# Preclinical Evaluation of Antiangiogenic Thrombospondin-1 Peptide Mimetics, ABT-526 and ABT-510, in Companion Dogs with Naturally Occurring Cancers

Anthony Rusk,[1] Evelyn McKeegan,[2] Fortuna Haviv,[2] Sandra Majest,[2] Jack Henkin,[2] and Chand Khanna[1]

**Abstract**

**Purpose:** The angiogenic phenotype of malignant cancers has been established as a target for cancer therapy. ABT-526 and ABT-510, two peptide mimetics of thrombospondin-1 (TSP-1), block angiogenesis *in vitro* and *in vivo* and slow tumor growth in mice. To guide the clinical development of these drugs, translational studies in dogs with naturally occurring cancers were initiated.

**Experimental Design:** A prospective open-label trial using ABT-510 or ABT-526 in pet dogs with measurable malignant spontaneously arising tumors. Endpoints included safety, pharmacokinetics, antitumor activity, and preliminary assessment of changes in circulating endothelial cell populations.

**Results:** Two-hundred and forty-two dogs were sequentially entered to this open-label trial. The elimination half-life for ABT-510 and ABT-526 was 0.7 and 0.8 h, respectively (range, 0.5-1 h). No dose-limiting toxicities were seen in any dogs ($N = 242$). Forty-two dogs receiving peptide had objective responses (>50% reduction in tumor size; $n = 6$) or significant disease stabilization. Most objective responses were seen after 60 days of exposure to the TSP-1 peptide. Antitumor activity was similar for both peptides and was seen in several histologies, including mammary carcinoma, head and neck carcinoma, soft tissue sarcoma, cutaneous T-cell lymphoma, and non – Hodgkin's lymphoma. Assessment of circulating endothelial cell populations in a small subset of dogs suggested that effective exposure to TSP-1 peptides may be associated with reductions in circulating endothelial cells.

**Conclusions:** These results support the safety and activity of ABT-526 and ABT-510 in dogs with naturally occurring malignant cancers. Data from this preclinical trial support the development of TSP-1 mimetic peptides as anticancer agents.

During cancer progression, endothelial cells are activated by growth factors, which result in new capillary development at the tumor site (angiogenesis) or the recruitment of endothelial cell progenitors from bone marrow (vasculogenesis; refs. 1–3). In healthy tissue, endothelial cell proliferation is controlled by a balance between protein factors that activate and antagonize endothelial cells. Malignant tumors promote the endothelial cell proliferation and organization required to create a supportive vasculature and release antiapoptotic factors that discourage endothelial cell death (4–6).

Drugs that target endothelial cells or the process of blood vessel formation may have the potential to prevent development of the vascular beds necessary for tumor growth. Support for this has been provided in preclinical models (7) and more recently in human cancer patients (8, 9). Natural protein inhibitors of angiogenesis include TSP-1, metallospondins, and proteolytic degradation products such as angiostatin, endostatin, kringle 5, and vasostatin (10). Pleiotropic inhibitors, such as thrombospondin-1 (TSP-1), are capable of blocking many aspects of endothelial cell activation initiated by most of the known growth factors. Although individual endothelial cell activators operate via specific cell receptors, all seem to have similar cell signaling pathways. A common mechanism has been suggested for the natural inhibitors in which each, using its unique receptor, leads to apoptosis of the activated endothelial cell (11–15). Because the inhibitors have no effect on quiescent endothelial cells, activation must be required to prime the cell for induction of an apoptotic program by these inhibitors.

TSP-1 was the first natural angiogenesis inhibitor to be discovered (16, 17). TSP-1 and the closely related TSP-2 have the ability to act broadly against a wide variety of proangiogenic growth factors, but their large molecular size and multifunctional nature have precluded their use as therapeutic agents (16, 18). Modified peptide segments of the antiangiogenic domain of TSP-1 containing D-amino acids have been shown to mimic the antiangiogenic action of TSP-1 (19). Recently, two

**Authors' Affiliations:** [1]Animal Clinical Investigation, LLC, Columbia, Maryland and [2]Abbott Laboratories, Abbott Park, Illinois

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**Requests for reprints:** Anthony Rusk, Animal Clinical Investigation, LLC, at Friendship Hospital for Animals, 4105 Brandywine St., NW, Washington, DC 20016. Phone: 410-419-0804; Fax: 202-363-7126; E-mail: trusk@animalci.com.



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further modified nonapeptides in this series, ABT-526 and ABT-510 (also called DI-TSP and DI-TSPa, respectively), have also been found to show antiangiogenic activity (13, 20). *In vitro* studies have shown that 0.5 to 10 nmol/L concentrations of these peptides act through antiangiogenic mechanisms. Specifically, they block the migration of human microvascular endothelial cells stimulated by any of several growth factors, abrogate endothelial cell proliferation and tube assembly in fibrin gels stimulated by vascular endothelial growth factor, and enhance apoptosis of activated endothelial cells (13, 20). *In vivo* studies have shown that both ABT-526 and ABT-510 slow tumor growth in syngeneic and xenograft mouse models and that, like TSP-1, these peptides increase the apoptotic index of tumor endothelial cells in orthotopic mouse models (11, 13, 20). ABT-510 has recently been shown *in vivo* to reduce circulating endothelial cell and endothelial cell progenitors in TSP-1 knockout mice and in mice bearing Lewis lung tumors (21). Because endogenous inhibitors have little inherent potential for toxicity, it should be possible for proteins or active peptide domains with similar mechanisms to restore and maintain antiangiogenic balance through multiple mechanisms. Although rapid destruction of tumor cells is unlikely, tumor stasis or slow regression may be more feasible. These long-term activities may be difficult to define in conventional mouse cancer models due to their rapid rates of tumor progression and the discordant background of human tumor cells and mouse endothelial cells in xenograft models. To better define the safety and explore the potential anticancer activity of the TSP-1 mimetic peptides, ABT-526 and ABT-510, a prospective, open-label nonclinical trial using both antiangiogenic peptides was undertaken in pet dogs with a number of distinct naturally occurring cancers. Both peptides were well tolerated and yielded surprising and dramatic regressions in a small number of dogs with measurable malignant tumors. Tumor regressions were primarily seen following extended (>30 days) exposures to TSP-1 peptides. Data from this nonclinical trial in pet dogs have been informative and have guided the developmental path of TSP-1 mimetic peptides for human patients.

## Materials and Methods

### TSP-1 mimetic peptides, ABT-526 and ABT-510

ABT-526 is a nonapeptide, *N*-Ac-Sar-Gly-Val-D-Ile-Thr-Nva-Ile-Arg-Pro-NHEt, where Sar and Nva are abbreviations for sarcosine and L-norvaline, respectively. ABT-510 is an enantiomer of ABT-526, a peptide of identical sequence wherein the D-isoleucine at position 4 was replaced with D-alloisoleucine (20). These two TSP-1 mimetic peptides were designed based on the heptapeptide fragment, Gly-Val-Ile-Thr-Arg-Ile-Arg, derived from the second type-1 repeat of TSP-1 (22). The optimization of potency and pharmacokinetics of the original heptapeptide has been reported (20). For nonclinical studies in pet dogs, ABT-526 and ABT-510 were provided in sterile serum-capped vials, each containing ABT-526 or ABT-510 solution dissolved in 5% sterile glucose.

### Cell lines

Human microvascular endothelial cells were obtained from Cambrex (Walkersville, MD).

Dog hemangiosarcoma cells were kindly provided by Dr. Doug Thamm (University of Wisconsin, Madison, WI). Dog blood outgrowth endothelial cells were provided by Dr. Robert Hebbel (University of Minnesota, Minneapolis, MN; ref. 22). Endothelial cells were grown in EGM2-MV medium (BioWhittaker). Dog hemangiosarcoma cells were grown in DMEM (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum and 1% penicillin-streptomycin.

### Endothelial chemotaxis assay

Cells were washed with PBS and cultured into endothelial basal medium containing only 5% fetal bovine serum and 1% penicillin-streptomycin for 18 h. The cells were then washed, trypsinized, and resuspended in Hanks buffer (no $Ca^{2+}$) at a concentration of $2 \times 10^6$/mL to $5 \times 10^6$/mL. Cells were incubated with calcein AM (Invitrogen) at room temperature in the dark for 30 min. Cells were then centrifuged and resuspended in endothelial basal medium containing 0.1% bovine serum albumin and 1% penicillin-streptomycin at $1 \times 10^6$/mL. A 96-well filter plate (NeuroProbe 101-8) was used according to the manufacturer's instructions. Briefly, 10 ng/mL vascular endothelial growth factor (R&D Systems, Minneapolis, MN) was prepared in endothelial basal medium/bovine serum albumin medium and added to the bottom chamber. Cells were added to the top chamber. The plates were incubated at 37°C, 5% $CO_2$ for 4 h. After 4 h, the free cells (unmigrated) were removed from top filter and the plate was read in a FLx800 BioTek fluoroscan at 485-nm excitation and 530-nm emission.

