**In vivo** Bioavailability and Pharmacokinetics of a *c-MYC* Antisense Phosphorodiamidate Morpholino Oligomer, AVI-4126, in Solid Tumors

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**Abstract**

Phosphorodiamidate morpholino oligomers (PMO) inhibit targeted gene expression by preventing ribosomal assembly, thereby preventing mRNA translation. AVI-4126, a PMO targeted against *c-MYC*, has been extensively characterized in multiple cancer and other disease models and is currently in human clinical trials. A phase I clinical study was conducted to address the issue of PMO bioavailability in malignant tumors surgically excised from patients with adenocarcinoma of prostate and breast 1 day after i.v. administration of a single dose of 90 mg AVI-4126 PMO. The study objectives were to evaluate safety, to determine AVI-4126 concentration in tissue samples of the tumors, and to examine the distribution of AVI-4126 (margin versus tumor core). Significant concentrations of intact PMO similar to the animal models were detected in both human prostate and breast tumor tissues with increased distribution in the tumor core for the vascular breast tumors. No serious adverse events (graded according to National Cancer Institute Common Toxicity Criteria) were reported. Another phase I study was conducted in normal human volunteers to assess AVI-4126 plasma pharmacokinetics following single i.v. administration of 90 mg AVI-4126. Data from both human studies indicated similar plasma concentration-time profile. These studies show PMO bioavailability in tumor tissue and establish the feasibility of using PMO targeting specific genes in human cancer clinical trials.

The *c-MYC* proto-oncogene, which is frequently overexpressed in human cancer, plays a critical role in the control of cell proliferation, differentiation, and apoptosis (1, 2). Several different strategies, including antisense-mediated inhibition of the gene, have been employed to develop novel drugs that seek to inactivate *c-MYC* for the treatment of cancer (2). AVI-4126, a phosphorodiamidate morpholino oligomer (PMO) targeting *c-MYC*, has an excellent safety profile in preclinical animal models and in human clinical trials (3). PMOs are neutrally charged antisense agents, wherein the deoxyribose moiety of DNA is replaced with a six-membered morpholine ring and the charged phosphodiester internucleoside linkage is replaced with phosphorodiamidate linkages (4, 5). PMO antisense molecules are steric blockers and inhibit gene expression by physically preventing binding or progression of splicing or translational machinery components (5–7). The neutral character of the PMO chemistry avoids a variety of potential significant limitations, including the binding of other cellular and extracellular proteins that is observed with charged oligonucleotide chemistries (8, 9).

The 20-mer AVI-4126 sequence was chosen based on favorable solubility, efficacy, and potency compared with >100 other antisense *c-MYC* PMOs targeted to various sites along the *c-MYC* 5′ untranslated region, the splice acceptor of the first intron, and around the translation start site. Previous studies have shown that AVI-4126 inhibits *c-MYC* translation in a sequence-specific manner in cancer cell lines in culture by steric blockade and missplicing of pre-mRNA, resulting in significant growth inhibition (10, 11). AVI-4126 has been observed to specifically down-regulate *c-Myc* protein, decrease cyst formation, and improve renal function in a murine model of polycystic kidney disease (12); causes growth arrest in a rat liver regeneration model (13); and is effective in preventing cardiac restenosis in multiple animal models (14, 15). The safety profile of the PMO chemistry, including that of AVI-4126, has been widely studied in animal toxicity models as well as in human clinical trials after systemic and local routes of administration (3, 16). No serious side effects (graded according to the National Cancer Institute Common Toxicity Criteria) have been reported. AVI-4126 antisense PMO has also shown promising efficacy in tumor models of lung cancer (17) and prostate cancer (3, 18, 19). Therefore, the purpose of this study was to characterize PMO bioavailability in solid tumor models in animals and in surgically excised malignant tumors from patients after single systemic AVI-4126 administration and to assess pharmacokinetics and safety.
Materials and Methods

Oligomers
AVI-4126 (c-MYC antisense PMO: 5′-AGCITGGAGGCGATCGTCCG-3′) and control scrambled (5′-ACTGTAGGGCCATCGTCCG-3′) morpholino oligomers were synthesized at AVI BioPharma, Inc. (Corvallis, OR) as described previously (4, 18). Purity was >95% as determined by reverse-phase high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption ionization time of flight mass spectrometry.

