Mediterranean Journal of Hematology and Infectious Diseases

Review Article

Treatment of Low-Blast Count AML using Hypomethylating Agents

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Competing interests: The authors have declared that no competing interests exist.

Abstract. In 2002, the WHO classification reduced the proportion of blasts in the bone marrow (BM) necessary for the diagnosis of acute myeloid leukemia (AML) from 30% to 20%, eliminating the RAEB-t subtype of myelodysplastic syndromes (MDS). However, this AML subtype, defined as low-blast count AML (LBC-AML, with 20-30% BM-blasts) is characterized by peculiar features, as increased frequency in elderly individuals and after cytotoxic treatment for a different primary disease (therapy-related), poor-risk cytogenetics, lower white blood cell counts, and less frequent mutations of *NPM1* and *FLT3* genes. The clinical course of this entity is often similar to MDS with 10-19% BM-blasts. The hypomethylating agents azacitidine and decitabine have been shown to induce responses and prolong survival both in MDS and LBC-AML. The role of these agents has also been demonstrated in AML with >30% BM-blasts, particularly in patients with poor-risk cytogenetics and in AML with myelodysplasia-related changes. Most recent studies are evaluating strategies to improve outcome, including combinations of hypomethylating agents with immune-response checkpoint inhibitors, which have a role in cancer immune surveillance. Efforts are also ongoing to identify mutations which may predict response and survival in these patients.

Keywords: AML, MDS, azacitidine, decitabine.

Citation: De Bellis E., Fianchi L., Buccisano F., Criscuolo M., Maurillo L., Cicconi L., Brescini M., Del Principe M.I., Di Veroli A., Venditti A., Amadori S., Arcese W., Lo-Coco F., Voso M.T. Treatment of low-blast count aml using hypomethylating agents. Mediterr J Hematol Infect Dis 2017, 9(1): e2017045, DOI: http://dx.doi.org/10.4084/MJHID.2017.045

Published: July 1, 2017 **Received: April 30, 2017** **Accepted: June 10, 2017**

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Introduction. The 2002 WHO classification reduced the proportion of blasts in the bone marrow (BM) necessary to the diagnosis of acute myeloid leukemia (AML) from 30% to 20%, due to the evidence that outcome in most of the patients with 20 to 30% BM-blasts was similar to that of patients with over 30% blasts.[1] However, in several cases, often leukopenic, as in leukemias evolving from a previous MDS, and in those with MDS-type karyotype abnormalities, the clinical course of 20-30% blast AML, the so-called low-blast count AML (LBC-AML), is less aggressive than that of *classical* AML. In this line, a recent retrospective analysis focusing on BM-blast percentage has been performed by the MD Anderson in 1654 patients with untreated AML or MDS with >10% of blasts. Patients had been diagnosed between 2000 and 2014, and treated with intensive induction therapy (IC), hypomethylating agents, or other regimens,

including low-intensity therapy. Characteristics of AML with 20–29% blasts were similar to those of MDS with 10-19% blasts, frequently including advanced age, poor-risk cytogenetics, lower WBC counts and rare occurrence of *NPM1* and *FLT3*-ITD mutations.[2] The authors identified three groups of patients with different proportion of BM-blasts at diagnosis (10-19%, 20-29%, and >30%): survival in patients below the age of 60 was similar for all *blast-groups* (p= 0.98). However, in patients aged 60-69 years, survival was similar in the groups with 10-19% and 20-29% blast, and significantly shorter in patients with greater than 30% blasts. The difference was lost in elderly patients, aged over 70 years, all characterized by very dismal prognosis. Multivariate analysis showed inferior survival associated with older age, poor-risk cytogenetics, therapy-related AML and proliferative disease (white blood cell counts, WBC> 25 $x10^9$/L, elevated LDH, presence of blasts in the peripheral blood, PB), independent of BM-blast counts.

