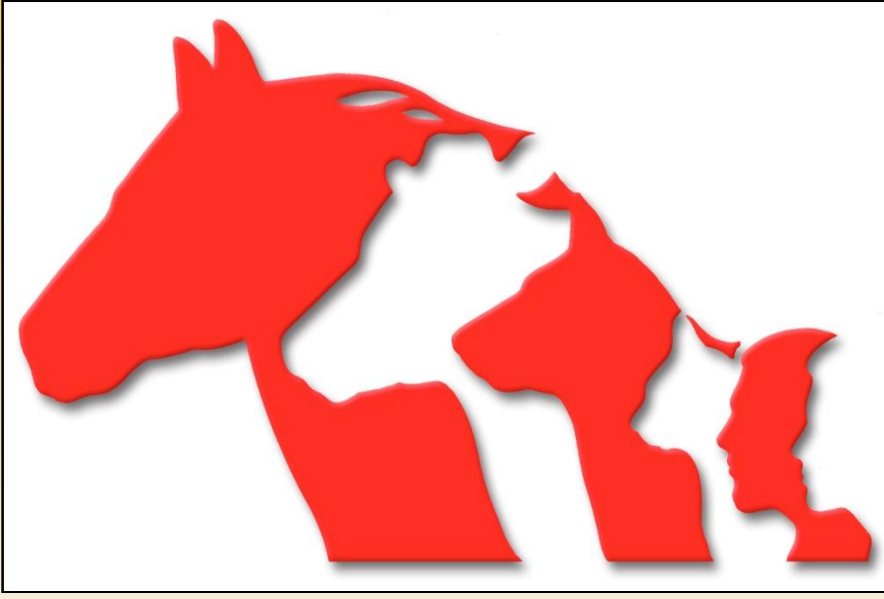


Phase I evaluation of STA-1474, a pro-drug of the novel HSP90 inhibitor ganetespiib (formerly STA-9090), in dogs with spontaneous cancer



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Abstract

Purpose: The novel water soluble compound STA-1474 is metabolized to ganetespiib (formerly STA-9090), a potent HSP90 inhibitor previously shown to kill canine tumor cell lines *in vitro* and inhibit tumor growth in the setting of murine xenografts. The purpose of the following study was to extend these observations and investigate the safety and efficacy of STA-1474 in dogs with spontaneous tumors.

Experimental Design: This was a Phase 1 trial in which dogs with spontaneous tumors received STA-1474 under one of three different dosing schemes. Pharmacokinetics, toxicities, biomarker changes, and tumor responses were assessed.

Results: Twenty-five dogs with a variety of cancers were enrolled. Toxicities were primarily gastrointestinal in nature consisting of diarrhea, vomiting, inappetence and lethargy. Upregulation of HSP70 protein expression was noted in both tumor specimens and PBMCs within 7 hours following drug administration. Measurable objective responses were observed in dogs with malignant mast cell disease (n=3), osteosarcoma (n=1), melanoma (n=1) and thyroid carcinoma (n=1), for a response rate of 24% (6/25). Stable disease (>10 weeks) was seen in 3 dogs, for a resultant overall biological activity of 36% (9/25).

Conclusions: This study provides evidence that STA-1474 exhibits biologic activity in a relevant large animal model of cancer. Given the similarities of canine and human cancers with respect to tumor biology and HSP90 activation, it is likely that STA-1474 and ganetespiib will demonstrate comparable anti-cancer activity in human patients.