### Nonclinical trial of ABT-526 and ABT-510 in pet dogs with naturally occurring cancers

***Study design.*** This prospective open-label, multicenter nonclinical trial evaluated the clinical efficacy of TSP-1 mimetic peptides (ABT-526 and ABT-510) in dogs with spontaneous, measurable, histologically confirmed malignant tumors treated between June 1, 2000, and August 1, 2004. Animals were evaluated at one of the following veterinary hospitals with the oversight of the Animal Clinical Investigation, LLC: Friendship Hospital for Animals, Washington, DC; Atlantic Veterinary Internal Medicine, Annapolis, MD; Dogs and Cats Veterinary Referral of Bowie, Bowie, MD; Gulf Coast Veterinary Diagnostic Imaging and Oncology, Houston, TX; Med Vet, Worthington, OH; New England Veterinary Oncology Group, Waltham, MA; Pet Emergency and Specialty Center, La Mesa, CA; San Francisco Veterinary Specialists, San Francisco, CA; Southpaws Veterinary Referral Center, Springfield, VA; Veterinary Oncology and Hematology Center, Norwalk, CT; and Veterinary Referral Associates, Gaithersburg, MD. Eligibility criteria included the following: measurable tumors (measurable by gross visualization, radiography, ultrasonography, or computed tomography or magnetic resonance imaging scan); grade 0 to 1 performance status (based on modified Eastern Cooperative Oncology Group performance score; Table 1); the expectation of survival of at least 30 days; no concurrent chemotherapy or radiation therapy; no chemotherapy or radiation therapy within 21 days of entry to trial; no initiation of corticosteroid or nonsteroidal anti-inflammatory therapy within 21 days of entry; and written informed consent obtained from all the dog owners before any study procedures were initiated.

***Study treatment protocol.*** Before initiation of treatment, animals underwent complete physical examination and clinical staging by WHO tumor-node-metastasis classification. Staging included complete blood count, serum biochemistry, urinalysis, and thoracic and abdominal radiographs. Additional diagnostic tests were done to complete tumor-node-metastasis staging as needed. Following staging, measurable lesions were identified and recorded for longitudinal assessment. On day 1, the first dose of ABT-526 or ABT-510 was administered s.c. by the investigator with the owner present. Pet owners were trained to administer s.c. injections thereafter. The administration regimen and schedule for ABT-526 and ABT-510 were as follows:

Dogs 1 to 26: ABT-526 12.5 mg BID, s.c.
Dogs 27 to 72: ABT-526 0.5 mg/kg BID, s.c.
Dogs 1 to 30: ABT-510 12.5 to 17.5 mg BID, s.c. (cases 11, 13, 17, 19, 20, 24, 25, 26, and 29 received 17.5 mg BID, s.c.).
Dogs 31 to 170: ABT-510 1.0 mg/kg QD or divided BID, s.c.

61 cases were treated with 1.0 mg/kg QD.
71 cases were treated with 1.0 mg/kg divided BID.

Follow-up examination on day 7 included physical examination, review of owner compliance history, and drug administration technique. On day 30 and every 30 days thereafter, reevaluation of measurable lesion(s) was undertaken. Response to therapy was recorded as defined below. Owner compliance was evaluated by questionnaire and reconciliation of returned empty ABT-526 or ABT-510 vials. Pet owners were given the right to withdraw their pets from therapy at any time during the treatment course.

***Pharmacokinetic evaluation of tumor-bearing dogs receiving ABT-526 or ABT-510.*** Serum TSP peptide analysis was carried out by liquid chromatography/mass spectrometry, with a quantitative detection limit of 5 ng/mL. Pharmacokinetic analysis of ABT-526 was undertaken in selected tumor-bearing dogs receiving the fixed dose/variable body weight treatment regimen (dogs 1-27) at the initiation of therapy and after 30 days of therapy. Serum samples were collected immediately before injection and at 0.25, 1.0, 2.0, 4.0, and 8 h postinjection. Pharmacokinetic analysis of ABT-510 was undertaken in a similar manner to ABT-526. Serum samples were collected from the first 10 dogs assigned to receive ABT-526. Samples were collected immediately before injection and at 0.25, 1.0, 2.0, 3.0, 4.0, and 5.0 h postinjection.

***Clinical assessment.*** Treatment-associated adverse events were evaluated at study days 7 and 30, and then every 30 days thereafter, by review of owner history and physical examination. Complete blood count, serum biochemistry, and urinalysis were done on all dogs if drug-induced adverse events were suspected. Owners were instructed to contact the investigator within 24 h of any adverse event. Adverse events, whether confirmed to be drug induced or not, were entered as part of each animal's veterinary medical record and included in the primary study notebook. Definitions for tumor responses were as follows.

Complete response was defined as the disappearance of all clinical evidence of measurable lesions. Partial response was considered to be a decrease of 50% or more in the sum of the products of measurements for measurable lesions, no increase of any size in any single lesion, and no evidence of the appearance of new lesions. Stable disease was defined as no response to treatment, a response less than a partial response without the appearance of new lesions, or worsening of clinical signs. Progressive disease was considered to be an unequivocal increase of 50% or more in the size of any measurable lesion or the appearance of any new lesions. Relapse was defined as the appearance of new lesions or reappearance of old lesions in animals with a previously complete response. In dogs with only a partial previous response, relapse was defined as an increase of at least 50% in the sum of the products of measurements of representative lesions over that obtained at the time of maximum response to treatment.

**Table 1.** Modified Eastern Cooperative Oncology Group performance score

| Grade | Description |
|---|---|
| 0 | Normal activity |
| 1 | Restricted activity; decreased activity from predisease status |
| 2 | Compromised; ambulatory only for vital activities; consistently defecates and urinates in acceptable areas |
| 3 | Disabled; must be force fed; unable to confine urination and defecation to acceptable areas |
| 4 | Dead |

***Detection of circulating endothelial cells.*** Validated detection of circulating endothelial cells in dogs was as recently described.[3] Whole blood was collected by venipuncture, stored in heparinized glass vials, and shipped overnight at 4°C for analysis. Cells were incubated with fluorescent antibodies to human CD146-FITC (Calbiochem, San Diego, CA), canine CD34-phycoerythrin (BD Biosciences, San Diego, CA), and canine CD45-allophycocyanin (Serotec, Raleigh, NC) along with a nuclear stain (LDS-751, Molecular Probes). The phenotypic analysis was done by switching CD106-phycoerythrin (human), Annexin V-phycoerythrin (BD Biosciences), or CD133-phycoerythrin (human; Miltenyi Biotec) for the CD34 stain. A panel of isotype control antibodies (BD Biosciences) was used to establish the negative control instrument settings. Stained whole blood then underwent RBC lysis and fixation using FACSLyse (DAKO) and was read on a BD FACSCalibur. The control gates were set such that a combination of all variables (FITC positive, phycoerythrin positive, nucleus positive, and allophycocyanin negative) was <0.01% of the total population and a combination of either FITC positive, nucleus positive, and allophycocyanin negative or phycoerythrin positive, nucleus positive, and allophycocyanin negative, was <0.25% of the total population. Control gating was done to ensure that all positive events (circulating endothelial cells) would be captured with a minimum of nonspecific events incorporated into the analysis. Fifty thousand events were run in duplicate for each measurement and data are reported as the average of these measurements. All phenotypic analysis was reported as a percentage of the total circulating endothelial cells ($CD146^+$, nucleus positive, and $CD45^-$) from a sample. Data were reported as the mean, SE, and ranges. Additionally, an absolute nucleated cell count for the blood sample was determined using the leukocount kit (BD Biosciences) to enable the final readout of circulating endothelial cells per microliter of blood. Statistical analyses to determine *P* values were done using the Student's *t* test using equal variances. Successful circulating endothelial cell analysis on blood collected at day 0 and after 30 days of ABT-510 therapy was available in 13 dogs. The assay was not available during treatment of dogs with ABT-526.

