A Phase I/II Open-Label Study of Molibresib for the Treatment of Relapsed/Refractory Hematologic Malignancies

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ABSTRACT

Purpose: Molibresib is a selective, small molecule inhibitor of the bromodomain and extra-terminal (BET) protein family. This was an open-label, two-part, Phase I/II study investigating molibresib monotherapy for the treatment of hematological malignancies (NCT01943851).

Patients and Methods: Part 1 (dose escalation) determined the recommended Phase 2 dose (RP2D) of molibresib in patients with acute myeloid leukemia (AML), Non–Hodgkin lymphoma (NHL), or multiple myeloma. Part 2 (dose expansion) investigated the safety and efficacy of molibresib at the RP2D in patients with relapsed/refractory myelodysplastic syndrome (MDS; as well as AML evolved from antecedent MDS) or cutaneous T-cell lymphoma (CTCL). The primary endpoint in Part 1 was safety and the primary endpoint in Part 2 was objective response rate (ORR).

Results: There were 111 patients enrolled (87 in Part 1, 24 in Part 2). Molibresib RP2Ds of 75 mg daily (for MDS) and 60 mg daily (for CTCL) were selected. Most common Grade 3+ adverse events included thrombocytopenia (37%), anemia (15%), and febrile neutropenia (15%). Six patients achieved complete responses [3 in Part 1 (2 AML, 1 NHL), 3 in Part 2 (MDS)], and 7 patients achieved partial responses [6 in Part 1 (4 AML, 2 NHL), 1 in Part 2 (MDS)]. The ORRs for Part 1, Part 2, and the total study population were 10% [95% confidence interval (CI), 4.8–18.7], 25% (95% CI, 7.3–52.4), and 13% (95% CI, 6.9–20.6), respectively.

Conclusions: While antitumor activity was observed with molibresib, use was limited by gastrointestinal and thrombocytopenia toxicities. Investigations of molibresib as part of combination regimens may be warranted.

Introduction

Bromodomains (BRD) are conserved modules located in chromatin-associated proteins, which act as epigenetic readers regulating chromatin-templated processes by the recognition of acetylated histones (1, 2). In particular, proteins containing BRDs regulate the transcription of many genes controlling growth, cell cycle progression, and differentiation (2–7). While many proteins contain BRDs (3), the BRD and extra-terminal (BET) family of proteins (BRD2, BRD3, BRD4, and BRDT; testes) have gathered interest as potential therapeutic targets for various cancers (3, 8). BET proteins contribute to both oncogenesis and treatment resistance in multiple solid and hematologic malignancies (9–11). BET BRD inhibition modulates c-Myc transcriptional function and transcriptionally represses the *MYC* proto-oncogene (12–14). *MYC* has been shown to cooperate with many oncogenic events to initiate tumorigenesis, and *MYC* overexpression and/or activation has been identified in more than half of human cancers (15). As such, small molecule BET inhibitors that use competitive binding to displace BET proteins from chromatin and counteract

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Translational Relevance

Molibresib is a selective, small molecule inhibitor of the bromodomain and extra-terminal (BET) protein family. The results of this open-label, two-part, Phase I/II study in 111 patients with hematological malignancies demonstrated that treatment with molibresib was tolerable, though its use was limited by gastrointestinal and thrombocytopenia toxicities. The most common adverse events were diarrhea ($n = 55$; 50%), nausea ($n = 51$; 46%), and thrombocytopenia ($n = 44$; 40%). Across the whole study, 6 patients achieved a complete response, and 7 patients achieved a partial response (objective response rate, 13%; 95% confidence interval, 6.9–20.6). This modest antitumor activity with molibresib monotherapy is consistent with emerging evidence that some (but not all) epigenetic therapies may need to be used as part of combination therapy to achieve maximal clinical benefit in relapsed/refractory myeloid disease and leukemia. As such, investigations into combinatorial approaches that use BET inhibition and other targeted therapies may be warranted.

their oncogenic effects were developed as potential anticancer agents (16).

Molibresib (GSK525762) is an orally bioavailable, selective, small molecule pan-BET family inhibitor (17) that has been evaluated in various solid tumor and hematological malignancies (18). A two-part, first-time-in-human (FTIH) study investigating the safety, efficacy, pharmacokinetics (PK), and pharmacodynamics (PD) of molibresib for the treatment of various solid tumor types has been conducted (115521; NCT01587703), in which limited efficacy and frequent gastrointestinal adverse events (AE) and thrombocytopenia were reported (18, 19). Data from this and other studies show that molibresib is rapidly absorbed and eliminated via metabolism by cytochrome P450 (CYP) 3A4 enzymes (20). It has two, active, equipotent major metabolites and induces its own metabolism, leading to reduced molibresib, but increased active metabolite exposure over time at doses of 60 mg and higher (18). Following on from the FTIH study, a population PK model was developed that included a semi-mechanistic liver compartment and that adequately described the PK of both molibresib and its two major metabolites (21).

Molibresib has consistently exhibited broad, antiproliferative activity *in vitro* in cancer cell lines and induces cytotoxicity in many hematological cancer models, including those derived from acute myeloid leukemia (AML; refs. 22–24), multiple myeloma (MM; refs. 12, 23, 25), and Non–Hodgkin lymphoma (NHL; ref. 23). It has also been shown to inhibit tumor growth and significantly enhance survival in MM and AML mouse models (22, 24, 25). Here, we report the results of a two-part Phase I/II study investigating the use of molibresib monotherapy for the treatment of hematological malignancies.

Patients and Methods

Study design

This was an open-label, two-part, Phase I/II study (116183; NCT01943851) conducted in 15 centers across Australia, South Korea, Spain, the UK, and the US. Part 1 was a dose escalation phase to determine the recommended Phase 2 dose (RP2D) or the maximum tolerated dose (MTD) of molibresib in patients with relapsed/refractory AML, MM, or NHL, as well as to assess the clinical efficacy, PK, and PD of molibresib. Based on interim data from Part 1 of the study, Part 2 was a dose expansion phase to investigate the safety, efficacy, and PK of molibresib at the RP2D or MTD in patients with relapsed/refractory myelodysplastic syndrome (MDS), as well as AML that has evolved from an antecedent MDS and cutaneous T-cell lymphoma (CTCL; Supplementary Fig. S1). Patients were enrolled with AML that was evolved from an antecedent MDS to maximize time on-study, because it reflects a more slowly progressing disease that maintains many of the clinical characteristics of AML.

The study was conducted in accordance with good clinical practice and the Declaration of Helsinki. Study approval was provided by each participating institution's Ethical Review Board and all patients provided written, informed consent before study participation.

Patients

Full inclusion and exclusion criteria are presented in Supplementary Methods S1. In brief, eligible patients were ≥18 years of age and had a diagnosis of one of the following hematologic malignancies (which had relapsed or were refractory to treatment): MM, AML, NHL (Part 1), MDS (as well as AML that has evolved from antecedent MDS), or CTCL (Part 2). Additional key inclusion criteria were an Eastern Cooperative Oncology Group performance score of ≤1 for Part 1 and ≤2 for Part 2, no clinically significant gastrointestinal abnormalities that may alter absorption, and adequate organ system function. Patients were excluded from the study if they had any protocol-defined concomitant conditions, including hematological malignancies associated with HIV or hepatitis B/C, solid tumor malignancies (both concurrent and historical, unless they had been disease-free for over 5 years), severe or uncontrolled infection, severe or uncontrolled systemic disease (e.g., respiratory, hepatic, renal, or cardiac disease/abnormalities), and symptomatic or untreated central nervous system disease. Patients were also excluded if they had experienced gastrointestinal bleeding within the past 3 months or hemoptysis within the past 7 days, were currently receiving another cancer therapy (exceptions are listed in Supplementary Methods S1), or had received anticoagulant therapy within the past 7 days.

Treatment

The starting dose of molibresib in Part 1 was 5 mg daily (QD), administered orally. An accelerated dose escalation design was then followed, including one patient per dose level (any tumor type; ≤2-fold increase in dose each time) until the first instance of Grade ≥2 drug-related, non-hematological toxicity in any single cohort. After that, a standard 3+3 design (3-week assessment period) was followed for separate tumor cohorts (AML, NHL, and MM) until the MTD was reached and/or the RP2D was established for each tumor type (up to a maximum dose of 200 mg; Supplementary Fig. S1). The decision to escalate was based on all available data, including emerging PK and safety data, investigator assessment, and recommended doses predicted using the Neuenschwander continual reassessment method (26). Dose adjustments were allowed to address tolerability and safety issues, and alternate dosing schedules (e.g., intermittent dosing) were permitted to manage toxicities.

