

The translational value of comparative oncology trials in client-owned dogs with spontaneous cancer: an overview of clinical trials initiated in dogs that resulted in phase I, II, or III clinical trials in human medicine

Translationele studies bij honden met kanker als brug naar het humane veld: een overzicht van klinische trials uitgevoerd bij honden die geleid hebben tot fase I, II, of III klinische studies in de humane geneeskunde

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ABSTRACT

Pet dogs with spontaneous cancer are a relevant translational model for human medicine. Several therapeutics evaluated in canine clinical trials progressed to clinical trials in humans. Promising results from comparative oncology trials in dogs provided good predictions of their safety and efficacy in human patients unlike many preclinical studies performed in murine models only. In this literature review, an overview is provided of clinical cancer studies initiated in pet dogs that have led to clinical trials in human patients and/or approval by the European or United States agencies responsible for drug approval. The results from these studies reveal the importance of clinical trials in dogs with spontaneous cancer that can pave the way for successful clinical trials in human cancer patients.

SAMENVATTING

Honden met kanker zijn relevante translationele modellen voor de humane geneeskunde. Verschillende geneesmiddelen getest in klinische studies bij honden hebben geleid tot klinische studies in de humane geneeskunde. De resultaten van deze studies hielpen om de veiligheid en werkzaamheid van producten bij humane patiënten te voorspellen, en dit beter dan preklinische studies die enkel bij muizen getest werden. In dit literatuuroverzicht worden de klinische studies weergegeven die aanvankelijk uitgevoerd werden bij honden met kanker en daarna geleid hebben tot klinische studies bij humane patiënten met kanker en/of tot therapiegoedkeuring door de regelgevende instanties van de Europese Unie of de Verenigde Staten van Amerika. De resultaten van deze studies tonen het belang aan van hoe klinische studies bij honden met spontane kanker een succesvolle brug kunnen vormen naar het humane veld.

INTRODUCTION

In cancer research, most preclinical studies are performed in rodents (Workman et al., 2010; Guo et al., 2024). Such experiments are valuable to identify therapeutic targets and to enhance early detection and intervention strategies (Guo et al., 2024). However, it is challenging in rodents to fully replicate the biological effects observed in humans (Nikanjam et al., 2022); as a result, differences in therapeutic outcome may arise. In those studies, rodents, in particular mice and rats, are inoculated with human cancer cells. These cancer cells are injected under the skin (i.e. subcutaneously) or in the organ or tissue similar to where the cancer develops in humans (i.e. orthotopically) (Lwin et al., 2018; Draheim et al., 2024; Guo et al., 2024). Orthotopic models provide a more reliable representation of human disease than subcutaneous models (Du et al., 2014; Fernandez et al., 2023). However, both are established in immune-deficient animals (Miyasaka et al., 2022; Stribbling et al., 2024). The complex interactions between cancer- and immune cells, as observed in humans, are therefore absent. Syngeneic immunocompetent rodents exist but can only be used with their own tumor types (Long et al., 2022; Nicotra et al., 2024). It is possible to genetically engineer mice to have a humanized immune system (Guil-Luna et al., 2020; Ménoret et al., 2024). However, in mice, human T-cells are educated in a mouse thymus and therefore trained to recognize mouse antigens instead of human antigens (Greenblatt et al., 2012; Guil-Luna et al., 2020). In addition, the production of B-cell antibodies in mice is suboptimal (Willinger et al., 2011; Guil-Luna et al., 2020). Both mice and rats can also be humanized upon xenograft transplantation of human lymphoid tissue or human pluripotent stem cells (Agarwal et al., 2020; Ma et al., 2024). However, challenges in cross-reactivity and functional reconstitution may remain (Chen et al., 2023; Ma et al., 2024). Furthermore, similar genes in rodents and humans may be regulated differently (Fischer, 2021; Gilbertson and Weinmann, 2021; Munro et al., 2022), further contributing to differences between both species.

In comparative oncology trials, spontaneous occurring cancers in companion animals have been investigated as relevant translational models for human medicine (Paoloni and Khanna, 2007). Clinical studies in cancer-bearing dogs and cats may help bridge the gap between preclinical rodent research and clinical trials in human medicine (Randall et al., 2013; Abma et al., 2016; Cannon, 2015; Dhawan et al., 2022; Giuliano, 2022). The histology, biology and genetic background of dogs are significantly closer than those of rodents to the histology, biology and genetic background of humans (Abma et al., 2016; Klosowski et al., 2023; Santiago-Rodriguez, 2024). Similarly, feline cancer types closely resemble human cancer types in their genetic, clinical, and histopathological characteristics

(Groll et al., 2021; Wong et al., 2021). Most cancers in dogs and cats also behave similarly to those seen in humans; all three species face similar environmental carcinogenic risks and have a competent immune system (Giuliano, 2022; Oh and Cho, 2023). While a direct comparison between the feline immune system and human immune system is yet to be investigated, a recent transcriptomic study has revealed that the immune system of dogs and humans is similar in their signaling pathways (i.e. TNF- α pathway, IL-2 and IL-6 pathways, T cell receptor signaling pathways, and RIG-I pathways) (Paoloni et al., 2015; Chow et al., 2024). Differences in their innate and adaptive immunity exist, but this does not diminish their value as translational models for human medicine (Lin, 1992; Chow et al., 2024). Rather, the immunological differences may provide guidance in selecting relevant immunological biomarker endpoints (Chow et al., 2024b). In addition to the previously listed advantages, the size of dogs and cats also enables the use of diagnostic and medical imaging techniques similar to those employed in human medicine. Moreover, pets have a shorter life-span than humans, which makes it possible to study long-term effects on survival within a shorter time (Gardner et al., 2016). Furthermore, clinical trials in pets present lower costs compared to human clinical trials, and differences in regulatory frameworks further expedite the process (Fürdös et al., 2015; Seyhan, 2019).

