Cancer Investigation, 30:415–421, 2012
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.3109/07357907.2012.675381

informa
healthcare

CLINICAL TRANSLATIONAL THERAPEUTICS

MG98, a Second-Generation DNMT1 Inhibitor, in the Treatment of Advanced Renal Cell Carcinoma

Robert J. Amato,[1] Joe Stephenson,[2,6] Sebastien Hotte,[3] John Nemunaitis,[4] Karl Bélanger,[5] Gregory Reid,[6] andRobert E. Martell[7]

Department of Internal Medicine, The University of Texas Medical School/ Memorial Hermann Cancer Center, Houston, Texas, USA,[1] Cancer Treatment Center, Greenville, South Carolina, USA,[2] Juravinski Cancer Center, Hamilton, Ontario, Canada,[3] Mary Crowley Research Center, Dallas, Texas, USA,[4] Hôpital Notre Dame du CHUM, Montreal, Quebec, Canada,[5] MethylGene, Inc., Montreal, Quebec, Canada,[6] Tufts Medical Center Cancer Center, Houston, Texas, USA[7]

Background: In carcinogenesis, methylation of DNA promoter regions results in inactivation of tumor-suppressing genes. MG98 was designed to inhibit DNA methyltransferases enzyme 1 production.

Methods: This multicenter study explored two schedules of MG98 with Interferon-α-2β to identify schedule and dose for patients with metastatic RCC.

Results: Doses of IFN 9 MIU/MG98 125 mg/m^2 for a continuous schedule and IFN 9 MIU/MG98 200 mg/m^2 for an intermittent schedule were considered the MTDs. Treatment resulted in one PR and eight SD.

Conclusion: MG98 combined with IFN was safe and resulted in clinical activity.

Keywords: Kidney cancer; Tumor suppressor gene; DNA methylation; CpG; DNMT; Interferon; MG98

INTRODUCTION

Renal cell carcinoma (RCC) represents 5% of epithelial cancer diagnosed annually in the United States, and 20–30% of RCC patients present with metastatic disease, which has a poor prognosis (1, 2).However, treatment options for patients have improved substantially over the last few years following FDA approval of sunitinib, sorafenib, pazopanib, temsirolimus, everolimus, and bevacizumab for use against advanced RCC. Despite such advances, the management of metastatic renal cancer remains a challenge. The ability to layer a new therapeutic agent on top of an existing therapy without any increased toxicity or reduced efficacy would be of great value. Metastatic RCC is strongly resistant to chemotherapy and radiotherapy, and a review of numerous chemotherapeutic treatments revealed a total response rate of 2–6% (3). Past clinical guidelines recommended immunotherapy, including interleukin-2 (IL-2) and/or interferon-alpha (IFN-α), for patients with metastatic disease while only a selected population may essentially benefit in terms of survival parameters (4). There has been a breakthrough in the exploration of new drugs targeting angiogenesis and other molecular mechanisms of tumorigenesis, such as inhibitors of mammalian target of rapamycin (mTOR) kinase (e.g., temsirolimus and everolimus); and multitarget inhibitors of multiple kinases, including receptors of Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor (PDGF) (e.g., sorafenib, sunitinib, and pazopanib; and monoclonal antibody to VEGF (i.e., bevacizumab) (5–11).

The above results are promising, yet the majority of patients eventually develop resistance. Thus, further research allowing improvement of the mRCC treatment options is a high priority.

Gene therapy offers a new approach for RCC treatment. Researchers have investigated the role of mutations in tumorigenesis, as well as epigenetic modifications that are not caused by alterations in the primary nucleotide sequence of DNA (12). One example of epigenetic modification inducing tumorigenesis is the methylation of DNA 65 promoter regions, which results in activation of tumor-suppressing genes, such as RASSF1A, VHL, and STAT (13). The DNA methylation pattern is normally controlled by interaction of *cis*- and *trans*-acting signals, as well as enzymes known as DNA methyltransferases (DNMT) with the DNMT1 enzyme playing a key role in maintenance methylation (14–19). In cancer cells, DNMT1 appears to be responsible for most DNA-methylating capacity (20, 21), and it is an attractive target for anticancer therapy (22). MG98, a second-generation antisense oligonucleotide molecule, was specifically designed to inhibit DNMT1 production with minimal toxicity by binding via Watson-Crick base pairing to a selected region within a target messenger ribonucleic acid (mRNA). By binding with DNMT1 mRNA MG98 interferes with its further processing and production of the enzyme DNMT1, thereby reducing its cellular concentrations. In preclinical studies, MG98 reinduced expression of a silenced gene as evidenced by p16^{ink4a}

