Receptor tyrosine kinase inhibitors

Receptor tyrosine kinases (RTKs) are a family of cell surface proteins which mediate cellular responses to soluble growth factors in the plasma or extracellular fluid. Examples of RTKs include the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and Kit. Upon binding to a growth factor, two RTK proteins will dimerize and activate one another in an ATP-dependent process termed autophosphorylation. Following autophosphorylation, activated RTKs interact with and phosphorylate other nearby membrane-associated proteins, initiating a cascade of protein activation within the cell, culminating in the activation of transcription factor proteins which initiate gene transcription. The genes expressed as a result of RTK activation result in increased cell proliferation, increased cellular protein synthesis, decreased sensitivity to apoptotic signals, and increased cellular motility.

Under normal physiologic conditions, the activity of RTKs is tightly controlled. However, RTK activity is frequently dysregulated in cancer. RTKs may be expressed by cancer cells in unusually high amounts, or may be mutated in such a way that activation occurs in the absence of binding to growth factors. Dysregulated RTK activity endows cancer cells with many of their hallmark capabilities, such as unrestricted proliferation, resistance to apoptosis, and the capacity for invasion and metastasis. A well-characterized example of RTK dysregulation in small animal cancer is mutation of the c-Kit gene in canine mast cell tumors. Activating mutations in the c-Kit gene render the corresponding Kit protein able to maintain an activated state in the absence of binding to its growth factor ligand, stem cell factor (SCF). Mast cell tumors bearing c-Kit mutations are typically higher grade, and are associated with higher rates of local recurrence and metastasis, and shorter survival times, following surgical removal.

During the course of their progression, many cancers become dependent upon aberrant RTK signaling for sustaining their survival; such cancers are often referred to as “oncogene-addicted.” Pharmacologic disruption of aberrant RTK signaling in oncogene-addicted cancers can lead to tumor regression. The past two decades have witnessed an explosive development of drugs targeted at aberrantly functioning RTKs in a variety of human cancers. RTKI therapy has also recently become available for cancer-bearing dogs and cats. In 2009, the U.S. Food and Drug Administration (FDA) approved toceranib (Palladia) for the treatment of recurrent or metastatic Patnaik grade 2 or 3 mast cell tumors in dogs. Shortly thereafter, in 2010, masitinib (Kinavet) received conditional FDA approval for the treatment of nonresectable Patnaik grade 2 or 3 mast cell tumors which have not been previously treated with radiation therapy or chemotherapy. It should be noted that the conditional approval of status of masitinib prohibits the use of this drug in an off-label fashion. However, since toceranib has received full FDA approval, veterinarians may use this drug in an off-label fashion, in accordance with the Animal Medicinal Drug Use Clarification Act (AMDUCA).

Over the past five years, toceranib has been used extensively to treat canine mast cell tumors. A pivotal study demonstrated that toceranib induced measurable tumor regression in 43% of recurrent or metastatic Patnaik grade 2 or 3 mast cell tumors in dogs. The median duration of tumor control afforded by toceranib treatment was approximately 4.5 months. Toceranib is labeled for dosing at 3.25 mg/kg PO every 48 hours. However, the drug appears to be better tolerated if dosed at 2.5-2.75 mg/kg PO every 48 hours or on a Monday-Wednesday-Friday dosing schedule. This reduction in dose from the label dose does not appear to reduce efficacy. Toceranib has also recently demonstrated efficacy against canine thyroid carcinoma, osteosarcoma, head and neck carcinomas, and apocrine gland adenocarcinoma of the anal sac.

Although toceranib is usually well-tolerated, approximately 20% of treated dogs experience severe side effects. The most common side effects are gastrointestinal, and include vomiting, diarrhea, melena, and hematochezia. Toceranib also may cause glomerular injury, resulting in proteinuria and hypertension. In dogs experiencing side effects of toceranib, the author typically recommends a 7 day drug holiday as well as symptomatic care as needed. Proteinuria and hypertension are usually manageable with angiotensin converting enzyme (ACE) inhibiting drugs, such as enalapril or benazepril. The author’s experience has been that, following an appropriate drug holiday, toceranib can be re-started at a lower dose (10-20% reduction) in dogs that experience side effects at standard doses.

