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Safety Evaluation of Combination Vinblastine and Toceranib Phosphate (Palladia®) in Dogs: a Phase I dose-finding study

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Abstract

Combining drugs with known single-agent activity that lack overlapping dose-limiting toxicities and exert antitumor activity through different mechanisms could improve clinical outcome. As toceranib and vinblastine meet these requisites, a phase I trial was performed on the combination in dogs with mast cell tumors. The dose-limiting toxicity for the simultaneous combination was neutropenia and the maximally tolerated dose was vinblastine (1.6 mg/m² every other week) concurrent with toceranib (3.25 mg/kg PO, every other day). This represents greater than a 50% reduction in dose-intensity for vinblastine (compared to single-agent use) and as such does not support this combination based on current drug combination paradigms. While a strict adherence to dose paradigms speaks against the combination, evidence of significant activity (71% objective response) and enhanced myelosuppression suggest additive or synergistic activity. A prospective randomized evaluation comparing this combination with standard single-agent treatments would seem prudent to interrogate this potential.

Mast cell tumors (MCT) are the most common cutaneous tumor in dogs, accounting for 16–21% all skin tumors (1). Several factors are known to be predictive of biological behavior and clinical outcome. Histological grade, clinical stage, location, and growth rate are amongst the most important prognostic factors recognized. (1–11).

In an attempt to improve outcome in dogs with high grade or advanced-stage MCT, combinations of local (surgery, radiation) and systemic (chemotherapy) therapy are recommended. Several chemotherapy agents have documented activity against MCT, including single agent vinca alkaloids (vincristine, vinblastine), CCNU, prednisone, hydroxyurea and tyrosine kinase inhibitors (masitinib, toceranib) (3,4,12–23). Multiagent therapy has also been evaluated with varying success (24–26). Medical treatment of macroscopic, advanced-stage disease generally results in modest and non-durable responses; however recent evidence suggests that multiagent protocols may provide higher response rates (27).

Currently, most successful cancer chemotherapy regimens in people follow the paradigm of multiple drugs given simultaneously to optimally treat cancer. Several criteria are theoretically requisite for simultaneous use of drugs in combination protocols (27,28).

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Firstly, individual drugs should have documented single-agent activity for the histology in question. Additionally, the antitumor activity of drugs chosen for use in combination should occur through different mechanisms of action. Further, the combination of drugs that have overlapping toxicities should be avoided. Finally, drugs should be used at their optimal dose and schedule (maximal dose intensity) allowing for the shortest time for recovery of the most sensitive normal target tissue.

Chemotherapy using vinblastine, a potent plant alkaloid extracted from the periwinkle plant, has shown to be safe and effective as a single-agent therapy (with or without prednisone) for the treatment of dogs with MCT, and is currently considered a standard of care treatment for this disease in the adjuvant setting or when surgery is not feasible (3,4,12–23). Vinblastine inhibits microtubule formation by binding to tubulin, disrupting the normal mitotic process. The dose-limiting toxicity of vinblastine in dogs is neutropenia, and while gastrointestinal adverse events are possible, they are usually mild and self-limiting. Previous literature suggests that the nadir for neutropenia occurs at 1 week in dogs and that most will tolerate weekly treatments at dosages ranging from 2.0 – 2.6 mg/ m², and biweekly dosages as high as 3.5 mg/m² (3, 4,12–23).

Toceranib (Palladia®) is a small molecule tyrosine kinase inhibitor (TKI). It is FDA-approved for use in dogs to treat canine cutaneous MCT. Toceranib's mechanism of action results from competitive blockade of the ATP binding site of several tyrosine kinase receptors (VEGFR, PDGFR and Kit), impairing phosphorylation and downstream signaling (17–20). When evaluated in a prospective, randomized, placebo-controlled study in dogs with nonresectable grade II and III MCT, the overall objective response rate was 42.8%, demonstrating that toceranib has single-agent biologic activity against canine MCTs (20). The dose-limiting toxicity was demonstrated to be gastrointestinal in origin.