So far, most research has been conducted in dogs, although cats are an even better translational model for human medicine for some cancer types (Adega et al., 2016). Yet, comparative oncology trials in cats remain scarce. Cats are generally more difficult to handle than dogs and, in addition, the availability of experimental cats to perform initial safety studies is limited. Safety studies are required before using any novel drug in cancer treatment (Gilbride and McGowan, 1999). In determining the safety of a compound, a dose-escalation study is performed in healthy animals. Following administration, animals are clinically examined at predetermined time points and monitored for any signs of adverse effects. Blood and/or urine is drawn to investigate potential unwanted effects on vital organs. In stress exhibited pets, some hematologic and biochemistry parameters might be altered (Beerda et al., 1997; Stella et al., 2013; Malancus et al., 2024), which may significantly compromise study validity. This could partially explain why less clinical trials are undertaken with cats.

Once the safety of the compound is established, clinical trials determine the therapeutic benefits in pets with cancer. In veterinary medicine, the main focus is quality of life (Jarvis, 2010; Fulmer et al., 2022). Therefore, patients are only enrolled in clinical trials when they have exhausted all standard treatment options or when no harm is expected for the patients themselves and the findings may lead to clinical improvements. To ensure informed decision-making, the

veterinarian in charge must discuss the benefits and possible side effects with the owners, and acquire their informed consent prior to starting the clinical trial (McKenna et al., 2023). Furthermore, similar to experimental studies, clinical studies in pets need prior approval by the local ethics committee, and an exemption is required by the government since client-owned animals are not purpose-bred¹. Strict regulations ensure that animal welfare is prioritized and that high ethical standards are upheld during the research process^{2,3}. The ultimate aim is to improve patient outcomes in both human and veterinary medicine. By conducting clinical trials in companion animals with cancer, valuable data can be gathered in veterinary patients that closely mirror human conditions, providing a more accurate prediction of human disease outcomes (Füördös et al., 2015). Moreover, clinical trials in veterinary medicine offer pets access to innovative therapies that are not yet available in human medicine.

Clinical research in pets is divided into two main research areas: agents developed for humans that are first tested in pets and repurposed agents. Repurposed agents are developed for veterinary patients but later used in humans.

In this literature review, a non-exhaustive overview of clinical trials is provided, conducted in dogs with spontaneous cancer that resulted in clinical trials and/or standard-of-care treatment in human medicine (Table 1).

ANTIANGIOGENIC THERAPIES

A rich vascular network is crucial to support tumoral growth, as cancer cells' rapid growth depends on nutrients and oxygen. Cancer cells release chemical molecules that induce the formation of new capillary networks, also known as angiogenesis (Lv et al., 2017). Antiangiogenic therapies inhibit the growth of neoplastic cells by preventing the formation of new blood vessels (Montemagno and Pagès, 2020; Lopes-Coelho et al., 2021). Tyrosine-kinase inhibitors (TKI) are antiproliferative therapeutic agents that can also have an antiangiogenic effect (Gotink and Verheul, 2010; Lawrence et al., 2012; Ammendola et al., 2021;). The TKI toceranib phosphate (Palladia[®]) inhibits the platelet derived growth factor-, vascular endothelial growth factor- and the colony stimulating factor-1 receptor (London et al., 2012). It was first developed for veterinary patients but, recently, it has been investigated in vitro as a therapeutic agent for human hepatocellular cell carcinoma, which could pave the way for human clinical trials (Qiao et al., 2023). The TKI masitinib has already progressed fur-

ther in this trajectory and inhibits the platelet derived growth factor- and fibroblast growth factor receptor (Dubreuil et al., 2009; Humbert et al., 2009).

Masitinib

In a study by Hanh et al. (2008), masitinib proved safe and effective in delaying tumor progression in dogs diagnosed with a non-resectable, non-metastatic grade II or grade III mastocytoma. Treatment with masitinib significantly prolonged the time to progression compared to placebo (75 days versus 118 days), and an even more pronounced effect was observed when dogs received masitinib as a first-line treatment (75 days versus 178 days). This was both observed in tumors with mutant KIT as well as a wild-type form of KIT (Hanh et al., 2008). The same year, the compound received approval from the European Medicine Agency (EMA) and reached the European veterinary market as Masivet[®], registered for treatment of canine mastocytoma (Marech et al., 2014). Conditional approval in the USA was granted by the Food and Drug Administration (FDA) between 2010 and 2015.

Following its introduction in veterinary medicine, masitinib was studied as a repurposed agent in human cancer patients with advanced pancreatic ductal adenocarcinoma (PDAC). In a study by Deplanque et al. (2015), the combination of the TKI masitinib with the chemotherapeutic gemcitabine revealed to be safe and effective in the treatment of PDAC. Prolonged survival rates were observed in patients with an up-regulated expression of the genetic liquid biomarker AXOC1 and those with extreme pain. The pain intensity in patients with PDAC is related to an increased presence of mast cells (Hoogerwerf et al., 2005; Yu et al., 2019). Masitinib targets macrophages and inhibits angiogenesis, thereby reducing inflammation. In addition, masitinib has a negative effect on tumor growth by inhibition of angiogenesis and cell division (Demir et al., 2013; Mora et al., 2020; Tu et al., 2023). Masitinib obtained orphan drug designation from the FDA (2009) and EMA (2023) for the treatment of pancreatic cancer, providing a first step towards marketing authorization. The Orphan Drug Act (USA, 1983) and Orphan Medicinal Products Regulation (Europe, 2000) are used to support and encourage sponsors to develop and evaluate new treatments that can prevent, diagnose or treat rare diseases.