Masitinib is also potentially useful for the treatment of mast cell tumors in some dogs. The recommended dose for this drug is 12.5 mg/kg PO every 24 hours. Side effects of masitinib are similar to those for toceranib, although gastrointestinal side effects generally are milder. Unfortunately, the conditional licensure of masitinib limits its utility in the management of mast cell tumors, and it cannot currently be used for the treatment of non-mast cell neoplasia.

There are few data available regarding the use of RTKIs in cancer-bearing cats. A small pilot study showed that toceranib was tolerable in cats at a dose of 3 mg/kg PO q 48 hrs, and had modest anti-tumor activity against some feline cancers, including injection site sarcomas and squamous cell carcinomas. Imatinib, a RTKI used extensively to treat human cancers, has also been shown to be safe in cats at a dose of 10 mg/kg PO q 24-48 hrs.
Canine melanoma vaccine

Oral malignant melanoma (OMM) is an extremely aggressive cancer which is both locally invasive and has highly metastatic. Common sites of metastasis include regional lymph nodes and the lungs. Local tumors within the oral cavity can be successfully managed with surgery and radiation therapy, but there has historically been no effective medical therapy for managing metastatic disease. Melanoma is notoriously resistant to traditional cytotoxic chemotherapy, and tumor responses to chemotherapy are typically brief and of limited benefit to patients.

The recent development of a DNA-based vaccine (Oncept™, Merial, Ltd.) offers new hope for providing effective systemic cancer control for dogs with OMM. Oncept consists of a circular plasmid DNA vector into which the gene for the human tyrosinase protein has been inserted. Tyrosinase is an enzyme specific to melanocytes which catalyzes the initial step in melanin synthesis. As tyrosinase is specific to melanocytes, immune responses elicited against tyrosinase will be directed only against melanocytes, and not at other normal cells. Oncept is delivered intramuscularly in the medial thigh using a pneumatic injection device. Once delivered, the plasmid DNA is taken up by antigen-presenting dendritic cells, in which the tyrosinase gene is transcribed and translated into the corresponding protein. The tyrosinase protein is then presented to immune effector cells, including cytotoxic T-lymphocytes, and an immune response is initiated against tyrosinase-expressing cells. Importantly, because Oncept contains human DNA, the associated protein is recognized as foreign by the canine immune system, allowing an effective anti-tyrosinase immune response to be mounted. Oncept has been shown to have an excellent safety profile, with the most common side effects being local reactions and/or hematomas at the injection site. Very rarely, vitiligo (pathological depigmentation) can also occur if normal melanocytes are targeted by the immune response.

In 2009, the U.S. Department of Agriculture (USDA) approved Oncept for the treatment of the treatment of locally controlled stage 2 or 3 canine oral malignant melanoma, which includes tumors 2 to >5 cm in diameter, with and without regional lymph node metastasis, but without distant visceral metastasis. The term “locally controlled” refers to tumors in which the primary tumor and all sites of nodal metastasis have been rendered undetectable following surgery and/or radiation therapy. The importance of achieving local tumor control prior to initiating therapy with Oncept cannot be over-emphasized. Survival times for dogs with macroscopic melanoma treated using Oncept are reduced by 50% relative to those for dogs with microscopic melanoma. Tumor shrinkage occurs only rarely in dogs treated with Oncept in the setting of measurable tumor burden. However, for dogs in which local control has been achieved, Oncept is reported to afford excellent median survival times: >939 days for dogs with stage 1 tumors, >908 days for dogs with stage 2 tumors, and >1,646 days for dogs with stage 3 tumors. These survival times compare very favorably to those for dogs historically treated with surgery and/or radiation alone, in which median survival times are typically on the order of 5-7 months. Notably, median survival time is only 239 days for dogs treated with Oncept for stage 4 tumors (i.e. those with macroscopic visceral metastases), which further demonstrates that Oncept is most effective for treating dogs with microscopic tumor burdens.

