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Safety and tolerability of guadecitabine (SGI-110) in patients with myelodysplastic syndrome and acute myeloid leukaemia: a multicentre, randomized, dose-escalation phase 1 study

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Contributors

MA and J-PI conceived and designed the study. J-PI, GR, DR, WS, CO, KY, RY, EG, KW, and HK enrolled and treated patients and gathered data. SN, PT, AO, YH, and WC gathered data. PT, YH, AO, JL, WC, J-PI and MA analysed and interpreted the data. JL, J-PI, HK, and MA wrote the report. All authors reviewed the report and approved the final version.

Declaration of interests

J-PI has served as a consultant for GSK, TEVA, Astex and Janssen, has served as an advisory board member for BI and has received research funding from Astex.

GR has received personal fees from AstraZeneca, Celgene, Boehringer Ingelheim, Roche, Novartis, Astex, Agios, Shire, and GlaxoSmithKline, and has received research funding from Astex.

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KY has served as a consultant for Methylogene; received honoraria from Celgene Canada; and received research grants from Astex Pharmaceuticals, Celator Pharmaceuticals, Roche, Karyopharm, Oncoethix, Genentech, Boehringer Ingelheim, Novartis Pharmaceuticals, and GlaxoSmithKline.

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Summary

Background—Guadecitabine (SGI-110) is a novel hypomethylating dinucleotide of decitabine (DAC) and deoxyguanosine that is resistant to degradation by cytidine deaminase.

Methods—This is a first-in-human pharmacokinetic (PK)- and pharmacodynamic (PD)-guided Phase 1 dose-escalation study in adults with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Patients with MDS or AML refractory to, or relapsed after, standard treatment were randomly assigned to one of two regimens of subcutaneous (SC) guadecitabine: Daily×5 or Once Weekly for three weeks. Stratification was based on disease (MDS vs. AML). Treatment assignment was not blinded. A Twice Weekly for three weeks regimen was later added to the study. All regimens were given in 28-day cycles. The primary objective was the safety profile of all regimens and the recommended dose and schedule for phase 2 by either maximum tolerated dose (MTD) or biologically effective dose (BED). All patients who received at least one treatment were included in the analyses. Enrollment is complete and all patients have finished treatment. This study is registered with ClinicalTrials.gov, number NCT01261312.

Findings—93 patients were treated (74 AML and 19 MDS): 44 on Daily×5 (3–125 mg/m²/d), 34 on Once Weekly (6–125 mg/m²/d), and 15 on Twice Weekly (60 and 90 mg/m²/d). Guadecitabine SC produced a longer exposure window and half-life, and lower C_{max}, of plasma DAC than intravenous DAC. The MTD was 90 mg/m² in MDS on the Daily×5 regimen but was not reached in AML or on the other regimens. The most common Grade 3 adverse events were febrile neutropenia (38/93, 41%), pneumonia (27/93, 29%), thrombocytopenia and anemia (23/93, 25% each), and sepsis (16/93, 17%). The most common serious adverse events (SAEs) were febrile neutropenia (29/93, 31%), pneumonia (26/93, 28%), and sepsis (16/93, 17%). Potent dose-related DNA demethylation occurred on the daily regimen, reaching a plateau at 60 mg/m² Daily×5 (designated as BED). Responses were seen in heavily pretreated patients including six responders (two complete response [CR], two CR with incomplete blood count recovery [CRI], one CR with incomplete platelet recovery [CRp], and one partial response [PR]) in AML patients and two marrow complete response (mCR) in MDS patients. Responders showed significantly more demethylation than non-responders.

Interpretation—Guadecitabine SC at 60 mg/m² Daily×5 is well-tolerated, easily administered, and biologically and clinically active in both MDS and AML; it warrants testing in phase 2 studies.

INTRODUCTION

Epigenetic changes associated with aberrant methylation of DNA in tumors have led to the investigation of hypomethylating agents (HMAs) such as azacitidine (AZA) and decitabine (DAC) in cancer therapy.¹ Both drugs have been approved for use in MDS in the USA and DAC is approved in the EU for elderly AML patients who are not candidates for intensive induction chemotherapy. While responses occur in as many as half of patients, relapse is inevitable, and patients with relapsed or refractory disease have short survival.² The mechanism of action of DAC and AZA requires DNA incorporation, making them S-phase specific drugs. This fact, combined with their very short half-life (less than 30 minutes), may limit their activity in patients, particularly those with less proliferative diseases. Strategies to increase cellular exposure to HMAs by prolonging exposure time may overcome resistance in some cases.