Introduction

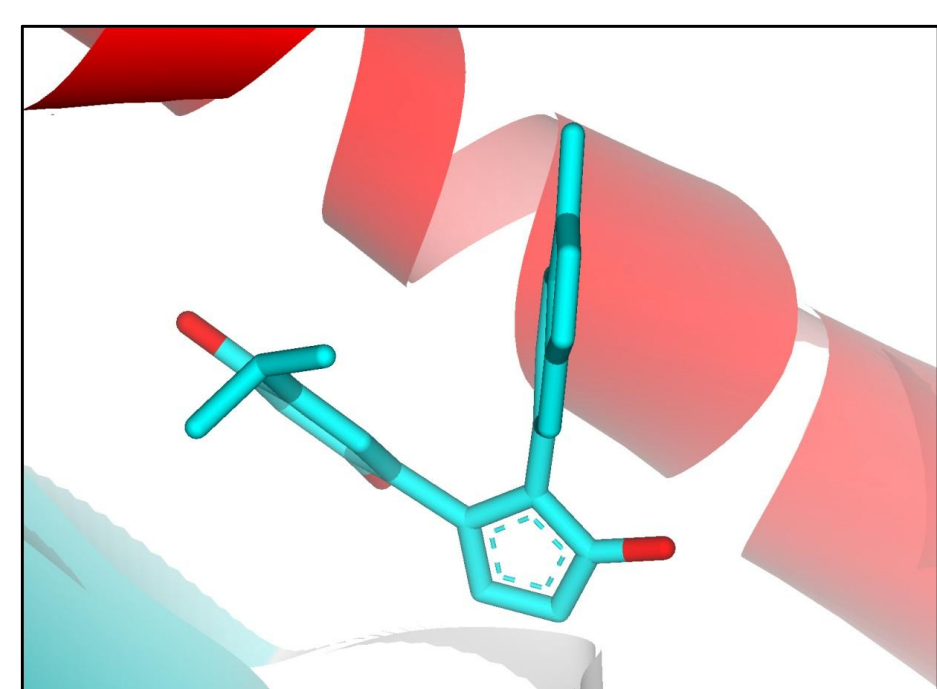
The first class of HSP90 inhibitors was based on geldanamycin a benzoquinone ansamycin antibiotic that binds to the N-terminal ATP-binding pocket of HSP90. Geldanamycin and its derivatives have a number of limitations including formulation challenges and side effects such as hepatotoxicity.

STA-1474 is a highly soluble prodrug of ganetespiib (formerly STA-9090), a novel resorcinol-containing compound unrelated to geldanamycin that binds in the ATP-binding domain at the N-terminus of HSP90 and acts as a potent HSP90 inhibitor.

In previous work, ganetespiib or STA-1474 induced growth inhibition, apoptosis, and inactivation and/or down-regulation of pKit/Kit, pMet/Met, pAkt/Akt, and pSTAT3 in canine osteosarcoma (OSA) and mast cell tumor (MCT) cell lines. Both ganetespiib and STA-1474 exhibited superior activity to 17-AAG. Ganetespiib inhibited tumor growth in MCT xenograft model and STA-1474 induced tumor regression, caspase-3 activation and downregulation of p-Met/Met and p-Akt/Akt in OSA xenografts.

The purpose of this clinical trial was to extend our *in vitro* and murine studies and investigate the safety and efficacy of STA-1474 in dogs with spontaneous tumors as a prelude to future clinical work in humans with cancer.

3D structure of ganetespiib inside the ATP binding pocket of HSP90 N terminus



Materials and Methods

Dogs with a variety of spontaneous tumors (Table 1) were administered STA-1474 using one of three dosing regimens (Table 2 and 3) for 4 consecutive weeks. Dogs were evaluated weekly or twice per week dependent on the regimen used. Toxicities were assessed based on the established VCOG-CTAE criteria (Table 3).

The response (stable disease, partial or complete response) in dogs with measurable disease was judged by the investigator on the basis of RECIST criteria.

Blood samples were drawn on day 1 of drug administration for ganetespiib pharmacokinetics, and PBMCs and tumor biopsies were obtained to assess modulation of HSP70 and HSP90.