Despite the impressive initial reports of Oncept’s efficacy, a recent study documented no significant improvement in survival for dogs treated with Oncept compared to dogs which did not receive the vaccine. While this recent study had many limitations, the author has also observed that Oncept seems to afford significant benefit for some dogs, but limited benefit for many others. It is possible that certain tumor-related factors, such as cell proliferation rate or inherent immunogenicity, may correlate with clinical response to Oncept. However, such factors have yet to be identified.

Metronomic chemotherapy

An important principle of traditional cytotoxic chemotherapy is that chemotherapy drugs must be administered at the maximally tolerated dose (MTD), which is defined as the dose which produces the greatest level of antitumor response at an acceptable level of side effects. A second important principle is that chemotherapy drug doses should be administered at the greatest possible frequency in order to maximize their effects against the tumor. The biological rationale for these therapeutic principles is that all clinically apparent cancers are composed of genetically heterogeneous cells, many of which have acquired mutations that make them inherently resistant to cytotoxic drugs. To overcome this inherent drug resistance, chemotherapy drugs must be given with the greatest possible intensity. However, the intensity at which chemotherapy drugs may be delivered is limited by the tolerance of sensitive organs, notably the bone marrow and the gastrointestinal tract. Toxic effects of chemotherapy to these organs produce clinical syndromes such as neutropenia (occasionally with sepsis), nausea, vomiting, and diarrhea, any of which may be life-threatening. These toxic effects limit the dose and frequency at which chemotherapy drugs can be safely administered, and long breaks in therapy must occur in order for the bone marrow, gastrointestinal tract, and other normal tissues to recover. Unfortunately, these limitations also reduce the therapeutic effectiveness of chemotherapy, and this accounts for the inability of chemotherapy to cure most disseminated cancers.

Metronomic chemotherapy is a novel way of administering chemotherapy which attempts to circumvent these limitations of MTD chemotherapy. Metronomic chemotherapy involves daily oral administration of low doses of chemotherapy, doses which are far below the MTD. At such low doses, metronomic chemotherapy is unlikely to have efficacy directly against tumor cells. Rather, metronomic chemotherapy is hypothesized to target rapidly-dividing endothelial cells which are constantly forming new blood vessels to sustain a tumor’s inexorable growth. Since these endothelial cells are genetically normal, they are much more sensitive to the
cytotoxic effects of chemotherapy drugs than are the cancer cells themselves. Thus, metronomic chemotherapy is a form of anti-
angiogenic chemotherapy. Recent data also support that metronomic chemotherapy may exert anticancer effects by helping to
strengthen natural immune responses against a tumor.

Metronomic chemotherapy has several advantages over MTD chemotherapy. First, because upregulated angiogenesis is a nearly
universal feature of cancer, metronomic chemotherapy is likely to have efficacy against a broad spectrum of tumor types. Second, the
low dose of metronomic chemotherapy limits its toxicity, and side effects of metronomic chemotherapy are infrequent and usually
mild. Third, metronomic chemotherapy is dosed orally, which is more convenient for pet owners than is repeated parenteral dosing at
a veterinary hospital. Despite these advantages, metronomic chemotherapy does have disadvantages. One disadvantage is that
objective tumor shrinkage following therapy is uncommon, and metronomic chemotherapy more frequently stabilizes tumor growth
for a period of time. Second, metronomic chemotherapy may take several months in order to demonstrate clinical benefit.
Additionally, while the acute toxicoses associated with metronomic chemotherapy are mild, its associated chronic toxicoses are poorly
characterized. Chronic (possibly permanent) injury to the bone marrow and induction of secondary malignancies are possible
concerns in patients on metronomic chemotherapy for periods of many months or years.

Despite these possible limitations, several recently published studies document the effectiveness of metronomic chemotherapy
against small animal cancers, and it represents an important area of ongoing clinical investigation. Commonly used metronomic
chemotherapy protocols include chlorambucil at a dose of 4 mg/m² PO every 24 hours, or combined cyclophosphamide at a dose 10-
15 mg/m² PO q 24 hrs with piroxicam at 0.3 mg/kg PO q 24 hrs.

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