Clinical Cancer Research

Phase Ib Study of Glasdegib, a Hedgehog Pathway Inhibitor, in Combination with Standard Chemotherapy in Patients with AML or High-Risk MDS

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Abstract

Purpose: This open-label, multicenter, dose-finding, phase Ib study (NCT01546038) evaluated the safety, pharmacokinetics, pharmacodynamics, and clinical activity of the novel Hedgehog pathway Smoothened inhibitor glasdegib (PF-04449913) in patients ($N = 52$) with acute myeloid leukemia (AML) or high-risk myelodysplastic syndrome (MDS).

Experimental Design: Glasdegib 100 or 200 mg was administered orally, once daily in 28-day cycles, in combination with low-dose cytarabine (arm A) or decitabine (arm B) to newly diagnosed patients considered not suitable for standard induction chemotherapy, and in combination with cytarabine/daunorubicin (arm C) to fit patients. The study followed a standard 3+3 dose-escalation design. The primary endpoint was dose-limiting toxicity (DLT). Ten additional patients were enrolled in expansion cohorts of arms A ($n = 23$) and C ($n = 22$) to confirm the recommended phase II dose (RP2D).

Results: No DLTs were observed in arms A and B; 1 DLT (grade 4 neuropathy) occurred in arm C. The most common treatment-related nonhematologic adverse events were mostly grades 1 and 2 in all arms. Muscle spasms, dysgeusia, and alopecia were generally mild. Overall, 16 patients (31%) achieved a complete remission (CR)/CR with incomplete blood count recovery. Note that 100 mg daily was selected as the RP2D for glasdegib in combination with standard chemotherapies in the absence of an estimated MTD in this setting.

Conclusions: Treatment with glasdegib in combination with standard chemotherapy was generally well-tolerated and consistent with prior findings, warranting further evaluation of glasdegib-based combinations in patients with AML or high-risk MDS. *Clin Cancer Res; 24(10); 2294–303. ©2018 AACR.*

Introduction

The Hedgehog (Hh) signaling pathway regulates cell differentiation and self-renewal in the developing embryo, and is typically silenced in adult tissues (1). Aberrant Hh signaling in the post-embryonic stage may result from mutations in key pathway genes, nonmutational mechanisms related to the secretion of Hh ligands, or signals from cells in the tumor microenvironment (2). Aberrant Hh signaling has been identified in a variety of human leukemias and specifically in leukemia stem cells (LSC; refs. 2, 3). Upregulation of components of the Hh pathway has been observed in chemoresistant acute myeloid leukemia (AML) cell lines and is thought to be mediated, in part, by B4GALT family members (4, 5). Thus, pharmacologic inhibition of the Hh pathway has been pursued as an antileukemia strategy. Inhibition of the Hh pathway resulted in decreased multidrug resistance and P-glycoprotein expression in AML cell lines (4). In addition, recent studies also suggest that Hh inhibition may sensitize cells to cytarabine (3), 5-azacitidine (6), or radiotherapy (7).

Glasdegib (PF-04449913), which targets the Hh pathway component Smoothened (SMO), is an oral inhibitor of the Hh pathway currently in clinical development for patients with myeloid malignancies (8–13). SMO inhibition by glasdegib was shown to reduce LSC populations in cells from AML patients, downregulate Hh target genes involved in the maintenance of LSCs, and significantly reduce tumor burden (9–12).

In phase I studies, the safety profile of glasdegib was consistent with that observed following administration of other Hh

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Translational Relevance

Aberrant Hedgehog pathway signaling has been identified in human leukemias and specifically in leukemia stem cells. Preclinical studies have shown that Smoothened (SMO) inhibition may sensitize leukemic cells to cytarabine and other agents. These findings provided a rationale for investigating combinations of the SMO inhibitor glasdegib with standard chemotherapies to reduce therapeutic resistance and leukemic persistence or progression. In this phase Ib study, we evaluated glasdegib in combination with low-dose cytarabine, decitabine, or standard (cytarabine/daunorubicin) chemotherapy in 3 noncomparative arms, in patients with acute myeloid leukemia or high-risk myelodysplastic syndrome. In these patient populations, the recommended phase II dose for glasdegib combined with standard chemotherapy is 100 mg daily. Addition of glasdegib to standard chemotherapy demonstrated a generally well-tolerated safety profile and favorable pharmacokinetics, supporting further evaluation in prospective clinical trials of glasdegib-based combinations for the treatment of patients with myeloid malignancies.

inhibitors (13–15). The MTD for single-agent glasdegib was estimated at 400 mg once daily for patients with myeloid malignancies, with 2 dose-limiting toxicities (DLT) observed at the 80-mg (grade 3 hypoxia and pleural effusion in a patient with pneumonia at study entry), and 600-mg (grade 3 peripheral edema) dose levels in 41 DLT-evaluable patients. Based upon safety, tolerability, pharmacodynamic analysis, and preliminary clinical activity, the recommended phase II dose (RP2D) for glasdegib monotherapy was determined to be 200 mg or lower, once daily (13).

Previous studies had demonstrated that SMO inhibition by glasdegib reduced expression of key, intracellular LSC regulators (e.g., GLI2), enhanced cell-cycle transit, and sensitized blast phase LSCs to tyrosine kinase inhibition *in vivo* (11, 16). These preclinical findings, together with the clinical activity demonstrated by single-agent glasdegib in patients with AML or myelodysplastic syndrome (MDS; ref. 13), warranted further evaluation of this novel Hh pathway inhibitor in combination with chemotherapeutic agents to reduce resistance and leukemic persistence or progression in these patient populations. Treatment with the Hh pathway inhibitors erismodegib/sonidegib or vismodegib in combination with hypomethylating agents or other chemotherapies is also being evaluated in early studies in patients with myeloid malignancies (17, 18).