Correspondence to: Robert J. Amato, DO, Department of Internal Medicine, The University of Texas Medical School/Memorial Hermann Cancer Center, 6410 Fannin, Suite 830, Houston, TX 77030, USA. email: Robert.Amato@uth.tmc.edu

Table 1. Definition of Dose Levels*

CONTINUOUS INFUSION SCHEDULE			INTERMITTENT INFUSION SCHEDULE		
Dose Level (DL)	MG98 Dose (mg/m^2/day)	Interferon Dose (MIU/day)	Dose Level (DL)	MG98 Dose (mg/m^2/day)	Interferon Dose (MIU/day)
DL 3	150	12	DL 3	200	12
DL 2.5	150	9	DL 2.5	200	9
DL 2	125	12	DL 2	160	12
DL 1.5	125	9	DL 1.5	160	9
DL 1	100	9	DL 1	120	9

*Shading indicates initial dose.

protein production; furthermore, it was demonstrated to cause a decrease in proliferation of a variety of growing cancer cells. Of note, in tumor cell lines moderately to highly resistant to IFN-α, pretreatment with MG98 for 8 days re-established sensitivity to the apoptotic effects of IFN-α (23). Further *in vitro* studies have been preformed to examine the effects of combination treatment with MG98 and IFN. These experiments examined the apoptotic effects IFN-α on pretreated RCC tumor cell lines that were moderately to highly resistant to IFN. Results show that pretreatment of these cell lines with MG98 (40 nM) for 8 days renders the cell more susceptible (or re-establishes sensitivity) to the apoptotic effect of both IFN-α-2β and β-2α. Cells pretreated with MG98 followed by IFN were as much as 30% TUNEL positive versus less than 10% TUNEL positive in cells treated with a mismatch oligonucleotide control. The observed sensitization of cells to IFN induced apoptosis after selective depletion of DNMT1 by MG98 suggests that silencing of genes participating in the cellular response to IFNs may contribute to resistance of renal cancer to IFN, and this resistance may be overcome by DNA demethylating agents (24). This dose-finding study explored two methods of MG98 administration (continuous intravenous (IV) infusion and an intermittent IV regimen), taken in combination with IFN-α-2β, to identify the administration schedule and dose. The study aimed to compare the drug toxicity and to assess both the clinical and biological activity of these two schedules.

MATERIALS AND METHODS

Study design and conduct

This was an open-label multicenter study from November 2004 to June 2006. Patients were assigned to either an intermittent MG98 treatment schedule administered twice weekly intravenously or a continuous MG98 7-day IV infusion. In both arms, the treatment was given in combination with IFN. Each tested regimen was given for at least two cycles, with each cycle comprised of 4 weeks; subsequent follow-up treatment could allow for as many cycles as was medically appropriate and/or tolerable. Disease assessment was performed every 8 weeks or earlier if clinically indicated. Initially in each regimen, patients were enrolled in cohorts of three with the starting dose considered intermediate based on previous clinical experience. The second stage of the study was intended to evaluate single agent IFN versus MG98 + IFN, but it was not initiated because of early closure of the study. This was due to a lack of patient accrual as a result of the development of molecular-targeted agents such as sorafenib, sunitinib, and everolimus.

Patients were assigned to predefined dose levels (Table 1) according to the following algorithm. When the three patients had completed one cycle of treatment, the toxicity profiles were assessed prior to opening accrual to the next cohort in order to determine the dose level for the next cohort and to choose the size of the next cohort (three or six) in each regimen, according to the pre-established protocol algorithm. When none of the first three patients experienced a dose-limiting toxicity (DLT), the dose of MG98 for the next cohort was increased to the next whole numbered dose level. In contrast, when $\geq$2 of the three patients experienced DLT, the dose level (of IFN and/or MF98) for the next cohort was reduced to the next incremental dose level. For each regimen, the optimal dose was to be defined as the combination of the MG98 and IFN doses that were best tolerated and caused DLT in $\leq$1/6 patients. DLT was defined as an adverse reaction (at least possibly related to MG98 and/or IFN) evaluated according to the NCI Common Terminology Criteria for adverse events, Version 3, and meeting any of the following criteria: absolute neutrophil count $<0.5 \times 10^9$/L lasting $\geq$5 days, febrile neutropenia, thrombocytopenia $<50 \times 10^9$/L, thrombocytopenic bleeding $\geq$grade 3, elevation of ALT of grade 3 on two determinations at least 7 days apart or any grade 4 ALT elevation, grade 4 vomiting despite maximal antiemetic therapy, any treatment delay, interruption or withdrawal of any patient due to a prolonged recovery (more than 2 weeks) from toxicity, and any nonhematological toxicity $\geq$ grade 3.