Results

Table 1: Subject Demographics

Characteristics	Total	1 hr 1x/wk	8 hr 1x/wk	1 hr 2x/wk
Number of dogs	25	12	6	7
Age				
Median	9	9	7.5	9
Range	2-14	5-14	4-9	2-11
Gender				
Male intact	1	1		
Male neutered	9	2	3	4
Female neutered	15	9	3	3
Tumor Type				
Osteosarcoma	10	5	3	2
Mast cell tumor	4	1	1	2
Thyroid carcinoma	3	1	2	
Lymphoma	3			3
Other carcinoma	2	2		
Nasal chondrosarcoma	1	1		
Oral melanoma	1	1		
Oral fibrosarcoma	1	1		
Prior Treatment				
Yes	18	8	6	4
No	7	4		3

Table 2: Mean PK parameters of ganetespiib (STA-9090)

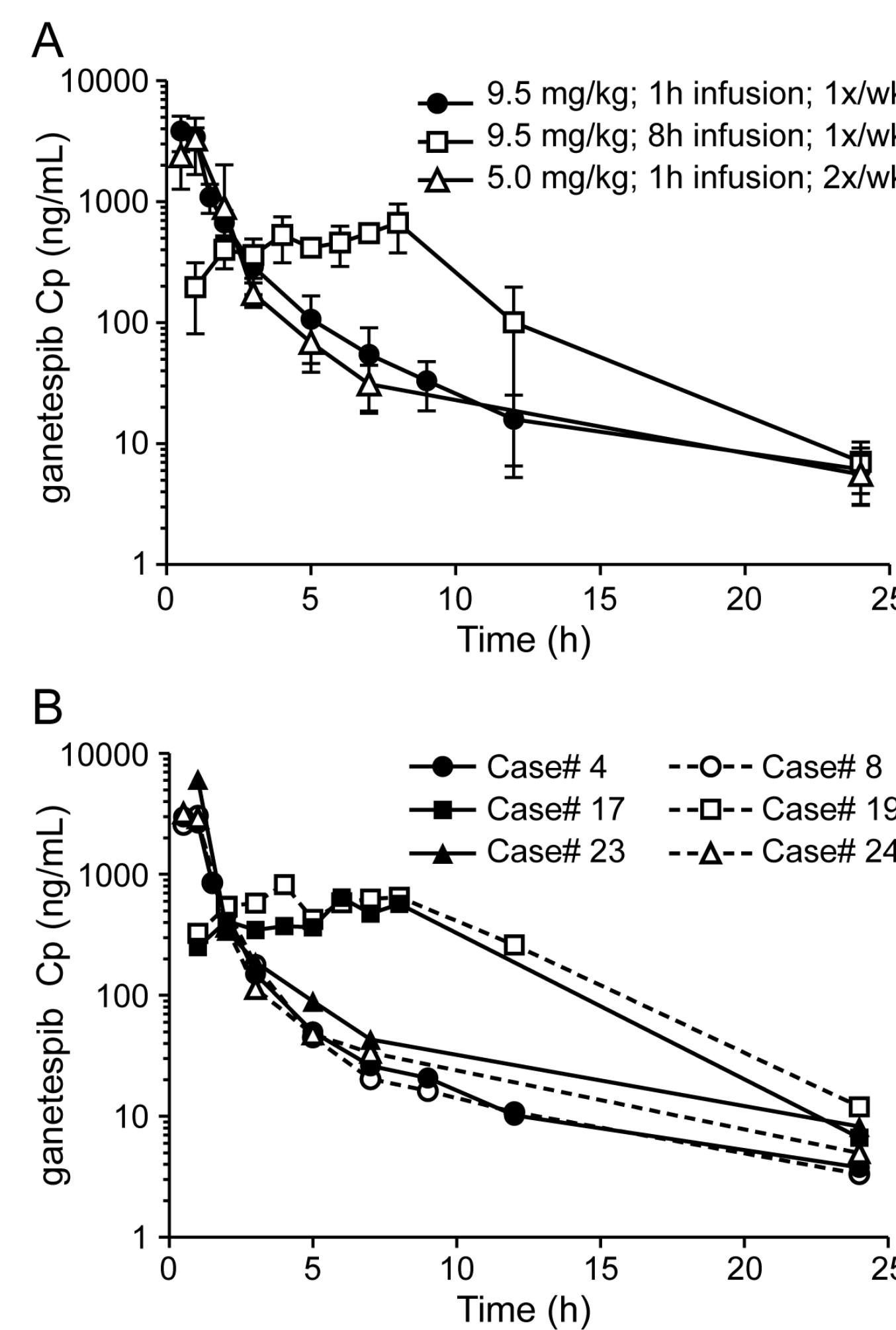
STA-1474 Dose (mg/kg)	Infusion (h)	Treatment Frequency	t _{1/2} (h)	Tmax (h)	Cmax (ng/mL)	AUC _{last} (ng/mL-h)
9.5	1	Once weekly	5.6	0.7	3982	5701
9.5	8	Once weekly	NR	5.7	802	5605
5	1	Twice weekly	6.1	1.1	3415	5013