In this trial, we investigated the safety, tolerability, pharmacokinetics (PK), pharmacodynamics, and clinical activity of glasdegib in combination with low-dose cytarabine (LDAC) or decitabine in patients with AML or high-risk MDS considered not suitable for standard induction chemotherapy (ICT) and in combination with cytarabine and daunorubicin in fit patients.

Patients and Methods

Study design

This was an open-label, phase IB, multicenter, dose-finding study of glasdegib in combination with LDAC (arm A) or decitabine (arm B) in patients with newly diagnosed AML or high-risk MDS who were not suitable for standard ICT, or in combination with standard ICT (cytarabine and daunorubicin; arm C) in fit patients. A standard 3+3 dose-escalation design was applied, for each arm separately, in 3- to 6-patient cohorts until identification of the MTD or maximum administered dose for each combination explored (Supplementary Information). Dose escalation continued until DLTs were observed in $\geq$2 patients treated at a dose level, indicating that the MTD had been exceeded for that arm. Additional patients ($n = 10$) were enrolled in expansion cohorts for arms A and C to confirm the RP2D.

The primary objective of the study was to determine the MTD and RP2D of glasdegib for each of the three combinations. The primary endpoint was first-cycle DLT (steady state is achieved with glasdegib after 8 days of therapy; ref. 13). Secondary endpoints included safety, complete response (CR), CR with incomplete blood count recovery (CRi), overall survival (OS), PK parameters, corrected QT (QTc) interval, and pharmacodynamic biomarkers.

The study was approved by the Institutional Review Board of each participating institution and complies with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. It is registered at ClinicalTrials.gov (NCT01546038). Patients provided written informed consent. All authors had access to the primary clinical data.

Patients

Adult patients (18 and older) were included in this study if they had newly diagnosed and previously untreated AML (*de novo* AML, AML evolving from MDS or other prior hematologic malignancy, or AML secondary to previous cytotoxic or radiotherapy) or refractory anemia with excess blast 2 high-risk MDS by the World Health Organization 2008 classification. Patients were allowed 1 prior regimen with a commercially available agent for prior hematologic disease, but no prior therapy for their AML.

Patients had to have adequate hepatic function [aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$3 $\times$ upper limit of normal (ULN), or AST and ALT $\leq$5 $\times$ ULN if liver function abnormalities were due to underlying malignancy] and renal function (serum creatinine $\leq$1.5 $\times$ ULN or estimated creatinine clearance $\geq$60 mL/min), and Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2.

Patients were excluded if they had t(9;22), acute promyelocytic leukemia; hyperleukocytosis (leukocytes $\geq 30 \times 10^9$/L) at screening (hydroxyurea or leukopheresis were allowed before and up to 1 week after first dose of glasdegib for control of rapidly progressing leukemia); known active leukemia in the central nervous system; received prior treatment with an Hh inhibitor or other investigational agents for hematologic malignancies; ongoing or recent cardiac disease [i.e., myocardial infarction, congenital long QT syndrome, torsades de pointes, clinically significant ventricular arrhythmia, or QTc interval > 470 msec using the Fridericia (QTcF) correction formula].

Patients with 1 or more of the following criteria, shown to predict mortality rates during administration of standard ICT (19), were considered not suitable for standard ICT and thus eligible for arm A or B: age $\geq$75 years, ECOG PS 2, serum creatinine >1.3 mg/dL, or severe cardiac disease [i.e., left-ventricular ejection fraction (LVEF) <45% by multigated acquisition or echocardiography at screening]. Patients without these criteria and meeting the remainder of the inclusion/exclusion criteria were considered able to receive standard ICT and eligible for arm C. Treatments for arms A and B were assigned according to the

following rules: if patients had received prior decitabine or azacitidine for their high-risk MDS or antecedent hematologic disease (AHD), they were eligible for arm A only; conversely, if patients had received prior LDAC for their high-risk MDS or AHD, they were eligible for arm B only. Patients who had not received prior decitabine, azacitidine, or cytarabine were eligible for enrollment in either arm A or B. Arm assignment operated by the sponsor followed an alternating allocation scheme.

Study treatment

In all 3 arms, glasdegib 100 or 200 mg was administered orally, once daily in 28-day cycles on a continuous basis for the duration of the study. The daily starting dose for glasdegib was 100 mg to be escalated to 200 mg or deescalated to 50 mg daily in newly enrolled patient cohorts, based on the DLT(s) observed. Intrapatient dose escalation was not permitted. Intrapatient dose reductions were allowed for treatment-related toxicities; following a dose reduction, the treatment dose could not be re-escalated. In arm A, patients received glasdegib from day 3 of cycle 1, in combination with LDAC 20 mg administered subcutaneously twice daily on days 1 to 10 of each 28-day cycle. In arm B, patients received glasdegib from day 2 of cycle 1, in combination with decitabine 20 mg/m^2 by i.v. infusion over 1 hour, for the first 5 days of each 28-day cycle. In arm C, patients received glasdegib from day 3 in combination with i.v. daunorubicin (60 mg/m^2 on days 1–3 of induction) and cytarabine (100 mg/m^2 on days 1–7 of induction), followed by consolidation (2–4 cycles of cytarabine 1 g/m^2 twice daily on days 1, 3, and 5 of each cycle). If required, a second induction cycle could be initiated as early as day 21 of cycle 1 at the same doses. Glasdegib was started at different times in different arms to allow collection of PK samples for the combination drugs administered alone (arms A and B) or to allow multiple dosing of glasdegib prior to collection of PK samples (arm C). Following consolidation, patients received single-agent glasdegib 100 mg administered daily, continuously as maintenance therapy for a maximum of 6 cycles. Treatment was administered for up to 1 year or until disease progression, unacceptable toxicity, or patient withdrawal from the study. Prophylactic use of granulocyte/granulocyte-macrophage colony-stimulating factors was not permitted during cycle 1 in arms A and B and during induction cycle(s) in arm C, but they could be used to manage treatment-emergent, complicated neutropenia according to institutional guidelines.