Despite the newer WHO classifications, the international prognostic scoring system (IPSS) has still been used for many years to stratify patients with MDS in two prognostic risk groups (low/Int-1 and Int-2/high, defined as lower- and higher-risk MDS, respectively).[3] The original IPSS score included RAEB-t (20-29% blasts), and this led to the inclusion of this patient subset in MDS protocols, in particular when using hypomethylating treatment (HMT). Most recently, the IPSS-Revised has been introduced, and this scoring system only classifies patients with MDS, up-to 19% BM blasts.[4]

The purpose of this review is to summarize most recent evidence on the outcome of LBC-AML, taking into account the introduction of HMT, and improved supportive care measures.

Azacitidine. The first randomized trial on the use of azacitidine (AZA, Vidaza, Celgene™) in MDS, the AZA-001 study, has been reported in 2009.[5] In this protocol, 113 patients with LBC-AML (20-34% BM-blasts at diagnosis) were included, with a median age of 70 years (range 50-83). They received standard dose azacitidine (AZA) versus a pre-selected CCR (conventional care regimens, including intensive chemotherapy, IC, or low-dose, LD, cytarabine, or best supportive care). Despite similar complete remission (CR) rates in the two groups (18% AZA vs 16% CCR), there was a significant benefit in terms of overall survival in patients who received AZA. Actually, 50% of patients treated with HMT, versus 16% of those treated with CCR were alive at two years from randomization.[6] Following these observations, the efficacy of hypomethylating agents was also assessed in AML, evaluating the relationship between response and baseline BM-blast counts.

In 2014, the AGMT-Study Group reported on efficacy and safety of azacitidine in a cohort of 302 AML patients including both patients with 20-29% and ≥30% BM-blasts, who had received at least one dose of azacitidine.[7] Overall response rate (ORR) was 48% in the total cohort, and 72% in patients evaluable according to MDS-IWG-2006 response criteria (after at least 2 AZA cycles), respectively. Median time to first response was 3.0 months: this corresponded to the best response in 69% of cases, though the median duration of response was 3.4 months (range 0.3-33.0). As a significant result, patients who achieved hematological improvement (HI platelets, and/or neutrophils, and/or erythrocytes) had significantly longer OS than those who did not (16.1 vs. 4.5 months, p=<0.001). This underlines the importance to continue HMT in the case of HI, regardless of bone marrow response. On the other hand, BM-blast counts did not significantly affect OS, both in the whole patient cohort and after excluding pre-treated patients.[7]

The international phase III AZA-AML-001 study was the first prospective, randomized study to evaluate efficacy and safety of azacitidine compared with CCR (BSC only, LD-cytarabine, or standard IC) in elderly patients (age ≥65 years), with newly diagnosed AML and ≥30% BM blasts.[8] Inclusion criteria included ineligibility for allogeneic stem cell transplantation (HSCT), intermediate- or poor-risk cytogenetics (NCCN 2009 criteria), ECOG ≤ 2 and white blood cell counts ≤15 × 10^9/L. A total of 481 patients were randomized (AZA n=241, CCR n=240). Median OS for patients receiving AZA and CCR was 10.4 and 6.5 months, respectively; stratified HR was 0.85 (95% CI, 0.69-1.03; *p*=0.1009). The survival benefit became significant in the pre-planned post-hoc Cox model, evaluating the time to subsequent treatment. The risk of death was reduced by 25% in the AZA arm, and median OS was prolonged by more than 5 months, compared with CCR (12.1 vs 6.9 months; stratified HR, 0.76; 95% CI, 0.60-

0.96; *p*=0.0190). Univariate OS analyses documented favorable trends for AZA therapy compared with CCR across all subgroups, with a statistically significant survival benefit in patients with poor-risk cytogenetics and in AML with myelodysplasia related changes, consistent with the positive results previously reported by several groups in patients with higher-risk MDS. No significant OS improvement was observed in therapy-related AML, but there was a positive trend for the azacitidine-treated group.