Patients entering Part 2 received oral molibresib at the RP2D until disease progression, withdrawal of consent, unacceptable AEs, or death.

Endpoints and assessments

Part 1

The primary safety endpoints in Part 1 were the incidence of AEs, serious AEs (SAE), dose-limiting toxicities (DLT), dose reductions or delays, withdrawals due to toxicities, and changes in safety assessments

(e.g., laboratory parameters, vital signs, and cardiac parameters). DLT criteria are outlined in Supplementary Methods S2.

Secondary endpoints included: (i) investigator-assessed overall response rate (ORR), defined as the percentage of patients who achieved a complete response (CR) or partial response (PR) using standard disease-specific response criteria (27, 28); and (ii) standard PK parameters for molibresib, its two active metabolites [measured together and termed the "active metabolite composite" (GSK3529246)], and total active moiety (TAM), including maximum plasma concentration (C_{max}), elimination half-life ($t_{1/2}$), and area under the concentration time curve from 0 to 24 hours (AUC_{0-24}).

Exploratory endpoints included PD parameters, as assessed by changes in the expression of genes regulated by BET inhibition.

Part 2

The primary endpoint in Part 2 was investigator-assessed ORR. For the MDS cohort (as well as AML evolved from antecedent MDS), this was defined as the percentage of patients achieving CR, marrow CR, CR with a platelet count $<100\times10^9$/L (CRp), CR with a platelet count $<100\times10^9$/L or neutrophil count $<1\times10^9$/L (CRi), or PR defined by the International Working Group criteria for MDS and AML, as appropriate (27, 28). For the CTCL cohort, this was defined as the percentage of patients who achieved a CR or PR lasting more than 4 months, as per global response criteria and the modified severity weighted assessment tool (International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organization for Research and Treatment of Cancer; ref. 29). Patients not meeting the criteria for CR, CRp, CRi, or PR were categorized as non-responders as prespecified in the study protocol.

Secondary endpoints included safety (AEs, SAEs, dose reductions or delays, withdrawals due to toxicities, and changes in safety assessments) and population PK parameters for molibresib and its active metabolite composite.

PK analysis

Blood samples for PK analysis of molibresib and its active metabolite composite in plasma were collected over 24 hours (following dosing) on week 1 day 1 and week 2 day 7; sparse sampling was used after repeated administration on week 1 days 2 and 5, week 2 day 6, week 3 day 1, week 7 day 1, and on day 1 for every 6 weeks thereafter. Sparse sampling was used in Part 2 at week 1 day 1, week 3 day 1, week 7 day 1, and on day 1 for every 6 weeks thereafter. Plasma concentrations of molibresib, its active metabolite composite, and TAM were quantified using a validated high-performance LC/MS-MS method (GSK data on file).

PD analysis

Details on sample collection, processing, and sequencing, including methodology for RT-qPCR analysis, differential expression analyses, and gene set enrichment analysis (GSEA) for RNA sequencing (RNA-seq), are provided in Supplementary Methods S3. In brief, the fold change for gene expression after molibresib dosing (relative to predose expression) was calculated for each target gene on the RT-qPCR panel. For RNA-seq, RNA was isolated from bone marrow aspirate (BMA) samples and a differential expression analysis performed using DESeq2 (version 1.20.0) and R (version 3.5.0). The *P* values were calculated using the Wald test with Benjamini–Hochberg multiple testing correction. GSEA, which determines whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states (e.g., phenotypes), was performed using Molecular Signatures Database, Hallmark gene sets, and the GSEA Preranked approach using GSEA software (version 3.0; refs. 30–32). Genes were ranked on the basis of $\log_2(FPKM_{post\text{-}dose}/FPKM_{pre\text{-}dose})$, and adjusted *P* values were calculated with subsequent Benjamini–Hochberg multiple testing correction. GSEA heatmaps were generated using gplots (version 3.0.1; ref. 33).

Sample size and statistical analysis

Sample size

For Part 1, the sample size was not driven by statistical considerations, but by the number of dose escalations required. No formal statistical hypotheses were tested, and all analyses were descriptive and exploratory. For Part 2, a sample size was determined using a traditional, 2-stage Green-Dahlberg design for each individual disease cohort to ensure that there was at least 85% power to detect a clinically meaningful effect in the primary efficacy outcome (34). Using the estimated sample size, a Bayesian predictive probability design was evaluated. In the MDS cohort, a sample size of 32 at the RP2D provided 87% power with a type 1 error of 0.034 to detect an ORR of 30% and exclude an ORR of 10%. In the CTCL cohort, a sample size of 37 at the RP2D provided 85% power with a type 1 error of 0.049 to detect an ORR of 40% and exclude an ORR of 20%.

Analysis populations

Safety and efficacy analyses were performed using the all-treated population, defined as all patients enrolled who received at least one dose of study treatment. The PK population (Part 1 or Part 2) consisted of all patients enrolled in the relevant study part who received at least one dose of study treatment and who provided viable blood samples for PK analysis and/or for whom a PK parameter was available. The PD population was defined as patients who received at least one dose of study treatment and for whom tumor biopsy material was obtained and analyzed for biomarkers.

Statistical analysis

Patient baseline characteristics and safety data were quantified using descriptive statistics and analyzed by Part 1, Part 2, Part 2 disease cohort, and the total study population. ORRs were summarized by Part 1, Part 2, Part 2 disease cohort, and the total study population using observed values and corresponding 95% confidence intervals (CI). In Part 1 of the study, molibresib, its active metabolite composite and/or TAM PK parameters were analyzed using conventional non-compartmental methods using Phoenix WinNonlin (Certara) and summarized using descriptive statistics. In Part 2, the PK parameters were analyzed using Bayesian population PK methods and the published population PK model (21).

Data availability

Within 6 months of this publication, anonymized individual patient-level data, along with supportive documents, such as annotated case report form, protocol, reporting and analysis plan, data set specifications, raw dataset, analysis-ready dataset, and clinical study report, will be made available for research proposals approved by an independent review committee. Research proposals should be submitted to www.clinicalstudydatarequest.com. A data sharing agreement will be required.

Results

Patients and baseline characteristics

In total, 111 patients were enrolled in the study (87 in Part 1 and 24 in Part 2). A total of 17 (15%) patients withdrew from the study.

Reasons for discontinuation included early-study termination due to limited molibresib efficacy in the CTCL cohort ($n = 8$; 7%), withdrawal of consent ($n = 6$; 5%), loss to follow-up ($n = 2$; 2%), and investigator discretion ($n = 1$; <1%; Supplementary Fig. S2).

Baseline demographics, clinical characteristics, and disposition for the total Part 1 population, individual Part 2 cohorts, total Part 2 population, and total study population are summarized in Supplementary Table S1 (individual Part 1 cohorts are summarized in Supplementary Table S2). Demographics and clinical characteristics were broadly similar across Part 1 and Part 2 of the study, although patients in Part 2 tended to be younger, had a shorter time since diagnosis, and were mostly females.

Expansion cohort selection and recommended Phase 2 dose

During Part 1 (dose expansion), molibresib doses from 5–120 mg QD were investigated. In addition to the cohorts detailed in **Table 1** (60–120 mg QD molibresib), dosing cohorts included 5 mg QD ($n = 1$), 10 mg QD ($n = 1$), 20 mg QD ($n = 1$), 30 mg QD MM ($n = 5$), 40 mg QD ($n = 1$), and 40 mg QD MM ($n = 4$). Emerging data from Part 1 indicated delayed clinical responses in patients with AML (requiring >4 weeks of treatment, sometimes >10 weeks), while 3 patients exhibited objective clinical responses and evidence of antecedent MDS (i.e., with a primary malignancy type of AML after MDS). In the NHL cohort, interim data suggested more robust efficacy in patients with CTCL compared with other NHL subtypes. No responses were observed in patients with MM. As such, MDS and CTCL were selected for dose expansion in Part 2. In Part 1, the MTD in AML cohorts (defined as a dose level at which patients experienced a >33% incidence rate of DLT) did not exceed 80–120 mg QD molibresib, though one DLT (a Grade 3 reduction in left ventricular ejection fraction) was reported with the 120 mg QD dose. The MTD in NHL cohorts did not exceed 60–80 mg QD molibresib, though one DLT (Grade 4 thrombocytopenia) was reported at 60 mg QD and there was a trend towards higher rates of thrombocytopenia with the 80 mg QD dose. Considering early clinical benefit was seen at doses of 60 mg QD or greater, a molibresib RP2D of 75 mg QD (for ease of administration based on available tablet strengths) was selected for patients with MDS and an RP2D of 60 mg QD was selected for patients with CTCL to potentially provide the best clinical efficacy while maintaining tolerability. Part 2 included 8 patients in the CTCL cohort (7 received 60 mg QD and 1 received 80 mg QD molibresib; due to a protocol deviation) and 16 patients in the MDS cohort (75 mg QD molibresib).

