Tyrosine Kinase Inhibitors in Veterinary Medicine

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Substantial progress in the field of molecular biology has permitted the identification of key abnormalities in cancer cells involving cell proteins that regulate signal transduction, cell survival, and cell proliferation. Such abnormalities often involve a class of proteins called tyrosine kinases that act to phosphorylate other proteins in the cell, tightly regulating a variety of cellular processes. A variety of small molecule inhibitors that target specific tyrosine kinases (known as tyrosine kinase inhibitors [TKIs]) have now been approved for the treatment of human cancer, and it is likely many more will become available in the near future. In some instances these inhibitors have exhibited significant clinical efficacy, and it is likely their biologic activity will be further enhanced as combination regimens with standard treatment modalities are explored. Although TKIs have been used extensively in humans, their application to cancers in dogs and cats is relatively recent. The TKIs Palladia (toceranib), Kinavet (masitinib), and Gleevec (imatinib) have been successfully used in dogs, and more recently Gleevec in cats. This article will review the biology of tyrosine kinase dysfunction in human and animal cancers, and the application of specific TKIs to veterinary cancer patients.

Keywords: cancer, inhibitor, kinase, target, therapy

Tyrosine kinases are proteins that phosphorylate other proteins on tyrosine residues. They are key players in normal cell signal transduction, acting to tightly regulate cell growth and differentiation. Tyrosine kinases bind adenosine triphosphate (ATP) and use this to add phosphate groups to key residues on themselves (termed "autophosphorylation") and on other molecules, resulting in the generation of intracellular signaling, ultimately leading to alterations in gene transcription that impact cell proliferation and survival (reviewed in[1]). This process is usually initiated in response to external signals generated from growth factors or other stimuli that begin the cascade of tyrosine phosphorylation. Protein kinases may be located at the cell surface, in the cytoplasm, or in the nucleus.

Those tyrosine kinases expressed on the cell surface often bind growth factors that regulate their activation. They are usually present as monomers on the cell surface and growth factor binding results in dimerization and autophosphorylation followed by initiation of kinase activity and phosphorylation of signaling intermediates.[2,3] Examples of receptor tyrosine kinases include Kit, Met, Axl, and epithelial growth factor receptor (EGFR), all of which are known to be dysregulated in particular forms of cancer.[4-7] In addition to regulating normal cell function, certain tyrosine kinase receptors are important in promoting the growth of tumor blood vessels, also known as tumor angiogenesis. These include vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and Tie1/2.[8-11] VEGFRs are expressed on vascular endothelium, and VEGF-VEGFR interactions are important for endothelial migration and proliferation.[8] PDGF and PDGFR are expressed in stroma and pericytes, and PDGF can promote angiogenesis.[10,11] Lastly, fibroblast growth factor is synergistic with VEGF to induce the expression of VEGF, thereby enhancing the process of angiogenesis.[10] With respect to the cytoplasmic kinases, many are not tyrosine kinases; rather, they are serine-threonine kinases that work similarly but phosphorylate other proteins on serine and threonine. Cytoplasmic tyrosine kinases include Src family members and Abl, among others.[12,13]

Dysregulation of Tyrosine Kinases in Cancer

An extensive characterization of the activation status of tyrosine kinases has been undertaken in human malignancies and is just beginning to be investigated in canine and feline tumors. Evidence suggests that in both human and veterinary patients, tyrosine kinases are often abnormally activated in malignant tumors. This may occur through mutation, overexpression, the generation of fusion proteins from chromosomal translocation, or autocrine loops of activation through coexpression of growth factor and receptor.[14] The consequence of this dysregulation is persistent cell signaling in the absence of appropriate negative regulation/growth factor stimulation, thereby inducing uncontrolled cell proliferation and survival.

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1527-3369/06/0604-0171\.00/0
doi:10.1053/j.tcam.2009.02.002