Guadecitabine (SGI-110) is a second generation novel HMA (2'-deoxy-5-azacytidyl- (3'→5')-2'-deoxyguanosine sodium salt)^{3–5} that is relatively resistant to cytidine

deaminase (CDA), the main enzyme responsible for DAC degradation. This report describes the first-in-human dose escalation study of guadecitabine SC using three schedules in patients with relapsed or refractory high-risk MDS or AML. This study was done to determine the safety profile of all regimens and establish the recommended dose and schedule for phase 2 by either maximum tolerated dose (MTD) or biologically effective dose (BED).

METHODS

Study design and participants

Study SGI-110-01 was an open-label, multicenter, dose escalation/expansion study in patients with MDS and AML, conducted at nine North American academic medical centers. The protocol was approved by the institutional review board/independent ethics committee (IRB/IEC) at each study center; all patients provided written informed consent. Eligible patients were at least 18 years of age with a confirmed diagnosis of MDS or AML that was refractory, relapsed, or unresponsive to standard treatment, including HMAs. Patients with acute promyelocytic leukemia were excluded; otherwise no one was excluded based upon histologic, cytogenetic or mutational status. Relapsed/refractory status was determined by the presence of ≥5% abnormal blasts in the bone marrow or blood after primary treatment with standard therapy for leukemia, with (relapsed) or without (refractory) a period of remission. MDS patients (including those with chronic myelomonocytic leukemia [CMML]) had to have an International Prognostic Scoring System (IPSS) score of intermediate-1, intermediate-2, or high risk having previously been treated with therapy. All patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, estimated minimum 3 month survival, and adequate organ functions. Patients with symptomatic CNS disease, other metastatic malignancy, active systemic infection, active graft versus host disease, or other medical conditions suggesting an estimated survival of less than 3 months were excluded.

Randomisation and masking

Patients were randomly assigned by a computer generated algorithm by Astex Data Management Group to one of two 28-day schedules: Daily×5 (Days 1–5) or Once Weekly (Days 1, 8, and 15). No masking was used. A third 28-day schedule, Twice Weekly (Days 1, 4, 8, 11, 15, and 18), was subsequently tested under an amendment. The randomized patients were stratified by disease. The randomization was dynamic in order to realize balance of the stratifying variable across cohorts. Dose escalation was based on a modified 3 + 3 design within each regimen (figure 1).

Procedures

Prior therapy had to have been concluded at least 2 weeks before randomization, except for nitrosoureas (6 weeks) and stem cell transplantation (8 weeks). The starting doses were 3 mg/m²/day SC on the Daily×5 regimen and 6 mg/m² SC on the Once Weekly regimen. The Twice Weekly regimen was added at the end of the study utilizing the two highest safe doses (60 and 90 mg/m²/day). A Safety Review Committee (SRC) reviewed at least the first course for safety, and reviewed pharmacokinetics (PK) data from three patients at each dose to

guide either escalation to the next dose level or the inclusion of up to six additional patients to better assess safety or PK. The SRC also reviewed DNA methylation as a pharmacodynamics (PD) marker for BED determination. Treatment continued as long as the patient benefitted from therapy with acceptable toxicity as determined by the investigator, guided by the dose-limiting toxicity (DLT) criteria. It was recommended that patients receive at least six cycles of therapy unless unacceptable toxicity or rapid progression occurred. Treatment delays and dose-reductions were allowed based on tolerability, per the discretion of the investigator, guided by the DLT criteria.

Dose escalation in the randomized regimens was guided by the historical PK of the commercially approved dose of 20 mg/m² IV infusions of DAC daily for five days. If no DLT was observed in at least three patients at a particular dose, the dose was doubled until 50% of the reported mean C_{max} of DAC IV (74 ng/mL) OR 50% of the mean AUC_{0-∞} of DAC IV (58 ng*h/mL) was reached. Subsequent escalations were increased according to a Fibonacci schema. The co-primary endpoints of the study were MTD and BED. All patients receiving treatment were included in the analyses which were done on an “as treated” basis. Enrollment is complete, all patients have finished treatment, and six are being followed for survival.

Outcomes

Pharmacokinetic assessment—PK of both the parent drug, guadecitabine, and its active metabolite DAC, were secondary endpoints. Plasma samples were collected on Course 1, Day 1, and Day 5 in the Daily×5 schedule; on Course 1, Day 1 and Day 8 of the Once Weekly schedule; and on Course 1, Day 1 of the Twice Weekly schedule. Samples were collected pre-dose, 15 min, 30 min, 60 min, 90 min, 2 hr, 4 hr, 6 hr, 8 hr, and 24 hr postdose. A validated^{6,7} liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method was used for determination of guadecitabine and DAC in K₂EDTA plasma with tetrahydrouridine for stabilization, with linear assay ranges of 1–200 ng/mL and 0.5–100 ng/mL, respectively. PK was assessed for each regimen, with PK parameters derived for each patient using a non-compartmental approach and using descriptive statistics for guadecitabine and DAC by cohort and regimen. Dose proportionality was tested using linear regression between dose and dose-adjusted parameter estimates.

