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Azacitidine in Fludarabine-Refractory Chronic Lymphocytic Leukemia: A Phase II Study

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Abstract

Because preclinical data suggested that hypomethylating agents might be effective in chronic lymphocytic leukemia (CLL), we conducted a phase II trial with azacitidine in patients with recurrent fludarabine-refractory CLL. Eight of 9 patients did not respond, and 1 patient experienced reduction of hepatosplenomegaly and a substantial increase in platelet count after 4 cycles of therapy. Further studies of azacitidine in CLL are warranted.

Background—Treatment of fludarabine-refractory disease in patients with chronic lymphocytic leukemia (CLL) remains a challenge. Because a recent genome-wide methylation analysis of CLL cells suggested that demethylation therapy might be beneficial in CLL, we conducted a phase II trial with the hypomethylating agent azacitidine in patients with recurrent fludarabine-refractory CLL.

Patients and Methods—Nine patients with recurrent fludarabine-refractory Rai stage IV CLL (median age, 74 years; range, 49–81 years) were enrolled. Azacitidine (75 mg/m^2) was administered by subcutaneous injection daily for 7 consecutive days every 3 to 8 weeks, and the data were analyzed at a median follow-up of 9 months (range 3–47 months).

Results—The trial was prematurely discontinued because of lack of response and slow accrual. The number of cycles administered ranged from 1 to 6. Three patients received 1 cycle, 3 patients received 2 cycles, and the remaining 3 patients received 4, 5, or 6 cycles. Side effects included grade 2 or 3 infectious episodes (resulting from immunosuppression and drug-induced neutropenia), diarrhea, rash, vomiting, anemia, and thrombocytopenia. One patient experienced reduction of hepatosplenomegaly and a substantial increase in platelet count after 4 cycles of therapy. However this response did not qualify as a partial response according to the National Cancer Institute International Workshop on CLL (NCI-IWCLL) criteria. At a median follow-up of 9 months after the start of azacitidine treatment, 3 patients (33%) who went on to receive other treatments were alive.

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Conclusions—Although no partial or complete responses occurred in these heavily pretreated patients, the encouraging response in 1 of these patients may warrant further studies to investigate the effects of azacitidine in CLL.

Keywords

Azacitidine; Chronic lymphocytic leukemia; DNA methylation

Introduction

Although frontline treatment for chronic lymphocytic leukemia (CLL) improved substantially with the introduction of fludarabine, cyclophosphamide, and rituximab (FCR), the treatment for fludarabine-refractory or recurrent disease is still a challenge.[1] In agreement with the MD Anderson data,[1] a randomized phase III study by the German CLL Study Group comparing responses to FCR and to fludarabine with cyclophosphamide found only that FCR showed a higher complete response rate (44% vs. 22%; P <.001), higher overall response rate (95% vs. 88%; P = .01), and longer median progression-free survival time (52 vs. 33 months; P < .001) than did fludarabine with cyclophosphamide alone.[2] However the response rates were lower in older patients and in those with advanced disease, and lower response rates to FCR were obtained in patients who were unable to complete more than 4 cycles of therapy.

Attempts to improve the outcomes of FCR treatment and provide a therapeutic regimen for patients whose CLL is refractory to fludarabine have been made in recent years. The combination of alemtuzumab and fludarabine compared with fludarabine alone was investigated in a randomized phase III trial in a minimally pretreated population with relatively favorable clinical characteristics[3]; the combination treatment had greater response and duration of remission rates without a significant benefit in overall survival. Alemtuzumab in combination with FCR has been tested in both a frontline setting for CLL[1] and in recurrent and/or refractory CLL in a population including heavily pretreated patients, 39% of whom had fludarabine-refractory disease, with a median of 3 previous treatment regimens.[4] The overall response rate was 67%, the complete response rate was 27%, and the median time to failure was 11 months. In a study in a frontline setting (which included 60 patients younger than 70 years), as in the study in a recurrent and/or refractory setting, only 42% of the patients completed 6 cycles of therapy. The complete response rate was 70%, and the median time to progression was 38 months. Another approach for heavily pretreated patients with advanced CLL has been attempted with single-agent ofatumumab. The overall response rates were similar to those with single-agent rituximab.[5] Thus a therapeutic regimen that is less toxic and easier to administer is still needed for patients with recurrent and/or refractory disease.