An assessment of safety data and biological and clinical activity was conducted after the completion of two cycles of treatment. Postcycle 2 data were collected as available. Safety assessments included adverse events, incidence of grade 3–4 toxicities, laboratory evaluations including PTT, vital signs, and physical examinations. The parameters of biological activity included the number of patients achieving complete response (CR), partial response (PR), stable disease (SD), or patients with progressive disease (PD). The overall objective response (OR), the duration of the objectively confirmed CR/PR, as well as progression-free survival (PFS), and overall survival (OS) were also considered in the clinical activity evaluation. All responses were defined as per Response Eval-

uation Criteria in Solid Tumors (RECIST) ((25), and the OR was based on the confirmed evaluations.

The study was conducted according to local laws and regulations relevant to the use of new therapeutic agents in the country of conduct. Study approval from the applicable Institutional Review Boards and the informed consent of each patient were obtained.

Patient population

The following inclusion criteria were specified: confirmed RCC of predominantly clear cell histology and radiologically documented disease; at least one site of disease that is unidimensionally measurable as defined by RECIST criteria; age >18 years; prior resection of the primary tumor required; postnephrectomy progression of disease; demonstrated disease progression; no more than three prior treatment regimens for advanced disease, ECOG performance status 0 or 1; no history of cardiac disease ≥class 2 according to the New York Heart Association criteria, and without clinically significant ECG abnormalities. Laboratory eligibility criteria required absolute granulocyte count $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L and applied restrictions toward range of serum creatinine, bilirubin, hepatic enzymes, protein in urine, and PTT/INR values. Patients with documented brain metastases or a history of any prior invasive malignancy, as well as Hepatitis B, C, or HIV, were excluded.

Treatment

Each treatment cycle occurred over a 4-week period. In the intermittent group, patients were treated with a 2-hr IV infusion of MG98 given twice per week on Monday and Thursday, for three weeks, followed by one week of rest. The continuous group of patients received MG98 in two 7-day continuous infusions, with each treatment week followed by one week of rest. Potential dose levels for MG98 are designated in Table 1 include an intermediate dose (DL2), a higher dose (DL3), and a lower dose (DL1). In addition, in both regimens, patients received IFN subcutaneously three days per week, typically Monday, Wednesday, and Friday, on an ongoing basis with an initial dose of 12 MIU/day (DL2 and 3) or 9 MIU/day (DL1.5 and 2.5). For each individual, the MG98 and/or IFN dose could be reduced during the cycle, depending on tolerability, with a maximum of two dose reductions for one or both agents. If further dose reductions were required, the patient was withdrawn from the study. Once the IFN dose was reduced it was not re-escalated. Toxicities were evaluated throughout the study on an ongoing basis.

Analysis

This dose-finding study was not intended to provide an adequately powered comparison between the two regimens. Rather, the goal was to identify the treatment schedule and dose for further testing. Thus, the sample size was chosen in order to provide reasonable confidence that a truly inferior arm is not likely to be selected. The decision on the regimen selection is to be made in a step-wise manner considering toxicity (specifically, the incidence of grade 3–4 toxicities, worst grade per patient per cycle) as the most important criterion. If the two schedules were equal in toxicity, then consideration would be given to the number of patients with early progression in each schedule. If these numbers were the same, then consideration would be given to DNMT1 mRNA levels as a surrogate marker of MG98 activity. For the purpose of the analysis, there were three cohorts defined for this study. The safety population and ITT populations included all patients who took their first dose of study medication: MG98, MG-98 with IFN, or IFN alone. The evaluable for response (ER) population included those ITT patients who completed at least one cycle and had their disease reassessed or who exhibited objective progression prior to the end of cycle 1. Patients withdrawn for reasons other than progression prior to completing their first cycle were considered not evaluable. The efficacy analyses were carried out using the "Observed Cases" method.

RESULTS

Patient disposition and treatment assignment

Two Canadian centers and four centers in the United States participated in the study. A total of 19 patients were enrolled and included in the ITT: 10 patients were included in the continuous regimen and 9 patients were included in the intermittent regimen. Patient characteristics are described in Table 2. A total of 17 patients completed the two cycles, while one patient experienced early progression at the end of the first cycle, and one patient expressed a desire to exit the study prematurely. Consequently, 18 patients were considered evaluable.