Table 3: Enrollment, toxicity and response by dose and regimen

Regimen	Dose (mg/kg)	No. of dogs	Response to therapy	Lethargy			Anorexia			Vomiting			Diarrhea		
				1	2	3	1	2	3	1	2	3	1	2	3
1 hr/wk	7.0	4	none	2											
	9.5	5	1 PR**												
	10.25	3	none		1	1									
8hr/wk	9.5	6	2 PR, 1 MR, 2 SD	2			5	4		3		4	4		
1hr/2x wk	5.0	7	2 PR	4	2		3	5				5	1	1	

PR=partial response, MR=mixed response, SD=stable disease. **Dog had extravasation resulting in extended drug exposure. Grade and frequency of adverse events in each category are shown for the first 4 cycles of STA-1474. There were no grade 4 or 5 adverse events.

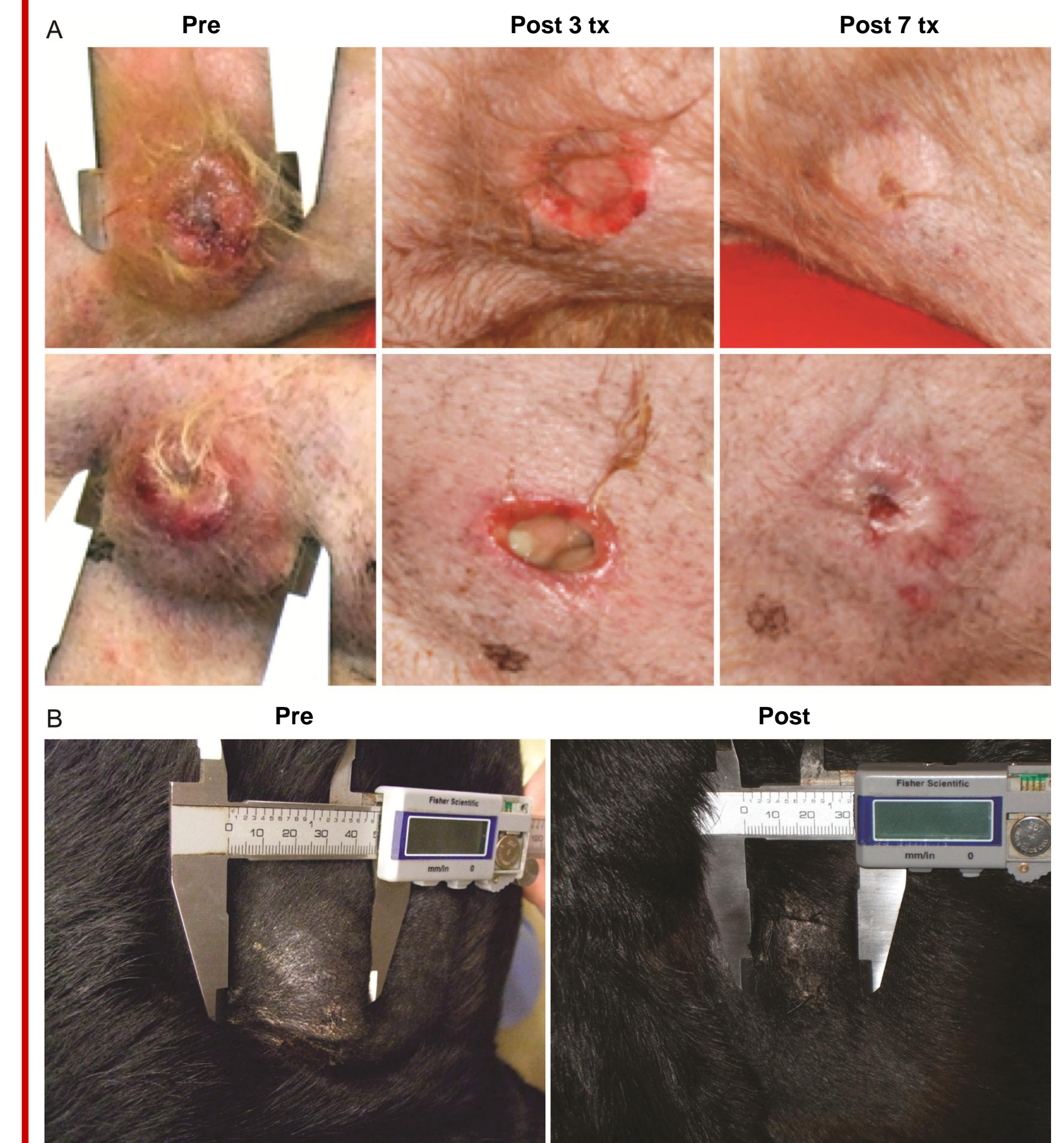
Pharmacokinetic analysis of STA-1474 following administration using three different dosing regimens



