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Review Article

Tyrosine Kinase Inhibitors; an Approach for Targeted Cancer Therapies-A Review

Arpita Shrivastav[1]* and Neeraj Shrivastava[2]

[1]Assistant Professor, Department of Veterinary Pharmacology Toxicology, College of Veterinary Science A.H. Rewa, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[2]Assistant Professor, Department of Veterinary Microbiology, College of Veterinary Science A.H. Rewa, NDVSU, Jabalpur, Madhya Pradesh, INDIA

*Corresponding Author: arpitavet@gmail.com

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Abstract

Targeted cancer therapies include drugs which target specific molecules involved in the growth of cancer cells. These therapies comprise of monoclonal antibodies that attack cell surface receptors, apoptosis-inducing drugs, angiogenesis inhibitors and enzyme inhibitors that enter cells and inhibit critical cell enzymes. Tyrosine kinase inhibitors are synthetic agents that inhibit signal transduction pathways and interfere with activation of enzymes tyrosine kinase in cancer cells. They compete for the ATP binding site of protein tyrosine kinase and reduce tyrosine kinase phosphorylation, thereby inhibiting cancer cell production. So even if the tumour is not getting reduced, its out-of-control growth has found to be changed. This may give regular chemo for a better chance to work. Slowing or stopping out-of-control growth may also help people live longer, even without adding other drugs. These drugs have made great progress in the treatment of cancer, but the problem of acquired resistance is still unavoidable, restricting the treatment of cancer. Currently, Thirteen tyrosine kinase inhibitors have been approved by Food and Drug Administration. Among them Imatinib, Masitinib, Pazopanib and Toceranib are used for the veterinary purpose Proper dosage regimens, defining of type of cancers and combination with the standard therapeutics are some of the challenges yet to be worked on.

Keywords: Receptor Tyrosine Kinases, NRTK, Targeted Therapies, Tyrosine Kinase Inhibitors

Introduction

In recent days for the treatment of cancer, special drugs or other substances are used which directly block the growth and spread of cancer by targeting those molecules which holds role in the spread and development of such abnormal growth. These molecules are also referred to as molecular targets or molecularly targeted drugs. Such type of therapy is known as Targeted cancer therapies.

In the body few proteins play a very crucial role in certain signal pathways which communicate among the various cells playing an important role in cancer cell division, cell movement and cell responses towards specific external stimuli, and apoptosis. To become cancerous, normal cells go through a process called carcinogenesis. These cancer cells may then grow into tumours or reproduce throughout a body system, as it happens in blood cancers. (American Cancer Society, 2011). If such signals regarding growth of cancer cells is blocked growth can be inhibited resulting in of cancer. As, a etiology of cancer is not only restricted to the chemicals, irradiation but certain changes at cellular and molecular level may also lead to such condition therefore if therapy is focused on these changes related specifically to the cancer such as blocking these signals that to distinguish and destroy cancer cells. Producing toxic substances directly to the cancer cells can also lead to its destruction. Such targeted therapies can be used alone or in combination with chemotherapy and radiation therapy (Murray *et al*., 2011). The drugs preferred for the targeted therapies are often grouped on the basis of their mechanism of action or their major site of action in the cell or body.

Types of Target Therapies

The different targeted therapies /drugs are:

1. Apoptosis-Inducing Drugs- These drugs produce change in the proteins related to cancer cells and leads to death of cells through apoptosis therefore called as apoptosis-inducing drugs. Eg- Cyclophosphamide etc

2. Angiogenesis Inhibitors- Angiogenesis is a normal healthy and physiological process which includes formation of new blood vessels. In case of cancer patients this process helps in the growth of tumour by providing good amount of blood supply and helps it to grow and spread. Angiogenesis inhibitors inhibit tumours to make new blood vessels, cut off their blood supply thus the growth and development of tumour also stops. Eg-Sunitinib, Sorafenib.

3. Enzyme Inhibitors- Enzymes are a special group of substances which help in performing certain biochemical reactions of our body. Some of the enzymes are also important in case of certain cancers where these enzymes act as signals. Enzyme inhibitors are among those targeted therapies which block or inhibit these enzymes. When the activity of these enzymes as well as special proteins related to it are blocked it hampers the growth and spread of cancer cells in the body. Eg-Bortezomib Romidepsin etc.

