Veterinary Oncology

REVIEW

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Comparative oncology in action: vignettes on small molecule development

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Abstract

Comparative oncology is the study of naturally-occurring cancer in companion (pet) animals, mainly dogs, and is a powerful tool in cancer research and drug development. Comparative oncology clinical trials are defined by their translational value to human cancer research through unique opportunities to evaluate in vivo target modulation, drug tolerability, pharmacokinetic-pharmacodynamic (PK-PD) relationships, and identification of translatable biomarkers for drug response. In this manuscript we review specific examples of how comparative oncology clinical trials, built upon and designed to supplement conventional preclinical datasets, have led directly to human clinical development and eventual approval. In doing so, provide a facile reference for those unfamiliar with canine comparative oncology trials and their link to human cancer drug development, inclusive of their purpose, design elements and data interpretation.

Keywords Comparative oncology, Drug development, Clinical trials, Small molecules

Introduction

The main components of drug development pertain to drug safety/tolerability, efficacy, and the mechanism of drug action on the target biology of interest [1, 2]. Descriptive reviews on how these individual datasets are created and refined are covered elsewhere through defined guidance toward regulatory approval [3]. Anti-cancer drug development typically begins with identification of drug target(s) that play a key role in a cancer-associated, preferably cancer-driving, pathway or mechanism. Access to publicly-available large-scale genomic, transcriptomic and clinical datasets derived from human cancer patients have significantly enabled novel target identification and assessment in recent years. Medicinal chemistry efforts then ensue to design a suite of molecules that inhibit or disrupt these pathways, using a variety of assays to demonstrate drug candidate(s) interact with the target and induce favorable anti-cancer effects with predicted acceptable pharmacokinetic (PK) properties [2]. Molecules can be screened using in silico and/or computational methods to rank the most promising candidates to proceed into more specific in vitro assays. Preclinical work involving animal models typically follows this workflow, focused on determining in vivo properties such as absorption/distribution/metabolism/excretion (ADME) parameters, toxicity, and efficacy against experimentally-induced disease. Clinical studies in patients, directed by regulatory guidance and review, ultimately determines if a new drug will gain approval for use in humans.

Comparative oncology studies carried out primarily in pet dogs have the power to complement and inform many stages of the drug development path by providing

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unique, supplementary datasets that ask and answer questions outside of and/or in parallel with the existing paradigm [4]. A key component of these studies is the credentialling of shared tumor biology between dogs and humans, which also supports studies that are histology-agnostic. Further, the data generated in pet dog studies has the power to inform future human studies through identification of responsive histologies outside of the primary target human population of interest and through the collection of high-value biologic specimens. These samples, which often comprise multi-timepoint matched sets of tumor tissue, normal/tumor-adjacent tissue, and blood components (serum, plasma, peripheral blood mononuclear cells (PBMCs)), support powerful studies that address target modulation in vivo within a spontaneously-developing tumor in an immune-competent host. Such studies enhance data generated in other experimental animal models and provide critical insight into the ideal dose, schedule and target patient population for follow-on human studies. The studies presented here provide specific examples of how the comparative approach has directly informed the development and use of three small molecules for treatment of human cancer patients.

Vignette #1: Assessment of CB-5339 in canine solid tumors and hematologic malignancies

Preclinical summary

The AAA (ATPases Associated with diverse cellular Activities) ATPase p97, also known as VCP (valosin-containing protein) has a well- described role in the ubiquitin proteasome system (UPS), where it chaperones subsets of proteins to the proteasome for degradation [5–7]. Targeting of protein homeostasis has become a clinically proven anti- cancer strategy since the introduction of proteasome inhibitors as a treatment for multiple myeloma [8, 9]. Although their development provided the rationale for targeting of the UPS, the clinical use of proteasome inhibitors resulted in the development of high rates of resistance and concern for toxicities such as peripheral neuropathy and thrombocytopenia, prompting efforts to design and develop alternate strategies [10, 11].

A second-generation inhibitor of VCP, CB-5339, was developed and systemically compared to its predecessor, CB-5083 [12–14]. CB-5339 exhibited comparable in vitro and in vivo potency profile, but with improved physiochemical, drug metabolism and PK properties. Preclinical efficacy of both CB-5083 and CB-5339 had been explored and confirmed in a variety of preclinical model systems, with an emphasis on hematologic malignancies such as acute myelogenous leukemia (AML) and multiple myeloma (MM) [15]. These studies demonstrate the kinetics of cell kill and relevant mechanisms, including induction of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) [16, 17]. This sensitivity has been confirmed through patient-derived samples as well as cell lines [18]. In general, CB-5339 is comparable to CB-5083 in activity and is active with different regimens of administration in vivo. In mouse models of MM and AML, activity is similar or better than what is observed with most agents.

