Phase I Study of MG98, an Oligonucleotide Antisense Inhibitor of Human DNA Methyltransferase 1, Given as a 7-Day Infusion in Patients with Advanced Solid Tumors

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Abstract

Purpose: To assess the safety and tolerability, pharmacokinetics, and early evidence of antitumor activity of escalating doses of MG98, an antisense oligonucleotide to DNA methyltransferase 1 (DNMT1), which has been shown to reduce CpG island methylation and allow reexpression of tumor suppressor genes in vitro.

Experimental Design: In this phase I, open-label study, patients with advanced solid malignancies were treated with escalating doses of MG98 administered as a continuous i.v. infusion over 7 days repeated every 14 days. Cohorts of three patients, which could be expanded to six patients, were studied. The maximum tolerated dose was defined as the highest dose at which no more than 33% of subjects experienced dose-limiting toxicity. Pharmacokinetic and pharmacodynamic parameters of MG98 were also characterized.

Results: Thirty-three patients were treated at doses of 100 to 250 mg/m²/d MG98. MG98 was well tolerated with mild fatigue and myalgia, dose-limiting toxicity was asymptomatic transaminitis, and the maximum tolerated dose was 200 mg/m²/d. One patient achieved a partial response and another prolonged disease stabilization. Plasma half-life of MG98 was short (2 hours), drug concentrations reaching a dose-dependent steady state during infusion with a volume of distribution equivalent to plasma volume. Suppression of DNMT1 expression was observed in 26 of 32 patients studied.

Conclusions: MG98 was well tolerated with early evidence of clinical activity. Proof of mechanism was observed and measurement of DNMT1 expression in peripheral blood mononuclear cells may be useful in future phase II development.

DNA methylation and hence reduced expression of tumor suppressor and growth regulatory genes have been proposed as both a tumor-initiating event and a potential cause of chemotherapy resistance in solid tumors (1, 2). Methylation of the CpG islands in the regulatory region of these genes has been shown to cause this reduced expression and has been proposed as a potential novel therapeutic target (3, 4).

Several strategies have been used to attempt to overcome DNA methylation and restore gene function. Decitabine, a cytidine antimetabolite analogue that is incorporated into DNA and inhibits DNA methyltransferase (DNMT), has entered phase I and II studies in combination with carboplatin (5) and has been approved as a single agent in hematologic malignancies (3, 6). Alternatively, antisense compounds have been developed, which reduce the expression of DNMT (7).

MG98 is a second-generation, 20-mer antisense compound with a phosphorothioate backbone. It is targeted to the 3′-untranslated region of the DNMT1 gene. In in vitro investigations, MG98 has been shown to suppress DNMT1 expression and to restore expression of the cyclin-dependent kinase inhibitor p16 (α-CDKN2A; ref. 8). However, in a previous study of MG98 infused continuously over 21 days, consistent suppression of DNMT1 expression could not be shown in the surrogate tissue, peripheral blood mononuclear cells (PBMC; ref. 9). A similar lack of effect was observed in a phase I study using an intermittent schedule of MG98 administration (10).

In this phase I study of MG98 administered as a 7-day continuous infusion every 14 days, DNMT1 expression was monitored in PBMC during two cycles of therapy. This article describes the clinical results of this study. In addition, global DNA methylation was determined to investigate the
potentially downstream pharmacologic effect of this antisense compound.

**Materials and Methods**

**Patient selection**

Patients gave written informed consent for all clinical and research aspects of the study according to national and institutional guidelines and the protocol was reviewed by central and institutional review boards.

Patients with relapsed or refractory histologically or cytologically confirmed advanced solid malignancies in which hypermethylation is thought to play a role and who had failed standard therapies were eligible. Appropriate tumor types were gastric, colonic, ovarian, breast, renal, and lung cancer and melanoma. Patients had measurable or evaluable disease and adequate hematologic function (platelet count $>10^9$/L or thrombocytopenic bleeding, and grade $\geq 3$ nonhematologic adverse events except alopecia, vomiting, or hypersensitivity reaction. Grade 4 vomiting despite maximal antiemetic therapy was considered a DLT. If, at any dose level, one DLT occurred in the first three patients treated, the cohort was expanded to a total of six patients. If DLT or toxicity that delayed dosing occurred in only one of six patients at a specific dose, an increased dose level was evaluated. If two or more patients experienced DLT or toxicity that delayed dosing, the next lower dose was expanded to a total of 12 evaluable (receiving at least two cycles of MG98) patients to establish the maximum tolerated dose (MTD). Patients had to meet all inclusion criteria and any toxicity had to have resolved to grade $\leq 1$ for retreatment.