Plasmid-based test system for screening phosphorodiamidate morpholino oligomer antisense activity
A fusion c-MYC luciferase construct (pCINeo-myc-LucΔA) was generated in the pCINeo expression vector (Promega, Madison, WI) as described previously (20). This plasmid features a T7 promoter capable of generating in vitro transcribed RNA from a cloned insert for use in cell-free rabbit reticulocyte in vitro translation reactions and a cytomegalovirus promoter for constitutive expression in mammalian cells. In vitro transcription was carried out with T7 Mega script (Ambion, Austin, TX).

Cell-free luciferase assay
In vitro translation was done by mixing rabbit reticulocyte lysate with known amounts of antisense, scrambled PMOs or vehicle (water) followed by addition of a known amount of the c-MYC/Luc RNA (~1 nmol/L final concentration). The Promega luciferase assay reagent protocol was followed. The percent inhibition of luciferase activity compared with control was calculated based on readings from a luminometer (Cardinal, Santa Fe, NM).

Cell culture
PC-3, LNCaP, DU145, human prostate cancer cell lines, and LLC1 murine Lewis lung cancer cell line were obtained from American Type Culture Collection (Rockville, MD). The prostate cancer cells were cultured in RPMI 1640 (Hyclone Laboratories, Logan, UT) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin.

Animals
Male athymic (Ncr nu/nu) nude mice and C57BL/6 mice (~4 weeks old) were obtained from Simonson Laboratories (Gilroy, CA) and were housed in sterile plastic cages at the Laboratory Animal Resources Facility at Oregon State University (Corvallis, OR). The athymic mice were housed in laminar airflow cabinets at the Laboratory Animal Resources Facility at Oregon State University (Corvallis, OR). The molds were wrapped in aluminum foil to protect from light and frozen in a −80°C freezer. Cryostat sections (5 μm) were cut at Oregon State University Veterinary Diagnostic Laboratory. Slides were air dried in the dark for 30 minutes and coverslipped mounted using Fluoromount-G mounting medium (Southern Biotechnology Associates, Birmingham, AL). The photomicrographs were taken with a Nikon Diaphot 300 microscope connected to an Olympus (Melville, NY) Magnafire SP-brand digital camera. The exposure times were kept constant for all fluorescent pictures at 25 seconds.

Flow cytometry of mouse tumor cells
Tumor cells from animals injected with fluorescein-labeled AVI-4126 were harvested by digestion with 0.1% collagenase (type I) and 50 mg/mL DNase as described previously (21). Dispersed cells were washed thrice with PBS and resuspended in fluorescence-activated cell sorting buffer. Flow cytometry was carried out at the Oregon State University Core Facility using the BD FACSCalibur cytometer (Becton Dickinson, Mountain View, CA) and data were analyzed using the FCS express software.

High-performance liquid chromatography detection of phosphorodiamidate morpholino oligomer in tumor and organ tissue
Tumor tissue, liver, and kidney lysates from AVI-4126-treated animals were prepared as described above and analyzed for presence of PMO by HPLC analysis as described (22). Briefly, a 10-μL aliquot (500 ng) of the internal standard PMO (15-mer whose sequence was derived from a 5′ truncation of AVI-4126) was added to all 250-μL aliquots of tumor, kidney, and liver lysate (0.2 g/mL) samples. Methanol (300 μL) was added to each sample and the tubes were vortexed. The tubes were centrifuged for 10 minutes using a high-speed centrifuge and supernatants were transferred to new Eppendorf tubes. The pellet was then washed with 100 μL Trit and the wash buffer was added to the supernatant. The supernatants were heated in a water bath at 70°C for 10 minutes. The samples were recentrifuged for 10 minutes and the supernatants were transferred to new Eppendorf tubes. Methanol was evaporated using a Speed vac (Savant, Farmingdale, NY) and the samples were finally transferred to clear shell vials and lyophilized after the addition of 100 μL deionized water to each vial. The lyophilized samples were reconstituted using 100-μL aliquots of 5′ fluorescent DNA (1.0 absorbance units/mL) whose sequence was complementary to that of AVI-4126 PMO. A set of AVI-4126 standards was prepared by spiking the PMO into 250-μL aliquots of blank rat plasma (10, 25, 50, 100, 250, 500, and 1,000 ng/250 μL plasma) along with the internal standard. The standards were extracted similarly. The samples were analyzed by injecting onto a Dionex DNA Pac PA-100 column (4 × 250 mm column, Dionex Corp., Sunnyvale, CA) using a Varian autosampler (AI-200) connected to a Varian HPLC pump (model 9010 inert) equipped with a Varian fluorescence detector (model 9075). The mobile phases [A: 0.025 mol/L Tris-HCl (pH 8); B: 0.025 mol/L Tris (pH 8)/1.0 mol/L NaCl] were prepared using HPLC-grade water and reagents and filtered through a 0.2 μm
filter before use. The gradient program employed was [90-10% B] at 0 minute and [55-45% B] at 20 minutes while the pump was held at a flow rate of 1.5 mL/min. The runs were monitored at excitation and emission wavelengths of 494 and 518 nm, respectively.