Characteristics of treatment

Initially, three patients were randomized to each treatment schedule at Dose Level two (DL2) (Table 1). Based on the DLT assessment and according to the preplanned protocol algorithm, the subsequent cohort of patients on the continuous regimen entered the study at DL1.5. In the intermittent regimen, one subsequent cohort was started at DL2.5, and another cohort was further increased to DL3.

Safety

The general toxicity profile of the MG98/IFN combination regimen with the severity representation is provided in Table 3. Side effect data collected among the institutions were based on the combination rather than the individual potential of each drug. Neutropenia, thrombocytopenia, reduction in white blood cells count, and fatigue appeared to be the most common Adverse Drug Reactions (ADRs) presenting with severity grade 3 or higher. Other frequently reported toxicities of the combination therapy, regardless of severity, were pyrexia, chills, anorexia, nausea, and vomiting (Table 3).

With regard to the number of related AEs, there was no significant difference between the regimens, but more severe neutropenias and thrombocytopenias were noted in the continuous regimen group. There was indirect evidence of better tolerability of the intermittent regimen as demonstrated by the greater number of patients who completed more cycles in the intermittent group compared to the con-

Table 2. Summary of Renal Cell Carcinoma Diagnosis (ITT Population)

		CONTINUOUS DOSING		INTERMITTENT DOSING		
n	All Subjects	Dose Level 1.5 (n = 7)	Dose Level 2 (n = 3)	Dose Level 2 (n = 3)	Dose Level 2.5 (n = 3)	Dose Level 3 (n = 3)
Pathological Diagnosis						
Clear Cell	16	6	3	3	2	2
Papillary Cell	1	0	0	0	1	0
Squamous Cell	1	0	0	0	0	1
Other	1	1	0	0	0	0
Median age at diagnosis (range)	59 (40-75)	61 (51-67)	69 (45-69)	59 (42-59)	40 (40-69)	59 (50-75)
Radical Nephrectomy (Nephrectomy with Regional Lymphadenectomy)	18	7	3	3[x]	3	3
Radiotherapy	4	0	2	1	0	1
Immunotherapy / Chemotherapy+	8	3	1	2	2	0
Surgery – non Nephrectomy++	7	3	1	1	1	1
Number of patients with poor prognostic factors (PPF)**						
0 PPF	8	5	2	1	0	0
1 PPF	6	1	0	1	1	2
2 PPF	3	1	1	1	1	0
3 PPF	1	0	0	0	0	1
4 PPF	0	0	0	0	0	0
Unknown	1	0	0	0	1	0

n, number of subjects.
[x]One patient had partial nephrectomy with regional lympadenectomy.
*TNM at initial diagnosis: T1N0M0 = 1; T1N0M1 = 1; T1N1M1 = 1; T2N0M0 = 3; T3N0M0 = 3; T3N2M1 = 1; TxNxM1 = 1; T3aN0M1 = 1; T3aN2M0 = 1; T3bN0M0 = 1; T3bN2M1 = 1; T3bNxM0 = 1; unknown = 3. 13 patients underwent lymphadenectomy at the time of nephrectomy.
+Prior systemic therapy included IL2, GMCSF, thalidomide, Avastin, interferon, Everolimus and ABT-510.
++Non-nephrectomy surgery included adrenalectomy; lung mass and node resection; lung mass resection; reduction of pathologic hip fracture; thoracotomy left upper lobe/ right thyroid removal; osteoplasty iliac bone; retroperitoneal tumor resection.
**Poor prognostic factors: <1y from dx; Hb <LLN; LDH>1.5ULN; Corrected Ca>10mg/dL

tinuous regimen (Table 4). In the intermittent treatment group, four patients completed four cycles, two patients completed eight cycles, and one patient finished nine cycles versus two, zero and zero patients in the continuous regimen, respectively.

In the continuous schedule at DL2 (IFN 12 MIU/MG98 125 mg/m^2), there were two patients out of three enrolled with DLT: thrombocytopenia and thrombocytopenia/neutropenia. The next lower dose level DL1.5 (IFN 9MIU/MG98 125 mg/m^2) had two DLTs out of seven patients enrolled, fever/chills and thrombocytopenia. In the intermittent schedule, there were no DLTs in three patients at DL2 (IFN 12 MIU/MG98 160 mg/m^2). At DL3 (IFN 12 MIU/MG98 200 mg/m^2), there were two DLTs in three patients: postinfusion reaction/shortness of breath/nausea/confusion and nausea/fatigue/weight loss/anorexia. DL2.5 was implemented for the intermittent cohort (IFN 9 MIU/MG98 200 mg/m^2), which resulted in no DLTs out of three patients. DL1.5 for the continuous schedule and DL2.5 for the intermittent schedule were considered the MTDs, however, due to early discontinuation of the study it was not possible to optimally characterize these dose levels.