Assessments

Safety. Safety evaluations included physical examinations, laboratory test results, 12-lead electrocardiograms, and monitoring of adverse events (AE), which were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

The following first-cycle AEs met the definition of DLT if considered by the investigator as possibly related to treatment with glasdegib in combination with chemotherapy: (a) grade ≥ 3 nonhematologic AEs (uncontrolled despite optimal medical management), excluding infection, fever, infusion-related AEs, electrolyte abnormalities, and ALT/AST elevations returning to grade ≤ 1 or baseline within 7 days; (b) prolonged myelosuppression (>42 days; absolute neutrophil count < 500/μL, or platelet count $< 10 \times 10^9$/L) in patients with a normal bone marrow (<5% blasts and no evidence of disease or dysplasia); (c) inability to deliver at least 80% of the planned study doses for all agents in a combination due to nonhematologic AEs; or (d) a treatment delay >28 days due to persistent, nonhematologic AEs. Patients were not evaluable for DLT if they had not received at least 80% of the planned treatment doses for reasons other than treatment-related toxicities.

Clinical activity. Efficacy endpoints were based on investigator assessment, using the modified International Working Group criteria (20, 21). Bone marrow evaluations were performed for patients in arms A and B: at screening, on day 1 of every third cycle (cycles 3, 6, 9, and 12), at initial hematologic recovery in the peripheral blood (absolute neutrophil count >1,000/μL; platelets $\geq$100,000/μL), at investigator's discretion, and at end of treatment (EOT); and for patients in arm C: at screening, induction day 21 (reinduction day 21, if required), at hematologic recovery in peripheral blood, day 21 of the consolidation final cycle, day 1 of maintenance cycles 3 and 6, and EOT.

Pharmacokinetics. Blood samples were collected from patients in each study arm for PK analysis of glasdegib and for LDAC/Ara-U in arm A, decitabine in arm B, and daunorubicin/daunorubicinol and cytarabine in arm C, at protocol-defined time points. The PK parameters, including maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), and area under the plasma concentration versus time curve (AUC), were calculated for each patient using noncompartmental analysis of plasma concentration–time data. The potential for coadministered drug(s) to affect glasdegib plasma PK was determined by examining glasdegib PK parameters in the absence and presence of the coadministered drug(s).

Molecular profiling and pharmacodynamic analyses. Assessments included analysis of genes frequently mutated in patients with AML or MDS (*CEBPA*, *DNMT3A*, *FLT3*, *IDH1*, *IDH2*, *KIT*, *NPM1*, *K-Ras*, *N-Ras*, *RUNX1*, *TET2*, and *WT1* genes), and serum concentration measurements of proteins potentially implicated in Hh pathway signaling and/or AML/MDS pathobiology. DNA samples extracted from frozen peripheral blood or bone marrow aspirates were analyzed by next-generation DNA sequencing using the Illumina MiSeq instrument. In a secondary assay, an amplicon-based approach was used to further characterize the FLT3 gene for the presence of an internal tandem duplication (ITD) mutation. Sera obtained from serial, peripheral blood samples were analyzed for a panel of 38 protein analytes (listed in Supplementary Information) using multiplexed immunoassays at Myriad RBM.

Statistical analysis

Descriptive statistics (count and percentage for categorical variables; mean and standard deviation, and median and range for continuous variables) were used throughout the study. The Kaplan–Meier method was used for the time-to-event analysis. For baseline gene mutation status, the Fisher exact test was used to assess differences in the proportions of gene mutations for response versus nonresponse comparisons by treatment arm. Statistical analyses were performed using SAS version 9.4 (SAS Institute).

Results

Patients

A total of 52 patients were enrolled in 14 U.S. centers between July 2012 and October 2013 and treated in 3 noncomparative

Table 1. Patient baseline characteristics

	Arm A Glasdegib + LDAC	Arm B Glasdegib + Decitabine	Arm C Glasdegib + Cytarabine/Daunorubicin
Patients, *n*	23	7	22
Males, *n* (%)	15 (65.2)	5 (71.4)	12 (54.5)
Females, *n* (%)	8 (34.8)	2 (28.6)	10 (45.5)
Median age, y (range)	76 (60–85)	75 (68–82)	59 (27–70)
Age ≥65 y, *n* (%)	22 (95.7)	7 (100)	7 (31.8)
Race, *n* (%)			
White	18 (78.3)	7 (100)	18 (81.8)
Black	1 (4.3)	0	3 (13.6)
Asian	1 (4.3)	0	0
Other	3 (13.0)	0	1 (4.5)
ECOG PS, *n* (%)			
0	3 (13.0)	2 (28.6)	9 (40.9)
1	12 (52.2)	2 (28.6)	13 (59.1)
2	8 (34.8)	3 (42.9)	0
Primary diagnosis, *n* (%)			
AML	20 (87.0)	5 (71.4)	20 (90.9)
MDS	3 (13.0)	2 (28.6)	2 (9.1)
Disease history, *n* (%)			
De novo	8 (34.8)	6 (85.7)	18 (81.8)
Secondary AML/MDS	15 (65.2)	1 (14.3)	4 (18.2)
Secondary AML/MDS, *n* (%)			
From prior hem. disease	13 (56.5)	1 (14.3)	4 (18.2)
From prior chemotherapy or radiotherapy	2 (8.7)	0	0
Stratification factors,[a] *n* (%)			
Good cytogenetic risk	3 (13.0)	2 (28.6)	6 (27.3)
Intermediate cytogenetic risk	11 (47.8)	3 (42.9)	10 (45.5)
Poor cytogenetic risk	9 (39.1)	2 (28.6)	6 (27.3)
Prior HMA treatment, *n* (%)	12 (52.2)[b]	0	0
Azacitidine	10 (43.5)	0	0
Decitabine	2 (8.7)	0	0

Abbreviations: hem, hematologic; HMA, hypomethylating agent.
[a]For AML, good risk = favorable; intermediate risk = intermediate-I and intermediate-II; and poor risk = adverse risk group.
[b]One of the 3 patients (33.3%) with MDS in arm A had received prior HMA therapy (azacitidine).

study arms: A (glasdegib 100 or 200 mg + LDAC, $n = 23$), B (glasdegib 100 or 200 mg + decitabine, $n = 7$), and C (glasdegib 100 or 200 mg + cytarabine/daunorubicin, $n = 22$; Table 1; Supplementary Table S1).