In preparation for a canine clinical trial, canine cancer cell line in vitro sensitivity assays as well as canine-in-mouse tumor xenograft studies were performed utilizing both CB- 5083 and CB-5339 to optimize inputs to trial-related PD assays and confirm efficacy [19]. This was done to ensure appropriate assay support was in place to interpret and translate the findings from the canine trial to eventual human trial designs. PD markers were chosen for their proximity to the actions of VCP. When VCP is inhibited, accumulation of proteosome-specific (K48) ubquitinated proteins occurs in the cytosol, with induction of ER stress and UPR leading to induction of expression of the transcription factor CHOP in the nuclear fraction of treated cells [16].

In support of a regulatory approval path as well as a comparative oncology clinical trial, data was generated in purpose-bred Beagle dogs and determined a No Adverse Event Level (NOAEL) of 7.5 mg/kg/day and Maximum Tolerated Dose (MTD) of 10 mg/kg/day, respectively. The initial dosing cohorts for pet dogs were designed with this information in hand, given that young, healthy purpose-bred dogs often tolerate higher doses and exposures of cytotoxic agents compared to aged companion dogs.

Comparative oncology trial

Questions

What are the safety, pharmacokinetics, and pharmacodynamic modulation features of CB-5339 treatment in dogs with spontaneous malignancies, including multiple myeloma? Can correlations be made between tolerable exposures of orally-administered CB-5339 in pet dogs to activation of the unfolded protein response (UPR) and/or clinical efficacy?

Study design

Study schema are given in Fig. 1. In COTC028, a comparative oncology clinical trial conducted by the National Cancer Institute's Comparative Oncology Trials Consortium (NCI-COTC), CB-5339 was administered orally once a day on days 1–4 and days 8 −11, followed by one week off, in 22-day cycles [19, 20]. Tumor biopsies were taken prior to CB-5339 administration (pretreatment assessment, conducted prior to Day 1), on Day 1–2 (tumor biopsy times 1 h, 6 h, 24 h after 1st oral dose), Day 8, and on Day 22 if stable disease or progressive disease (SD/PD) was present. PBMCs were collected at the same