Mean ganetespiib plasma concentration – time profile for each dosing regimen

Representative ganetespiib plasma concentration – time profiles for two patients in each dosing regimen

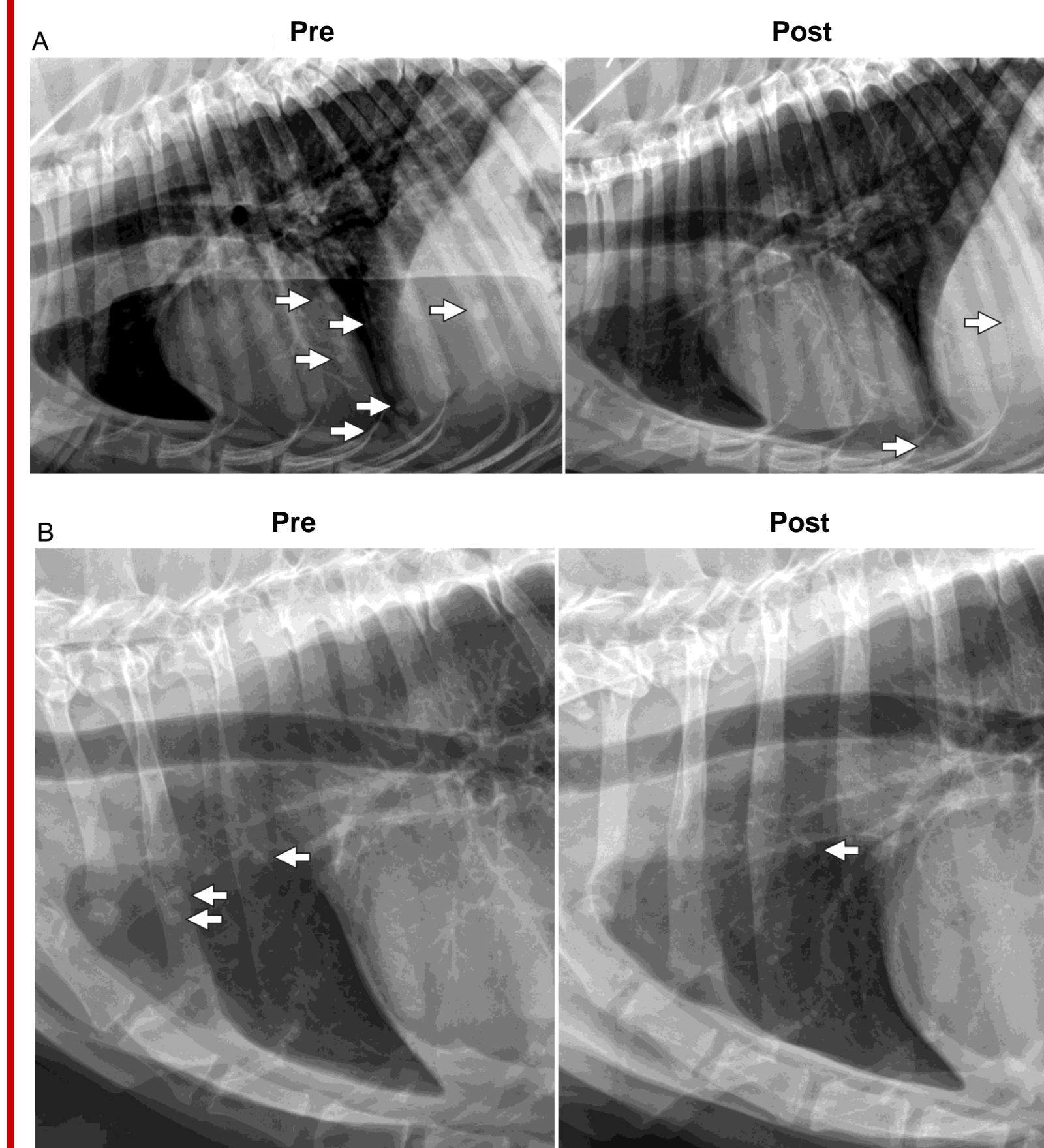
Response of cutaneous mast cell tumors to STA-1474 treatment