Based on the enzymes they block they are classified as:

a. Tyrosine kinase inhibitors
b. MTOR (Mammalian target of Rapamycin) inhibitors
c. Proteosome inhibitors
d. Growth factor inhibitors
e. Signal-transduction inhibitors
f. Multi-targeted kinase/multikinase inhibitor.

Tyrosine Kinases

There are two families of tyrosine kinases-

1. Receptor tyrosine kinases /Transmembrane receptor kinases
2. Cytoplasmic non-receptor kinases (NRTK)

Receptor Tyrosine Kinases /Transmembrane Receptor Kinases

The enzymes that catalyse phosphoryl transfer to tyrosine residues in protein substrates, using ATP as a phosphate

donor, are the protein tyrosine kinases, Receptor tyrosine kinases (RTKs) are essential components of signal transduction pathways that mediate cell-to-cell communication. These enzymes receive extracellular signals with the help of ligand binding site which in turn stimulates activation of cytoplasmic domain and transmit signals from the membrane receptors to inside the cell, fix themselves with the cell membranes by hydrophobic domain site. The RTK family includes, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptors, fibroblast growth factor receptors (FGFRs), vascular endothelial growth factor receptors, hepatocyte growth factor/scatter factor [HGF/SF] receptor, ephrin receptors, and the insulin receptor. Receptor tyrosine kinase are usually present as monomers on the cell surface, their binding with growth factor results in dimerization and autophosphorylation followed by initiation of kinase activity and phosphorylation of signalling intermediates. (Madhusudan and Ganesan 2004). The activation process displays two essential stages. The first stage relies on the dimerization of receptors which results in their conformation modification. In the second stage, TKs are auto phosphorylated which is modulated by regulatory ligands. Autophosphorylation initiate a series of phosphorylation reactions which activate various proteins it continues till the signal reaches the nucleus and results in changes of expression of specific targeted genes (Schlessinger, 2000).

Cytoplasmic Non-Receptor Kinases (NRTK)

Cytoplasmic non-receptor tyrosine kinases (nRTKs) are enzymes catalyses the transfer of a phosphate group from a nucleoside triphosphate donor, ATP, to a tyrosine residue in proteins. These receptors are characterized by c-ABL, relics in an inactive state by intramolecular autoinhibition with the support of certain inhibitor proteins and lipids. Later these nonreceptor tyrosine kinase are activated by diverse intracellular signals through dissociation of inhibitors, causing dimerization and autophosphorylation, and through trans-phosphorylation by other kinases. Later signals of these receptors are terminated through the action of tyrosine phosphatases that hydrolyze tyrosyl phosphates and by the induction of inhibitory molecules (Etten, 2003; Krause and Etten 2005) Unlike the receptor tyrosine kinases (RTKs), non-receptor tyrosine kinases are cytoplasmic enzymes (Weiss and Littman, 1994). Several non-receptor tyrosine kinases have been recognized in human cells. These kinases are similar in mechanism to receptor kinases which have mutual catalytic domains. Each catalytic domain of tyrosine kinase contains a specific ATP binding site also act as phosphate residue donor and a substrate binding site that transfers phosphate residues from ATP.

Classification of Tyrosine-Kinase Inhibitors

1. BCR-ABL Tyrosine Kinase Inhibitors
2. Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors
3. Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitors
4. Platelet-Derived Growth Factor Inhibitors

BCR-ABL Tyrosine Kinase Inhibitors **–**Imatanib, Nilotinib, Dasatinib

In chronic myeloid leukemia characteristic cytogenetic abnormality seen in 90 per cent of the people whereas 15 to 30 per cent of such people suffer from acute lymphoblastic leukemia (ALL) called Philadelphia chromosome. Such type of cancer occur due to abnormal Bcr-Abl fusion protein. This translocation results in the formation of the BCR-ABL oncogene by the fusion of BCR gene on chromosome 22 and the ABL tyrosine kinase gene located on chromosome 9. It is known as the 9; 22 translocation or Philadelphia chromosome (Ph). This fusion results in the expression of two forms of protein-tyrosine kinases: p190 (BCR-ABL) and p210 (BCR-ABL). Imatinib specifically inhibit or kill proliferating myeloid cell lines containing BCR-ABL minimally harmful to normal cells.