Response to treatment

Best response to the combination treatment of MG98 and IFN included no CR, one PR (5.6% of all patients), eight SD (44.4%), and nine PD (50%) (Table 5). The patient with PR had a history of T3bN0M0 disease at diagnosis. At time of study entry, the patient had one out of four poor prognostic factors and had multiple lung metastases, several of which were 3 cm in diameter. The time to first documented progression on study was 34.3 weeks for this patient. There was one more patient, whose response was evaluated by the investigators as PR, but this was not confirmed as an OR. In one additional patient, whose metastatic sites included lung, bone, and mediastinum, there was observed a clinical advantage with respect the pain reduction, improvement in ECOG status, prolonged time to progression (382 days), and greater than expected survival (554 days) from the time of treatment.

For patients treated with MG98 as a continuous infusion, the best response was SD in 50% of patients (three and two patients at DL1.5 and DL2, respectively) and PD in 50%. The intermittent schedule resulted in PR in one patient (12.5% of patients from the intermittent group DL2.5), which occurred after 8 weeks of treatment and lasted for 26.3 weeks. Also, in the intermittent treatment group, 37.5% of patients achieved

Table 3. Number of Subjects Reporting Treatment Related Adverse Events* by Highest Reported Grade (Safety Population)

		CONTINUOUS DOSING										INTERMITTENT DOSING														
		Dose Level 1.5 (n = 7) Grade					Dose Level 2 (n = 3) Grade					Dose Level 2 (n = 3) Grade					Dose Level 2.5 (n = 3) Grade					Dose Level 3 (n = 3) Grade				
System Organ Class	Toxicity	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Blood and lymphatic system disorders	Anaemia	7	0	0	0	0	1	0	1	1	0	3	0	0	0	0	2	1	0	0	0	3	0	0	0	0
Gastrointestinal disorders	Nausea	4	2	1	0	0	0	1	2	0	0	1	1	1	0	0	0	0	3	0	0	0	0	2	1	0
	Vomiting	4	2	1	0	0	2	1	0	0	0	2	0	1	0	0	3	0	0	0	0	1	1	0	1	0
General disorders and administration site conditions	Asthenia	7	0	0	0	0	3	0	0	0	0	3	0	0	0	0	2	0	0	1	0	1	0	2	0	0
	Chills	5	2	0	0	0	0	3	0	0	0	1	1	1	0	0	2	1	0	0	0	1	0	1	1	0
	Fatigue	2	2	2	1	0	1	0	2	0	0	1	2	0	0	0	1	0	1	1	0	0	1	0	2	0
	Influenza like illness	6	0	0	1	0	3	0	0	0	0	3	0	0	0	0	2	0	1	0	0	2	1	0	0	0
	Pyrexia	4	2	1	0	0	0	3	0	0	0	1	1	1	0	0	3	0	0	0	0	1	1	1	0	0
Investigations	Alanine aminotransferase increased	5	0	1	1	0	2	1	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0
	Neutrophil count decreased	4	0	1	2	0	1	0	1	1	0	1	0	1	0	1	3	0	0	0	0	3	0	0	0	0
	Platelet count decreased	3	2	0	1	1	0	1	0	2	0	2	0	1	0	0	2	1	0	0	0	3	0	0	0	0
	Weight decreased	6	1	0	0	0	2	0	1	0	0	2	1	0	0	0	2	1	0	0	0	2	1	0	0	0
	White blood cell count decreased	7	0	0	0	0	1	0	1	1	0	2	0	0	1	0	3	0	0	0	0	3	0	0	0	0
Metabolism and nutrition disorders	Anorexia	3	4	0	0	0	2	0	1	0	0	3	0	0	0	0	3	0	0	0	0	1	0	1	1	0
	Decreased appetite	5	2	0	0	0	3	0	0	0	0	3	0	0	0	0	2	0	1	0	0	2	1	0	0	0
Nervous system disorders	Headache	7	0	0	0	0	2	1	0	0	0	1	1	1	0	0	3	0	0	0	0	2	0	1	0	0
Respiratory disorders	Dyspnoea	7	0	0	0	0	3	0	0	0	0	3	0	0	0	0	2	0	1	0	0	1	0	1	1	0
Vascular disorders	Hypertension	6	0	0	1	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0

*Includes adverse events considered possibly, probably or definitely related AND reported by at least four subjects or by at least one subject with a grade of 3 or higher

SD (one and two patients at DL2 and DL3, respectively) and 50% had PD at the confirmed best response. There was noted no appreciable difference in the investigators' best response evaluations between the two treatment regimens. Of the 18 evaluable patients, 12 patients (66.7%) developed PD by the end of the study.

