Receptor Tyrosine Kinases and Cancer in Dogs

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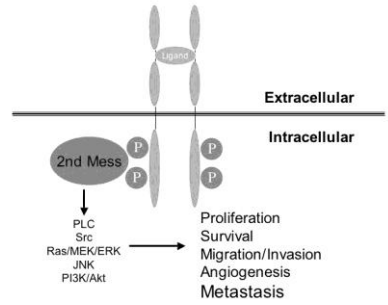
Cancer is a disease characterized by dysregulated growth, abridged cell death, and enhanced cell migration, invasion and angiogenesis. While the molecular mechanisms responsible for this phenotype are very diverse, one class of molecule that has been receiving a great deal of recent attention as a target for therapy are the receptor tyrosine kinases (RTKs). These are cellular receptors for extracellular growth factors that allow communication from the extracellular milieu to the cell interior, mediating functions such as growth, survival, invasion and angiogenesis. Multiple RTKs are expressed by almost all tumor cells, but they can play variable roles in the pathogenesis of the disease.

Most RTKs exist as monomers, which dimerize upon contact with the appropriate ligand, inducing a conformational shift that allows phosphorylation of tyrosine residues in the intracellular domain. This then triggers several different intracellular second messenger cascades, culminating in altered gene expression and, often, a more malignant phenotype (Fig. 1).

Figure 1. Diagram of a generic receptor tyrosine kinase



There are several new cancer drugs currently available on the human market that act by inhibiting signaling through one or more RTKs, and many more are in late-stage clinical development (see below). In addition to their potential for off-label use in veterinary cancer patients, RTK inhibitors specifically labeled for use in veterinary patients are now available. Thus, an understanding of this class of protein and the drugs designed to inhibit RTK signaling will be critical as veterinary oncology advances in the next decade.

RTK activation

Under most normal circumstances, activation of RTKs occurs only when the receptor encounters the appropriate ligand in the extracellular milieu. However, there are a variety of mechanisms by which RTKs can be inappropriately activated. These include: (1) Production of the ligand by the tumor cells themselves (autocrine stimulation); (2) Overexpression of the RTK, leading to spontaneous dimerization; (3) Mutations in the RTK, leading to constitutive activation in the absence of bound ligand. Any of these alterations can result in inappropriate signaling through the receptor, leading to an enhanced oncogenic phenotype.

RTK inhibition and human cancer

One of the first tyrosine kinases to be definitively implicated in human tumorigenesis and successfully inhibited was the ***bcr-abl*** fusion protein (the product of the so-called Philadelphia chromosome translocation), present in many cases of chronic myelogenous leukemia (CML). This fusion protein fuses the activation domain of the Bcr protein to the kinase domain of the Abl protein, rendering it active and capable of signaling at all times, providing an unending stimulus for cell proliferation and protection against cell death (apoptosis). Given the dependence on this fusion kinase for proliferation and survival in CML cells, an effort was made to find a drug capable of specifically inhibiting this signaling. This culminated in the discovery of a small molecule called STI571 (also called imatinib mesylate or Gleevec). This molecule was capable of potently inhibiting signaling through the Abl kinase, resulting in diminished CML cell proliferation and enhanced cell death. This molecule showed excellent antitumor activity and a good safety profile in humans with CML, and is currently approved for that indication.

Since the successful approval of imatinib, many additional RTK inhibitors have been approved for use in human cancer **(Table 1)**.

