Gemtuzumab ozogamicin in combination with vorinostat and azacitidine in older patients with relapsed or refractory acute myeloid leukemia: a phase I/II study

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ABSTRACT

Epigenetic therapeutics such as the histone deacetylase inhibitor, vorinostat, and the DNA methyltransferase I inhibitor, azacitidine, enhance gemtuzumab ozogamicin efficacy *in vitro*. We therefore investigated vorinostat/azacitidine/gemtuzumab ozogamicin in 52 adults aged 50 years or over with acute myeloid leukemia requiring therapy for first relapse (remission duration ≤12 months) or primary refractory disease in a phase I/II trial. Vorinostat and gemtuzumab ozogamicin were escalated step-wise during the phase I portion of the trial. Vorinostat (400 mg/day orally from Days 1-9), azacitidine (75 mg/m^2/day intravenously or subcutaneously from Days 1-7), and gemtuzumab ozogamicin (3 mg/m^2/day intravenously on Days 4 and 8) were identified as the maximum tolerated dose. Among the 43 patients treated at this dose, 10 achieved a complete remission and 8 achieved a complete remission with incomplete blood count recovery, for an overall response rate of 41.9% (exact 95% confidence interval (CI): 27.0-57.9%). Four of these 18 patients (2 with complete remission and 2 with complete remission with incomplete blood count recovery) had persistence of minimal residual disease by flow cytometry at the time of best response. Four patients died within 28 days of treatment initiation. Median overall survival for the 18 patients achieving complete remission/complete remission with incomplete blood count recovery was significantly longer than for those 21 patients who failed therapy but lived at least 29 days after treatment initiation (224.5 days (range 70-798) *vs.* 95 days (range 36-900); *P*=0.0023). These data indicate that vorinostat/azacitidine/gemtuzumab ozogamicin has activity in this difficult-to-treat acute myeloid leukemia patient subset. (*ClinicalTrials.gov: identifier 00895934*).

Introduction

Acute myeloid leukemia (AML) in older adults remains a difficult-to-treat disease, with most patients either relapsing after initial achievement of a complete remission (CR) or having primary refractory disease, yielding a 5-year relative survival rate of 10% or less for those over 65 years of age.[1] Thus, novel strategies are needed for these patients, especially for those with poor performance status (PS) who are at high risk of life-threatening or fatal toxicities from intensive therapy, and those who are at high risk of relapse based on cytogenetic and molecular characteristics and presence of minimal residual disease (MRD) despite being in CR. Because of well-defined cell surface antigens and easy tumor accessibility, considerable effort has been directed at the therapeutic exploitation of monoclonal antibodies, with a paradigm being gemtuzumab ozogamicin (GO), an immunoconjugate between an anti-CD33 antibody (hP67.6) and a toxic calicheamicin-γ^1 derivative.[2,3] However, when used alone in patients with relapsed or refractory AML, remission rates have typically not exceeded 25%.[2,3] Research has, therefore, focused on developing novel means to improve GO therapy.

Following CD33-mediated cellular uptake and subsequent intracellular cleavage from hP67.6, the calicheamicin-γ1 moiety causes DNA damage, apoptosis, and cell death.[2,3] This mechanism suggests that drugs that augment the DNA damage response and lower the apoptotic threshold could improve GO efficacy. Histone deacetylase (HDAC) inhibitors such as vorinostat are of interest in this regard as they function synergistically or additively with conventional anti-AML chemotherapeutics, partly due to lowering of the apoptotic threshold within tumor cells.[4,5] Indeed, recent *in vitro* studies indicate that HDAC inhibitors potently augment DNA intercalation of the calicheamicin-γ^1 derivative and enhance DNA degradation and GO efficacy.[6]

Likewise, DNA methyltransferase I inhibitors such as azacitidine or decitabine have been demonstrated to enhance GO efficacy *in vitro*,[7,8] possibly through lowering of the apoptotic threshold, and early clinical studies have indicated activity in patients with AML.[9-11] These studies, together with the improved clinical activity reported when HDAC inhibitors are used with DNA methyltransferase I inhibitors,[12] prompted a phase I/II study with vorinostat and azacitidine as chemosensitizers for GO in the treatment of primary refractory AML or AML in first relapse (remission duration ≤12 months) requiring first salvage therapy.