Several studies suggested that epigenetic silencing plays a role in the pathogenesis of CLL. For example, methylation of zeta-chain–associated protein kinase 70 (ZAP-70) on C-334 downregulates ZAP-70 expression,[6] and promoter methylation of the death-associated protein kinase 1 (DAPK1) is thought to play a pathogenetic role in heritable predisposition to CLL.[7] We recently performed a genome-wide methylation analysis of patients with CLL and identified 280 new potential targets of aberrant DNA methylation.[8] Most patients had 8 to 11 methylated genes, and no patient had fewer than 5 methylated genes. This was consistent with the hypermethylation phenotype reported in other leukemias, including acute lymphoid and myeloid leukemias.[8] These and other studies suggested that CLL might respond to the hypomethylating agent azacitidine, which has been used with success to treat myelodysplastic syndrome and acute myeloid leukemia. In patients with low-risk myelodysplastic syndrome, azacitidine induced a 30% to 40% hematologic

improvement.[9,10] DNA methylation profiles were evaluated during the first 2 cycles and reported elsewhere.[8]

We studied the safety and efficacy of azacitidine in patients with CLL. The activity of azacitidine in CLL has not been established; therefore our study was restricted to heavily pretreated patients with fludarabine-resistant disease.

Patients and Methods

We conducted a single-institution phase II study to determine the safety and efficacy of azacitidine in patients with fludarabine-resistant CLL. Patients with Richter transformation were eligible. Institutional review board approval and written informed consent from patients were obtained. The diagnosis of CLL was established according to the World Health Organization classification criteria and clinically staged according to the Rai staging system.

Baseline creatinine and bilirubin levels and liver function test results were obtained 7 days before azacitidine treatment, and a bone marrow aspirate was obtained < 1 month before treatment initiation. A negative pregnancy test result was required for women of child-bearing potential. Women of childbearing potential agreed not to become pregnant during treatment, and men agreed not to father a child during treatment.

Eligibility criteria included a bilirubin level of < 2 mg/dL, a serum aspartate aminotransferase or alanine aminotransferase level of less than twice the normal level, a creatinine level of < 2 mg/dL, and adequate cardiac function, excluding New York Heart Association class III or IV heart failure. Exclusion criteria included breastfeeding or pregnancy, known or suspected hypersensitivity to azacitidine or mannitol, and the presence of uncontrolled intercurrent illness, infection, or advanced malignant hepatic tumors. Noncompliance with study requirements excluded entry into or prompted removal from the study.

A detailed physical examination was conducted before every cycle, and peripheral blood counts were conducted once or twice weekly during the first cycle and then every 2 to 4 weeks during treatment. Azacitidine was administered at a dosage of 75 mg/m^2 subcutaneously daily for 7 days. Premedication for nausea and vomiting was given. The treatment was repeated every 3 to 8 weeks and could be continued as long as the patient continued to benefit or for up to 1 year. If no beneficial effect or toxicity was observed after 2 cycles, the dose could be escalated to 100 mg/m^2. For subsequent cycles to be administered, a patient's platelet count had to be > 30,000/μL. Dose reductions to 50 or 25 mg/m^2 were used for prolonged myelosuppression of more than 60 days with evidence of hypocellular bone marrow. A minimum of 1 full cycle was required for a patient to be included in evaluations of efficacy or toxicity.

The primary endpoints of the trial were the complete response and partial response rates. The National Cancer Institute International Workshop on Chronic Lymphocytic Leukemia (NCI-IWCLL) criteria were used to define responses. The study was designed to assess response rate after enrollment of 12 patients. If 2 or more patients responded, 18 additional patients would have been enrolled.

Results

The study was discontinued after 9 patients enrolled because of a lack of efficacy and slow accrual. The clinical characteristics of the patients are summarized in Table 1. Six men and 3 women were treated. Their median age was 74 years (range, 49–81 years). At the start of

treatment, all patients were transfusion dependent and had Rai stage IV CLL. Two patients had previously been diagnosed with Richter transformation, and we confirmed the diagnosis in 1 of them. All patients had good kidney and liver function; the median creatinine level was 1.1 mg/dL (range, 0.8–1.7 mg/dL), the median bilirubin level was 0.6 mg/dL (range, 0.3–1.2 mg/dL), and the median serum alanine aminotransferase level was 12 IU/L (range, 12–22 IU/L).

