Published in final edited form as:

J Surg Res. 2011 December ; 171(2): 684–690. doi:10.1016/j.jss.2010.05.066.

High Antimetastatic Efficacy of MEN4901/T-0128, a Novel Camptothecin Carboxymethyldextran Conjugate

Huaiyu Ma, MD¹, Zhijian Yang, MD¹, Satoshi Okuno, MD², Takayuki Kawaguchi, MD², Shigeo Yagi, PhD¹, Michael Bouvet, MD³, and Robert M. Hoffman, PhD^{1,3,4}

¹AntiCancer, Inc., San Diego, CA

²Tanabe Seiyaku Co., Ltd., Saitama, Japan

³Department of Surgery, University of California San Diego, San Diego, CA

Abstract

The antimetastatic activity of a novel camptothecin conjugate. MEN4901/T-0128, in which 7-ethyl-10-aminopropoxy-camptothecin (T-2513) is bound to a biodegradable carboxymethyldextran via a Gly-Gly-Gly linker, was observed in this study. High antimetastatic activity of MEN4901/T-0128 was demonstrated in a clinically-relevant orthotopic mouse model of human colon cancer. MEN4901/T-0128 and irinotecan were compared for anti-metastatic activity as well as efficacy against the primary tumor. An imageable, metastatic model was made by surgical orthotopic implantation (SOI) of the green fluorescent protein (GFP)-expressing HT-29 tumor in nude mice. MEN4901/T-0128 and irinotecan were administered intravenously at various doses and schedules. MEN4901/T-0128, with treatment beginning on day 49 after SOI, was highly effective on lymph node metastasis as well as against the primary tumor. Both GFP imaging and histology demonstrated a markedly lower metastatic incidence of lymph nodes in all MEN4901/T-0128 treated mice compared to irinotecan-treated and untreated mice. At the most efficacious dose of MEN4901/T-0128, only 1 of 12 animals had lymph node metastasis compared to 19 of 24 in the control group. The present study demonstrates the principle that when a camptothecin is conjugated to an appropriate polymer, the drug can become extremely effective with important clinical potential for antimetastatic therapy, a most urgent need.

Keywords

camptothecin; polymer conjugate; nude mice; orthotopic model; metastasis; efficacy

Introduction

Camptothecin (CPT) analogues with improved water solubility, CPT-11 and topotecan, have emerged as important antitumor agents (1–4). CPT-11 has been available in Japan since 1994 for the treatment of colorectal, cervical, ovarian, uterine, gastric, breast, and lung cancer. In the United States, CPT-11 is presently used for the treatment of advanced

© 2010 Elsevier Inc. All rights reserved.

⁴Address correspondence to: Michael Bouvet, MD, UCSD Moores Cancer Center, 3855 Health Sciences Dr. #0987, La Jolla, CA 92093-0987 Tel: 858-822-6191 Fax: 858-822-6192, mbouvet@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

colorectal adenocarcinoma. However, the main clinical adverse effects of the drug are myelosuppression and gastrointestinal toxicity, especially severe diarrhea. These side effects are closely related to its pharmacokinetic properties (5,6).

In an attempt to overcome the side effects and improve the pharmacological profile of camptothecin drugs, MEN4901/T-0128 has been designed as a macromolecular camptothecin prodrug. MEN4901/T-0128 comprises the novel CPT analogue 10-(3V-aminopropoxy)-7-ethyl-(20S)-CPT (T-2513) and carboxymethyldextran, which are linked via a triglycine spacer (7,8). The evaluation of the *in vivo* antitumor activity of MEN4901/T-0128 against a panel of human tumors implanted subcutaneously in nude mice has demonstrated enhanced antitumor activity and a broad range of therapeutic doses. Pharmacokinetic analysis showed that MEN4901/T-0128 had an extended plasma half-life with sustained tumor levels, which may explain the superior activity of MEN4901/T-0128 *in vivo* (9). However, these subcutaneous-transplant models generally do not metastasize and could not evaluate the anti-metastatic activity of MEN4901/T-0128.

Steeg et al (10) have recently reviewed the urgent need for anti-metastasis drugs. They pointed out, for example, that Paez-Ribes et al (11) and Ebos et al (12) showed that inhibition of angiogenesis reduced primary tumor growth and microvessel density, but paradoxically, accelerated invasion and metastasis.

