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Combination therapies of hypomethylating agents for elderly patients with acute myeloid leukemia

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Abstract

Older patients with acute myeloid leukemia (AML) are encumbered with poor long-term outcomes due to patient and disease characteristics. Hypomethylating agents (HMAs), acting as DNA methyltransferase (DNMT) inhibitors, have been established as a new treatment option, but they have been associated with relatively low response rates (15%–20% complete remission) when administered separately for treating elderly with AML. However, appropriate combination therapies with decitabine or azacitidine have flourished. The results of randomized trials of various combinations of HMAs with chemotherapy, histone deacetylase inhibitors, monoclonal antibodies, immunomodulatory agents, kinase inhibitors, or bexarotene are summarized.

Keywords:
acute myeloid leukemia, elderly, azacitidine, decitabine, combination therapies

Introduction

Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy, characterized by abnormal proliferation of progenitor cells. Age is a fundamental risk factor, with median overall survival (OS) <6 months for elderly patients with AML receiving intensive therapy [1]. Several measurable and immeasurable factors, including poor performance status, adverse cytogenetics, genetic mutations, and complications, are responsible for poor prognosis [2-5].

DNA methylation has been demonstrated to silence tumor suppressor transcription, contributing to the occurrence of hematopoietic disease [6]. Synthesized in the 1960s, the hypomethylating agents (HMAs) azacitidine (AZA) and decitabine (DAC) play a role in reactivating the silenced genes and inducing the differentiation of leukemia cells [7, 8]. Treatment with AZA or DAC in elderly patients with AML was investigated in clinical trials. Through the analysis in patients with low bone marrow blast count (20%-30%) receiving AZA 75 mg/m^2 or a conventional care regimen (CCR) [best supportive care (BSC), low-dose cytarabine, or intensive chemotherapy], an improved OS (24.5 *v*ersus 16 months) and similar morphologic complete remission (CR) (18% versus 16%) were confirmed in the AZA arm [9]. A survival advantage was also obtained from the AZA-AML-001 study, designed for elderly patients with AML with blasts of > 30% (10.4 versus 6.5 months) [10]. DAC given at 15 mg/m^2/8 h for 3 consecutive days showed a relatively higher CR rate (15% versus 0%) but nondifferential OS (8 *versus* 6 months) when compared to BSC [11]. Another dosage, which was defined as 20 mg/m^2 for 5 days, resulted in 17.8% CR, compared to 7.8% with BSC or low-dose cytarabine, and a modest 2-month improvement of the median OS (7.7 versus 5.5 months) [12]. In all mentioned studies, the CR rate ranged from 15% to 20% using either of the administration schedule. Therefore, appropriate combination therapies of HMAs have been initiated. In this review, we evaluated the safety and efficacy of combined regimens in the treatment of elderly patients with AML.

Combined with chemotherapy

In a previous in vitro experiment, Neil et al. [13] suggested a synergistic effect of cytarabine (Ara-C) and AZA in L210 leukemic mice. This might be due to deoxycytidine kinase (dCK) inactivity related to Ara-C resistance. AZA was shown to induce dCK, phosphorylating Ara-C to its active compound Ara-CTP and restoring cell sensitivity toward Ara-C [14]. Standard induction chemotherapy in elderly patients with AML remains a "3+7" regimen characterized by the combination of intermediate-dose Ara-C administered for 7 days with an anthracycline for 3 days. Two randomized studies investigated AZA in conjunction with DA for treatment of elderly patients with AML *in vivo*. A pilot study aimed at elderly de novo AML patients with a leukocyte count of < 20,000/μl revealed a CR rate of 50% and median OS of 8.8 months [15]. However, large sample data from the AML-AZA trial failed to demonstrate favorable results, with nonsignificant shorter OS in the AZA/DA arm compared to a DA monotherapy arm (15 versus 21 months), which might be in part explained by imbalances in high-risk cytogenetics, lactate dehydrogenase (LDH) levels, and unfavorable performance status in the combination arm [16]. Adverse events (AEs) were also more common in the combination arm. The addition of AZA to DA increased toxicity and did not provide benefits. It was noted that the treatment effect was associated with the sequence and the time intervals between the doses of each agent. Additionally, the optimal schedule was AZA after Ara-C at a time when tumor DNA synthesis had maximally recovered [13]. Therefore, an optimal administration schedule and further clinical trials need to be further surveyed and studied.