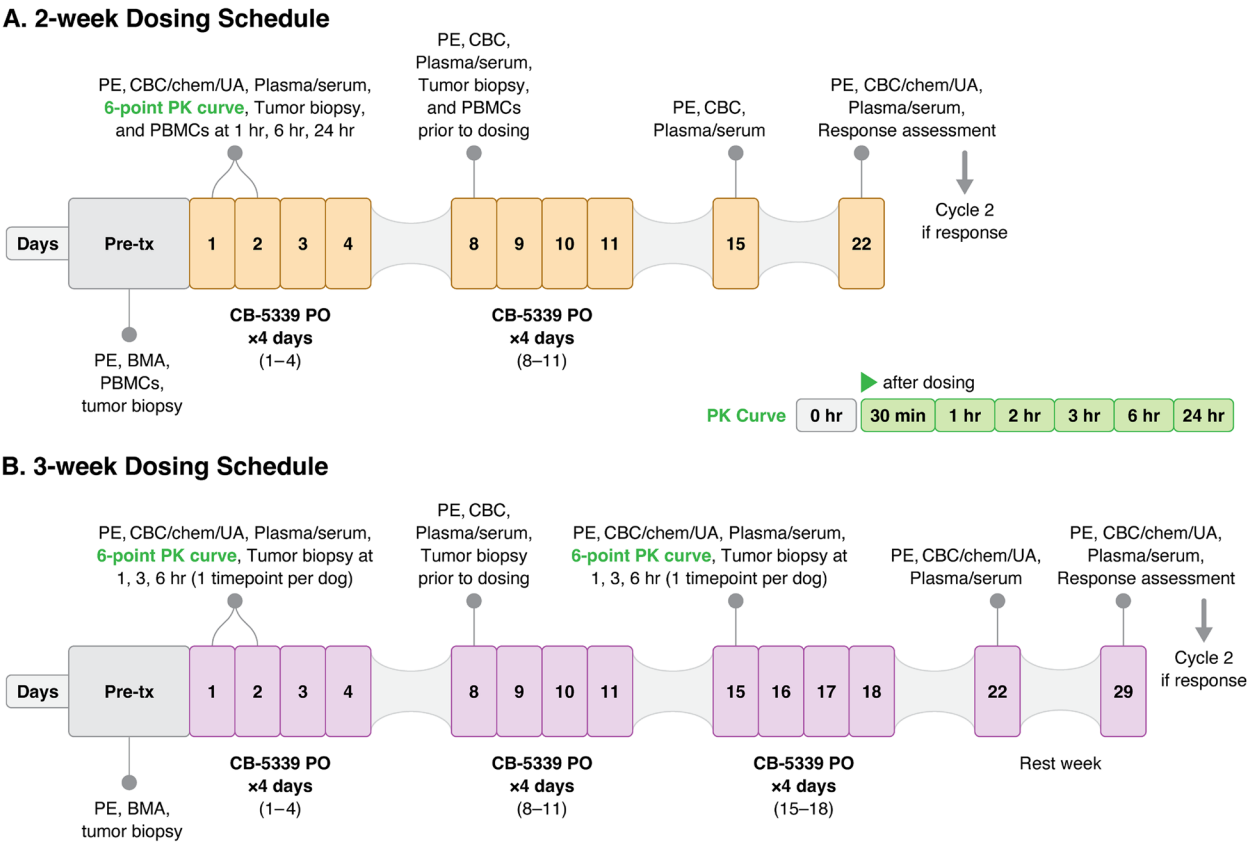

Fig. 1 Comparative oncology assessment of CB-5339 in tumor-bearing dogs. **A** depicts the 2-week dosing schedule, which was subsequently expanded into a 3-week schedule (**B**). PE: physical exam, CBC: complete blood count, UA: urinalysis, BMA: bone marrow aspirate, PBMCs: peripheral blood mononuclear cells, PO: per os/oral dosing, PK: pharmacokinetics

timepoints as all tumor biopsies. Blood samples for PK analysis were collected prior to administration Day 1 and then 30 min, 1, 2, 3, 6 and 24 h after agent administration on Day 1 during cycle 1 only. Clinical assessments determined that dogs with SD or PD on Day 22 would exit the study; dogs with CR or PR could continue for optional cycles of therapy if desired. PD responses were assessed in tumor tissue and PBMCs using a validated K48 immunoassay and qPCR method for induction of CHOP to determine engagement of the UPS, and link to PK at matched timepoints to explore PK/PD relationships [19].

Deliverables

A total of 24 pet dogs with a variety of malignancies were enrolled over a 2-year period, with assessment of 2 different drug formulations (active pharmaceutical ingredient (API) as the free-base powder in capsule vs. human clinical formulation). CB-5339 was generally well-tolerated, although adverse events were noted, mainly related to gastrointestinal effects (inappetence, nausea, and vomiting). Through a stepwise dose and schedule escalation schema, we determined the maximum tolerated dose to be 7.5 mg/kg when administered orally on a 4-days on, 3-days off schedule per week for 3 consecutive weeks. PK/PD data suggested a relationship between exposure and modulation of targets related to induction of the unfolded protein response, but not to tolerability of the agent. An efficacy signal was detected in 33% (2/6) dogs with multiple myeloma, consistent with a mechanism of action relating to induction of proteotoxic stress in a tumor type with abundant protein production. Flexibility in the trial schema allowed exploration of both 2- and 3-week exposure schedules [19].

Human use: how was it informed by canine comparative oncology data? This agent was developed jointly by Cleave Therapeutics and the National Cancer Institute's Novel and Experimental Therapeutics (NExT) program, through which engagement with the NCI-COTC was possible for conduct of the canine trial described herein [20]. Several important lessons were learned from this trial, including the observation that PBMCs are not a suitable surrogate for tumor tissue during drug exposure to monitor the PD response in vivo, which has direct

implication for sample collection plans in human trials of this agent. We also determined iatrogenic hemorrage during biopsy collections of tumor tissue could interfere with certain PD assay readouts, indicating that additional methods should be considered for future trials. The efficacy signal observed in naturally-occurring canine multiple myeloma was a central element that helped garner enthusiasm for licensing of the agent for ongoing clinical development and for initiation of first-in-human trials in patients with hematologic malignancies. In a Phase 1 clinical trial in patients with acute myeloid leukemia and myelodysplastic syndrome, the drug was well tolerated in 55 patients and demonstrated signs of clinical activity (NCT04402541).

Current drug status Additional clinical trials of CB-5339 in humans with AML and MM are planned. CASI Pharmaceuticals has continued the clinical development of this agent.

Vignette #2: Assessment of KPT-335 (verdinexor) in canine solid tumors and lymphoma

Preclinical summary

XPO1 is a nuclear exportin that regulates a wide range of cellular processes through its transport of hundreds of proteins and multiple RNA species (mRNAs, microRNAs, etc.) out of the nucleus [21–23]. Given that several known tumor suppressors and growth regulatory proteins (i.e., p53, p21, among many others) are substrates of XPO1, it is widely believed that aberrant protein localization mediated by XPO1 (i.e., export of proteins that require nuclear localization for function) contributes to tumor development and progression [21, 24, 25]. Moreover, overexpression of XPO1 has been documented in several human cancers including carcinomas, sarcomas, and hematologic malignancies, often correlating with a poor prognosis [21, 26, 27]. Accordingly, the functional consequences XPO1 overexpression are believed in part to be driven by forced aberrant localization of proteins; enhanced export of multiple tumor suppressor proteins impairs their ability to constrain tumor cell growth. Consequently, therapeutic targeting of XPO1 is believed to have potential value across a multitude of tumor types.