Table 1. RTK inhibitors approved for human use

Name	Class	Targets	Indication
Imatinib (Gleevec)	Small molecule	Bcr-Abl, Kit, PDGFR	Chronic myelogenous leukemia (CML), gastrointestinal stromal tumor (GIST)
Trastuzumab (Herceptin)	Monoclonal antibody	HER2 (erbb2)	Breast cancer
Gefitinib (Iressa) Erlotinib (Tarceva)	Small molecules	EGFR	Lung cancer
Cetuximab (Erbitux)	Monoclonal antibody	EGFR	Colorectal cancer
Bevacizumab (Avastin)	Monoclonal antibody	VEGFR2	Colorectal cancer
Sorafenib (Nexavar)	Small molecule	Kit, FLT3, VEGFR2/3, PDGFR	Renal cell carcinoma (RCC)
Sunitinib (Sutent)	Small molecule	Kit, FLT3, VEGFR1-3, PDGFR, CSF-1R, RET	RCC, GIST
Dasatinib (Sprycel)	Small molecule	Bcr-Abl, Src, KIT, EPHA2, PDGFR	CML, ALL
Nilotinib (Tasigna)	Small molecule	Bcr-Abl, Kit, PDGFR	CML
Lapatinib (Tykerb)	Small molecule	EGFR, HER2	Breast cancer
Panitumumab (Vectibix)	Monoclonal antibody	EGFR	Colorectal cancer
Pazopanib (Votrient)	Small molecule	VEGFR1-3, PDGFR, KIT	Renal cell carcinoma
Axitinib (Inlyta)	Small molecule	VEGFR1-3, PDGFR, KIT	Renal cell carcinoma
Crizotinib (Xalkori)	Small molecule	ALK / Met	Lung cancer with ALK mutation
Pegaptanib (Macugen)	RNA aptamer	VEGF	Age-related macular degeneration (AMD)
Ranibizumab (Lucentis)	Monoclonal antibody fragment	VEGF	AMD
Ruxolitinib (Jakafi)	Small molecule	JAK	Myelofibrosis
Vemurafenib (Zelboraf)	Small molecule	BRAF	Melanoma

Rtks and angiogenesis

There are a group of RTKs that mediate the growth of new blood vessels (angiogenesis) in tumors. These include receptors for vascular endothelial growth factor (VEGF), basic fibroblast growth factor, the angiopoietins, and others. As in tumor cells themselves, signaling through these RTKs are of prime importance in the growth and maintenance of tumor vasculature, and therapies designed to inhibit signaling through these angiogenic growth factor receptors (e.g. bevacizumab, sorafenib, sunitinib) are showing great promise in human clinical trials, and veterinary clinical trials using toceranib (Palladia), which targets VEGF receptor as well as KIT (see below), have been completed.

RTKs and veterinary cancer

Feline vaccine-associated sarcoma and PDGFR

Recent work has demonstrated that a majority of feline vaccine-associated sarcomas (VAS) express the RTK **PDGFR**, the receptor for platelet-derived growth factor (PDGF). Additionally, PDGF stimulates the proliferation of feline VAS cells in culture and protects VAS cells from the antiproliferative and pro-apoptotic effects of doxorubicin. Furthermore, inhibition of PDGFR signaling with imatinib eliminates the oncogenic effects of PDGF and inhibits tumor growth in a nude mouse model of VAS. Therapy with imatinib has been pursued in a small number of tumor-bearing cats to date, and it appears to be tolerated at doses approaching those associated with antitumor activity in humans.

RTKs and canine osteosarcoma

A variety of RTKs have been identified in canine osteosarcoma (OSA) cells, including PDGFR, HER2 (one of several receptors for epidermal growth factor), IGF-1R (the receptor for insulin-like growth factor 1), and MET (the receptor for hepatocyte growth factor). IGF-1R and MET have been shown to mediate important oncogenic functions such as proliferation, anti-apoptosis, invasion, motility and chemoresistance in canine OSA cells, and interestingly, constitutive activation of the MET receptor has been demonstrated in canine OSA as well. Furthermore, MET activity can be inhibited in canine OSA and other canine tumor cells by a small molecule *in vitro*. A randomized, placebo controlled trial in dogs with OSA using a somatostatin analog designed to reduce IGF-1 levels demonstrated no difference between dogs receiving chemotherapy and somatostatin analog and dogs receiving chemotherapy and

placebo, however serum IGF-1 concentrations were reduced by less than half in the treated dogs. It is quite possible that more potent inhibition might result in different results.

A more complete list of RTKs identified in veterinary cancer and levels of evidence for their utility as targets for therapy is presented in Table 2.