As Steeg et al (10) state, the emphasis on preclinical testing of new compounds including anti-angiogenesis drugs relies on responses of subcutaneous or orthotopic primary tumors. In the few instances when preclinical compounds, were tested for both primary tumor size and metastasis, they often had discordant results. According to Steeg et al, (10) for example, cyclophosphamide inhibited primary lung adenocarcinoma but promoted metastasis to the lung and liver, (13) and the Hsp90 inhibitor 17-AAG inhibited primary breast cancer but promoted metastasis to the bone (14). Vandetanib, a vascular endothelial growth factor receptor (VEGFR) inhibitor, reduced the growth of primary fibrosarcomas, but had no effect on their metastasis (15). Some agents have been shown to have effects on primary tumor size, but still had the capacity to inhibit metastasis (11–24).

Surgical orthotopic implantation (SOI) models have most features of clinical metastatic cancer (25). Cancer cell lines have been stably transfected with the jellyfish *Aequorea victoria* green fluorescent protein (GFP) in order to track metastases in at ultra-high resolution and externally image metastases in the SOI models (26–28). Effective antimetastatic drugs can be discovered and evaluated in the SOI models utilizing human tumor cell lines as well as patient tumors (25).

The main objective of the present study was to test the ability of MEN4901/T-0128 to inhibit metastasis in an SOI model of human colon cancer expressing GFP.

Materials and Methods

Test agents

MEN4901/T-0128 (Lot #: M0086151A) was synthesized and provided by Medicinal Chemistry Research Laboratories, Tanabe Seiyaku Co., Ltd. (Saitama, Japan). A detailed characterization of MEN4901/T-0128 has been published previously (8). Irinotecan (Lot #: EQBZJ14 and EQAZF13) was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). MEN4901/T-0128 and irinotecan were stored at room temperature and protected from light. MEN4901/T-0128 was formulated in physiologic saline at concentrations of 88, 44 and 22 mg/ml. Irinotecan was formulated in physiologic saline to 4 and 2 mg/ml. Two test agents were prepared half an hour before injection.

Human colon cancer cell line

Human colon cancer cell line HT-29, obtained from the American Type Culture Collection (Rockville, MD) was maintained in DMEM (Irvine Scientific, Irvine, CA) supplemented with heat-inactivated 10% FBS (Gemini Biologic Products, Calabasas, CA), 2mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B (Life Technologies, Inc., Grand Island, NY). The cells were incubated at 37°C in 5% CO₂.

Retroviral GFP transduction of tumor cells

For GFP gene transfection, 25% confluent HT-29 cells were incubated with a 1:1 precipitated mixture of GFP retroviral supernatants of PT67 packaging cells and RPMI 1640 (GIBCO Life Technologies, New York, NY) containing 10% FBS (Gemini Biological Products) for 72 h. Fresh medium was replenished at this time. Cells were harvested by trypsin-EDTA 72 h after transduction and subcultured at a ratio of 1:15 into medium, which contained 200 µg/ml G418 to select for high GFP expression, since the GFP retrovirus also contained the neomycin resistance gene. The level of G418 was increased to 400 µg/ml stepwise. Clones stably expressing GFP were isolated with cloning cylinders (Bel-Art Products, Pequannock, NJ) with the use of trypsin-EDTA. The high-GFP-expressing cells were then amplified and transferred by conventional culture methods. High GFP-expression clones were then isolated in the absence of G418 for 10 passages to select for stable expression of GFP.

Animals

Female NCr nu/nu mice, 4-week old, were purchased from Taconic, Germantown, NY. The animals were maintained in a HEPA filtered environment in a Micro-VENT full ventilation rodent housing system (Allentown Caging Equipment Co., Allentown, NJ). They were housed 5 per cage and acclimatized for 18 days and an inspection was performed before transplantation to ensure their suitability for the study. The animals were fed with autoclaved laboratory rodent diet (PMI Nutrition International Inc., Brentwood, MO). All animal studies were conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals under assurance number A3873-1. The Animal Ethics Committee of TANABE Seiyaku Co., Ltd. approved the experimental protocol concerning animal welfare.