XPO1 small molecule inhibitors were initially developed in the early 1980s. The first of these was leptomycin B, [28] which demonstrated anti-tumor activity in vitro and in mouse tumor models through covalent and irreversible binding to XPO1 [29]. Unfortunately, a Phase 1 study in people demonstrated little clinical activity and the trial was terminated early due to a high rate of malaise and anorexia [30]. Leptomycin B analogues were subsequently developed that had greater potency with a marked reduction in toxicity [31, 32]. However, the requirement for intravenous administration limited clinical development. To address challenges associated with previous XPO1 inhibitors, novel, drug-like, orally bioavailable, small-molecule Selective Inhibitors of Nuclear Export (SINE) that bind to XPO1 at the reactive site Cys 528 residue were designed by Karyopharm Therapeutics including KPT-335 (verdinexor) and KPT-330 (selinexor) [33]. Slowly reversible with a $t_{1/2}$ of ~ 24 h, they result in functional inactivation of XPO1 protein and transient proteosome mediated degradation. SINE were shown to induce apoptosis and block proliferation in an array of cancer cell lines with activity in several different mouse cancer models [21, 23, 33]. Importantly, SINE demonstrated good oral bioavailability and tolerability in mice.

Prior to engaging in canine studies, several SINE (KPT-185, KPT-214 and KPT-335) were studied in vitro against an array of canine cancers using primary tumor cells and tumor cell lines to better understand their biologic activity. Both KPT-185 and KPT-335 reduced the viability of the diffuse large B cell lymphoma (DLBCL) canine cell line CLBL1, as well as primary canine DLBCL samples at low nanomolar concentrations of drug [34]. Additionally, KPT-214 inhibited the growth of canine mast cell tumor, osteosarcoma and melanoma cell lines (one each) with IC_{50} values ranging from 70–450 nM [34]. In vitro studies of KPT-335 were expanded to include a larger cohort of canine melanoma cell lines, demonstrating growth inhibition with IC_{50} values ranging from 71–330 nM [35]. In this study, KPT-335 inhibited colony formation, promoted upregulation and nuclear localization of p53 and p21, and induced apoptosis in the melanoma lines at drug concentrations in the nanomolar range. Lastly, although expression of XPO1 protein was reduced in the presence of KPT-335, a compensatory upregulation of XPO1 mRNA was noted. Additional studies of KPT-335 were undertaken in canine osteosarcoma, mammary cancer and transitional cell cancer tumor cell lines, demonstrating dose dependent growth inhibition, activation of caspase 3 and 7, enhanced expression of p53 and p21, and nuclear localization of p53 [36, 37].

Prior to beginning client owned canine studies, an initial assessment of KPT-335 pharmacokinetics was undertaken in healthy beagle dogs given a single dose of drug at 1.5 mg/kg after being fed a meal [34]. This dose was determined based on prior work with KPT-330 in healthy beagle dogs. Blood samples were obtained over a 48 h time period and analyzed for plasma KPT-335 showing the mean T_{max} was approximately 4 h with a C_{max} of approximately 250 ng/ml, and an average AUC of 1800 ng/ml and therefore adequate for target inhibition. A subsequent target animal safety study confirmed that KPT-335 given orally is well absorbed following a meal

in dogs and achieves therapeutic levels (> 0.5 to 1.0 μM) with doses of 1 to 3 mg/kg.

Comparative oncology trial

Questions

What are the safety, pharmacokinetics, and biologic activity of KPT-335 in dogs with cancer? What strategies can be employed to mitigate adverse events associated with XPO1 inhibition?

Study design

Study schema are given in Fig. 2. A standard 3 + 3 Phase I clinical trial of KPT-335 was performed in dogs with lymphoma, mast cell tumor or osteosarcoma to identify the maximum tolerated dose and drug related toxicities. Cohort expansion was planned if both safety and biologic activity were observed with in a specific tumor type/dose. Building upon these findings, a Phase II clinical trial was undertaken in dogs with naïve and relapsed B or T cell lymphoma. Pharmacokinetic analysis was undertaken in a subset of dogs.