Safety

In total, 4 patients in the dose expansion cohorts experienced a DLT: one increase in alanine aminotransferase and blood bilirubin in the same patient [$n = 1/18$ (6%), 60 mg QD NHL cohort]; one decrease in ejection fraction [$n = 1/6$ (17%), 120 mg QD AML cohort]; one case of thrombocytopenia [$n = 1/18$ (6%), 60 mg QD NHL cohort]; and one case of sialadenitis [$n = 1/16$ (6%), 75 mg QD MDS cohort].

Across the full study population ($N = 111$), 11 (10%) patients experienced a maximum Grade 1 or 2 AE, and 100 (90%) experienced a maximum Grade 3 or more AE. The most common AEs were diarrhea ($n = 55$; 50%), nausea ($n = 51$; 46%), and thrombocytopenia ($n = 44$; 40%; **Table 1**), and the most common Grade 3+ AEs (occurring in >10% of patients overall) were thrombocytopenia ($n = 41$; 37%), anemia ($n = 17$; 15%), febrile neutropenia ($n = 17$; 15%), pneumonia ($n = 15$; 14%), hyperglycemia ($n = 14$; 13%), and increased blood bilirubin ($n = 12$; 11%). A total of 88 (79%) patients experienced an SAE (Supplementary Table S3), the most common of which were thrombocytopenia ($n = 23$; 21%), pneumonia ($n = 14$; 13%), and febrile neutropenia ($n = 12$; 11%). A total of 42 (38%) patients experienced an SAE that was considered related to treatment. The only treatment-related SAEs (any grade) occurring in >1 patient were thrombocytopenia ($n = 22$; 20%), neutropenia ($n = 3$; 3%), increased alanine aminotransferase, cardiac failure, decreased appetite, diarrhea, hematuria, and pyrexia (each $n = 2$; 2%). AEs by grade, disease, and dose cohort (60–120 mg QD doses in Part 1) are summarized in Supplementary Table S4.

The most common AEs leading to dose reduction, interruption, or treatment discontinuation are summarized in **Table 2**. Across the full study population ($N = 111$), 68 (61%) patients required dose interruptions, 28 (25%) required dose reductions, and 33 (30%) needed to permanently discontinue study treatment. In most cases, AEs were resolved by dose interruptions. Diarrhea ($n = 11$; 10%) and thrombocytopenia ($n = 11$; 10%) were the only AEs that led to dose interruptions in ≥10% of patients; thrombocytopenia ($n = 17$; 15%) was the only AE that led to dose reduction in ≥10% of patients; the only AEs leading to permanent discontinuation of treatment in >3% of patients were sepsis ($n = 5$; 5%) and dysgeusia ($n = 4$; 4%).

In Part 2, 6/8 (75%) patients in the CTCL cohort and 5/16 (31%) patients in the MDS cohort experienced AEs leading to dose reductions. No patients in the CTCL cohort and 9/16 (56%) patients in the MDS cohort experienced AEs leading to treatment discontinuation.

Across the full study population ($N = 111$), 87 (78%) patients died during the study (including during the time from last treatment). None were considered related to the study treatment.

Efficacy

Treatment responses are summarized in **Table 3** and Supplementary Table S5; the ORRs for Part 1, Part 2, and the total study population were 10% (95% CI, 4.8–18.7), 25% (95% CI, 7.3–52.4), and 13% (95% CI, 6.9–20.6), respectively. In Part 1, one patient with diffuse large B-cell lymphoma (DLBCL) achieved a durable CR in the 80 mg NHL cohort ($n = 1/7$; 14%), one patient achieved CRp in the 120 mg QD AML cohort ($n = 1/6$; 17%), and one patient achieved CRi in the 100 mg AML cohort ($n = 1/16$; 6%). In addition, 4 patients with AML achieved a PR ($n = 4/45$; 9%), as did 2 patients with NHL ($n = 2/25$; 8%). In Part 2, one patient achieved a CR in the 75 mg QD MDS cohort ($n = 1/16$; 6%), one patient achieved a bone marrow CR in the 75 mg QD MDS cohort ($n = 1/16$; 6%), one patient achieved CRi in the 75 mg QD MDS cohort ($n = 1/16$; 6%), and one patient achieved a PR in the 75 mg QD cohort ($n = 1/16$; 6%). No patients in the Part 1 MM or Part 2 CTCL cohorts achieved a CR or PR.

To provide further insights into the characteristics of the responses observed during the study, case vignettes for 3 exemplar patients are presented. In one patient (69 years of age; Patient 6), molibresib was initiated (60 mg QD) after failure of azacitidine therapy for AML with myelodysplasia-related changes. The patient had an adverse-risk molecular profile, including a complex monosomal karyotype and mutations in *IDH1* and *TP53*. Molibresib treatment was well tolerated; the patient experienced a Grade 3 febrile neutropenia event 35 days after starting molibresib, which resolved with treatment 2 days later and did not require molibresib dose adjustments or interruptions. Although no immediate decrease in disease burden was noted with molibresib (**Fig. 1A**; Day 41), the patient chose to continue with treatment. Subsequent bone marrow assessments showed a progressive reduction in blast percentage, ultimately achieving the best blast percentage, mutational burden, and histological responses after 98 days (**Fig. 1A** and **B**) and a documented PR at 99 days. The patient subsequently progressed with increasing marrow blast counts after 134 days of molibresib treatment.

Table 1. Summary of AEs occurring in >10% of the total study population (60–120 mg QD doses).

