Phase I and pharmacologic study of the human DNA methyltransferase antisense oligodeoxynucleotide MG98 given as a 21-day continuous infusion every 4 weeks*

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Summary

Purpose: MG98 is a second generation phosphorothioate antisense oligodeoxynucleotide which is a highly specific inhibitor of translation of the mRNA for human DNA McTase I (DNMT 1). This phase I study examined the toxicity and pharmacologic profile of MG98 administered as a continuous 21-day intravenous infusion every 4 weeks. Patients and methods: Fourteen patients with solid cancers received a total of 25 cycles of MG98 at doses ranging from 40 to 240 mg/m²/day. Steady-state concentrations of MG98 were measured as were several pharmacodynamic assessments including mRNA of the target gene, DNMT1, in PBMC. In addition, other potential surrogate markers of drug effects were explored, including hemoglobin F, Vimentin and GADD45. Results: Dose limiting effects were drug-related reversible transaminase elevation and fatigue seen at doses of 240, 200 and 160 mg/m²/day. The dose level of 80 mg/m²/day was felt to be safe and tolerable when delivered on this schedule. No evidence of antitumor activity was observed. Although pharmacokinetic analysis revealed that at the higher dose levels, mean Css values of MG98 were approximately 10-fold times the IC₅₀ values associated with target inhibition in vitro, the extent of MG98 penetration into target tumors in this trial was not determined. No consistent, dose-related changes in correlative markers including DNMT1 mRNA, hemoglobin F, Vimentin and GADD45, were observed. Conclusions: This schedule of MG98 given as a 21-day continuous intravenous infusion every 4 weeks was poorly tolerated in the highest doses; therefore, further disease-site specific evaluation of the efficacy of this agent will utilize a more favorable, intermittent dosing schedule. Pharmacodynamic evaluations undertaken in an attempt to explore and validate the biological mechanisms of MG98 did not show dose-related effects.

Introduction

Cytosine methylation is well recognized as a post-replicative covalent modification of the genome, catalyzed by the enzyme DNA 5-cytosine McTase I [1]. The methylation process can alter epigenetic inheritance in contrast to genetic inheritance which effects changes in DNA sequence [2,3]. DNMT1 functions by transferring a methyl group from the ubiquitous methyl donor S-adenosyl methionine to the 5-position of cytosines residing in the dinucleotide sequence cytosine-guanine (CpG) [4]. Methylated

cytosines are found almost exclusively in the dinucleotide CpG, and approximately 80% of cytosines in this doublet are methylated in vertebrates [5], with the distribution being dependent on cell and tissue types as well as developmental stage [5–8].

When CpG dinucleotides lie within the promoter regions of genes, their increased methylation generally correlates with transcriptional silencing, but this inverse relationship has not been demonstrated for methylation in the transcribed parts of genes [9]. The precise mechanism by which hypermethylation represses gene transcription is unclear, but theories include interference with the binding of transcription factors to regulatory sequences, modification of chromatin structure which blocks access by transcription factors, or attraction of methylated-DNA-binding proteins such as MeCP1 or MeCP2 that suppress gene expression [3,10,11]. In some cancer cells, the promoter regions of tumor suppressor genes are hypermethylated such that their transcription is inhibited, resulting in carcinogenesis and tumor progression [10,12]. Multiple studies have shown aberrant DNA methylation patterns in tumor cells [10,13] and methylation also seems important in determining tumor response to chemotherapeutic agents. Hypermethylation of the promoter of the mismatch repair gene hMLH1 has been shown to induce cisplatin resistance in ovarian cancer [14]. In contrast, inactivation of the DNA-repair enzyme O6-methylguanine-DNA methyltransferase, through hypermethylation of its promoter, increases glioma sensitivity to alkylating agents [15]. Thus, methylation appears key target for anticancer therapy.

Antisense oligonucleotides are synthetic nucleic acids designed to hybridize to a selected region within a target mRNA transcript. Gene expression is impeded through destruction of the duplex by the endonuclease RNase H, or via steric inhibition of translation [16]. MG98 is a second generation phosphorothioate antisense oligodeoxynucleotide targeted to the 3’ untranslated region of the DNMT1 mRNA. Second generation agents compare favorably against their first generation predecessors with greater resistance against degradation by nucleases, longer plasma and tissue half-lives, better affinity for their mRNA targets, and improved therapeutic index [17–20]. MG98 is thus expected to be a potent inhibitor of the human DNA McTase.

Preclinical evaluation of MG98 in human cancer cell lines revealed IC50 values of 50–70 nM for the inhibition of DNMT1 mRNA [21]. In nude mice bearing human non-small cell (A549) and colon (Caco205) cancer xenografts, MG98 produced significant tumor growth delay and regression, as compared to mismatch and saline controls [21]. Frequent administration was more active than attenuated dosing. Based on these data, two phase I clinical trials of MG98 were initiated under the auspices of the National Cancer Institute of Canada Clinical Trials Group. The present study examined the results of MG98 administered as a 21-day continuous intravenous infusion given every 28 days. The objectives of the study were to establish the MTD of MG98 using this schedule, to determine the safety, toxicity, PK and PD profiles, and to describe any preliminary evidence of antitumor activity. Pharmacodynamic evaluations were performed to examine mRNA levels of the target gene, DNMT1, in PBMC. In addition, several other possible surrogate markers of drug effects were explored: HbF levels and expression of Vimentin and Gadd45, genes which were found to be upregulated by MG98 in preclinical assays.

Patients and methods

Patient selection

Key eligibility criteria included: (1) histologically or cytologically documented incurable advanced and/or metastatic solid tumor; (2) age ≥ 18 years; (3) ECOG PS ≤ 2 and life expectancy of at least 3 months; (4) ANC ≥ 1.5 × 10^9/L, platelets ≥ 100 × 10^9/L, bilirubin ≤ 1.25 times UNL, AST ≤ 3 times UNL (≤ 4 times UNL if documented liver metastases), serum creatinine ≤ 1.25 times UNL, urine protein < 2+ or ≤ 500 mg protein/24 h, normal aPTT; (5) up to 3 prior chemotherapy regimens; (6) no chemotherapy or investigational therapy within 3 weeks (no high dose chemotherapy within 12 months) and recovery from the acute toxic effects of prior therapies; (7) no known hypersensitivity to oligodeoxynucleotides; (8) no concomitant therapy with therapeutic warfarin or heparin; (9) no active brain or leptomeningeal metastases; and (10) no pregnant or lactating women. All patients gave written informed consent according to institutional and federal guidelines before treatment.

Study design

The starting dose of MG98 in this study was 40 mg/m²/day, administered as a 21-day continuous intravenous infusion followed by a 7-day rest period. Treatment cycles were repeated every 28 days. Dose selection was based on a 21-day i.v. infusion toxicology study