Imatinib also reduced the formation of BCR-ABL positive colonies by approximately 95% *in vitro*, in later stages of the disease resistance is often experienced. Nilotinib and dasatinib are designed to overcome imatinib resistance in CML. Nilotinib is a selective Bcr-Abl inhibitor, which is more potent (20 fold) than imatinib against wild type Bcr-Abl and is also active against 32 of 33 imatinib-resistant Bcr-Abl mutants (Fleur *et al.*, 2011). Bosutinib is a dual inhibitor of Abl and Src kinases (Keller *et al.*, 2010; Isfort *et al.*, 2014). Bosutinib has a high anti-proliferative activity, can inhibit the proliferation and survival of CML cells Doan V, Wang A, (Prescott 2015). However, Bcr-Abl products have multiple effect, a single pathway of inhibition cannot completely eliminate the malignant proliferation of tumor cells, so this product is only efficient rather than special effects of anti-cancer drugs (Roy *et al.*, 2015)

Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors–Erlotinib, Lapatinib, Canertinib, Gefitinib

The EGFR signal transduction pathways have been implicated in the regulation of various neoplastic processes, including cell cycle progression, inhibition of apoptosis, tumor cell motility, invasion, and metastasis. EGFR activation also stimulates vascular endothelial growth factor (VEGF), which is the primary inducer of angiogenesis. Overexpression of EGFR and Her2 is a factor of poor prognosis in a variety of malignancies, including breast cancer, ovarian cancer, and lung cancer. Gefitinib is a selective EGFR-TKI, which is usually expressed in epithelial-derived solid tumors. inhibits mutated EGFR and is registered for treatment of NSCLC. Inhibition of EGFR tyrosine kinase activity can prevent tumor growth, metastasis and angiogenesis and increase tumor cell apoptosis

Erlotinib, is preferred over gefitinib in some of the patient group with NSCLC, lung tumours might be resistant to erlotinib, metastases of these tumours to the brain can be sensitive to the drug. It is used for third-line treatment of locally advanced or metastatic NSCLC after a previous failure of at least one chemotherapy regimen in some of the patient group with NSCLC, lung tumours. Erlotinib with gemcitabine used for first-line treatment of locally advanced unresectable or metastatic pancreatic cancer

Lapatinib, which in addition to EGFR inhibits ErbB2. Lapatinib is a reversible dual inhibitor of EGFR and HER2. Lapatinib can inhibit both EGFR and HER2 tyrosine kinases Canertinib can inhibit all EGFR group.Canertinibcan inhibitall EGFR group.

Vascular Endothelial Growth Factor (VEGF) Tyrosine Kinase Inhibitors-Semaxanib

VEGF is secreted by almost all solid tumours and tumor-associated stroma in responseto hypoxia. It is highly specific for vascular endothelium and regulates both vascular proliferation and permeability. Excessive expression of VEGF levels correlates with increased microvascular density, cancer recurrence, and decreased survival (Qinlian *et al.,* 2018)

VEGF act on high affinity of VEGFR1 and VEGFR2 in the vascular endothelial cells both have different signal transduction pathways (Ariotti *et al.,* 2015; Xu *et al.,* 2015). VEGFR2 shows the biological effect of VEGF, which is closely linked to cell chemotaxis, cell division and act in recombination (Pfister *et al.,* 2015). VEGFR1 bind strongly to VEGF, (Abbasi *et al.,* 2015). VEGFR3 is highly expressed in the blood vessels of the embryonic vessels, veins and lymphatic vessels, but after the development of the fetus, VEGFR3 only in the lymphoid endothelial cells. In a variety of tumor course, VEGFR3 induced tumor lymph angiogenesis, promoting tumor lymph node invasion and lymph node metastasis. VEGFR3 plays an important role in helping cellular viability and blocking VEGFR3 signaling hinders this ability, which may induce autophagy Semaxanib competitively blocks ATP binding to the tyrosine kinase domain of VEGFR2, and thereby tumor angiogenesis, inhibiting the growth of xenografts established from a variety of human cancers (Laura *et al.,*2002)

Platelet-Derived Growth Factor Inhibitors– Leflunomide, Sorafenib

Platelet-derived growth factor (PDGF) signals through a cell surface tyrosine kinase receptor (PDGFR) to stimulate various cellular functions, including growth, proliferation, and differentiation (Sedlacek, 2000).