Median patient age was similar in arms A and B (76 and 75 years, respectively), and expectedly younger (59 years) in arm C. Most patients had a diagnosis of AML: 87%, 71%, and 91% of patients in arms A, B, and C, respectively (Table 1). A total of 65% of patients in arm A, 57% in arm B, and all patients in arm C had baseline ECOG PS score of 0 to 1. Cytogenetic risk group (19) was classified as poor risk in 39%, 29%, and 27% of patients in arms A, B, C, respectively (Tables 1 and 2).

Safety

Treatment with glasdegib was generally well tolerated in all three combinations studied. One of the 26 patients evaluable for DLT across all dose-escalation groups (28 enrolled patients) experienced a DLT (arm C/glasdegib 100 mg) of grade 4 polyneuropathy, which resolved with treatment discontinuation (Table 3). The AEs observed during the study were as expected by treatment arm. The most common treatment-emergent all-causality AEs were constipation (43.5%), diarrhea (43.5%), nausea (43.5%), and febrile neutropenia (39.1%) in arm A; nausea (71.4%), back pain (57.1%), and neutropenia (57.1%) in arm B; and nausea (77.3%), diarrhea (72.7%), constipation (59.1%), febrile neutropenia (54.5%), and muscle spasms (54.5%) in arm C (Table 4).

In arm A, the most common treatment-related AEs were nausea (39.1%), dysgeusia (26.1%), and diarrhea, muscle spasms, neutropenia, and thrombocytopenia (each 21.7%; Supplementary Table S2). The majority of these AEs were grade 1 or 2. The most frequently reported grade 3 to 4 AEs across treatment arms were hematologic AEs (Supplementary Tables S2–S4). Grade 3 fatigue ($n = 3$) was the only treatment-related, nonhematologic grade 3 to 4 AE reported in ≥2 patients in arm A. The most frequent cause of death in arm A, as in arms B and C, was disease progression. Seven (30.4%), 1 (14.3%), and 1 (4.5%) patients, respectively, died ≤28 days following last dose of study treatment; and 14 (60.9%), 5 (71.4%), and 9 (40.9%) patients, respectively, died in follow-up (>28 days after last dose of study treatment). Three patients in arm A died of treatment-related acute respiratory distress syndrome, acute myocardial infarction with congestive heart failure, and multiple organ dysfunction syndrome ($n = 1$ each).

In arm B, the most common treatment-related AEs were neutropenia (57.1%) and alopecia, anemia, nausea, and thrombocytopenia (each 42.9%; Supplementary Table S3). Two patients had treatment-related, nonhematologic grade 3 to 4 AEs, including grade 3 pneumonia and grade 3 dyspepsia ($n = 1$ each).

In arm C, the most common treatment-related AEs were nausea (68.2%), diarrhea (50.0%), muscle spasms (45.5%), febrile

Table 2. Patient stratification (disease risk) by treatment group

N = 52	Arm A Glasdegib + LDAC		Arm B Glasdegib + Decitabine		Arm C Glasdegib + Cytarabine/ Daunorubicin	
Disease risk	**100 mg**	**200 mg**	**100 mg**	**200 mg**	**100 mg**	**200 mg**
Patients, *n*	17	6	4	3	16	6
Cytogenetic risk,[a] *n* (%)						
Good	3 (17.7)	0	1 (25.0)	1 (33.3)	4 (25.0)	2 (33.3)
Intermediate	6 (35.3)	5 (83.3)	2 (50.0)	1 (33.3)	8 (50.0)	2 (33.3)
Poor	8 (47.1)	1 (16.7)	1 (25.0)	1 (33.3)	4 (25.0)	2 (33.3)
Prognostic factors for AML, *n* (%)	15	5	2	3	14	6
Favorable	3 (20.0)	0	1 (50.0)	1 (33.3)	2 (14.3)	2 (33.3)
Intermediate-I	2 (13.3)	1 (20.0)	0	0	5 (35.7)	0
Intermediate-II	3 (20.0)	4 (80.0)	1 (50.0)	1 (33.3)	3 (21.4)	2 (33.3)
Adverse	7 (46.7)	0	0	1 (33.3)	4 (28.6)	2 (33.3)
Prognostic factors for MDS, *n* (%)	2	1	2	0	2	0
Good	0	0	0	0	2 (100)	0
Intermediate	1 (50.0)	0	1 (50.0)	0	0	0
Poor	1 (50.0)	1 (100)	1 (50.0)	0	0	0
MDS IPSS score, *n* (%)	2	1	2	0	2	0
1.5–2	2 (100)	0	1 (50.0)	0	0	0
≥2.5	0	1 (100)	1 (50.0)	0	2 (100)	0

Abbreviation: IPSS, International Prognostic Scoring System.
[a]For AML, good risk = favorable; intermediate risk = intermediate-I and intermediate-II; and poor risk = adverse risk group.

neutropenia (36.4%), and dysgeusia (31.8%; Supplementary Table S4). Treatment-related, nonhematologic grade 3 to 4 AEs reported in ≥2 patients included grade 3 pyrexia ($n = 2$). None of the patients in arms B and C experienced treatment-related grade 5 AEs.