Deliverables

A total of 17 dogs with lymphoma (naive or relapsed), mast cell tumor or osteosarcoma were enrolled in the Phase I study [34]. The maximum tolerated dose of KPT-335 was identified as 1.75 mg/kg 3x/week, although biologic activity was noted at 1 mg/kg. Clinical benefit including partial response to therapy (PR, $n = 2$) and stable disease (SD, $n = 7$) was demonstrated in dogs with lymphoma for a duration of 35–256 days. Based on these findings, a cohort expansion of 6 dogs with lymphoma was added, using 1.5 mg/kg KPT-335 3x/week; clinical benefit was documented in 4 dogs for a duration of 35–354 days. Adverse events noted included anorexia, weight loss, vomiting and diarrhea. These were managed with supportive care, dose modulation and administration of low dose prednisone to stimulate appetite. Lastly, a quality of life metrics tool showed that dogs maintained quality of life. In the Phase II study, 58 dogs with naive or relapsed B-cell or T-cell lymphoma were enrolled across 10 different study sites. KPT-335 was administered orally in one of three dosing groups (1.5 mg/kg 3x/week, 1.25 mg/kg 3x/week, and 1.25–1.5 mg/kg 2x/week) [38]. For

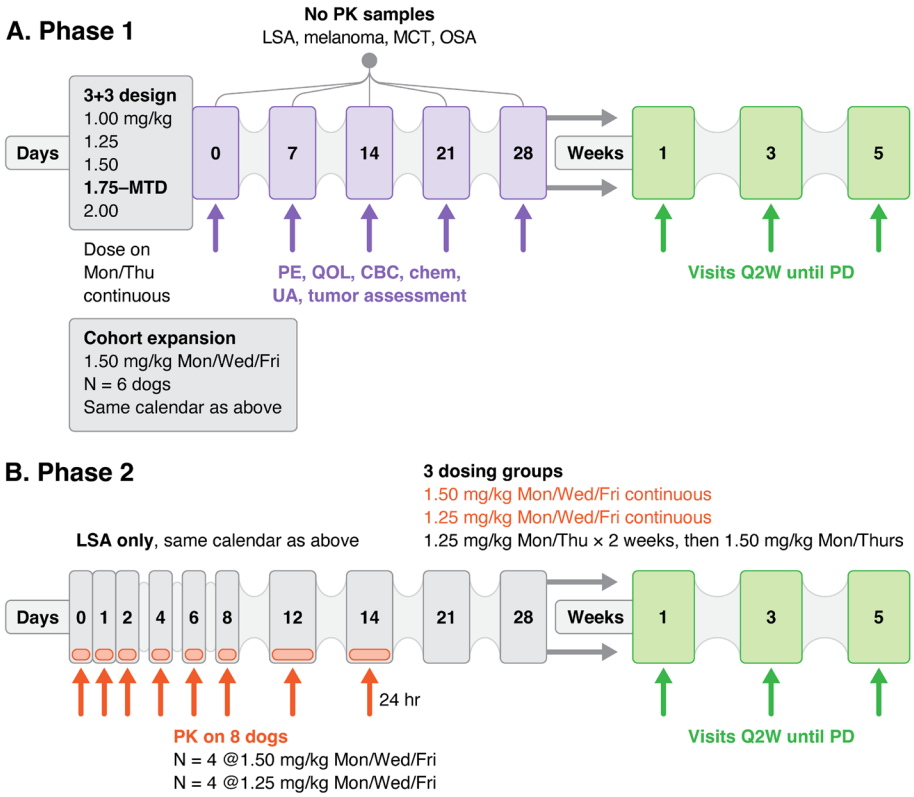

Fig. 2 Comparative oncology assessment of KPT-335 in tumor-bearing dogs. Two distinct Phase 1 studies were performed to explore tolerability and efficacy in dogs with a variety of cancer types. In Phase 2, a focused study in dogs with lymphoma (LSA) was conducted to explore several dosing methods and schedules with PK data gathered form a subset of patients. MCT: mast cell tumor, OSA: osteosarcoma, PE: physical exam, QOL: quality of life assessment survey, CBC: complete blood count, chem: serum chemistry panel, UA: urinalysis, Q2W: every 2 weeks, PD: progressive disease, PK: pharmacokinetics

all dogs, the objective response rate (CR/PR) was 37% (20/54) in the evaluable patient population; dogs with T cell lymphoma had a higher objective response rate (71%). The most common adverse events across all dose groups were consistent with the Phase I study (anorexia, weight loss, vomiting, lethargy and diarrhea) and were manageable using dose modulation and low dose prednisone. Seven dogs receiving KPT-335 at 1.5 mg/kg ($n=4$) or 1.25 mg/kg ($n=3$) 3x/week underwent pharmacokinetic analysis. For all dogs, the mean Cmax was 278 ng/ml with a mean AUC of 1970.6 ng*t/ml, Tmax of 5.3 h and $T_{1/2}$ of 5 h. Both doses achieved KPT-335 plasma levels sufficient for XPO1 inhibition.