## Results

**In vitro** ***activity of ABT-526 and ABT-510 against canine and human endothelial cells.*** To show biological activity of TSP-1 mimetic peptides in canine cells, *in vitro* activity of ABT-526 and ABT-510 was assessed against canine and human endothelial cells using a vascular endothelial growth factor–induced endothelial cell chemotaxis assay. Both TSP-1 mimetic peptides responded similarly to the vascular endothelial growth factor–induced endothelial cell chemotaxis assay (not shown). Table 2 shows suppression of vascular endothelial growth factor–induced endothelial cell chemotaxis and TSP-1 mimetic peptide suppression of endothelial cell chemotaxis in dog and human endothelial cells. Inhibition of canine and human endothelial cell chemotaxis after exposure to ABT-510 at 1,000 pmol/L was 62% and 53%, respectively. Inhibition of chemotaxis was carried out in a medium depleted of protein and growth factors so that endothelial cell chemotaxis inhibition reflected both cell type responsiveness and intrinsic affinity. These *in vitro* ABT-510 concentrations are relevant because *in vivo* plasma concentrations measured over the first 4 h after injection range from 50 to 500 nmol/L in mice, dogs, and humans. Furthermore, at these relative exposures, both canine and human endothelial cell chemotaxis was inhibited by 75% and 79%, respectively. These data support the relevance and biological activity of TSP-1 mimetic peptides against canine

[3] E. McKeegan et al., in review.

**Table 2.** TSP-1 peptide mimetics are biologically active against canine endothelial cells

| ABT-510 (pmol/L) | Percentage of chemotaxis inhibition* | | |
|---|---|---|---|
| | HMVEC | Dog BOEC | Dog DEN-HAS |
| 10,000 | 70 ± 5 | 79 ± 8 | 99 ± 1 |
| 1,000 | 53 ± 7 | 62 ± 11 | 78 ± 4 |
| 100 | 30 ± 5 | 53 ± 5 | 78 ± 7 |
| 10 | <10 | 34 ± 1 | 63 ± 18 |
| 1 | <10 | <10 | 57 ± 8 |
| 0.1 | <10 | <10 | <10 |

Abbreviations: HMVEC, human microvascular endothelial cells; BOEC, blood outgrowth endothelial cell; DEN-HAS, canine hemangiosarcoma cell line.
*Mean percentage of endothelial cell chemotaxis inhibition, normalized against untreated cells (±SE for triplicate experiments). Each experiment was repeated at least twice.

endothelial cells *in vitro*. Interestingly, the canine hemangiosarcoma cell line (a malignant endothelial cell or endothelial cell progenitor) was more resistant to the chemotaxis inhibition than either canine or human endothelial cells (Table 2).

***Population of evaluable tumor-bearing dogs.*** Seventy-four dogs were enrolled in the study to receive ABT-526, and 168 dogs were enrolled in the study to receive ABT-510. Of these, 58 dogs were able to receive ABT-526 for at least 30 days, and 122 dogs were able to receive ABT-510 for at least 30 days; these cases constituted the evaluable population (population that received treatment). The most common cause of early withdrawal from the study was tumor progression, which significantly affected the quality of life. No dogs discontinued therapy as result of treatment-associated adverse events. The tumor histologies (Table 3) represented in the ABT-526–evaluable population included carcinoma ($n$ = 21), lymphoma ($n$ = 17), sarcoma ($n$ = 14), melanoma ($n$ = 4), and other ($n$ = 2). Tumor histologies represented in the ABT-510–evaluable population included carcinoma ($n$ = 40), lymphoma ($n$ = 16), sarcoma ($n$ = 54), melanoma ($n$ = 5), and other ($n$ = 7). Fifty-four dogs treated with ABT-526 and 93 dogs treated with ABT-510 had received prior chemotherapy, surgery, or radiation therapy before receiving either ABT-526 or ABT-510 (Table 3). All dogs, irrespective of past therapy, had measurable disease at the time of study enrollment and had not received past therapy for at least 21 days.

***Pharmacokinetic analysis in tumor-bearing dogs.*** Pharmacokinetic studies after the first dose and after 30 days of ABT-526 therapy revealed an ABT-526 half-life of 48 min (range, 33-60 min) with no drug detectable (detection limit 10 ng/mL) in serum 8 h after injection (Fig. 1). A similar single-dose pharmacokinetic pattern was seen in dogs treated with ABT-510 (Fig. 2). The half-life of ABT-510 was 44 min (range, 36-44 min). The pharmacokinetic profile in dogs remained stable after 1 month of treatment for both TSP-1 peptides, with no induction of metabolism through 30 days of exposure to the drug (data not shown).

***Adverse events reported in tumor-bearing dogs.*** No significant treatment-related adverse effects were observed in any dog. A single dog with multiple cutaneous mast cell tumors developed signs of weakness and lethargy 30 days after ABT-526 therapy was initiated; however, these signs could not be separated from those of disease progression. Four dogs underwent surgical procedures during either ABT-526 or ABT-510 therapy without adverse effects on wound healing. Surgical incisions included midline laparotomy and nephrectomy, midline laparotomy and splenectomy, surgical repair of wound dehiscence that developed before ABT-526 therapy, and an incisional biopsy. No animals showed clinical evidence of joint discomfort or arthritis exacerbated by drug treatment. Most dogs entered to this study were older dogs, many of which would be expected to have clinical or subclinical osteoarthritis. The development of keratitis or progressive conjunctivitis was not observed in any dog.

***Clinical response.*** Responses in measurable lesion(s) in the evaluable ABT-526–treated population of 58 dogs (Tables 3 and 4) included three complete responses (100% tumor regression), seven partial responses (>50% tumor regression), and nine cases of stable disease determined to be significant based on the natural biology of each disease and the progression pattern before study entry. Objective responses (50-100% tumor regression) of malignant measurable tumors in dogs receiving TSP-1 mimetic peptides provided direct evidence of the anticancer activity of these agents, because each dog serves as its own treatment control. The notable objective responses are as follows.

ABT-526 dog 5, an 8-year-old male golden retriever, with an incompletely resected recurrent grade 3 fibrosarcoma of the soft tissue overlying the left elbow (Fig. 3), treated with 12.5 mg ABT-526 BID. The primary tumor had recurred following incomplete resection twice before presentation to the study. At presentation, histologically confirmed metastases were noted in the left axillary and left prescapular lymph node (estimated tumor volume in lymph nodes was 700 $cm^3$). Thoracic radiographs showed at least three pulmonary metastases. Metastatic soft tissue sarcomas in dogs are poorly sensitive to systemic chemotherapy and are expected to progress within 30 to 45 days of presentation (23, 24). No significant change in disease status was noted after 30 days of ABT-526 therapy.

**Table 3.** Description of evaluable ABT-526– and ABT-510–treated dogs

| | Evaluable ABT-526 dogs ($n$ = 58) | Evaluable ABT-510 dogs ($n$ = 122) |
|---|---|---|
| Histology | | |
| Carcinoma | 21 | 40 |
| Lymphoma | 17 | 16 |
| Sarcoma | 14 | 54 |
| Melanoma | 4 | 5 |
| Other cancer | 2 | 7 |
| Previous therapy | | |
| Surgery | 20 | 39 |
| Chemotherapy | 37 | 53 |
| Radiation therapy | 12 | 18 |
| Combination surgery, chemotherapy or radiation | 19 | 20 |
| None | 11 | 32 |

NOTE: The evaluable population of dogs are those that are able to receive ABT-526 or ABT-510 for at least 30 days.