Patient #19 had recurrent grade 3 mast cell tumors. This patient received STA-1474 on the 1 hour infusion protocol 2x/week and experienced a PR of the cutaneous lesions after 7 treatments

Patient #24 had a large mast cell tumor and received STA-1474 on the 1 hour infusion protocol 2x/week. He underwent a PR of the cutaneous lesions after 4 treatments

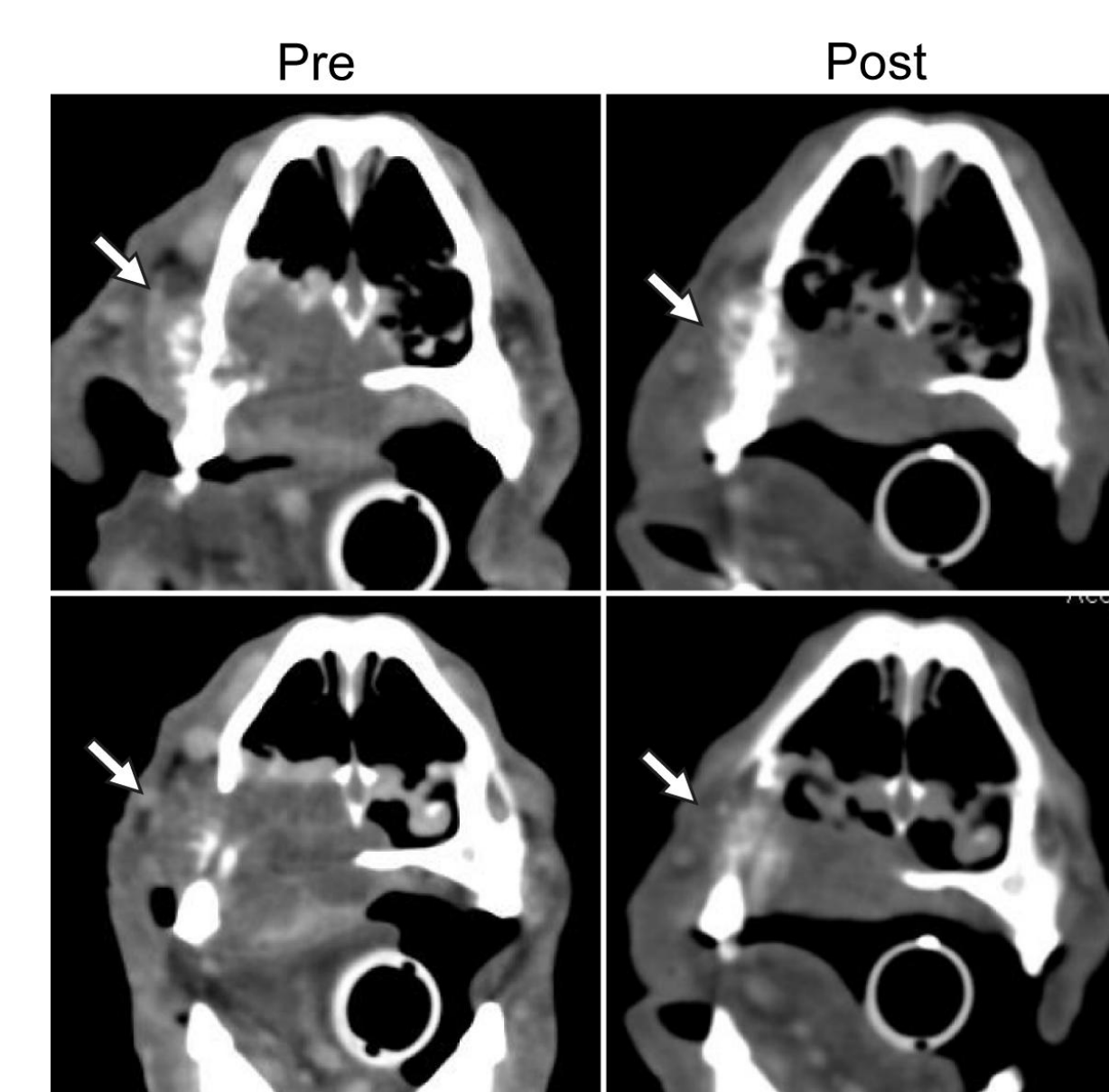
Response of metastatic thyroid carcinoma and osteosarcoma to STA-1474 treatment