As vinblastine and toceranib both have documented single-agent activity against canine MCT, possess different mechanisms of action and dose-limiting toxicity profiles; they meet the theoretical requisites for effective simultaneous combination in dogs with MCT. The hypothesis under interrogation in this trial was that toceranib could be safely administered on an every other day schedule simultaneously with a standard weekly protocol of vinblastine, at dosages known to have single-agent activity against MCT in dogs. The aims of the study were two-fold. The primary aim was to determine the maximum tolerated dose of toceranib that can be administered in tumor-bearing dogs, when given concurrently with a standard vinblastine dosing regimen, through the use of a standard phase I dose-finding trial design. A secondary aim was to determine the adverse event profile of toceranib and vinblastine in combination and compare to that of each drug when used alone. While efficacy is not a primary end-point for dose-finding trials, the study did evaluate antitumor activity of the combination protocol.

METHODS

Patient selection

Client-owned dogs with one or more histologically confirmed measurable recurrent or naïve MCT, who were not scheduled for surgical resection based on nonresectability or owner preference were considered for enrollment. There were no histologic grade requirements, and any age, weight, gender or breed with satisfactory health (VCOG-CTCAE constitutional health of 0 [normal activity] or 1 [mild lethargy over baseline]) were eligible (29). The clinical protocol was approved by the contributing institutions' Animal Care and Use Committees and prior to enrollment and written informed consent was obtained from owners. Patients receiving concurrent chemotherapy, corticosteroid therapy or radiation therapy were excluded. Prior surgery, radiation therapy, chemotherapy (other than

vinblastine or toceranib) and corticosteroid therapy were allowed if recurrence or progression was documented following treatment and a minimum washout period (1 month for radiation, chemotherapy; 72 hours for corticosteroid therapy) had occurred prior to study initiation. Concurrent use of non-steroidal antiinflammatory agents was not allowed. Prior to treatment initiation (baseline), a physical examination, complete blood count (CBC), biochemistry profile, urinalysis, and tumor measurements were performed. While complete clinical staging of disease was recommended (i.e., thoracic radiographs, abdominal ultrasound, regional node assessment), measurability of tumor lesions (up to five target lesions) was the only tumor-related requirement for protocol entry.

Study design and treatment protocol

A standard open-label Phase I dose-cohort (3+3) escalation design (28) was employed to assess the safety of combination vinblastine and toceranib in companion dogs with spontaneous MCTs. All dogs were scheduled to receive vinblastine at a starting dose of 2.3 mg/m² (IV bolus) weekly (cohort 1; table 1) for a total of 4 treatments. Patients were also prescribed diphenhydramine (2 mg/kg PO, T.I.D.) and omeprazole (0.5–1 mg/kg PO, once daily) to avoid the effects of possible MCT degranulation. Toceranib was scheduled to be administered at a starting dose of 2.25 mg/kg PO (cohort 1), every other day concurrent with vinblastine treatment and then for a minimum of 7 days after the 4th vinblastine treatment. As per the standard 3 + 3 phase I cohort design, 3 dogs were initiated in each cohort and observed for dose-limiting toxicities (DLT). A DLT was defined as grade 3 for any adverse events with the exception of neutropenia, where a grade 3 neutropenia that resolved prior to the next scheduled treatment was not deemed dose limiting. Any grade 4 neutropenia was deemed a DLT. If no DLTs were observed in the first cohort of dogs following the 4 weeks of treatment, a second cohort was treated on the same regimen at the higher dose. If a DLT was observed in 1 dog, the cohort was expanded to 6 dogs. If two or more DLTs were noted in any initial or expanded cohort, no further dose escalations were performed and it was considered that the maximally tolerated dose (MTD) was exceeded. Toceranib dose escalation was planned in 0.25 mg/kg increments until the maximally tolerated dose (MTD) was established or until the FDA label dose (3.25 mg/kg, every other day) was achieved, whichever was lower.