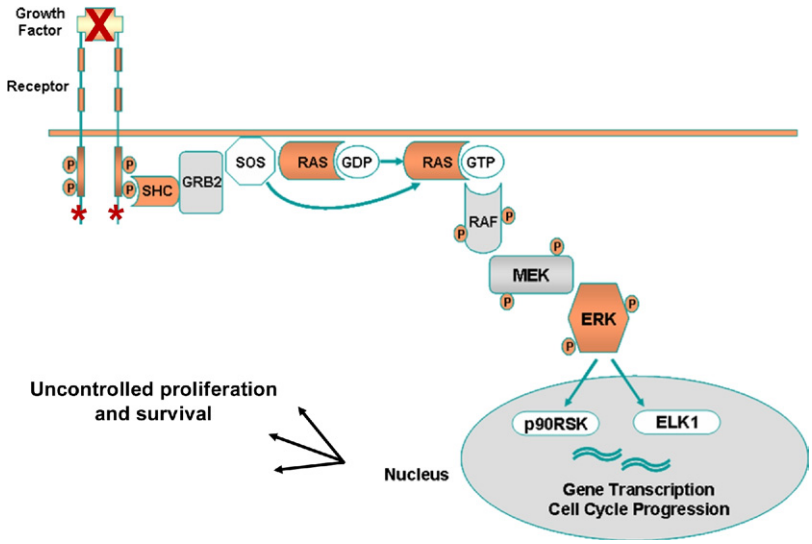

Figure 1. Tyrosine kinase dysfunction in cell signaling. Receptor tyrosine kinases normally exist on the cell surface as monomers. The growth factor (ligand) for these receptors will bind the monomers, inducing dimerization and subsequent autophosphorylation and downstream signaling and regulated cell growth and survival. In the presence of an activating mutation, the growth factor is no longer needed for receptor activation. Instead, the receptor is constitutively autophosphorylated, resulting in unregulated downstream signaling, thereby promoting uncontrolled cell proliferation and survival.

There are now several well-characterized instances of tyrosine kinase dysfunction in human cancers (Fig 1). Perhaps the most studied example is that of the Bcr-Abl fusion protein found in approximately 90% of human patients with chronic myelogenous leukemia (CML).[15,16] The generation of the fusion protein occurs through chromosomal translocation and induces constitutive activation of the cytoplasmic tyrosine kinase Abl. Given the high prevalence of this specific mutation in CML, it represents a unique target for therapeutic intervention. Another tyrosine kinase for which mutations in human cancer have been characterized is Kit, a receptor normally expressed on hematopoietic stem cells, melanocytes, in the central nervous system, and on mast cells.[17] Dysregulation of Kit has been identified in several human cancers including systemic mastocytosis,[18] acute myelogenous leukemia,[19] and gastrointestinal stromal tumors (GISTs)[20,21] through a variety of different mutations that induce activation of Kit in the absence of growth factor (stem cell factor) stimulation. Another example is the presence of a point mutation in receptor tyrosine kinase EGFR in a subset of human primary lung tumors that induces prolonged signal transduction after stimulation by its ligand EGF. This excessive signal transduction promotes uncontrolled growth and survival. Interestingly, this mutation is primarily found in patients who have never smoked but have developed a particular histopathologic subset of lung cancer, bronchoalveolar carcinoma.[22]

Although tyrosine kinase dysfunction has been extensively studied in human oncology, it is far less characterized in veterinary oncology. As previously mentioned, Kit is a receptor found on mast cells, and Kit signaling is required for the differentiation, survival, and function of mast cells.[17,23-28] Mutations in Kit have been demonstrated to occur in systemic mastocytosis in people and these mutations lead to excessive signaling, resulting in loss of growth control.[29-35] Several authors have identified the presence of Kit mutations in dog mast cell tumors (MCTs), and these also result in uncontrolled signaling.[36-39] In the majority of affected dogs, the Kit mutations consist of internal tandem duplications (ITDs) in the juxtamembrane domain of Kit (encoded by exons 11-12). This region of Kit is responsible for negatively regulating receptor activation, and evidence suggests that the ITDs disrupt the structure of this domain, resulting in a loss of this function.[40] More recently, activating mutations have also been identified in the extracellular domain of Kit in a small number of MCTs,[41] and these also appear to promote uncontrolled tumor growth. Up to 30% of all dog MCTs may carry Kit mutations, and these have been shown to be significantly associated with tumor grade: mutations are rarely identified in well-differentiated tumors, whereas approximately 35% of poorly differentiated tumors carry an ITD.[39,42] Furthermore, the mutations have been associated with local recurrence and decreased survival. Mutations in juxtamembrane domain of Kit have also been found in canine GISTs and are nearly identical to those present in the human disease.[43]