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The primary objective of this study was to determine the vorinostat dose with the most favorable efficacy and toxicity when combined with azacitidine and GO.

Methods

Study population

Patients aged 50 years or over with AML other than acute promyelocytic leukemia[13] with an ECOG PS of 0-3 and adequate cardiac, liver, and kidney function were eligible if they required either first salvage chemotherapy for primary refractory disease (as defined as persistent disease after 1 or 2 courses of conventional curative-intent chemotherapy) or relapsing disease with a duration of first CR of less than 12 months after at least one course of curative-intent chemotherapy. Patients with prior hematopoietic cell transplantation (HCT) were eligible if relapse occurred 6-12 months post-transplant. Cytogenetic risk-group assignment was made according to the modified United Kingdom Medical Research Council/National Cancer Research Institute criteria.[14] Treatment responses were defined according to standard criteria.[15,16] MRD was assessed by multiparametric flow cytometry using the institution's routine methodology; any level of MRD was considered MRD^{pos}. The protocol (*ClinicalTrials.gov: identifier 00895934*) was approved by the institutional review board of both participating institutions, and patients gave written informed consent in accordance with the Declaration of Helsinki.

Treatment plan

In phase I, the starting dose level (level 1) used azacitidine 75 mg/m^2/day intravenous (i.v.) or subcutaneously (s.c.) on Days 1-7, vorinostat 200 mg/day orally on Days 1-9, and GO 3 mg/m^2 i.v. on Day 8. At dose levels 2 and 3, the daily vorinostat dose was increased to 300 mg and 400 mg, respectively, while keeping the other drug doses unchanged. At dose level 4, GO 3 mg/m^2 was given on Days 4 and 8 while keeping the other drug doses unchanged from level 3. If necessary, hydroxyurea was given to reduce the leukocyte count to less than 25 x 10^9/L before beginning study therapy, but was discontinued prior to initiation of treatment with vorinostat and azacitidine. If there was clear evidence of persistent leukemia (≥20% blasts, no hypocellularity) on Day 15, the first cycle was repeated. In all other patients, a second cycle was begun if peripheral blood counts had recovered and/or all toxicities had resolved to grade 2 or under. Patients were removed from study if there was disease progression. Otherwise, patients came off study if a partial remission was not achieved after cycle 3, or if a CR or CR with incomplete peripheral blood count recovery (CRi) was not achieved after cycle 6.

Statistical considerations

A "3+3" dose escalation design for phase I and a Simon minimax two-stage design for phase II were used,[17] with a response rate of 17% considered unacceptably low based on historical data of similarly treated patients (*EH Estey, unpublished observation, 2013*) and a response rate of 34% considered acceptably high. Dose-limiting toxicities (DLTs) were defined as: 1) any grade 3 non-hematologic toxicity lasting over 48 h that results in a delay of more than seven days of the subsequent treatment cycle, with the exception of febrile neutropenia or infection; 2) any grade 4 or over non-hematologic toxicity, with the exception of febrile neutropenia/infection or constitutional symptoms if recovery to grade 2 or under within 14 days; and 3) prolonged myelosuppression in the absence of persistent disease. Cumulative toxicities were assessed after 2 treatment cycles. Efficacy was determined as the best response achieved during study treatment.