Safety evaluation

Patients were evaluated seven days after each of the 4 vinblastine treatments and a complete physical examination, CBC, and tumor measurement(s) were performed. At the seven-day recheck following the 4th vinblastine treatment a urinalysis and biochemistry profile were also performed. Clients were required to fill out quality of life forms at each visit, and adverse events were recorded. Adverse events were graded using the Veterinary Cooperative Oncology Group- Common Terminology Criteria for Adverse Events v1.0 (29). Patients with grade 3 or higher adverse events or those with milder events, but perceived to be unacceptable by the owner, received reduced subsequent doses of vinblastine or toceranib (20% reduction). When tolerated, dose escalations were carried out following the protocol (table 1). Disease progression or signs attributable to disease were not considered adverse events.

After completing the 4 scheduled weeks of the study, patients were allowed to continue receiving vinblastine and/or toceranib therapy, or were eligible for surgical resection of their tumor(s) if deemed surgical at that point.

Antitumor response assessment

While antitumor response is not a primary endpoint of phase I trials, activity was evaluated prior to vinblastine treatments 2–4, and monthly thereafter, based on modified RECIST v1.1

criteria. The longest diameter of the target lesion(s) was recorded. A complete response (CR) was defined as complete regression of the disease; partial response (PR) was defined as at least a 30% decrease in the sum of the target tumor longest diameters; stable disease (SD) was defined as neither CR, PR nor progressive disease. Progressive disease (PD) was defined as >20% increase in the sum of the target tumor longest diameters or the appearance of a new lesion. Dogs that developed PD were withdrawn from the study and further treatment options were offered.

RESULTS

Fourteen dogs were enrolled for completion of the trial, 9 at the University of Wisconsin-Madison, 4 at Ohio State University, and 1 at the University of Missouri. Patient demographics are described in table 2. Eight patients had treatment-naïve tumors. Two had previous surgery with incomplete margins but no local recurrence, and enlarged metastatic regional lymph nodes were chosen as target lesions. Four dogs had previous surgery and presented with recurrence; of these, one dog received CCNU therapy 5 months prior to recurrence, and one dog had received prior prednisone therapy which was discontinued 72 hours before initiating the protocol. Mean target lesion size was 3.89 cm (longest diameter; median 3.65- range 0.9–7.4cm). Seven of eleven fully staged dogs had regional lymph node metastasis (4 grade III, 3 grade II tumors), 1 had evidence of splenic involvement (grade III primary), and 3 staged negative beyond their primary tumor. One dog had mild eosinophilia, which was not present on subsequent CBC. Two dogs had mildly elevated liver enzymes (ALP, ALT, AST) at study entry; one improved from baseline values when reevaluated four weeks later, the other dog had advanced disease at enrollment, was euthanized 1 week later due to progression and the elevated liver values were attributed to hepatic metastasis which was confirmed at necropsy.

Dose and safety evaluation

Adverse events are summarized in table 3. Dose-limiting (grade 3 or 4) adverse events were observed at higher combination doses of vinblastine and toceranib, however, no deaths were noted with concurrent administration in any cohort. Both dogs in the first cohort experienced dose-limiting adverse events (grade 4 febrile neutropenia). As these adverse events were hematologic in origin and most consistent and attributable to vinblastine therapy based on its known single-agent profile, dose modifications of vinblastine were instituted for subsequent cohorts rather than toceranib modification as planned in the original design. Two of three dogs in the second cohort also developed a grade 4 febrile neutropenia, after which a second 20% vinblastine dose reduction was applied for cohort 3. In all patients, body temperatures normalized after 24 - 48 hours of supportive care in the hospital. This included the addition of broad-spectrum antibiotics and fluid support. No dogs developed cumulative myelosuppression. No further vinblastine dose reductions were necessary, however, in all cohorts, neutrophil count recovery was not adequate (>1500/ μ l) to allow weekly continuation of vinblastine treatment and it became necessary to modify treatment schedules such that vinblastine would be given every two weeks in all subsequent cohorts (table 1).

It was established in cohort 3 that a safe and feasible dosage of vinblastine is 1.6 mg/m² given every other week when administered in combination with toceranib at a dosage of 2.25 mg/kg every other day. Vinblastine dosage was maintained at this point and toceranib escalations were reinstituted as previously planned for subsequent cohorts. In the final cohort (cohort 5), no dose-limiting adverse events were observed, confirming the FDA label dose for toceranib of 3.25 mg/kg EOD as safe in combination with a maximal dose of 1.6 mg/m² vinblastine every other week.