Therapy-related myeloid neoplasms (t-MN), including myelodysplastic syndromes and acute myeloid leukemia (t-MDS and t-AML) are associated to clinical and biologic unfavorable prognostic features, including changes in DNA methylation levels. Due to the association with exposure to DNA-damaging agents, including chemo- and radiotherapy, and the possible common pathways of leukemic transformation, these diseases have been grouped together and included in the WHO classification of AML since 2002.[1,9] A multicenter retrospective study was conducted by our group in 50 patients (34 t-MDS and 16 t-AML) that received azacitidine as induction treatment.[10] Overall response rate was 42% (CR: 21%, Partial Remission, PR: 4.2% and HI: 16.7%) and was obtained after a median of 3 cycles (range 1–6). Stable disease (SD) was documented in 31% of patients. Median overall survival was 21 months (range 1–53.6+) from azacitidine start, and was significantly better in patients with BM blasts <20% and in t-AML patients with normal karyotype, consistent with the known important prognostic role of cytogenetics. Comparing the efficacy of azacitidine in 196 *de novo* MDS/LBC AML, vs 58 t-MN, we did not observe any survival differences (median 16.9 vs 16.2 months, respectively, p= 0.1997), sustaining the activity of AZA in the t-MN setting, independent of the previous history of cytotoxic treatment (Fianchi & Voso, unpublished).

Recently, the efficacy of azacitidine was compared with that of intensive chemotherapy in elderly patients with AML secondary to a previous MDS, myeloproliferative neoplasm, or prior cytotoxic exposure.[11] t-AML accounted for 45% of cases. Median BM-blast count was 30% (range 25-62) and 50% (range 27-82) in the azacitidine and IC groups, respectively (p<0.0001). In this study, there was no significant survival difference comparing chemotherapy and azacitidine (9.6 vs 10.8 months, respectively, p = 0.899). Adjusted time-dependent analyses showed that survival was indeed similar up to 1.6 years post-treatment. After this time-point, patients who received chemotherapy had a lower risk of death compared to those who received azacitidine (adjusted HR 0.61, 95%CI: 0.38-0.99, at 1.6 years).

Decitabine. The hypomethylating agent Decitabine (DAC, Dacogen, Janssen), has been initially approved in the United States for previously treated and untreated *de novo* and secondary MDS, included in the intermediate-1 to high-risk IPSS groups.[12] In Europe, it has been approved in 2012 in patients with newly diagnosed *de novo* or secondary AML, according to the WHO classification, who are not candidates for standard IC.

An international, multicenter, randomized, open-label, phase III trial conducted by Kantarjian *et al*., compared efficacy and safety of decitabine with physician's treatment choice (LD-cytarabine or best supportive care) in a cohort of 485 elderly patients, of a median age of 73 years (64-91), with newly diagnosed *de novo* or secondary AML, and poor- or intermediate-risk cytogenetics.[13] The decitabine schedule was 20 mg/m^2 per day as a 1-hour intravenous infusion, for five consecutive days, every 4 weeks. Significantly improved remission rates were observed with decitabine versus physician's treatment choice, with 17.8% CR or CRp (CR with incomplete platelet recovery), vs 7.8%; respectively (P = 0.001). Despite the fact that the survival difference was not significant at the 2009 cut-off year, mature survival data collected in 2010 showed that there was a significantly improved OS for patients treated with decitabine (nominal p = 0.037). The trend towards a benefit for decitabine treatment was more clearly observed in patients ≥ 70 years old, with *de novo* AML, over 30% baseline BM-blasts, intermediate- or poor-risk cytogenetics, and ECOG PS 2, versus 0 to 1.[13] Similar results have been reported by Bhatnagar *et al*. in a retrospective analysis on 45 previously untreated AML patients, judged unfit for intensive chemotherapy, and treated with a 10 day-decitabine schedule. The ORR was 42%, with 31% CR, and 11% CR with incomplete count recovery. The response rate was higher in patients with lower pre-treatment BM-blasts counts (42%),

as compared to patients with higher BM-blasts (p = 0.01).[14]