Pharmacokinetic study
A phase I single center, open label, and dose escalating study was conducted to evaluate the safety (3) and pharmacokinetics of AVI-4126 given i.v. in normal healthy male and female subjects. Protocol specifications and a detailed time and events schedule were prepared by MDS Harris (Lincoln, NE) with local ethics committee approval. Five dose levels were evaluated with six normal healthy subjects enrolled in each cohort after screening medical history, examination, and laboratory tests had shown no clinically significant abnormalities. Cohorts 1 (three males and three females), 2 (two males, four females), 3 (three males, three females), 4 (two males, four females), and 5 (four males and two females) received 1, 3, 10, 30, and 90 mg AVI-4126, respectively, given as a slow (10-15 seconds) i.v. bolus/push. The subjects in all five dose levels were in the fed state. They were confined to the clinic through the 72-hour post-dose events. The pharmacokinetic variables were studied for the highest dose (90 mg). Blood draw was conducted at the following time points: 0.033, 0.083, 0.167, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours following AVI-4126 administration.

Pharmacokinetic analysis
The data were evaluated with the assistance of the computer program PKCALC as published previously (23). The i.v. plasma concentrations measured were fit to Eq. A (24):

\[
C(t) = A e^{-\alpha t} + B e^{-\beta t}
\]

where \( C(t) \) is the plasma concentration at time \( t \), \( A \) and \( B \) are intercept terms, \( \alpha \) is a distribution rate constant, and \( \beta \) is an elimination rate constant. All data are expressed as mean ± SE as determined by the computer program Instat 2 (GraphPad, San Diego, CA). Graphs were prepared with Prism version 3.0 (GraphPad).

Phase I study of AVI-4126 localization in human solid tumors
In an exploratory analysis, we sought to characterize the localization of AVI-4126 in human solid tumors.

Patients. Adult, good performance status (Eastern Cooperative Oncology Group PS 0 or 1) patients with cytologically or histologically confirmed adenocarcinoma of the breast, colon, prostate, or non-small cell lung cancer who had a tumor at least 1.0 x 1.0 cm and were scheduled for surgical resection were eligible. Patients could not have metastases, active infection, or another serious medical condition; could not be pregnant or nursing; could not have received an investigational agent within 30 days or any prior chemotherapy, hormonal therapy, or radiation therapy; and had to have adequate hematologic, renal, and hepatic function (hemoglobin ≥ 11 g/dL for men and ≥ 10 g/dL for women; serum creatinine ≤ institutional upper limits of normal; serum bilirubin < upper limits of normal; aspartate aminotransferase < 1.5 x upper limits of normal). All patients provided signed informed consent and the study was approved by the Institutional Review Board of the Oregon Health and Science University.

Procedures. Pretreatment evaluation included history and physical examination, urinalysis, serum pregnancy test, complete blood count with automated differential, and serum chemistries. AVI-4126 (90 mg) was given by a slow i.v. push (-10-15 seconds) 16 to 30 hours before the scheduled surgery. Vital signs were monitored before and 30 minutes after dosing, before hospital discharge, and 7 to 14 days later. Complete blood count and serum chemistries were obtained before hospital discharge and again 7 to 14 days later. EDTA-stabilized plasma was collected for AVI-4126 concentration analysis immediately before AVI-4126 dosing, 30 minutes later, before anesthesia, and after anesthesia recovery. Adverse events were recorded 30 minutes after dosing and again 7 to 14 days later. Tissue specimens that included the core and margin of the tumor as well as adjacent normal tissue were collected with the goal of measurements of the concentration and distribution of AVI-4126.