	Part 1									Part 2			
n (%)	60 mg QD AML (*n* = 8)	60 mg QD NHL (*n* = 18)	60 mg QD MM (*n* = 3)	75 mg QD AML (*n* = 8)	80 mg QD[a] (*n* = 1)	80 mg QD AML (*n* = 7)	80 mg QD NHL (*n* = 7)	100 mg QD AML (*n* = 16)	120 mg QD AML (*n* = 6)	60 mg QD CTCL (*n* = 7)	75 mg QD MDS (*n* = 16)	80 mg QD CTCL (*n* = 1)	Total (*N* = 111)
Diarrhea	3 (38)	7 (39)	0	4 (50)	1 (100)	4 (57)	3 (43)	11 (69)	3 (50)	2 (29)	11 (69)	1 (100)	55 (50)
Nausea	3 (38)	6 (33)	2 (67)	4 (50)	0	6 (86)	2 (29)	8 (50)	3 (50)	2 (29)	11 (69)	0	51 (46)
Thrombocytopenia	0	16 (89)	2 (67)	3 (38)	0	0	7 (100)	1 (6)	0	3 (43)	5 (31)	1 (100)	44 (40)
Blood bilirubin increased	3 (38)	4 (22)	0	4 (50)	1 (100)	3 (43)	1 (14)	11 (69)	3 (50)	0	3 (19)	0	34 (31)
Fatigue	1 (13)	6 (33)	1 (33)	1 (13)	0	4 (57)	3 (43)	5 (31)	1 (17)	4 (57)	1 (6)	0	29 (26)
Decreased appetite	2 (25)	3 (17)	2 (67)	3 (38)	0	2 (29)	1 (14)	5 (31)	1 (17)	2 (29)	5 (31)	0	28 (25)
Dysgeusia	3 (38)	2 (11)	0	2 (25)	0	3 (43)	1 (14)	6 (38)	1 (17)	3 (43)	6 (38)	1 (100)	28 (25)
Hyperglycemia	1 (13)	1 (6)	0	3 (38)	0	1 (14)	4 (57)	7 (44)	3 (50)	2 (29)	4 (25)	0	28 (25)
Vomiting	1 (13)	3 (17)	2 (67)	0	1 (100)	2 (29)	1 (14)	4 (25)	1 (17)	3 (43)	7 (44)	0	26 (23)
Pyrexia	1 (13)	5 (28)	2 (67)	1 (13)	0	2 (29)	1 (14)	4 (25)	2 (33)	0	2 (13)	0	24 (22)
Anemia	0	6 (33)	1 (33)	2 (25)	1 (100)	1 (14)	3 (43)	3 (19)	0	0	2 (13)	1 (100)	22 (20)
INR increased	3 (38)	2 (11)	0	2 (25)	0	1 (14)	0	8 (50)	3 (50)	0	1 (6)	0	21 (19)
Hypokalemia	3 (38)	1 (6)	1 (33)	2 (25)	1 (100)	1 (14)	0	4 (25)	3 (50)	0	2 (13)	0	20 (18)
Cough	2 (25)	1 (6)	1 (33)	2 (25)	0	2 (29)	1 (14)	3 (19)	0	1 (14)	2 (13)	1 (100)	18 (16)
Febrile neutropenia	4 (50)	1 (6)	0	2 (25)	0	3 (43)	0	4 (25)	3 (50)	0	0	0	18 (16)
Pneumonia	1 (13)	1 (6)	0	1 (13)	1 (100)	3 (43)	2 (29)	5 (31)	2 (33)	0	0	0	18 (16)
Abdominal pain	2 (25)	3 (17)	2 (67)	1 (13)	0	1 (14)	0	3 (19)	1 (17)	0	4 (25)	0	17 (15)
Epistaxis	1 (13)	2 (11)	1 (33)	0	0	3 (43)	1 (14)	2 (13)	1 (17)	0	3 (19)	0	16 (14)
Dyspnea	2 (25)	2 (11)	1 (33)	1 (13)	0	1 (14)	1 (14)	2 (13)	0	0	2 (13)	0	15 (14)
APTT prolonged	0	5 (28)	0	1 (13)	1 (100)	1 (14)	0	4 (25)	2 (33)	0	0	0	14 (13)
Constipation	2 (25)	2 (11)	1 (33)	1 (13)	1 (100)	0	1 (14)	3 (19)	0	1 (14)	1 (6)	0	14 (13)
Rash	1 (13)	3 (17)	0	1 (13)	0	1 (14)	2 (29)	2 (13)	0	0	3 (19)	0	14 (13)
Asthenia	1 (13)	3 (17)	0	3 (38)	0	0	2 (29)	0	0	1 (14)	1 (6)	1 (100)	12 (11)
Hypomagnesemia	1 (13)	1 (6)	1 (33)	1 (13)	1 (100)	1 (14)	0	2 (13)	0	0	2 (13)	0	12 (11)
Peripheral edema	1 (13)	2 (11)	0	1 (13)	0	0	0	3 (19)	2 (33)	1 (14)	1 (6)	0	12 (11)
Dizziness	0	1 (6)	1 (33)	1 (13)	0	0	1 (14)	3 (19)	1 (17)	0	3 (19)	0	11 (10)
Hypotension	2 (25)	0	0	2 (25)	1 (100)	0	0	1 (6)	1 (17)	0	2 (13)	0	11 (10)
Urinary tract infection	1 (13)	2 (11)	1 (33)	0	0	1 (14)	0	1 (6)	1 (17)	0	3 (19)	0	11 (10)

Abbreviations: AE, adverse event; AML, acute myeloid leukemia; APTT, activated partial thromboplastin time; CTCL, cutaneous T-cell lymphoma; INR, international normalized ratio; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, Non–Hodgkin lymphoma; QD, once daily.

[a]One patient was listed in a separate 80 mg QD cohort due to a change in the electronic case report form data capture.

Table 2. Summary of AEs, SAEs, and AEs leading to dose reduction, interruption, or discontinuation (60–120 mg QD doses).

	Part 1									Part 2			
n **(%)**	**60 mg QD AML (*n* = 8)**	**60 mg QD NHL (*n* = 18)**	**60 mg QD MM (*n* = 3)**	**75 mg QD AML (*n* = 8)**	**80 mg QD[a] (*n* = 1)**	**80 mg QD AML (*n* = 7)**	**80 mg QD NHL (*n* = 7)**	**100 mg QD AML (*n* = 16)**	**120 mg QD AML (*n* = 6)**	**60 mg QD CTCL (*n* = 7)**	**75 mg QD MDS (*n* = 16)**	**80 mg QD CTCL (*n* = 1)**	**Total (*N* = 111)**
Any AE	8 (100)	18 (100)	3 (100)	8 (100)	1 (100)	7 (100)	7 (100)	16 (100)	6 (100)	7 (100)	16 (100)	1 (100)	111 (100)
AEs related to treatment	6 (75)	18 (100)	3 (100)	6 (75)	0	6 (86)	7 (100)	13 (81)	5 (83)	7 (100)	16 (100)	0	97 (87)
Any SAE	6 (75)	12 (67)	2 (67)	8 (100)	1 (100)	6 (86)	7 (100)	14 (88)	6 (100)	5 (71)	12 (75)	1 (100)	88 (79)
SAEs related to treatment	2 (25)	11 (61)	1 (33)	4 (50)	0	2 (29)	6 (86)	5 (31)	1 (17)	2 (29)	5 (31)	0	42 (38)
Fatal SAEs	2 (25)	1 (6)	1 (33)	3 (38)	0	2 (29)	0	8 (50)	2 (33)	0	2 (13)	0	21 (19)
AEs leading to dose interruptions in ≥10% of patients overall													
Any AE	5 (63)	7 (39)	2 (67)	7 (88)	1 (100)	6 (86)	5 (71)	10 (63)	6 (100)	5 (71)	9 (56)	1 (100)	68 (61)
Diarrhea	0	0	0	0	0	0	2 (29)	0	0	1 (14)	5 (31)	0	11 (10)
Thrombocytopenia	0	3 (17)	2 (67)	0	0	1 (14)	0	2 (13)	1 (17)	1 (14)	1 (6)	0	11 (10)
AEs leading to dose reductions in ≥2% of patients overall													
Any AE	0	8 (44)	0	0	0	0	5 (71)	2 (13)	1 (17)	6 (86)	5 (31)	0	28 (25)
Thrombocytopenia	0	7 (39)	0	0	0	0	5 (71)	1 (6)	0	2 (29)	1 (6)	0	17 (15)
Dysgeusia	0	0	0	0	0	0	0	0	0	1 (14)	1 (6)	0	2 (2)
Fatigue	0	0	0	0	0	0	0	0	0	2 (29)	0	0	2 (2)
AEs leading to permanent discontinuation of treatment in >2% of patients overall													
Any AE	3 (38)	3 (17)	0	2 (25)	0	3 (43)	1 (14)	8 (50)	2 (33)	0	9 (56)	0	33 (30)
Sepsis	0	0	0	0	0	0	0	3 (19)	1 (17)	0	1 (6)	0	5 (5)
Dysgeusia	0	0	0	0	0	0	0	0	0	0	4 (25)	0	4 (4)
Nausea	0	0	0	0	0	0	0	0	0	0	3 (19)	0	3 (3)
Pneumonia	0	0	0	0	0	1 (14)	0	2 (13)	0	0	0	0	3 (3)
Respiratory failure	0	0	0	1 (13)	0	1 (14)	0	1 (6)	0	0	0	0	3 (3)
Decreased appetite	0	0	0	0	0	0	0	1 (6)	0	0	2 (13)	0	3 (3)
Thrombocytopenia	0	1 (6)	0	0	0	0	0	0	0	0	1 (6)	0	3 (3)

Abbreviations: AE, adverse event; AML, acute myeloid leukemia; CTCL, cutaneous T-cell lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, Non–Hodgkin lymphoma; QD, once daily; SAE, serious adverse event.
[a]One patient was listed in a separate 80 mg QD cohort due to a change in the electronic case report form data capture.

Table 3. Overview of clinical responses in patients receiving molibresib.

