Phase I study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia

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Short title: MGCD0103 in leukemia and MDS
ABSTRACT

MGCD0103 is an isotype-selective inhibitor of histone deacetylases (HDACs) targeted to isoforms 1, 2, 3 and 11. In a Phase I study in patients with leukemia or myelodysplastic syndromes (MDS), MGCD0103 was administered orally 3 times weekly without interruption. Twenty-nine patients with a median age of 62 years (range 32-84 years) were enrolled at planned dose levels (20, 40, and 80 mg/m^2). The majority of patients (76%) had acute myelogenous leukemia (AML). In all, 24 of 29 patients (83%) had received ≥ 1 prior chemotherapy (range 0-5), and 18 of 29 patients (62%) had abnormal cytogenetics. The maximum tolerated dose was determined to be 60 mg/m^2, with DLTs of fatigue, nausea, vomiting, and diarrhea observed at higher doses. Three patients achieved a complete bone marrow response (blasts ≤ 5%). Pharmacokinetic analyses indicated absorption of MGCD0103 within 1 h and an elimination half-life in plasma of 9 ± 2 h. Exposure to MGCD0103 was proportional to dose up to 60 mg/m^2. Analysis of peripheral white cells demonstrated induction of histone acetylation and dose-dependent inhibition of HDAC enzyme activity. In summary, MGCD0103 was safe and had anti-leukemia activity which was mechanism-based in patients with advanced leukemia.
INTRODUCTION

Despite many advances in the management of acute leukemia, patients with acute myelogenous leukemia (AML) who are refractory to conventional therapy, or who have relapsed after conventional therapy, have a poor prognosis for survival. In addition, the inability to tolerate or benefit from induction chemotherapy due to advanced age or co-morbidities is associated with poor clinical outcomes. Novel therapeutic strategies focusing on tumor-related alterations in chromatin structure and epigenetic silencing are currently being explored.

In eukaryotic cells, histone acetylation/deacetylation has an important role in the control of gene transcription regulation. Transcriptionally active genes are characterized by hyperacetylated chromatin, while repressed genes are typically in a hypoacetylated state. This process is mediated by a complex interplay of proteins with histone acetyltransferases and deacetylases. Histone deacetylases (HDACs) are divided into 4 general classes. Class I includes HDACs 1, 2, 3 and 8. Class II HDACs 4, 5, 6, 7, 9 and 10. Class III includes Sirt 1 to 7; the latter group of enzymes is not targeted by HDAC inhibitors. Class IV includes HDAC 11, which is distinct among the other classes.

Small molecule inhibitors of HDACs are a novel therapeutic class of drugs with anticancer potential. Although not fully understood, the clinical activity of these inhibitors is thought to be mediated in part by induction of histone acetylation, resulting in a permissive or more open chromatin configuration and potential reactivation of aberrantly suppressed genes (e.g. tumor suppressor genes). The changes in gene expression lead to inhibition of cell proliferation, induction of apoptosis and/or cell differentiation.

HDAC inhibitors can be grouped into different subclasses, such as hydroxamic acids and aminophenylbenzamides based on their chemical structure. MGCD0103 is an isotype-
specific aminophenylbenzamide that was synthesized through medicinal chemistry optimization of small molecule HDAC inhibitors. MGCD0103 inhibits HDAC isotypes 1, 2, 3, and 11\textsuperscript{14,15}. This greater selectivity allows for targeting of the HDAC isotypes that are thought to be linked to cancer. Indeed, the number of genes with expression induced by MGCD0103 is dramatically smaller than that induced by non-specific hydroxamate HDAC inhibitors, yet efficacy in preclinical cancer models is maintained or increased (Fournel et al, submitted). Therefore, we hypothesize that the selective targeting of specific HDAC isotypes by MGCD0103 may improve the therapeutic window in cancer patients.

Preclinical studies have demonstrated that MGCD0103 is orally bioavailable with significant \textit{in vitro} antineoplastic activity at sub-micromolar concentrations against a broad spectrum of human cancers, including various leukemia cell lines and xenografts \textsuperscript{16}(Fournel et al submitted). Of importance, the half life of histone acetylation after MGCD0103 exposure in animals and in patient peripheral blood mononuclear cells (PBMCs) appears to outlasts pharmacokinetic (PK) exposure\textsuperscript{16}. This prolonged pharmacodynamic (PD) effect appears to allow for less frequent dosing of MGCD0103. Based on this information, we conducted this open-label, nonrandomized, dose-escalation, multi-center Phase I trial of oral MGCD0103 administered three times a week in patients with leukemia and myelodysplastic syndromes (MDS).
MATERIALS AND METHODS

The objectives of the study were to assess the safety and tolerability of increasing doses of MGCD0103 when administered to patients with acute or chronic leukemia or MDS, and to determine the maximum tolerated dose (MTD) and dose limiting toxicities (DLTs). Secondarily, the clinical activity, effects on several potential clinical biomarkers, and PK characteristics of the compound were also evaluated in this patient population. This study was approved by all IRBs at MD Anderson Cancer Center, Jewish General Hospital and Princess Margaret Hospital. The study was performed following the principles of the Declaration of Helsinki. The study is registered at clinicaltrials.gov as NCT00324129.

