Treatment of Acute Myeloid Leukemia with 20-30% Bone Marrow Blasts

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Abstract. The transition of patients with ≥20% <30% bone marrow (BM) blast from the FAB category of myelodysplasia to the family of acute myeloid leukemia (AML) according to the recent WHO classification has not resolved the argument as to whether the natural history and responsiveness to therapy of these diseases is comparable to that of AML with > 30% BM blast. These controversies are even more manifest when it comes to elderly patients in whom concern for intensive chemotherapy (IC) related toxicity is the critical determinant for the therapeutic choice. In fact, due to concerns of treatment-related morbidity and mortality associated with delivery of IC, approximately only 30% of all patients ≥ 65 years are considered eligible for this approach. Therefore, a great deal of attention has been dedicated to alternative agents such as hypomethylators (azacitidine and decitabine). Actually, these agents have shown efficacy with reduced toxicity when administered to elderly patients with 20-30% BM blasts and not eligible for IC. In the present review, we will discuss the clinical results achieved in the treatment of elderly patients with 20%-30% BM blasts AML using intensive chemotherapy (IC) or hypomethylating agents. Overall, our survey of the literature suggests that only controlled, randomized, clinical trials will answer the question as to whether hypomethylating agents has the potential to substitute for IC even in elderly patients with an optimal functional status.

Introduction. Since 1982, “refractory anemia with excess of blasts in transformation” (RAEB-t) has been recognized as a discrete entity in the French-American-British (FAB) classification for myelodysplastic syndrome (MDS). In the FAB member’s proposal, RAEB-t should have represented an intermediate category with features transitional between acute leukemia and MDS.¹ In fact, at the same time the FAB members also revised the definition of acute myeloid leukemia (AML) with a threshold for the diagnosis being set at 30% bone marrow (BM) blast infiltration. Based on this, a diagnosis of RAEB-t is made when blast percentage is ≥ 20% <30% in BM, and/or > 5% in peripheral blood (PB). Additionally, it was
acknowledged that the presence of Auer rods in granulocytic precursors was consistent with the diagnosis of AREB-t irrespective of BM and PB blast count. A large number of studies have demonstrated the prognostic impact of FAB classification and the poor outlook of patients with RAEB-t whose median overall survival (OS) was about 6 months, not significantly different from that of patients with AML. Nevertheless, a number of studies have also shown that RAEB-t is a rather heterogeneous entity with the major subjects of contention being identified in the prognostic role of Auer rods and PB blast count. In 1999, the World Health Organization (WHO) published a revised classification of MDS based on which RAEB-t was abolished. Actually, because of the similarities in survival between AREB-t and AML, the panelists regarded as appropriate the reduction to 20% of the BM blast threshold for the diagnosis of AML, ratifying “de facto” AREB-t removal from MDS family. While the agreement about recognizing RAEB-1 and RAEB-2 was unanimous due to the demonstrated impact on prognosis of such a distinction, getting rid of RAEB-t was not painless. In fact, some authors argued that the inferior median OS of patients with RAEB-t was not an acceptable reason to classify and treat them as AML and suggested that clinico-biologic features other than the mere blast count might contribute to the poor outcome. The main points of controversy pertained the karyotype alterations of AML, in particular translocations, which are not so frequent in RAEB-t and the appropriateness to deliver an AML-like treatment to patients who have indolent RAEB-t and therefore might not necessitate it. Moreover, the observation that some cases of AML initially present with MDS-related features implies a previous, sub clinical MDS phase and a pathogenesis different from other cases of AML lacking dysplasia. Therefore, the 3rd edition of WHO classification included a new AML subtype called “AML with multilineage dysplasia” (AML-MLD). In this category, patients were included with 20% or more blasts, and with: 1) morphologic dysplasia in 50% or more of at least two cell lineages, or 2) evolution from a previous MDS or myelodysplastic/myeloproliferative (MDS/MPN) neoplasm. The creation of this new category mitigated in part the disappointment resulting from the elimination of RAEB-t. Indeed, some authors assume that the prognostic power of such a category is questionable if based only on features of multilineage dysplasia; therefore they suggest that disease outcome is better established when multilineage dysplasia (MLD) is associated with genetic abnormalities. Conversely some other authors emphasize the clinical utility of this category even when defined by the sole morphology. As a matter of fact, WHO now includes in this category also patients with specific myelodysplasia-related cytogenetic abnormalities regardless of whether or not multilineage dysplasia is morphologically documented (“AML with myelodysplasia-related changes”, AML-MRC).

Finally, argument still remains as to whether the natural history and responsiveness to therapy of patients with 20%-30% BM blasts is comparable to that of patients with >30% BM blast AML.

Based on this background, we will discuss in the present review the clinical results achieved in the treatment of patients with 20%-30% BM blasts AML using intensive chemotherapy (IC) or hypomethylating agents.