Qin et al. [17] designed an in vitro experiment combining Ara-C with DAC in human leukemia cell lines HL60, ML-1, RAji, and Jurkat. The authors found through measurements of the half-maximal inhibitory concentration (IC_{50}) that the combination of DAC and Ara-C showed an additive or synergistic induction of cell death in all cell lines. In addition, a sequential schedule with DAC followed by Ara-C was valid.

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Granulocyte colony-stimulating factor (G-CSF) priming induced G0/G1 phase leukemia cells into an S phase, enhancing cell response to DAC. Then, DAC (15 mg/m^2 D1–5) plus CAG (termed D-CAG, comprising Ara-C [10 mg/m^2, q12h, D3–9], aclarubicin [10 mg/day, D3–6], and G-CSF [300 μg/day, D0–9]) was designed to treat older AML patients [18]. Overall response rate (ORR) was achieved by 70 patients (82.4%), and CR was 64.7% after the first cycle. The median OS for all patients was 10 months (1-42 months), with a 1-year OS probability of 46.23%. This regimen was well tolerated, with induction mortality of only 4.4%. The results verified that the D-CAG regimen was superior to DAC monotherapy when compared with historical data.

Homoharringtonine (HHT), an alkaloid from Chinese natural plants, was considered a potential treatment for leukemia. Geng et al. [19] verified the enhanced effect of DAC plus HHT for inhibiting the viability of AML-derived Kg-1a cells. In a randomized Chinese study, the CR rate was statistically different between three treatment arms (DAC alone: 21.7%; HAG alone: 48.1%; and combination therapy: 58.2%) [20]. The addition of DAC to HAG did not increase toxicities and early mortality. No statistical difference in terms of OS was observed, which might be due to various consolidation therapy regimens.

Combined with immunotherapy

DAC was shown to act as a sensitizer to immunotherapy, eliciting an antitumor cytotoxic T-lymphocyte (CTL) response by inducing cluster of differentiation 80 (CD80) expression [21]. The immunocompromised status of AML had an impact on the antileukemia activities of DAC-based chemotherapy. Because of this, there should be an attempt to combine DAC-based chemotherapy with haploidentical lymphocyte infusion (HLI) or human leukocyte antigen (HLA)-mismatched stem cell microtransplantation (SC-MST) to overcome this limitation. A multicenter phase II trial infusing HLI followed by 36 hours after the last dose of D-CAG showed a CR of 55.2% and ORR of 86.2% after the first cycle [22]. Superior survival time was present, with a median OS of 18.2 months and 2-year OS and disease-free survival (DFS) rate of 59.6% and 36.9%, respectively. AEs were limited to < 5% incidence of most grade III–IV events, except for febrile neutropenia. Consistent results were demonstrated in leukemic mice. Combination therapy of D-CAG plus SC-MST produced a better CR rate of 81.8% and ORR rate of 86.4% after the first cycle [23]. There was no ratio difference between the cytogenetic and molecular subgroups. The median OS for all patients was 17 months, with a 2-year OS of 34.8%, and it was also closely related to the treatment cycles of SC-MCT. This regimen was well tolerated, with rare nonhematological toxicities. Early mortality (within 1 month) was only 4.3%, and no graft versus host disease (GVHD) was observed.

Lenalidomide, an immunomodulatory agent, is capable of regulating gene expression and phosphatase activity [24]. The combination therapy of sequential AZA and lenalidomide demonstrated encouraging clinical activity in previously untreated elderly AML patients [25]. In a phase II study, 52 older untreated patients were administered with AZA 75 mg/m^2 D1–7, followed by escalating doses (5 mg, 10 mg, 25 mg, and 50 mg) of lenalidomide D8–28 of each cycle every 6 weeks [26]. The ORR was 40%, but the median OS was 5 months. Median OS was longer for responders compared with nonresponders (17.25 versus 3.75 months). The combination therapy led to limited grade ≥ 3 toxicities and an early mortality of 17%. Another phase II study reported the outcome of a combination regimen in poor-risk previously treated elderly AML patients [27]. After a median of two treatment cycles (1-11), the response rate was 25%, and responders showed a greater survival advantage. Neutropenic fever was the most common serious AE.

To summarize, the combination of HMAs with immunotherapy enhanced the antileukemic effect over a single regimen, and it was considered effective and safe for elderly AML patients, especially untreated ones.