Masitinib has also been evaluated in human patients with gastrointestinal stromal tumors (GISTs) (Le Cesne et al., 2010; Adenis et al., 2014; Kelly et al., 2021), for which the standard treatment is imatinib (Kanda et al., 2024). Imatinib targets the c-KIT receptor and the platelet derived growth-factor receptor, from which mutations are present in most GISTs

¹ Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) in dogs and cats

² European Union: Directive 2010/63/EU; Regulation 2019/6/EU; Directive 2024/1262/EU

³ USA: Institutional Animal Care and Use Committee (NIH, USA); FDA-2023-D-2654; Animal Welfare Act 4 Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) in dogs and cats

Table 1 Clinical trials conducted in dogs with cancer followed by clinical trials in human medicine.

Therapy class	Generic name and development code	Brand name and species for which developed	Species studied	Study ID number	Trial phase	Sample size	Cancer type	Clinical trial number	References			
Antiangiogenic	Masitinib (AB110)	D: Masivet® H: Kinavet® D: Masipro®	D	N/A	phase III	Tx: 161; P: 26	MCT grade II/III	N/A	(Hahn et al., 2008)			
			H	N/A	phase III	Tx: 106; P: 26	MCT grade II/III	N/A	(Hahn et al., 2010)			
				AB07001	phase I/II	Tx: 40; P: 0	Solid tumors	NCT01506336	(Soria et al., 2009)			
				AB12004	phase II	Tx: 23; Tc: 21	GIST	NCT02009423	(Adenis et al., 2014)			
				AB04016		7 ^a		NCT00998751	*			
				AB04030		30 ^b		2008-000973-40	*			
				AB11002	phase III	450 ^c		2011-001790-41	*			
				AB04030		258 ^c		NCT01694277	*			
				AB04030		335 ^a		NCT00812240	*			
				AB07012	phase IV/III	Tx: 175; P: 187	Pancreatic cancer	NCT00789633	(Deplanque et al., 2015)			
				AB12005	phase III	Tx: 244; Tc: 135		NCT03766295	(Ezenfis and Hermine, 2021)			
			Anti-inflammatory	Piroxicam (CP16171)	H: Feldene®	D	N/A	phase III ^a	134	Melanoma	NCT01280565	(AB Science, 2019)
H	N/A	phase IV/III ^b				248	Ovarian cancer	NCT02490488	*			
	AB12010	phase II/III ^b				219	Colorectal	NCT03556956	*			
	AB04019	phase II ^b				24	Refractory multiple myeloma	NCT00866138	*			
	AB06002	phase III ^a				147		NCT01470131	*			
	N/A	phase I				Tx: 62	Multiple tumor types	N/A	(Knapp et al., 1992)			
	N/A	phase II				Tx: 34	TCC of the bladder	N/A	(Knapp et al., 1994)			
	N/A	Retrospect				Tx: 7	Anaplastic breast cancer carcinoma	N/A	(Carlos et al., 2009)			
	N/A	pilot study				Tx: 13; P: 13	TCC of the bladder	N/A	(Dhawan et al., 2010)			
	N01CN85186	phase IV/III				Tx: 76; P: 70	NMI bladder cancer	NCT00006124	(Sabichi et al., 2011)			
	2401	phase II				Tx: 58; Tc: 66	NMI bladder cancer	NCT02343614	(Pagliarulo et al., 2015)			
Inhalation Chemo therapy	5-Fluorocil ^d	H: Adrucil® H: Efidex® H: Carac® H: Taxol® H: Abraxane® H: Adriamycin® H: Rubex® H: Doxorubicin® H: Rubex® H: Doxorubicin® H: Rubex®				D	N/A	pilot study	Est. 15	MI bladder cancer	NCT02885974	*
			H	N/A	phase III	Tx: 1763; P: 876	HER-2 (i.e. ERBB2) negative breast cancer	NCT02429427	(Coombes et al., 2021)			
				N/A	pilot study	Tx: 19	Primary and metastatic lung cancer	N/A	(Tatsumura et al., 1993)			
				N/A	PoP study	Tx: 9 (DOX only) Tx: 6 (PTX only) Tx: 13 (DOX/PTX)			(Hershey et al., 1999)			
				000088	phase I	Tx: 53	Primary and metastatic lung cancer	NCT00020124	(Otterson et al., 2007)			
				99-049	phase I/II	Tx: 34 (dose level 1) Tx: 9 (dose level 2)		NCT00004930	(Otterson et al., 2010)			
				N/A	validation	Tx: 28	Solid tumors	N/A	(Fidel et al., 2015)			
				BB-001	phase I ^b	Tx: 21	Skin cancer	NCT02097875	*			
				BB-002	phase I ^b	Tx: 17	Brain cancer (glioma)	NCT02234297	*			
			Fluorescence guided surgery	Tozaleriside ^e	N/A	D	N/A	validation	Tx: 28	Solid tumors	N/A	(Fidel et al., 2015)
						H	BB-001	phase I ^b	Tx: 21	Skin cancer	NCT02097875	*