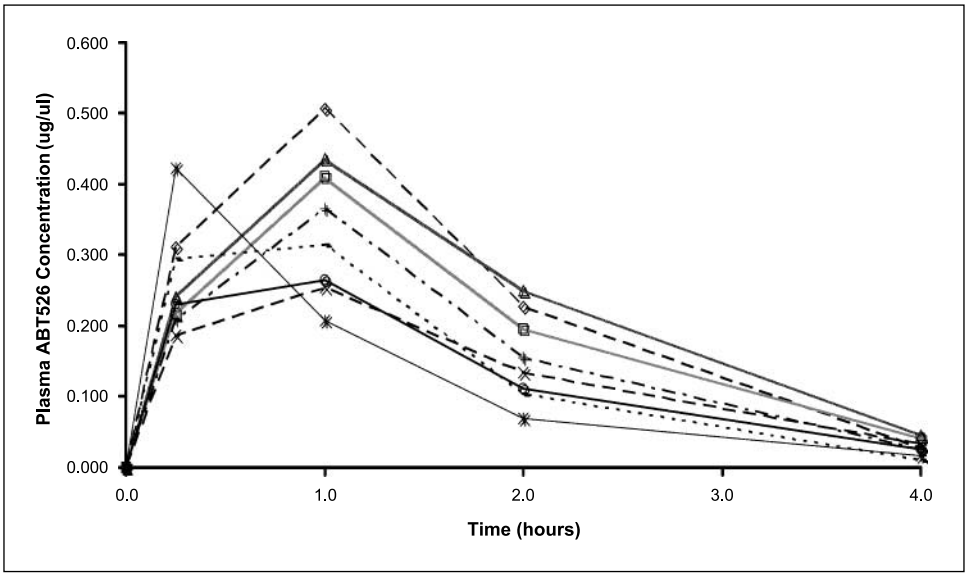


**Fig. 1.** Serum concentrations of ABT-526 (μg/mL) obtained by pharmacokinetic analysis over a 4-h period after the initial s.c. injection of the study drug on day 1 in eight dogs with spontaneously occurring tumors.

After 60 days of therapy, mild regression of lymph node and pulmonary metastases was noted. Continued therapy with ABT-526 over 210 days resulted in a near-complete regression of pulmonary metastases (90% response), a complete regression of prescapular lymph node, stable disease of the axillary lymph node (35% regression), and stable disease of the ulcerated primary site. Eventual progression of the primary tumor and pulmonary metastases prompted euthanasia at >270 days after initiation of peptide treatment. Responses seen at the effaced prescapular lymph node and axillary lymph node persisted despite progression in the primary tumor and pulmonary metastases.

ABT-526 dog 7, an 8-year-old male miniature poodle with generalized cutaneous lymphoma (mycosis fungoides). Cutaneous lesions manifested as generalized scaling with patchy alopecia, erythema pruritus, and mild dermal ulceration distributed over the trunk and perineum. Previous anticancer therapy included cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP)–like chemotherapy and retinoid therapy to which the cutaneous lesions were refractory. No effective treatments for mycosis fungoides exist following development of resistance of systemic chemotherapy or retinoid therapy (23, 24). Following the discontinuation of retinoid and chemotherapy, with appropriate washout, ABT-526 therapy was initiated and resulted in partial regression of all lesions within 30 days. Complete regression of all lesions was noted after 60 days of ABT-526 therapy (Fig. 4) with the exception of mild scaling at the perineum that persisted through day 150. Beginning at 150 days of therapy, progressive recurrence of cutaneous lesions, including generalized scaling, erythema, and pruritus were noted, prompting discontinuation of ABT-526 therapy.

ABT-526 dog 9, a 12-year-old male Labrador retriever cross with multicentric, high-grade, B-cell non–Hodgkin's lymphoma, treated with 12.5 mg ABT-526 BID. Past therapy included CHOP-like chemotherapy that provided a decrease in lymph node size but no complete remission. Additional chemotherapy included a combination of dacarbazine and doxorubicin chemotherapy that was not associated with sustained reduction in lymphoma burden. Based on the natural history of chemoresistant lymphoma, it was not likely that additional rescue chemotherapy would be effective in

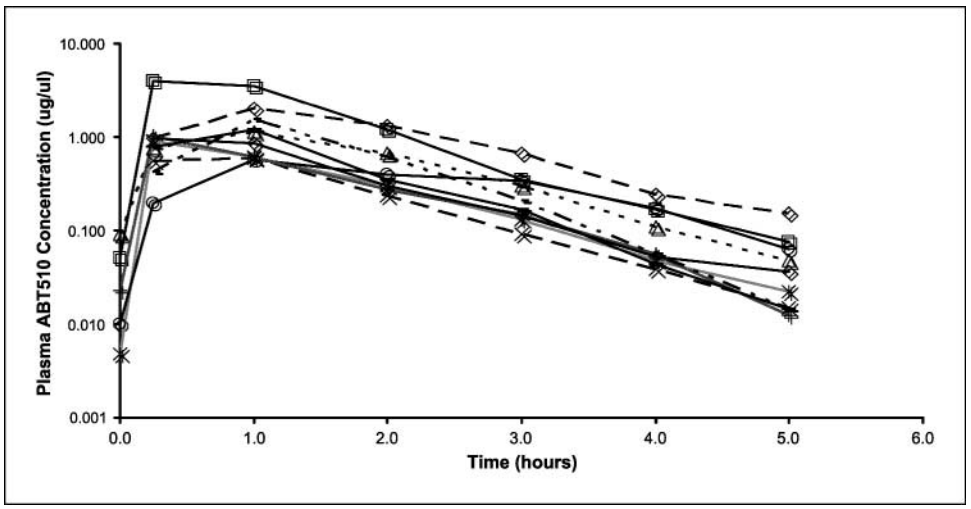


**Fig. 2.** Serum concentrations of ABT-510 (μg/mL) obtained by pharmacokinetic analysis over a 5-h period after the initial s.c. injection of the study drug on day 1 in 10 dogs with spontaneously occurring tumors.

**Table 4.** Responding population of dogs treated with ABT-526

| Breed/study no. | Tumor histology | TNM stage | Response | Response duration (d) |
|---|---|---|---|---|
| Labrador retriever | Nasal adenocarcinoma | $T_{3b}N_0M_0$ | CR | 165 |
| Corgi mix | Cutaneous lymphoma | Cutaneous | PR | 185 |
| Labrador retriever mix | Nasal carcinoma (SCC) | $T_{3b}N_1M_0$ | PR | >100 |
| Cocker spaniel | NHL | IIIb | PR | 60 |
| Golden retriever | Soft tissue sarcoma | $T_{(sx)}N_1M_1$ | PR | >270 |
| Doberman mix | NHL | IIIa | PR | 90 |
| Miniature poodle | Cutaneous lymphoma | Cutaneous | PR | 150 |
| English cocker spaniel | GI adenocarcinoma (apocrine gland) | $T_{(sx)}N_2M_0$ | CR | >195 |
| Labrador retriever mix | NHL | IIIa | PR | >1,310 |
| Rottweiler mix | Oral carcinoma (SCC) | $T_2N_0M_0$ | PR | >1,090 |
| Sheltie mix | Mammary adenocarcinoma | $T_{(sx)}N_1M_0$ | SD | >900 |
| Golden retriever | NHL | IVa | SD | 90 |
| Cairn terrier | Cutaneous basal cell carcinoma | $T_{(sx)}N_1M_0$ | SD | 90 |
| Shetland sheepdog mix | Mammary adenocarcinoma | $T_{(sx)}N_2M_0$ | SD | 90 |
| Cocker spaniel | NHL | IIIa | SD | 60 |
| Border collie | Thyroid adenocarcinoma | $T_{2b}N_0M_0$ | SD | 180 |
| Golden retriever | Abdominal carcinoma GI | $T_2N_1M_0$ | SD | 150 |
| Labrador retriever mix | Basal cell carcinoma | $T_{2b}N_0M_0$ | SD | 90 |
| Airedale | Tracheal chondrosarcoma | $T_{(sx)}N_0M_1$ | SD | 180 |

Abbreviations: SCC, squamous cell carcinoma; NHL, non–Hodgkin's lymphoma; GI, gastrointestinal tract; TNM, tumor-node-metastasis; CR, complete response; PR, progressive disease; SD, stable disease.

this dog. Accordingly, chemoresistance and decreased quality of life prompted entry to the ABT-526 preclinical trial. At the time of entry, the measurable lesion was defined as a single cytologically confirmed mandibular lymph node (lymph node volume, 58 $cm^3$). Prescapular lymph nodes were concurrently enlarged at presentation. Through 90 days of ABT-526 therapy, a gradual regression of the mandibular lymph node and complete regression of enlarged prescapular lymph nodes was noted. Complete regression of the previously measurable mandibular lymph node was noted by day 120. This response waxed and waned between a coplete response and a partial response (lymph node volume, 15 $cm^3$) through 420 consecutive days of therapy. An incisional lymph node biopsy done at day 420 was histologically consistent with a low-grade lymphoma. This dog was free of progression, at the time of data analysis, 1,310 days after starting ABT-526.