Pharmacodynamic assessment—The co-primary endpoint was BED, as determined by DNA demethylation. Whole blood samples for demethylation assays were collected weekly during the first course. Global DNA methylation was measured by the long interspersed nuclear element (LINE-1) methylation assay as previously reported.⁸ Gene-specific DNA methylation changes were measured using the *INSL6* (Insulin-Like 6) gene, which is nearly completely methylated in normal blood.⁹ We expressed changes in DNA methylation after treatment as relative change (%), computed by the following formula: 100*([Methylation on a given post-treatment day] – [Baseline methylation])/[Baseline methylation]. BED was defined as the lowest guadecitabine dose that achieved a maximum LINE-1 hypomethylation in at least three successive dose levels. The magnitude of LINE-1 demethylation was also compared between patients categorized as responders versus non-responders using the Mann-Whitney test for quantitative data and the Fisher’s exact test for

qualitative data. Primer sequences, methodology and assay conditions for LINE-1 and *INSL6* pyrosequencing are included in supplemental table 1.

Efficacy assessments—Clinical response, hematologic improvement, overall survival, and response duration were secondary endpoints. Clinical response and hematologic improvement for patients with MDS were determined according to the 2006 International Working Group (IWG) recommendations.¹⁰ Clinical response for patients with AML was based on revised recommendations of the 2003 IWG.¹¹ Overall survival was calculated from the first day of treatment to the day of death or last contact if still alive. Response duration was calculated from the day first observed to the day progression was noted. After the initial screening bone marrow aspirate, the frequency of follow-up bone marrow aspirates was left to the investigator. Complete blood counts and differential were performed weekly for blast percent, granulocyte, platelet, and hemoglobin measurements. If a response was noted on blood counts a bone marrow aspirate was to be performed immediately to confirm and at 2 month intervals while responding. Transfusion independence required 56 days without receiving a platelet or erythrocyte transfusion, after having required them during the previous 28 days.

Safety assessment—The MTD was a co-primary endpoint. Patient-reported and investigator-observed (through physical examinations, clinical hematology and laboratory tests, and ECGs) adverse events were collected and categorized according to the Common Terminology Criteria for Adverse Events (CTCAE version 4.0) criteria. DLTs were defined using CTCAE grades and were considered related to guadecitabine if they could not be reasonably explained by underlying disease, intercurrent illness, or concomitant medications. DLTs included any study drug-related Grade 3 or 4 non-hematologic toxicity except Grade 3 or 4 nausea or vomiting that was controllable by anti-emetics or Grade 3 or 4 diarrhea controllable by optimal therapy (Grade 3 laboratory investigations other than serum creatinine, bilirubin, AST, or ALT were not considered a DLT unless they were associated with clinical manifestations); study-drug related Grade 4 thrombocytopenia or neutropenia and febrile neutropenia not present at study entry that did not resolve within seven days and that was not related to underlying disease; prolonged myelosuppression or pancytopenia with a hypocellular bone marrow and no marrow blasts lasting for six or more weeks that was not related to disease progression; and any toxicity that resulted in cycle 2 treatment delay of more than four weeks. MTD was defined as the highest dose for which fewer than 33% of patients experienced a DLT during cycle 1 of guadecitabine administration. This study is registered with ClinicalTrials.gov, number NCT01261312.

Statistical analysis

This is a Phase 1 dose escalation trial and most data are observational. Incidence rates are calculated for clinical response and adverse events. Confidence intervals (95%) based on binomial distributions are provided when appropriate. Analysis specifics regarding PK and PD are discussed in those methods sections.

Role of the funding source

Representatives of the study sponsor (MA, PT, YH, AO, SN, and JL) were involved in the study design, data collection, data analysis, data interpretation, and writing of the report. All had access to the raw data. The corresponding author (J-PI) also had full access to all data in the study including the raw data and had final responsibility for the decision to submit for publication. SU2C provided support for the corresponding author's laboratory studies.

RESULTS

Patient Characteristics and Dose levels

From 04 January 2011 to 11 April 2014, 93 patients (19 with MDS and 74 with AML) participated in this study: 66 were randomized, 32 in the Daily×5 regimen and 34 in the Once Weekly regimen; 12 were treated in a seventh cohort of the Daily×5 regimen and 15 in the Twice Weekly regimen (figure 1). One patient who received no treatment was excluded from analyses. All treated patients were included in the pharmacokinetic analyses; 77 treated patients were included in the pharmacodynamic analyses. The patients not analysed lacked sufficient samples. Median followup of patients was 27 months with an interquartile range of 14 months.