Results

Between September 2009 and November 2012, 52 eligible adults with a median age of 64.8 (range 50.2-78.9) years were enrolled in this study (Table 1). Twenty-nine of these patients had primary refractory disease at the time of enrollment; these patients had received a variety of treatments before they entered this study, including single courses of cytarabine and an anthracycline ("7+3"; n=13), repeat courses of 7+3 (n=4), 1 or 2 courses of high-dose cytarabine-containing regimens (n=11), or clofarabine/low-dose cytarabine (n=1). All patients received study therapy and completed at least one course of induction therapy. Nineteen patients only received one course of therapy, 20 received 2 courses of therapy, 11 received 3 courses of therapy, and 2 received 4 courses of therapy. In the vast majority of courses, study drugs were administered in the outpatient facilities of the study institutions.

Phase I

The initial 15 patients (8 males, 7 females), median age 65.7 (range 50.2-69.8) years, with either primary refractory disease (n=6) or first relapse (n=9; median duration of first CR: 6 months) were enrolled on the dose escalation portion of this study and received a median of 2 (range 1-3) cycles of therapy.

Toxicity

As summarized in Table 2, no DLTs occurred at dose levels 1-3. In contrast, one DLT of death due to sepsis and respiratory failure occurred at dose level 4 after cycle 1, defining this level as maximum tolerated dose (MTD).

Table 1. Characteristics of study cohort.

Parameter	n=52
Median age (range), years	64.8 (50.2-78.9)
Male gender, n. (%)	29 (55.8%)
Disease status, n. (%)	
Primary refractory	29 (55.8%)
Relapse	23 (44.2%)
Median CR duration before relapse (range), months*	3 (1-72)
Performance status, n. (%)	
0	4 (7.7%)
1	39 (75.0%)
2	8 (15.4%)
3	1 (1.9%)
Cytogenetic risk group, n. (%)	
Favorable	2 (3.8%)
Intermediate	32 (61.5%)
Adverse	18 (34.6%)
Laboratory findings at baseline, median (range)	
WBC (x 10^9L)**	2.8 (0.3-26.2)
Hemoglobin (g/dL)	9.7 (2.9-13.0)
Platelets (x 10^9L)	37 (8-486)
Creatinine (mg/dL)	0.9 (0.5-1.8)
Total Bilirubin (mg/dL)	0.5 (0.2-2.2)
SGOT (U/L)	22 (10-66)
SGPT (U/L)	27 (7-80)

**1 patient treated during phase 1 (dose level 2) was erroneously enrolled on this study despite a prior CR duration >12 months; **5 patients received hydroxyurea prior to study therapy initiation.*

Table 2. Dose escalation scheme, best responses, and dose-limiting toxicities during phase I.

Dose level	Azacitidine (i.v./SC, D1-7)	Vorinostat (PO, D1-9)	GO (3 mg/m², i.v.)	Patients (n)	Best responses*	Dose-limiting toxicities
1	75 mg/m²	200 mg	D8	3	RD, CRi/MRD, RD	None
2	75 mg/m²	300 mg	D8	3	RD**, RD, CRi/MRD	None
3	75 mg/m²	400 mg	D8	3	MLFS, CR, CRi/MRD	None
4	75 mg/m²	400 mg	D4 and D8	6	DA, RD, RD, CRi, CR, RD	n=1 (sepsis/respiratory failure)

*CR: complete remission; CRi: CR with incomplete peripheral blood count recovery; CYTO: persistent cytogenetic abnormalities in routine karyotyping; DA: death in aplasia; MLFS: morphological leukemia-free state; MRD: minimal residual disease by flow cytometry or cytogenetics; RD: resistant disease. **This patient achieved a CRi in peripheral blood and bone marrow but had persistent leukemia cutis.*

Efficacy

Among these 15 patients, 2 achieved a CR without flow cytometric evidence of MRD (13.3%; exact 95%CI: 1.7-40.5%); 4 additional patients (26.7%) met morphological criteria for CRi, with 3 of them having either MRD by flow cytometry (n=2) or persistently abnormal findings by routine karyotyping (n=1), for a total response rate (CR/CRi) of 6 of 15 (40.0%; exact 95%CI: 16.3-67.7%). Nine patients (60.0%) either had resistant disease (RD) (n=8, 53.3%) or experienced death in aplasia (DA) (n=1, 6.7%) on Day 28.