Dog 10, a 3-year-old Rottweiler cross male with a rostral maxillary squamous cell carcinoma with invasion into the left oral cavity (Fig. 5), was treated with 12.5 mg ABT-526 BID. No other treatment had been received before the ABT-526 therapy. Tumor progression and invasion of the rostral maxilla was noted through the first 60 days of therapy (23, 24). Tumor progression and bleeding from this oral lesion was noted between days 30 and 60 of therapy. After 90 days of continuous therapy, a marked regression of the oral mass was noted. Incisional biopsy of the primary tumor site at 420 days revealed microscopic persistence of squamous cell carcinoma at the site of the original tumor. No recurrence of this oral mass has been noted after 1,185 days on therapy. This sustained response is considered to be highly significant given the expectation that oral squamous cell carcinoma would be associated with progressive primary tumor growth and invasion within 60 days of presentation.

ABT-526 dog 11, a 9-year-old female Sheltie with a history of a malignant mammary tumor resected completely. This dog presented for entry to the ABT-526 preclinical study following regional lymph node metastases from the mammary tumor had progressed despite treatment with doxorubicin and then carboplatin chemotherapy. In the first 60 days of therapy, there was mild progression of the lymph node metastases. Subsequent monthly evaluations over 210 days revealed disease stabilization. Needle aspirate of the measurable lymph node showed necrotic debris and viable malignant mammary carcinoma cells. Continued stabilization of disease over 60 days was observed. Lymph node resection was done at day 270. Histologic evaluation of the resected lymph node revealed malignant mammary carcinoma. ABT-526 was continued for 30 days following surgery. This dog was free of disease and recurrence at the time of data analysis (900 days) following initiation of ABT-526 therapy. Dogs that present with metastatic mammary tumors that are not responsive to cytotoxic chemotherapy have a grave prognosis typically associated with disease progression at initial and secondary metastatic sites, including, liver, lung, and bone (23, 24).

Of the 10 dogs with complete or partial responses to ABT-526, eight dogs have had relapse of their disease while on continuous therapy. Significant stable disease was observed in nine dogs (Tables 3 and 4).

Responses in measurable lesion(s) in the evaluable ABT-510–treated population of 122 dogs (Tables 4 and 5) included 3 complete responses, 6 partial responses, and 14 dogs with stable disease determined to be of significant duration. Notable objective responses included the following:

ABT-510 dog 1, a 6-year-old female spayed golden retriever, with a history of relapsed non-Hodgkin's lymphoma treated before the study with multiagent cyclical chemotherapy. Lymphoma relapse was seen 12 months after induction with CHOP-like chemotherapy. The site of relapse was intra-abdominal mesenteric lymph nodes confirmed cytologically

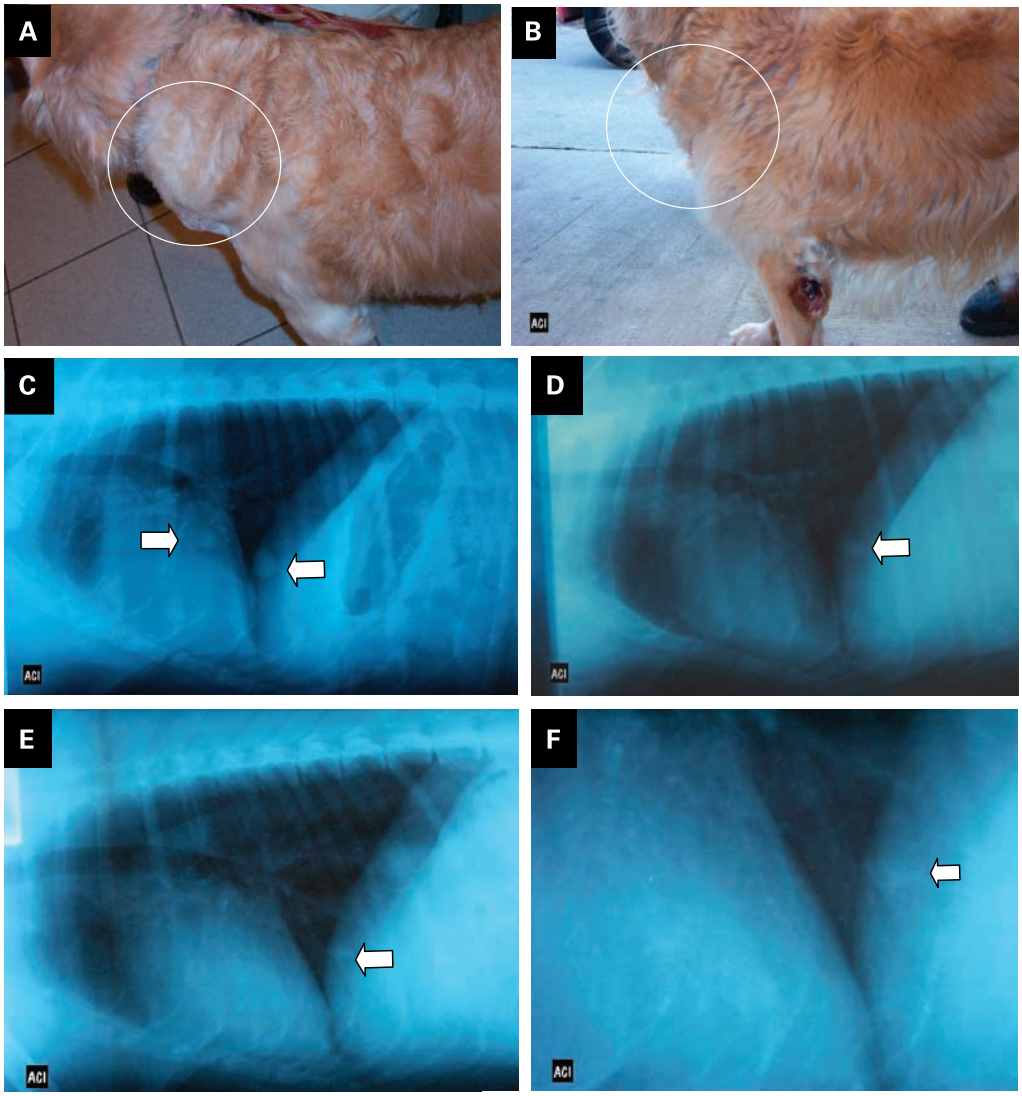


**Fig. 3.** ABT-526 peptide of TSP-1 resulted in objective regression in a dog with metastatic soft tissue sarcoma. *A,* histologically confirmed regional prescapular lymph node metastasis from a minimally resected soft tissue sarcoma of the elbow before TSP-1 therapy (*within white circle*). *B,* a complete regression of this prescapular lymph node metastasis was seen after 60 d of treatment; image was captured at day 210 (*within white circle*). *C,* pulmonary metastasis identified on lateral thoracic radiographs before initiation of therapy (*arrows*). *D,* regression of pulmonary metastases was noted after 60 d of therapy. Seventy-five percent regression of pulmonary metastasis seen after 150 d of treatment. *E* and *F,* further regression to 90% (regression of remaining pulmonary metastasis occurred through 240 d of treatment). *F,* magnified radiographic image of metastatic site.

as lymphoma. A partial response to 12.5 mg ABT-510 BID was noted after 30 days of therapy. A complete response to ABT-510 was identified at day 90 and continued until recurrent mesenteric lymph nodes were found at day 180. The dog continued ABT-510 therapy until day 240 with slow progression in mesenteric lymph nodes noted. At day 240, the dog discontinued ABT-510 therapy and was then euthanized due to disease progression.

ABT-510 dog 2, a 9-year-old female spayed Doberman, with a history of grade 2 maxillary fibrosarcoma associated with the zygomatic arch. The dog received palliative radiation therapy, which resulted in a partial response. After no further radiation response was seen, treatment with 12.5 mg ABT-510 BID was initiated and tumor staging was done every 30 days via digital photography and examination. The total tumor volume at study initiation was 21.0 $cm^3$. No response to therapy was seen through 90 days of continuous ABT-510 therapy. A partial response to ABT-510 was identified at day 120 (tumor volume, 10 $cm^3$) and continued until day 240 (tumor volume, 18.0 $cm^3$). No changes in the dose of ABT-510 were made; however, a second response to therapy was noted at day 270 (tumor volume, 2.6 $cm^3$). ABT-510 therapy was continued until day 970 at which time tumor progression was noted (tumor volume, 35.0 $cm^3$), and the dog discontinued ABT-510 therapy. Coincidentally, a laparotomy and splenectomy were done for a benign splenic hematoma at day 580, while receiving ABT-510, without complications or delays in wound healing.