PDGF signalling is initiated by binding of the growth factors to the extracellular ligand-binding domains of the receptors, leading to the dimerization of the receptors and subsequent phosphorylation of the tyrosine residues in the intracellular domain (Kelly *et al.*, 1991).Both receptor types are overexpressed in several solid tumours as well as in the surrounding stroma (Sedlacek, 2000).

Leflunomide are a small molecule inhibitor of PDGFR-mediated phosphorylation is converted to its principal metabolite, which interferes with de novo pyrimidine synthesis.

Sorafenib is a new drug Raf kinase and VEGFR inhibitor that inhibits tumor cell proliferation and angiogenesis. Even though initially established as a Raf kinase inhibitor, it was later found to inhibit various other kinase receptors, like VEGFR, EGFR, and PDGFR kinases.

Oncogenic Activation of Tyrosine Kinases

In cancer conditions tyrosine kinase activity is dys-regulated. When there is some chromosomal abnormality like in blood cell cancer receptor or non -receptor tyrosine kinase fuses with partner protein and results in oligomerization of tyrosine kinase without ligand binding this in-turn promotes auto-phosphorylation and abnormal activation of receptors.

Abnormal TK activation can increase the survival, proliferation and cytotoxic drug resistance of malignant cells, and in tumours it can increase angiogenesis, invasiveness, and metastatic potential. Activation of receptor and cytoplasmic tyrosine kinases induces the phosphorylation reaction cascade including enzyme autophosphorylation or phosphorylation of cytoplasmatic substrates and adaptor proteins. In chronic myeloid leukaemia a tetramerization domain in BCR overcomes auto-inhibition of ABL catalytic activity through oligomerization and autophosphorylation resulting in the increased tyrosine kinase activity of BCR/ABL and in phosphorylation of several cellular substrates. Activation of a number of signal pathways by BCR/ABL leads to malignant transformation by interfering with control of cell proliferation and differentiation (Madhusudan and Ganesan, 2004).

In acute myeloid leukemia (AML) there is mutation in the Fms-like tyrosine kinase 3 (FLT3) receptor which make this Tyrosine Kinase active in the absence of ligand. In non-small-cell lung cancers (NSCLC) small deletions and point mutations in the kinase domain of epidermal growth factor receptor (EGFR) increase the sensitivity of the receptor to its ligand and alter receptor signalling.

Tyrosine kinases also stimulate angiogenesis processes directly involved in the growth of the cancer. Oncogenic tyrosine kinases disrupt regular cells processes which cause pathological phenotype development it appears that these specific proteins with tyrosine kinase activity might be attractive targets for effective cancer therapy. In certain types of cancers tyrosine kinase receptors show variation in signal regulation. As these changed/defective signals generated by kinase can be transferred to the nucleus, where induced target gene expression results in a cell response (e.g.: cells division).

Mechanism of action of Tyosine Kinase Inhibitors

When ligand bind to the extracellular domain tyrosine kinase receptor is activated, oligomerized, autophosphorylated within the activation loop of the kinase. It further changes position of certain amino acid residues, increasing the catalytic activity of the enzyme. Autophosphorylation generates binding sites for signaling proteins, recruiting them to the membrane, and activating multiple signaling pathways (Schlessinger, 2000). Tyrosine kinase inhibitors block the ATP binding site of kinases, acting as reversible or irreversible competitive inhibitors (Wakeling, 2005; Wanebo *et al*., 2006; Shchemelinin *et al*., 2006). In the absence of ATP binding, the kinase is not able to phosphorylate itself or initiate downstream signalling. It interferes with protein kinase signalling pathways occupied in gene transcription, DNA synthesis, and cellular growth (e.g. epidermal growth factor, platelet derived growth factor, nerve growth factor, fibroblast growth factor). Both inhibit proliferation and induce apoptosis. Most kinase inhibitors ATP competitive and present one to three hydrogen bonds to the amino acids located in the hinge region of the target kinase, thereby mimicking the hydrogen bonds that are normally formed by the adenine ring of ATP (Liu, & Gray, 2006).