Results
AVI-4126 activity in a plasmid-based in vitro screening system.
To ascertain the sequence specificity of the PMO, AVI-4126, a plasmid-based test system was used for cell-free screening. A fusion construct, pCiNeo-myc-LucΔA, was generated by subcloning a small segment of the c-MYC gene that includes the AUG translation start site followed by luciferase into the pGNeo expression vector. In vitro translation was done by mixing the rabbit reticulocyte lysate with known amounts of antisense, scrambled oligomers or vehicle (water) followed by addition of a known amount of the c-MYC/Luc in vitro transcribed RNA. The relative inhibition of luciferase activity in the presence of various concentrations of PMO compared with the vehicle control was calculated based on the readings from a luminometer. The data in Fig. 1 reveal a dose-dependent and specific inhibition of luciferase activity, IC50 of 100 nmol/L in the presence of increasing concentrations of the antisense c-MYC PMO (AVI-4126) compared with the scrambled PMO control.

Differential bioavailability of AVI-4126 in murine solid tumors models. Three different tumor models with varying degrees of vascularity (LLC1 syngeneic lung tumor, LNCaP prostate xenograft, and PC-3 prostate xenograft) were employed to explore the relationship between tumor blood supply and accumulation of AVI-4126 in the tumor. In each case, a single dose (300 µg/mouse) of AVI-4126 was given to the mice i.p. and/or i.t. The tumors were resected 24 hours after AVI-4126.

![Fig. 1. Plasmid-based test system for screening AVI-4126 PMO sequence specificity and antisense inhibition of c-MYC mRNA in the region from 5' untranslated region in to the AUG translation start site with the luciferase reporter gene. In vitro translation was done by mixing rabbit reticulocyte lysate with various concentrations of vehicle (water), antisense, or scrambled PMOs in the presence of c-MYC luciferase RNA. Points, mean (n = 3) to reveal the luciferase light units in the lysates normalized for protein content to show specificity of the antisense c-MYC PMO in comparison with a 76% identical but scrambled sequence; bars, SE.](image-url)
scrambled PMO, or saline administration and subjected to flow cytometric analysis, preparation of cryosections, and tumor lysates. Significant AVI-4126 bioavailability was detected up to 24 hours after a single i.p. administration in the LLC1 lung syngeneic tumors (Fig. 2) and LNCaP prostate xenograft tumors, both of which are highly vascular (Fig. 3) Immunoblot analysis of the respective tumor lysates (Figs. 2A and 3A) revealed specific inhibition of target c-Myc protein levels in the AVI-4126 PMO-treated tumors compared with the control groups. Quantitation of AVI-4126 PMO levels in the tissue lysates from the tumors was carried out by HPLC analysis. The elution order of each chromatogram is AVI-4126, the internal standard, and the excess fluoresceinated DNA probe. The peak corresponding to full-length AVI-4126 was readily observed in both LLC1 (Fig. 2B and C) and LNCaP (Fig. 3B and C) tumor tissue samples as shown in the representative chromatograms. This analytic technique is capable of resolving peaks resulting from (N - 1)-mers and truncated versions of AVI-4126, but neither was detected in the tissue or tumor lysates. Significant levels of AVI-4126 PMO were detected, which correlated with decreased c-Myc protein levels in the mouse prostate tissue isolated from the LLC1 s.c. tumor model following either i.p. or i.t. AVI-4126 administration (Fig. 4). In contrast, AVI-4126 bioavailability in the tumor core was minimal for the relatively avascular PC-3 s.c. prostate xenograft tumors following i.p. AVI-4126 administration as shown in Fig. 5D. Immunoblot analysis (Fig. 5A) of the PC-3 tumor lysates revealed significant inhibition of c-Myc levels and AVI-4126 accumulation in the tumor following i.t. administration but not following i.p. administration. Table 1 summarizes the HPLC-based quantitative analysis of AVI-4126 PMO levels in the various experimental tumor models. In situ tissue photomicrographs of the tumor center and periphery and flow cytometric analysis of the harvested tumor cells following i.p. or i.t. administration of a fluorescent-tagged AVI-4126 PMO in the vascular LLC1 model versus the avascular PC-3 tumor model showed a similar trend (Fig. 6).
AVI-4126 plasma pharmacokinetics. Two separate studies are being reported herein. One is a complete AVI-4126 plasma pharmacokinetic study in normal human subjects following a single bolus i.v. dose of 90 mg (n = 5). Blood samples were collected at various time points as indicated in Fig. 7. The second study was conducted in patients undergoing resection surgery for prostate or breast tumors and the blood samples were collected at 30 minutes and 24 hours following single i.v. administration of 90 mg AVI-4126 (n = 2). AVI-4126 PMO levels in plasma levels were detectable up to the last time point examined following administration: 72 hours (the limit of detection of the analytic assay is 10 ng/mL). The data revealed absence of metabolic breakdown as only full-length 20-mer test agent was recovered in plasma at all time points in both studies. The data from both studies indicate a good correlation and a similar plasma concentration-time profile. The calculated r² value of the nonlinear regression analysis of the 90 mg dose group in the pharmacokinetic study (clinical study 1) was 0.8389 and changed to 0.8351 on inclusion of data from the bioavailability study (clinical study 2). The plasma concentration-time curve (Fig. 7) has at least two distinct phases. The curve has a relatively rapid distribution phase followed by a slower elimination phase. The pharmacokinetic data were best fit with a two-compartment model. The analysis revealed a volume of distribution equal to 266.1 ± 64.1 mL/kg and a clearance rate of 16.8 ± 4.6 mL/h/kg. The distribution half-life for AVI-4126 was 1.0 ± 0.1 hours, whereas the elimination half-life was 11.2 ± 1.6 hours.