Combined with histone deacetylase inhibitors

Histone deacetylase inhibitors (HDACis) have been proven to induce leukemia cell differentiation and prevent proliferation *via* blocking the cell cycle at the G1 phase [28]. Current published HDACis include valproic acid (VPA), vorinostat, entinostat, and panobinostat.

VPA, a short-chained fatty acid, was demonstrated to promote growth inhibition, apoptosis induction, and reactivation of p57KIP2 and p21CIP1 in leukemic cell lines HL-60 and MOLT4 when combined with DAC [29]. However, in vivo, the combination regimen did not show antileukemia activity. A phase 1/2 and a phase 1 study revealed that the addition of VPA led to neurotoxicity, whose severity depended on the dose [30, 31]. A phase II study compared the efficacy and safety of DAC monotherapy with the combination therapy [32]. Among 62 patients with AML, 58 (93.5%) were aged ≥ 60 years. A statistically higher CR rate and an approximate 2-month modest improvement of the median OS were observed in the DAC monotherapy arm. The incidence of AEs was higher with the combination arm, particularly neurotoxicity. This unfavorable outcome may be due to the use of a particularly weak HDACi and the administration scheduling. VPA, AZA, plus all-trans-retinoic acid (ATRA) were also evaluated as induction therapies. The result from a phase 1/2 study was favorable, with a response rate of 52% for elderly patients with untreated AML [33]. A phase II study enrolling 65 patients with median age of 72 years (50-87 years) revealed that the best responses were 14 CR and 3 PR (26%). Median OS was 12.4 months for the whole cohort and 18.1 months for the untreated AML/myelodysplastic syndrome (MDS) group [34]. The use of HDACi was considered to restore sensitivity to ATRA, and AZA could enhance the activity of ATRA, inducing hypomethylation and reexpression of the retinoic acid receptor-beta2 (*RAR-b2*) gene [35, 36]. It is feasible that the combination of HMAs with HDACi sensitizes ATRA-resistant AML to the effects of ATRA. Vorinostat, an orally administered synthetic HDACi, has a dual ability of inhibiting both histone and protein deacetylase. A phase I dose-escalation study determined the maximum administered dose (MAD) of DAC and vorinostat at 20 mg/m^2 for 5 days and 400 mg/day for 14 days, respectively. For untreated AML, patients receiving concurrent therapy showed a higher response rate (46% versus 14%) compared to those with sequential therapy. However, more serious AEs were reported in the concurrent arm [37].

Panobinostat, a pan-HDACi, was combined with AZA for AML and high-risk MDS patients unfit for intensive chemotherapy [38]. During the phase Ib stage, the MTD dose of panobinostat was 30 mg. Then,

in the phase II stage, patients with AML receiving panobinostat 30 mg showed an ORR of 31% (9/29) and an OS of 8 months after a median follow-up of 13 months. Most frequently, grade III–IV AEs contained febrile neutropenia (22%), nausea (17%), infection (17%), dyspnea (17%), and fatigue (13%).

Entinostat exerted antileukemia activity through the inhibition of class I HDAC enzymes. However, in a phase 2 MDS/AML trial, entinostat presented negative results. Patients with AML receiving AZA alone showed longer median OS (7.1 versus 5.3 months) than those receiving combination therapy after a median follow-up of 30 months [39].

Taken together, it is suggested that epigenetic therapy of AML using HDACi and HMAs warrants further investigation. The result from an in vitro study designed by Momparler et al. [40] showed that a triple combination of DNA methylation, histone methylation, and histone deacetylation induced a remarkable synergistic antineoplastic effect against human AML cells, providing a possible better treatment platform.

Combined with monoclonal antibodies

Gemtuzumab ozogamicin (GO), a humanized anti-CD33 monoclonal antibody, was shown to be effective in elderly AML patients. The reason for the combination of AZA and GO was non-cross-resistant mechanisms and AZA strengthening the efficacy of GO through the increase of the CD33 expression and the decrease of the p-glycoprotein expression on the leukemic blasts. In 2008, results showing a CR rate of 70% and median OS of 10 months were verified in a pilot trial combining hydroxyurea (HU) 1500 mg, AZA 75/m^2, and low-dose GO 3 g/m^2 [41]. Then, a phase II study applied a consistent schedule for elderly patients with untreated AML as induction therapy [42]. Those achieving CR then received four cycles of AZA alone as one consolidation treatment. Patients in the good- or poor-risk cohorts classified by age and Eastern Cooperative Oncology Group (ECOG) showed a CR rate of > 30% and similar median OS of 11 months. The early mortality rate was 7.2% and 13%, respectively. The results demonstrated that this combination therapy was a possible option for elderly patients, especially poor-risk patients. Walter et al. [43] reported the efficacy and safety of GO, AZA, plus vorinostat for elderly patients with relapsed/refractory AML. The results of an MAD schedule of GO 3 mg/m^2 D4–8, vorinostat 400 mg/day D1–9, and AZA 75 mg/m^2 D1–7 yielded a CR rate of 23.3%. Responders lived longer than those who failed therapy but lived at least 1 month (7.48 months versus 3.17 months). The treatment was well tolerated, with 1-month mortality rate of 9.3%.