To try to increase treatment efficacy, a 10-day DAC schedule was explored in a phase II clinical trial using single-agent decitabine, in patients aged over 60 years, with previously untreated AML.[15] The ORR was 64%, including 47% CR and 17% CR with incomplete count recovery, with no difference according to karyotype. This pilot study showed promising results in terms of response and overall survival in elderly AML patients treated with the prolonged decitabine-schedule, regardless of blast count at diagnosis. Another important result was the demonstration that toxicity was similar to the 5-day schedule.[15]

Similar data were reported by Ritchie et al.,[16] who treated 52 patients using the 10-day Decitabine schedule, for at least one induction cycle. After achieving CR, most patients continued with the 5-day schedule, until toxicity or disease progression. The CR rate was 46% and the median OS was 318 days, while the median number of cycles required to achieve a response was 2 (1-4 cycles). Also in this study, the 10-day schedule was well tolerated, with toxicities similar to the 5-day schedule.

Treatment Combinations. One of the major pitfalls of HMT is the low proportion of complete and partial remission rates and the short duration of response. Combination treatments have been attempted to improve outcome. Combinations with histone-deacetylase inhibitors have not been shown to significantly improve efficacy of HMT, and have had scarce success both in terms of response and incidence of side-effects.[17] Zhao et al. studied efficacy and safety of decitabine, associated with thalidomide, versus decitabine monotherapy in elderly patients with MDS. A 2-year survival benefit was demonstrated, but only in the high risk-MDS group, with a median OS of 50.6% in DAC-thalidomide treated patients versus 40.2% in DAC-monotherapy patients (P<0.05).[18]

The combination of HMT with immune-checkpoint inhibitors (ICI) is a promising approach.[19] The PD-1 pathway has a role in immune surveillance and is composed by a co-stimulatory receptor primarily expressed on activated T-cells (PD-1), and its ligands, that are primarly expressed on tumor cell surface (PD-L1 and PD-L2). Binding of PD-1 to its ligands PD-L1 and PD-L2 inhibits effector T-cell function and this interaction can suppress immune surveillance and permit neoplastic growth.[20,21] Evaluation of expression of PD-1 pathway proteins in patients with myeloid neoplasms showed increased expression of PD-L1, PD-L2, PD-1 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) in CD34+ peripheral blood cells from patients with MDS, LMMC and AML**.** Expression of PD-L1 was significantly higher in MDS and CMML, compared to AML. In patients undergoing Decitabine, these genes were upregulated due to demethylation of PD-1, particularly in patients resistant to therapy, compared with those who achieved a hematologic response. This indicates that PD-1 signaling may be involved in MDS pathogenesis and mechanisms of resistance to hypomethylating agents.[22] Ørskov et al. identified a correlation between PD-1 promoter demethylation and increased PD-1 expression in PB T-cells of patients with MDS, following consecutive cycles of AZA, that resulted into significantly worse ORR (8% vs. 60%, p = 0.014), and shorter OS (p = 0.11). A significantly higher baseline methylation level of the PD-1 promoter was observed in T-cells of non-responding patients also when compared to healthy controls (p = 0.023).[23] The HMT/ICI combination may increase treatment response due to demethylation and re-activation of genes related to interferon signaling, antigen presentation and inflammation, which may favour the activity of ICI.[24]

Another potential target, shown to be overexpressed in CD34+ cells of patients with AML, is the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), which plays a role in therapy resistance.[25] Venetoclax (ABT-199) is an orally bioavailable, selective BCL-2 inhibitor that has been used in older, newly diagnosed AML patients not eligible for intensive chemotherapy. Treatment combinations of Venetoclax with HMT showed promising results, with 76% ORR in 39 AML patients treated in a phase 1b study.[26]

New Hypomethylating Agents. The novel hypomethylating agent guadecitabine (SGI-110, GDAC), is a dinucleotide of decitabine and deoxyguanosine, characterized by extended DAC activity due to resistance to deamination. A multicenter phase II study evaluated the efficacy of GDAC in patients with IPSS intermediate-2 or high-risk MDS, CMML and LBC-AML, refractory or relapsed after standard HMT

treatment. GDAC was given at 60mg/m^2/d subcutaneously, for 5 days, every 4 weeks, until progression, death or absence of response after 6 cycles. The ORR was 16% and the tolerance to GDAC was comparable to that of AZA or DAC. Median OS from protocol inclusion was 6.7 months (IC95% [5.6-11.8]) and was significantly shorter in pts with high IPSS (HR=2.1, 95%CI, 1.04-4.20, p=0.04), and with very poor IPSS-R cytogenetics (HR=4.3, 95%CI, 2.0-9.1, p=0.0015).[27]