Gastrointestinal adverse events were generally mild to moderate and attributable primarily to toceranib based on its known event profile. All resolved after temporary discontinuation (1 week) of toceranib therapy; toceranib dose reductions were applied in 3 dogs when quality of life was perceived as unsatisfactory by owners, despite not reaching a grade 3 dose-limiting level.

Mild to moderate weight loss was noted in six dogs. Neuromuscular signs consisting of limb weakness/stiffness were also noted in 2 dogs. In the first dog, signs persisted despite discontinuation of toceranib for 1 week, but did not worsen after reinitiating therapy. In the second dog, lameness resolved after a 1 week toceranib holiday. Six dogs developed mild (grade 1–2) liver enzyme increases from baseline and two dogs in early cohorts developed transient grade 3 ALT increases concurrent with grade 4 febrile neutropenia in early cohorts which resolved after treatment for febrile neutropenia. One dog completed the study without having experienced any adverse events.

Response assessment

Response to combination vinblastine and toceranib therapy was determined by caliper measurements of disease when possible, or ultrasonographic assessment of tumor size when indicated, using modified RECIST v1.1 criteria. Complete responses occurred in two dogs (14%) and partial response was observed in 8 dogs (57%) for a best overall observed response rate of 71%. While disease in 3 other dogs remained stable for the 4-week study period, the study length was temporally inadequate to define these as SD based on RECIST v1.1 criteria. Only one dog developed progressive disease while on protocol.

While duration of response is beyond the scope of a phase I study of this design, it can be noted that 3 dogs maintained a PR for at least 3 months in subsequent follow-up and both dogs that achieved a CR are still disease free at their last recheck (80 days and 62 days after study entry). Of these, one is receiving no further therapy and the other is receiving maintenance toceranib and prednisone on alternating days.

DISCUSSION

Dogs enrolled in this trial were generally representative of the overall population of client-owned dogs with advanced-stage macroscopic MCT in terms of age, breed, gender, tumor presentation and grade. No novel adverse events were noted in dogs receiving the two-drug combination that would not have been predicted based on the known event profiles for each drug used as a single-agent. However, the intensity of myelosuppression, specifically neutropenia, was found to be unexpectedly high following simultaneous combination when compared to each drugs known adverse event profile when used alone. This resulted in our determination that the maximally tolerated dose and schedule for the combination is vinblastine administered at 1.6 mg/m² every other week with toceranib used at the FDA label dose (3.25 mg/kg PO, every other day).

Two dogs in each of the first two cohorts experienced dose-limiting grade 4 neutropenia and all dogs in the remaining cohorts experienced grade 1 – 3 neutropenia at day 7 that necessitated extension of vinblastine dosing intervals to every other week. As neutropenia represents the established DLT for vinblastine in dogs and the starting cohort dose of toceranib was already at the low end of that documented to result in anti-MCT activity in phase I/II trials when used as a single agent (17), a decision was made to decrease vinblastine in subsequent cohorts rather than decrease the toceranib dose. This strategy was later confirmed as increasing the dose of toceranib did not exacerbate neutropenia in cohort 4 and 5 when the vinblastine dose was held constant.

The 1.6 mg/m² every other week dosing regimen established as maximal for vinblastine in this combination equates to a dose intensity (DI), defined as mg/m²/week regardless of schedule, of 0.8 mg/m²/wk. This represents a 54 – 71% reduction in DI when compared to that of published vinblastine monotherapy (DI of 1.75 – 2.6 mg/m²/wk) (3, 4,12–23). This degree of DI reduction required for the simultaneous combination is contrary to the current paradigm of only combining anticancer drugs if their simultaneous use allows maintaining DI known to be effective in the single-agent setting.