Patient Selection

Patients enrolled in this study were ≥18 years of age with a diagnosis of relapsed or refractory AML, chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), or MDS (WHO classification criteria). Patients > 60 years of age with previously untreated AML or MDS, who refused or were not candidates for induction chemotherapy, were also eligible. Patients were required to have an ECOG performance status score of ≤ 2; have adequate hepatic function (total bilirubin ≤ 2 mg/dL; have aspartate aminotransferase [AST] or alanine aminotransferase [ALT] ≤ 3 x the upper limit of normal); and have adequate renal function (serum creatinine ≤ 2.0 mg/dL or a calculated creatinine clearance > 50 mL/min; or proteinuria < 2+ on urine dipstick. Patients were excluded from the trial if any of the following criteria were present: other active malignancies or suspicion of central nervous system involvement; pregnancy or breast feeding; serious intercurrent illnesses, medical conditions, or other medical history (including known HIV or hepatitis B or C), which, in the investigator’s opinion, would be
likely to interfere with a patient’s participation in the study or interpretation of the results; treatment with any investigational drug within 30 days prior to study initiation; concurrent treatment with other experimental drugs or anti-cancer therapy; known hypersensitivity to any of the components of MGCD0103; and prior treatment with known HDAC inhibitors. The study was conducted at 3 centers in North America: Princess Margaret Hospital, Toronto, ON, Jewish General Hospital, Montréal, PQ, and MD Anderson Cancer Center, Houston TX. The study was approved by the local institutional ethics committee of each institution. All patients signed informed consent forms as per institutional guidelines.

**Study Treatment**

The active pharmaceutical ingredient of MGCD0103 was prepared by Torcan (Toronto, Canada), and the drug product was prepared by Patheon (Toronto, Ontario, Canada). Finished drug product was provided in 2 mg, 10 mg, and 25 mg gelcaps. Patients were instructed to take the intended dose orally 3 times per week on an every other day schedule with 200 mL of a low pH beverage, e.g., carbonated drinks. A cycle was defined as 21 days. The initial dose level was 20 mg/m².

The MTD was defined as the maximum dose at which less than 2 out of 6 patients experienced a DLT. If 1 of 3 patients experienced a DLT at a specified dose level, 3 more patients were treated at that dose to confirm that the DLT was not observed in > 1 of 6 patients. If 0 of 3 or 1 of 6 patients experienced a DLT, accrual began at the next higher dose level. All patients that received 1 dose of drug were evaluable for the safety and PK assessments, and all toxicity assessment for DLT occurred with respect to the first cycle of therapy. For the purpose of DLT and MTD determination each patient was counted once, at their initial assigned dose. All patients who received at least 1 cycle of therapy were eligible for response assessment unless
disease progression had occurred. Patients were allowed to receive supportive and palliative care as clinically indicated throughout the study. The following treatments were not permitted during the study: other anti-cancer treatment including chemotherapy and radiotherapy, other investigational therapy or anti-neoplastic agents, or growth factor support for prophylactic use or as a substitute for a scheduled dose reduction.

Toxicities were graded according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0. Leukemia-specific blood/bone marrow toxicity was used to classify hematological toxicity. Non-hematologic DLT was defined as any drug-related ≥ Grade 3 non-hematologic toxicity, except for Grade 3 nausea, vomiting, or diarrhea associated with suboptimal premedication and/or management, ≤ Grade 3 ALT for > 7 consecutive days, or any drug-associated toxic effect leading to 2 or more missed doses per cycle, or any drug-associated toxic effect resulting in the delay of the subsequent cycle by > 7 consecutive days. Hematologic DLT was defined as prolonged myelosuppression after therapy administration defined by an absolute neutrophil count of ≤ 500 k/μL and a platelet count of ≤ 30 k/μL with a bone marrow cellularity ≤ 5% without evidence of leukemia involvement lasting for more than 42 days.

Response criteria

Response was assessed using bone marrow aspirates collected pre-treatment and before the end of cycle 2, or as clinically indicated. A complete response (CR) required an absolute neutrophil count of ≥ 1 x 10^9/L, platelet count of ≥ 100 x 10^9/L, no blasts in the peripheral blood, bone marrow cellularity of ≥ 20% with normal trilineage maturation, bone marrow blasts ≤ 5% and absence of extramedullary involvement. PR was considered if there was normalization of peripheral blood counts as for CR, and the complete disappearance of peripheral blasts was
observed with > 5%, but < 25% blasts in the marrow. A complete marrow response was considered if marrow blasts were ≤5% independent of peripheral counts.