Immunotherapy	IL-2 ^d	N/A	D	→	N/A	BB-003	phase I ^f	Tx: 0	Sarcoma	NCT02464332	N/A		
			H		N/A	BB-004	phase I	Tx: 29	Pediatric CNS tumors	NCT02462629	*		
					N/A	BB-005	phase I ^b	Tx: 30	Breast cancer	NCT02496065	*		
					N/A	RG-1122110	phase I/II ^e	Est.: 15	Oral cancer	NCT05316688	*		
					N/A	IIT2020-09-Yu-BBIS1001	phase II ^e	Est.: 55	Adult CNS tumors	NCT04743310	*		
					N/A	N/A	phase II	Tx: 11	Metastatic lung Cancer	N/A	(Khanna et al., 1997)		
					N/A	N/A	phase I/II	Tx: 9	Locally advanced or metastatic sarcoma or refractory solid tumors	N/A	(Skubitz and Anderson, 2000)		
					N/A	N/A	phase II	Tx: 15	Stage-IV (M1b and M1c) melanoma	N/A	(Posch et al., 2014)		
					H. {no brand}	D	→	N/A	Melanoma	N/A	(Paoloni et al., 2015)		
					H. {no brand}	H		110225	Metastatic solid tumors	Tx: 59	(Strauss et al., 2019)		
Xenogeneic DNA vaccines	NHS-IL-12 ^d	H. {no brand}	H	→	H. {no brand}	110225	phase I	Tx: 23	Advanced solid tumors	NCT01417546	(Toney et al., 2023)		
					H. {no brand}	MS 201781_0031	phase I	Tx: 52	Locally advanced, unresectable, or metastatic solid tumors	NCT02994953	(Strauss et al., 2023)		
						200045	phase I/II	Tx: 50	HPV ¹⁶ - and HPV ¹⁸ -solid tumors resistant to ICB treatment	NCT04287868	(Floudas et al., 2025)		
						200045	phase I/II ^b	Tx: 51	HPV ¹⁶ local, advanced or metastatic cancer	NCT04287868	*		
					D. Oncepti®	D		N/A	phase I	Tx: 9 (3/dose)	Advanced malignant melanoma	N/A	(Bergman et al., 2003)
									validation	Tx: 3	Advanced malignant melanoma		(Liao et al., 2006)
									phase I prospective and control retrospect.	Tx: 58 Tc (historical): 53	Oral malignant melanoma		(Grosenbaugh et al., 2011)
									retrospect.	Tx: 58	Digit malignant melanoma		(Manley et al., 2011)
					H. {no brand}	H	→	N/A	phase I	Tx: 9 (3/dose)	Melanoma	N/A	(Wolchok et al., 2007)
					H. Cozaar®	H		07-003	phase I	Tx: 21	Melanoma	NCT00471133	(Yuan et al., 2013)
TIME modifying therapies	Losartan ^f	H. Cozaar®	D	→	N/A	N/A	phase I/II	Tx (safety data): 28 Tx (clinical data): 8	Osteosarcoma	N/A	(Regan et al., 2022)		
			H			18-2740.cc	phase I ^c	Est.: 41	Pediatric and adult relapsed/refractory osteosarcoma	NCT03900793	*		

^a Terminated due to sponsor portfolio prioritization, ^b Completed but no publications (yet) available, ^c ongoing clinical trial, ^d no development code available, ^e BLZ-100 under development, ^f study did not include patients and was withdrawn, * no published references, D: dog, H: human, MCT: mastocytoma, GIST: gastrointestinal stromal tumor, TCC: transitional cell carcinoma, NMI: non-muscle invasive, MI: muscle invasive, Adv. NSC: advanced non-small-cell, CNS: central nervous system, ICB: immune checkpoint blockade, PoP: proof-of-principle, Tx: treatment group, Tc: control group in which conventional therapy was applied, P: placebo group, Est.: estimated number of patients and N/A: not available or not found. Arrow: studies in dogs followed by studies in humans.

(Kelly et al., 2021). Most patients, however, develop imatinib resistance after 20-24 months (Serrano et al., 2019). Previous studies revealed the added value of masitinib for imatinib-resistant GISTs (Le Cesne et al., 2010; Adenis et al., 2014), but larger clinical trials are needed to confirm these results. The University Medical Centre Groningen (UMCG) is currently recruiting patients for a phase III study comparing the safety and efficacy of masitinib and imatinib in patients with GIST (EudraCT Number: 2011-001790-41), and to assess the safety and efficacy of two different doses of masitinib in the treatment of human cancer patients with GIST (EudraCT Number: 2008-000973-40). Furthermore, various other studies were initiated in human cancer patients (Table 1), but, for now, only the outcome in non-resectable metastatic melanoma patients has been described (AB Science, 2019), with the overall response rate (ORR) being 39.1% in first-line treatment and 33.3% regardless of the treatment line.

ANTI-INFLAMMATORY THERAPEUTICS

Non-steroidal, non-selective cyclooxygenase (COX) inhibitors relieve inflammation and pain by inhibiting COX1 and/or COX2; both present in normal tissues. COX-1 expression is constant and mostly found in blood vessels, interstitial cells, smooth muscle cells, platelets and mesothelial cells but only rarely found in parenchymal cells. In contrast, COX-2 expression in healthy tissue is predominantly found in parenchymal cells and only occasionally in interstitial cells, endothelial cells and smooth muscle cells (Zidar et al., 2009). COX-2 catalyzes the conversion of arachidonic acid to prostaglandin E2 (PGE2). PGE2 contributes to inflammation, neurological pain, and promotes the proliferation and metastasis of tumor cells (Mathews, 1996; Jiang and Dingleline, 2013; Pu et al., 2021). Different genetic polymorphisms in COX-2 have been associated with cancer (Menter et al., 2010). Furthermore, COX-2 is frequently expressed in various cancer types, including lung cancer (Brabender et al., 2002), liver cancer (Yang et al., 2011), gastrointestinal cancer (Venkatachala and Rajendran, 2017), breast cancer (Solanki et al., 2018), and head and neck cancer (Zhu et al., 2020).