	Part 1 total population[a] (*n* = 87)	**Part 2 MDS cohort (*n* = 16)**	**Part 2 CTCL cohort (*n* = 8)**	**Part 2 total population (*n* = 24)**	**Total study population (*N* = 111)**
ORR, % (95% CI)	10 (4.8–18.7)	25 (7.3–52.4)	0	25 (7.3–52.4)	13 (6.9–20.6)
Best response, *n* (%)[b]					
CR	1 (1)	1 (6)	0	1 (4)	2 (2)
mCR	0	1 (6)	0	1 (4)	1 (<1)
CRi/CRp	2 (2)	1 (6)[c]	0	1 (4)	3 (3)
PR	6 (7)	1 (6)	0	1 (4)	7 (6)
SD	9 (10)	1 (6)	5 (63)	6 (25)	15 (14)
Progressive disease	23 (26)	—	—	—	23 (21)
Not evaluable[d]	23 (26)	8 (50)	3 (38)	11 (46)	34 (31)
No response[e]	23 (26)	3 (19)	0	3 (13)	26 (23)

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete response; CRi, CR but platelet count <100 x 10^9/L or neutrophil count <1 x 10^9/L; CRp, CR but platelet count <100 x 10^9/L; CTCL, cutaneous T-cell lymphoma; mCR, marrow CR; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, Non–Hodgkin lymphoma; ORR, objective response rate; PR, partial response; QD, once daily; SD, stable disease.
[a]Part 1 of the study included patients receiving therapeutic doses of molibresib (60–120 mg QD) with AML (*n* = 46), MM (*n* = 3), and NHL (*n* = 25).
[b]Unconfirmed best objective responses are reported for MDS and confirmed (duration at least 4 months) best objective responses are reported for CTCL cohorts.
[c]Patient in Part 2 who previously had MDS (and was classified to the MDS cohort), which evolved to AML and was therefore assessed using AML criteria (using CRi/CRp).
[d]Patients with unknown/not evaluable or missing best objective response data are assumed to be non-responders and are included in the denominator for the percentage calculation.
[e]Patients not meeting the criteria for CR, CRp, CRi, or PR were categorized as non-responders as prespecified in the study protocol.

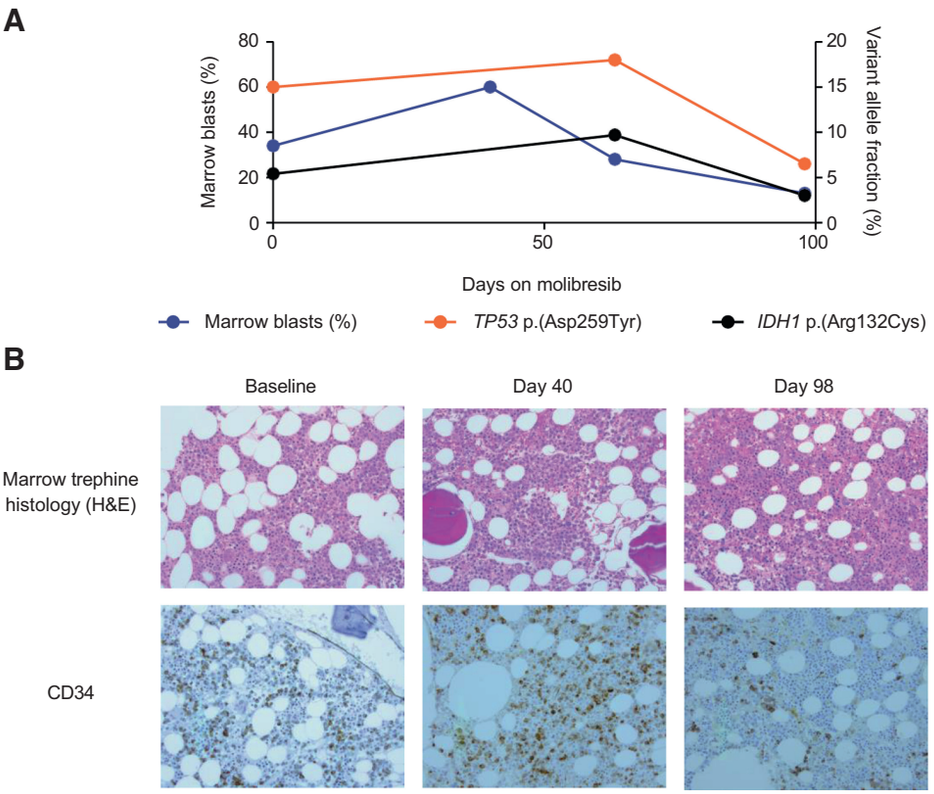


Figure 1.

A, Serial bone marrow blasts and mutation burden during molibresib therapy (60 mg QD). There was an initial rise in bone marrow blasts and mutational burden in *IDH1* Arg132Cys and *TP53* Asp259Tyr during molibresib treatment, but these decreased by day 98. **B,** Serial bone marrow trephine assessments showing a reduction in blasts by H&E stain (top) and by CD34 IHC (bottom), as well as evidence of regenerative erythropoiesis at day 98 of molibresib treatment. H&E, hematoxylin and eosin.

A second patient (63 years of age; Patient 1) with prior MDS showed no response to azacitidine treatment, progressed to AML with complex cytogenetics (including a *TP53* mutation, R248W), and did not respond to subsequent salvage therapy with a novel investigational nucleoside analog. The patient then initiated molibresib treatment (120 mg QD), which was tolerated with some dose interruptions required for incidences of dysgeusia. After 2 months of treatment, the bone marrow was shown to be hypocellular with peripheral blood cytopenia. After 4 months of treatment, the bone marrow showed no (0%) blasts and 5% cellularity, with a recovery of the absolute neutrophil count to 2.94×10^9/L and hemoglobin levels to 10.2 g/dL. After this, the patient required treatment interruption due to progressive fungal pneumonia and experienced a relapse after 6 weeks.

Lastly, a 37-year-old patient with NHL (DLBCL histology) was enrolled in the study just over 2 years after their cancer diagnosis of widespread and extranodal stage IV disease (Supplementary Fig. S3). The tumor exhibited high CD10 and *MYC* expression (70%), was classified with a germinal center B-cell phenotype at initial presentation, and was refractory to R-CHOP and DHAP therapy before responding to FGIV therapy alternating with HyperCVAD-B. This response was consolidated with a lomustine, cytarabine, etoposide, and cyclophosphamide autologous stem cell transplant, but the patient subsequently relapsed with bony disease within 3 months. At trial entry, the patient had a single extranodal site of disease in the left anterior superior iliac spine. After 1 month of molibresib treatment (80 mg QD), the lesion increased in size, but treatment continued as the patient remained asymptomatic. After 16 weeks of molibresib treatment, the patient was in complete remission (Supplementary Fig. S3), which was maintained for a total of 53 weeks. The patient subsequently relapsed in the central nervous system and died from progressive disease despite further chemotherapy and CD19 CAR T-cell therapy.