Among treatment-related AEs known to be associated with Hh pathway inhibition, muscle spasms, mostly of grades 1 to 2, were reported in 21.7%, 28.6%, and 45.5% of patients in arms A, B, and C, respectively. Treatment-related alopecia was reported in 8.7%, 42.9%, and 27.3%, and dysgeusia in 26.1%, 28.6%, and 31.8% of patients in arms A, B, and C, respectively (Supplementary Tables S2–S4). In general, alopecia and dysgeusia were observed continuously during treatment and did not resolve (patients were followed for AEs only until 28 days after dose or the start of nonprotocol treatments).

The median duration of treatment with glasdegib was 35 days (2–378 days) in arm A, 77 days (11–567 days) in arm B, and 59.5 days (14–539 days) in arm C. The median number of treatment cycles was 2 (1–14) in arm A, 3 (1–17) in arm B, and 2 (1–18) in arm C. A total of 10 patients in arm C received an allogeneic hematopoietic stem cell transplant (alloHSCT). Five (22.7%) patients in arm C received at least 1 cycle of maintenance therapy (glasdegib 100 mg), whereas 4 of 5 patients received ≥6 cycles of maintenance and 1 patient discontinued at maintenance cycle 2, after nearly 7 months of dosing, due to AEs.

Across study arms, 6 and 30 patients had dose reductions or dose delays/interruptions, respectively (Supplementary Table S5); 15 patients permanently discontinued treatment due to treatment-related AEs (Supplementary Table S6). Reasons for permanent treatment discontinuations reported in >1 patient in each arm included grade 3 to 4 febrile neutropenia in 2 (8.7%) patients in arm A (in 1 patient related to disease under study and in the other to study treatment) and grade 1 to 2, treatment-related vomiting in 2 patients in arm C.

Pharmacokinetics

The plasma PK of glasdegib administered daily at 100 mg was dose proportional and in line with expected plasma exposures (maximum plasma concentration and AUC). The exposures for glasdegib at steady state were also comparable across treatment arms (Supplementary Table S7). At the RP2D, the median time to maximum concentration of glasdegib ranged from 1.3 to 1.8

Table 3. DLTs by treatment group

Patients enrolled[a] *N* = 28	Arm A Glasdegib + LDAC		Arm B[a] Glasdegib + Decitabine		Arm C Glasdegib + Cytarabine/Daunorubicin	
	100 mg	**200 mg**	**100 mg**	**200 mg**	**100 mg**	**200 mg**
Patients, *n*	3	6	4	3	6	6
Evaluable for DLT[b], *n* (%)	3 (100)	5[c] (83.3)	4 (100)	2[c] (66.7)	6 (100)	6 (100)
Reported DLTs, *n* (%)	0	0	0	0	1 (16.6) Grade 4 polyneuropathy[d]	0

[a]A total of 28 patients were enrolled in the dose-escalation cohorts. Arm B was not fully enrolled due to a protocol amendment, which required all patients treated at 200 mg to be dose reduced to 100 mg, and no further patients enrolled in the 200-mg cohort.
[b]Twenty-six patients were evaluable for DLT across all dose-escalation groups.
[c]Per protocol, 2 patients were not evaluable for DLT: 1 patient (arm A) died from disease progression before receiving at least 80% of the planned dose, and 1 patient (arm B) declined to continue study treatment for reason other than an adverse event.
[d]Resolved following treatment discontinuation.

Table 4. Treatment-emergent all-causality adverse events

Adverse Event[a]	Arm A Glasdegib +LDAC $n = 23$		Adverse event[b]	Arm B Glasdegib + Decitabine $n = 7$		Adverse event[c]	Arm C Glasdegib + Cytarabine/ Daunorubicin $n = 22$	
n (%)	Total	G3–5	*n* (%)	Total	G3–5	*n* (%)	Total	G3–5
Any	23 (100)	20 (87.0)	Any	7 (100)	6 (85.7)	Any	22 (100)	19 (86.4)
Constipation	10 (43.5)	0	Nausea	5 (71.4)	0	Nausea	17 (77.3)	0
Diarrhea	10 (43.5)	1 (4.3)	Back pain	4 (57.1)	0	Diarrhea	16 (72.7)	0
Nausea	10 (43.5)	1 (4.3)	Neutropenia	4 (57.1)	4 (57.1)	Constipation	13 (59.1)	0
Febrile neutropenia	9 (39.1)	9 (39.1)	Alopecia	3 (42.9)	0	Febrile neutropenia	12 (54.5)	12 (54.5)
Dysgeusia	8 (34.8)	0	Anemia	3 (42.9)	2 (28.6)	Muscle spasms	12 (54.5)	1 (4.5)
Fatigue	8 (34.8)	5 (21.7)	Asthenia	3 (42.9)	0	Headache	11 (50.0)	0
Neutropenia	7 (30.4)	5 (21.7)	Constipation	3 (42.9)	0	Pyrexia	11 (50.0)	4 (18.2)
Thrombocytopenia	7 (30.4)	7 (30.4)	Decreased appetite	3 (42.9)	0	Vomiting	9 (40.9)	0
Muscle spasms	6 (26.1)	1 (4.3)	Fatigue	3 (42.9)	0	Dysgeusia	8 (36.4)	0
Peripheral edema	6 (26.1)	0	Thrombocytopenia	3 (42.9)	3 (42.9)	Fatigue	8 (36.4)	2 (9.1)
Pyrexia	6 (26.1)	1 (4.3)				Hypokalemia	8 (36.4)	1 (4.5)
						Peripheral edema	8 (36.4)	0
						Hypocalcemia	7 (31.8)	0
						Pain in extremities	7 (31.8)	0

NOTE: G3–G5, grade 3–5.
[a]Reported in >25% of patients.
[b]Reported in >2 patients.
[c]Reported in >30% of patients.

hours in arm A and from 1.03 to 2.00 hours in arm B, following multiple dosing. No evidence of drug–drug interactions was noted between glasdegib and LDAC. Although limited data were available for arm B, exposures for glasdegib appeared similar with and without decitabine. In arm C, the plasma exposure for glasdegib, although lower on induction day 3, most likely due to steady state not having been achieved rather than drug–drug interactions, was as expected by induction day 10 (Supplementary Table S7).