It is likely that toceranib sensitizes the myeloid compartment to the effects of vinblastine by a process that is not yet understood. This is not a novel phenomenon and has been seen when tyrosine kinase inhibitor therapy and cytotoxic chemotherapy have been combined in human clinical trials (30–33). In people, similar findings were observed when combining gefitinib and vinorelbine or gemcitabine. High rates of grade 3–4 adverse events, including 72% of people developing neutropenia, were observed on this combination, leading to early closure of the study (30). Similar observations were made when erlotinib and vinorelbine were combined in a phase I study which resulted in 50% grade 3–4 neutropenia and 25% febrile neutropenia that precluded adequate delivery of therapy (31). Further, studies combining vinca alkaloids with EGFR inhibitors demonstrated unacceptable myelosuppression (32, 33). It has been suggested that one potential mechanism for this hematologic toxicity may be that both agents are metabolized by CYP3A4 enzymes, resulting in prolonged exposure to the active drugs (33, 34). The cytochrome P450 hepatic enzyme system is thought to play a major role in toceranib metabolism (35) and the label insert advises against co-administration of toceranib with strong inhibitors of CYP3A4 enzyme systems. While this is plausible, pharmacokinetic modeling of the combination would be necessary to substantiate this interaction. As toceranib affects several cellular signal transduction pathways involved in cell cycling, proliferation and apoptosis, other mechanisms of synergistic myelosuppression could also be involved. For example, this class of drug does inhibit colony stimulating factor-1 receptor, and while toceranib did not result in clinically significant neutropenia (i.e., grade 3 or 4) and was not dose-limiting in the field trial (20), this effect may exacerbate the level of neutropenia when combined with cytotoxic drugs. Importantly, several TKIs have been shown to inhibit ATP-binding cassette (ABC) multidrug transporters (MDR-ABC proteins) and the pattern of MDR-ABC transporter-TKI interactions may also help to understand the general pharmacokinetics and toxicities of new TKIs used in combination with standard cytotoxic drugs (36). In particular, sunitinib, a TKI that was co-developed along with toceranib which targets identical TKI receptors has been shown to inhibit these multidrug transporter systems (37,38). Finally, a similar c-kit inhibiting TKI, imatinib mesylate has been shown to interact with MDR-ABC proteins in hematopoietic stem cells (39). Taken in totality, such TKI-MDR interactions may explain the exacerbation of myelosuppression noted with the combination under study here and warrant further interrogation.

While the extent and durability of antitumor activity is not a primary objective of phase I dose-finding trials of this design, the combination of vinblastine /toceranib produced objective clinical response in 71.4% of dogs and some were durable. Responses were observed in all dosing cohorts evaluated. This response rate is higher than that reported in two studies using single agent vinblastine at 2 mg/m² (12% objective response rate) (16, 40) and also higher than that reported in the phase II field trial of single agent toceranib (20). While this response rate appears to compare favorably to that reported for single-agent vinblastine or toceranib, the degree to which activity relates to each agent alone, or the potential synergistic anti-tumor activity of the combination would require a prospective randomized clinical trial comparing single and combination vinblastine and toceranib therapies. Based on the significant response rate and enhanced myelosuppression observed with the combination, one could theorize that the combination, even with the requirement

for decreased DI of vinblastine, may result in additive or synergistic antitumor response that may have clinical utility. The previously discussed interaction between TKI agents and MDR-ABC proteins that may be at play in the enhanced myelosuppression observed with the combination may also theoretically result in synergistic interactions between toceranib and MDR-substrate cytotoxics and warrants further *in vitro* and *in vivo* interrogation. A prospective randomized clinical trial, comparing single and combination treatment arms would be required to confirm or refute any additive or synergistic antitumor activity from the combination. Until such a study is performed, there is insufficient information to recommend the combination over sequential use of vinblastine followed by maintenance toceranib, which is currently employed by many veterinary oncologists for similar staged macroscopic MCT in dogs.