***Changes in circulating endothelial cells during TSP-1 peptide therapy.*** Toward the completion of accrual to this study, the detection of canine circulating endothelial cells became feasible. Accordingly, a small subset of dogs (13) assigned to receive ABT-510 therapy had pretreatment, and day 30 circulating endothelial cell analysis was done. Within this population of dogs, three were included in the evaluable population and 10 dogs had early disease progression that resulted in their removal from study at or before day 30 of ABT-510 therapy. In the 10 dogs that experienced early disease progression, no significant changes in total mean circulating endothelial cells or circulating endothelial cell subtypes were seen between pretreatment and day 30 (Fig. 6). In the three dogs that continued on ABT-510 therapy for longer than 30 days, there was a significant decrease in mean total circulating endothelial cell numbers, $CD106^+$ (endothelial precursor) circulating endothelial cells, $CD133^+$ (endothelial precursor) circulating endothelial cells, and an increase in Annexin V–positive (apoptotic endothelial cells) circulating endothelial cells from pretreatment to the day 30 sample collection time (Fig. 6).

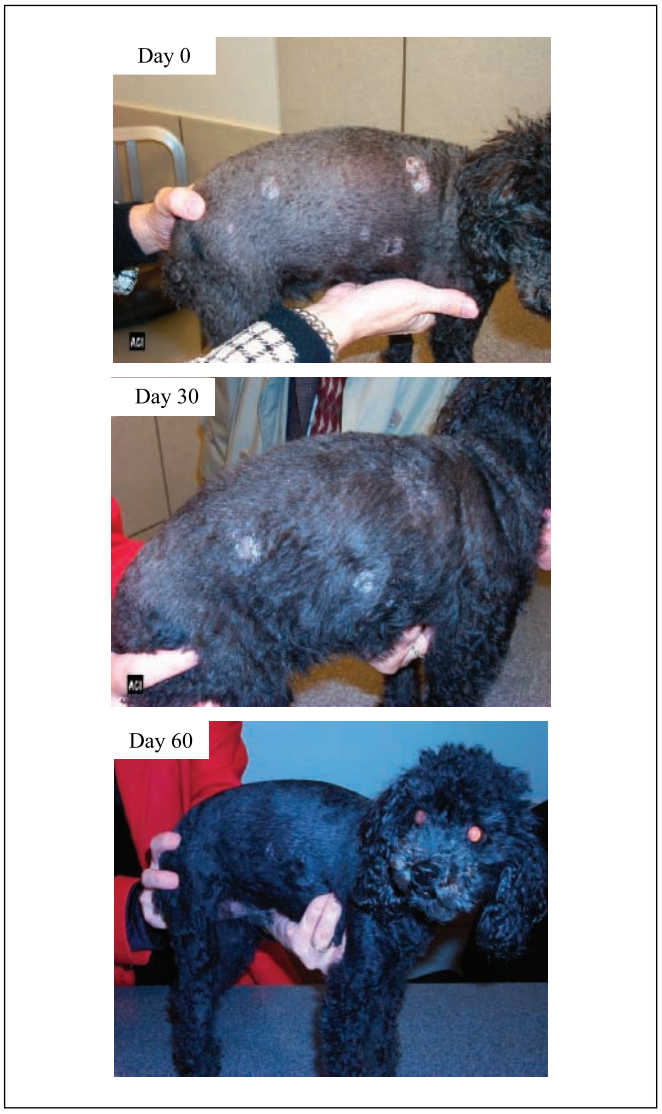


**Fig. 4.** ABT-526 peptide of TSP-1 resulted in regression of cutaneous lymphoma (mycosis fungoides) lesions in an 8-y-old toy poodle. ABT-526 therapy was initiated following CHOP-like chemotherapy and retinoid therapy to which the cutaneous lesions were refractory. Partial regression of diffuse scaling and erythema was noted at day 30 with complete regression of most lesions at day 60. Progression of scaling, erythema, and pruritus was noted at day 150, prompting discontinuation of ABT-526 therapy.

## Discussion

ABT-526 and ABT-510 antiangiogenic therapies, administered s.c. to pet dogs with naturally occurring cancers, resulted in the objective regression of measurable lesions in 19 of 180 evaluable dogs and significant disease stabilization in 23 of 180 evaluable dogs. Objective responses and significant disease stabilization required continuous and extended exposures to TSP-1 peptides and were seen in many tumor histologies. Treatment with ABT-526 or ABT-510 was well tolerated. Pharmacokinetic assessment in tumor-bearing dogs suggested rapid clearance of peptides following s.c. dosing. Response to therapy could not be predicted based on initial tumor burden, stage of disease (primary or metastatic lesions), or past treatments. The consistency of pharmacokinetic data among dogs suggests that it was unlikely that response was related to different clearance between animals, especially because these peptides were cleared as simple cleaved products in urine without secondary metabolism. Circulating endothelial cells were successfully detected in dogs treated with ABT-510. Preliminary data suggested that decreases in circulating endothelial cell numbers may be informative and associated with responding versus nonresponding individuals. Results from this study have been used to develop follow-up studies of TSP-1 mimetic peptides in dogs with specific cancer histologies, including lymphoma and sarcoma.[4] Data from the presented nonclinical studies in pet dogs with naturally occurring cancer have been integrated into the developmental path of the TSP-1 mimetic peptides as new human cancer drugs and will continue to be useful in the design of future trials of TSP-1 peptides for human cancer patients.

ABT-526 and ABT-510 (DI-TSP, DI-TSPa; Abbott Laboratories) are capped nonapeptides based on the linear TSP-1 heptapeptide sequence, . . .Gly-Val-Ile-Thr-Arg-Ile-Arg. . ., from within the second properdin repeat.

ABT-526 contains a D-isoleucyl substitution for the first L-isoleucine (22), and the internal Arg residue is replaced by the unnatural amino acid, norvaline. The sequence, structure, preclinical pharmacokinetics, and preclinical anticancer activity have been recently reported (13, 20). The mechanism for the anticancer activity of these TSP-1 peptide mimetics includes antiangiogenic and antivasculogenic effects, as well as the promotion of death signals in tumor endothelial cells. The *in vivo* antiangiogenic activity of these TSP-1 mimetic peptides has been supported by corneal pouch neovascularization assays and through demonstration of TSP-1 peptide induction of endothelial cell apoptosis in orthotopic murine models (13, 20). No direct cytotoxicity has been associated with ABT-526 or ABT-510 *in vitro*.[5] Rodent cancer models have been used effectively, as discussed above, to understand basic principles that underlie the activity and biology of TSP-1

[4] Please see companion article, A. Rusk, et al. Cooperative activity of cytotoxic chemotherapy with antiangiogenic thrombospondin-1 peptides, ABT-526, in pet dogs with relapsed lymphoma, in this issue.

[5] J. Henkin, unpublished observation.