Clinical activity

The proportion of all patients with CR/CRi based on investigator-reported best overall responses is presented in Table 5. Two patients in arm A [8.7%; 80% confidence interval (CI), 2.3%–21.5%], 2 patients in arm B (28.6%; 80% CI, 7.9%–59.6%), and 12 patients in arm C (54.5%; 80% CI, 38.9%–69.5%) achieved a CR/CRi.

Investigator-reported, best overall responses observed in patients with AML are summarized in Supplementary Table S8. In this patient population, a clinically beneficial response [defined as CR, CRi, morphologic leukemia-free state, partial response (PR), or PR with incomplete blood count recovery (PRi)] was observed in 2 (10%) patients in arm A, 3 (60%) in arm B, and 12 (60%) in arm C. In MDS, 2 (66.7%) patients in arm A and all patients in arms B ($n = 2$) and C ($n = 2$) had a clinically beneficial response [CR or marrow CR (mCR)].

The estimated median OS was 4.4 (80% CI, 2.5–6.6) months in arm A [21 (91.3%) deaths], 11.5 (80% CI, 4.5–17.4) months in arm B [6 (85.7%) deaths], and 34.7 (80% CI, 14.5–not reached) months in arm C [10 (45.5%) deaths; Table 5]. Ten patients are still being followed for survival at data cut-off. The median OS was 27.2 months in arm C after censoring at the date of transplant for patients ($n = 10/22$) who received an alloHSCT.

Molecular profiling and pharmacodynamic analyses

Results of the baseline mutational analyses performed on bone marrow and/or peripheral blood samples obtained from individual patients ($n = 52$) are listed in Supplementary Table S9.

Table 5. Complete responses as best overall response and OS

AML + MDS patients	Arm A Glasdegib + LDAC	Arm B Glasdegib + Decitabine	Arm C Glasdegib + Cytarabine/Daunorubicin
All patients, *n*	23	7	22
CR/CRi,[a] *n* (%), [80% Exact CI[b]]	2 (8.7) [2.3–21.5]	2 (28.6) [7.9–59.6]	12 (54.5) [38.9–69.5]
Median OS, months (80% CI)[c]	4.4 (2.5–6.6)	11.5 (4.5–17.4)	34.7 (14.5-NR)
AML, *n*	20	5	20
CR, *n* (%)	1 (5.0)	1 (20.0)	10 (50.0)
CRi, *n* (%)	0	1 (20.0)	1 (5.0)
MDS, *n*	3	2	2
CR, *n* (%)	1 (33.0)	0	1 (50.0)
CRi, *n* (%)	0	0	0

Abbreviation: NR, not reached.
[a]Investigator-reported, confirmed responses.
[b]Using exact method based on binomial distribution.
[c]Based on the Brookmeyer and Crowley method.

Table 6. Baseline gene mutations in responders and nonresponders by treatment arm[a]

		Arm A Glasdegib + LDAC $n = 9/23$[c] n (%)		Arm B Glasdegib + Decitabine $n = 1/7$ n (%)		Arm C Glasdegib + Cytarabine/Daunorubicin $n = 12/22$ n (%)	
Gene[b]	Mutation status	Responder	Nonresponder	Responder	Nonresponder	Responder	Nonresponder
CEBPA	Mutated	0	3 (33.3)	0	0	2 (16.7)	0
	Nonmutated	0	6 (66.7)	0	1 (100)	9 (75.0)	1 (8.3)
DNMT3A	Mutated	0	2 (22.2)	0	0	0	0
	Nonmutated	0	7 (77.8)	0	1 (100)	11 (91.7)	1 (8.3)
FLT3	Mutated	0	1 (11.1)	0	0	2 (16.7)	0
	Nonmutated	0	8 (88.9)	0	1 (100)	9 (75.0)	1 (8.3)
FLT3-ITD	Mutated	0	0	0	0	1 (8.3)	0
	Nonmutated	0	9 (100)	0	1 (100)	10 (83.3)	1 (8.3)
IDH1	Mutated	0	1 (11.1)	0	0	0	0
	Nonmutated	0	8 (88.9)	0	1 (100)	11 (91.7)	1 (8.3)
IDH2	Mutated	0	0	0	0	2 (16.7)	0
	Nonmutated	0	9 (100)	0	1 (100)	9 (75.0)	1 (8.3)
KIT	Mutated	0	0	0	1 (100)	0	0
	Nonmutated	0	9 (100)	0	0	11 (91.7)	1 (8.3)
KRAS	Mutated	0	1 (11.1)	0	0	0	0
	Nonmutated	0	8 (88.9)	0	1 (100.0)	11 (91.7)	1 (8.3)
NPM1	Mutated	0	0	0	0	4 (33.3)	0
	Nonmutated	0	9 (100)	0	1 (100)	7 (58.3)	1 (8.3)
NRAS	Mutated	0	5 (55.6)	0	0	1 (8.3)	0
	Nonmutated	0	4 (44.4)	0	1 (100)	10 (83.3)	1 (8.3)
RUNX1	Mutated	0	1 (11.1)	0	0	1 (8.3)	0
	Nonmutated	0	8 (88.9)	0	1 (100)	10 (83.3)	1 (8.3)
TET2	Mutated	0	3 (33.3)	0	0	1 (8.3)	0
	Nonmutated	0	6 (66.7)	0	1 (100)	10 (83.3)	1 (8.3)
WT1	Mutated	0	0	0	0	0	0
	Nonmutated	0	9 (100)	0	1 (100)	11 (91.7)	1 (8.3)

Abbreviations: MLFS, morphologic leukemia-free state; SD, stable disease.