**Pharmacokinetic evaluation**

The plasma concentration measurements for MGCD0103 were obtained from patients who received oral doses of 20, 40, 60, 70, or 80 mg/m² three-times weekly for 3 weeks (1 cycle). Total daily doses ranged from 36 mg to 170 mg. Blood samples for evaluation of MGCD0103 PK were collected during cycle 1 on Day 1 and Day 12 prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 hour post dose, and on Day 3 of cycle 1 prior to dosing. Whole blood was collected into a 5 mL sodium heparin Vacutainer tube and centrifuged at 3,500 rpm for 10 minutes at 4 °C. Plasma was aliquoted and stored at ≤−40 °C. Samples were analyzed using a validated HPLC/MS method with a detection limit of 0.5 ng/mL. Plasma concentrations of MGCD0103 versus time profiles were generated, and PK parameters were derived using noncompartmental methods with WinNonlin® Professional (Pharsight Corp., Mountain View, California). The following PK parameters were determined: maximum times (T_{max}, h) and maximum concentrations (C_{max}, ng/mL) of drug in plasma, area under the plasma concentration-time curve (AUC_{0-24h}, ng*h/mL), and elimination half-life of drug in plasma (t_{1/2}, h).

**Isolation of human mononuclear cells**

Blood samples were obtained pre-treatment and on Days 1, 3, 8, and 11 of cycle 1; Days 1 and 8 of cycle 2 and 4, and at the end of treatment. Whole blood was collected in sodium heparin tubes and shipped at ambient temperature to a central laboratory at MethylGene within 24 hour. Human PBMCs were separated using standard procedures.

**Histone acetylation**
Histone acetylation analysis, by enzyme-linked immunosorbant assay (ELISA), was performed using nuclear lysate or acid precipitated extracts from patient peripheral white cells. Isolated peripheral white cells for nuclear lysate extraction were washed in PBS and then lysed on ice for 10 minutes in Buffer A (10 mM Tris-HCl, pH 8.0, 1.5 mM MgCl₂, 5 mM KCl, 0.5% NP-4, protease inhibitors, and sodium butyrate). After centrifugation at 350 x g for 15 minutes, the nuclear pellet was washed in Buffer A and then lysed in nuclear lysis buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 1% NP-40, 1 mM EDTA, 10% glycerol, protease inhibitors, and sodium butyrate). Samples were sonicated and centrifuged, and the nuclear lysate was transferred to a fresh tube. Isolated peripheral white cells for acid extraction were lysed in Buffer A (10 mM Tris-HCl, pH 8.0, 1.5 mM MgCl₂, 5 mM KCl, 0.5% NP-4, protease inhibitors, and Sodium Butyrate). After centrifugation at 350 x g for 15 minutes, the nuclear pellet was washed in Buffer A and resuspended in cold water. Nonhistone proteins were precipitated with 3.3% H₂SO₄ for 1 hour, then cleared by centrifugation. Acid soluble proteins were recovered by overnight acetone precipitation and resuspended in H₂O.

Sandwich ELISAs from nuclear lysate extracts were performed for determination of histone protein acetylation. ELISA plates (black Maxisorp, Nunc #437111) were coated with anti-pan-Histone antibody (Chemicon, Millipore, Billerica, MA MAB052,) for 2 h at room temperature. The plates were washed with PBS and blocked with 1% BSA + 0.1% TritonX-100 in PBS. Nuclear lysate extracts (5 μg) were incubated in the plate with rabbit anti-acetyl-H3 antibodies (Millipore, Billerica MA, 06-599). Detection was with HRP-conjugated goat anti-rabbit antibody (Sigma, Oakville, Ontario A-0545). The HRP substrate Amplex-Red (Molecular Probes, Invitrogen, Burlington Ontario, A12222) was used according to the manufacturer’s instructions. ELISAs from purified histones: black plates (Maxisorp, Nunc
were coated with antihistone antibodies (Chemicon, Millipore, Billerica MA, product H11-4) and blocked with 1% BSA + 0.1% TritonX-100 in PBS. For the H3Ac ELISA, purified histones (2µg) were incubated in the plate with rabbit anti-acetyl-H3 (Upstate, Millipore, Billerica MA, 06-599); for the total H3 ELISA, purified histones (0.5µg) were mixed with rabbit anti-H3 (Abcam, Cambridge MA, product ab1791). For both H3Ac and H3, the detection antibody was HRP-coupled goat anti-rabbit (Sigma, Oakville Ontario, A-0545). The HRP substrate Amplex-Red (Molecular Probes, Invitrogen, Burlington Ontario, A12222) was used according to the manufacturer’s instructions.