MG98 was supplied by Vernalis in 5 or 10 mL single-use vials containing 50 mg MC98 and 40 mg lactose monohydrate or 200 mg MG98 and 160 mg lactose, respectively. Vials were reconstituted immediately before use with 2 or 8 mL of 0.9% sterile sodium chloride, giving a final concentration of 25 mg/mL in both cases. The drug was administered by ambulatory infusion pump via central venous access.

**Assessments**

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Treatment-related toxicities were those toxicities considered by the investigators to be unknown, possibly, probably, or definitely related to MG98. A physical examination, concurrent medication profile, assessment of performance status, and routine laboratory studies were done before treatment and at least weekly after treatment. Routine laboratory studies included a complete blood count, differential white blood count, serum electrolytes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, $\gamma$-glutamyltransferase, lactate dehydrogenase, serum creatinine, urate, and creatine phosphokinase. A medical history and pregnancy tests (in women of childbearing potential) were done before enrollment. Pretreatment studies also included an electrocardiogram, chest X-ray, and radiological studies for evaluation of all measurable and evaluable sites of disease. Appropriate tumor markers were assessed. Radiologic studies for disease status were repeated after every two or more patients experienced DLT or toxicity that delayed dosing, the next lower dose was expanded to a total of 12 evaluable (receiving at least two cycles of MG98) patients to establish the maximum tolerated dose (MTD). Patients had to meet all inclusion criteria and any toxicity had to have resolved to grade $\leq 1$ for retreatment.

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Sterile H2O, Taqman Universal PCR Master Mix, primers and probe for manufacturer’s instructions. cDNA was prepared using a standard Taqman each PBMC sample using RNeasy (Qiagen) according to the manufacturer’s instructions. RNA was isolated from whole blood using a standard kit (Qiagen) at the same sample times as whole blood DNA from each patient sample were measured by high-performance liquid chromatography. The degree of DNA methylation was expressed as a ratio of methylated cytosine to unmethylated cytosine based on chromatographic peak area. Briefly, genomic DNA was isolated from whole blood using a standard kit (Qiagen) at the same sample times as the DNA from the study participants. DNA was heated to 100°C followed by digestion with nuclease and alkaline phosphatase at 42°C and 37°C, respectively. Digested samples were analyzed by high-performance liquid chromatography on a Supelcosil LC18 15 cm × 4.6 mm, 3 µm column; the mobile phase was 50 mmol/L potassium phosphate (pH 4.0)/2.5% methanol/methanol (50:50) at a flow rate of 1 mL/min. 2’-Deoxycytosine and 5-methyl-2’-deoxycytosine had retention times of 5.3 and 10.5 min, respectively, and were quantified by UV absorbance at 285 and 276 nm.

**Pharmacokinetic studies**

Plasma samples for pharmacokinetic analysis of MG98 and the n-1 and n-2 metabolites were collected on cycles 1 and 2 for all study participants. Samples were taken before dose; 2, 4, and 6 h after starting the infusion; and 0.5, 1, 2, 3, and 4 h after stopping the 7-d infusion. A validated liquid chromatography-mass spectroscopy method, linear over the range 0.2 to 10 µg/mL, was used to analyze the samples as reported previously (12).

**DNMT1 expression measurements.** DNMT1 expression was determined in PBMCs using quantitative reverse transcription-PCR. Samples of PBMC were obtained using Lymphoprep before treatment and at 3, 7, and 10 d after the start of each cycle. Firstly, RNA was isolated from each PBMC sample using RNeasy (Qiagen) according to the manufacturer’s instructions. DNA was prepared using a standard Taqman kit. To each sample or standard was added the appropriate quantity of sterile H2O, Taqman Universal PCR Master Mix, primers and probe for DNMT1, and primers and probe for the internal standard (β-actin). Samples and standards were placed on ice before transfer to a 96-well plate. All samples from each patient were analyzed in triplicate on each plate, which also included a reference standard prepared in bulk from MCF-7 cells. Samples from each patient were analyzed three times (three plates) to ensure reproducibility and robustness of the data. Plates were analyzed on a GeneAmp 5700 real-time PCR machine (Applied Biosystems). Baseline and threshold values of cycle threshold (Ct) were set manually for each run. An iterative scheme for the exclusion of outliers on each plate was used, with any Ct value >40 automatically excluded. In calculating the standard curve, replicate analyses were removed if the predicted value differed by >6% from the actual value. In addition, single replicate values that differed by more than 1 Ct unit from the other two replicates were excluded, provided these latter two replicates were within 1 Ct unit of each other. Average rate of standard or sample failure per plate was <5%. Ct values for each sample were normalized to that of β-actin for the same sample, and within a plate, rDNMT1 expression values were normalized to that of the external standard prepared from MCF-7 cells. Data for each patient were plotted against time for each cycle of therapy for which there were samples. For each cycle of therapy, the degree of suppression of DNMT1 expression in PBMC (nadir value) was expressed as a percentage of the pretreatment value.