For the two randomized regimens, doses were at least doubled from the 3 mg/m²/d (Daily×5) and 6 mg/m²/d (Once Weekly) doses until the dose of 36 mg/m²/d based on AUC of the active metabolite DAC after guadecitabine SC administration. Doses were subsequently more conservatively escalated up to 125 mg/m²/d for both regimens. The Twice Weekly regimen was evaluated at the end of the study using only two dose levels: 60 and 90 mg/m²/d.

The three groups were well balanced according to baseline demographic criteria, except the initial median bone marrow blast percentage in the Daily×5 group (42%), which was twice the value in the other two groups (19% and 20%; table 1). ECOG status was 1 in 66%; the median age was 70 years (range, 29–86 years). The mean number of prior regimens was 3.3 (median: 3.0), and the mean bone marrow (BM) blast proportion was 38% (median: 24%). In patients with MDS, 13 (68%) had an IPSS score of intermediate-2 or high risk. In patients with AML, 31 (42%) had poor risk cytogenetics and 25 (34%) had secondary AML. Prior treatment with azacitidine or DAC had been administered to all patients with MDS and to 44 (59%) patients with AML. Fifteen AML patients were primary refractory to standard induction therapy to anthracycline and ara-C, and five were primary refractory to HMAs. Five patients were treated in first relapse, four after an initial response to an HMA. The remaining patients were initially responsive to induction and received multiple lines of therapy prior to the study as characterized in table 1.

Pharmacokinetics

There were no differences in PK profiles between the regimens or between days in a particular regimen (table 2). Plasma levels for both guadecitabine and DAC persisted for at least eight hours after SC administration at higher doses and no accumulation was evident for either. Thus, total AUC per cycle would be projected to be the same for a cumulative

total dose regardless of schedule. This also permitted PK analysis to be performed by dose for the entire population, regardless of regimen.

The plasma PK profile of guadecitabine after SC injection showed rapid absorption, with $T_{\max}$ reached within approximately 0.83 to 2.02 hours (figure 2).^{12–14} The active metabolite DAC was detectable shortly after the appearance of guadecitabine in plasma, achieving $T_{\max}$ in approximately two hours (table 3). The lasting presence of guadecitabine in plasma up to eight or more hours resulted in the continuous appearance of DAC delivering an exposure window that also lasted ~11–12 hours (based on extrapolation from guadecitabine doses 36 mg/m²). The mean elimination half-life ($T_{1/2}$) was in the range of 0.59 to 1.44 hours for guadecitabine and 1.23 to 1.79 hours for DAC. The $T_{1/2}$ for DAC after guadecitabine SC administration is considerably longer than the $T_{1/2}$ of 0.25 to 0.6 hour reported for DAC after IV infusion¹⁵ and is likely due to continuous slow appearance of DAC from guadecitabine in plasma for the duration of the guadecitabine exposure window. Evaluation of plasma exposures for guadecitabine and DAC on a molar basis suggested the conversion is very efficient.

Daily AUC exposures of the parent drug guadecitabine appeared to increase in near-dose-proportional fashion from 18 to 125 mg/m². Daily AUC exposures for the active metabolite DAC increased slightly more than dose-proportionally from 3 to 125 mg/m². After guadecitabine 60 and 90 mg/m² doses, DAC AUC exposures approached or exceeded those reported for IV DAC after 20 mg/m² 1-hour infusion (99.3 and 158 ng*hr/mL versus 115 ng*hr/mL,¹⁴ respectively). DAC $C_{\max}$ levels after guadecitabine 60 and 90 mg/m² doses were less than one-third of those of DAC 1-hour IV infusion at 20 mg/m² (27.8 and 43.0 ng/mL versus 147 ng/mL,¹⁴ respectively).

Pharmacodynamic Determination of BED

Guadecitabine effects on DNA methylation, as measured by LINE-1, were evaluated in 77 patients (35 treated in the Daily×5 regimen, 30 in the Once Weekly regimen, and 12 in the Twice Weekly regimen). Pharmacodynamic effects of guadecitabine were dose- and schedule-dependent (figure 3). Peak LINE-1 demethylation compared to baseline reached up to 39%, generally on Day 8 after Daily×5 regimen, and correlated with *INS16* demethylation in a subset of patients with both LINE-1 and *INS16* assessments available ($R=0.90$, $P<.0001$) (supplemental figure 1). A dose-related decrease in LINE-1 methylation on Day 8 was observed in all patients after treatment with the Daily×5 regimen at doses between 18 and 125 mg/m²/d (figure 3a). Maximum average demethylation of ~25% was reached at 60 mg/m²/d with no further decrease in methylation at higher doses. The BED for the Daily×5 regimen was therefore established at 60 mg/m². The mean maximal demethylation in the Once Weekly regimen did not exceed ~8% from baseline (figure 3b). Maximal demethylation after the Twice Weekly regimen was usually delayed (observed on Day 15) and reached approximately ~15–20% after the 60 and 90 mg/m²/d doses (figure 3c). Figure 3d compares three dose schedules that deliver generally equivalent amounts of total drug and demonstrates the strong effect of dose-schedule on demethylation. PK-PD evaluation between PK exposures and extent of LINE-1 demethylation was performed (data not shown) and showed correlation between PK exposures (AUC) and percent change in