Human use: how was it informed by canine comparative oncology data? The canine studies confirmed a signal of biologic activity in hematologic malignancies (lymphoma), determined a tolerable dosing regimen (2x/week), and identified effective strategies to mitigate toxicities secondary to the use of SINE compounds. The safety profile of verdinexor, marketed for canine use under conditional FDA-CVM approval as Laverdia-CA1 in tumor-bearing pet dogs, was included as supplemental information in the initial Investigational New Drug designation by the FDA for selinexor. The canine studies identified the primary toxicities associated with XPO-1 inhibition (nausea, vomiting, hyporhexia), strategies to mitigate this adverse event (administration of steroids with the inhibitor) and the dosing regimen (twice per week). This dosing strategy with steroids was used in the subsequent human clinical trial of selinexor in human patients with diffuse large B cell lymphoma (DLBCL), which led to accelerated FDA approval in 2020 [39].

Current drug status KPT-330 (XPOVIO, selinexor) is FDA approved for the treatment of relapsed multiple myeloma (2019) and diffuse large B cell lymphoma (2020) in humans.

Vignette #3: PAC-1 therapy in canine CNS malignancies

Preclinical summary

Procaspase-3 in cancer

The cleavage of procaspase-3 (PC-3) to caspase-3 represents a critical node in the intrinsic and extrinsic apoptotic cascade, as this executioner caspase catalyzes the hydrolysis of hundreds of protein substrates [40, 41], which ultimately leads to cell death. Given the irreversibility of apoptosis, a survival advantage is bestowed upon tumor cells that have the ability to circumvent programmed cell death through mutation and dysregulation of apoptotic proteins [42]. These perturbations in cellular death signaling are effectively "breaks" in the apoptotic circuitry. Consequently, several anticancer strategies have focused on small molecule inhibition of these mutated proteins [43, 44], with the rationale for resensitizing cancer cells to apoptotic signaling. An orthogonal, yet complementary, approach involves the small molecule-mediated activation of proapoptotic proteins, such as PC-3. Based on the downstream location of PC-3 in the apoptotic cascade relative to frequently mutated proteins [45], the rarity of PC-3 mutations in cancer [46], and the robust expression of the PC-3 enzyme in a number of hematopoietic and solid tumor histologies [47], the small molecule-mediated activation of PC-3 has been hypothesized to be a rational and innovative anticancer strategy [48]. In 2006, the first procaspase activating compound (PAC-1) was discovered by high throughput screening [49], and its translational potential as a novel strategy for treating human cancer patients has been advanced through traditional (rodent) and spontaneous (pet dog) tumor modeling.

Through a detailed series of chemical synthesis, in vitro cell culture, and in vivo experiments, we have found the PAC-1 class of compounds to be a promising anticancer strategy. We have characterized PAC-1 to be a blood–brain barrier penetrant, small molecule, pro-apoptotic activator of PC-3, which possesses favorable pharmacokinetics, tolerability, and synergistic activities when combined with conventional treatments across diverse preclinical rodent tumor models [50–54]. Importantly, in rat and mouse tumor experiments PAC-1 reproducibly enhances the cytoreductive activities of systemic chemotherapy, ionizing radiation, small molecule inhibitors, and novel pro-apoptotic inducing agents. To accelerate the advancement to human Phase I clinical trials, PAC-1 has been systematically evaluated in both healthy research dogs and pet dogs with naturally occurring cancers. When orally administered daily to purpose-bred research dogs, PAC-1 demonstrates predictable oral bioavailability, pharmacokinetics, and does not cause hematologic or biochemical toxicities [55]. Preclinical Investigational New Drug data generated in Beagle dogs determined the NOAEL to be 6.5 mg/kg/day when administered daily for 21 days followed by a 7-day washout period per treatment cycle, and repeated for 3 cycles. Importantly, peak plasma concentrations of PAC-1 achievable in rodents and dogs (~10 μM) superimposes with in vitro concentrations sufficient to selectively induce death of PC-3 overexpressing cancer cell lines (≤10 μM). In pet dogs with spontaneously arising cancers, daily oral PAC-1 dosed at 10 mg/kg (range 7.5–12.5 mg/kg) combined with MTD systemic chemotherapeutics (doxorubicin, temozolomide, or hydroxyurea) or ionizing radiation have been evaluated in the disease

settings of T-cell lymphoma, metastatic osteosarcoma, glioma, and meningioma [51, 52, 56, 57].