Surgical orthotopic implantation (SOI)

HT-29-GFP tumor stock was established by subcutaneously injecting 5×10^6 HT-29-GFP cells to the flank of nude mice. The tumor was maintained in nude mice subcutaneously as tumor stock prior to being used for SOI. On the day of SOI, the tumor was harvested from the subcutaneous site and placed in RPMI-1640 medium. Strong GFP expression of the HT-29-GFP tumor tissue was confirmed by fluorescence imaging. Necrotic tissues were removed and viable tissues were cut into 1-mm³ pieces. Test animals were transplanted by SOI using the tumor tissue fragments harvested from the stock tumors. The animals were anesthetized by injection of 0.02 ml of a solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. The surgical area was sterilized using iodine solution and alcohol. An incision approximately 1.5 cm long was made along the left lateral abdomen of the nude mouse using a pair of sterile scissors. After the abdomen was opened, the cecum was exposed and the serosa of the transplantation site was stripped. Two to three fragments of tumor tissue (one cubic millimeter each) were sutured adjacent to each other onto the colon (cecum) with sterile 8-0 surgical sutures (nylon, Ethicon Inc., NJ) to generate one primary tumor. The abdomen was closed with sterile 6-0 surgical sutures (silk, Ethicon Inc.). All surgical procedures and animal manipulations were conducted under HEPA-filtered laminar-flow hoods with a 7× microscope (Olympus) (25,29).

Drug Treatment

When the estimated mean tumor volume reached 66 mm^3 and lymph node metastasis was confirmed by GFP imaging on day 49 post-SOI, all mice were randomized into 7 treatment groups of 12 mice each. 24 animals were used in the untreated control group. All drug administration was by tail vein injection. Mice in **Group-1** received a single injection of MEN4901/T-0128 at a dose of 1760 mg/kg on day 49 post-SOI. Mice in **Group-2** received two injections of MEN4901/T-0128 at a dose of 880 mg/kg on days 49 and 56 post-SOI. Mice in **Group-3** received a single injection of MEN4901/T-0128 at a dose of 880 mg/kg on day 49 post-SOI. Mice in **Group-4** received two injections of MEN4901/T-0128 at a dose of 440 mg/kg on days 49 and 56 post-SOI. Mice in **Group-5** received four injections of MEN4901/T-0128 at a dose of 440 mg/kg on days 49, 53, 57 and 61 post-SOI. Mice in **Group-6** received four injections of irinotecan at a dose of 80 mg/kg on days 49, 53, 57 and 6 post-SOI. Mice in **Group-7** received four injections of irinotecan at a dose of 40 mg/kg on days 49, 53, 57 and 61 post-SOI. Mice in **Group-8** served as a negative control and did not receive treatment. See Table 1 for treatment conditions for each group. All animals were dosed at a rate of 0.1 ml/5 sec for MEN4901/T-0128 and 0.025 ml/5sec for irinotecan.

Tumor measurement and physical observation of mice

Each animal was checked at least twice a day from initiation of treatment for mortality or signs of morbidity. Primary tumor volumes and body weights for each animal were measured twice a week. Tumor volumes were measured with sliding calipers (Scienceware, Pequannock, NJ) and calculated using the formula $(L \times W^2) \times \frac{1}{2}$, where L and W represent the larger and smaller diameters, respectively. All surviving tumor-bearing animals were sacrificed on day 71 after tumor implantation. The tumor was removed from each mouse and weighed at autopsy. The tumor growth inhibition rate (IR) was calculated using the formula: $\text{IR} (\%) = (1 - T/C) \times 100$, where T and C are the mean tumor weight of treatment and control groups, respectively. Regression efficacy refers to the mean estimated tumor volume at the end of the experiment being smaller than that at the initiation of treatment.

GFP imaging

During the study, the animals were placed in the primary in the same position for each time point for non-invasive imaging of the primary tumor and metastasis. At the end of the study, all mice were sacrificed and open images of tumors and metastasis expressing GFP was acquired. Primary tumors and all metastases were carefully imaged. A Leica fluorescence stereo microscope model LZ12 (Leica Microsystems, Inc., Bannockburn, IL) equipped with a mercury lamp and a 50-W power supply was used. Selective excitation of GFP was produced through a D425/60 band-pass filter and a 470 DCXR dichroic mirror. Emitted fluorescence was collected through a long-pass filter (GG475; Chroma Technology, Brattleboro, VT) on a Hamamatsu C5810 3-chip cooled color charge-coupled device camera (Hamamatsu Photonics, Bridgewater, NJ). Images were processed for contrast and brightness and analyzed with the use of IMAGE PRO PLUS 3.1 software (Media Cybernetics, Silver Spring, MD). High-resolution images of 1024×724 pixels were captured directly on an IBM PC or continuously through video output on a high-resolution Sony VCR, model SLV-R1000 (Sony, Tokyo).