Piroxicam

The non-steroidal agent piroxicam was developed for human patients with rheumatoid arthritis pain and osteoarthritis pain (Pitts and Proctor, 1978; Dessain et al., 1979; Zizic et al., 1985), and approved for medical use in 1979 (EMA, 1979; FDA, 1982). In 1984, piroxicam revealed to decrease tumor burden in rats with intestinal tumors (Pollard and Luckert, 1984). In a case series study in human medicine by Breaux et al. (1989), the reduction of lung metastases in hu-

man patients receiving piroxicam was described. In that study, piroxicam treatment resulted in complete remission (CR) in one patient and 'minor regressions' in five other patients. The results from that study, combined with two promising case studies obtained in veterinary medicine, led to a phase I dose escalation study (range: 0.5mg/kg-1.5mg/kg) of the single-agent piroxicam in dogs with spontaneous cancer, followed by an additional group of dogs treated with the conventional dose for pain relief (0.3mg/kg, q24h). Partial anti-tumor response (PR) was observed 8/14 patients with various epithelial tumors (Knapp et al., 1992). Thereafter, a phase II clinical trial was performed in 34 dogs with bladder transitional cell carcinoma (0.3mg/kg q24h) (Knapp et al., 1992; Knapp et al., 1994). Promising outcomes were observed in 70% of all patients (CR: 2/34 dogs, PR: 4/34 dogs, stable disease (SD) for at least 56 days: 18/34 dogs) (Knapp et al., 1994). Thereafter, clinical trials were initiated in human cancer patients with celecoxib, a drug very similar to piroxicam, but more selective towards COX-2. The tumor cell apoptosis observed in humans was similar to that seen in dogs (Dhawan et al., 2010). In a randomized phase III clinical trial by Kelly et al. (2019), celecoxib was compared with a prospective control group, in a larger cohort of human patients with translational cell carcinoma of the bladder. Results from that study revealed that celecoxib increased the time to recurrence significantly in cancer patients where the cancer had grown into the bladder wall but had not yet reached the muscle layer and/or spread to other places in the body (Kelly et al., 2019). Findings evaluated in dogs with mammary gland tumors may also inspire clinical studies in humans with similar tumor types (Carlos et al., 2009). A retrospective study by Carlos et al. (2009) revealed increased survival rates in seven dogs with inflammatory mammary tumors receiving single-agent piroxicam treatment compared to three dogs receiving intravenous chemotherapy (overall survival 128-238 days versus 6-30 days). In human patients, celecoxib was evaluated in HER-2 (i.e. ERBB2) negative early-stage breast cancer patients with risk of relapse after conventional therapy protocols (Coombes et al., 2021). The treatment cohort celecoxib (n=1763) was prospectively compared with a placebo group (n=876). In that study, the treatment effect of celecoxib was examined between subgroups defined by menopausal status (premenopausal, perimenopausal, premenopausal versus postmenopausal), nodal involvement, and chemotherapy use (neoadjuvant versus adjuvant versus none). No significant differences were observed between all groups (hazard ratio: 0.97 (P = 0.75), confidence interval (0.8-1.17) (Coombes et al., 2021). However, in 665 patients who did not receive chemotherapy prior to exposure to celecoxib, the hazard ratio of tumor recurrence reduced by 35% (hazard ratio: 0.65 (P=0.035), confidence interval (0.41-1.04)) (Coombes et al., 2021; Harris and Schwartzbaum, 2022). ERBB2-negative breast can-

cer is often associated with significant lower amounts of pro-inflammatory cytokines than observed in ERBB2-positive breast cancer patients and healthy women (Muraro et al., 2011). Indeed, patients who may benefit most from anti-inflammatory compounds such as celecoxib are those of whom the breast cancer has a pronounced inflammatory infiltrate (Coombes et al., 2021). These results underscore the importance of further testing single-agent celecoxib treatment in patients with early-stage breast cancer who were not previously treated by chemotherapy (Coombes et al., 2021; Harris and Schwartzbaum, 2022), and in patients with inflammatory breast cancer (i.e. ERBB2-positive breast cancer).

CHEMOTHERAPY

Inhalation chemotherapy

While most chemotherapy protocols with chemotherapeutics in dogs are derived from clinical protocols in human medicine, the concept of inhalation chemotherapy was first applied in dogs (Shevchenko and Resnik, 1968; Otterson et al., 2007). Proof-of-concept studies were conducted in patients with primary or metastatic lung cancer with the chemotherapeutic 5-fluorouracil (5FU), and doxorubicin (DOX) or paclitaxel (PTX) (Tatsumura et al., 1993; Hershey et al., 1999). The inhalation studies with 5FU, DOX and PTX focused on the therapeutic benefit of inhalation chemotherapy and the mechanisms behind it. The doses applied were based on the dosing of intravenous chemotherapy with the same compounds (Tatsumura et al., 1993; Hershey et al., 1999). No adverse effects were observed with 5FU and PTX (Tatsumura et al., 1993; Hershey et al., 1999). Pneumonitis and fibrosis were observed in 3/28 dogs receiving DOX. However, the dose of DOX was not adjusted per body surface area in these dogs (Hershey et al., 1999). Patients with inoperable epithelial lung cancer treated by 5FU, received a mixture of 125mg 5FU and 1-2ml bromhexine hydrochloride vaporized by a supersonic nebulizer, twice a day, two to three days per week. The response rate was determined based on tumor volume. Promising results were obtained with 5FU during this treatment period (CR: 2/10 dogs, PR: 4/10 dogs) (Tatsumura et al., 1993). Patients treated by DOX or PTX received newly developed inhalation solutions of either PTX or DOX, once every two weeks. In these patients, response to therapy was measured upon radiographic interpretation of the tumor nodule number and volume, taken before each treatment event. A total of six biweekly therapies were scheduled per patient unless additional therapy was warranted. Alterations in compounds were offered when dogs failed to achieve a response after two consecutive treatments with a particular agent (Hershey et al., 1999). In contrast to 5FU, efficacy was low with DOX (CR: 0/18 dogs, PR: 4/18 dogs) and PTX (CR: 1/15 dogs, PR: 1/15