**Global DNA methylation.** Methylated cytosines in genomic DNA from each patient sample were measured by high-performance liquid chromatography. The degree of DNA methylation was expressed as a ratio of methylated cytosine to unmethylated cytosine based on chromatographic peak area. Briefly, genomic DNA was isolated from whole blood using a standard kit (Qiagen) at the same sample times as the DNA from the study participants. DNA was heated to 100°C followed by digestion with nuclease and alkaline phosphatase at 42°C and 37°C, respectively. Digested samples were analyzed by high-performance liquid chromatography on a Supelcosil LC18 15 cm × 4.6 mm, 3 µm column; the mobile phase was 50 mmol/L potassium phosphate (pH 4.0)/2.5% methanol/methanol (50:50) at a flow rate of 1 mL/min. 2’-Deoxycytosine and 5-methyl-2’-deoxycytosine had retention times of 5.3 and 10.5 min, respectively, and were quantified by UV absorbance at 285 and 276 nm.

**Results**

**Clinical.** Thirty-four adult patients with advanced solid tumors were recruited and 33 received at least one dose of study drug. One patient’s condition deteriorated before dosing. The treated population comprised 21 male and 12 female

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**Table 2. Summary of dose levels explored and number of cycles administered**

<table>
<thead>
<tr>
<th>Dose (mg/m²/d)</th>
<th>100</th>
<th>125</th>
<th>160</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients treated</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Total no. cycles administered</td>
<td>17</td>
<td>12</td>
<td>10</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>Median no. cycles (range)</td>
<td>5 (1-9)</td>
<td>2 (1-9)</td>
<td>3 (1-3)</td>
<td>2 (1-4)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>No. patients experiencing DLT (any cycle)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>No. patients requiring dose reduction</td>
<td>3—160 mg/m²/d</td>
<td>3—200 mg/m²/d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One patient received only 4 d of infusion and was replaced.

† Dosing at this level was discontinued after two of the first five patients treated experienced DLT.

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**Table 3. Drug-related toxicity by dose level in any cycle (worst grade observed)**

<table>
<thead>
<tr>
<th>Dose (mg/m²/d)</th>
<th>100 mg/m²/d</th>
<th>125 mg/m²/d</th>
<th>160 mg/m²/d</th>
<th>200 mg/m²/d</th>
<th>250 mg/m²/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 4)</td>
<td>(n = 18)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transaminitis</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Elevated alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

NOTE: Includes toxicity assigned by investigators as definitely, probably, and unknown drug association; only toxicity in cycle 1 considered definitely or probably associated with investigational agent was used to evaluate DLT.
adults with a median age of 58 (range, 36-76) and good performance status (ECOG performance status 0:1:2 = 7:25:1), all patients bar one being Caucasian in origin. The most common tumor types were colorectal cancer (7 patients, 21%), upper gastrointestinal tumors (8 patients, 24%), and pancreatic/biliary (4 patients, 12%). Patient demographics are summarized in Table 1. Only 18 (55%) patients had primary surgery for their disease, this relatively low proportion reflecting the preponderance of upper gastrointestinal cancers in the patient population. Sixteen (49%) patients had received prior radiotherapy and all 33 patients had received previous chemotherapy with a median of 2 (range, 1-9) cycles; the number of patients treated and the number of cycles at each dose level are summarized in Table 2. Three of the five patients who received MG98 at 250 mg/m²/d required a dose reduction to 200 mg/m²/d for transaminitis (three patients) and thrombocytopenia (one patient). Three patients in the expanded cohort (200 mg/m²/d) also had dose reductions due to transaminitis, fatigue, and elevation of alkaline phosphatase (one patient each). Details of all toxicities are given below.