**Cytogenetics and Genetic Abnormalities.** In 2008, the WHO classification created a new category called AML-MRC with or without morphological MLD associated. By designing this category which encompasses cases of AML characterized by MDS-like features, the experts tried to work-out the issue of RAEB-T elimination since the vast majority of AML-MRC present with a 20% to 30% BM blasts infiltration. A further element of interest relates to the observation that MLD features are frequently associated with an adverse karyotype. A recent German study, compared 408 adult patients diagnosed as AML-MRC or AML not otherwise specified (NOS). MLD pattern significantly correlated with pre-existing MDS and MDS related cytogenetic changes. AML NOS and AML-MRC associated with MLD features had a superior EFS and OS compared with AML-MRC diagnosed for history of MDS or for MDS related cytogenetics. Table 1 report the cytogenetic abnormalities needed for a diagnosis of AML-MRC when 20% or more PB or BM blasts are present: although balanced chromosomal aberrations can be detected, these abnormalities include mainly unbalanced karyotype lesions. Patients who are categorized in this subgroup and have a normal karyotype should be analyzed for FLT3, NPM1, and CEBPA mutations, frequency of which is approximately 20%, 30% and 9%, respectively, in de novo AML-MRC with MLD features and approximately 7%, 12% and 7%, respectively, in AML-MRC supervening after a previous history of MDS or with MDS related cytogenetics. As a consequence of such a complex scenario some authors proposed to omit the group of patients classified as AML-MRC only on the basis of MLD features.

The close biological and clinical relationship between AML with 20-30% of BM blasts and high risk MDS, including the efficacy of hypomethylating agents and the poor response to conventional chemotherapy,
leads to the hypothesis that epigenetic deregulation might be implicated in their pathogenesis. A series of single locus studies have demonstrated that several genes are silenced in association with the methylation of their promoter. These include genes participating into cell-cycle regulation, apoptosis, adhesion and motility, and other pathways. Among these, CDKN2B (p15) hypermethylation is frequently found in RAEB-t or AML supervening after MDS and is associated with old age, deletions of 5q and 7q, and a poor prognosis. Such a hypermethylation was calculated in rates from 0% in low-risk MDS, to 30% in high-risk MDS, up to 75% in AML transformed from MDS. Recent advances in technologies such as high-resolution single nucleotide polymorphism (SNP) array and next-generation sequencing have led to the identification of somatic mutations in epigenetic as well as post-translational histone modifications regulators. Somatic mutations affect genes that encode proteins regulating DNA cytosine methylation, hydroxymethylation and demethylation. DNA methyltransferase 3A (DNMT3A) mutation is thought to promote gene hypomethylation but it is not yet known which genes are altered. Its frequency in AML is approximately 12-22% and is associated with poor prognosis. Mutations in Tet methylcytosine deoxegenerate 2 (TET2) alter conversion of 5-methylcytosine (5-mC) to 5-OH-methylcytosine (5-hmC), an intermediate event producing gene demethylation. In AML, it occurs at an estimated frequency of 7-23% and it is associated with poorer prognosis in patients with favorable cytogenetics or cytogenetically normal AML (CN-AML). Mutations in isocitrate dehydrogenase 1/2 (IDH1/2), result in a neomorphic, 2-hydroxyglutarate-driven enzymatic activity which serves as a competitive inhibitor of TET family of enzymes. Studies of a large cohort of AML patients demonstrated that IDH mutations frequency is 15-33% and that IDH and TET2 mutations are mutually exclusive. Yet, results on prognostic effects in AML are divergent.

Post-translational histone modifications cause mutations in histone-modifying enzymes. Enhancer of Zeste Homolog-2 (EZH2) is the main member of Polycomb group (PcG) proteins which are transcriptional repressors regulating cell differentiation. Mutations of EZH2 are rare in AML and are associated with a worse OS in MDS. It is unclear whether mutations of Addition of Sex Combs-like 1 (ASXL1) confer a loss or a gain of function. It has been suggested that ASXL1 loss of function results in disarrangement of the transcriptionally repressive H3K27 thrymethylation with consequent increase of HOXA gene expression. ASXL1 mutations frequency is approximately 5% in AML and it is associated with poor prognosis. Finally, Mixed-Lineage Leukemia (MLL) is a member of a multiprotein complex that mediates the methylation of H3K4 within the promoter region of genes. MLL can be affected by mutations that result in partial tandem replication (PTD) which in turn can boost the levels of H3K4me3 at the level of target genes. MLL-PTD frequency is 4-7% and is associated with poor prognosis in AML and CN-AML. MLL is also involved in translocations at the locus (11q23); such alterations are frequent in infant leukemia and therapy-related AML (frequency 10-15%). This translocation is frequently linked with acquisition of H3K79 methyltransferase activity.

**Table 1. Cytogenetic abnormalities sufficient for diagnosis of AML-MRC.**

<table>
<thead>
<tr>
<th>Complex karyotype*</th>
<th>Unbalanced abnormalities</th>
<th>Balanced abnormalities</th>
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<tbody>
<tr>
<td>-7 or del(7q)</td>
<td>(t(11;16)(q23;p13.3) **</td>
<td>(t(3;21)(q26.2;q22.1) **</td>
</tr>
<tr>
<td>-5 or del (5q)</td>
<td>(t;13)(p36.3;q21.1) **</td>
<td>(t;21)(p21;q23) **</td>
</tr>
<tr>
<td>i(17q) or t(17p)</td>
<td>(t;5)(q12;p33) **</td>
<td>(t;5)(q33;q12) **</td>
</tr>
<tr>
<td>-13 or del(13q)</td>
<td>(t;5)(q17;q33;p13) **</td>
<td>(t;5)(q10;q33;q21)</td>
</tr>
<tr>
<td>del(11q)</td>
<td>(t;3)(q5;q25;q34)</td>
<td></td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(9q)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>idic(X)(q13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three or more unrelated abnormalities, none of which are included in the “AML with recurrent genetic abnormalities” subgroup; such cases should be categorized in the appropriate cytogenetic group.

** Abnormalities that most commonly occur in “therapy-related AML”: the latter should be excluded before using such abnormalities as evidence for diagnosis of AML with myelodysplasia-related changes.