LINE-1 demethylation from baseline for doses up to the BED of 60 mg/m² (Daily×5 regimen). At doses higher than 60 mg/m², PK exposures continued to increase while LINE-1 demethylation plateaued.

Safety outcomes

The systemic AE profile of guadecitabine was similar to that previously reported for IV DAC. As the myelosuppression and resultant infections overlap with the complications of AML and MDS, attribution and tolerability decisions were left to the judgment of the investigators, guided by DLT definitions described in the methods. The most common AEs regardless of grade and relationship were febrile neutropenia (39/93, 42%), injection site events (39/93, 42%), diarrhea (36/93, 39%), and pneumonia (33/93, 35%) (supplemental table 2). The most common guadecitabine-related AEs regardless of grade and relationship were injection site events (39/93, 42%), mucosal events (27/93, 29%), thrombocytopenia (15/93, 16%), anemia (12/93, 13%), fatigue (12/93, 13%) and neutropenia (10/93, 11%). Injection site pain reactions were mostly Grade 1 and were managed by slow injection and ice packs. Nausea, vomiting, and diarrhea occurred with a Grade 2 incidence of 6%, 3%, and 4%, respectively, and a Grade 3 incidence of 1%, 2%, and 3%, respectively. These adverse events were easily controlled by first-line agents and were usually not recognized as drug-related. The most common Grade 3 AEs regardless of relationship to guadecitabine were febrile neutropenia (38/93, 41%), pneumonia (27/93, 29%), thrombocytopenia and anemia (23/93, 25% each), and sepsis (16/93, 17%) (supplemental table 3). The most common guadecitabine-related Grade 3 AEs occurring in >1 patient were thrombocytopenia (13/93, 14%), anemia (10/93, 11%), neutropenia (9/93, 10%), leukopenia (7/93, 8%), febrile neutropenia (6/93, 7%) and sepsis (4/93, 4%) (table 4). The median time to pneumonia was 30 days with 85% of pneumonias occurring during the first two cycles of guadecitabine and sporadically thereafter. Six patients experienced a second pneumonia 12–69 days after the first. Serious adverse events (SAEs) were experienced by 69/93 (74%) patients caused by the following events in more than 5%: febrile neutropenia (29/93, 31%), pneumonia (26/93, 28%), sepsis (16/93, 17%) and cellulitis (6/93, 6%). Guadecitabine-related SAEs were observed in 8/93 (9%) patients, six experiencing one or more of febrile neutropenia, pneumonia or sepsis, one with myocardial ischemia and one with dysphagia. The incidence of the AEs was similar across regimens. Two DLTs were observed during cycle one of treatment, both in patients with MDS receiving the 125 mg/m² dose in the Daily×5 regimen. In one DLT, treatment was temporarily suspended for a 73-year-old white woman who had Grade 4 thrombocytopenia and Grade 4 neutropenia; treatment resumed at 60 mg/m². The other DLT was in a 70-year-old white woman who had Grade 4 thrombocytopenia, Grade 4 febrile neutropenia, and *Fusobacterium* sepsis, which led to death. There were no DLTs in patients with AML. Thus, as two of three MDS patients experienced DLT in the 125 mg/m² Daily×5 regimen, the MTD was 90 mg/m². The MTD was not reached in the AML patients with no DLT in nine patients at the Daily×5 125 mg/m² dose. Dosing delays (% per cycle) were unusual (20% Daily×5, 9% Once Weekly, and 19% Twice Weekly). Similarly, doses were seldom reduced (2% Daily×5, 2% Once Weekly, and 19% Twice Weekly).

Clinical activity

Twelve patients showed evidence of clinical activity (table 5). Of the 74 patients with AML, six (four on the Daily×5 regimen and two on the Once Weekly regimen) had clinical responses: two CRs, one CRp, two CRi, and one PR. The patient with a PR as best response was treated for 581 days before responding and had the longest on-study drug period of 787 days. Of the 19 MDS patients treated, six (two on the Daily×5 regimen, three on the Once Weekly regimen, and one on the Twice Weekly regimen) had clinical benefit including two patients with marrow complete response (mCR), and four hematological improvement (HI) responses (three single lineage HI and one bi-lineage HI). Most responders continued treatment until progression. Four patients (two AML and two MDS) initially dependent on platelet transfusions were independent for a median of 161 days (70–542). Five patients (three AML and two MDS) became RBC transfusion independent for a median of 84 days (72–533).