Given favorable blood–brain barrier penetrant properties, we focused on PAC-1's broader applicability for the treatment of various brain cancer malignancies, and specifically leveraged naturally occurring and comparative brain tumors in pet dogs [58, 59] for PC-3 activating strategies. Towards this goal, we performed large-scale validation of PC-3 as a druggable target across 650 human and canine brain tumor samples, evaluated the prognostic significance of PC-3 expressions in glial tumors, tested sensitivity of immortalized glioma cell lines to PAC-1 under biologically achievable conditions, and the cytoreductive activity of PAC-1 alone and in combination with ionizing radiation therapy in the intracranial GL261 murine model [52, 54]. Through the inclusion of pet dogs with either glioma or meningioma, we performed preliminary feasibility studies showing the tolerability of combining PAC-1 with multimodality therapies including ionizing radiation, temozolomide, and hydroxyurea [52, 57].

Comparative oncology trial

Questions

What is the tolerability of combining oral PAC-1 with combinatorial therapies inclusive of ionizing radiation and systemic chemotherapies in pet dogs with spontaneous malignant glioma or meningioma?

Study design

Study schema are given in Fig. 3. Dogs had baseline MRI of primary central nervous system neoplasms following pre- or concurrent treatment with corticosteroids to quantify tumor volume with minimal peritumoral edema. Serial MRI was performed at scheduled timepoints to quantify radiologic response following PAC-1 therapy alone or in combination with conventional therapies. In dogs with glioma, oral PAC-1 was administered daily for a total duration of 84 consecutive days and single agent activity, as well as combinatorial activity with 2 cycles of oral temozolomide (100 mg/m^2 for 5 consecutive days, 28 day cycle) and full course definitive radiation therapy (48 Gy, 3 Gy fractions × 16) were evaluated. As a single agent, 28-day treatment with PAC-1 achieved stabilization of disease, however, objective response rates (1 CR, 2 PR) with PAC-1 in combination with temozolomide and ionizing radiation were achieved [52]. In pet dogs with meningioma, the combination of oral PAC-1 concurrently administered with either hydroxyurea or temozolomide was evaluated. No significant cytoreductive activity was identified with oral PAC-1 and hydroxyurea, however, PAC-1 combined with temozolomide exerted modest cytoreductive activities (1 PR, 2 SD) after 6 weeks of therapy [57].

Deliverables

A total of 9 pet dogs with glioma or meningioma were evaluated for the safety and cytoreductive activity of oral PAC-1 alone or in combination with conventional brain cancer therapies including ionizing radiation, temozolomide, or hydroxyurea. These preliminary findings identified PC-3 to be a potentially druggable target for naturally occurring brain cancers, and underscored the safety of PAC-1 and feasibility to combine with standard-of-care therapies. These small scale comparative oncology studies provided foundational data to accelerate the evaluation of PAC-1 towards human Phase 1 clinical trials.

Human use: how was it informed by canine comparative oncology data? The blood–brain barrier penetrant properties, lack of apparent toxicities and potential efficacy when combined with conventional therapies demonstrated in pet dogs with brain cancers supported FDA's 2016 decision to grant orphan drug status for PAC-1 in the treatment of glioblastoma multiforme. Additionally, these findings in pet dogs further supported the conductance of a Phase I clinical trial combining oral PAC-1 with temozolomide for recurrent anaplastic astrocytoma and glioblastoma multiforme in adults (NCT03332355). The safety profile of PAC-1 in tumor-bearing dogs was included as supplemental information in the initial Investigational New Drug designation by the FDA, which paved the path forward to advance PAC-1 into first-in-human clinical trials. The safety and activity of PAC-1 in combination with traditional interventions for CNS pathologies, such as ionizing radiation, temozolomide, and hydroxyurea, provided some real-life assurances that PAC-1 could be incorporated into conventional backbone therapies without added toxicity.

Current drug status Completion of a Phase I clinical trial with single-agent PAC-1 (NCT03332355, component 1) has been completed [60], as well as evaluating combination of PAC-1 with temozolomide (NCT03332355, component 2) in patients with recurrent anaplastic astrocytoma or glioblastoma multiforme [61]. Additionally, oral PAC-1 in combination with entrectinib for the treatment of metastatic uveal melanoma has been clinically evaluated in a Phase Ib trial (NCT04589832) [62]. In the Phase I trial (component 1), a total of 48 patients were enrolled with 33 patients being evaluated for dose limiting toxicities. At the highest dose evaluated, oral PAC-1 at 750 mg daily showed provocative activity in drug-refractory pancreatic neuroendocrine tumors (PNET), with 5 patients achieving either partial response