Progression free and overall survival

While the trial was not designed to assess PFS or OS in the first stage of the study, these were estimated. PFS was 15.6 and 10.1 weeks in the intermittent and continuous treatment groups, respectively. Of 19 patients in the ITT population, five from the continuous group and four from the intermittent schedule had died, resulting in an estimated median survival of 41.3 weeks (28.3 weeks and 75 weeks for the continuous and intermittent schedule, respectively).

DISCUSSION

The evidence for an association between methylation and RCC is convincing. DNA methylation appears to play a critical role in the regulation of gene transcription, and offers an alternate mechanism of genetic mutation whereby expression of a gene can be silenced. RCC is an example of an instance in which hypermethylation in CpG islands and gene promoters are prevalent. For example, hypermethylation of the promoter of the tumor suppressor gene von Hippel-Lindau (VHL) has been described.

The tumor suppressor genes silenced by methylation in RCC include RASSF1A (26), VHL (27–30), and members of the STAT family (STAT1, STAT2, and STAT3). In experimental models, the DNMT inhibitor azacitidine has been shown to restore VHL expression in cell lines and xenografts and to reduce xenograft tumor size (27). Regional DNA hypermethylation and DNMT1 protein overexpression have been demonstrated in patients with RCC (30). DNA hypermethylation has also been correlated with higher histologic grade, infiltrative growth, and vascular involvement, and has been shown to be associated with poor recurrence-free survival in these patients (30).

Molecules that target, bind, and inhibit DNMTs would appear to be excellent candidates for therapeutic use against RCC. This would include antisense oligodeoxynucleotides designed to bind, via Watson-Crick base pairing, to selected regions within a target messenger RNA (mRNA). One promising antisense inhibitor of human DNMT1 is MG98. By binding to DNMT1 mRNA, this second-generation, 20-nucleic acid oligonucleotide prevents further processing of the mRNA and reduces cellular levels of DNMT1. Two particular chemical traits of MG98 enhance its plasma half-life: (a) the substitution of naturally occurring phosphodiester linkages with phosphorothioate linkages, and (b) the inclusion of 2′-0-methyl modifications that increase the molecule's affinity for its mRNA target and reduce itstoxicity (31).

In vitro, MG98 has been shown to downregulate DNMT1 expression in a variety of cancer cells by reducing, in dose-

Table 4. Extent of MG98 Exposure (Safety Population)

	CONTINUOUS DOSING		INTERMITTENT DOSING		
	Dose Level 1.5 (n = 7)	Dose Level 2 (n = 3)	Dose Level 2 (n = 3)	Dose Level 2.5 (n = 3)	Dose Level 3 (n = 3)
Cycles Completed					
1	7	3	3	3	3
2	7	3	2	3	2
3	2	1	2	1	1
4	1	1	2	1	1
5	1	0	1	1	0
6	1	0	1	1	0
7	1	0	1	1	0
8	0	0	1	1	0
9	0	0	1	0	0
Dose Reduction Needed	5	3	3	2	2
Planned Dose per Patient per Cycle mg/m^2	1750	1750	960	1200	1200
Total Dose Received per Patient mg/m^2					
Mean,SD	6798 ± 5208	4675 ± 1500	3470 ± 2946	3320 ± 1950	2120 ± 1244
Median	4655	4725	2980	2400	2400
(min, max)	(2275, 17731)	(3150, 6150)	(800, 14054)	(2000, 5560)	(760, 3200)
Percent Planned Dose Received					
Mean, SD	121 ± 43	111 ± 57	79 ± 3.6	80 ± 21	77 ± 20
Median	108	90	78	83	67
(min, max)	(65, 179)	(67.5, 176)	(77, 83)	(58, 100)	(63, 100)
Total Dose Received (All patients, all cycles) mg/m^2	43806	14024.5	10410	9960	6360
Planned Dose (All patients, all cycles) mg/m^2	35000	14000	13440	14400	8400
Percent Planned Dose Received	125.2%	100.2%	77.5%	69.2%	75.7%

n, number of patients.