Prior therapy with HMAs had been given to all the MDS patients and two of the AML patients who responded in this study. Responses were seen across most dose levels and at all three treatment schedules. In AML, the lowest dose associated with a response was 36 mg/m²/d. Overall, 60 AML patients were treated at doses 36 mg/m²/d. Of these, six (10%) patients responded and all were characterized by a maximum LINE-1 demethylation of more than 10% relative to baseline, regardless of treatment regimen (table 5). Responders showed significantly greater demethylation at both day 8 and day 15 (figure 4, supplemental figure 2). Response rate was 0/33 in patients with less than 10% peak demethylation compared to 6/26 for those with 10% peak demethylation or greater (P=.0051). In MDS, the lowest dose associated with a response was 6 mg/m²/d. Overall, 18 patients were treated at doses 6 mg/m²/d. Of these, 6/93 (33%) patients responded and four responders showed >10% peak demethylation. Overall, responders showed significantly greater demethylation than the nonresponders (figure 4, supplemental figure 2). Two patients with MDS responded despite having <10% peak demethylation, and these had the shortest response duration of all MDS responders.

DISCUSSION

Research in context

Evidence before this study

Reports of clinical trials and review articles agree upon the limitations of first generation HMAs (azacitidine and decitabine), which have been studied and used in the clinic for more than 20 years. This is particularly true in solid tumors where only sporadic activity is seen and this has been attributed in part to the short half-life of the drugs.

Guadecitabine was designed to extend the in vivo exposure of its active metabolite, decitabine.

Added value of this study

In this first-in-human study, guadecitabine was found to be safe and showed improved PK exposure and PD demethylation over what has been reported in first generation

HMA. Guadecitabine induced clinical responses in heavily pretreated patients including patients with prior exposure to other HMAs. A biologically effective dose that is lower than the MTD is recommended for phase 2 studies.

Implications of all the available evidence

Guadecitabine's enhanced PK and PD may improve clinical outcomes in patients with hematologic malignancies and make it useful in solid tumors.

In this phase 1 study, we show that the BED of guadecitabine in relapsed/refractory MDS and AML is 60 mg/m² SC daily×5 every 28 days, which is lower than the MTD and is recommended for phase 2 studies. Guadecitabine was generally safe and showed improved PK exposure over what has been reported for first generation HMAs. In addition, guadecitabine induced clinical responses in heavily pretreated patients (including patients with prior exposure to other HMAs) and LINE-1 demethylation correlated with clinical responses. First generation HMAs may have been limited by their brief half-lives and necessity for susceptible target cells to be in S-phase, as they require incorporation into DNA to inhibit DNA methyltransferase.^{15,16} Guadecitabine showed a PK profile of its active metabolite, decitabine, distinct from that of DAC IV. While the corresponding mean C_{max} after 60 or 90 mg/m² was less than 1/3 that of 20 mg/m² IV DAC, the effective apparent T_{1/2} of DAC formed from guadecitabine was several times longer than IV DAC, resulting in comparable AUC exposures to IV DAC. Perhaps of greatest interest, the decitabine PK profile showed an exposure window of approximately 11–12 hours (extrapolated), which is more than double that of IV DAC. The reduced C_{max} may result in less peak-related cytotoxicity. We did not directly compare guadecitabine to SC DAC due to the lack of published PK data. With an aqueous preparation, the PK of DAC SC would be expected to follow the IV fairly closely, but with a lower C_{max}.

The use of PK and PD evaluation by cohort allowed assessment of various schedules and doses by their biological effectiveness. LINE-1 DNA demethylation after treatment in the first cycle demonstrated that the Daily×5 regimen was associated with the most potent demethylation. Demethylation reached a plateau at 60 mg/m²/d thus establishing the BED for guadecitabine. The majority of patients treated on this study had prior HMA exposure, and resistance mechanisms may alter PD after guadecitabine treatment. In the ongoing phase 2 studies, it will be important to ascertain whether demethylation (and indeed the BED) is influenced by prior HMA exposure. The most common AEs were injection site events, mostly Grade 1 pain, and myelosuppression and its sequelae. DLTs were not encountered until the 125 mg/m² dose only for the Daily×5 regimen in MDS patients. Thus, as predicted by in-vitro and previous in-vivo studies, the BED is substantially lower than the MTD for this drug.