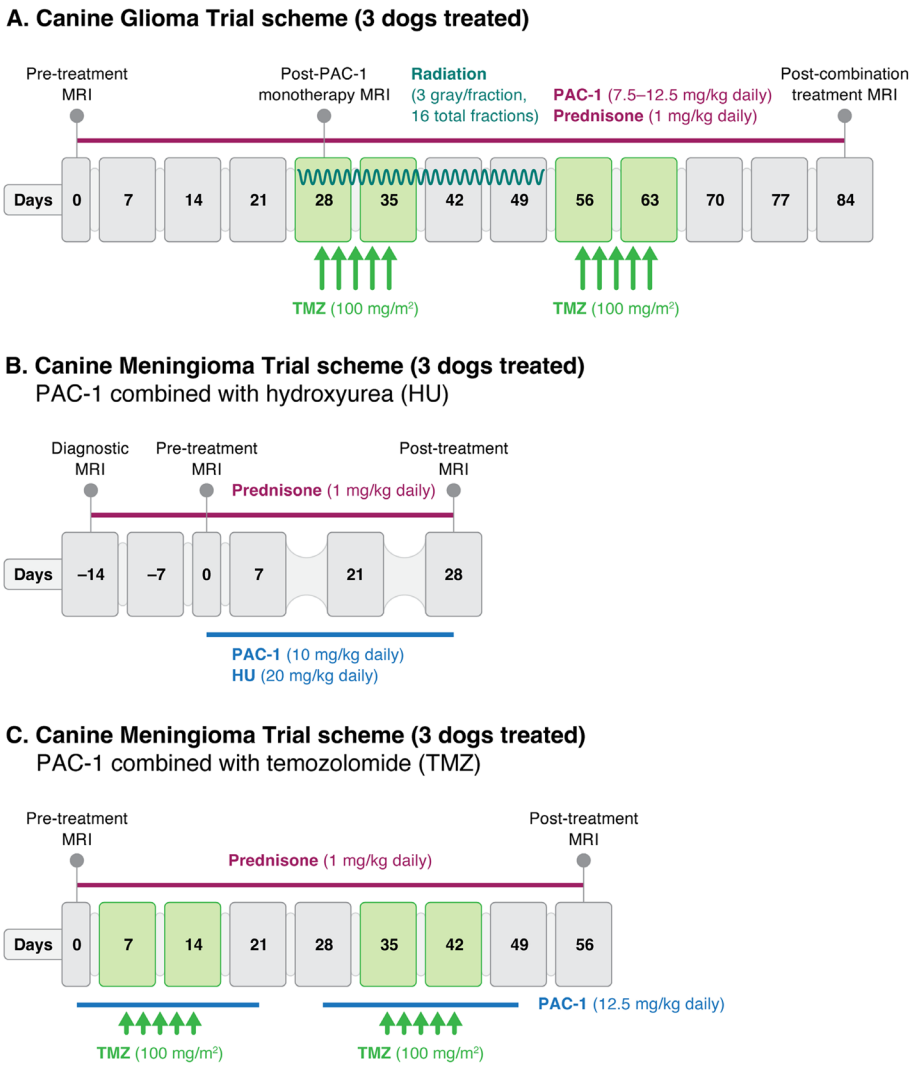

Fig. 3 Comparative oncology assessment of PAC-1 in tumor-bearing dogs. Three different clinical studies were conducted to assess PAC-1 in either canine glioma or meningioma in either the single agent (**A**) or combination (**B** and **C**) setting. MRI: magnetic resonance imaging, HU: hydroxyurea, TMZ: temozolomide

($n=2$) or durable stable disease ($n=3$) [60]. In the Phase I trial (component 2), a total of 18 patients with recurrent anaplastic astrocytoma or glioblastoma multiforme were evaluated. Two patients achieved partial response and an additional 2 patients maintained stable disease [61]. Systems Oncology has continued the clinical development of PAC-1 and intends to explore PNET indications based upon Phase I findings.

Conclusions

Comparative oncology clinical trials provide valuable supplemental data for evaluation of novel anti-cancer agents and are also a mechanism for assessment of combinatorial strategies and repurposing of existing agents for new indications/patient populations. The flexibility in comparative oncology trial designs supports expeditious investigations into altered dosing schedules that are informed by interim analysis of clinical and biologic data. For additional information on shared molecular features between specific human and canine cancers, as well as the ethical and animal welfare requirements for comparative oncology trials, several references are available [63–65].

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Authors' contributions
AL, CL, PJH, and TMF wrote the main manuscript text. CM prepared Figs. 1–3. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
TMF and PJH are both co-founders of Vanquish Oncology that licensed PAC-1 to Systems Oncology for development and commercialization. AL is co-Editor-in-Chief of this journal, and recused herself from all decisions about this paper.

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