dogs). Tumor types for which response with DOX or PTX has been demonstrated, included osteosarcoma (n=3), liposarcoma (n=1) and undifferentiated sarcoma (n=1). However, the authors did not specify to which treatment group these patients belonged. For two other cases, the authors revealed that in one dog with mammary carcinoma pulmonary metastases, a 47% reduction in tumor volume was observed with PTX (Hershey et al., 1999). Furthermore, one dog with widespread metastatic liposarcoma, which previously did not have any benefit from DOX, experienced long-term stabilization of disease on PTX inhalation therapy (495 days). The reported feasibility of inhalation chemotherapy in dogs led to a phase I dose-escalation study in humans with metastatic lung cancer. The human study included DOX because of its broad anti-tumor efficacy (Otterson et al., 2007), although promising results in veterinary medicine were mainly obtained with 5FU (Tatsumura et al., 1993; Hershey et al., 1999). No drug-related systemic toxicity was observed with DOX doses ≤ 7.5 mg/m² in patients with primary or metastatic lung cancer (Otterson et al., 2007). However, in a phase I/II study on DOX inhalation therapy (6mg/m²) by Otterson et al. (2010), it was suggested not to move forward with DOX due to logistic and safety challenges as well as lack of efficacy.

FLUORESCENCE-GUIDED SURGERY

Clinical trials evaluating fluorescence-guided surgery (FGS) aim to determine whether novel fluorescent ligands can enhance surgeons' ability to accurately identify tumor margins and detect disseminated cancer cells. For FGS, an intravenous fluorescent contrast agent is used that is expected to accumulate at the level of the tumor. The ultimate aim of FGS is guiding surgeons in intraoperative decision-making during intraoperative tumor margin assessment and/or enabling the localization of metastatic disease (Hernot et al., 2019; Stewart and Birch, 2021).

Contrast agent BLZ-100

Targeting contrast agents consist of a ligand (i.e. monoclonal antibody, nanobody or peptide) conjugated to a fluorescent dye. The ligands bind proteins overexpressed on tumor cells. The aim of targeting contrast agents is to provide surgeons with more accurate tumor delineation for intraoperative decisions compared to non-targeting fluorescent dyes (Debie and Hernot, 2019; Hernot et al., 2019).

BLZ-100 (thozuleristide) consists of a synthetic chlorotoxin derived from the venom of a scorpion, conjugated to the non-targeting FDA/EMA approved contrast agent indocyanine green (Lyons et al., 2002; Fidel et al., 2015). The natural chlorotoxin binds tumors on cholesterol-rich lipid rafts, such as annexin A2 and metalloproteinase 2 (MMP2) (Yamada et al.,

2021). In 2015, results were published from a preclinical study on toxicity in mice, and preliminary results on safety, drug-target efficacy and tumor-to-background ratio in a clinical setting in dogs with various solid tumors (Fidel et al., 2015). A phase II/III clinical trial in human cancer patients with oral squamous cell carcinoma is currently ongoing and is estimated to finalize in 2027 (NCT05316688).

IMMUNOTHERAPY

Besides surgery, chemotherapy and radiotherapy, immunotherapy has emerged as a fourth crucial pillar in combating cancer. In immunotherapy, the patient's own immune system is utilized to fight off cancerous cells, and thereby it offers a natural treatment approach for metastatic cancer patients (Ling et al., 2022). To apply immunotherapy, monoclonal antibodies, checkpoint inhibitors, cytokines or small molecules are used. Additionally, vaccines can be made from the patient's own tumor cells (i.e. allogeneic vaccines) or from another individual from the same species (i.e. xenogeneic vaccines). Furthermore, it has recently become possible to engineer synthetic receptors that function to redirect lymphocytes, most commonly T-cells, to recognize and eliminate cells expressing a specific target antigen (i.e. CAR-T therapy) (Sternier and Sternier, 2021). The final aim of immunotherapy is to boost the natural immune system's ability to eliminate malignant cancer cells (Zhang and Zhang, 2020; Verma et al., 2023).