The oral formulation of azacitidine CC-486 may also represent an effective alternative approach to patients with MDS, CMML and AML. CC-486 was evaluated in 3 phase 1-2 studies, including patients who had previously received standard HMT (50% of patients with AML). Five of 13 patients (38%) refractory to prior HMT, responded, including 1 patient with AML who achieved CR. The ORR was 35% and no significant difference in ORR and in the rate of specific responses (CR, PR, CRi, HI and transfusion independence) was observed between patients with MDS, CMML or AML. Similar response rates were achieved in patients who relapsed or were refractory to prior HMT, suggesting that HMT failure does not preclude future response to CC-486.[28]

Prognostic Factors for HMT in AML. Although available data on HMT in elderly patients with AML show a benefit of these agents in terms of overall survival and response, the treatment is demanding for patients and care-givers. In fact, affected patients are usually elderly subjects with frequent comorbidities, and they need repeated admissions to outpatient care units.

Research currently aims at identifying somatic mutations that could be useful to predict response to HMT. Bejar *et al*. sequenced 40 genes recurrently mutated in myeloid malignancies in the BM-DNA from 213 MDS patients collected before treatment with azacitidine or decitabine.[29] The overall response rate of 47% was not different between agents. None of the mutations was predictive of response *per se*, but *TET2* mutations predicted a significantly higher response rate to HMT (when at over 10% variant allele fraction), compared to wild-type *TET2*. Response rates were highest in the subset of *TET2*-mutant patients without clonal *ASXL1* mutations (OR 3.65, P = .009). On the other hand, mutations of *TP53* were a negative predictor of survival (P= .002) and identified a particularly poor prognostic subgroup in patients with complex karyotype, with a median survival of only 0.9 years, compared to 1.3 years in patients with complex cytogenetics and no *TP53* mutations (P= .003). In this last subgroup, survival was not different from that of patients with other karyotype abnormalities (median 1.8 years, P = .28). This suggests that the adverse prognostic value ascribed to complex karyotype is largely induced by its frequent association with *TP53* mutations, also during HMT. These data partially constrast with more recent reports on the prognostic role of *TP53* mutations in the context of HMT. Muller-Thomas *et al*., showed that *TP53*-mutated patients had a higher probability of response to AZA, compared to *TP53*-WT patients.[30] This difference was more pronounced in MDS.

This has been confirmed by a recent paper by Welch *et al*., in patients with MDS or AML treated with 20 mg/m^2 Decitabine for 10 days.[31] Response rates were higher in patients with unfavorable cytogenetics than in patients with intermediate-risk or favorable-risk cytogenetics (67% vs. 34%, P<0.001), and in *TP53*-mutated, compared to *TP53*-WT patients (21 of 21 [100%] vs. 32 of 78 [41%], P<0.001). Furthermore, *TP53*-mutated allelic burden significantly decreased after four serial 10-day courses of decitabine. Although responses were not durable, overall survival rates of *TP53*-mutated patients were similar to those of patients with intermediate-risk AML, who received the same treatment schedule. These data indicate that HMT may significantly modify the prognostic impact of adverse genetic alterations, particularly *TP53* mutations. However, these data need to be confirmed in larger, prospective studies.

Conclusions. In general, low-blast count AML present clinical characteristics similar to MDS, not only in terms of low proliferation rates and MDS features, but also of prevalence of monosomal and complex karyotypes, and TP53 mutations, which are usually poor prognostic factors for response to chemotherapy. These features may explain the improved outcome of LBC-AML using HMT, indicating the need for specific classification of this AML subtype, according not only to BM-blast proportion, but also to the presence of MDS-type somatic mutations.[9]

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