Histologic analysis

After the open imaging, the primary tumor and the mesentery LNs, lung, liver and spleen were collected from all animals and were fixed in 10% buffered neutral formalin solution, embedded in paraffin, and sectioned. Sections were stained with H&E. All slides were carefully reviewed using a Nikon microscope.

Statistical analysis

The Dunnett's test with an α equal to 0.05 (two-sided) was used to compare mean tumor weight between the treated and untreated groups. The Fisher's exact test was used to compare metastatic frequencies to the LNs and distant organs between the treated and untreated groups. The difference was considered statistically "significant" when $P < 0.05$, whereas difference levels with $P < 0.01$ were considered "highly significant."

Results

Antimetastatic efficacy of MEN4901/T-0128

The activity of MEN4901/T-0128 and irinotecan against the primary tumor and metastasis were evaluated in a surgical orthotopic implantation animal model of HT-29 human colon cancer cell line. Tumor-bearing mice were treated with different doses of MEN4901/T-0128 and irinotecan at different schedules. At necropsy, all animals were opened and GFP imaging was performed to examine metastasis. Metastases in mesentery lymph nodes, diaphragm, abdominal and thoracic cavity were detected by GFP imaging among the tumor-bearing animals (Figure 1). As shown in Table 1, GFP imaging demonstrated a lower metastatic incidence of lymph nodes in all MEN4901/T-0128 treated groups compared to the irinotecan-treated and untreated groups ($P \leq 0.001$). The incidence of metastases in the diaphragm, abdominal and thoracic cavity of all treated and untreated animals were too low to be statistically analyzed.

For histological examination of metastasis, the lung, liver, spleen, and lymph nodes were collected and H&E staining was performed (Figure 2). As shown in Table 1, significant differences of metastatic incidence to lymph nodes were observed by histology. All MEN4901/T-0128-treated animals showed lower lymph node metastases than irinotecan-treated and untreated animals. Furthermore, in 4/24 untreated, 1/12 MEN4901/T-0128 (440 mg/kg, q7d $\times$ 2) and irinotecan-low-dose treated animals, lung metastases were found, whereas no lung metastasis was found in all other MEN4901/T-0128 and irinotecan-high-dose treated animals.

At the most efficacious dose of MEN4901/T-0128, only 1 of 12 animals had lymph node metastasis compared to 19 of 24 in the control group (Table 1).

Efficacy of MEN4901/T-0128 on the primary tumor

Primary tumor volumes were measured with sliding calipers twice a week during the treatments. All surviving tumor-bearing animals were sacrificed on day 71 after tumor implantation. The primary tumors were removed and weighed, and compared between the treated groups and untreated group using the Dunnett's test. As shown in Table 2 and Figure 1, all MEN4901/T-0128 treated groups had significantly smaller tumor volumes at different time points and tumor weights at the end of the study than the irinotecan and untreated group ($P < 0.001$). The IRs of MEN4901/T-0128 treatments were 80.4% in **Group-1** (1760 mg/kg, single), 81.5%, in **Group-2** (880 mg/kg, q7d $\times$ 2), 70.7%, in **Group-3** (880 mg/kg, single), 80.4% in **Group-4** (440 mg/kg, q7d $\times$ 2), and 84.8% in **Group-5** (440 mg/kg, q4d $\times$ 4). All MEN4901/T-0128 treated groups except **Group-3** (880 mg/kg, single) were markedly effective and the tumor growth was significantly suppressed. Tumor regression was observed in all animals treated with MEN4901/T-0128. On the other hand, irinotecan used as a positive control partially inhibited tumor growth only at high doses (80 mg/kg, q4d $\times$ 4).

Effect of drug treatment on body weight

As shown in Table 3, body weight changes were observed in all treated and untreated tumor-bearing animals. However, more body weight losses were found in MEN4901/T-0128 and irinotecan-treated animals compared to the untreated control. A nadir of 9.5% and 4.8% reduction in body weight, which occurred seven days after initiation of treatment, was observed in animals receiving MEN4901/T-0128 at a single dose of 1760 mg/kg and 880 mg/kg, respectively. A nadir of 4.3% and 8.1% body weight loss, which occurred 11 days after initiation of treatment, was seen in animals receiving MEN4901/T-0128 at two doses of 880 mg/kg and 440 mg/kg, respectively. Body weight loss in MEN4901/T-0128 treated animals recovered by two weeks after initial treatment. More than 10% reduction in body weight at nadir was observed in animals receiving MEN4901/T-0128 at four doses of 440 mg/kg and irinotecan-treated animals. Body weight loss in those animals recovered slowly. Because of a slight reduction in body weight in the untreated animals (2.2% body weight loss at nadir), body weight loss in the treated groups may have been partly due to cachexia induced by the tumors.