Cytokine IL-2

In a combined safety and efficacy study by Khanna et al. (1997), human recombinant IL-2 liposome inhalation therapy was initiated in eleven dogs with pulmonary metastases (n=8), primary lung carcinoma (n=2), and primary oral melanoma (n=1) after a safety study in purpose-bred dogs. In dogs with cancer, only minimal toxicity was observed limited to mild coughing immediately after inhalation; no allergic reactions or changes in pulmonary function were associated with the therapy (Khanna et al., 1997; Khanna and Vail, 2005). Complete regression of all metastatic disease was observed in 2/11 dogs with metastatic osteosarcoma after twice-daily inhalation up to 15 and 30 days, respectively (SD for >420 days and >700 days, respectively). Furthermore, SD was observed in one dog with a primary lung carcinoma (280 days) and in one dog with a primary oral melanoma (>180 days) (Khanna et al., 1997). The clinical study in dogs led to a phase I inhalation therapy study with IL-2 liposomes in human patients with lung metastases (Skubit and Anderson, 2000; Khanna and Vail, 2003;). A phase II study by Posch et al. (2024) was initiated in melanoma patients, either as treatment for lung me-

tastases without surgery, or as postsurgical treatment to prevent recurrence of lung metastases after surgical removal. No significant results were obtained due to the small number of patients in this study (n=15). Two patients dropped out before the first evaluation. However, 9/15 patients showed clinical response (PR: 4/15, SD for >7 months: 5/13, progressive disease: 4/15 patients). In these nine patients, IL-2 inhalation therapy reduced the size of pulmonary melanoma metastases with unresectable disease and none of the patients showed recurrence of lung metastases during the treatment period (median 24.5 months). The authors concluded that inhalation therapy with IL-2 might offer an effective treatment option for patients with unresectable melanoma lung metastases (Posch et al., 2014).

Cytokine NHS-IL12

The cytokine NHS-IL12 was generated by conjugating two IL-12 heterodimers to the monoclonal human IgG1 antibody NHS76. The NHS76 antibody binds exposed DNA/Histone complexes often exposed in necrotic regions of tumors, thereby directing IL-12 to these regions (Paoloni et al., 2015). A phase I dose-escalation study in canine patients with malignant melanoma defined a narrow therapeutic (0.8 mg/m²) window for subcutaneous systemic injection of NHS-IL12; higher doses resulted in grade 4 or grade 5 adverse effects⁴. Promising signs of therapeutic activity during the dose-escalation study were used to inform the design of a phase I clinical trial in human cancer patients (Paoloni et al., 2015). The safety study in humans with metastatic solid tumors was initiated, based on the strong genetic similarity between canine- and human IL-12 (Strauss et al., 2019; Pantelyushin et al., 2021). Subsequently, an exploratory study by Toney et al. (2023) was published on the optimal treatment regimen for immune activation. Stronger immune activation was observed when NHS-IL12 was administered every two weeks rather than every four weeks (Toney et al., 2023). In another phase I study, the combination of NHS-IL12 with the checkpoint inhibitor avelumab was evaluated in patients with advanced solid tumors (Strauss et al., 2023), whereas in a non-randomized phase I/II study, the combination of NHS-IL12 with avelumab and the human papillomavirus HPV vaccine (PDS0101) was evaluated. The triple combination revealed notable response rates in patients with ICB-naïve or ICB-resistant HPV16⁺ tumors (Floudas et al., 2025) and has led to a phase II trial that is estimated to be finalized in 2026 (NCT04287868).

Xenogeneic DNA vaccines

Oncept[®] is a xenogeneic human tyrosinase DNA vaccine directed towards the tyrosinase-antigen, pres-

⁴ Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) in dogs and cats

ent on canine melanoma tumor cells. Because of the similar homology between canine and human tyrosinase, Oncept[®] can target canine tyrosinase and induce an immune response towards oral melanoma cells (Liao et al., 2006). After Oncept[®] was evaluated in a combined safety and preliminary clinical efficacy trial in dogs (Bergman et al., 2003), it received approval from the United States Department of Agriculture in 2009, as the first DNA vaccine for the treatment of dogs with stage II and III oral malignant melanoma (Bergman et al., 2003; Grosenbaugh et al., 2011; Pellin, 2022). Before the human tyrosinase vaccine was commercially available, the safety of murine xenogeneic tyrosinase DNA vaccine was investigated in a clinical trial in 58 dogs with malignant melanoma of the digit. A retrospective study was published after Oncept[®] became commercially available. The study revealed the murine xenogeneic tyrosinase vaccine was safe in dogs with digit melanoma (Manley et al., 2011). A phase I safety and immunogenicity trial with xenogeneic murine tyrosinase and human tyrosinase DNA vaccines was initiated in human cancer patients with melanoma. Intramuscular (IM) injection was well tolerated in all patients with transient grade 1 injection site reactions (Wolchok et al., 2007). That study was followed up by another phase I study evaluating the safety and efficacy of *in vivo* electroporation to enhance IM delivery of the xenogeneic murine tyrosinase DNA vaccine. The vaccine could be safely administered via *in vivo* electroporation to melanoma patients. Tyrosine-reactive CD8⁺ T-cell responses were observed in 6/15 patients receiving 5 immunizations in a 1.5mg dose cohort (Yuan et al., 2013). Another study was initiated in 40 patients with stage III/IV melanoma focusing on the safety and efficacy of a poly-epitopic xenogeneic DNA cancer vaccine prepared from murine B16 and LLC murine melanoma cell lysates (Seledtsov et al., 2006). Delayed hypersensitivity skin reactions showed a remarkable level of immune-reactivity, especially toward B16 melanoma antigens. A longer median overall survival was observed among xeno-vaccinated patients compared to historical controls, and patients with stronger hypersensitive skin reactions had better clinical outcomes (Seledtsov et al., 2006; Shariati et al., 2024). However, the attention has shifted toward vaccines encoding chimeric antigens since few studies have shown the immune response against DNA vaccines encoding xenogeneic antigens to be robust enough to significantly alter the clinical course of cancer patients (Shariati et al., 2024).