**Whole cell HDAC enzyme activity**

Whole cell HDAC enzyme assays were performed in 96 well plates (Corning Inc, Lowell, Ca, Costar 3694) by seeding 8 x 10^5 isolated peripheral white cells per well, in a 50 µL reaction volume. These cells were incubated with 0.3mM Boc-Lys(ε-Ac)-AMC (Bachem, Torrance CA, I-1875), a membrane permeable HDAC substrate. After 1 h at 37 °C with 5% CO₂, the reaction was quenched with 1 µM TSA (BioMol, Plymouth Meeting PA, GR-309), cells were lysed with 1% NP-40, and the deacetylated product was cleaved with 1:60 diluted Fluor-de-Lys-Developer™ (BioMol, Plymouth Meeting PA, KI-105). The reaction was allowed to develop for at least 15 min at 37°C with 5% CO₂, then the fluorescent signal was detected at Ex 360, Em 470, with a cutoff of 435 on a fluorometer (Molecular Devices, Sunnyvale CA, GeminiXS). A standard curve of Boc-Lys-AMC (Bachem, Torrance CA, I-1880) allowed the conversion of fluorescent signal into micromoles of deacetylated product.

**Statistical Methods**
PK parameters were calculated based on actual blood sample collection times.

MGCD0103 plasma concentrations and PK parameters were summarized using descriptive statistics.
RESULTS

Patient characteristics

In all, 29 patients were enrolled in this study between February 2005 and July 2006. Patient characteristics are shown in Table 1. All 29 patients were eligible for the safety and PK analyses; only 23 patients were eligible for response assessment due to intercurrent illness (3 patients), toxicity (1 patient), or investigator/patient request (2 patients). Patients not eligible for the efficacy evaluation were removed from the study early during the first cycle of treatment. PK parameters were calculated for 27 patients. The most common diagnosis was AML in 22 patients (76%), followed by MDS in 5 patients (17%). Two other patients presented with either ALL or CML. Twenty-four (83%) patients had > 1 previous chemotherapy regimen including 4 patients (14%), who had also undergone allogeneic stem cell transplantation. Median age was 62 years (range 32 to 84 years). Cytogenetics were diploid in 11 of 29 (38%) patients, and abnormal in the 18 of 29 (62%) patients, including 2 patients with t(8;21) and 1 with t(9;22).

MGCD0103 dose escalation and toxicities

The most frequently reported adverse events were fatigue (22 / 29 patients, 75.9 %), nausea (20 / 29 patients, 69%), diarrhea (18 / 29 patients, 62%), vomiting (14 / 29 patients, 48.3%), and dyspnea (13 / 29 patients, 44.8%). The number of drug-related toxicities during cycle 1 of therapy by dose of MGCD0103 is shown in Table 2. No DLTs were observed with 20 or 40 mg/m² doses. Grade 3 diarrhea, vomiting, and/or fatigue/weakness were observed in 3 of 4 patients receiving a dose of 80 mg/m² three times per week. Therefore, 80 mg/m² was considered to have exceeded the MTD. A dose of 60 mg/m² three times per week was then evaluated. Three new patients were enrolled at 60 mg/m²/day with no significant toxicities observed. The 60 mg/m² cohort was expanded to include 8 additional patients. No significant toxicities were
observed in any patient at this dose level. Therefore, a new cohort was opened at 70 mg/m² per protocol. Of 6 patients treated at this dose level, 3 experienced DLTs (mucositis, acid reflux/gastritis, hip/leg pain in 1 patient each). Two of the patients treated at 80 mg/m² continued therapy at a reduced dose of 60 mg/m² without further significant toxicity.

Non-hematological drug-related adverse events are also listed in Table 2. Fatigue (7 patients) and abdominal pain (2 patients) were the most common Grade 3 (severe) non-hematological drug-related toxicities observed in patients receiving MGCD0103. Grade 3 nausea, vomiting, or diarrhea occurred in 1 patient each. There were no Grade 4 (life-threatening) nonhematological drug-related adverse events.

Clinical activity

Twenty-three patients were evaluable for efficacy. One patient receiving 60 mg/m² and 2 patients initially receiving 80 mg/m² followed by a dose reduction to 60 mg/m² achieved a complete bone marrow response (Table 3). The time to response was 1-2 cycles, and responses lasted for 1-3 cycles. No responses were observed in patients in dose groups receiving 20 mg/m² and the 40 mg/m² MGCD0103 three times a week. A summary of the characteristics of the patients who responded to MGCD0103 are also listed in Table 3. Of the three responders, 1 patient had refractory anemia with excess blasts (RAEB) and had received no prior therapy, and the other 2 patients had refractory AML and had received prior chemotherapy. For 2 of the responding patients the cytogenetics were diploid, and were complex for the other responding patient. The 2 patients initiated at the 80 mg/m² dose of MGCD0103 were subsequently reduced to 60 mg/m² for the majority of their treatment period due to gastrointestinal toxicity. The total duration of therapy ranged from 4-5 cycles for responders. Therapy was eventually discontinued in the responding patients for disease progression or lack of correction of peripheral blood counts.
All 3 responding patients exhibited histone acetylation and HDAC inhibition in peripheral blood cells.