AVI-1426 bioavailability and safety in human solid tumors. A 57-year-old woman with invasive ductal carcinoma of the breast (T₁N₀M₀, grade 3 of 3) and a 59-year-old man with an adenocarcinoma of the prostate (T₃N₀M₀, Gleason grade 3 + 4) were given 90 mg (equivalent to 1 mg/kg according to patient weight) AVI-4126 by a slow i.v. push (~10-15
Table 1. Summary of HPLC-based quantitative analysis of AVI-4126 PMO levels detected in different tumor xenograft models or normal mouse prostate 24 hours after administration of AVI-4126 PMO

<table>
<thead>
<tr>
<th>Model</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>PMO recovered (µg/g)</th>
<th>Tissue weight average (mg)</th>
<th>PMO recovered (µg/tissue)</th>
<th>PMO recovered (% of total dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC1 s.c. xenograft</td>
<td>i.p.</td>
<td>15</td>
<td>0.62 ± 0.04</td>
<td>0.3</td>
<td>0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>DU145 s.c. xenograft</td>
<td>i.p.</td>
<td>15</td>
<td>2.35</td>
<td>0.25</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>LNCaP s.c. xenograft</td>
<td>i.p.</td>
<td>15</td>
<td>2.85 ± 0.1</td>
<td>0.23</td>
<td>NA</td>
<td>0.65</td>
</tr>
<tr>
<td>PC-3 s.c. xenograft</td>
<td>i.p.</td>
<td>15</td>
<td>Below detection limit</td>
<td>0.1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PC-3 s.c. xenograft</td>
<td>i.t.</td>
<td>15</td>
<td>6 ± 3.7</td>
<td>0.08</td>
<td>0.48</td>
<td>0.16</td>
</tr>
<tr>
<td>Mouse prostate</td>
<td>i.p.</td>
<td>15</td>
<td>1.4</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Mouse prostate</td>
<td>i.t.</td>
<td>15</td>
<td>Below detection limit</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

The data revealed significant tissue accumulation of AVI-4126 in both breast and prostate tissues. The breast tumor had increased accumulation in the tumor core compared with more uniform distribution in the surrounding normal tissue, tumor periphery, and tumor core in the prostate tumor.

**Discussion**

The current study provides a critical comparison of PMO bioavailability between experimental animal models and human subjects in solid tumors following parenteral administration. We report here that PMO accumulation, dosing, and efficacy studies in animal tumor models can lead to reasonable prediction of human tumor bioavailability, although the number of patients studied is small.
In vitro activity in a plasmid-based screening system identified an IC₅₀ of 100 nmol/L for AVI-4126 c-MYC antisense PMO. The HPLC analysis revealed a concentration of 720 ng/g in the breast tumor core, which represents a molar value of ~103 nmol/L. This concentration is practically identical to the IC₅₀ value generated from the in vitro studies. These findings suggest that the dose tested produces tissue concentrations of AVI-4126 in a human breast tumor that would be expected to inhibit the target. Not surprisingly, PMO bioavailability increases with greater tumor vascularity in animal models of cancer. The same relationship may hold true in human solid tumors (the breast carcinoma was microscopically more vascular than the prostate cancer), but further testing is needed. The present study provided not only new information about AVI-4126 plasma pharmacokinetic profile in human subjects following i.v. administration but also, by examining AVI-4126 in target tissue, suggests that the current dose is pharmacodynamically relevant. Higher than plasma concentration in the breast tumor suggests tissue accumulation and may allow for less frequent dosing interval than dictated by plasma pharmacokinetics alone. For less vascular tumors like prostate, frequent dosing or higher doses might be necessary.