Discussion

T-0128 comprises a CPT analogue T-2513 covalently bound to CM dextran by a triglycyl linker and has improved therapeutic efficacy (8). T-0128 has demonstrated a strong antitumor activity against a panel of human tumor subcutaneously implanted in nude mice that were mostly refractory to CPT-11, T-2513, and topotecan (9). These studies could not evaluate antimetastatic efficacy, since they involved subcutaneously-growing tumors which do not metastasize. The present study investigated antimetastatic efficacy of MEN4901/T-0128 as well as efficacy on the primary metastatic fluorescent human colon cancer orthotopic tumor model. This orthotopic tumor model utilizes surgical orthotopic implantation (SOI) of tumor tissue at the site of the organ of origin. The organ-specific site provides the implanted tumor with an optimal environment for growth and progression (30). Because of its clinical-like patterns and high incidence of metastasis, this model better reflects the clinical feature of human cancer and has demonstrated a high correlation of drug response compared to that in the clinic (31,32).

The present study used GFP tumor imaging and histology to evaluate the antimetastatic efficacy of MEN4901/T-0128 in the orthotopic model. Imaging was used for antimetastatic evaluation since this technology allows tumor growth and metastasis to be visualized and tracked during any stages of tumor progression (33). Both imaging and histological examination demonstrated much stronger antimetastatic efficacy of MEN4901/T-0128 than irinotecan, indicating antimetastatic potential in the clinic.

As summarized in Table 2, MEN4901/T-0128 given as repeated treatments showed a marked activity. In contrast, a single treatment of MEN4901/T-0128 had less effective antitumor activity unless a higher dose was used, indicating repeated treatment regimens were superior to single bolus drug administration. Furthermore, all MEN4901/T-0128 treated animals had tumor regression efficacy against the primary human colon tumor, which was consistent with antitumor activity in human tumor subcutaneous xenografts (9).

The toxicity of MEN4901/T-0128 was assessed by body weight loss of the treated animals. Less body weight loss was observed in the animals receiving MEN4901/T-0128 with single and q7d $\times$ 2 schedules as compared to the animals given a q4d $\times$ 4 schedule, indicating increasing toxicity with more frequent administration.

Previously published studies have added to our knowledge of the mechanism of action of MEN4901/T-0128 (7–9). CPT is a potent, antitumor agent acting mainly through inhibition

of topoisomerase I during the S-phase of the cell cycle. Although CPT shows potent antitumor activity *in vitro* and *in vivo*, its low water solubility and untoward toxicity have severely restricted its clinical applications. Linking CPT to a high molecular weight anionic polymer enhances solubility and improves distribution to the tumor through enhanced permeability and retention due to the leakiness of tumor vasculature in comparison with that of normal tissues (8,9,34). Furthermore, Harada et al have shown that T-0128 is taken up by tumor macrophages and the liberated T-2513 kills tumor cells; therefore MEN4901/T-0128 is active *in vivo* but not *in vitro* against cancer cells in culture (7).

The present study demonstrated ultra-high antimetastatic efficacy of MEN4901/T-0128 as well as regression activity against the primary tumor with a broad range of therapeutic doses in clinical-like human colon orthotopic tumor model. The data suggest that MEN4901/T-0128 is a promising candidate in clinical trials for the treatment of solid cancer. The study shows that a clinically-relevant metastatic orthotopic model of cancer can identify the antimetastatic activity of a novel compound.