Other kinases currently under investigation for their potential role in canine cancers include EGFR and Met, among others. EGF stimulation of 2 malignant mammary lines could inhibit apoptosis induced by serum starvation or doxorubicin treatment (D Thamm, personal communication, May 2008). Furthermore, the cell lines demonstrated enhanced chemotaxis and VEGF production in response to EGF. Studies from our laboratory have shown that similar to the case of

human osteosarcoma (OSA), canine OSA cell lines aberrantly express the receptor tyrosine kinase Met, and simulation of Met by its ligand hormone growth factor induces scattering, migration, and colony formation of the canine OSA lines.[44]

Tyrosine kinase dysfunction has not been well investigated in feline cancers. Vaccine-associated sarcoma (VAS) cell lines were shown to express PDGFRβ that was phosphorylated after PDGF exposure.[45] Furthermore, PDGF induced a protective effect on VAS cells after treatment with doxorubicin or carboplatin. These studies support the notion that PDGFR may promote the growth and survival of VAS in vivo and thus be an appropriate target for therapeutic intervention with targeted approaches. Recently, mutations in Kit have been identified in feline MCTs.[46] Although no mutations were identified in the juxtamembrane or catalytic domains of Kit,[47] mutations in exon 8 have been found and are believed to promote unregulated Kit stimulation in these tumor cells. Therefore, Kit is likely a relevant target for therapy in feline mast cell disease.

Tyrosine Kinase Inhibitors

Although several strategies exist for targeting protein kinases, the most successful approach to date has been the use of a class of drugs termed "small molecule tyrosine kinase inhibitors" (TKIs). These typically work by blocking the ATP-binding site of kinases, essentially acting as competitive inhibitors that may be reversible or irreversible.[48-50] In the absence of ATP binding, the kinase is not able to phosphorylate itself or initiate downstream signaling. To develop inhibitors specific for particular proteins, the ATP-binding pockets of many kinases have been characterized to permit the design of inhibitors that exhibit activity against a restricted set of kinases, thereby limiting off-target effects (ie, inhibition of other nontarget kinases). Such inhibitors are often easy to synthesize in large quantities, are orally bioavailable, and can readily enter cells to gain access to the intended target.

Perhaps the most successful small molecule kinase inhibitor developed to date is Gleevec (imatinib mesylate, Novartis, Basel, Switzerland), an orally administered drug that blocks the activity of the cytoplasmic kinase Abl. This drug was designed specifically to target the constitutively active Bcr-Abl fusion protein found in human patients with CML.[15,16] Numerous clinical trials of Gleevec have been completed in patients with CML that confirm substantial clinical activity.[51-56] For those individuals in the chronic phase of CML, Gleevec induces a remission rate close to 95%, and most patients remain in remission for more than 1 year. Unfortunately, remission rates are much lower for patients in blast crisis (20%-50%), lasting on average less than 10 months. Resistance to Gleevec is primarily a consequence of mutations in the ATP-binding pocket that prevent appropriate binding of the inhibitor, although Bcr-Abl gene amplification can also overwhelm the ability of Gleevec to inhibit activity.[57,58]

Gleevec also binds to the ATP-binding pocket of the receptor tyrosine kinase Kit. As previously discussed, GISTs often possess activating mutation in Kit and are known to be resistant to chemotherapy.[59,60] Clinical trials of Gleevec for the treatment of GISTs induced responses in 50% to 70% of patients, far better than the 5% response rate treatment with a variety of chemotherapeutics. Additionally, a small number of GISTs do not possess Kit mutations, but instead have activating mutations in PDGFRα; these patients also respond to Gleevec because the drug is known to inhibit phosphorylation of PDGFR as well.[61] Based on the high response rate of GISTs to Gleevec, it has become standard-of-care therapy for affected individuals.

The TKI Sutent (SU11248, sunitinib, Pfizer, New York, NY, USA), was developed as an antiangiogenic agent and blocks the activity of the split kinase receptor tyrosine kinase family including VEGFR, PDGFR, and FGFR; it also inhibits Kit activation because Kit is a member of the split kinase family.[62] Like Gleevec, Sutent sits in the ATP-binding pocket of these receptor tyrosine kinases, thereby inhibiting both autophosphoryation and downstream signal transduction. Sutent has activity against several cancers including neuroendocrine, colon, and breast cancers, and is currently under investigation in a variety of combination regimens to treat these and other cancers.[62] Definitive efficacy was further demonstrated in patients with Gleevec-resistant GIST (61% demonstrated disease regression or stable disease lasting longer than 4 months),[63] and renal cell carcinoma that had failed interleukin-2 and/or interferon therapy (40% achieved a partial response, whereas an additional 25% experienced stable disease).[62] Sutent is now considered standard of care for most patients with metastatic renal cell carcinoma.