Comorbidities and Treatment Choice in Elderly Patients with AML. Although patients aged ≥ 60 years are prone to experience greater treatment related toxicity than younger subjects and, therefore, to have a shorter overall survival as a consequence of such toxicity, there is evidence that selected older adults can benefit from intensive chemotherapy delivery. Based on this, it became clear that age alone may be an inadequate measure of patients’ eligibility to intensive treatments, whereas Karnofsky and ECOG PS scales are subjective and may lack sensitivity in capturing meaningful impairments in physical functions. Based on this, multidimensional geriatric assessments have been developed by interrogating host-specific clinical characteristics such as cognitive, physical and psychological function with the aim to predict vulnerability to chemotherapy. Such an approach, more reliably than PS scales, allows for those who should be addressed to alternative strategies (e.g. hypomethylating agents) to be identified. An exploratory experience demonstrated a clear-cut...
correlation between geriatric assessment and tumour aggressiveness and those cognitive, psychological and physical impairments were observed across all cytogenetic subsets. A further step in this line of research is to evaluate, prospectively, the correlation between such a risk assessment, survival parameters, treatment toxicity and early mortality in order to implement approaches of individualized treatments.

**Intensive Chemotherapy.** AML is a common disease of the adult age with a peak incidence between 65 and 70 years. According to recent epidemiological data, ≥ 75% of patients with AML are ≥ 60 years old. In this age-range, AML is an incurable disease with less than 10% of patients being alive at 2 years. Such a dismal outcome has been traditionally explained by the concurrence of co-morbidities and biologically poor-risk AML features. Although co-morbidities can hamper delivery of IC, and in spite of reluctance of physicians to expose older patients to the toxic effects of the anti-leukemic therapy, population-based studies have demonstrated that IC prolongs survival and ameliorates quality of life in all age groups, as compared to palliative therapy. Nevertheless, only about one-third of elderly patients receive IC with low-dose cytosine arabinoside (ARAc), farnesyl inhibitor tipifarnib and gentuzumab ozogamicin (GO) being identified as potential alternatives. As a consequence of this situation, the optimal remission induction and post-consolidation therapy in elderly patients has not yet been determined. In this section we will review the most recent advances in standard treatment of elderly AML with a special focus on patients with 20%-30% BM blast count. In all of the considered studies, diagnosis of AML grounded on WHO criteria. However, since the amount of BM blast infiltration did not emerge as a prognosticator either in univariate or multivariate analysis, we assume that most of the clinical results reported below may be extended to the specific subset of elderly patients with 20%-30% BM blast. This is in line with the results of two seminal studies by Estey and Bernstein, either agreeing that a diagnosis of RAEB-t or AML did not have any impact on the effect of IC. Estey and co-workers treated 106 patients affected with RAEB-t and 372 with AML by delivering a combination of high dose ARAc, idarubicine (IDA) and fludarabine or topotecan. Complete remission (CR) rate and event free survival (EFS) were identical (66% and 7 months, respectively; P=NS for both). Bernstein and co-workers, treated 33 patients with RAEB-RAEB-t according to 1984-1992 CALGB AML protocols achieving similar outcomes in terms of CR rate (79% and 68%, P=NS) and EFS (11 and 15 months, P=NS). *Induction intensification.* Table 2 summarizes the most recent studies exploring the effect of IC in elderly AML. In all of the studies, eligibility criteria required at least 20% BM blast infiltration with the exception of National Cancer Research Institute (NCRI) and HOVON-SAKK studies in which also patients with >10% BM blasts were included. Lowenberg has recently reported on a randomized trial comparing high-dose (90 mg/m² for three days) versus conventional dose (45 mg/m² for three days) daunorubicin (DNR), given as an induction regimen in association with standard-dose ARAc. The study recruited 813 elderly patients with previously untreated AML. Despite a significantly superior CR rate in the high-dose DNR arm (64% vs 54%, P=.002), no difference was observed in terms of OS and EFS. Induction death rate was similar (11% vs. 12%, P=NS). A post-hoc analysis demonstrated that the patients who benefited the most by escalated DNR were those aged 60 to 65, and that the beneficial effect took place in terms of CR frequency (73% vs 51%) and 2-year EFS and OS duration (29% vs 14%, P=.002 and 38% vs 23%, P<.001, respectively). Increasing age, poor PS, presence of splenomegaly or extramedullary disease, high white blood cell count (WBCc) and unfavorable cytogenetic were associated with a short duration of OS and EFS. However, no impact of BM blast infiltration on overall outcome was demonstrated. The results of the HOVON-SAKK study were not confirmed in the ALFA-9801 study. The backbone of this protocol was the comparison between high-dose DNR (80 mg/m² for 3 days) versus standard-dose (12 mg/m² for 3 days) or escalated dose (12 mg/m² for 4 days) of IDA in 468 AML patients aged 50-70 years. Neither high-dose DNR nor high-dose IDA gained any significant superiority over IDA given at a standard dose. In multivariate analysis, the age of 60 years came out as an independent prognosticator for OS, WBCc and cytogenetics for OS and EFS; induction death rate was similar across the two treatment arms (6% vs 8% vs 6%, P = NS, respectively). The NCRI and HOVON-SAKK experience indicates that anthracycline dose escalation is feasible and does not endanger elderly patients more than conventional approaches; however definitive advantage in terms of OS and EFS is not yet demonstrated.