Phase II

Based on the parameters used in the Simon minimax two-stage design (*Online Supplementary Appendix*), a total of 43 patients were targeted to receive study therapy at the recommended phase II dose level. Thus, following establishment of a phase II dosing schedule for vorinostat, azacitidine, and GO, an additional 37 patients were enrolled and received treatment at dose level 4.

Efficacy

As summarized in Table 3, 10 of these 43 patients (23.3%, exact 95%CI: 11.8-38.6%) achieved CR, and 8 patients (18.6%; exact 95%CI: 8.4-33.4%) achieved CRi, for a CR/CRi rate of 18 of 43 (41.9%; exact 95%CI: 27.0-57.9%). Of note, 4 of these 18 patients (2 each with CR and CRi, respectively) had persistence of MRD at the time of best response. Eleven of these 18 patients achieved their best response after one cycle of therapy, whereas 6 required 2 cycles, and one patient required 3 cycles of study therapy; thus, the vast majority of patients achieving a positive response did so within the first 2 cycles of therapy. Among the 6 patients requiring 2 treatment courses to achieve CR/CRi, 3 received course 2 early (i.e. after disease assessment on Day 15) because of clear evidence of persistent leukemia after the initial treatment cycle. Of the 24 patients who had response assessment data available and were categorized as resistant, 14 were removed from study therapy after one course of therapy because of early death (n=2), clear evidence of early disease progression (n=4), patient decision (n=1), or physician decision (n=7); 7 patients were removed after 2 courses (including 4 patients who died from persistent disease and one patient who discontinued therapy because of toxicity), 2 after 3 courses (including one patient who died from persistent disease), and one patient after 4 treatment courses (because of patient decision), respectively.

Toxicity assessment

As anticipated, besides grade 3-4 cytopenias, infectious complications were the most common grade 3 or more adverse events (Table 4). A total of 4 patients, all treated at dose level 4, died within 28 days of treatment initiation. In all 4 patients, deaths were related to infectious complications in the setting of persistent AML. One patient treated at dose level 4 died from infectious complications on Day 39 after start of study therapy with persistent pancytopenia; at autopsy, the bone marrow was found to be hypocellular without evidence of residual AML.

Table 3. Overall treatment response.

Parameter	All patients (n=52)	Patients treated at MTD (n=43)
Best response after induction therapy, n. (%)		
CR	11 (21.2%)	10 (23.3%)
Without MRD	9 (17.3%)	8 (18.6%)
With MRD	2 (3.8%)	2 (4.7%)
CRi	11 (21.2%)	8 (18.6%)
Without MRD	6 (11.5%)	6 (14.0%)
With MRD	5 (9.6%)	2 (4.7%)
Total response rate (CR+CRi)	22 (42.3%)	18 (41.9%)
Morphological leukemia-free state	1 (1.9%)	0 (0%)
Resistant disease	28 (53.8%)	24 (55.8%)
Death in aplasia	1 (1.9%)	1 (2.4%)
Early death*	4 (7.7%)	4 (9.3%)
N. of induction cycles to CR/CRi, n. (%)		
1	15 (68.2%)	11 (61.1%)
2	6 (27.3%)	6 (33.3%)
3	1 (4.5%)	1 (5.6%)

**Death within 28 days of initiation of study therapy.*

Post-study therapy of responders

Among the 18 patients who achieved a CR or CRi after treatment at the phase II dose level, 13 were taken off protocol to receive additional, more intensive consolidative chemotherapy, including 12 who were programmed to undergo allogeneic HCT. The remaining 5 patients discontinued study therapy because of patient preference (n=2), unacceptable toxicity (n=1), disease recurrrence (n=1), and death while in remission (n=1).