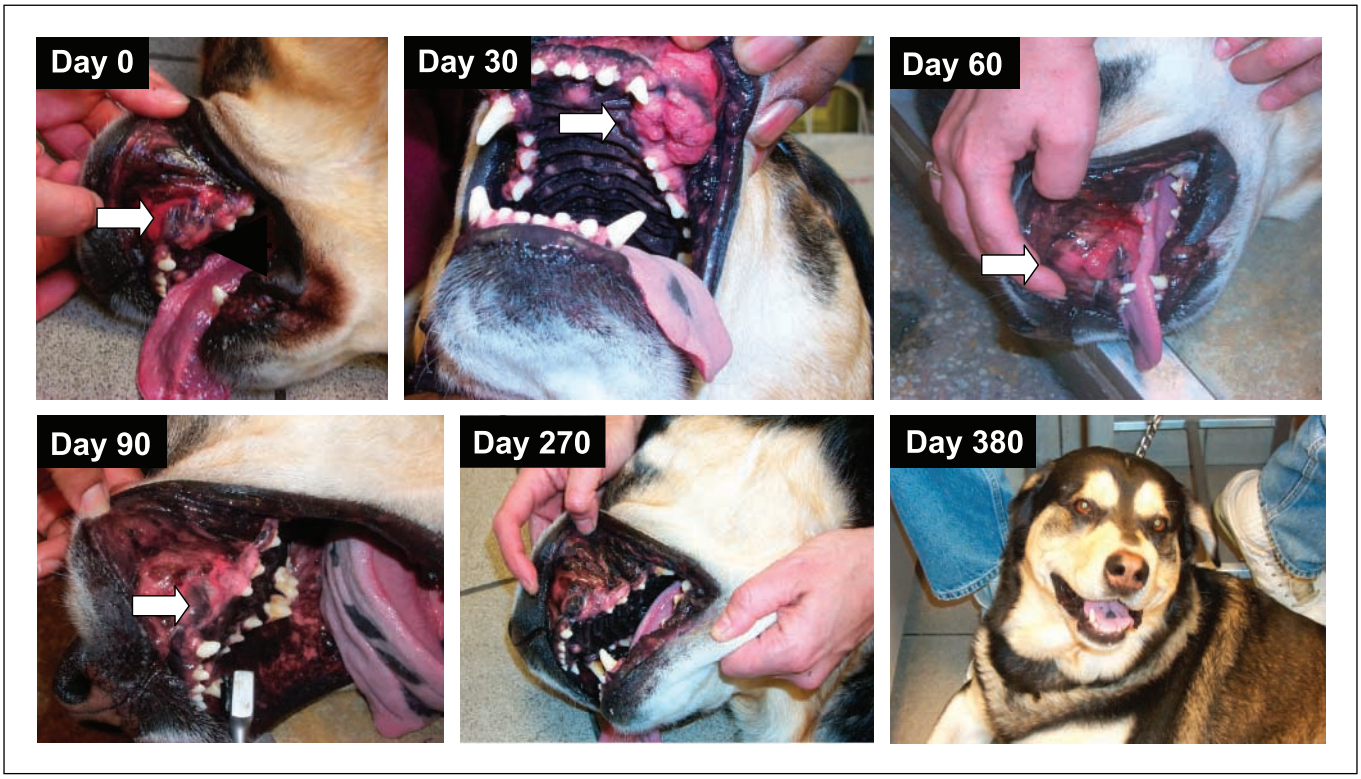


**Fig. 5.** ABT-526 peptide of TSP-1 resulted in the regression of an invasive rostral maxillary squamous cell carcinoma in 10-y-old Rottweiler cross (*white arrows*). Loss of the left maxillary canine tooth was noted at the time of presentation (*open arrowhead*). Progression of the mass was noted through the first 60 d of therapy with daily s.c. ABT-526, with evidence of hemorrhage from the tumor site (*open arrow*). Complete regression of the measurable mass occurred after 90 d of treatment. Incisional biopsy at the previous tumor site conducted at day 270 revealed microscopic evidence of squamous cell carcinoma despite complete regression of the measurable lesion. The dog remains on daily TSP-1 therapy, asymptomatic, free of measurable tumor, over 3 y after initial diagnosis.

peptide mimetics as cancer drugs. However, the translation of these results to human clinical trials has been complicated by the differences between these murine cancer models and cancer seen in human patients that are characterized by a gradually evolved complex interaction between tumor, host, and microenvironment. Unlike transplantable mouse cancers, human cancers are associated with intratumoral heterogeneity in both tumor genetics and tumor-host interactions, and interindividual variability of these factors in patients even with the same cancer diagnosis. Furthermore, as a class, anti-angiogenic therapies, which rely on largely noncytotoxic and therefore slower mechanisms of action, have been difficult to assess in the more conventional rapidly progressive mouse models of cancer. An underused group of nonclinical animal models for cancer drug development include companion (pet) animals, primarily dogs, which develop naturally occurring malignancies. Cancers in companion animals may answer many of the questions that are difficult to answer in conventional animal (mouse) studies and human clinical trials. The inclusion of pet animals with cancer in studies of cancer drug development and cancer biology has been reviewed elsewhere (24). A number of features of companion animal (pet dog) cancers contribute to their value as models of cancer in humans (25, 26). Pet dogs are large outbred animals with strong genetic similarities to humans. Companion animal cancers share tumor biology and behavior with human cancers and in some cases have identical tumor histology, cancer genetics, and response rates to conventional chemotherapy. By their nature, companion animal cancers are characterized by interpatient and intratumoral heterogeneity, the development of recurrent or resistant disease, and metastasis to relevant distant sites. In these ways, companion animal cancers capture the "essence" of the problem of cancer in humans. These features make dogs with naturally occurring cancer a potentially valuable additional model to assist in the development of cancer drugs, particularly for drugs with antiangiogenic mechanisms.

For the reasons articulated above, studies with TSP-1 mimetic peptides as anticancer drugs in companion animals with cancer were initiated. *In vitro* studies showed biological inhibitory activity of ABT-526 and ABT-510 against canine and human endothelial cell chemotaxis at concentrations $\geq$10 ng/mL (10 nmol/L). These data suggested that the inclusion of pet dogs with cancer in studies of TSP-1 peptide anticancer activity was rational. Extrapolating from mouse tumor models, it was predicted that efficacy could be observed by divided doses of TSP-1 peptides given at 1 mg/kg/d i.v. or s.c. This was based on providing at least 3-h exposure above a threshold plasma peptide concentration of 100 ng/mL. This minimum exposure for 3 to 4 h was achieved in beagle research dogs receiving s.c. doses of 0.5 mg/kg of either peptide (20). Pharmacokinetic studies using a fixed delivered peptide dose to tumor-bearing dogs with varying body weight showed rapid clearance of ABT-526 in tumor-bearing dogs. This fixed dose analysis, done in the first 26 dogs entered for study, allowed the definition of a per kilogram dosing schedule used thereafter for dogs receiving ABT-526 and ABT-510. Similar considerations were applied to

starting minimal daily doses in phase I and II human clinical trials of ABT-510 (27).

The population of evaluable dogs entered for study was representative of the types of cancers seen in the pet population. These canine cancers, for the most part, share significant biological similarities to the same cancers seen in human patients. By proportion, dogs develop a greater number of sarcomas and lymphomas and relatively fewer carcinomas than humans. The common cancers seen in pet dogs and the features that define them as models for human cancers have been recently reviewed (24). The fact that these dogs have the systemic disease and consequences of cancer make them valuable not only as models of the anticancer activity of novel agents but also to validate toxicity and pharmacokinetic studies commonly undertaken in colony bred, non–tumor-bearing, healthy research dogs. It was possible that the systemic effects of cancer and the associated changes in circulating cytokines and inflammatory mediators seen in aged tumor-bearing dogs would result in differences in pharmacokinetics from that seen in colony bred, generally younger, research dogs. For these reasons, it is possible that tumor-bearing dogs may be more predictive of toxicities and pharmacokinetics seen in human cancer-bearing patients receiving new agents in phase I human trials (24). In spite of these potential differences, the tumor-bearing pet dogs receiving ABT-526 and ABT-510 showed pharmacokinetics that were very similar to results from beagle dogs (20). Furthermore, both ABT-526 and ABT-510 were well tolerated in all tumor-bearing dogs both in the acute and chronic settings. Interestingly, potential adverse effects associated with the long-term use of antiangiogenic agents in a geriatric population, at risk for arthritis, were not seen. Wound healing in a small number of dogs was also not compromised during treatment with these agents. These data are consistent with a growing body of evidence that suggests that the biology of angiogenesis in normal and noncancer tissues is unique and distinct from angiogenesis in cancer (28). These data suggest the possibility that long-term safe use of antiangiogenic agents in similarly at risk and aged populations of human cancer patients is feasible.

Dogs entering this clinical trial had a variety of cancer histologies, stage, or disease distributions (localized, regional, and metastatic), and prior therapies. As part of the eligibility criteria for therapy, all dogs were required to have measurable disease. In this setting, it was not expected that tumor regression would be a likely outcome for a single-agent antiangiogenic therapy. As such, we attempted to define the progression of disease before entry such that it would be possible to define the disease stabilization as an outcome in dogs. This assessment, coupled with an understanding of the natural history for the cancers diagnosed in these dogs, allowed "significant disease stabilization" to be defined in 23 dogs receiving therapy. It should be noted that tumor regression of <50% was seen in many of these cases with significant disease stabilization; however, these regressions did not meet the definition of a partial response and as such were reported as stable disease. Nonetheless, whether these cases can be classified as drug-associated significant disease stabilization may be disputed or debated by experts in the field (29). The objective responses seen in 19 dogs are less disputable and were surprising. These responses included a variety of cancer histologies, including mammary carcinomas, head and neck carcinomas, non–Hodgkin's lymphoma, and several soft tissue sarcomas. In each case, the natural history of these cancers in dogs can include disease progression at local, regional, and distant sites (23). No clinical variable measured in this study, including cancer histology, stage of disease, tumor size, tumor location, or past therapy, could predict response to TSP-1 mimetic peptides. In part, this lack of prediction may be the result of the small groups of dogs in each subgroup; however, notable responses were seen in dogs with lymphoma and sarcoma. Interestingly, the reduction in circulating endothelial cell number and subtypes in a small subset of dogs, able to continue on therapy for over