References

1. Cersosimo RJ. Irinotecan: a new antineoplastic agent for the management of colorectal cancer. *Ann Pharmacother.* 1998; 32:1324–1333. [PubMed: 9876815]
2. Cersosimo RJ. Topotecan: a new topoisomerase I inhibiting antineoplastic agent. *Ann Pharmacother.* 1998; 32:1334–1343. [PubMed: 9876816]
3. Hsiang YH, Liu LF. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res.* 1988; 48:1722–1726. [PubMed: 2832051]
4. Slichenmyer WJ, Rowinsky EK, Donehower RC, Kaufmann SH. The current status of camptothecin analogues as antitumor agents. *J Natl Cancer Inst.* 1993; 85:271–291. [PubMed: 8381186]
5. Chabot GG. Clinical pharmacokinetics of irinotecan. *Clin Pharmacokinet.* 1997; 33:245–259. [PubMed: 9342501]
6. Takimoto CH, Wright J, Arbuck SG. Clinical applications of the camptothecins. *Biochim Biophys Acta.* 1998; 1400:107–119. [PubMed: 9748525]
7. Harada M, Imai J, Okuno S, Suzuki T. Macrophage-mediated activation of camptothecin analogue T-2513-carboxymethyl dextran conjugate (T-0128): possible cellular mechanism for antitumor activity. *J Control Release.* 2000; 69:389–397. [PubMed: 11102679]
8. Okuno S, Harada M, Yano T, Yano S, Kiuchi S, Tsuda N, Sakamura Y, Imai J, Kawaguchi T, Tsujihara K. Complete regression of xenografted human carcinomas by camptothecin analogue-carboxymethyl dextran conjugate (T-0128). *Cancer Res.* 2000; 60:2988–2995. [PubMed: 10850447]
9. Fujita F, Koike M, Fujita M, Sakamoto Y, Okuno S, Kawaguchi T, Yano S, Yano T, Kiuchi S, Fujiwara T, Kudoh S, Kakushima M. MEN4901/T-0128, a new camptothecin derivative-carboxymethyl dextran conjugate, has potent antitumor activities in a panel of human tumor xenografts in nude mice. *Clin Cancer Res.* 2005; 11:1650–1657. [PubMed: 15746070]
10. Steeg PS, Anderson RL, Bar-Eli M, Chambers AF, Eccles SA, Hunter K, Itoh H, Kang Y, Matrisian LM, Sleeman JP, Theodorescu D, Thompson EW, Welch DR. Preclinical drug development must consider the impact on metastasis. *Clin Cancer Res.* 2009; 15:4529–4530.
11. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell.* 2009; 15:220–231. [PubMed: 19249680]
12. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell.* 2009; 15:232–239. [PubMed: 19249681]
13. Man S, Zhang Y, Gao W, Yan L, Ma C. Cyclophosphamide promotes pulmonary metastasis on mouse lung adenocarcinoma. *Clin Exp Metastasis.* 2008; 25:855–864. [PubMed: 18766303]
14. Price JT, Quinn JM, Sims NA, Vieusseux J, Waldeck K, Docherty SE, Myers D, Nakamura A, Waltham MC, Gillespie MT, Thompson EW. The heat shock protein 90 inhibitor, 17-allylamino-17-demethoxygeldanamycin, enhances osteoclast formation and potentiates bone