**New agents as monotherapy or combination therapy.** The combination of standard chemotherapy and multi-drug resistance (MDR) modulators has been explored in large international trials. Nevertheless, both the NCRI AML14 trial and the ECOG 3999 trial failed to demonstrate any advantage from the administration of the MDR modulators PSC833 or Zosuquidar combination with chemotherapy. The lack of benefit of these agents suggested that they were either not effective inhibitors of MDR or that MDR...
Clofarabine was also tested in combination with other drugs, especially ARAc and hypomethylating agents. 37 Clofarabine 20 mg/m² for 5 days and subcutaneous ARAc 20 mg twice daily for 10 days achieved an encouraging response rate of 66%. Median OS and relapse free survival (RFS) was 12.7 and 14.1 months, respectively, median OS of responding patients was 24.2 months and induction mortality low (7% at 8 weeks) with manageable toxicities. Upcoming randomized phase 3 trials will compare clofarabine and standard induction therapy in elderly patients with AML. The postulated activity in poor prognostic groups such as those of patients with adverse cytogenetic makes this drug particularly attractive for the management of patients with low BM blast count, therefore post-hoc analysis of this category will be very welcome once the above mentioned protocols are closed.

**Table 2: Intensive regimens for elderly AML**

<table>
<thead>
<tr>
<th>Study</th>
<th>Age (range)</th>
<th>N</th>
<th>Induction and post-remission</th>
<th>CR</th>
<th>ED</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOVON-SAKK</td>
<td>67 (60-83)</td>
<td>813</td>
<td><strong>INDUCTION</strong></td>
<td>P=0.002</td>
<td>P=NS 12%</td>
<td>30% (2-yrs)</td>
</tr>
<tr>
<td>ALFA-9801</td>
<td>60 (50-70)</td>
<td>478</td>
<td>DNR 80 mg/m² x 3d vs IDA 12 mg/m² x 4d + ARAc 200 mg/m² x 7 days POSTREMISSION ARAc 1 g/m² q 12 h x 4d + induction anthracycline x 2d</td>
<td>P=NS 70% vs 83% vs 78%</td>
<td>P=NS 8% vs 3% vs 6%</td>
<td>38% (2-yrs)</td>
</tr>
<tr>
<td>ALFA-9803</td>
<td>72 (65-85)</td>
<td>429</td>
<td><strong>INDUCTION</strong></td>
<td>57%</td>
<td>10%</td>
<td>27%</td>
</tr>
<tr>
<td>AML HD98</td>
<td>65 (61-78)</td>
<td>329</td>
<td>ICE 2 cycles + ATRA POSTREMISSION HAM 1 cycle Random IEiv 1 cycle or 1-year oral maintenance therapy IEpo</td>
<td>46%</td>
<td>-</td>
<td>24% (4-yrs)</td>
</tr>
<tr>
<td>AML14</td>
<td>67 (44-88)</td>
<td>1273</td>
<td><strong>INDUCTION</strong></td>
<td>54%</td>
<td>18%</td>
<td>13% (5-yrs)</td>
</tr>
</tbody>
</table>

Abbreviations: CR = complete remission; ED = early death; OS = overall survival; ARAc = cytosine arabinoside; DNR = daunorubicin; SCT = stem cell transplantation; GO = gemtuzumab ozogamicin; IDA = idarubicine; ICE = (idarubicin 12 mg/m² i.v. days 1 and 3, cytarabine 100 mg/m² cont. i.v. days 1–5, etoposide 100 mg i.v. days 1 and 3); HAM = cytarabine 0.5 g/m²/12 h i.v., days 1–3; mitoxantrone 10 mg/m² i.v., days 2 and 3; IEiv = idarubicin 12 mg/m² i.v. days 1 and 3, etoposide 100 mg/ m² i.v. days 1–5; IEpo = idarubicin 5mg p.o. days 1, 4, 7, 10, 13; etoposide 100mg p.o. days 1 and 13; repeated on day 29 for 12 courses; DAT = Daunorubicin 50 or 35 mg/m² IV days 1, 2, 3 IV, Cytosine Arabinoside 100 or 200 mg/m² IV 12-hourly on day 1–10, Thioguanine 100 mg/m² oral 12-hourly days 1–10; MIDAC = Mitoxantrone 8 mg/m² IV on days 1, 2 and 3 Cytosine Arabinoside 500 mg/m² 2 by 2-h infusion 12-hourly, days 1, 2 and 3.
some conclusion may be deduced by the analysis of the largest published studies. Neither high-dose ARAc\textsuperscript{38} nor post-remission chemotherapy (Table 3). NCRI-AML11 trial demonstrated that 4 intensive consolidation cycles added no survival advantage when compared to 1 cycle of moderate intensity chemotherapy.\textsuperscript{41} ALFA9803 and AML-HD98 reached divergent conclusions. The former\textsuperscript{42} demonstrated a benefit in terms of OS (56\% vs 37\%, \(P=0.030\)) and disease free survival (DFS) (28\% vs 17\%, \(P=0.040\)) for elderly patients receiving 6 months of low-dose therapy as compared with those undergoing a single intensive consolidation cycle. At the opposite, HD98 study\textsuperscript{43} showed a survival benefit for those patients receiving 2 intensive multi-drug consolidation therapies as compared with those treated by mild oral maintenance (median OS 22.3 vs 14.3 months, \(P=0.04\)). These discrepant results may be explained by the different composition of the patients’ population in the AML-HD98 study such as the over-representation of intermediate risk karyotypes and the few patients randomized in the consolidation arm. Actually, a major drawback of the clinical trials involving elderly patients consists in the high withdrawal rate that results in a less than expected number of patients receiving the assigned therapy. This may be troublesome when it comes to data analysis and interpretation even if intent to treat approach is used. It is our opinion that consolidation cycles should be administered to all eligible patients even if the randomized trials have not provided the formal and definitive demonstration of the superiority of such an option. In line with this, recent reports suggest that, for patients aged 60-65 years, reduced intensity stem cell transplantation (RIC) represents the logical an option. In line with this, recent reports suggest that, for patients aged 60-65 years, reduced intensity stem cell transplantation (RIC) represents the logical and natural extension of the eligibility to intensive chemotherapy. In these reports, it was proven that age up to 70 years doesn’t seem to affect the outcome of transplant so that RIC can be regarded as a valid option in this category of patients.\textsuperscript{44,46} The largest series published to date entail data from 1036 AML patients aged 50 to 70 years who were in first CR. Of these 1036 patients, 152 underwent allogeneic SCT and 884 were given consolidation chemotherapy. In the group treated by transplant, the cumulative incidence of relapse was significantly lower (22\% vs 62\%) and OS significantly longer (62\% vs 51\%, \(P=.012\)) than in that of patients receiving consolidation courses.\textsuperscript{47}