Metabolism of Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors are small molecules and are in contrast to monoclonal antibodies able to pass through the cell membrane (Imai and Takaoka, 2006). Small-molecule inhibitors are largely hydrophobicand can easily enter the cell where they can interact with the intracellular domain of receptors and intracellular signalling molecules. As a result, small-molecules kinase inhibitors are able to block the activation of various downstream signalling pathways intracellularly. Salt form of Tyrosine kinase inhibitors can be given orally, and are also being administered to patients at a fixed dose once or twice daily, because the changeability in pharmacokinetics of these agents is not expressively affected by weight.Some of the tyrosine kinase inhibitors are metabolized by the liver primarily by cytochrome-P enzymes. The primary metabolite is further metabolized by CYP3A4 to its secondary inactive metabolite. Elimination is primarily via faeces.

Clinical Efficacy

The NCCN clinical practice guidelines for oncology that how each of the tyrosine kinase inhibitors should be used in their respective indications and recommends participation in an ongoing clinical trial as the best management for any cancer. The comparative data evaluating imatinib suggest dasatinib and nilotinib are more effective than imatinib in improving response rates in the treatment of CML; however, no differences in overall survival were reported between treatment groups. Outcomes appeared to vary depending on type of cancer being treated, severity of disease, and previous treatment(s) received. Overall, chemotherapy with tyrosine kinase inhibitors should be guided by clinical practice guidelines and individual patient characteristics.

Adverse Drug Reactions

Cancer therapy is associated with severe and possibly life-threatening adverse reactions. The most common adverse events reported with the tyrosine kinase inhibitors include significant gastrointestinal side effects (especially diarrhoea), hypertension, myelosuppression, and dermatologic reactions. Therapeutic monitoring with all of the tyrosine kinase inhibitors is important as each of the individual agents have differing end targets and is associated with significant risks. These effects are magnified with multi targeted inhibitors compared with those with a very narrow spectrum of kinase inhibition. Both Toceranib and Mastinib can induce anorexia, vomiting, diarrhea, and gastrointestinal bleeding in treated patients. In addition to toxicities typical of antineoplastic therapies, both Toceranib and Mastinib have the capacity to cause unique side effects. Toceranib is known to induce a mild, neutropenia. Additionally, a small proportion of Toceranib-treated dogs will develop localized muscle cramping, readily treated with nonsteroidal anti-inflammatory drugs, tramadol. Interestingly, dogs that develop this toxicity do not appear to be predisposed to further episodes once therapy is reinitiated. Mastinib induce a protein-losing nephropathy, haemolytic anaemia. Imitanib 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, imitanibcan induce an idiosyncratic hepatotoxicity in a subset of dogs. In the 2 reports of dogs treated with imitanib no hepatotoxicity was noted, although the duration of treatment tended to be short. Additionally, no obvious gastrointestinal or biochemical toxicities were noted, suggesting that imitanib 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 imatinib is indeed sufficiently orally bioavailable in this species at a dose of 10 mg/kg.

Cellular Resistance to Tyrosine Kinase Inhibitors

Resistance has become a serious problem in anticancer therapy in reference to the tyrosine kinase inhibitors. Several mechanisms are responsible for this resistance which could also extend other kinase inhibitors:

a. Active efflux across the cell surface are increased reducing the intracellular concentration of the compound through over-expression of p-glycoprotein efflux transporters through MDR-1 gene.
b. Concentration of free fraction of the kinase inhibitor available to the cancer cell reduce by over-expression of α-1 acid glycoprotein.
c. Over-expression of metabolic enzymes such as prostaglandinendoperoxide synthase 1/cyclooxygenase 1 (PTGS1/COX1) involve in the metabolism of certain drugs like Imitanib.
d. Activation of alternative biochemical signalling pathways to bypass the effect of the kinase inhibitor.
e. CML positive patients with Philadelphia chromosome in blast crisis phase have shown increased activity of Bcr/Abl despite Imatinib treatment due to amplification of the oncogene Bcr-Abl, with the subsequent increase of the production of Bcr-Abl. Recent studies have shown mutations in the ATP binding domain which may affect Bcr/Abl protein conformation resulting in Imatinib resistance. down-regulation of the expression by small interfering RNA (siRNA) or antisense strategies for *bcr/abl*mRNA can also be used but these approaches differ in efficiency
f. Mutations in the primary structure of the kinase,mutations around the ATP pocket are reported to alter the binding properties through which the inhibitor interacts, or introduces a new steric restriction.These two phenomena are reported to reduce the therapeutic activity of the inhibitor. For suppressing mutant resistance, researchers have recently come up with several strategies in drug design. These approaches include combination of more potent compounds, disabling the obstructions introduced by the mutations; stabilization of the inactive conformation of the kinase with a Type II inhibitor; design of new molecules by *hybrid-design*, or application of a combinatorial