Major clinical responses were observed in patients with refractory AML when adequate LINE-1 demethylation (>10%) was achieved regardless of regimen, but only at doses approaching the BED or higher (≥ 36 mg/m²/d). The six responses in MDS occurred regardless of dose, regimen, or extent of LINE-1 demethylation in patients who had all previously received HMA. However, the two responses with low levels of hypomethylation induction were relatively short lived. Phase 2 studies are underway in MDS and AML

evaluating dose response for 60 and 90 mg/m²/d using the Daily×5 regimen. The prolonged exposure time of decitabine after guadecitabine SC administration may also prove beneficial in solid tumors which are less proliferative with a lower S-phase index. Studies in solid tumors (ovarian cancer in combination with carboplatin, and hepatocellular carcinoma) are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Dose escalation schema

Note that cohort 7 was enrolled without randomization, as there was no corresponding cohort in the Weekly regimen arm.

Figure 2. Concentration-Time Profile of Guadecitabine (A) and Decitabine (B) after Guadecitabine SC Injection

Mean concentration (ng/mL)-Time Profiles by Dose (regardless of regimen) on Days 1, 5, or 8 of Cycle 1. DAC IV simulation based on 1-h infusion with 20 mg/m² dose

A. Guadecitabine

B. Decitabine

DAC IV simulation was based on data as reported by Blum and colleagues,¹⁰ Oki and colleagues,¹¹ and the Dacogen package insert.¹²

Figure 3. Average LINE-1 demethylation compared to baseline after guadecitabine SC doses
 Average LINE-1 demethylation for Daily×5 regimen (A), Once Weekly×3 regimen (B), Twice Weekly×3 regimen (C) and the three dose schedules with approximately equivalent total doses (D). In D, total doses were 300 mg/m² for Daily×5, 375 mg/m² for Once Weekly and 360 mg/m² for Twice Weekly. Dosing days indicated by ▲.

Figure 4.
Patterns of demethylation of responders vs. non-responders

Table 1

Demographic and baseline characteristics by regimen

Characteristics	Daily×5 (N=44)	Once Weekly (N=34)	Twice Weekly (N=15)	Total (N=93)
Median age, years, n (range)	68 (36–86)	69 (29–83)	72 (51–84)	70 (29–86)
Male sex, n (%)	25 (57)	26 (76)	12 (80)	63 (68)
Race, n (%)				
White	39 (89)	31 (91)	14 (93)	84 (90)
Black or African American	2 (5)	3 (9)	1 (7)	6 (6)
Asian	2 (5)	0	0	2 (2)
Other	1 (2)	0	0	1 (1)
Clinical characteristics, n (%)				
ECOG				
0	10 (23)	7 (21)	4 (27)	21 (23)
1	28 (64)	24 (71)	9 (60)	61 (66)
2	6 (14)	3 (9)	2 (13)	11 (12)
No. of prior regimens				
1	4 (9)	2 (6)	8 (53)	14 (15)
2	15 (34)	14 (41)	1 (7)	30 (32)
3–5	16 (36)	15 (34)	5 (33)	36 (39)
6–9	9 (20)	3 (9)	1 (7)	13 (14)
Mean (SD)	3.7 (2.1)	3.2 (1.5)	2.4 (1.9)	3.3 (1.9)
Median (range)	4.0 (1–9)	3.0 (1–7)	1.0 (1–7)	3.0 (1–9)
Prior decitabine or azacitidine (patients)	27 (61)	23 (68)	13 (87)	63 (68)
Disease				
AML	35 (80)	28 (82)	11 (73)	74 (81)
MDS	9 (20)	6 (18)	4 (27)	19 (19)
BM Blast (%)				
Mean (SD)	45.0 (31.0)	31.9 (28.9)	33.3 (28.8)	38.5 (30.3)
Median (range)	42.0 (2–98)	19.0 (1–95)	20.0 (2–89)	24.0 (1–98)
WBC ($\times 10^9/L$)				
Mean (SD)	10.7 (15.7)	5.1 (5.5)	11.9 (19.7)	8.9 (13.9)
Median (range)	3.0 (0.1–70)	2.5 (0.6–21)	3.2 (0.1–69)	2.8 (0.1–70)

Table 2

PK parameters for guadecitabine, mean (±SD)

Dose (mg/m ²)	N ^a	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-inf} (ng*hr/mL)	T _{1/2} (hr)
3	4 (8)	0.83 (0.36)	11.2 (8.13)	19.3 (9.88)	0.59 (0.17)
6	6 (11)	1.02 (0.54)	13.8 (8.5)	23.1 (13.5)	0.63 (0.26)
9	4 (8)	1.17 (0.9)	26.9 (12.9)	55.1 (13.2)	1.32 (0.93)
18	8 (16)	1.39 (0.64)	45 (26.4)	103 (38.4)	0.84 (0.50)
36	12 (24)	2.02 (1.26)	101 (123)	227 (95.6)	1.24 (0.93)
60	19 (27)	1.64 (0.84)	118 (39.2)	376 (110)	1.31 (0.82)
90	13 (25)	1.37 (0.93)	151 (65.2)	440 (162)	1.04 (0.54)
125	18 (35)	1.91 (1.00)	200 (80.2)	699 (228)	1.44 (0.91)

^aN denotes number of subjects (number of sampling occasions on D1 and D5 or D8). The number of sampling occasions was less for some parameters.