**Pharmacokinetics**

Plasma MGCD0103 concentrations were measured serially up to 24 h after dosing on Day 1 to obtain single dose profiles, and on Day 12 to obtain “steady-state” profiles. PK data were available from all 27 patients on Day 1 and from 13 patients on Day 12. MGCD0103 plasma concentration-time profiles for Day 1 are presented in Figures 1A and 1B, and for Day 12 in Figures 1C and 1D. MGCD0103 was rapidly absorbed. On Day 1, the maximum plasma concentrations occurred between 0.5 hour and 1 hour postdose. The drug concentration decreased biphasically and remained quantifiable at 24 hour. Similar profiles were observed following dosing on Day 12.

MGCD0103 plasma PK parameters are presented in Figure 2 and Supplemental Table 1. C$_{\text{max}}$ values and exposure to drug (AUC) increased with dose up to the 60 mg/m$^2$ dose level. Although data were sparse at the higher dose levels, C$_{\text{max}}$ and AUC values appeared to plateau or, in some cases, even decrease following doses of 70 and 80 mg/m$^2$. The drug had an elimination half-life in plasma of 9 ± 2 h. Median T$_{\text{max}}$ ranged from 0.5 hour to 1.2 hour after single or multiple doses of drug, while mean t$_{1/2}$ values ranged from 7 to 11 hour. Mean clearance was CL/F = 123 ± 47 L/hour (%CV = 38) on Day 1. Individual patient C$_{\text{max}}$ (Figures 2A and 2B), AUC$_{(0-24h)}$ (Figures 2C and 2D), t$_{1/2}$ (Figures 2E and 2F), and T$_{\text{max}}$ (Figures 2G and 2H) values are plotted versus dose (expressed in mg/m$^2$ and in mg). These plots (Figures 2A-D) show a general trend of an increase in exposure as a function of dose, and illustrate the apparent plateau or downward trend at the higher dose levels. T$_{1/2}$ and T$_{\text{max}}$ (Figures 2E-H) appear independent of dose, as would be expected. Some inter-patient variability was evident.
An evaluation of PK exposure across all MGCD0103 Phase I studies was performed (data not shown) comparing predicted exposure (based on actual dose given) with body surface area (BSA). There was a minor trend toward inverse correlation between exposure and BSA. However, this was insignificant relative to the inter-patient variability and is felt not to be clinically significant to predicting a given patient's likely exposure. In this evaluation, patients with a very low BSA (BSA ≤ 1.6) were not more likely to have excess drug exposure, while larger patients (BSA ≥ 2.2) were not more likely to be under-exposed. When the pharmacokinetic parameters AUC, or Cmax were compared in patients not experiencing first cycle DLT with patients experiencing first cycle DLT (Figures 3 A, B), a clear correlation was not observed. However, the first cycle DLT in patients did correlate with the total daily dose they received (Figure 3C).

**Histone acetylation and inhibition of whole cell HDAC enzyme activity**

Histone H3 acetylation levels and whole cell HDAC enzyme activity were measured pre- and post-treatment in peripheral white cells as potential PD markers for MGCD0103 activity. In cycle 1, there was induction of average histone H3 acetylation observed 24 hour post an initial dose of MGCD0103, with maximal levels at 60 mg/m², and these levels reached a plateau following administration of a dose of 60 mg/m², the MTD (1.3-1.5-fold induction, Figure 4A). For all groups over 40 mg/m², 1/3 patients of each group were positive for induction. The average percent inhibition of HDAC enzyme activity during cycle 1 was increased in a dose-dependent manner up to the 40 mg/m² dose (group at 20 mg/m² vs group at 40 mg/m² p=0.085), where it reached a plateau at approximately 20-25% inhibition as compared to the baseline (Figure 4B).
DISCUSSION

In preclinical studies, several structurally diverse HDAC inhibitors, including MGCD0103\textsuperscript{16}; depsipeptide\textsuperscript{17}; hydroxamic acid HDAC inhibitors (SAHA [Vorinostat, Zolinza\textsuperscript{11,18,19}, LAQ824\textsuperscript{20}, and PXD101\textsuperscript{21}]; and aminobenzamide HDAC inhibitors (CI-994\textsuperscript{22,23} and MS-275\textsuperscript{12}) have been found to have potent antitumor activities, tumor specificity, and promising therapeutic potential in early-phase clinical trials. SAHA is a Class I and Class II non-specific hydroxamic acid HDAC inhibitor approved by FDA for the treatment of cutaneous manifestations of advanced refractory cutaneous T-cell lymphoma\textsuperscript{24}. Phase I clinical trials with these agents have determined that the more common toxicities with HDAC inhibitors are fatigue, gastrointestinal side effects and dose-related transient cytopenias and, to a lesser extent in a subset of these agents, cardiac toxicity (e.g., QTc prolongation). At the present time, several HDAC inhibitors are in development both in leukemias and other indications. Structurally and at the biological level, these agents are quite diverse, and it is not currently known whether one class of agents is better than others.