therapy of different anti-neoplastics.

g. T790M mutation will increase the tyrosine kinase activity, enhance tumorigenicity (Kim et al.,2012) patients with such type of resistance show slow progress of disease. If withdrawn immediately, the disease has the possibility of outbreak, and targeted therapy is still effective after interruption of treatment, which may be due to drug-resistant tumor cells still exist in a certain proportion of cells sensitive to EGFR-TKI, but the exact mechanism is not clear.

Tyrosine Kinase Inhibitors in Veterinary Medicine

Imitanib

It 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. A recent study demonstrated some response to therapy in 10/21 dogs treated with Imatinib; the objective response rate was 100% in dogs whose MCTs possessed a Kit ITD (Isotani *et al.,* 2008) Another study reported partial responses to therapy in 3 dogs with systemic mast cell disease treated with Imatinib. (Marconato *et al*., 2008) A phase I clinical trial evaluating the toxicity of Imitanib was performed in 9 cats with a variety of tumors. ((Lachowicz *et al*., 2005) Doses of 10 to 15 mg/kg were well tolerated with no evidence of hematologic toxicity and only mild gastrointestinal toxicity.

Toceranib Phosphate

It is currently approved for the treatment of MCTs in dogs; however, the drug also appears to have biological activity against other tumours in dogs such as mammary carcinoma, soft tissue sarcoma and anal gland adeno carcinoma. Dogs affected with MCTs with activating mutations in KIT were approximately twice as likely to respond to toceranib as those without the mutation.

Masitinib

Masitinib is a potent and selective tyrosine kinase inhibitor active, orally bioavailable in vivo, and has low toxicity. Mastinib treated dogs showed 15-16% response suggesting that mastinib has biologic activity in dogs and cats.

Masitinib, a selective TKI, has previously been shown to enhance the anti-proliferative effects of gemcitabine in human pancreatic cancer, showing the potential of this drug as a ‘chemosensitizer’ In an exploratory study in canine cell lines, the ability of masitinib to sensitize lines to doxorubicin, vinblastine or gemcitabine was investigated. Masitinib strongly sensitized histiocytic sarcoma cells to vinblastine, as well as osteosarcoma and mammary carcinoma cells to gemcitabine. In addition, several cell lines were sensitized to doxorubicin. However, this was at doses of masitinib that could not be achieved in patients. These data establish proof-of-concept that masitinib in combination with chemotherapeutic agents can generate synergistic growth inhibition in various canine cancers, possibly through chemosensitization. However, extensive work on how this could be achieved at safe dosing needs to established. Recently, a phase I study was conducted by to evaluate the safety and dose limiting toxicity (DLT) of combination protocol with vinblastine and toceranib phosphate in canine mammary tumours. The DLT for the simultaneous combination in this study was found to be neutropaenia. The maximally tolerated dose was vinblastine at 1.6 mgm−2 every other week, concurrent with toceranib at 3.25 mg kg−1orally every other day. This represents greater than a 50% reduction in dose intensity for vinblastine (compared with single-agent use) and as such does not support this combination based on current drug combination paradigms. Although, a strict adherence to dose paradigms speaks against the combination, evidence of significant activity (71% objective response) and enhanced myelo-suppression suggest additive or synergistic activity. A prospective randomized evaluation comparing this combination with standard single agent treatments would seem prudent to interrogate this potential. The immunomodulatory effects of the TKI toceranib, as a single agent or in combination with metronomic CYC, have not been previously investigated in dogs. In one trial, TKIs and metronomic dosing of cyclophosphamide (CYC)were investigated for improvement in tumour control by suppression of regulatory T cells (Treg) and restoration of T cell-mediated immune responses in mice and humans and results have shown administration of toceranib significantly decreased the number and percentage of Treg in the peripheral blood of dogs with cancer. Dogs receiving toceranib and CYC showed a significant increase in serum concentrations of IFN-α , which was inversely correlated with Treg numbers after 6 weeks of combination treatment. In addition to antitumor effects,