Disease relapse and overall survival of responders

Of the 18 patients achieving a CR/CRi at the phase II dose level, 5 experienced disease relapse after a CR duration of 1.2, 2.6, 4.0, 4.0, and 4.8 months, respectively; 3 died while in remission after a CR duration of 1.5, 3.2, and 4.3 months (2 of which after allogeneic HCT); and 10 are

in ongoing remission after 2.2, 4.6, 5.1, 9.8, 10.3, 11.1, 12.0, 16.1, 19.6, and 23.3 months, respectively. Among the patients treated at the phase II dose level, median overall survival for the 18 patients achieving CR/CRi was significantly longer than for those 21 patients who failed therapy but lived at least 29 days after treatment initiation (7.4 (range 2.3-26.2) *vs.* 3.1 (1.2-29.6) months; log rank P=0.0023).

Relationship between response and disease status/risk

Interestingly, our data indicate that the likelihood of achieving CR/CRi did not strongly depend on the disease status of the patients, i.e. whether a patient presented with relapsed or primary refractory disease. In fact, among the patients who were treated at dose level 4 and lived for at least 29 days after treatment (i.e. did not experience early death), 12 out of 18 (66.7%) patients achieving CR/CRi had primary refractory disease whereas the remaining patients had relapsed disease; this proportion appears relatively similar to that seen in patients who failed study treatment, where 13 of 21 (61.9%) had primary refractory disease. However, this analysis has to be interpreted with caution, as many patients with primary refractory disease entered this trial after having received only one course of the prior treatment regimen. On the other hand, among the 42 patients treated at the phase II dose level who could be assessed for response (i.e. excluding the patient who died in aplasia), those who achieved CR/CRi appeared to have a more favorable cytogenetic risk profile (favorable risk: n=1; intermediate risk: n=13; adverse risk: n=4) than those who were resistant (intermediate risk: n=12; adverse risk: n=12). Unfortunately, mutation testing of the leukemias was not done in many patients at the time of initial diagnosis or at relapse, thus preventing any meaningful correlative analyses between response and molecular risk profile.

Discussion

During the initial clinical testing of GO as monotherapy, patients have typically received 2 doses of 9 mg/m^2 14 days apart.[2,3] However, recent studies have demonstrated that lower doses of GO (3-6 mg/m^2), at least when combined with conventional chemotherapeutics, have clinical efficacy in some patients with AML.[2,3] Interestingly, studies with radiolabeled anti-CD33 antibodies indicated that saturation of CD33 binding sites is generally achieved with doses around 5 mg/m^2.[18,19] Furthermore, early studies indicated that surface CD33 levels return to pre-treatment levels within 72 h after anti-CD33 antibody administration despite internalization and modulation.[20,21] These two observations suggested the possibility that repeated administrations of lower doses of GO every three days may enhance intracellular delivery of the toxic calicheamicin-γ^1 derivative relative to the biweekly administration schedule. Indeed, the use of fractionated doses of GO (i.e. GO at 3 mg/m^2 given on Days 1, 4 and 7) has been pioneered by the French ALFA group and revealed a favorable efficacy/toxicity profile.[22-24] For example, among 57 patients with AML in first relapse, GO monotherapy given in fractionated doses resulted in 15 (26%) CRs and 4 (7%) CRs with incomplete platelet recovery (CRps). Interestingly, relative to our study, the study by Taksin *et al*. included adults of all ages requiring therapy for AML in first relapse with a CR duration of at least three and up to 18 months,[22] i.e. included patients with a slightly higher expected response rate with conventional salvage chemotherapeutics than our cohort.

In the present study, our intent was to combine vorinostat and azacitidine with GO and utilize this targeted agent in a fractionated administration schedule if tolerat-

Table 4. Tolerability and safety of study therapy.