The c-MYC gene, the cellular homologue of the avian myelocytic leukemia virus, is implicated in a large number of human solid tumors, leukemias, and lymphomas as well as in a variety of animal neoplasias (25, 26). Overexpression of c-MYC associated with uncontrolled cell proliferation is a frequent genetic event in androgen-refractory adenocarcinoma of the prostate. Notably, androgen deprivation therapy has been observed to result in c-MYC gene amplification associated with increased cell proliferation (27, 28). Similarly, c-MYC is frequently overexpressed in breast cancer and has been identified to be up-regulated by estrogen stimulation of hormone-dependent breast cancer cells (29). Thus, an inhibition strategy for c-MYC is likely to provide an attractive therapeutic modality for multiple neoplastic settings (2).

The application of antisense strategy (30) as therapeutic agents in oncology has come a long way in the last two decades with various agents currently in clinical trials (31–33). The most widely used antisense chemistry includes the phosphorothioates, where the oligonucleotide backbone is stabilized by the substitution of sulfur for oxygen at the phosphorous of the

![Fig. 7. Plasma concentration-time curve of AVI-4126 following single bolus i.v. administration of 90 mg in human test subjects. The data points in study 1 are from blood samples collected at various time points from normal human subjects following single i.v. AVI-4126 administration (n = 5 per time point). Those from study 2 are blood samples collected at 30 minutes and 24 hours post i.v. administration of AVI-4126 in patients undergoing prostate or breast resection surgery (n = 2).](image)

![Fig. 8. Representative photomicrographs of the resected breast and prostate tumors. At low power (original magnification, ×40, left), the breast carcinoma shows areas of central necrosis and fibrosis. A closer view of the tumor edge (×100, right) reveals numerous capillaries (arrowheads) and a medium-sized vein (arrow). A medium-power image of the prostate adenocarcinoma (×200, left) shows perineural invasion and extension of the tumor into adjacent fat. The architecture of the glands viewed at higher power (×200, right) consists of a mixture of intermediate-grade (Gleason pattern 3) and higher-grade (Gleason pattern 4) patterns.](image)
molecule (34). The first and only antisense drug (Formiviren, ISIS Pharmaceuticals, Inc., Carlsbad, CA) approved by the U.S. Food and Drug Administration is a phosphorothioate used for the treatment of cytomegalovirus-induced retinitis in AIDS patients. Although phosphorothioates are relatively resistant to enzymatic degradation and exhibit greater nuclease stability than the phosphodiester oligonucleotides, they are digested intracellularly (35). The phosphorothioates have also been shown to nonspecifically bind to cellular components and these interactions potentially interfere with the translational process (36, 37). On the other hand, PMO chemistry and unique RNase H–independent mechanism of action seem to offer stable, nontoxic, and specific translational inhibitors. Functional efficacy of multiple PMO antisense targets ranging various genes, including c-MYC, have been shown in preclinical cancer models (10, 38–40). I.t. administration of AVI-4126 in the avascular PC-3 xenograft tumors decreased tumor burden by ~75% compared with control treatments (3). In a Lewis lung carcinoma murine model, a combination regimen in which cisplatin was given on days 2 and 13 and 15 followed by AVI-4126 treatment on days 6 to 12 and 17 to 23 inhibited tumor growth significantly in comparison with cisplatin alone (17). Similar potentiation of antitumor activity of AVI-4126 by i.p. administration was also observed in a novel combination strategy involving inhibition of both c-MYC and β-human chorionic gonadotropin using PMOs in a prostate cancer model (18). Thus, AVI-4126 is a promising new agent that might be used alone or in combination with either hormonal or cytotoxic therapies in solid tumors.

AVI-4126 has been tested in multiple clinical trials and shown to have an excellent safety profile. The commonly observed side effects like increase in APTT, complement C3a, anemia, decrease in platelet count, and hypotension related to various phosphorothioates (35, 41, 42) were not observed with the AVI-4126 PMO in human clinical trials via bolus i.v., local, and s.c. routes of administration. Pharmacokinetic studies in human subjects and animal models have also established the feasibility of once-daily dosing (16). Currently, efficacy of AVI-4126 is being tested as a cancer therapeutic in clinical studies, which will include not only assessment of tissue PMO concentration but also, whenever feasible, assessment of target expression before and after administration of AVI-4126. In summary, this is the first study that has characterized PMO bioavailability in solid tumors and reveals the potential of PMOs as a clinically viable strategy in cancer therapeutics.

### References


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