This study demonstrates that MGCD0103 administered orally three times a week is safe and active in patients with relapsed or refractory leukemia. Dose-limiting toxicities included primarily fatigue, nausea, vomiting and diarrhea, a symptom cluster that has been observed previously with this class of agents\textsuperscript{11}. These toxicities, however, were non-life threatening and were effectively addressed by dosing delay and dose reduction. There were no Grade 4 drug-related adverse events noted in this study. The MTD was 60 mg/m\textsuperscript{2} with this dosing regimen, which is approximately equivalent to a 110 mg flat dose. The findings that the PK reached a plateau at higher doses, and that the incidence of DLTs tracked most closely with total daily dose
rather than PK exposure, collectively suggest that unabsorbed drug may be limiting at very high doses.

We also demonstrated that MGCD0103 had favorable PK properties in this patient population with this dosing schedule. The drug was rapidly absorbed within 1 hour after oral administration, with a median T\text{max} ranging from 0.5 hour to 1.2 hours after single or multiple doses. Importantly, the drug had a long elimination half life in plasma of 9 hours, and remained quantifiable at 24 hours after a single dose. Drug exposure increased with doses up to 60 mg/m\textsuperscript{2}, and then did not increase significantly with further increases in dose. PK exposure at the 60 mg/m\textsuperscript{2} dose level exceeded the efficacious exposure in mouse xenograft models\textsuperscript{16}. These properties, along with the biological effect of prolonged histone acetylation, allowed for the thrice weekly dosing schedule.

The PD activity of HDAC inhibition was explored in this study. In addition to the traditional method of measuring histone acetylation in cell lysates, a novel whole cell enzyme assay was developed in order to monitor HDAC activity in peripheral white cells obtained from the clinical setting\textsuperscript{25}. This assay used a cell-permeable substrate with a fluorescent read-out, thus allowing evaluation of HDAC activity in live-cell populations. Considering the intricate complexity of transcriptional complexes that are disrupted in the process of measuring histone acetylation by traditional methods, this novel whole-cell HDAC activity assay maintains the integrity of these transcriptional and other protein-DNA complexes, and significantly contributes to the armamentarium of tools to study the PD effects of HDAC inhibitors. This assay has been validated in a variety of systems, and in patients with solid tumors, HDAC enzyme inhibition was dose-dependent and correlated well with drug accumulation in plasma\textsuperscript{16,25}. The true HDAC inhibition in peripheral white cells in leukemia patients may in fact be underestimated, as the
apoptosis of peripheral blast cells may reduce the cell population most sensitive to drug treatment, particularly at time points following prolonged exposure to drug. Once further validated, this method could be adapted into a high throughput clinical system to follow HDAC activity in real time and help facilitate therapeutic decision-making. Other assays exist, however, that could be used to assess HDAC inhibitory activity such as Western blotting\textsuperscript{26,27}, ELISA\textsuperscript{19} or new assays\textsuperscript{28}. From the cumulative data published so far, it appears that histone acetylation is universal with these potent HDAC inhibitors, but that histone acetylation does not show a simple correlation with response\textsuperscript{19,26,27}. It should be noted that in this study, we did not analyze histone H4 acetylation. The results of the assays used for pharmacodynamic analysis are difficult to correlate with the pharmacokinetic characteristics of the drug, and it appears that there is a dissociation between the PD properties and the PK characteristics of MGCD0103 (Li Z et al, manuscript submitted) and other HDAC inhibitors\textsuperscript{19}. This PD/PK dissociation has implications for clinical trial development, as other schedules (daily or more prolonged exposure) may be beneficial. Studies examining these issues are ongoing. It is possible that cell selection, for example CD34 positive cells, may provide a more precise assessment of molecular effects secondary to therapy. Although using these cells is possible, prior Phase I studies conducted by our group have failed to detect differences between peripheral and bone marrow biomarker expression in patients with AML or high risk MDS\textsuperscript{19,26,27}.