dependent and highly specific fashion, the cellular concentrations of mRNA (50% inhibitory concentration [IC50] 18–70 nmol/L) and protein (IC50 18–45 nmol/L) (31). In other *in vitro* studies, MG98 treatment has been shown to reactivate silenced tumor suppressor genes (e.g., $p16^{ink4a}$) in several cancer cell lines and, at concentrations of 25–76 nmol/L, to inhibit the proliferation of growing cancer cells. Supporting these *in vitro* findings are *in vivo* observations of MG98 induced, dose-dependent reductions in tumor growth in both human colon and murine lung tumor xenograft models. Although daily dosing led to the greatest reductions in tumor volume, twice or even once-weekly dosing also caused significant reductions.

In this study, the combination treatment for mRCC with MG98 and IFN revealed manageable safety profile that appeared to be better tolerated in the intermittent schedule. With regards to efficacy, PR was observed in one patient. One patient had unconfirmed PR, and one additional patient had symptomatic improvement associated with prolonged SD. This was observed in the intermittent group.

Given the above efficacy results and relatively acceptable tolerance, it may be worthwhile to continue investigations comparing MG98 in a combination with other agents in those tumor types that are known to have hypermethylation as an important causative effect of tumorigenesis, such as RCC. Recently, the approach to mRCC treatment has quickly evolved in view of the availability of new agents, such as sorafenib, sunitinib, pazopanib, temsirolimus, everolimus, and bevacizumab. Due to the promising results of PFS and OS in randomized studies (6–11), these drugs have been recently approved by the FDA, which allows expanding therapy options for patients with limited alternatives. Since RCC is one of

Table 5. Summary of Best Response, Investigator-Assessed – Confirmed Responses Only* (ER Population)

		CONTINUOUS DOSING		INTERMITTENT DOSING		
n (%)	All Subjects	Dose Level 1.5 (n = 7)	Dose Level 2 (n = 3)	Dose Level 2 (n = 2)	Dose Level 2.5 (n = 3)	Dose Level 3 (n = 3)
Complete Response	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Partial Response	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)
Stable Disease	8 (44.4%)	3 (42.9%)	2 (66.7%)	1 (50.0%)	0 (0.0%)	2 (66.7%)
Progressive Disease	9 (50.0%)	4 (57.1%)	1 (33.3%)	1 (50.0%)	2 (66.7%)	1 (33.3%)

n, number of subjects.
*Confirmation of Complete or Partial Response only. Confirmation of Stable and Progressive Disease not required.

the most therapy resistant tumors, development of new treatments is highly warranted. One innovative approach could be investigating the MG98 activity in combination with a molecular-targeted agent.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