- metastasis of a human breast cancer cell line. *Cancer Res.* 2005; 65:4929–4938. [PubMed: 15930315]
15. Padera TP, Kuo AH, Hoshida T, Liao S, Lobo J, Kozak KR, Fukumura D, Jain RK. Differential response of primary tumor versus lymphatic metastasis to VEGFR-2 and VEGFR-3 kinase inhibitors cediranib and vandetanib. *Mol Cancer Ther.* 2008; 7:2272–2279. [PubMed: 18687659]
 16. Bergamo A, Masi A, Dyson PJ, Sava G. Modulation of the metastatic progression of breast cancer with an organometallic ruthenium compound. *Int J Oncol.* 2008; 33:1281–1289. [PubMed: 19020762]
 17. Biswas S, Guix M, Rinehart C, Dugger TC, Chytil A, Moses HL, Freeman ML, Arteaga CL. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest.* 2007; 117:1305–1313. [PubMed: 17415413]
 18. Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, Karikari C, Alvarez H, Iacobuzio-Donahue C, Jimeno A, Gabrielson KL, Matsui W, Maitra A. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res.* 2007; 67:2187–2196. [PubMed: 17332349]
 19. Kishida Y, Yoshikawa H, Myoui A. Parthenolide, a natural inhibitor of Nuclear Factor-kappaB, inhibits lung colonization of murine osteosarcoma cells. *Clin Cancer Res.* 2007; 13:59–67. [PubMed: 17200339]
 20. Koto K, Horie N, Kimura S, Murata H, Sakabe T, Matsui T, Watanabe M, Adachi S, Maekawa T, Fushiki S, Kubo T. Clinically relevant dose of zoledronic acid inhibits spontaneous lung metastasis in a murine osteosarcoma model. *Cancer Lett.* 2009; 274:271–278. [PubMed: 18986762]
 21. Magenta G, Borenstein X, Rolando R, Jansis M. A. Rosiglitazone inhibits metastasis development of a murine mammary tumor cell line LMM3. *BMC Cancer.* 2008; 8:47. [PubMed: 18261208]
 22. Manni A, Washington S, Craig L, Cloud M, Griffith JW, Verderame MF, Texter LJ, Mauger D, Demers LM, Harms JF, Welch DR. Effects of alpha-difluoromethylornithine on local recurrence and pulmonary metastasis from MDA-MB-435 breast cancer xenografts in nude mice. *Clin Exp Metastasis.* 2003; 20:321–325. [PubMed: 12856719]
 23. Steeg PS, Theodorescu D. Metastasis: a therapeutic target for cancer. *Nat Clin Pract Oncol.* 2008; 5:206–219. [PubMed: 18253104]
 24. Titus B, Frierson HF Jr, Conaway M, Ching K, Guise T, Chirgwin J, Hampton G, Theodorescu D. Endothelin axis is a target of the lung metastasis suppressor gene RhoGDI2. *Cancer Res.* 2005; 65:7320–7327. [PubMed: 16103083]
 25. Hoffman RM. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. *Invest New Drugs.* 1999; 17:343–359. [PubMed: 10759402]
 26. Hoffman RM, Yang M. Whole-body imaging with fluorescent proteins. *Nat Protoc.* 2006; 1:1429–1438. [PubMed: 17406431]
 27. Hoffman RM, Yang M. Color-coded fluorescence imaging of tumor-host interactions. *Nat Protoc.* 2006; 1:928–935. [PubMed: 17406326]
 28. Hoffman RM, Yang M. Subcellular imaging in the live mouse. *Nat Protoc.* 2006; 1:775–782. [PubMed: 17406307]
 29. Rho YS, Lee KT, Jung JC, Yoon C, An Z, Hoffman RM, Chang SG. Efficacy of new platinum analog DPPE in an orthotopic nude mouse model of human colon cancer. *Anticancer Res.* 1999; 19:157–161. [PubMed: 10226537]
 30. Furukawa T, Kubota T, Watanabe M, Kuo TH, Nishibori H, Kase S, Saikawa Y, Tanino H, Teramoto T, Ishibiki K, et al. A metastatic model of human colon cancer constructed using cecal implantation of cancer tissue in nude mice. *Surg Today.* 1993; 23:420–423. [PubMed: 8324335]
 31. Fu XY, Besterman JM, Monosov A, Hoffman RM. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci U S A.* 1991; 88:9345–9349. [PubMed: 1924398]
 32. Ji Y, Hayashi K, Amoh Y, Tsuji K, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Bouvet M, Hoffman RM. The camptothecin derivative CPT-11 inhibits angiogenesis in a dual-color imageable orthotopic metastatic nude mouse model of human colon cancer. *Anticancer Res.* 2007; 27:713–718. [PubMed: 17465193]

33. Hoffman RM. Visualization of GFP-expressing tumors and metastasis in vivo. *Biotechniques*. 2001; 30:1016–1022. 1024-1016. [PubMed: 11355337]
34. Maeda H, Bharate GY, Daruwalla J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. *Eur J Pharm Biopharm*. 2009; 71:409–419. [PubMed: 19070661]

Figure 1. Antimetastatic efficacy of MEN4901/T-0128 in the orthotopic HT-29-GFP human colon tumor model

Typical fluorescence optical images at necropsy obtained from animals treated with MEN4901/T-0128, Irinotecan and untreated animals. Fluorescence optical imaging was performed using a Hamamatsu C5810 3-chip cooled color charge-coupled device camera with a D425/60 band-pass excitation filter and GG475 long-pass emission filter as described in the Materials and Methods. **A:** Animal from **Group-1** (MEN4901/T-0128 1760 mg/kg, single). **B:** Animal from **Group-2** (MEN4901/T-0128 880 mg/kg, q7d $\times$ 2). **C:** Animal from **Group-3** (MEN4901/T-0128 880 mg/kg, single). **D:** Animal from **Group-4** (MEN4901/T-0128 440 mg/kg, q7d $\times$ 2). **E:** Animal from **Group-5** (MEN4901/T-0128 440 mg/kg, q4d $\times$ 4). **F:** Animal from **Group-6** (Irinotecan 80 mg/kg, q4d $\times$ 4). **G:** Animal from **Group-7** (Irinotecan 40 mg/kg, q4d $\times$ 4). **H:** Animal from **Group-8** (Untreated). White solid arrows indicates primary tumor in the colon. Orange solid arrows indicate mesentery lymph node metastasis.