[a]AML responders: patients with investigator-reported best overall response of CR, CRi, MLFS, PR, PRi. MDS responders: patients with investigator-reported best overall response of CR, mCR, PR, and SD.

[b]Twelve genes analyzed centrally as described in the Patients and Methods section with results reported in the clinical database: *CEBPA*, CCAAT-enhancer binding protein α; *DNMT3A*, DNA (cytosine-5-)-methyltransferase 3 α; *FLT3*, Fms-related tyrosine kinase 3; *FLT3-ITD, FLT3*-internal tandem duplication mutation; *IDH1*, isocitrate dehydrogenase (NADP+) 1; *IDH2*, isocitrate dehydrogenase (NADP+) 2; *KIT*, tyrosine-protein kinase Kit/CD117; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *NMP1*, nucleophosmin or nucleolar phosphoprotein B23; *NRAS*, neuroblastoma RAS viral oncogene homolog; *RUNX1*, runt-related transcription factor 1; *TET2*, Tet methylcytosine dioxygenase 2; *WT1*, Wilms tumor 1. Statistical significance in comparison of responders with nonresponders was determined using the Fisher exact test. $P = 1.000$ for all evaluable comparisons.

[c]n = evaluable/enrolled patients.

Mutations were detected in 11 of the 12 genes analyzed. Clinically beneficial responses (defined as investigator-reported, best overall response of CR, CRi, morphologic leukemia-free state, PR, or PR with incomplete blood in AML, and of CR, mCR, PR, or stable disease in MDS) were observed in patients in arm C with diverse mutational profiles, including mutations in the *CEPBA* ($n = 2$), *FLT3* ($n = 2$), *IDH2* ($n = 2$), *NPM1* ($n = 4$), *NRAS* ($n = 1$), *RUNX1* ($n = 1$), and *TET2* genes ($n = 1$; Table 6).

All 52 treated patients were evaluable for serum biomarker analysis. These analyses focused on cytokines, as previous studies had demonstrated that aberrant cytokine expression is nearly universal in AML/MDS and that some cytokines exhibit potential prognostic and/or predictive value or are implicated in AML/MDS pathobiology (including IL6, IL8, IL10, VEGF, and TNFα; refs. 22–27). Moreover, Hh pathway signaling has been shown to modulate the expression of proinflammatory molecules such as TNFα, IL1β, and IL6 (28). Changes in serum cytokines over time were assessed by treatment arm. In general, minimal or inconsistent changes were evident in circulating cytokines following treatment. However, a number of cytokines exhibited statistically significant (2-sided $P < 0.05$ based on Wilcoxon signed rank test) and pronounced modulation from baseline with treatment in arm C. These included IL8 [median 2.5-fold higher at induction day 10 (d10)], brain-derived neurotrophic factor (BDNF, median 1.2 ng/mL at baseline, with decrease to 0.2 ng/mL at d10), IL5 (median 0.0 pg/mL at baseline, with increase to 99 pg/mL at d10), VEGF (median 50% decrease at d10), monocyte chemoattractant protein-1 (MCP-1, median 3.5-fold higher at d10), matrix metalloproteinase (MMP)-3 (median 2.5-fold higher at induction day 3), and interferon-inducible T-cell α chemoattractant (ITAC, mean 163.7 pg/mL at baseline, with decrease to 13.8 pg/mL at d10; median = 0.0 ng/mL at both time-points).

Modest, but statistically significant, associations with response were detected in arm C for stromal cell–derived factor 1 (SDF-1) and MMP-3. Serum concentrations of SDF-1 were lower in responders versus nonresponders at baseline (median 2,275 vs. 3,275 pg/mL, $P = 0.0265$) and on induction day 3 after dose (median 2,510 vs. 3,260 pg/mL, $P = 0.0464$). MMP-3 serum concentrations were lower in responders versus nonresponders at induction lead-in after dose (median 8.9 vs. 10.5 ng/mL, $P = 0.0185$; Supplementary Fig. S1). In addition, there was a nonsignificant trend in arm C, suggesting that baseline IL6 concentrations were higher in responders than in nonresponders (median 6.90 and 0.00 pg/mL, respectively, $P = 0.0717$). Similar trends

were observed in arms A and B for baseline IL6 and SDF-1, although statistical significance was not assessed within these arms ($n < 5$ for responders and/or nonresponders). In arm A, median IL6 baseline concentrations were 3.20 (0.0–11.0) and 0.00 (0–232) pg/mL in responders and nonresponders, respectively. In arm B, median SDF-1 baseline concentrations were 1,720 (1,440–3,190) and 4,045 (2,860–5,230) pg/mL in responders and nonresponders, respectively.

Discussion

In this phase IB study, the orally available SMO inhibitor glasdegib combined with LDAC, decitabine, or standard (cytarabine/daunorubicin) ICT in three noncomparative arms appears safe in the treatment of patients with newly diagnosed AML or high-risk MDS.

Treatment with glasdegib in combination with standard chemotherapies was generally well tolerated. The AEs reported in fit patients and in patients not suitable for standard ICT were manageable and as expected for these populations during chemotherapy and Hh pathway inhibitor therapy (29–33). No DLTs were observed in arms A and B and only 1 DLT (grade 4 neuropathy) occurred in arm C, which resolved following treatment discontinuation. The MTD was not reached in arm A, B, or C. The most common, treatment-related, nonhematologic AEs were mostly grades 1 and 2 in all arms. As expected, the most frequently reported grade 3 to 4 treatment-related AEs in arms A and B were neutropenia and thrombocytopenia; and in arm C, febrile neutropenia, anemia, and thrombocytopenia. All these AEs were consistent with disease-related complications of AML and MDS, and standard-of-care therapies for these diseases. Treatment-related muscle spasms, dysgeusia, and alopecia were observed in this and prior studies of glasdegib, and with other inhibitors of the Hh pathway, suggesting that these AEs are drug class-related and consistent with the mechanism of action of Hh pathway inhibition (13, 17, 29–33). Consistent with our findings, the majority of these AEs were reported as grade 1 or 2 in severity. Muscle spasms, considered the most frequent related AE, were observed in up to 49% to 76% of patients, usually occurring in the early stages of treatment. Conversely, it was noted that treatment-related alopecia may develop gradually over time and persist in a proportion of patients, particularly those with basal cell nevus syndrome (31). Comorbidities and combination or concomitant treatments may also influence the development of alopecia.