Combined with kinase inhibitors

FLT3 tyrosine kinase inhibitor

AML with internal tandem duplication (ITD) mutations in the FLT3 kinase gene (FLT3/ITD) frequently predicted poor prognosis. Chang *et al*. [44] verified that a combination therapy of FLT3 inhibitor and HMAs confers synergistic antileukemic effects on cell apoptosis, differentiation, and growth inhibition, which provides a novel therapeutic approach.

Midostaurin (MS), an oral inhibitor, had shown a therapeutic effect for wild-type and mutated FLT3 AML [45]. Superior anti-AML activity was displayed in cultured and primary FLT3-ITD-expressing AML cells administered with sequential treatment with DAC and MS [46]. A phase I study testing the efficacy and safety of AZA plus MS verified modest clinical activity [47]. Of 14 available patients, three patients achieved CR and two patients achieved hematology improvement. The median OS was 6 months.

Sorafenib, another FLT3 inhibitor, was applied to FLT-3-mutant AML cells MV4-11 in association with a DAC in vitro experiment [48]. The treatment significantly improved growth inhibition relative to either agent alone. In a phase II study, relapse/refractory or elderly AML patients received AZA at a dose of 75 mg/m^2 for 7 days and sorafenib 400 mg twice daily. The modest outcome was a response rate of 46% and CR of 27% [49].

Aminopeptidase inhibitor

Tosedostat is an oral aminopeptidase inhibitor showing significant antileukemic activity [50]. Mawad *et al*. [51] performed a clinical trial using tosedostat with Ara-C or DAC in elderly patients with untreated AML or high-risk MDS. Thirty-four patients were randomized to receive tosedostat 120 mg D1-21 or 180 mg D1–35 accompanied by either Ara-C 1 g/m^2 or DAC 20 mg/m^2 D1-5. In the tosedostat/DAC arm, nine patients (53%) achieved CR/CR with incomplete count recovery (CRi). Patients receiving treatment of tosedostat 120 mg/m^2 and DAC showed a median OS of 16.7 months. The grade III–IV AEs with incidence rate of > 10% were febrile neutropenia, fever, pneumonia, and sepsis. Only one patient died within 4 months after starting treatment.

Proteasome inhibitor

Preclinical studies in AML cells showed that bortezomib induced miR-29b upregulation, which was associated with a clinical response to DAC [52]. Thus, a phase I trial of bortezomib and DAC enrolled 10 elderly poor-risk AML patients. DAC 20 mg/m^2 D1–10 plus bortezomib (escalated up to 1.3 mg/m^2 on Days 5, 8, 12, and 15) as an induction therapy showed a CR/CRi rate of 50% in elderly AML patients. Nonetheless, a bortezomib-related neuropathy developed after repetitive cycles [53].

Combined with bexarotene

Bexarotene, the retinoid X receptor agonist, has been demonstrated to be active in non-M3 AML and to induce myeloid differentiation *in vivo* [54]. A phase I study enrolled 19 elderly or relapsed AML patients receiving DAC 20 mg/m^2 followed by bexarotene 100 mg/m^2 or 200 mg/m^2 or 300 mg/m^2 D1–5 as induction therapy. Though the combination therapy resulted in a modest outcome (CR: 5.2%; PR:15.8%), it was well tolerated [55].

Conclusion

HMAs improve the outcome in elderly AML patients but are associated with relatively low response rates. It has been shown that HMA

combination therapy provides a good treatment platform. Attractive outcomes have been obtained using a combination of HMAs with CAG chemotherapy, immunomodulatory therapy, monoclonal antibodies, and proteasome inhibitors, with other regimens warranting further investigation.

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Conflict of interest

The authors state no conflicts of interest.

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