Table 3
PK Parameters for decitabine formed after guadecitabine SC injection, mean (±SD)

Dose (mg/m ²)	N ^a	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-inf} (ng*hr/mL)	T _{1/2} (hr)
3	4 (8)	1.45 (0.8)	1.64 (0.83)	4.62 (2.58)	1.23 (0.37)
6	6 (11)	1.26 (0.33)	3.28 (1.48)	8.91 (3.49)	1.36 (0.55)
9	4 (8)	1.51 (0.48)	3.1 (0.92)	11.2 (4.71)	1.76 (0.65)
18	8 (16)	1.83 (0.93)	10.4 (6.12)	30.7 (14.6)	1.27 (0.83)
36	12 (24)	2.56 (1.56)	17.0 (8.33)	66.3 (20.7)	1.61 (1.04)
60	19 (27)	2.11 (0.91)	27.8 (20.7)	99.3 (50.5)	1.79 (0.89)
90	13 (25)	1.86 (0.79)	43.0 (19.2)	158 (57.9)	1.37 (0.70)
125	18 (35)	2.38 (1.04)	64.2 (24.4)	264 (91.8)	1.69 (1.03)

^aN denotes number of subjects (number of sampling occasions on D1 and D5 or D8). The number of sampling occasions was less for some parameters.

Table 4

Treatment-Emergent Drug-Related Adverse Events of Grade 3 in >1 Patient

Event	Adverse Events with Grade 3 [n (%)] of patients]				
	Daily×5 (N=44)	Once Weekly (N=34)	Twice Weekly (N=15)	Total (N=93)	
Any related event	18 (41)	8 (24)	8 (53)	34	(37)
Hematologic Events:					
Thrombocytopenia	7 (16)	2 (6)	4 (27)	13	(14)
Anaemia	5 (11)	3 (9)	2 (13)	10	(11)
Neutropenia	5 (11)	1 (3)	3 (20)	9	(10)
Leukopenia	3 (7)	3 (9)	1 (7)	7	(8)
Febrile neutropenia	3 (7)	1 (3)	2 (13)	6	(7)
Non-hematologic Events:					
Sepsis	3 (7)	0	1 (7)	4	(4)

Table 5

Responder profiles

Dose (mg/m ²)	Regimen	Cytogenetic Risk (AML) IPSS Risk (MDS)	Baseline		HMA Exposure (Response)	Response Status	Duration of Response (days)	Survival (days)	Max LINE-1 %Demeth- ylation (C1 D8-22)
			WBC K/uL	BM Blast (%)					
AML									
36	Daily×5	Intermediate	9	35	None	CRi	350	581	13
60	Once Weekly	Poor	2-6	8	D (UNK)	CR	558	585	23
60	Daily×5	Intermediate	3-7	16-2	None	CR	213	892	35
60	Daily×5	Poor	2-3	35	D (NR) A (NR)	CRi	47	168	23
90	Daily×5	Intermediate	2-7	17-6	None	PR	196	826	35
125	Once Weekly	Not evaluable	5-7	22	None	CRp	42	251	13
MDS									
6	Once Weekly	High risk	2-1	16	A (PR) D (NR)	HI	100	236	4
18	Daily×5	High risk	2-6	8	A (PR) D (CR) L/D (PR)	mCR	119	441	19
60	Twice Weekly	Not available	21-1	3	A (NR)	HI	106	540	34
90	Once Weekly	High risk	5-3	22-6	A/E (PR)	HI	81	259	4
90	Once Weekly	Intermediate Risk-1	1-3	2	A (PR)	HI	133	873	24
125	Daily×5	Intermediate Risk-2	3-2	17	D (NR) L/A (NR)	mCR	178	666	38

A, azacitidine; BM, bone marrow; CR, complete response; CRi, CR with incomplete blood count recovery; CRp, CR with incomplete platelet recovery; D, decitabine; E, entinostat; HMA, hypomethylating agent; Int, Intermediate; L, lenalidomide; NR, no response; PB, peripheral blood; PR, partial response; Uneval, unevaluable; UNK, unknown; WBC, white blood cell count.