Table 2. Receptor Tyrosine Kinase Expression, Function and Inhibition in Veterinary Cancer

RTK	Ligand	Tumor Type	Level of Evidence
KIT	Stem Cell Factor	Canine mast cell tumor, canine hemangiosarcoma	Expressed on most MCT and HSA: ~30% of MCT carry a functional activating mutation. Small molecules capable of inhibiting signaling and antitumor effects *in vitro* and in patients have been identified.
		Feline mast cell tumor	Expressed on most MCT. Some functional mutations identified, and some clinical responses documented to inhibitors.
MET	Hepatocyte Growth Factor/Scatter Factor	Multiple canine tumors, including osteosarcoma, melanoma, histiocytic sarcoma, MCT	Expressed in most tumors of the histotypes examined. Constitutive activation detected in osteosarcoma. Receptor is functional and signaling inhibitable *in vitro* with small molecules.
IGF- 1R	Insulin-like Growth Factor-1	Canine osteosarcoma, melanoma	Expressed in most tumors of the histotypes examined. Receptor is functional and signaling inhibitable *in vitro* with small molecules.
EGFR	Epidermal Growth Factor, Transforming Growth Factor-α, Others	Canine mammary, lung, transitional cell, and nasal carcinoma, feline oral SCC	Expression documented most tumors of the histotypes examined. Receptor is functional in canine mammary carcinoma and signaling inhibitable *in vitro* with small molecules.
HER2	Epidermal Growth Factor, Transforming Growth Factor-α, Others	Canine and feline mammary carcinoma, canine osteosarcoma	Expressed in a large minority of canine and feline mammary tumors, appears overexpressed in some canine osteosarcomas. Coreceptor for EGFR so may contribute to biologic effects of EGF in mammary carcinoma.
PDGFR	Platelet-derived growth factor	Canine osteosarcoma and hemangiosarcoma, feline vaccine-associated sarcoma	Expressed in canine osteosarcoma. Expressed, functional and inhibitable with small molecules *in vitro* and in mouse models of vaccine-associated sarcoma.
VEGFR2	Vascular endothelial growth factor	Canine hemangiosarcoma, melanoma, nasal carcinoma Tumor vasculature	Expressed in some melanoma and nasal carcinomas and most HSA evaluated. Some evidence of functionality in canine HSA cells. Increased concentrations of ligand detected in serum of HSA patients.
FGFR1 and 2	Basic fibroblast growth factor	Canine hemangiosarcoma Tumor vasculature	Expressed in most HSA evaluated. Functional in canine HSA cells. Increased concentrations of ligand in urine of HSA patients.
TrkA	Nerve growth factor	Canine osteosarcoma	Expressed in most OSA. Blockade of TrkA induces apoptosis.

Canine mast cell tumor and KIT

The majority of canine (and human) mast cell neoplasms express the tyrosine kinase growth factor receptor KIT, and a large minority of canine MCT (20-50% depending on the study) possess a mutation in the *c-kit* gene coding for the KIT protein. (KIT expression and *c-kit* mutations have also been identified in feline MCT recently). This gene codes for a transmembrane protein that serves as the receptor for the growth factor stem cell factor, important in the maturation of normal mast cells and other hematopoietic cells. Mutations can render KIT active even in the absence of bound stem cell factor. In other words, these mutations mean that the cells are receiving signals to proliferate and survive when they normally would not, leading to unchecked growth. New molecules have been developed that inhibit signaling through the KIT tyrosine kinase, and these compounds are able to interfere with the proliferation of

canine MCT *in vitro*. The 2 veterinary-approved molecules in this class are toceranib (Palladia, Pfizer) and masitinib (Masivet/Kinavet, AB Science).

Following encouraging *in vitro* and early-phase clinical studies with toceranib, a multi-center, placebo-controlled, double-blind, randomized study of toceranib was performed in dogs with recurrent or metastatic grade II or III MCT. Dogs were randomized to receive oral toceranib 3.25 mg/kg or placebo every other day for 6 weeks in the blinded phase. Thereafter, eligible dogs received open-label toceranib. The overall response rate in toceranib-treated dogs (n = 86) was 37.2% (7 complete response, 25 partial response) versus 7.9% (5 partial response) in placebo-treated dogs. Among the toceranib treated responders, the median duration of objective response and time to tumor progression was 12.0 weeks and 18.1 weeks, respectively. Interestingly, dogs whose MCT harbored activating mutations in the *c-kit* gene were roughly twice as likely to respond to toceranib than those with wild-type *c-kit* (60% vs 30%). The efficacy observed in this study led to the full approval of toceranib by the U.S. FDA.