**Toxicity.** The 7-day infusion of MG98 every 14 days was generally well tolerated, treatment-related toxicities being generally mild (Common Toxicity Criteria grade ≤2) fatigue, headache, and myalgia. DLT at the highest dose level explored (250 mg/m²/d) was grade 3 transaminitis (three patients) and grade 3 thrombocytopenia (one patient). The patient who developed grade 3 thrombocytopenia and transaminitis on cycle 1 had a dose reduction for cycle 2 but again developed grade 3 elevation of transaminases. However, in the two patients where transaminitis was the sole toxicity, this was observed in cycle 2 and a subsequent cycle was successfully given at a reduced dose. Although the cycle 2 DLT did not meet the protocol definition, the investigators felt this dose was the MTD and the dose level below (200 mg/m²/d) was expanded until 12 additional patients had received two cycles of drug. Within this expanded cohort of 18 patients, 3 patients developed grade 3 transaminitis on the first cycle and 3 different patients on subsequent cycles. The MTD for the regimen of a 7-day infusion of MG98 every 14 days is 200 mg/m²/d. Table 3 summarizes all drug-related toxicity observed (including that assessed by investigators as “possibly related” or “unknown”).

**Pharmacokinetics.** Pharmacokinetic data for MG98 and for the two major metabolites n-1 and n-2 (representing removal of successive nucleotides from the oligonucleotide) are shown in Fig. 1. MG98 concentrations remained at a dose-dependent steady-state level during the 7-day infusion period, with concentrations of n-1 and n-2 around 10% and 5%, respectively, of the parent oligonucleotide. The half-life of MG98 was short (2 hours; Table 4) and independent of dose. The area under the plasma concentration time curve increased from 210 ± 91 µg/L h at 100 mg/m² to 560 ± 219 µg/L h at 250 mg/m². Clearance did not significantly vary significantly with dose level, with an overall mean ± SD of 0.9 ± 0.3 L/h. Volume of distribution was small, approximating plasma volume 3.13 ± 0.91 L. The IC₅₀ value for MG98 against DNMT1 activity is 45 nmol/L (13), which greatly exceeded by the steady-state concentrations of 600 nmol/L (4 mg/L) seen at the higher doses in this phase I study.

**Pharmacodynamics.** Of the 34 patients enrolled on the study, DNMT1 data were available for 32 patients. Of these, 26 showed suppression of DNMT1 expression compared with baseline in at least one cycle of administration. Measurement of pretreatment expression of DNMT1 in 32 patients gave a median rDNMT1 value of 0.602 (range, 0.024-3.84).

Examples of DNMT1 expression levels for two representative patients are given in Fig. 2. Although there was some degree of variation in rDNMT1 observed among the replicate plates for each patient, within each plate a consistent pattern of decline in DNMT1 expression was observed. Figure 2A and B shows mean data over two cycles for two patients treated at 100 and 200 mg/m²/d, respectively. The data for two cycles of treatment in patients treated at the five dose levels in this study are summarized in Table 4. There was no difference in the degree of suppression of DNMT1 in PBMC between dose levels.

![Fig. 1. Mean plasma concentration time data for MG98 (A) and for the two major metabolites n-1 (B) and n-2 (representing removal of successive nucleotides from the oligonucleotide, C).](image-url)
Although DNMT1 expression was decreased in at least one cycle of treatment with MG98 in most patients, the time course of suppression and subsequent recovery differed among the patients. In some, a decrease in rDNMT1 was apparent at day 3 or 4 of treatment, whereas in others an effect was observed only after 7 days of treatment. Whereas DNMT1 expression recovered quickly in some patients, with a return to pretreatment values on day 8 or even day 7, others showed a suppression of expression lasting into the pretreatment sample for cycle 2.

Comparing the lower dose levels (100 and 125 mg/m²/d) with the two higher dose levels (200 and 250 mg/m²/d), the higher doses were associated with a more rapid decrease in DNMT1 expression in cycle 1 (median time to nadir, 3 versus 8.5 days; P = 0.0086). A similar difference between the high and low doses was also seen in cycle 2 (median time to nadir, 7 versus 14 days; P = 0.0175). In half of the patients studied, across all dose levels, an apparent rebound increase in DNMT1 expression was seen in the pretreatment sample for cycle 2.