Clinical activity with MGCD0103 was observed in this population of patients with refractory, relapsed acute leukemia and MDS. Three patients on the study achieved a complete bone marrow response at doses of 60 mg/m\textsuperscript{2} and above, suggesting a possible dose response at these levels. Of significance, 2 of the 3 responding patients had been previously treated with chemotherapy for refractory AML. The time to response observed in this study was 1-2 cycles,
with the duration of response ranging from 1-3 cycles. All 3 patients had histone acetylation and HDAC inhibition as measured by the PD assays, but these limited observations could not necessarily be statistically correlated with response. Although preliminary, the single agent activity of this oral, isotype specific HDAC inhibitor in such a high risk, refractory population is notable and should be investigated further in efficacy trials.

In summary, we demonstrate that MGCD0103 is safe, and has clinical activity when administered orally as a single agent in patients with heavily pretreated AML, and in a patient with untreated INT-1 MDS. It is important to emphasize that MGCD0103 is highly selective for HDACs 1, 2, 3 and 11, with negligible ability (IC_{50} > 10 \mu M) to inhibit class II HDACs. Since preclinical work demonstrates distinct biologic sequelae from different spectra of HDAC inhibition and a significant body of evidence implicates HDAC 1 in cancer, we hypothesize that an isotype-selective HDAC inhibitor will improve the therapeutic window, thus allowing greater efficacy for a given amount of toxicity when HDACs are targeted. The PK properties of MGCD0103 allow for administration in a thrice weekly schedule and maintenance of the biological effect of histone acetylation. Further preclinical and clinical studies with MGCD0103 alone and in combination with other cytotoxic and or targeted therapies will help define the best timing and possible synergistic activity. Subsequent controlled studies will be necessary to characterize the magnitude of this activity.

**Conflict of interest:** AK, JL, MD, T-A P, ZL, JMB, GR, and RM are employees of MethylGene and EL is an employee of Pharmion. Both companies are developing MGCD0103 in conjunction and therefore have financial ties to the data presented here.
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Author contribution: GGM and MM designed the study, wrote the manuscript, treated patients on study, and analyzed data. SA, JC, ZE, HK, WMN, and WM treated patients on study and analyzed data. HY, AK, JL, MD, TAP, ZL, JMB, GR, EL performed correlative studies and/or analyzed data. RM designed the study, analyzed data and wrote the manuscript.
# TABLES

**Table 1: Patient Characteristics (n = 29)**

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</tr>
<tr>
<td>Prior Treatment</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2 [0-5]</td>
</tr>
<tr>
<td>Untreated</td>
<td>5 (17)</td>
</tr>
<tr>
<td>1</td>
<td>8 (28)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>16 (55)</td>
</tr>
</tbody>
</table>

*AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; ALL = acute lymphocytic leukemia; MDS = myelodysplastic syndromes*
Table 2: Non-hematological Drug-related Toxicities in Patients Treated with MGCD0103

<table>
<thead>
<tr>
<th>MGCD0103 (mg/m²)</th>
<th>N°. of patients</th>
<th>N°. patients with 1st Cycle DLT</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>11</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Most Common (≥ 10%) Drug-Related Non-Hematological Toxicities (n=29)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Total</th>
<th>N°. patients by worst Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Anorexia</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Total number of treatment cycles in the study was 56; the median number of treatment cycles was 1 (range 1-6). Other drug-related grade 3 non-hematologic toxicities occurring in 2 or fewer patients included gastrointestinal reflux disease in 2 patients and mucosal inflammation, abdominal distension, arthralgia, bacteremia, constipation, gastritis, hematuria, lower GI hemorrhage, and extremity pain occurring in 1 patient each. No Grade 4 or 5 toxicities were reported.
Table 3: Clinical Response to MGCD0103

<table>
<thead>
<tr>
<th>MGCD0103 (mg/m²)</th>
<th>N</th>
<th>Evaluable* (N⁰)</th>
<th>Marrow Response (N⁰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>11</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>23</td>
<td>3</td>
</tr>
</tbody>
</table>

*Evaluable: >1 week treatment, Marrow Response = Complete bone marrow response, blasts ≤ 5%

Characteristics of Patients Responding to MGCD0103

<table>
<thead>
<tr>
<th></th>
<th>76</th>
<th>52</th>
<th>58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>76</td>
<td>52</td>
<td>58</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Disease</td>
<td>MDS; INT-1</td>
<td>AML</td>
<td>AML</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Diploid</td>
<td>Complex, 43-46, X, -X, add (3)(q35), +10, t(11;12)(p13;p12)</td>
<td>Diploid</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>None</td>
<td>• Ida + Ara-C</td>
<td>• Decitabine</td>
</tr>
<tr>
<td>Treatment course</td>
<td>80 mg/m² → 60 mg/m²</td>
<td>80 mg/m² → 60 mg/m²</td>
<td>60 mg/m²</td>
</tr>
<tr>
<td>Time to response</td>
<td>2 Cycles</td>
<td>2 Cycles</td>
<td>1 Cycle</td>
</tr>
<tr>
<td>Response duration</td>
<td>No repeat aspirate</td>
<td>3 Cycles</td>
<td>1 Cycle</td>
</tr>
<tr>
<td>On study duration</td>
<td>4 Cycles</td>
<td>5 Cycles</td>
<td>4 Cycles</td>
</tr>
<tr>
<td>Max HDAC inhibition†</td>
<td>23%</td>
<td>63%</td>
<td>38%</td>
</tr>
<tr>
<td>Max histone acetylation†</td>
<td>149%</td>
<td>337%</td>
<td>338%</td>
</tr>
</tbody>
</table>