**Table 5.** Responding population of dogs treated with ABT-510

| Breed | Tumor histology | TNM stage | Response | Response duration (d) |
|---|---|---|---|---|
| Golden retriever | NHL | IVa | CR | 240 |
| Doberman | Oral fibrosarcoma | $T_2N_0M_0$ | PR | 970 |
| Norfolk terrier | Oral fibrosarcoma | $T_2N_0M_0$ | CR | >450 |
| Standard poodle | Hemangiosarcoma | $T_{(sx)}N_0M_1$ | PR | 530 |
| Golden retriever | Lingual/labial hemangiosarcoma | $T_2N_0M_1$ | PR | 570 |
| Labrador retriever | Cutaneous T-cell lymphoma | Cutaneous | PR | 75 |
| Dalmatian | Hemangiopericytoma | $T_2N_0M_0$ | CR | >450 |
| Labrador retriever mix | NHL | IIIa | PR | >150 |
| Labrador retriever mix | Multicentric cutaneous plasmacytomas | $T_2N_0M_1$ | PR | >120 |
| Labrador retriever mix | Cutaneous B-cell lymphoma | Cutaneous | SD | 140 |
| Shepherd mix | Metastatic insulinoma | $T_{(sx)}N_0M_1$ | SD | 240 |
| Scottish terrier | Thyroid adenocarcinoma | $T_2N_0M_0$ | SD | 150 |
| Dachshund | NHL | IIIa | SD | 62 |
| Labrador retriever mix | Transitional cell carcinoma | $T_2N_0M_0$ | SD | 420 |
| Shetland sheepdog | Fibrosarcoma | $T_{(sx)}N_0M_1$ | SD | 453 |
| Springer spaniel | Papillary carcinoma, nasal | $T_{3b}N_0M_0$ | SD | 120 |
| Labrador retriever mix | Cutaneous plasmacytoma | $T_2N_0M_1$ | SD | 90 |
| Maltese | Transitional cell carcinoma | $T_2N_0M_0$ | SD | 270 |
| Shepherd mix | Thyroid adenocarcinoma | $T_3N_0M_0$ | SD | >210 |
| Shepherd mix | Thyroid carcinoma | $T_{2b}N_0M_0$ | SD | >270 |
| Australian cattle dog mix | Rhabdomyosarcoma | $T_3N_0M_0$ | SD | >120 |
| Puli | Anaplastic transitional cell carcinoma | $T_2N_0M_0$ | SD | >90 |
| Fox terrier | Prostatic adenocarcinoma | $T_2N_0M_0$ | SD | >120 |

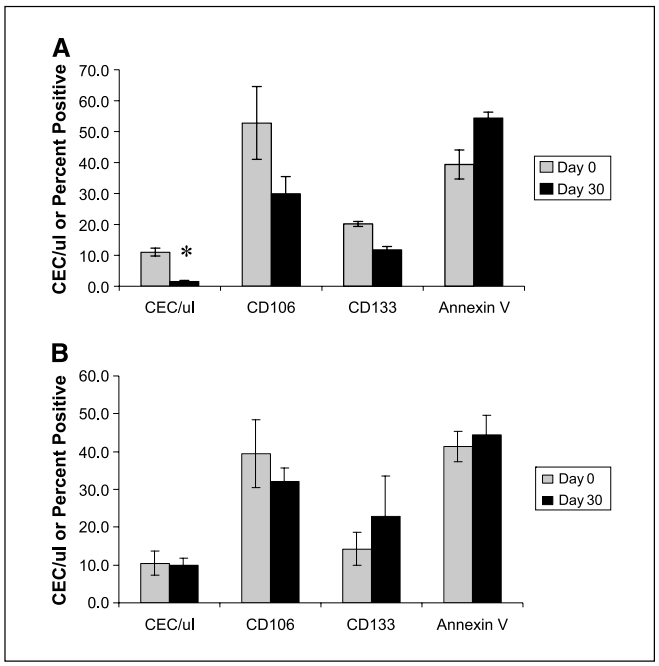


**Fig. 6.** Changes in circulating endothelial cells (*CEC*) in a small subset of dogs receiving ABT-510 therapy. *A,* dogs included in the evaluable population, based on absence of significant disease progression in the first 30 d of study, in which circulating endothelial cell data was available ($n = 3$), had a decrease in total mean circulating endothelial cells from day 0 to 30 of ABT-510 therapy (*, $P < 0.05$). *B,* no changes in circulating endothelial cell numbers were seen in the 10 dogs, in which day 0 and day 30 circulating endothelial cell data was available, and which had significant disease progression at or before 30 d of therapy. Cells were incubated with fluorescent antibodies to human CD146 (Calbiochem), canine CD34 (BD Biosciences), and canine CD45 (Serotec) along with a nuclear stain the phenotypic analysis was done by switching CD106, CD133 (human), or Annexin V for the CD34. Stained whole blood then underwent RBC lysis and fixation using FACSLyse (DAKO) and was read on a BD FACSCalibur. A panel of isotype control antibodies (BD Biosciences) was used to establish the negative control instrument settings.

30 days, were distinct from the changes in circulating endothelial cells seen in dogs with nonresponsive and rapidly progressive disease. The small sample numbers are insufficient to draw any definitive conclusions but suggest the usefulness of similar studies in tumor-bearing dogs to validate the use of circulating endothelial cells as a biomarker of exposure and response to antiangiogenic therapies. Studies are currently under way in tumor-bearing dogs to define tumor and host-associated biomarkers that may help define responsive and responding subpopulations. These biomarker studies in tumor-bearing dogs will be valuable in the design of early human studies that will rely on similar correlative end points.

A potentially important and informative finding from these studies was that extended exposures to TSP-1 mimetic peptides was necessary for objective responses to be seen. The minimum duration needed for response is not known but seems to be at least 30 days, and, in some cases, as long as 60 days. This may be particularly important in the design of early-phase human clinical trials with these and similar agents and may suggest that some disease progression through 60 days of therapy may occur before an eventual and potentially objective response is seen in patients. Evidence of delayed responses to TSP-1 mimetic peptides has been suggested in the early reports from clinical trials with these agents in humans (27). The opportunity to obtain serial tumor biopsies from pet dogs receiving TSP-1 mimetic peptides may contribute to our understanding of the mechanisms of this delayed response to therapy; such studies are under way. An important feature of the responses that were documented is the persistence of microscopic cancer at the sites of complete and partial regression of lesions. More extensive rebiopsy schedules are feasible in pet dog studies and should be included in future studies to confirm and better define this observation. If substantiated, these data suggest that a requirement for long term and potentially indefinite exposure to TSP-1 mimetics, even in patients with complete tumor regressions, will be needed. Not surprisingly, the notion that antiangiogenic therapy may represent a treatment modality that is not confounded by the emergence of resistance was not supported by data from this trial. The vast majority of dogs experiencing significant disease stabilization, partial responses, or complete responses eventually had disease progression while continuously receiving ABT-526 or ABT-510 therapy. The mechanisms associated with this resistance to TSP-1 mimetic antiangiogenic therapy are not known, but may include tumor cell elaboration of additional proangiogenic signals that bypass the TSP-1 antiangiogenic effect or through the release of circulating inhibitors that bind TSP-1 or block the presumed receptor (CD36) for TSP-1 and the mimetic peptides. It is possible, although less likely, that modulation of the TSP-1 receptor expression by tumor endothelial cells or modulation of signaling associated with CD36 in tumor endothelial cells may result in TSP-1 mimetic resistance. The importance of defining treatment approaches that combine TSP-1 mimetic peptides with conventional cytotoxic chemotherapy, other antiangiogenic treatments, or novel targeted therapies is underlined by these findings.

Data from this study suggest that ABT-526 and ABT-510 may be nontoxic, long-term treatment agents for cancer. Future human clinical trials with these agents should consider the potential need for extended drug exposures before measurable tumor responses are seen in patients, that some responses may be seen after initial disease progression, and that continued long-term use of these agents is predicted to be well tolerated. Additional nonclinical studies, including tumor-bearing pet dogs, are needed to define the determinants of the responsive patient.

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Clinical Cancer Research

# Preclinical Evaluation of Antiangiogenic Thrombospondin-1 Peptide Mimetics, ABT-526 and ABT-510, in Companion Dogs with Naturally Occurring Cancers

Anthony Rusk, Evelyn McKeegan, Fortuna Haviv, et al.

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