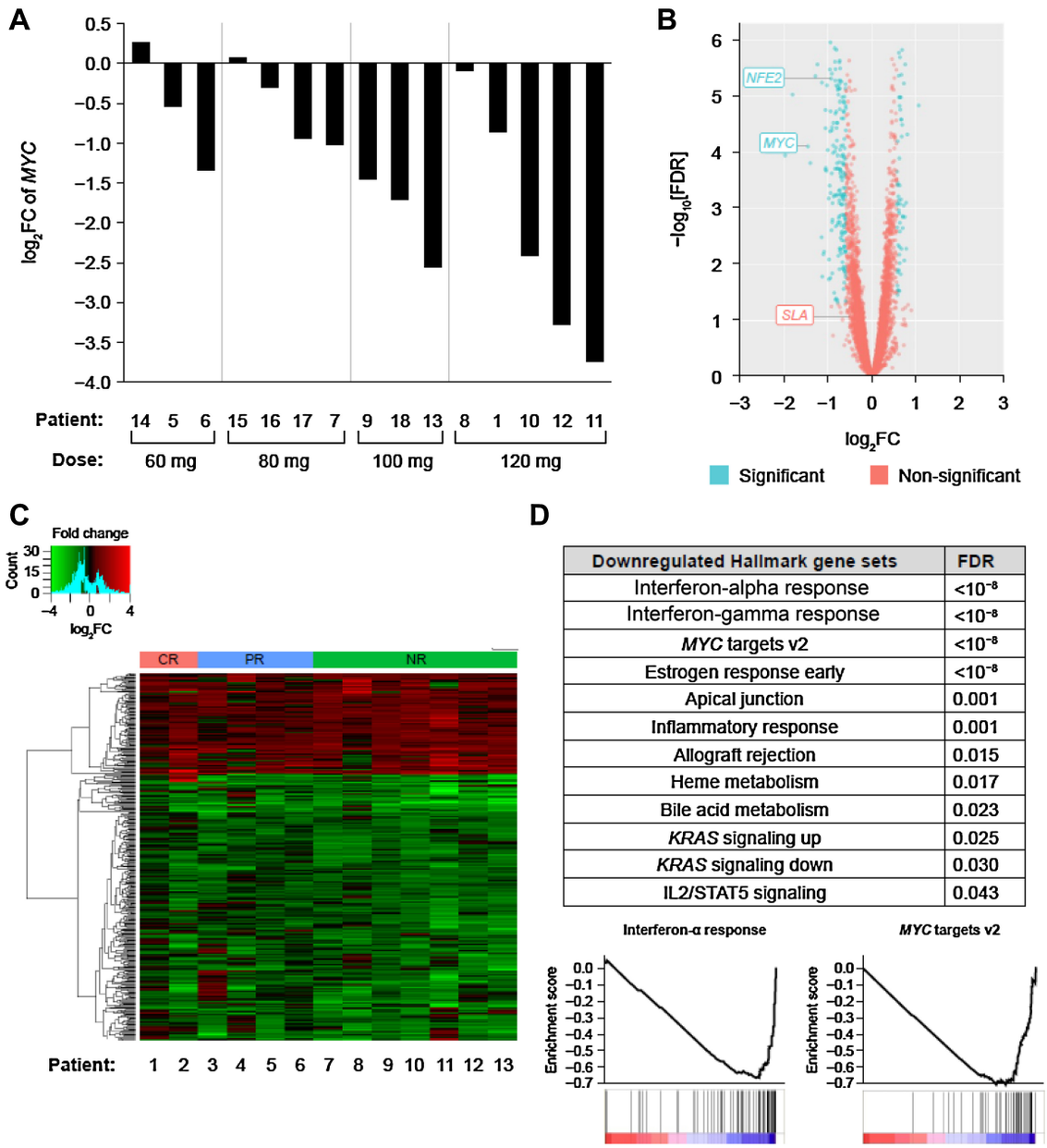
PK

The PK of molibresib following oral administration of 5–120 mg was characterized by rapid absorption, with a C_{max} occurring mostly within 2 hours and rapid elimination, with an average $t_{1/2}$ of 3–6 hours (Supplementary Fig. S4). Derived PK parameters for therapeutic doses (60–120 mg QD) in Part 1 of the study are presented in Supplementary Table S6. Although there was much variation in exposure between the cohorts, the overall trend was towards an increase in the average single- and repeat-dose exposure (AUC_{0-24}), with increasing molibresib doses. At therapeutic doses, molibresib exposure (AUC_{0-24}) appeared to decrease with repeated administration, whereas the active metabolite composite exposure increased, leading to a small change in TAM exposure. Compared with the AML and NHL cohorts, single-dose molibresib exposure at 60 mg appeared lower in patients with MM.

In Part 2 of the study, the population PK model (as described by Krishnatry and colleagues; ref. 21) adequately described the PK of molibresib, active metabolite composite, and TAM. Exposure metrics (AUC_{0-24}) were similar to those observed in Part 1.

PD and RNA-seq

Gene expression changes associated with molibresib treatment were evaluated in subsets of patients in the Part 1 dose escalation AML cohort ($n = 30$). The timing of BMA sample collection, at screening and post-dose, is presented in Supplementary Table S7. Gene expression analyses (Supplementary Methods S2) revealed several hundred differentially expressed genes associated with



Downregulated Hallmark gene sets	FDR
Interferon-alpha response	$<10^{-8}$
Interferon-gamma response	$<10^{-8}$
MYC targets v2	$<10^{-8}$
Estrogen response early	$<10^{-8}$
Apical junction	0.001
Inflammatory response	0.001
Allograft rejection	0.015
Heme metabolism	0.017
Bile acid metabolism	0.023
KRAS signaling up	0.025
KRAS signaling down	0.030
IL2/STAT5 signaling	0.043

Figure 2.

A, RT-qPCR to measure *MYC* expression was performed on bone marrow aspirate samples collected at screening and post-molibresib treatment. Bars represent the $\log_2$-fold change of *MYC* expression after treatment relative to screening. **B,** Volcano plot graphing the $\log_{10}$(FDR) versus the $\log_2$(fold change) for 7,978 genes that are expressed (>3 FPKM) in all analyzed samples. Blue dots indicate genes that pass the significance threshold of an FDR <0.05 and >1.5-fold change, whereas pink dots do not meet both criteria. **C,** A heatmap of all genes that are significantly differentially expressed when pooled across all 13 patients. Each column corresponds to the $\log_2$(fold change) for each patient. The bar at the top of the heatmap corresponds to clinical response, indicating CR (pink), PR (blue), or NR (green). **D,** GSEA results when examining the average fold change across all 13 patients. Twelve gene sets were significantly downregulated (FDR <0.05). Two example enrichment plots show the enrichment score (black line) across all genes, with black bars, indicating genes within the interferon alpha response and MYC target v2 gene sets, respectively. AML, acute myeloid leukemia; CR, complete response; FC, fold change; FDR, false discovery rate; FPKM, fragments per kilobase per million reads; GSEA, gene set enrichment analysis; NR, non-responder; PR, partial response; RT-qPCR, reverse transcription and quantitative polymerase chain reaction.

molibresib treatment across the evaluated doses (60–120 mg QD; **Fig. 2A–D**). The RT-qPCR analysis performed on 15 patients using a seven-gene panel assay (in which expression of six was expected to decrease and one was expected to increase upon pan-BET inhibition) showed that molibresib treatment was associated with a ≥2-fold downregulation of *MYC* in 8 of 15 patients, with a trend for greater downregulation at higher doses (**Fig. 2A**). However, there was no apparent association between *MYC* mRNA inhibition (primarily from the week 1 post-dose assessment) and clinical response in 15 patients with available data. Decreased gene

expression was also observed for the other five downregulated PD biomarkers, whereas *SERTAD1* gene expression was increased more than 2-fold in 10 out of the 15 patient samples across the evaluated doses (Supplementary Table S8). Subsequent RNA-seq analysis was performed on 13 of these patients to examine the global effects of molibresib treatment on gene expression. This analysis identified significant differential expression of 398 genes across all 13 patients (>1.5-fold change with an FDR of <0.05), with a majority (289/398; 72.6%) being downregulated (**Fig. 2B** and **C**). GSEA was performed to identify small but consistent changes across the annotated Hallmark gene sets, identifying 12 downregulated sets (**Fig. 2D**).

In addition to analyzing all patients, non-responders (NR) and responders (CR + PR) were analyzed as separate cohorts to identify differentially expressed genes associated with response to molibresib treatment. Heatmaps show that a large number of genes exhibited significant differential expression in NRs (884) compared with responders (107), despite the similar number of patients in each cohort (**Fig. 3A** and **B**). Although there was a marked