Another orally active small molecule TKI that has been successful in treating human cancers is Gefitinib (Iressa, Astra Zeneca, Wilmington, DE, USA). This drug inhibits EGFR signaling and, like Gleevec, acts as a competitive inhibitor of ATP binding.[64,65] The EGFR family is an attractive target for therapy because several cancers including breast, lung, and bladder carcinomas overexpress one or more family members.[5] In human patients, Iressa has demonstrated clinical activity in nonsmall-cell lung cancer, with 12% to 20% of patients experiencing complete or partial responses and an additional 30% to 40% of patients experiencing stable disease.[5,64] The likelihood of response to Iressa and other EGFR inhibitors is dependent on the presence of a point mutation in EGFR that induces prolonged signal transduction after stimulation by its ligand EGF.

Tyrosine Kinase Inhibitors in Veterinary Medicine

Limited data exist on the clinical efficacy of small molecule inhibitors in veterinary medicine. In part, this is due to fact that targets for therapeutic intervention are not clearly defined for most canine or feline cancers. Additionally, many of the human TKIs are currently cost-prohibitive, preventing their widespread use. Recently, Gleevec has been used to

Table 1. Tyrosine Kinase Inhibitors in Dogs and Cats

TKI	Targets	Tumor Types	Species	Dose	References
Palladia (Toceranib)	Kit, VEGFR, PDGFR, Flt-3	MCTs, sarcomas, carcinomas, melanoma, myeloma	Dogs	3.25 mg/kg EOD	66, 67, 68
Kinavet (Masitinib)	Kit, (PDGFR)	MCTs	Dogs	12.5 mg/kg SID	69
Gleevec (Imatinib)	Kit, Abl, PDGFR	MCTs, sarcomas	Dogs, cats	5 to 10 mg/kg SID	45, 70, 71, 72

Abbreviations: TKI, tyrosine kinase inhibitors; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; MCT, mast cell tumor; EOD, every other day; SID, once a day.

treat cancers in dogs and cats, and 2 TKIs (Palladia, Pfizer; and Kinavet, AB Science, Short Hills, NJ, USA) have undergone registrational studies in dogs with MCTs. These studies are discussed below, and their general use is summarized in Table 1.

Palladia (Toceranib Phosphate, SU11654)

The first evaluation of TKIs in veterinary patients involved a phase I clinical trial exploring the safety and efficacy of the novel multitargeted TKI Palladia (SU11654).[66] This orally biovailable compound is similar to Sutent, exhibiting potent inhibitory activity against members of the split-kinase receptor family, including VEGFR, PDGFR, and Kit, and was therefore predicted to have both antiangiogenic and direct antitumor activity. This study enrolled 57 dogs with a variety of cancers including carcinomas, sarcomas, MCTs, melanomas, and lymphomas, among others. As previously discussed, a substantial proportion of dog MCTs possess activating mutations in Kit, so it was predicted that responses would be observed frequently in dogs with these tumors. Measurable objective responses were documented in 16 dogs for an overall response rate of 28%. Stable disease for >10 weeks was seen in an additional 15 dogs for a resultant overall biological activity of 54%. Responding histologies included sarcomas, carcinomas, melanomas, and MCTs. As expected, the highest response rate was observed in MCTs, with 10/11 dogs with Kit mutations exhibiting partial/complete responses (n = 9) or stable disease (n = 1). Of the 11 dogs with MCTs that did not have a Kit mutation, a biologic response rate of approximately 30% was observed, suggesting that some dogs without obvious Kit dysfunction may also benefit from Palladia therapy, possibly because of inhibition of VEGFR signaling on malignant mast cells. The exact mechanism of the observed response in other tumor types is not entirely clear, but it is likely that Palladia's effects on VEGFR and PDGFR promoted an antiangiogenic effect that contributed to tumor regression in some cases. This study provided the first evidence that multitargeted TKIs can exhibit broad activity against a variety of spontaneous malignancies in canine patients with cancer.