Parameter	Grade 3-4	Grade 5
Fever, infection		
Device-related infection	1	–
Febrile neutropenia	39	–
Hepatitis, viral	1	–
Infections, other	8	–
Lung infection	2	–
Proctitis	1	–
Sepsis	7	2
Sinusitis	2	–
Upper respiratory tract infection	1	–
Cardiac		
Heart failure	1	–
Left ventricular systolic dysfunction	2	–
Myocardial infarction	1	–
Sinus tachycardia	1	–
Supraventricular tachycardia	2	–
Gastrointestinal		
Abdominal pain	1	–
Diarrhea	3	–
Gastric hemorrhage	1	–
Nausea	3	–
Vomiting	2	–
General		
Death, not otherwise specified	–	1
Fatigue	1	–
Fever	3	–
Infusion-related reactions	1	–
Injection site reaction	2	–
Investigations		
Alanine aminotransferase increase	1	–
Alkaline phosphatase increase	1	–
Aspartate aminotransferase increase	1	–
Weight loss	1	–
Metabolism and nutritional		
Acidosis	1	–
Anorexia	2	–
Dehydration	1	–
Hypokalemia	3	–
Hyponatremia	1	–
Hypophosphatemia	1	–
Respiratory		
Aspiration	3	–
Cough	1	–
Hypoxia	3	–
Pneumonitis	1	–
Pulmonary hypertension	2	–
Respiratory failure	3	–
Vascular		
Hypertension	3	–
Hypotension	7	–
Thromboembolic event	1	–
Other		
Allergic reaction	2	–
Confusion	1	–

Table summarizing grade 3-5 non-hematologic effects considered as definitively, probably, or possibly related to study treatment by the investigator that were experienced by the 43 patients treated at the MTD over a total of 84 treatment cycles.

ed. Our findings indicate that such a combination is indeed clinically feasible, even in older adults with previously treated AML. With a combined CR/CRi rate of approximately 42% among the individuals treated at the MTD, this combination results in an encouraging response rate in a group of patients with an expected CR rate of 15-20% with intensive salvage regimens based on historical data. Importantly, our data indicate that, in a significant subset of patients, more than one cycle of therapy is required to reach the best response and achieve CR or CRi. In our study, only approximately 60% of the responders achieved a CR/CRi after the initial treatment cycle. As several of the study patients categorized as resistant were removed from the study after one or 2 treatment courses based on physician or patient preference but in the absence of overt disease progression, rather than only after 3 courses that the protocol would have allowed, our estimate of the overall response rate may be an underestimation. The results of our triplet combination trial also appear favorable relative to recently reported, preliminary results for the doublet decitabine/non-fractionated GO (5 CR/CRi among 28 treated patients [18%]) in relapsed/refractory AML with a CR duration of less than one year,[25] or the doublet azacitidine/non-fractionated GO (6 CR/CRp among 24 treated patients [25%]) in relapsed AML.[26] Of course, as this was a single-arm clinical study, the relative contribution(s) of individual drugs to the clinical efficacy of vorinostat/azacitidine/GO is unknown, and only a better-controlled trial would ultimately be able to determine whether vorinostat/azacitidine indeed augment GO efficacy, as hypothesized from available *in vitro* data.

It is worthy of note that, among the patients who achieved either a CR or CRi in this trial, a large proportion (12 of the 18 patients [67%] treated at the MTD) were able to undergo allogeneic HCT. Since allografting is often the only curative treatment strategy in relapsed/refractory AML, combined therapy with vorinostat, azacitidine, and GO may thus provide an important bridge-to-transplant option for these patients.

Taken together, our study indicates that GO in combination with vorinostat and azacitidine has encouraging anti-AML activity in older adults with relapsed/refractory AML. These data would support further clinical testing of this combination in patients with newly diagnosed AML.