Patient #14 had a locally recurrent thyroid carcinoma with metastatic disease to the lungs. This patient received STA-1474 on the 8 hour infusion protocol and experienced a PR of both the locally recurrent and metastatic disease. Arrows point to the pulmonary nodules.

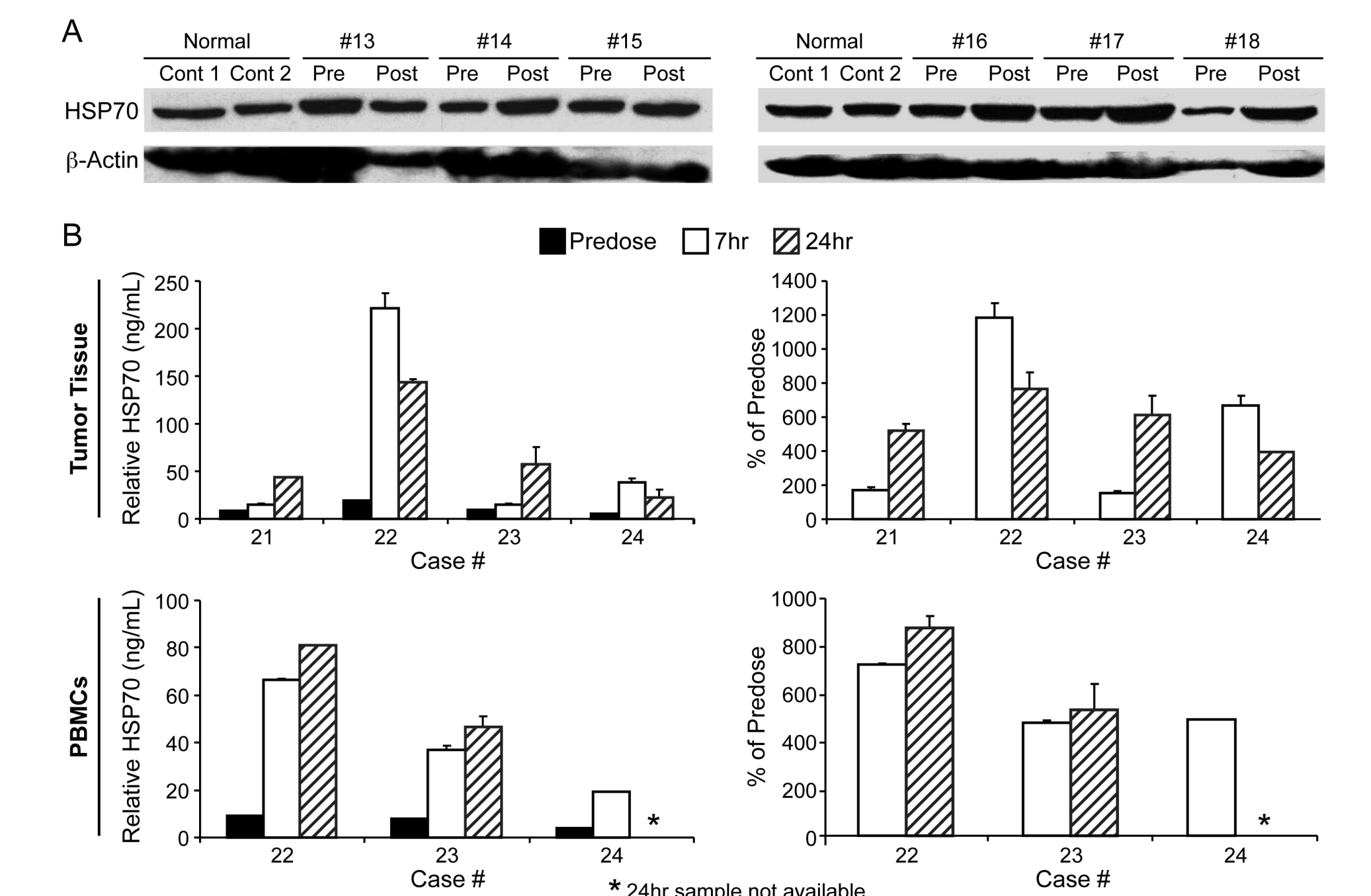
Patient #18 had metastatic osteosarcoma to the lungs following amputation and chemotherapy for long bone osteosarcoma. This patient received STA-1474 on the 8 hour infusion protocol and experienced a PR. Arrows point to the pulmonary nodules.

Serial CT demonstrating regression of oral malignant melanoma following treatment with STA-1474



Patient # 5 had an oral malignant melanoma that had invaded into the nasal cavity. During the 4th treatment cycle with STA-1474, an extravasation event occurred resulting in altered drug pharmacokinetics. A marked decrease in the oral mass was observed 7 days later and a subsequent CT scan confirmed a PR to therapy. Shown are two representative matched CT images of tumor before and after treatment.

Analysis of HSP70 expression in dogs before and after treatment with STA-1474



A) PBMCs were collected from normal control dogs (cont 1 and 2) and study dogs before and 24 hrs after treatment with STA-1474 at 9.5 mg/kg over 8 hours. Western blotting was performed for HSP70 and beta-actin.

B) Tumor biopsies and PBMCs were collected from dogs treated with 5 mg/kg STA-1474 over 1 hr before treatment and at 7 and 24 hours post treatment. Protein lysates were generated and an ELISA was performed to detect HSP70. Results are expressed as HSP70 protein (ng/ml) and percent of baseline.

Conclusions

- Adverse events associated with STA-1474 administration were primarily gastrointestinal in nature; no hematologic or biochemical toxicity was observed.
- Objective responses to therapy were associated with sustained blood levels of ganetespiib between 200-600 ng/ml for 8-10 hours.
- Upregulation of HSP70, a surrogate marker for HSP90 inhibition, occurred within 7 hours of STA-1474 administration in both tumors and PBMCs.
- These data laid the groundwork for the current clinical studies of ganetespiib in humans with cancer and further support the use of dogs with spontaneous tumors as pre-clinical models for human drug development.