Limitations of the current study are similar to those inherent in phase I dose-finding trials in general. As numbers are small and dose-intensity of cohorts vary, a more accurate assessment of response rates, response durability, and long-term adverse event profiles would await larger phase II or III assessments. That being said, observed antitumor activity is likely somewhat more accurate in this study as only de-escalations of vinblastine occurred rather than dose-escalations as is more common in phase I trials when below-MTD cohorts are prevalent early in trial. The lack of consistent clinical staging was an additional minor limitation, as this would likely only lead to under-staging and since adverse event profiles are known to be generally higher in more advanced disease, this would not have resulted in an underestimation of events. Finally, no attempt was made to interrogate the Kit mutation status of tumors in this trial. While this certainly may affect response rates for MCT in general, activity is not the primary endpoint for this study design.

CONCLUSIONS

The maximally tolerated dose and schedule for simultaneous combination of vinblastine and toceranib for the treatment of macroscopic canine MCT is vinblastine administered at 1.6 mg/m² every other week with toceranib used at the FDA label dose (3.25 mg/kg PO, every other day). It was demonstrated that this simultaneous combination produces significant myelosuppression that requires at least a 54% reduction in the dose-intensity of vinblastine based on single-agent use. Such a dose reduction does not support the use of this combination based on the current paradigm of drug combinations. That being said, the combination protocol does appear to have significant activity and is generally well tolerated and there is a suggestion of additive or synergistic activity when the agents are combined. Therefore, a prospective randomized evaluation comparing the combination with more dose-intense single-agent treatment arms should be completed before the authors recommend the combination over single-agent or sequential combination use of vinblastine and toceranib.

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TABLE 1

Scheduled Treatment Cohorts

COHORT	Vinblastine Dose (mg/m ² /wk* or mg/m ² /every other week ^b)	Palladia Dose (mg/kg EOD)	Dogs Treated	Number of Dogs Experiencing Dose-limiting Adverse Events	Best Response Observed
1	2.3*	2.25	2	2 [¶]	1SD/1PR
2	2.0*	2.25	3	2 [¶]	1PD/1PR/1CR
3	1.6 [#]	2.25	3	0	3PR
4	1.6 [#]	2.75	3	0	1SD/1PR/1CR
5	1.6 [#]	3.25	3	0	1SD/2PR

[¶]All dose-limiting adverse events represented Grade 4 neutropenia.

TABLE 2**Patient Demographics**

Characteristics		Value
Age (y)		
	Mean	7.7
	Median	8
	Range	3–12.5
Weight (kg)		
	Mean	25.3
	Median	22.7
	Range	11.2–41.8
Gender		
	Male intact	2
	Male neutered	3
	Female intact	1
	Female spayed	8
Tumor location		
	Limbs	7
	Dorsum of nose	3
	Cheek	1
	Oral cavity	1
	Lateral thorax	1
	Pinna	1
Tumor grade (12/14)		
	I	0
	II	7
	III	5
Mitotic Index – Mitoses per 10 high power field (10/14)		
	5	5
	>5	5
Recurrence		
	Yes	4
	No	10
Prior chemotherapy		
	Yes	1
	No	13
Clinical stage		
	I	3
	II	4

Characteristics		Value
	III	4
	IV	3

TABLE 3

Adverse Events by Grade and Cohort

AE Classification	Grade	COHORT				
		1 (n=2)	2 (n=3)	3 (n=3)	4 (n=3)	5 (n=3)
Neutropenia	1			1		1
	2			1		
	3		1	1	1	1
	4	2	2			
Anemia	1		1			
	2					
	3		1			
Thrombocytopenia	1			1	1	
	2					
	3					
Anorexia	1		1		1	1
	2	1				1
	3					
Vomiting	1		2	1	2	
	2					1
	3					
Nausea	1					
	2	1				
	3					
	3					
Diarrhea	1		1	1	1	
	2		1			1
	3					
Weight loss	1		1	2		1
	2				1	
	3					

AE Classification	Grade	COHORT				
		1 (n=2)	2 (n=3)	3 (n=3)	4 (n=3)	5 (n=3)
Lethargy	1				1	1
	2	1			1	
	3					
Neuropathy/lameness	1					1
	2					
	3		1			
Elevated ALP	1					
	2		1		1	
	3				1	
Elevated ALT	1				1	1
	2				1	
	3	1	1			
Epistaxis	1		1			
	2					
	3					