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, et al. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations), National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2009_pops09/, based on November 2011 SEER data submission, posted to the SEER web site, April 2012.
2. Walter RB, Appelbaum FR, Estey EH, Bernstein ID. Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. Blood. 2012;119(26): 6198-208.
3. Cowan AJ, Laszlo GS, Estey EH, Walter RB. Antibody-based therapy of acute myeloid leukemia with gemtuzumab ozogamicin. Front Biosci (Landmark Ed). 2013;18(4): 1311-34.
4. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006;5(9): 769-84.
5. Bots M, Johnstone RW. Rational combinations using HDAC inhibitors. Clin Cancer Res. 2009;15(12):3970-7.
6. ten Cate B, Samplonius DF, Bijma T, de Leij LF, Helfrich W, Bremer E. The histone deacetylase inhibitor valproic acid potently augments gemtuzumab ozogamicin-induced apoptosis in acute myeloid leukemic cells. Leukemia. 2007;21(2):248-52.
7. Balaian L, Ball ED. Cytotoxic activity of gemtuzumab ozogamicin (Mylotarg) in acute myeloid leukemia correlates with the expression of protein kinase Syk. Leukemia. 2006;20(12):2093-101.
8. Kurimoto M, Matsuoka H, Hanaoka N, Uneda S, Murayama T, Sonoki T, et al. Pretreatment of leukemic cells with low-dose decitabine markedly enhances the cytotoxicity of gemtuzumab ozogamicin. Leukemia. 2013;27(1):233-5.
9. Nand S, Godwin J, Smith S, Barton K, Michaelis L, Alkan S, et al. Hydroxyurea, azacitidine and gemtuzumab ozogamicin therapy in patients with previously untreated non-M3 acute myeloid leukemia and high-risk myelodysplastic syndromes in the elderly: results from a pilot trial. Leuk Lymphoma. 2008;49(11):2141-7.
10. Chowdhury S, Seropian S, Marks PW. Decitabine combined with fractionated gemtuzumab ozogamicin therapy in patients with relapsed or refractory acute myeloid leukemia. Am J Hematol. 2009;84 (9):599-600.
11. Bayraktar UD, Domingo GC, Schmit J, Pereira D. Azacitidine combined with gemtuzumab ozogamicin in patients with relapsed/refractory acute myeloid leukemia. Leuk Lymphoma. 2011;52(5): 913-5.
12. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, Yang H, Rosner G, Verstovsek S, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. Blood. 2006;108(10):3271-9.
13. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-51.
14. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116(3):354-65.
15. Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-9.
16. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453-74.
17. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989;10(1):1-10.
18. Scheinberg DA, Lovett D, Divgi CR, Graham MC, Berman E, Pentlow K, et al. A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. J Clin Oncol. 1991;9(3):

478-90.
19. Appelbaum FR, Matthews DC, Eary JF, Badger CC, Kellogg M, Press OW, et al. The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. Transplantation. 1992;54(5):829-33.
20. Caron PC, Jurcic JG, Scott AM, Finn RD, Divgi CR, Graham MC, et al. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. Blood. 1994;83(7):1760-8.
21. van der Velden VHJ, te Marvelde JG, Hoogeveen PG, Bernstein ID, Houtsmuller AB, Berger AS, et al. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: in vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. Blood. 2001;97(10):3197-204.
22. Taksin AL, Legrand O, Raffoux E, de Revel T, Thomas X, Contentin N, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia. 2007;21(1):66-71.
23. Farhat H, Reman O, Raffoux E, Berthon C, Pautas C, Kammoun L, et al. Fractionated doses of gemtuzumab ozogamicin with escalated doses of daunorubicin and cytarabine as first acute myeloid leukemia salvage in patients aged 50-70-year old: A phase 1/2 study of the acute leukemia French association. Am J Hematol. 2012;87 (1):62-5.
24. Castaigne S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie JN, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379 (9825):1508-16.
25. Daver N, Kantarjian HM, Garcia-Manero G, Estrov Z, Konopleva M, Burger JA, et al. Decitabine and gemtuzumab ozogamicin in acute myelogenous leukemia and high-risk myelodysplastic syndrome. Blood. 2012;120(21):abstract 3619.
26. Ball ED, Medeiros BC, Balaian L, Roque T, Corringham S, Rajwanshi R, et al. Update of a phase I/II trial of 5-azacytidine prior to gemtuzumab ozogamicin (GO) for patients with relapsed acute myeloid leukemia with correlative biomarker studies. Blood. 2010; 116(21):abstract 3286.