In the absence of an estimated MTD, glasdegib 100 mg daily was selected as the RP2D in combination with standard chemotherapy regimens, based on the observed tolerability profile for the combination with LDAC or intensive chemotherapy, evidence of modulation of Hh signaling pathway activity, favorable PK characteristics, and to account for the increase in glasdegib exposures observed on coadministration with strong cytochrome P3A4 inhibitors, such as azoles (34, 35).

In this dose-finding, phase 1b trial designed to assess safety, PK, and pharmacodynamics of glasdegib in combination with standard therapy for AML, the response rates do not appear significantly different than expected with standard treatments alone. A more formal assessment of the contribution of glasdegib to the clinical activity of standard chemotherapy regimens requires randomized studies which are ongoing. In this intent-to-treat analysis, 7 of 23 patients in arm A and 1 of 7 patients in arm B were registered as treatment failures despite failure to complete first on-study bone marrow response evaluation due to AE, death, or withdrawal, and this may have detracted from outcomes.

Glasdegib inhibits the Hh signaling pathway and may act as anti-LSC therapy. In this vein, it is hoped that the anticipated clinical benefit would be preventing disease relapse and improving OS, whereas improvement in response rate via tumor debulking may remain modest. Consistent with this hypothesis, the mOS of previously untreated (for AML) patients in arm C who received glasdegib plus ICT was 34.7 months, which compares favorably with similar, historical controls (36). In arm A, the mOS of 4.4 months with glasdegib plus LDAC was modest, yet included over 50% of patients with previous HMA failure for AHD and approximately 30% of patients who died prior to approximately 1 month follow-up after conclusion of therapy. This poor-risk population may have influenced mOS, and potential survival benefit would need to be confirmed in prospective randomized trials. Following initial dose escalation in the decitabine arm, the protocol was amended to concentrate on LDAC as the more globally accepted backbone therapy for patients with high-risk MDS or AML not suitable for standard ICT. The combination of glasdegib with the hypomethylating agent azacitidine is currently being further studied.

Analysis of gene mutation profiles in responders versus nonresponders in this small study did not indicate that response could be predicted by any particular baseline mutation profile, suggesting that clinical benefit from treatment with glasdegib in combination with standard chemotherapy is not restricted to specific disease clones. Early trends indicative of lower SDF-1 and MMP3 levels and higher IL6 concentrations were detected at baseline in responders versus nonresponders. The observed association between elevated serum levels of SDF-1 and lack of response to treatment is interesting, because SDF-1 and its cognate receptor CXC chemokine receptor 4 (CXCR-4) have been implicated in the pathobiology of AML and in resistance of AML cells to chemotherapy and kinase inhibitors (37–39). Furthermore, in pancreatic cancer cells, SDF-1/CXCR-4 signaling has been shown to directly regulate expression of sonic Hh, whereas in medulloblastoma, sonic Hh may modulate CXCR4 signaling by altering its subcellular localization (40, 41). Hence, the association between elevated SDF-1 and lack of response in this study could potentially reflect cross-talk between SDF-1/CXCR4 and Hh pathway signaling.

In general, minimal or inconsistent changes were evident in circulating cytokines with treatment. Although not compared with a standard ICT arm, a number of cytokines showed statistically significant and pronounced modulation from baseline with treatment in arm C. These included IL5 and ITAC. IL5 mediates eosinophil differentiation, maturation, activation, and survival, and the basis for its induction in this study has not yet been elucidated (42). ITAC is a ligand of the seven-transmembrane span receptor CXCR7 and the basis for its suppression in this study has not been elucidated, but it may reflect immunomodulatory effects of chemotherapy (43). Interestingly, SDF-1 is also a ligand of CXCR7. These preliminary results coupled with the potential correlation of SDF-1 baseline concentrations, and response suggests the potential association of CXCR7-SDF-1/ITAC and/or CXCR4-SDF-1 signaling with response to treatment.

Although most agents are focused on improving response rates and tumor control or eradication, both AML and MDS are heterogeneous diseases with multiple molecular aberrations, making targeted therapy difficult. The impact of Hh inhibition

on LSC growth and maintenance may represent a novel mechanism of action complementary to existing therapeutic strategies for these diseases, particularly given the recently demonstrated association of high scores for a 17-gene LSC expression signature and poor outcomes in AML (44). The addition of glasdegib to LDAC, decitabine, or standard ICT in the treatment of patients with AML or high-risk MDS shows a consistent safety and PK profile, supporting further evaluation in these patient populations.

Disclosure of Potential Conflicts of Interest

M.R. Savona reports receiving commercial research grants from Astex, Incyte, Sunesis, and Takeda, holds ownership interest (including patents) in Karyopharm, and is a consultant/advisory board member for Astex and Celgene. D.A. Pollyea reports receiving commercial research grants from Agios and Pfizer, and is a consultant/advisory board member for AbbVie, Agios, Argenx, Celgene, Celyad, Curis, Pfizer, and Servier. J. Lancet holds ownership interest (including patents) in Astellas, BioSight, Celgene, Janssen R&D, and Jazz Pharmaceuticals. M.N. Shaik and A.D. Laird hold ownership interest (including patents) in Pfizer. J.E. Cortes reports receiving commercial research grants from Astellas, Celgene, Daiichi, Jazz, Novartis, and Pfizer, and is a consultant/advisory board member for Astellas, Daiichi, Jazz, Novartis, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

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