Subsequent to FDA approval, considerable clinical experience with toceranib has been amassed by U.S. oncologists. Important observations in this post-approval phase include the high incidence of gastrointestinal toxicity in dogs treated with the label-indicated dosage and schedule: most U.S. oncologists currently utilize a dose of 2.75 mg/kg every-other-day or Monday-Wednesday-Friday, which appears well tolerated by the majority of dogs. Gastrointestinal toxicity, in the form of inappetance, weight loss, diarrhea, and occasionally vomiting or melena, are the most common adverse effects, and are generally manageable with symptomatic therapy, drug holidays and dosage reductions as necessary. Other adverse effects reported include mild to moderate leucopenia, and occasional muscle pain or leukotrichia.

A clinical trial of similar design was recently completed with masitinib in dogs with recurrent or unresectable MCT. Masitinib was administered at a dose of 12.5 mg/kg daily. This study demonstrated significantly improved time to progression in masitinib-treated versus placebo-treated dogs, and again, response rate and outcome was improved in dogs with MCT harboring *c-kit* mutations. Gastrointestinal adverse effects (vomiting or diarrhea) were most common but were mild in the vast majority of cases (usually grade 1 or 2) and self-limiting. Myelosuppression can also occur, particularly neutropenia, although in most cases this is mild. A small percentage of dogs developed a protein-losing nephropathy leading to edema. Increases in urea and creatinine were seen in some dogs, although this was not clear if it was drug-related or not – however caution is recommended when using this drug in patients with pre-existing renal disease. Hemolytic anemia was also seen as a rare occurrence in the study.

Practical monitoring of patients on RTKIs

1. Clinicians and owners should be vigilant to monitor patients for adverse effects, as described above. Monitoring of complete blood counts, serum biochemistry profiles and urinalysis is suggested at baseline, after 2 weeks of therapy and monthly thereafter. Dose reductions, drug holidays or supportive care, in the form of gut protectants, may be indicated.

A variety of unanswered questions exist regarding the use of RTK inhibitors such as toceranib and masitinib for the treatment of animal cancer. These include: (1) Can they be given to cats safely? The answer to this appears to be yes, but evidence of efficacy is limited to a small number of cats that have been treated with imatinib. (2) Are there other tumors where they might be efficacious? Anecdotal or early evidence of efficacy has been reported for toceranib for the treatment of diverse diseases including multiple myeloma, soft-tissue sarcoma, metastatic osteosarcoma and carcinomas of the mammary gland, thyroid and anal sac; similarly, there are reports of antitumor responses to masitinib in dogs with T-cell lymphoma. In these other tumor types, efficacy may be as a result of the targeting of other RTKs important in cancer, such as PDGFR or VEGFR2. (3) Are they effective for the postoperative treatment of incompletely resected or "high-risk" MCT? This has not been studied. Further complicating this question, it is not at all clear how long these drugs should be continued in the postoperative setting. (4) Can they be used together with chemotherapy or radiation therapy? This is not clear, however preliminary evidence suggests that toceranib can be combined safely with coarsely fractionated (palliative) radiation therapy, with encouraging antitumor activity. Early reports suggest additive toxicity when toceranib is combined with traditional cytotoxic chemotherapeutic agents such as VBL or CCNU, which suggest that significant dosage reductions of the cytotoxic agent may be necessary if the drugs are to be safely combined. Early abstracts suggest that masitinib/chemotherapy combinations (doxorubicin/carboplatin) may be tolerated at chemotherapy doses approaching the maximum tolerated dose. There is also interest in investigating the combination of RTKIs and low dose continuous ("metronomic") chemotherapy.

In conclusion, receptor tyrosine kinases represent an intriguing and potentially very powerful target for therapy in canine and feline malignancy. New drugs targeting these RTKs are being introduced at an ever-increasing pace, and it is virtually assured that this exciting new form of treatment will find an important place alongside surgery, radiation therapy and chemotherapy in the veterinary cancer clinician's armamentarium.

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