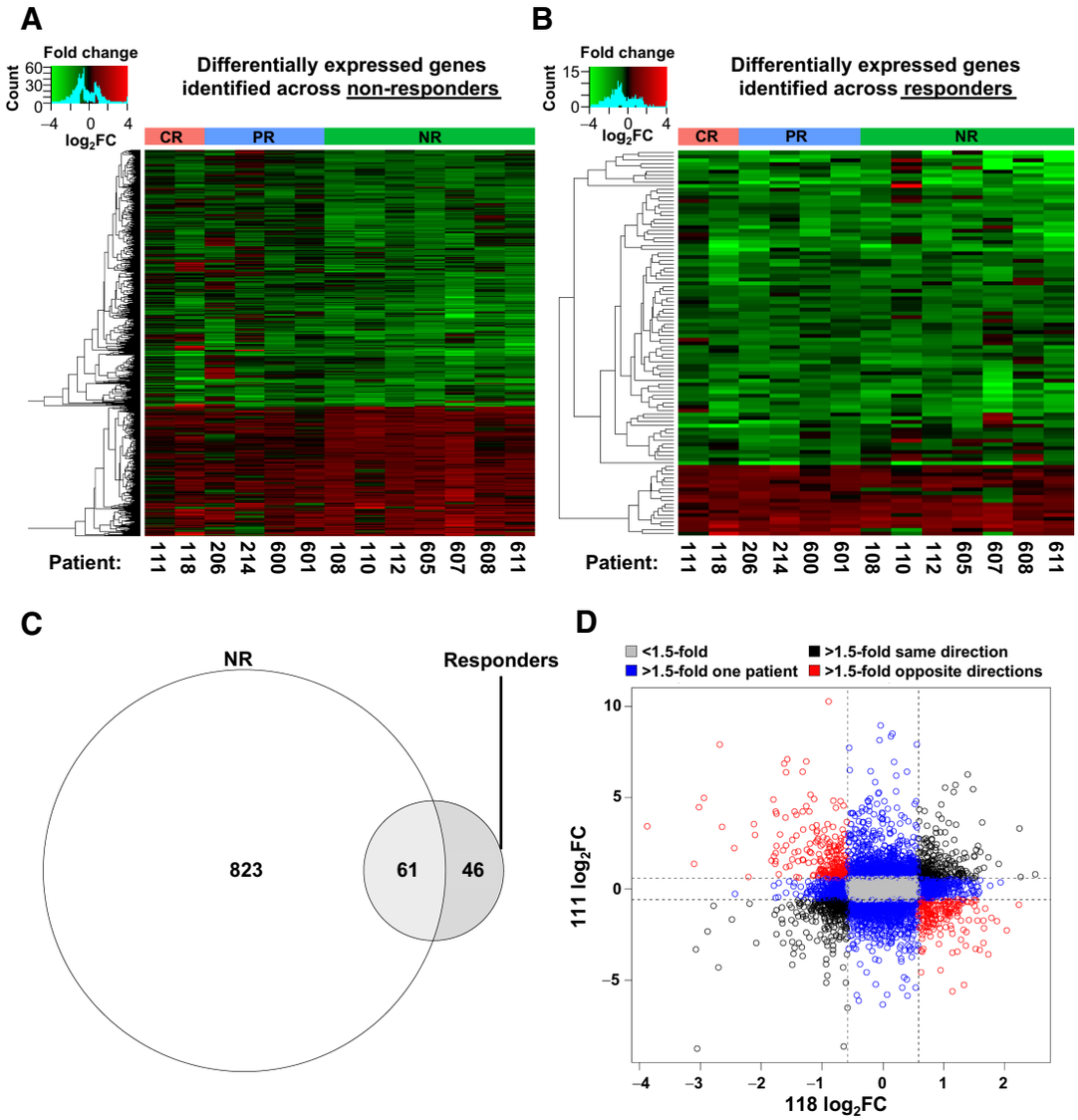


Figure 3.

A and **B,** Heatmaps of individual patients' change in gene expression following molibresib treatment (log_2; fold change) for genes that were identified as differentially expressed when pooled across all NRs **(A)**, or a clinical response (CR and PR; **B**). Genes are defined as differentially expressed if they reach a 1.5-fold change with an FDR <0.05. The bar at the top of the heatmap corresponds to clinical response, indicating CR [pink; Patient 1 with CRp, Patient 2 with CRi), PR (blue), or NR (green)]. **C,** Venn diagram showing the number of differentially expressed genes in responders, non-responders, or both. **D,** Scatterplot showing the fold change in gene expression for the 2 patients who achieved CR. Genes are categorized as unchanged in both patients (gray), differentially expressed in one patient (blue), differentially expressed in the same direction in both patients (black), or differentially expressed in both patients, but in the opposite direction (red) if they exhibited >1.5-fold change (dotted lines). CR, complete response; CRi, CR but platelet count $<100\times10^9$/L or neutrophil count $<1\times10^9$/L; CRp, CR but platelet count $<100\times10^9$/L; FDR, false discovery rate; NR, non-responder; PR, partial response.

difference in the number of differentially expressed genes, there were 61 shared genes between NRs and responders (**Fig. 3C**). The 2 patients with AML (Part 1) who achieved a CR (one CRi and one CRp) exhibited distinct molecular responses to molibresib shortly after initiation of dosing (post-treatment biopsies taken within 1 week of treatment initiation, all pre- and post-blast cell percentages 72%–88%). Despite both patients achieving a CR, there was only a weak correlation (Spearman rho = 0.125) of gene expression fold change between them (**Fig. 3D**).

GSEA revealed many downregulated gene sets following treatment with molibresib (Supplementary Fig. S5A; AML cohort). One patient who achieved a CR was the exception to this trend, exhibiting many upregulated gene sets (Supplementary Fig. S5A). GSEA across longitudinal samples from 3 patients (one CRi and two PRs, with the second CRp included for reference) revealed that later in the study (after week 15), the 2 patients with PR had expression patterns that were similar to the early expression patterns of the patient who achieved a CRi (Supplementary Fig. S5B).

Of note, the allograft rejection gene set, with a gene expression signature that may reflect immune activation, was significantly downregulated (FDR < 0.05) at week 1 for two PR samples and six NR samples (Supplementary Fig. S5A). In contrast to NRs, the expression of this gene set is significantly upregulated early in the 2 CR patients, and interestingly, gene set expression was also seen to increase over time when monitored in the longitudinal samples for the 2 patients who achieved PRs (week 13 for Patient 5; week 15 for Patient 6). These data suggest an evolving gene expression pattern in the PR samples, which at the time of best clinical response more closely approximates that seen in the CR samples (Supplementary Fig. S5B). This contention was further supported by assessment of the genes driving GSEA enrichment (using a leading edge analysis; Supplementary Fig. S5C), which indicated that subsets of this gene set were differentially regulated in a time-dependent manner, rather than one set of genes undergoing subsequent down and then upregulation.

Discussion

This study shows that molibresib has no unexpected toxicities and was generally tolerated, with most SAEs being unrelated to study treatment. Similar to findings from the FTIH study (19), the most common events limiting molibresib dosing were gastrointestinal AEs and thrombocytopenia. Overall, the safety profile of molibresib in the total study population was consistent with those of the individual tumor cohorts (AML/NHL cohorts in Part 1; MDS and CTCL cohorts in Part 2).

Molibresib exhibited antitumor activity, including in patients with poor-risk molecular and clinical features. Overall, 6 patients achieved a CR, CRp, bone marrow CR, or CRi [AML/NHL (DLBCL)/MDS cohorts], and 7 patients achieved a PR (AML/NHL/MDS cohorts). However, no responses were observed in patients with MDS or CTCL in Part 2 of the study. Combined with the efficacy findings from Part 2 of the FTIH study, where no CRs and only two PRs were reported in various solid tumor types (N = 102; ref. 19), it is clear that molibresib displays only a modest antitumoral effect in these patient populations.

As a single-agent epigenetic therapy, the findings presented here are consistent with emerging evidence that some (but not all) epigenetic therapies may need to be used as part of combination therapy to achieve maximal clinical benefit in relapsed/refractory myeloid disease and leukemia (35–38). Considering the limited clinical efficacy at the RP2D of molibresib, it appears that *in vivo* tolerability may limit the ability to fully achieve the antitumor activity that has been demonstrated in preclinical studies using translational models, cell lines, xenograft models, and/or murine models of AML, NHL, and MM (12, 23–25). Furthermore, modeling of molibresib/metabolite exposures suggests that higher exposures of molibresib are tolerated in mice compared with humans (unpublished data). As the majority of published preclinical efficacy data for BET inhibitors in AML models are at doses at/near the MTD, it is possible that the disconnect in efficacy is due to the differential tolerability for molibresib in preclinical models compared with patients in this study. Nevertheless, it is possible that additive or synergistic combinatorial approaches (with adapted BET inhibitor dosing) may provide the most promising therapeutic approach. It has been hypothesized that combining two or more epigenetic agents may provide improved clinical benefit versus monotherapy (35); this hypothesis is being investigated with different epigenetic agents in several clinical trials (i.e., NCT03843528 and NCT03263936; refs. 39, 40). Combinations of epigenetic agents with immunotherapies or targeted therapies are also being investigated (NCT03825367 and NCT03848754; refs. 41, 42).

Consistent with the findings of previous studies of molibresib (including the FTIH study; refs. 18, 21), molibresib PK was characterized by rapid absorption, with a C_{max} occurring within approximately 2 hours and a $t_{1/2}$ of approximately 3–6 hours. Compared with patients with AML and NHL, single-dose molibresib exposure (AUC_{0-24}) at 60 mg appeared lower in patients with MM, though this may be due to the small number of patients in the MM cohort. Molibresib exposure also appeared to decrease with repeated administration, whereas active metabolite exposure increased, leading to a modest change in TAM exposure and indicating auto-induction of metabolism. Consistent with previous findings (18), the average single- and repeat-dose exposures for molibresib generally increased with increasing molibresib doses. Overall, the PK of molibresib and total active metabolite in Part 2 of the study were adequately described using a previously derived population PK model for molibresib (21).