these data support further investigations into the immunomodulatory effects of toceranib, administered alone or in combination with CYC in dogs with cancer. In one prospective clinical multicentre trial, tolerability of combination hypo fractionated radiation therapy together with toceranib phosphate was investigated in non-resect able MCTs. Toceranib was administered for 1 week before initiating RT, consisting of 24 Gy delivered in 3 or 4 fractions. The overall response rate was 76.4, with 58.8% of dogs achieving a complete response and 17.6% a partial response. The median time to best response was 32 days, and the median progression-free interval was 316 days. The overall median survival time was not reached with a median follow-up of 374 days. Response rates and durations were higher than those reported for toceranib as a single-agent treatment for MCT only be done under the supervision of an experienced cancer chemotherapy physician (Keller *et al.*, 2004)

Combination Therapies

These therapies where one of the tyrosine kinase inhibitor is combined with any chemotherapeutic agent has shown some synergistic effect in the treatment regimes. Masitinib given with some chemotherapeutic agents has delivered synergistic growth inhibition in various canine cancers, possibly through chemosensitization. A phase I study was conducted by to evaluate the safety and dose limiting toxicity(DLT) of combination protocol with vinblastine and toceranib phosphate in canine MCTs. The DLT for the simultaneous combination in this study was found to be neutropaenia. In one trial, tyrosine kinase inhibitors and metronomic dosing of cyclophosphamide (CYC)were investigated for improvement in tumour control by suppression of regulatory T cells (Treg) and restoration of T cell-mediated immune responses in mice and humans and results have shown administration of toceranib significantly decreased the number and percentage of Treg in the peripheral blood of dogs with cancer. When toceranib and CYC was administered in dogs it showed a substantial increase in serum concentrations of IFN-α, which was inversely correlated with Treg numbers after6 weeks of combination treatment. Besides the antitumor effects, data support further surveys into the immunomodulatory effects of toceranib, administered alone or in combination with CYC in dogs suffering from cancer. In one prospective clinical multicentre trial, tolerability of combination hypofractionated radiation therapy together with toceranib phosphate was investigated in non-resectable MCTs. The overall response rate of Toceranib when given 1 week before initiating RT was 76.4, with 58.8% of dogsachieving a complete response and 17.6% a partial response. The average time for maximum response was32 days, and the average progression-free pause was 316 days. The overall average survival time was not reached with an intermediate follow-up of 374 days. Response rates and durations were higher than those reported for toceranib as a single-agent treatment for MCT only be done under the supervision of an experienced cancer chemotherapy physician.

Conclusion

Advances in molecular biology have led to novel anticancer drugs that target the specific abnormalities responsible for tumor progression. In addition to the clinical approval of variety of latest drugs, efficient approaches for the growth of effective and discriminatory inhibitors with necessary properties have become recognized. Cancer therapy is associated with severe and possibly life-threatening adverse reactions. Although the tyrosine kinase inhibitors have numerous adverse effects, they have improved tolerability compared to other common chemotherapy agents, At the moment it is difficult to compare Masitinib and Toceranib as the trials that have been conducted have targeted different patient's populations, and no 'head-to head' trial has been conducted. Inhibition of signal transduction has become a feasible and striking path in biomedical cancer research grounded on the discovery of a huge number of somatic mutations in many different types of cancer that lead to liberalized growth signal transduction and subsequent unusual growth, incursion, tumor-derived angiogenesis and metastasis. In these three situations it has been conceivable to mode potent and selective tyrosine kinase inhibitors that prevent the catalytic sites of these receptors thereby inhibiting tumor growth. Several challenges remain for such tyrosine kinase inhibitors 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.

Conflict of Interests

There is no conflict of interest.

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