In terms of the downstream effects of DNMT1 depletion, global methylation of DNA was quantified as the percentage of methylated to total cytosine in genomic DNA (expressed as a percentage). Mean pretreatment percentage for cycle 1 was 3.0 ± 0.5, with values of 3.3 ± 0.4, 2.6 ± 0.6, and 3.2 ± 0.4 on days 3, 7, and 8, respectively. Similar data were obtained in cycle two, with no significant or consistent decrease in DNA methylation on treatment with MG98.

**Antitumor activity.** Evidence of activity was observed with this schedule of MG98. One patient with esophageal cancer achieved a partial response and an additional patient with a gastrointestinal stromal tumor (with confirmed disease progression at study entry) showing very prolonged disease stabilization. This latter patient received nine cycles of MG98, at this point developing renal obstruction related to postsurgical fibrosis and was withdrawn from study for ureteric stenting. His disease remained stable for >3 years. He subsequently was diagnosed with an early-stage adenocarcinoma of the esophagus and died of treatment-related complications. Two further patients received four and five cycles of treatment, respectively (esophageal and biliary carcinomas).

**Discussion**

Methylation of CpG islands and subsequent suppression of expression of tumor suppressor genes has been identified as an important mechanism in oncogenesis and also in resistance to chemotherapy (14, 15). Several inhibitors of DNMTs have been developed and evaluated in clinical trials (2, 3, 16, 17). Inhibitors of DNMTs, such as azacytidine and decitabine, act on all DNMT isoforms, including DNMT3α and DNMT3β. In addition, these nucleoside inhibitors are incorporated into DNA and so may act by multiple mechanisms in exerting an antitumor effect (18–20). Decitabine has been shown to induce demethylation of the MAGEA CpG island in PBMCs, buccal cells, and tumor biopsies, as well as elevation of HbF expression (21). Using an antisense approach to blocking the action of DNMT1 has the potential advantage of specificity of

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**Table 4. Pharmacokinetic parameters (mean ± SD) for MG98 and summary of rDNMT1 data for each dose level**

<table>
<thead>
<tr>
<th>Dose level (mg/m²/d)</th>
<th>AUC(0-inf) (µg/L/h)</th>
<th>Half-life (h)</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with DNMT1 decrease</td>
<td>rDNMT1 % decrease</td>
<td>No. with DNMT1 decrease</td>
<td>rDNMT1 % decrease</td>
</tr>
<tr>
<td>100</td>
<td>210 ± 91</td>
<td>5.48 ± 7.48</td>
<td>3/3</td>
<td>63, 41, 17</td>
</tr>
<tr>
<td>125</td>
<td>200 ± 92</td>
<td>1.1 ± 0.4</td>
<td>3/3</td>
<td>54, 61, 81</td>
</tr>
<tr>
<td>160</td>
<td>332 ± 85</td>
<td>3.1 ± 1.5</td>
<td>1/1</td>
<td>61</td>
</tr>
<tr>
<td>200</td>
<td>507 ± 218</td>
<td>2.1 ± 0.9</td>
<td>14/18</td>
<td>53 (9-96)</td>
</tr>
<tr>
<td>250</td>
<td>560 ± 219</td>
<td>2.0 ± 0.8</td>
<td>3/5</td>
<td>38, 66, 14</td>
</tr>
</tbody>
</table>

**Note:** rDNMT1 data for 200 mg/m²/d dose level given as median (range). Other dose levels show all data in consistent order between the two cycles.

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**Fig. 2.** DNMT1 expression levels for two representative patients. A and B. Degree of variation in rDNMT1 observed among the replicate plates for two patients at 100 or 200 mg/m²/d. Y axis, rDNMT1 in peripheral blood lymphocytes relative to β-actin expression.
action in terms of DNA methylation and with regard to other DNMT isoforms. Preclinical studies with MG98 showed a consistent effect of decreasing DNMT1 expression and restoration of previously methylated gene targets.

This study reports the evaluation of the safety and toxicity of a prolonged i.v. infusion of an inhibitor of DNMT, MG98, and also showed for the first time in patients consistent suppression of target gene expression.