After this phase I study, an additional project was completed to evaluate the effect of Palladia on the activity of its target Kit in canine MCTs.[67] Tumor biopsies and blood samples were obtained from dogs with MCTs before and 8 hours after administration of a single oral dose of drug. Palladia blood concentration and levels of phosphorylated Kit were assessed in tumor biopsies by Western blot. Inhibition of Kit phosphorylation was documented in most tumors and directly correlated to both Palladia blood level as well as the presence of a Kit ITD. This was the first target modulation study of its kind performed in which a direct association could be made between blood level of a TKI, actual inhibition of the specific target in vivo, and an antitumor response.

Subsequently, a placebo-controlled, randomized, registrational study of Palladia was performed in dogs with nonresectable grade II and III MCTs.[68] During the blinded phase of the study, the objective response rate in Palladia-treated (n = 86) dogs was 37.2% (7 complete response [CR], 25 partial response [PR]) versus 7.9% (5 PR) in placebo-treated (n = 63) dogs. Of 58 dogs that received Palladia after placebo escape, 41.4% (8 CR, 16 PR) experienced an objective response. The overall objective response rate for all 145 dogs receiving Palladia was 42.8% (21 CR, 41 PR). As expected, dogs with Kit mutations were much more likely to respond to Palladia than those without Kit mutations (82% vs 55%). Also, those dogs without lymph node metastasis had a higher response rate than those with lymph node involvement (67% vs 46%). Interestingly, tumor grade did not affect the likelihood of response to therapy, although it did impact the time to progression, with grade III MCTs failing earlier than grade II MCTs. These data confirmed that Palladia has biologic activity against canine MCTs and suggests that the addition of Palladia to MCT treatment regimens may improve overall outcome.

Kinavet (Masitinib)

Kinavet is another TKI that primarily targets Kit and possibly PDGFR as well. An open-label phase II study of Kinavet was completed in dogs with grade II and III MCTs. Of 13 dogs treated, there were 2 complete responses, 2 partial responses, and stable diseases in an additional 2 dogs; the drug was well tolerated (S. Axiak and coworkers, VCS 2006, personal communication). Subsequent to this study, a randomized, double-blind, placebo-controlled phase III clinical trial of Kinavet was performed in more than 200 dogs with nonmetastatic grade II or III MCTs.[69] Although the overall response rate was not significantly different between placebo- and Kinavet-treated dogs (15% vs 16%), there was a significant difference in time to progression between the 2 groups (75 vs 118 days), suggesting that Kinavet has biologic activity in

MCTs. Although dogs with MCTs possessing Kit mutations did not experience a significantly greater response to therapy when treated with Kinavet (20%) compared with placebo (10%), they did experience a significantly longer time to progression. This was more pronounced in dogs with MCTs possessing Kit mutations. These data support the notion that the biologic activity of Kit inhibitors is greatest in the setting of activating mutations.

Gleevec (Imatinib Mesylate)

Gleevec has been used in dogs primarily to treat canine MCTs. However, it is known to induce hepatotoxicity in a proportion of dogs; this hepatotoxicity appears to be idiosyncratic in nature, resulting in elevations in ALT and ALP that necessitate discontinuation of therapy (London, personal communication). A recent study demonstrated some response to therapy in 10/21 dogs treated with Gleevec; the objective response rate was 100% in dogs whose MCTs possessed a Kit ITD (n = 5).[70] No dogs were observed to exhibit hepatotoxicity, although the duration of treatment was relatively short in most patients because several owners elected discontinuation of therapy because of cost. Another study reported partial responses to therapy in 3 dogs with systemic mast cell disease treated with Gleevec.[71] Two dogs survived for 117 and 159 days, and the third was alive after 75 days; no Gleevec-induced hepatotoxicity was noted in these 3 dogs.

Although Gleevec may induce hepatotoxicity in dogs, it is apparently well tolerated in cats. A phase I clinical trial evaluating the toxicity of Gleevec was performed in 9 cats with a variety of tumors.[72] Doses of 10 to 15 mg/kg were well tolerated with no evidence of hematologic toxicity and only mild gastrointestinal toxicity. However, no pharmacokinetic analyses were performed and no pharmacokinetic/pharmacodynamic relationship has been established for inhibition of feline Kit with Gleevec. Recently, a cat with systemic mastocytosis was treated with Gleevec at a dose of 10 mg/kg.[46] The cat exhibited a complete response to therapy at 5 weeks of treatment with no obvious toxicity. Interestingly, the malignant mast cells possessed a mutation in exon 8 of Kit, likely resulting in constitutive activation of Kit and subsequent promotion of uncontrolled mast cell tumor growth.