ACKNOWLEDGMENT

The study was sponsored by MethylGene Inc.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Costa LJ, Drabkin HA. Renal cell carcinoma: new developments in molecular biology and potential for targeted therapies. Oncologist 2007;12:1404–1415.
3. Yagoda A, Petrylak D, Thompson S. Cytotoxic chemotherapy for advanced renal cell carcinoma. Urol Clin North Am 1993;20:303–321.
4. Bleumer I, Oosterwijk E, De Mulder P, Mulders PF. Immunotherapy for renal cell carcinoma. Eur Urol 2003;44:65–75.
5. Dutcher JP, Szczylik C, Tannir N, Benedetto P, Ruff P, Hsu A, Berkenblit A, Thiele A, Strahs A, Feingold J. Correlation of survival with tumor histology, age, and prognostic risk group for previously untreated patients with advanced renal cell carcinoma (adv RCC) receiving temsirolimus (TEMSR) or interferon-alpha (IFN). J Clin Oncol 2007;25(18S):5033.
6. Amato RJ, Jac J, Giessinger S, Saxena S, Willis JP. A phase II study with a daily regimen of the oral mTOR inhibitor RAD001 (everolimus) in patients with metastatic clear cell renal cell cancer. Cancer 2009; ebpub 20 Mar.
7. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon-alfa (IFN-α) as first-line treatment of metastatic renal cell carcinoma (mRCC): updated results and analysis of prognostic factors. J Clin Oncol 2007;25(18S):241S.
8. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM; TARGET Study Group. Sorafenib in advanced clear-cell renal-cell carcinoma. New Engl J Med 2007;356:125–134.
9. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. New J Med 2003;349:427–434.
10. Escudier B, Koralewski P, Pluzanska A, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Moore N. A randomized, controlled, double-blind phase III study (AVOREN) of bevacizumab/interferon-α2a vs placebo/interferon- α2a as first-line therapy in metastatic renal cell carcinoma. J Clin Oncol 2007;25(18S):3.
11. Hutson TE, Davis ID, Machiels JP, De Souza PL, Rottey S, Hong BF, Epstein RJ, Baker KL, McCann L, Crofts T, Pandite L, Figlin RA. Efficacy and safety of pazopanib in patients with metastatic renal cell carcinoma. J Clin Oncol 2010;28(3):475–480.
12. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. New Engl J Med 2003;349:2042–2054.
13. Szyf M. The DNA methylation machinery as a target for anticancer therapy. Pharmacol Ther 1996;70:1–37.
14. MacLeod RA, Rouleau J, Szyf M. Regulation of DNA methylation status by the ras signaling pathway. J Biol Chem 1995;270:11327–11337.
15. Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A, MacLeod AR. DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. Nat Genet 2003;33:61–65.
16. Kautiainen TL, Jones PA. DNA methyltransferase levels in tumorigenic and nontumorigenic cells in culture. J Biol Chem 1996;261:1594–1598.
17. Szyf M. DNA methylation properties: consequences for pharmacology. Trends Pharmacol Sci 1994;15:233–238.
18. Battaglia C, Uzzo RG, Dulaimi E, Ibanez de Caceres I, Krassenstein R, Al-Saleem T, Greenberg RE, Cairns P. Promoter hypermethylation of tumor suppressor genes in urine from kidney cancer patients. Cancer Res 2003;63:8695–8699.
19. Yeivin A, Razin A. Gene methylation patterns and expression. EXS 1993;64:523–568.
20. Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 2002;416:552–556.
21. Rhee I, Jair KW, Yen RW, Lengauer C, Herman JG, Kinzler KW, Vogelstein B, Baylin SB, Schuebel KE. CpG methylation is maintained in human cancer cells lacking DNMT1. Nature 2000;404:1003–1007.
22. Agrawal S, Iyer RP. Perspectives in antisense therapeutics. Pharmacol Ther 1997;76:151–160.
23. Frederic J. Reu, Mark W. Fox, Mamta Chawla-Sarkar, Normand Beaulieu, Douglas W. Leaman, A. Robert Macleod, Ernest C. Borden. Genes involved in sensitization of renal cancer cells to interferon-induced apoptosis after selective depletion of DNA methyltransferase-1 by antisense oligonucleotide (MG98). Proc Amer Assoc Cancer Res. Volume 45, 2004. Abstract #1609.
24. Reu FJ, Beaulieu N, Mama CS, Macleod RA, Borden EC. Selective depletion of DNA methyltransferase-1 by antisense (MG98) sensitized renal cancer cells to interferon-induced apoptosis. Clin Cancer Res 2003;9 (December Suppl. 16): Abstract #167.
25. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–216.
26. Battagli C, Uzzo RG, Dulaimi E, Ibanez de Caceres I, Krassenstein R, Al-Saleem T, Greenberg RE, Cairns P. Promoter hypermethylation of tumor suppressor genes in urine from kidney cancer patients. Cancer res 2003;63:8695–8699.
27. Alleman WG, Tabios RL, Chandramouli GV, Aprelikova ON, Torres-Cabala C, Mendoza A, Rogers C, Sopko NA, Linehan WM, Vasselli JR. The in vitro and in vivo effects of re-expressing methylated von Hippel-Lindau tumor suppressor gene in clear cell renal carcinoma with 5-aza-2'deoxycytidine. Clin Cancer Res 2004;10:7011–7021.
28. Clifford SC, Prowse AH, Affara NA, Buys CH, Maher ER. Inactivation of the von Hippel-Lindau (VHL) tumour spuppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: evidence for a VHL-independent pathway in clear cell renal tumourigenesis. Genes Chromosomes Cancer 1998;22:200–209.
29. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarra JR, Linehan WM. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 1994;9700–9704.
30. Arai E, Kanai Y, Ushijima S, Fujimoto H, Mukai K, Hirohashi S. Regional DNA hypermethylation and DNA methyltranserase (DNMT) 1 protein overexpression in both renal tumors and corresponding nontumorous renal tissues. Int J Cancer 2006;119:288–296.
31. Agrawal S, Iyer RP. Perspectives in antisense therapeutics. Pharmacol Ther 1997;76:151–160.