Tumor microenvironment modifying immunotherapies

The compound losartan was initially developed as blood pressure medication for humans but also has an effect on the tumor microenvironment (TME) (Byyny,

1995; Ammons et al., 2022;). Losartan prevents monocyte recruitment to the TME and suppresses tumor growth (Datta et al., 2017; Ammons et al., 2022). Recently, a clinical trial in dogs has revealed that a combination of high dose losartan with the veterinary TKI toceranib phosphate provided better clinical outcomes than toceranib alone in dogs with metastatic osteosarcoma (Regan et al., 2022). However, it is noteworthy that a ten-fold higher dose of losartan was needed to induce its anti-cancer effects compared to its use as a blood-pressure agent. A phase I/b clinical trial investigating the optimal dose of losartan combined with the human sister analogue of toceranib phosphate, sunitinib, is currently open for human cancer patients with osteosarcoma and is estimated to finalize in 2027 (NCT03900793).

DISCUSSION

In this non-exhaustive literature review, an overview is provided of published (proof-of-concept) studies initiated in client-owned dogs with spontaneous cancer, which provided added value to initiate clinical trials in human cancer patients. The results of these studies provide evidence of the importance of comparative oncology trials for human medicine.

At the Faculty of Veterinary Medicine (Ghent University), various clinical trials in dogs with spontaneous cancer have also been initiated with the ultimate aim to start clinical trials in human medicine, but have not yet reached this stage: IL-12 electrogene therapy (EG) (Celsion-EGEN, Inc, Huntsville, AL, USA) revealed to be immunostimulatory and anti-angiogenic in nature but did not provide persistent tumor regression (Cicchelero, et al., 2017). Combined IL-12 EG and metronomic chemotherapy with cyclophosphamide resulted in enhanced anti-angiogenic effects and slower tumor progression with improved quality of life, compared to IL-12 EG alone (Cicchelero et al., 2017). Trials at the Faculty of Veterinary Medicine (Ghent University) focusing on novel targeting agents for FGS demonstrated that the targeting contrast agent DA364 (Bracco Imaging SpA, Colliere Giacosa, Italy) has potential to reveal residual disease; however, larger clinical trials are necessary to evaluate the sensitivity and specificity in cancer detection (Favril et al., 2020). Bracco Imaging is currently further investigating the compound. An ongoing FGS study includes the use of a humanized anti-EGFR nanobody 7D12 conjugated to the near-infrared dye s775z for intraoperative tumor delineation in dogs with EGFR-expressing tumors after a promising safety study in dogs (do Valle et al., 2023). The applicability of the compound for *ex vivo* margin assessment is being evaluated in both human and canine tumor specimens (KOTK⁵). Furthermore, during FGS, a focus is set on novel imaging techniques such as fluorescence

⁵ https://www.komoptegenkanker.be/projecten?contains=dog&type=222&cancer_type=All®ion=Al

lifetime (FLT) imaging. A clinical FLT imaging trial in pets with spontaneous cancer (dogs and cats) was started in early 2025, with the non-targeting contrast agent Indocyanine Green, and a novel macroscopic imaging system (VUB *tauCAM*TM), developed by the Department of Electronics and Informatics (Vrije Universiteit Brussel) (ETRO.RDI) (FWO⁶). Other ongoing research studies at the Faculty of Veterinary Medicine (Ghent University) involve the search for common canine hemangiosarcoma and human angiosarcoma biomarkers (FWO), the evaluation of novel treatments for acute lymphoblastic leukemia in dogs with lymphoma (KOTK), and another comparative study to evaluate a novel drug to prevent lung metastases in triple negative breast cancer by studying cats with high-grade mammary adenocarcinoma (KOTK).

CONCLUSION

By conducting clinical trials in pets prior to initiating clinical trials in humans, information can be acquired on which cancer type appears to be the most responsive to a novel drug, to which extent a novel therapeutic agent kills cancer cells or slows down cancer growth, or how well the agent can create contrast between cancer cells and surrounding healthy tissue during surgery. Furthermore, clinical trials in pet dogs can give insight into whether the compound ensures

a longer life expectancy with increased quality of life (Milevoj et al., 2020). These results can be acquired in a shorter time and with lower costs due to different regulatory frameworks in veterinary- and human medicine (Füüdös et al., 2015). While standardizing protocols across veterinary and human clinical trials can be challenging due to these differences, conducting clinical research in pets with cancer holds great potential for informing the practice and progress in both veterinary- and human medicine (Webb et al., 2024).

Ongoing clinical trials for veterinary cancer patients in Europe can be found on the Oncowaf website (<https://oncowaf.be/en/ClinicalTrials/searchResults>), whereas an overview of veterinary clinical trials in the USA can be found on the website of the National Cancer Institute (<https://ccr.cancer.gov/comparative-oncology-program/trials>).

REFERENCES

A complete reference list can be obtained from the authors.



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⁶ <https://www.fwo.be/nl/>

Smaken verschillen: kat en hond

Mully Gatowny had een kat
Die beschuit met muizen vrat
Vleer- of veld maakte niks uit
Het ging alleen om de beschuit

Mully Gatowny's laatste duit
Gaffie aan beschuiten uit.
Toen zaidie dattie 't welletjes vond,
Verzoop de kat en kocht een hond

En die vrat alles wattie vond,
Tot platgetreden paardenstront
Paardenkeutels, paardenvijgen,
Hij kon er niet genoeg van krijgen.

Fragment uit Mully Gatowny in 'Literary Larycook in Dutch and Doubledutch'
1977. John O'Mill, Andries Blitz, Amersfoort.