Four patients had a diploid karyotype, 1 patient had an 11q deletion in 1 metaphase, and fluorescence in situ hybridization analysis detected an 11q deletion. Two patients had complex cytogenetic abnormalities including a 17p deletion. In another patient with a near-normal karyotype, fluorescence in situ hybridization analysis revealed a *TP53* mutation and *LAMP1* gene deletion. One patient had 13q and 5q deletions, compatible with therapy-related myelodysplastic syndrome.

All patients had disease refractory to fludarabine. One patient had received only 1 previous treatment (with FCR), whereas 3 patients had received 3 previous treatments, 3 patients had 4 previous treatments, 1 patients had 7 previous treatments, and 1 patient had 8 previous treatments. Two patients had relapsed disease after hematopoietic stem cell transplantation. The median time from initial diagnosis to treatment with azacitidine was 84 months (range, 31–168 months), and the last follow-up was a median of 9 months (range, 3–47 months) after the start of azacitidine treatment.

The number of cycles of azacitidine administered ranged from 1 to 6. Three patients received only 1 cycle, 3 patients received 2 cycles, and the remaining 3 patients received 4, 5, or 6 cycles. The patient who received 4 cycles attained a response, with improvements in white blood cell count and hemoglobin level apparent after 1 cycle, resulting in stabilization of disease. However this patient did not attain a partial response according to the NCI-IWCLL response criteria. At baseline, his white blood cell count was 85.5/μL, his hemoglobin level was 9.5 g/dL, and his platelet count was 102,000/μL. He had hepatosplenomegaly; his spleen could be palpated 9 cm below the left costal margin and his liver could be palpated 4 cm below the right costal margin. He had cervical, axillary, and inguinal lymph-adenopathy, with lymph node size ranging from 0.5 to 1 cm. After 1 cycle, his white blood cell count was 71.1/μL, his hemoglobin level was 10.1 g/dL, and his platelet count was 81,000/μL. His lymph nodes became nonpalpable, and his spleen shrank so that it was palpable only to 7 cm below the left costal margin and his liver was palpable only to 2 cm below the right costal margin. After 4 cycles, his white blood cell count and hemoglobin level remained stable, and his platelet count rose to 291,000/μL. Two days after starting the 5th cycle, he was admitted to the hospital for pneumonia; azacitidine treatment was discontinued and the patient was removed from the study. After recovering from pneumonia, he was treated with lenalidomide plus rituximab and most recently with ofatumumab. He had some improvement and was alive at his last follow-up.

The most common side effects of azacitidine were grade 2 or 3 infectious episodes resulting from immunosuppression and neutropenia. Grade 2 and 3 events are listed in Table 2.

As expected, all the patients' peripheral blood counts dropped after treatment with azacitidine, and infections were the most common adverse event, leading to treatment discontinuation in most patients. Otherwise, azacitidine was well tolerated. One patient required a 25% dose reduction because of atrial fibrillation and a positive stress test that could be attributed to a β-blocker dose reduction.

At a median follow-up of 9 months after the start of azacitidine treatment, 3 patients (33%) were alive. However, all 3 of these patients underwent other treatments after discontinuation of azacitidine.

Discussion

In this phase II clinical trial, azacitidine was administered to 9 patients with fludarabine-refractory CLL, most of whom had been heavily pretreated. Only 1 of those patients attained a response, which did not meet the NCI-IWCLL partial response criteria, and the study was discontinued because of the lack of response and slow accrual.

Aberrant DNA methylation is an epigenetic phenomenon involved in silencing tumor suppression genes by altering the accessibility of DNA to transcriptional regulators through methylation of cytosine in the CpG dinucleotide in DNA. This mechanism is reversible in vitro and in vivo using demethylating agents such as azacitidine.[9,10] Azacitidine is a nucleoside analogue that inhibits ribonucleotide reductase[11] and possesses hypomethylating activity. Previous studies showed that drug-induced DNA hypomethylation peaks at 15 days after azacitidine administration[9] and returns to baseline levels by the end of each cycle. Several treatment cycles are therefore required to obtain a durable response.[9,10] Most of our patients received only 1 or 2 cycles of therapy—too few cycles to accomplish a clinical response.

In a study of azacitidine plus valproic acid in patients with advanced cancers, DNA hypomethylation and histone acetylation were attained, and stable disease was observed for a median of 6 months (range, 4–12 months) in 25% of patients. However no complete or partial responses were observed,[12] suggesting that attaining a hypomethylation state does not result in clinical remission, which may explain the lack of response in our patients as well.