GSEA results showed a consistent downregulation of gene sets when analyzing all patients with AML together, similar to previous findings in patients with solid tumors and consistent with BET proteins promoting gene transcription (19), though the effect of molibresib on specific gene expression appears to vary by patient and response to treatment. The heterogeneity in gene expression changes among the responders may indicate that multiple molecular responses to molibresib treatment contribute to clinical benefit.

The main limitation of this study was the small number of clinical responses, making analysis of efficacy outcomes challenging. Nevertheless, clinical responses were observed, with similar response rates to those reported for other epigenetic therapies (i.e., those that target non-oncogene epigenetic dependencies in cancers) such as DOT1L and LSD1 inhibitors (37, 38, 43). The modest efficacy observed with these single-agent therapies may be due to transcriptional plasticity, as they exert their clinical effect by altering gene expression programs rather than inducing cellular catastrophe (e.g., by the direct induction of apoptosis; ref. 7). As there can be multiple routes to activating or inhibiting gene expression, single-agent efficacy may be limited and combination approaches with other targeted therapies may prove to be the most effective. Similarly, our evaluation of the molecular mechanisms of response is limited by the small number of patients who provided adequate samples for evaluation (15 patients in total, of whom samples for longitudinal genomic analysis were only available for 3 patients). Further

research will therefore be required to elucidate the potential mechanism of action in greater detail.

In conclusion, treatment with molibresib was tolerable, although its use was limited by gastrointestinal and thrombocytopenia toxicities. Given that antitumor activity was observed in some patients receiving molibresib monotherapy, investigations into combination approaches that use BET inhibition and other targeted therapies may be warranted.

Authors' Disclosures

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Authors' Contributions

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References

1. French CA. Small-molecule targeting of BET proteins in cancer. Adv Cancer Res 2016;131:21–58.
2. Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. Oncogene 2007;26:5521–7.
3. Muller S, Filippakopoulos P, Knapp S. Bromodomains as therapeutic targets. Expert Rev Mol Med 2011;13:e29.
4. Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert JP, Barsyte-Lovejoy D, et al. Histone recognition and large-scale structural analysis of the human bromodomain family. Cell 2012;149:214–31.
5. Dey A, Nishiyama A, Karpova T, McNally J, Ozato K. Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. Mol Biol Cell 2009;20:4899–909.
6. Yang Z, He N, Zhou Q. Brd4 recruits P-TEFb to chromosomes at late mitosis to promote G1 gene expression and cell cycle progression. Mol Cell Biol 2008;28:967–76.
7. Dawson MA. The cancer epigenome: concepts, challenges, and therapeutic opportunities. Science 2017;355:1147–52.
8. Manzotti G, Ciarrocchi A, Sancisi V. Inhibition of BET proteins and histone deacetylase (HDACs): crossing roads in cancer therapy. Cancers 2019;11:304.

9. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. Nat Rev Cancer 2012;12:465–77.
10. Padmanabhan B, Mathur S, Manjula R, Tripathi S. Bromodomain and extra-terminal (BET) family proteins: new therapeutic targets in major diseases. J Biosci 2016;41:295–311.
11. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. N Engl J Med 2012;367:647–57.
12. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011;146: 904–17.
13. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011;478:529–33.
14. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 2011; 478:524–8.
15. Gabay M, Li Y, Felsher DW. MYC activation is a hallmark of cancer initiation and maintenance. Cold Spring Harb Perspect Med 2014;4:a014241.
16. Perez-Salvia M, Esteller M. Bromodomain inhibitors and cancer therapy: from structures to applications. Epigenetics 2017;12:323–39.
17. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, et al. Suppression of inflammation by a synthetic histone mimic. Nature 2010;468: 1119–23.
18. Piha-Paul SA, Hann CL, French CA, Cousin S, Braña I, Cassier PA, et al. Phase 1 study of molibresib (GSK525762), a bromodomain and extra-terminal domain protein inhibitor, in NUT carcinoma and other solid tumors. JNCI Canc Spectr 2019;4:pkz093.
19. Cousin S, Blay JY, Brana Garcia I, de Bono JS, Le Tourneau C, Moreno V, et al. Safety, pharmacokinetic, pharmacodynamic and clinical activity of molibresib for the treatment of NUT carcinoma and other cancers: results of a phase I/II open-label, dose escalation study. Int J Cancer 2022;150: 993–1006.
20. Riddell K, Patel A, Collins G, Zhou Y, Schramek D, Kremer BE, et al. An adaptive physiologically based pharmacokinetic-driven design to investigate the effect of itraconazole and rifampicin on the pharmacokinetics of molibresib (GSK525762) in healthy female volunteers. J Clin Pharmacol 2021;61: 125–37.
21. Krishnatry AS, Voelkner A, Dhar A, Prohn M, Ferron-Brady G. Population pharmacokinetic modeling of molibresib and its active metabolites in patients with solid tumors: a semimechanistic autoinduction model. CPT Pharmacometrics Syst Pharmacol 2021;10:709–22.
22. Bell CC, Fennell KA, Chan YC, Rambow F, Yeung MM, Vassiliadis D, et al. Targeting enhancer switching overcomes non-genetic drug resistance in acute myeloid leukaemia. Nat Commun 2019;10:2723.
23. Wyce A, Matteo JJ, Foley SW, Felitsky DJ, Rajapurkar SR, Zhang XP, et al. MEK inhibitors overcome resistance to BET inhibition across a number of solid and hematologic cancers. Oncogenesis 2018;7:35.
24. Dawson MA, Gudgin EJ, Horton SJ, Giotopoulos G, Meduri E, Robson S, et al. Recurrent mutations, including NPM1c, activate a BRD4-dependent core transcriptional program in acute myeloid leukemia. Leukemia 2014;28: 311–20.
25. Chaidos A, Caputo V, Gouvedenou K, Liu B, Marigo I, Chaudhry MS, et al. Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. Blood 2014;123:697–705.
26. Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Stat Med 2008;27:2420–39.
27. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108: 419–25.
28. Cheson BD, Bennett JM, Kopecky KJ, Buchner 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: 4642–9.
29. Olsen EA, Whittaker S, Kim YH, Duvic M, Prince HM, Lessin SR, et al. Clinical end points and response criteria in mycosis fungoides and Sezary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. J Clin Oncol 2011;29:2598–607.
30. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst 2015;1:417–25.
31. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–50.
32. Broad Institute Inc. GSEA: gene set enrichment analysis. [Accessed 2021 Dec 1]. Available from: https://www.gsea-msigdb.org/gsea/index.jsp.
33. Gregory RW, Ben B, Lodewijk B, Robert G, Wolfgang H, Andy L, et al. Various R programming tools for plotting data. R package version 3.0.1. Current version (3.1.1). 2016. [Accessed 2021 Sep 12]. Available from: https://CRAN.R-project.org/package=gplots.
34. Green SJ, Dahlberg S. Planned versus attained design in phase II clinical trials. Stat Med 1992;11:853–62.
35. Fennell KA, Bell CC, Dawson MA. Epigenetic therapies in acute myeloid leukemia: where to from here? Blood 2019;134:1891–901.
36. Morel D, Jeffery D, Aspeslagh S, Almouzni G, Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours—past lessons and future promise. Nat Rev Clin Oncol 2020;17:91–107.
37. Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, Savona MR, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood 2018;131:2661–9.
38. Salamero O, Montesinos P, Willekens C, Perez-Simon JA, Pigneux A, Recher C, et al. First-in-human phase I study of iadademstat (ORY-1001): a first-in-class lysine-specific histone demethylase 1A inhibitor, in relapsed or refractory acute myeloid leukemia. J Clin Oncol 2020;38:4260–73.
39. ClinicalTrials.gov. Vorinostat dose-escalation after allogeneic hematopoietic cell transplantation. 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT03843528
40. ClinicalTrials.gov. Epigenetic reprogramming in relapse/refractory AML. 2017. Available from: https://clinicaltrials.gov/ct2/show/NCT03263936
41. ClinicalTrials.gov. Nivolumab in combination with 5-azacytidine in childhood relapsed/refractory AML. 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT03825367.
42. ClinicalTrials.gov. Pracinostat and gemtuzumab ozogamicin (PraGO) in patients with relapsed/refractory acute myeloid leukemia. 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT03848754
43. Wass M, Gollner S, Besenbeck B, Schlenk RF, Mundmann P, Gothert JR, et al. A proof-of-concept phase I/II pilot trial of LSD1 inhibition by tranylcypromine combined with ATRA in refractory/relapsed AML patients not eligible for intensive therapy. Leukemia 2021;35:701–11.