**Hypomethylating Agents.** IC may not be the most appropriate option for elderly patients who have a poor performance status, co-morbid conditions, deficient BM reserve and for whom a significant treatment-related morbidity and mortality can be anticipated.\textsuperscript{41,48} These observations boosted search for alternative therapies that, although less intensive, could retain the potential of inducing CR, prolonging survival, and preserving quality of life. Emerging research has showed that silencing of key genes critical to growth, differentiation, angiogenesis, signalling, and DNA repair is mediated by aberrant DNA hypermethylation and/or histone deacetylation.\textsuperscript{50-52} Aberrant DNA methylation has been demonstrated to occur in stem cell compartment of MDS and AML\textsuperscript{53} and, since it is believed to be reversible, it represents an attractive therapeutic target for DNA methyltransferase (DNMT) inhibitors such as azacitidine and decitabine.\textsuperscript{54}

**Azacitidine.** Azacitidine is a pyrimidine nucleoside analogue of cytidine which is phosphorylated intracellularly to generate its active form azacitidine triphosphate; this phosphorylation is mediated by uridine-cytidine kinase. In turn, azacitidine is incorporated either into RNA, interfering with the synthesis of nucleic acids and proteins and, to a less extent, into DNA.\textsuperscript{55-56} The drug inhibits DNMT, therefore inducing hypomethylation and transcription of quiescent genes, restoring cancer-suppression functions and induction of cellular differentiation. Finally, there is evidence of a direct cytotoxic effect on BM abnormal hematopoietic cells.\textsuperscript{57}

This drug has been approved in United States (USA) for treatment of MDS of all FAB-defined subtypes and in the European Union (EU) for the treatment of patients not eligible for hematopoietic stem cell transplantation with: int-2- or high-risk MDS; chronic myelomonocytic leukemia with 10–29% bone marrow blasts without myeloproliferative disease; and WHO-classified AML with 20–30% blasts and multilineage dysplasia.

The evidence of the efficacy of azacitidine in patients with 20–30% BM blast AML and multilineage dysplasia comes from two large phase III studies and from registry programmes for compassionate-use surveys.

**Phase III clinical trials.** The phase II Cancer and Leukemia Group B (CALGB) trials (CALGB 8421 and 8921) demonstrated the efficacy of intravenous (iv) and

<table>
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<tr>
<th>Group</th>
<th>Study</th>
<th>Post remission Therapy</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>MRC 41</td>
<td>AML-11</td>
<td>1 vs 4 cycles</td>
<td>No difference</td>
</tr>
<tr>
<td>HOVON 39</td>
<td>AML-43</td>
<td>GO vs observation</td>
<td>No difference</td>
</tr>
<tr>
<td>ALFA 42</td>
<td>9803</td>
<td>6 months non-intensive vs 1 intensive cycle</td>
<td>Better DFS and OS at 2y</td>
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subcutaneous (sc) azacitidine in patients with FAB-defined MDS. The following phase III trial (CALGB 9221) compared sc azacitidine with supportive care in patients with MDS diagnosed according to the FAB classification criteria. Overall, 309 patients were included in the 3 studies; 268 were treated with azacitidine (220 received sc azacitidine at the dose of 75 mg/m² daily for 7 consecutive days) and approximately 60% of them were ≥ 65 years old. When WHO criteria were applied, 103 patients were reclassified as AML: 25 in CALGB 8421, 26 in CALGB 8921, 52 in CALGB 9221 (27 in the azacitidine arm, 25 in the supportive care arm, 13 of whom were treated with azacitidine after the observation phase), for a total of 91 patients treated with azacitidine. By using the “International Working Group” (IWG) response criteria for MDS, among the 91 patients treated with azacitidine the ORR (CR + partial remission + haematological improvement) was 36% and the median duration of response 7.3 months (range, 2.2 to 25.9 months). When the analysis was focused on the phase III trial CALGB 9221, the median OS of patients in the azacitidine arm was 19.3 months vs 12.9 months of those in the supportive care arm (Table 4). Although CR rate was lower (9%) than what expected with IC, the median OS of 19.3 months was comparable to that obtainable with chemotherapy, suggesting that azacitidine may alter the natural history of the disease regardless of the achievement of CR. Additionally, there was no increase of infections or haemorrhage in the azacitidine group beyond what expected for AML patients.