Induction of global hypomethylation is probably a marker of the biological activity of a given drug but does not predict clinical activity. Specific gene methylation studies may identify better markers of response. We recently reported that aberrant DNA methylation is common in CLL and has potential prognostic and therapeutic value.[8] We found that treatment of CLL with azacitidine resulted in decreased methylation of the genes *LINE*, *DLX4*, and *SALL1* in peripheral blood CLL cells. *IgVH* mutational status and ZAP70 expression were not associated with specific methylation profiles. Multivariate analysis showed that methylation of *LINE* and *APP* was associated with shorter overall survival ($P = .045$ and $P = .0035$, respectively).[8] Similar results were reported for acute myeloid leukemia and myelodysplastic syndrome.[10] Although preclinical data suggested that hypomethylating agents might be beneficial in CLL, current clinical trials using the available hypomethylating agents yielded disappointing results. Like in our study, a recent phase I trial with the demethylating agent decitabine revealed no significant activity in patients with CLL, and dose-limiting myelosuppression and infectious complications prevented dose escalation.[13] Whether nonmyelosuppressive demethylating agents are clinically active in CLL will have to be determined when these agents become available.

Although none of our patients responded to treatment according to the NCI-IWCLL criteria, our patient population consisted mostly of heavily pretreated patients with advanced disease (Rai stage IV) who received too few cycles for us to expect a significant clinical response. Of 9 patients, 6 received 1 or 2 cycles of therapy. Of the 3 patients who received 4 cycles or more, 1 experienced improvement. The drug induced a reduction in peripheral blood counts but otherwise was relatively well tolerated, and no unexpected adverse events were

observed. Therefore further studies in fludarabine-refractory patients with CLL with less advanced disease may be warranted.

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Clinical Practice Points

- Our study was designed to address the question of whether the demethylating agent Azacitidine is active in fludarabine refractory chronic lymphocytic leukemia (CLL), for which no standard therapy is currently available.
- A previous study with another demethylating agent Decitabine yielded negative results.
- In this small study found that Azacitidine treatment was beneficial in one patient with relapsed CLL, suggesting that further clinical trials of Azacytidine in CLL are warranted.

Table 1

Patient Characteristics

Patient No.	Sex	Age	WBCs, 10^9/L	Lymphocytes %	Hb, g/dL	Platelets, 10^9/L	Rai Stage	B_2M, mg/dL	ZAP70	*IgVH* Mutation	CD38	Cytogenetic Abnormalities	No. of Previous Treatments	Status at End of Study
1	M	55	0.3	66	10.4	14	IV	5	NA	NA	Negative	46, XY, Del(11) (q13q23)	3	Dead
2	F	49	58.9	99	8.4	51	IV	8.5	NA	NA	Negative	46, XX, del(11) (p11.2p15), del(17) (p12)	8	Dead
3	F	62	5.5	72	11.8	176	IV	4.7	NA	NA	Negative	46, XX	1	Alive
4	M	76	5.6	46	11.7	56	IV	4.3	Positive	NA	Negative	46, XY	4	Alive
5	M	74	85.5	91	9.5	102	IV	2.7	NA	NA	Negative	46, XY	4	Alive
6	F	71	8.5	85	10.7	72	IV	2.4	NA	NA	Negative	46XX	3	Dead
7	M	79	11.2	64	11.5	108	IV	10.1	NA	NA	Negative	45XY, t(1,10) (p13, q26), add(6) q,(13) +12,−13, add(16) (q13), −17	7	Dead
8	M	81	66.1	100	9.7	61	IV	8.4	NA	NA	Negative	45-46, XY, add(4) (q35) [cp3]	3	Dead
9	M	76	3.7	27	10.7	38	IV	9.1	NA	NA	High	45-46, XY, del(5) (q13q33), del(13) (q12q14) [cp7]	4	Dead

Abbreviations: B_2M = β_2-microglobulin; Hb = hemoglobin; NA = not available; WBCs = white blood cells.

Table 2

Adverse Events

Grade 2 or 3 Adverse Event	No. (%)
Hematologic	
Thrombocytopenia	1 (11)
Anemia	1 (11)
Neutropenia	3 (33)
Nonhematologic	
Diarrhea	2 (22)
Skin rash	3 (33)
Infections	6 (67)
Fever	2 (22)
Pneumonia	3 (33)
Vomiting	1 (11)