsacrine is dissolved in dimethylacetamide (DMA). As DMA can interact with plastics and rubber, glass syringes must be used; Codan syringes, mentioned under point 5 can be used  
4. Glass syringes can be cleaned with acetone.  
5. Amsacrine-DMA solution can if necessary be transferred to the vial with lactic acid by means of a 2 cc plastic syringe, trademark “CODAN”, if the amsacrine solution is kept in this syringe for not longer than 10 minutes. 20 cc “CODAN” syringes can, if necessary, be used to transfer diluted amsacrine-lactic acid solution to 5% glucose infusion solution if this solution does not stay longer than 30 minutes in the syringe.  
6. Glass infusion bottles or bags with rubber stops must not be used, as an interaction between amsacrine solution and rubber stops cannot be excluded.

10.6.3 SHELF-LIFE  
3 years.  
An infusion solution of maximal 400 mg amsacrine in 500 cc 5% glucose, stored in PVC or polythene infusion bags and prepared conform above instructions will be stable for 48 hours at room temperature and protected from light.  

10.6.4 SPECIAL PRECAUTIONS FOR STORAGE  
Do not store above 25 °C.  
Do not store in the refrigerator; do not freeze.  

10.6.5 NATURE AND CONTENTS OF CONTAINER  
Box containing 6 glass (type 1) ampoules of Amsidine and 6 glass (type 1) vials of lactic acid.  

10.6.6 INSTRUCTIONS FOR USAGE AND HANDLING AND DISPOSAL  
General  
As with other toxic substances extreme caution should be used in preparation and administration of the product. Precautions should be taken in order to prevent exposure to personnel during preparation and administration.  

Method of handling  
During preparation, preferably in vertical laminair airflow cabinet, protective gloves, mouth mask and spectacles should be used, during administration protective gloves (polyethylene with sterile rubber gloves on top). If Amsacrine-solution gets in contacts with the skin or the mucosa, the contact place should be washed immediately and thoroughly with soap and water. After accidental contact with amsacrine during preparation acute systemic toxicity can occur (nausea, vomiting, headache, feeling of general malaise, urticaria).  

Preparation of an intravenous solution  
Amsacrine is formulated as two sterile liquids that are aseptically combined prior to use. Each ampoule contains 1.5 ml of amsacrine solution in N, N-dimethylacetamide (50 mg amsacrine per ml). Each vial contains L-lactic acid (42.9 mg) in water for injections (up to 15 ml). Exactly 1.5 ml of the solution from the ampoule is removed by aid of a graduated glass syringe and immediately added to the vial with L-lactic acid (see section 6.2 incompatibilities). Mix by thoroughly shaking. The resulting orange-red solution contains Amsidine 5 mg/ml.
Because of phlebitis that may occur at doses greater than 70 mg/m², Amsidine must be diluted in 500 ml 5% glucose solution and infused over 60 to 90 minutes in PVC or polythene infusion bags and PVC administration sets.

Method of removal
Any unused product, any items that come into contact with the product and waste material must be disposed of in accordance with local requirements.

10.7 MARKETING AUTHORIZATION HOLDER
NordMedica A/S
Jægersborg Alle 164
DK-2820 Gentofte
Denmark

10.8 MARKETING AUTHORIZATION NUMBER(S)
RVG 09084

10.9 DATE OF FIRST AUTHORISATION/ RENEWAL OF THE AUTHORIZATION
May 12th 1982.

10.10 DATE OF REVISION OF THE TEXT
Last complete revision: September 2004
Last partial revision of section 7: June 2010
Based on IPI of July 18th 2003.