Because the survival advantage with azacitidine was established in patient subgroups (higher-risk patients) or after a landmark analysis to eliminate the confounding effect of the crossover design of the CALGB-9221 study, an additional trial was designed to confirm the OS benefit. The AZA-001 trial was an international, randomized, phase III study of azacitidine 75mg/m²/day for 7 days of each 28-day cycle, for at least 6 cycles, compared with the three most common conventional regimens (CCR) in Int-2 and high-risk MDS; prior to randomization, investigators were required to preselect patients to the CCR considered most appropriate: the choice was between supportive care (BSC), low-dose ARAc (LDAC) 20 mg/m² for 14 days every 28 days for at least 4 cycles, or IC consisting of an induction with ARAc 100–200 mg/m²/day for 7 days plus 3 days of DNR 45–60 mg/m²/day or IDA 9–12 mg/m²/day or mitoxantrone 8–12 mg/m²/day. 62 A total of 358 patients were included in the study and 113 (30%) had a diagnosis of AML with trilineage dysplasias (Table 4). Of these 113, 55 were assigned to the azacitidine arm and 58 to the CCR (27 BSC, 20 LDAC and 11 IC). Median age of those treated on azacitidine was 70 years (range 52-80), median BM blast percentage was 23% (range 20-34%), 69% and 25% of the patients had intermediate and adverse cytogenetic risk group, respectively. The CR rate in azacitidine, LDAC and IC arm was 18%, 15% and 55%, respectively. Median OS in the azacitidine and CCR groups was 24.5 and 16.0 months, respectively (P=.004) and the 2-year OS rates were 50% and 16%, respectively (P=.001). When the analysis was broken down according to each CCR group, median OS was significantly superior in the azacitidine group versus BSC (19 vs 13 months, respectively, P=.03) but no survival advantage was found versus LDAC group (median overall survival 24.5 vs 17.0 months, respectively) or IC group. Results from comparison of azacitidine versus IC should be considered with extreme caution because the IC group accounted only for 11 patients. The AZA-001 trial provided some evidence of azacitidine-associated benefits. In fact, azacitidine prolongs survival despite a lack of response; it is very well tolerated with nearly all adverse events occurring during the first two cycles of

### Table 4. Summary of clinical trials examining azacitidine in monotherapy for untreated WHO-defined acute myeloid leukemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>AML with 20-30% blasts (n)</th>
<th>Median Age (yrs)</th>
<th>ORR (%)</th>
<th>CR rate (%)</th>
<th>2-year OS (% of patients)</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silverman</td>
<td>91</td>
<td>91</td>
<td>66</td>
<td>36</td>
<td>9</td>
<td>NA</td>
<td>19.3</td>
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<tr>
<td>Fenaux</td>
<td>55 CCR 58</td>
<td>Aza 55 CCR 58</td>
<td>70</td>
<td>NA</td>
<td>18</td>
<td>16</td>
<td>24.5</td>
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<tr>
<td>Seymour</td>
<td>38 CCR 49</td>
<td>Aza 12 CCR 18</td>
<td>78</td>
<td>58</td>
<td>39</td>
<td>55</td>
<td>10.8</td>
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<tr>
<td>Thepot</td>
<td>138</td>
<td>44</td>
<td>73</td>
<td>21</td>
<td>14</td>
<td>18</td>
<td>10.2</td>
</tr>
<tr>
<td>Maurillo</td>
<td>82</td>
<td>16</td>
<td>77</td>
<td>48</td>
<td>19</td>
<td>13</td>
<td>9</td>
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</tbody>
</table>