† Inhibition of HDAC activity and histone acetylation were measured in peripheral white cells collected from the patients (see Methods).
FIGURE LEGENDS

Figure 1: **MGCD0103 plasma concentrations profiles.** Blood samples for evaluation of MGCD0103 pharmacokinetics were collected during cycle 1 on Day 1 and Day 12 prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h post dose. Samples were analyzed using a validated HPLC/MS method. Dosing of MGCD0103 was 2 times per week. A, B. Plasma concentration-time profiles Day 1, n = 27 patients; C, D. Plasma concentration-time profiles Day 12, n = 13 patients.

Figure 2: **Relationships of MGCD0103 Cmax and AUC with dose.** Pharmacokinetic parameters were derived using noncompartmental methods with WinNonlin® Professional (Pharsight Corp., Mountain View, California). A. Individual $C_{max}$ versus Dose in mg/m² or Dose in mg (B) for Day 1 and 12. C. Individual $AUC_{(0-24)}$ versus Dose in mg/m² or Dose in mg (D) for Day 1 and 12. E. Individual $T_{1/2}$ versus Dose in mg/m² or Dose in mg (F) for Day 1 and 12. G. Individual $T_{max}$ versus Dose in mg/m² or Dose in mg (H) for Day 1 and 12.

Figure 3 A, B and C: **Correlation of AUC, Cmax and Total Daily Dose with DLT.** Individual patients are plotted along the X axis sorted by AUC (3A), Cmax (3B) or Total Daily Dose (3C). Patients with no DLT during the first cycle of treatment are represented by an X; patients who experienced a DLT during the first cycle are represented by a filled circle.

Figure 4: **Induction of histone acetylation and inhibition of histone deacetylase activity.** Blood samples from patients were obtained pre-treatment and on Days 1, 3, 8 of cycle 1. Whole blood was collected in sodium heparin Vacutainer tubes and shipped at ambient temperature to a
central laboratory at MethylGene within 24 h. Peripheral white cells were isolated using standard procedures. A. Average histone H3 acetylation. Induction of histone acetylation was analyzed using an enzyme-linked immunosorbant assay (ELISA). Non-biased data with standard error are shown. B. Average percent inhibition of HDAC enzyme activity. Whole cell HDAC enzyme assays were performed using 8 x 10^5 isolated peripheral white cells per well, which were incubated with 0.3mM Boc-Lys(ε-Ac)-AMC, a membrane permeable HDAC substrate. After 1 h at 37 °C with 5% CO2 the reaction was quenched with 1 μM TSA, cells were lysed with 1% NP-40, and the deacetylated product was cleaved with Fluor-de-Lys developer. The reaction was allowed to develop for at least 15 min at 37°C with 5% CO2, then the fluorescent signal was read at Ex 360, Em 470, with cutoff of 435nm on a fluorometer. Non-biased data with standard error are shown.
REFERENCES

Figure 1

**Figure 1 A:** Single Dose MGCD0103 Plasma Concentration-time Profiles on Day 1 following an Oral Dose of MGCD0103 (linear scale)

**Figure 1 B:** Single Dose MGCD0103 Plasma Concentration-time Profiles on Day 1 following an Oral Dose of MGCD0103 (semi-log scale)

**Figure 1 C:** Plasma Concentration-time Profiles for Day 12 following an Oral Dose of MGCD0103 (linear scale)

**Figure 1 D:** Plasma Concentration-time Profiles for Day 12 following an Oral Dose of MGCD0103 (semi-log scale)
Figure 3

A: Correlation of AUC with First-Cycle DLT

B: Correlation of Cmax with First-Cycle DLT

C: Correlation of Total Daily Dose with First-Cycle DLT
Figure 4

A. Induction of Histone H3 Acetylation

B. Inhibition of Histone Deacetylase Enzyme Activity
Phase I study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia

Guillermo Garcia-Manero, Sarit Assouline, Jorge Cortes, Zeev Estrov, Hagop Kantarjian, Hui Yang, Willie Mae Newsome, Wilson H. Miller, Jr, Caroline Rousseau, Ann Kalita, Jianghong Liu, Marja Dubay, Tracy-Ann Patterson, Zuomei Li, Jeffrey M. Besterman, Gregory Reid, Eric Laille, Robert E. Martell and Mark D. Minden

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