Previous clinical studies with MG98 that have used either intermittent (2-hour infusion twice weekly for 2/3 weeks) schedules or a continuous infusion over 21 days have identified MTDs of 360 mg/m² (10) and 80 mg/m²/d (9), respectively. The 7-day continuous infusion schedule used here, administered on alternate weeks, resulted in a MTD of 200 mg/m²/d. This schedule allowed delivery of a higher dose intensity of MG98 per week (700 mg/m²/wk) compared with the other schedules that delivered 480 and 560 mg/m²/wk, respectively. MG98 was well tolerated clinically.

In those previous studies (10, 22), although similar investigations of DNMT1 expression in PBMC were done, no consistent suppression of DNMT1 expression was observed. It is possible that this lack of clear signal was due to technical factors in sample handling. In both these studies, blood samples were kept for 24 hours either on ice or at “ambient temperature” before isolation of PBMC and RNA extraction. It is possible that this led to degradation of RNA or loss of PBMC, rendering the data inconsistent. In this study, samples were held on ice at all times and PBMCs were isolated and frozen to −80°C within 30 minutes of sampling.

Perhaps for this reason, depletion of DNMT1 RNA was observed in most of the patients studied. The degree of suppression of DNMT1 expression varied among the patients and even in the same patient sampled on successive cycles. There was no indication of a dose-response relationship in the data, with all patients at the two lowest dose levels showing evidence of DNMT1 suppression. However, the dose range examined in this study was relatively narrow (2.5-fold), as previous phase 1 studies of different schedules had allowed the use of a relatively high starting dose and this may explain the lack of a dose-response relationship. There is a suggestion from the pharmacodynamic data that time to nadir of DNMT1 expression might be an alternative dose-dependent phenomenon, with the reduction being more rapid at the higher dose levels.

The time course of suppression of DNMT1 expression mapped to the duration of infusion of MG98, but some individuals showed a delay in response toward the end of the infusion period, whereas others had decreased DNMT1 expression in the first posttreatment sample. The duration of effect was also variable, although DNMT1 expression frequently returned to pretreatment values after completion of the MG98 infusion. Pharmacokinetic data on MG98 in this study indicated that the drug rapidly attains steady-state concentrations in plasma during the continuous infusion but that elimination is also rapid after the end of infusion. Comparison of DNMT1 expression levels in PBMC with the pharmacokinetics of MG98 indicates that antisense effects are observed long after systemic elimination of the majority of the drug.

Comparison of the data from this study with previous clinical studies of antisense compounds shows that the degree and time course of suppression of the target gene was analogous to that seen here. A similar schedule of administration of the Bcl-2 antisense compound G3139, continuous infusion over 7 days on a 21-day cycle, produced suppression of Bcl-2 expression in CD138-positive bone marrow aspirates (23). A similar time course of suppression and recovery of expression was seen following a short infusion of a liposomal formulation of an antisense to c-Raf (24).

The anticipated downstream effect of suppression of DNMT1 expression would be a reduction in the overall degree of DNA demethylation. Indeed, this has been shown for the DNMT inhibitor decitabine, which can produce up to 50% decrease in the relative amount of methylated cytosine, between 6 and 12 days after a dose (25). The background levels of methylated cytosine found in the present study were similar to those reported previously and showed no decrease during or after MG98 treatment. It may be that cytosine methylation is not dependent on DNMT activity in nonproliferating tissues such as PBMC. The level of cytosine methylation recorded here is similar to that reported for bone marrow mononuclear cells (26). Investigation of changes in the methylation status of specific genes, such as p15 or p16, was not possible in the translational research samples obtained in this study. Such an analysis, which could confirm preclinical evidence for targeted activity (8), would be important to consider in future clinical trial design.

Overall, the data presented here indicate that MG98 was well tolerated and there were indications of potential clinical benefit. This oligonucleotide has a consistent effect on the expression of DNMT1 in PBMCs. Measurement of DNMT1 expression in PBMC may be a useful surrogate tissue in future phase II studies of this drug. It remains to be seen whether this class of agent will have sufficient activity as a single agent or may be better placed in combination with other biological or cytotoxic agents.

Disclosure of Potential Conflicts of Interest

G. Reid, J. Besterman, and R. McLeod are employed by MethylGene, Inc. H. Calvert, I. Judson, and L. Siu received clinical research support costs to support the clinical trial. Alan Boddy received research support for the pharmacodynamic assay laboratory research.

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References


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Ruth Plummer, Laura Vidal, Melanie Griffin, et al.


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