Abbreviations: ORR = overall response rate; CR = complete remission; OS = overall survival; Aza = azacitidine; CCR = common conventional regimens; LDAC = low dose cytarabine; IC = intensive chemotherapy; NA = not available; NR = not reached
treatment and the rate of infections and hospitalization are significantly lower than in patients receiving CCR. However, it should also be considered that the median OS in the CCR arm was atypically long therefore suggesting bias in patient selection. In particular, the exclusion of patients with high WBCc-AML, who are known to have a worse prognosis, sounds critical. In order to answer this issue, a phase III trial is currently being conducted in patients aged ≥65 years, with untreated de novo or secondary AML. A subsequent AZA-001 post-hoc analysis focusing on patients aged ≥75 years confirmed the previous finding of an OS advantage for the azacitidine arm (table 4). Compassionate use of azacitidine. In addition to data from phase III studies, early trials and registry programs can help extrapolate the efficacy of azacitidine in patients with AML. In the French azacitidine compassionate use program (ATU), azacitidine was given to previously untreated or relapsed/refractory AML patients in the same dose and schedule as in the AZA-001 trial. In a first retrospective analysis (Table 4), 138 elderly untreated patients with a median age of 73 years were evaluated. Of these 138, 44 (32%) had 20-29% BM blasts infiltration, 61 (44%) had an adverse karyotype; 65 (47%) and 30 (22%) had secondary and therapy related AML, respectively. ORR and CR were 21% and 14%, respectively; 2-year survival was 18%, median survival 10.2 months. Unfavourable karyotype and a WBCc > 10×10⁹ leukocytes/L but not the percentage of BM blasts, were associated with a poor prognosis. A second retrospective analysis included 184 patients with relapsed/refractory AML: median age was 64 years, 15 (8%) patients had 20-29% BM blasts infiltration, 55 (30%) an adverse karyotype, 57 (31%) and 15 (8%) secondary and therapy related AML, respectively. ORR and CR were 11% and 7%, respectively; 1-year survival was 29.1%, median OS 7.8 months. In a recent analysis carried out in 282 patients treated with azacitidine, with high or intermediate-2 risk MDS and AML <30% blasts (22% of the series = 62), factors independently associated with lower response rates were previous LDAC treatment, BM blasts >15%, and cytogenetic abnormalities. Complex karyotype predicted a significantly shorter duration of response whereas performance status >2, intermediate- and poor risk cytogenetics, presence of circulating blasts and red blood cell transfusion-dependency >4 units/8 weeks were independent factors associated with a shorter duration of OS. An Italian retrospective study on 82 patients with AML treated with azacitidine in a compassionate program setting, showed similar results (table 4); in fact, in 35 untreated AML patients (46% of them with 20-29% of BM blasts), ORR and CR were 48% and 19%, respectively, 2-year survival 13% and median OS 9 months; in multivariate analysis, the chance of obtaining a response was significantly associated with a WBCc < 10×10⁹/L. In 47 patients with pre-treated AML (36% of them with 20-29% of BM blasts), ORR and CR were 19% and 11%, respectively, 2-year survival 7%, median survival 7 months. Other studies suggested that a diagnosis of de novo AML and BM blast count ≤45% on day 15 of the first treatment cycle or platelet doubling after the first azacitidine cycle are useful predictors of response to azacitidine. Combination therapy with azacitidine. Several combinations of azacitidine with other agents have been explored in order to improve results obtained with azacitidine monotherapy. To date, no randomized trial has compared the efficacy of azacitidine in combination versus azacitidine alone. Azacitidine has been challenged in combination with valproic acid, ATRA, thalidomide or thalidomide but ORR and CR (50% and 20%, respectively) were not significantly different from those achieved using azacitidine monotherapy. Association therapy of azacitidine and the histone deacetylase inhibitor vorinostat also exhibited promising activity in patients with AML. In a phase I trial conducted in 28 patients with AML and MDS, 10 (83%) of the 12 with AML/high-risk MDS reached a CR. Combination of azacitidine and lenalidomide is another approach eagerly investigated. Forty-two untreated patients with a median age of 74 years (range 62-86) received azacitidine 75mg/m² for 7 days followed by escalating doses (5 mg, 10 mg, 25 mg and 50 mg) of lenalidomide daily for 21 days starting on day 8 of a 28-day cycle. Patients remained on therapy until disease progression, unacceptable toxicity or completion of 12 cycles. ORR was 40% (CR + CRi rate 28%). Duration of CR/CRi was 28 weeks (range, 4- >104 weeks), therapy-related acute myeloid leukemia and high comorbidity index anticipated a short duration of response. More recently, phase II studies were carried out to explore the role of azacitidine as a maintenance therapy after conventional IC. In a first trial, 23 out of 60 AML/high risk MDS patients who achieved CR after a cycle of DNR plus ARAc, were given azacitidine at 60 mg/m² for 5 days every 28 days until relapse or unacceptable toxicity. The median age was 68 years, the median duration of response was 13.5 months and in 30% of the patients a sustained CR, lasting longer than 20 months, was observed. In a second trial, 46 patients (33 AML and 13 high-risk MDS) were included. The median age was 66 years, azacitidine was delivery at a dose of 60-75 mg/m² for 5 days every 28 days and the median number of cycles administered was 5.5. Thirty-three patients achieved CR/CRi and two partial remission,
Decitabine. Although even decitabine (5-aza-2'-deoxycytidine) is a pyrimidine analogue of cytidine with inhibitory effects towards DNMT, it characterizes for some pharmacokinetic differences compared to azacitidine. Once given iv, it undergoes a rapid deamination so that its half-life is relatively short. Cellular uptake of decitabine takes place through a nucleoside transport mechanism and, once into the cellular environment, it is phosphorylated by deoxycytidine kinase into 5-aza-2'-deoxycytidine-5'-triphosphate. Decitabine is incorporated exclusively into DNA where it binds covalently DNMT1 therefore depleting the cells of this enzyme. At low doses, decitabine can cause AML cell lines to differentiate and enter apoptosis whereas at high concentrations it has a cytotoxic effect, possibly leading to generation of alkali-labile DNA strands. In USA and EU, decitabine is approved for the treatment of previously treated and untreated, de novo and secondary MDS of all FAB subtypes and intermediate 1/2 and high-risk IPSS groups.

Phase II-III clinical trials. Clinical trials investigating the role of decitabine as a first-line therapy in newly diagnosed AML of elderly patients are underway; available data from concluded clinical trials are summarized in Table 5.

An initial multicenter phase II study of low dose of decitabine was conducted in elderly patients with newly diagnosed AML. The drug was administered intravenously at the dose of 20 mg/m²/day for 5 days, every 4 weeks. Median age was 74 years and 18 (33%) patients had BM blasts <30%. As a whole, ORR and CR rate were 25% and 24%, respectively. The authors observed a slight increase of CR rate to 28% when patients with BM blasts <30% were extrapolated. Similarly to azacitidine studies, even in this decitabine trial no responses were observed in patients with a peripheral absolute blast count greater than 10x10⁹/L. Median OS was 7.7 months and subgroup analysis demonstrated that patients with stable disease had an OS longer than the one of no responders. The most common toxicities, myelosuppression, febrile neutropenia and fatigue, were all manageable. Of interest, the efficacy of decitabine was not compromised in patients with poor-risk cytogenetics, a subgroup often associated with inferior outcomes.