Another feline tumor type that may benefit from Gleevec is vaccine-associated sarcoma (VAS). As previously mentioned, VAS cell lines were shown to express PDGFRβ, and Gleevec was shown to block PDGF-induced phosphorylation in these cells.[45] Additionally, Gleevec significantly inhibited the growth of VAS tumors in murine xenografts and reversed the protective effect of PDGF on doxorubicin and carboplatin-induced growth inhibition. These studies support the notion that PDGFR may promote the growth and survival of VAS in vivo and thus may be an appropriate target for therapeutic intervention using targeted approaches.

Clinical Toxicities of Tyrosine Kinase Inhibitors

Nearly all anticancer therapeutics exhibit some spectrum of clinical toxicities. In human medicine, various degrees are acceptable given an expectation of benefit from the treatment. In veterinary medicine, quality of life is a significant factor in decision making regarding various therapeutics. Similar to chemotherapeutics, the TKIs often induce toxicities that target normal tissues, likely because of the effects of chronic inhibition of receptors expressed on normal cells that require these pathways for cell survival/proliferation under normal homeostatic conditions. These effects are magnified with multitargeted inhibitors compared with those with a very narrow spectrum of kinase inhibition. Therefore, in many respects, TKIs should be viewed as similar to chemotherapeutics with respect to prevention and management of clinical effects.

Both Palladia and Kinavet can induce anorexia, vomiting, diarrhea, and gastrointestinal bleeding in treated patients.[66,68,69] Such clinical toxicities appear to be magnified in the setting of malignant mast cell disease in which circulating levels of histamine are often elevated, potentially exacerbating any drug-induced gastric/intestinal ulceration. Treatment for mild gastrointestinal toxicities primarily involves the initiation of supportive care (antacids/proton pump inhibitors, antidiarrheal drugs). Should the toxicities be significant, a treatment break is warranted, and an alteration in dose or schedule may be indicated. As with toxicities associated with standard chemotherapeutics, it is important to immediately recognize gastrointestinal toxicities secondary to TKIs so appropriate management can be initiated.

In addition to toxicities typical of antineoplastic therapies, both Palladia and Kinavet have the capacity to cause unique side effects. Palladia is known to induce a mild, nonlife-threatening neutropenia in a subset of treated patients (generally not lower than 1500 cells/μL). This does not seem to predispose dogs to bacterial infection, and the neutropenia often resolves over time. Additionally, a small proportion of Palladia-treated dogs will develop localized muscle cramping, readily treated with nonsteroidal anti-inflammatory drugs, tramadol, or a drug holiday. Interestingly, dogs that develop this toxicity do not appear to be predisposed to further episodes once therapy is reinitiated. Kinavet has been shown to induce a protein-losing nephropathy in a small subset of patients, the origin of which is not clear. Lastly, a syndrome of hemolytic anemia was noted in 2.5% of the MCT patients after Kinavet therapy. Again, the mechanism for this toxicity is not known.

Gleevec has not been widely used in dogs and cats, and no long-term treatment studies have been undertaken to characterize any potential toxicities. As previously discussed, Gleevec can induce an idiosyncratic hepatotoxicity in a subset of dogs. In the 2 reports of dogs treated with Gleevec, no hepatotoxicity was noted, although the duration of treatment tended to be short. Additionally, no obvious gastrointestinal or biochemical toxicities were noted, suggesting that Gleevec is likely to be well tolerated in dogs that do not experience hepatotoxicity. No significant toxicities were noted in cats in a small pilot study, although cats were not treated for long periods, and pharmacokinetic analyses were

not performed to confirm that Gleevec is indeed sufficiently orally bioavailable in this species at a dose of 10 mg/kg.

Conclusions

Dysfunction of tyrosine kinases occurs frequently in human cancers, and recent work indicates that a similar pattern of dysfunction will be observed in dog and cat cancers. Several inhibitors of tyrosine kinases are now available for use in human cancer therapy, and many of these have exhibited significant clinical activity. TKIs have only recently entered the arena of veterinary oncology, but the success of registrational studies for Palladia and Kinavet indicate that TKIs will soon become available to treat veterinary cancer patients. Several challenges remain for such TKIs in veterinary oncology including defining cancers in which they are most likely to be effective, establishing regimens that reduce their toxicities, evaluating their biologic activity in the microscopic disease setting, and investigating how to combine them with standard therapeutics such as radiation therapy and chemotherapy.

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