Figure 2. H&E staining of the tissue sections for metastasis assessment in the orthotopic HT-29 human colon tumor model

Typical histology from the animals with primary HT-29 tumor (A), mesentery lymph nodes metastasis (B) and lung metastasis (C).

Table 1

Efficacy of MEN4901/T-0128 on LN metastasis

Group No.	Treatment	Metastasis ^a			
		GFP imaging	<i>p</i> ^b	Histology	<i>p</i> ^b
1	MEN4901/T-0128 1760 mg/kg, single	2	< 0.001	1	< 0.001
2	MEN4901/T-0128 880 mg/kg, q7d × 2	1	< 0.001	1	< 0.001
3	MEN4901/T-0128 880 mg/kg, single	3	< 0.001	1	< 0.001
4	MEN4901/T-0128* 440 mg/kg, q7d × 2	4	0.001	3	0.007
5	MEN4901/T-0128 440 mg/kg, q4d × 4	2	< 0.001	1	< 0.001
6	Irinotecan 80 mg/kg, q4d × 4	5	0.002	4	0.021
7	Irinotecan 40 mg/kg, q4d × 4	9	0.136	8	0.433
8	Untreated	19	-	16	-

^aMetastatic incidence.

^bAll treated groups compared to Group 8 (untreated controls) by Fisher's exact test.

Table 2

Efficacy of MEN4901/T-0128 on primary tumor growth.

Group No.	Treatment	Tumor weight (g) (Mean $\pm$ SD)	P-value **	IR (%)
1	MEN4901/T-0128 1760 mg/kg, single	0.18 $\pm$ 0.11	< 0.001	80.4
2	MEN4901/T-0128 880 mg/kg, q7d $\times$ 2	0.17 $\pm$ 0.16	< 0.001	81.5
3	MEN4901/T-0128 880 mg/kg, single	0.27 $\pm$ 0.15	0.001	70.7
4	MEN4901/T-0128* 440 mg/kg, q7d $\times$ 2	0.18 $\pm$ 0.08	< 0.001	80.4
5	MEN4901/T-0128 440 mg/kg, q4d $\times$ 4	0.14 $\pm$ 0.07	< 0.001	84.8
6	Irinotecan 80 mg/kg, q4d $\times$ 4	0.40 $\pm$ 0.25	0.013	56.5
7	Irinotecan 40 mg/kg, q4d $\times$ 4	0.71 $\pm$ 0.60	0.703	22.8
8	Untreated	0.92 $\pm$ 0.85	-	-

* Eleven animals were used for evaluation in this group

** All treated groups compared to Group 8 by the Dunnett's test

Table 3

Effect of MEN4901/T-0128 on body weight

Group No.	Treatment	Body weight (g) on day 0 ^a (Mean ± SD)	Body weight (g) on day of nadir (Mean ± SD)	BWL _{max} ^c (%)
1	MEN4901/T-0128 1760 mg/kg, single	22.2 ± 2.3	20.1 ± 2.0 (day 7) ^b	9.5
2	MEN4901/T-0128 880 mg/kg, q7d × 2	23.2 ± 1.9	22.2 ± 1.9 (day 11)	4.3
3	MEN4901/T-0128 880 mg/kg, single	23.1 ± 2.2	22.0 ± 2.5 (day 7)	4.8
4	MEN4901/T-0128* 440 mg/kg, q7d × 2	23.5 ± 1.6	21.6 ± 1.1 (day 11)	8.1
5	MEN4901/T-0128 440 mg/kg, q4d × 4	23.4 ± 2.7	20.6 ± 2.5 (day 11)	12.0
6	Irinotecan 80 mg/kg, q4d × 4	24.2 ± 1.5	21.5 ± 2.4 (day 11)	11.2
7	Irinotecan 40 mg/kg, q4d × 4	22.0 ± 2.1	19.8 ± 2.6 (day 11)	10.0
8	Untreated	22.3 ± 2.1	21.8 ± 2.2 (day 11)	2.2

^aInitiation of treatment^bValues in parentheses correspond to days after initiation of treatment.^cMaximum body loss (%) from the mean body weight at the initiation of treatment.