In another study, 53 elderly patients, not candidates for or refusing IC, received an induction 10-day regimen of decitabine at 20mg/m² followed by 4–5 day cycles. Median age was 74 years (range 60–85 years), although the median BM blast infiltration was 52% (range 20-92%) an exact estimate of patients with BM blasts <30% was not retrievable from the study. With a median of 3 delivered cycles of therapy, the CR rate was 47% and subgroup analyses demonstrated comparable CR rates regardless of age, cytogenetics, presenting WBCc and whether the AML was de novo or evolved from an antecedent hematologic disorder. Median overall survival was 12.7 months. The clinical study was also paralleled by a translational research which documented that the higher the levels of miR-29b the more frequent the responses to decitabine. A multicenter, randomized phase III trial comparing decitabine versus investigator’s treatment choice (TC = supportive care or LDAC) enrolled 485 elderly patients with AML. Approximately one fourth of the patients had BM blasts <30%, 71% were ≥ 70 years of age, 35% had secondary AML and 36% had poor-risk AML. In those randomized to the experimental arm, decitabine was given at the dose of 20 mg/m² per day as a 1-hour iv infusion for five consecutive days, every 4 weeks. Although decitabine arm achieved a significantly higher CR rate compared to TC arm

Table 5. Summary of clinical trials examining decitabine in monotherapy for untreated WHO-defined acute myeloid leukemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>Regimen</th>
<th>AML with 20-30% blasts (n)</th>
<th>Median Age (yrs)</th>
<th>ORR (%)</th>
<th>CR rate (%)</th>
<th>Median survival (months)</th>
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<tbody>
<tr>
<td>Cashen ⁸⁰</td>
<td>55</td>
<td>20 mg/m²/day for 5 days every 4 wks</td>
<td>18</td>
<td>74</td>
<td>25</td>
<td>28</td>
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<tr>
<td>Blum ⁸¹</td>
<td>53</td>
<td>20 mg/m²/day for 10 days followed by 4-5 day cycles every 4 wks</td>
<td>13</td>
<td>74</td>
<td>64</td>
<td>47</td>
<td>12.7</td>
</tr>
<tr>
<td>Kantarjian ⁸²</td>
<td>238 237</td>
<td>20 mg/m³/day for 5 days every 4 wks vs SC or LDAC</td>
<td>65</td>
<td>73</td>
<td>20.3</td>
<td>18</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
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<td>58</td>
<td>73</td>
<td>11.5</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: ORR = overall response rate; CR = complete remission; SC = supportive care; LDAC = low dose cytarabine; wks = weeks
(17.8% vs 7.8%, respectively) at the planned clinical cutoff date no differences in terms of OS were seen (7.7 vs 5 months, respectively). Only a subsequent post hoc analysis came out with a significant better OS in favor of patients treated in decitabine arm (P = 0.037).

**Combination therapy with decitabine.** Studies using decitabine in association with other agents in patients with AML are few and very preliminary; therefore, firm conclusions cannot be drawn. In the attempt to silence multiple pathways involved in epigenetic changes, decitabine at 20 mg/m² daily for 10 days or at 20 mg/m² daily for 5 days has been given together with escalating doses of valproic acid or vorinostat. This strategy gained ORR of 44% and 36%, respectively. Still ongoing clinical trials include decitabine in combination with bexarotene (NCT01001143), midostaurin (NCT01130662), bortezomib (NCT01420926) and sapacitabine (NCT01303796). A Phase 3, open-label, randomized clinical trial is recruiting elderly patients with newly diagnosed AML to receive either ARAc/anthracycline-based induction regimen followed by ARAc consolidation therapy or clofarabine single agent as induction and consolidation therapy. Patients are then randomized to decitabine maintenance therapy for 12 months or observation (NCT01041703).

**Conclusions.** Overall, the current literature indicates that AML outcome in elderly patients remains disappointing. Physicians’ anxiety about treatment-related morbidity and mortality potentially associated with delivery of IC causes that approximately only one third of all patients aged ≥ 65 years are considered eligible for this approach. Moreover, the percentage of treated patients decreases progressively with each advancing decade of life, with only 6% of patients over the age of 85 receiving IC. Therefore, in recent years an ever more growing attention has been paid to development of effective and tolerable therapies for elderly patients. In this view, the discovery of the hypomethylating power of old drugs has led to the hope of cure with minimal toxicity for specific categories of elderly patients such as those not eligible for IC and with 20-30% BM blasts plus multilineage dysplasia. The clinical trials of azacitidine have demonstrated the feasibility of an outpatient administration and the favourable toxicity/efficacy ratio with lower incidence of complications and hospital admissions compared to IC. At the same time, we are not yet in the position to recommend that hypomethylating agents substitute for IC in elderly patients with an optimal functional status. This issue will hopefully be addressed in future controlled, randomized clinical trials.

**References:**


PMid:11167795

Christiansten DH, Andersen MK, Pedersen-Bjergaard J. Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor prognosis in therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 2003;17(9):1813–9. http://dx.doi.org/10.1038/sj.leu.2403054 PMid:12970781

PMid:15581048


PMid:11167795


PMid:15581048


Rowe JM, Closer to the truth in AML. Blood 2009 113:4129-4130; http://dx.doi.org/10.1182/blood-2008-12-192856 PMid:19406994


Chevallier P, Blaise D, Milpied N, et al. Reduced intensity conditioning (RIC) allogeneic stem cell transplantation for...
citidine

ed, open
citidine cycle is a promising predictor
rman LR, et al. Effects of
rvival compared with conventional care

PMid:9988526


68. Polleya DA, Zehnder J, Coutre S, et al. Sequential azacitidine


