New Chemotherapy Agents in Veterinary Medicine

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Advances in the medical treatment of cancer are constantly arriving. The challenge for the veterinary practitioner is to adapt new therapeutic agents developed in human oncology to the needs of companion animal patients with similar malignancies, or with mutational pathways to the human counterpart disease. The adaptation of traditional anticancer agents, the cytotoxic drugs, is well understood. Often the matter is as simple as discovering doses used in canine models in pre-clinical pharmacology/toxicology studies to arrive at an acceptable clinical dose for use in canine cancer patients. However, the issue with modifying drug doses in cats is a bit more challenging, since liver metabolism of drugs is quite different in this species. The more challenging aspect of adapting new therapies to veterinary oncology is the fact that many of the advances recently achieved in human oncology encompass biologic agents, such as monoclonal antibodies, that have been specifically engineered to work in human patients. Thus, the canine and feline immune systems would be expected to reject these agents as foreign proteins. Also, newly developed small molecular pathway interactive agents are difficult to apply on a routine clinical basis, as these drugs often require a molecular profile of the individual patient's tumor to be able to determine the applicability of a given drug to the patient's tumor. Finally, all new agents are costly, and the economics of veterinary medicine will often make such agents impractical simply from a cost perspective. Still, studies of newer agents are ongoing and merit discussion.

Traditional Cytotoxic Agents – ***While not new in human medicine, the following anticancer agents with traditional cytotoxic mechanisms of action have been used increasingly at Michigan State University.***

Ifosfamide was synthesized in the mid-1960's as an isomer of cyclophosphamide. Initial studies with ifosfamide indicated severe dose-limiting urothelial toxicity. Concurrent administration of the free radical scavenger 2-mercaptoethanesulfonate (mesna) has resulted in the safe utilization of the compound in oncologic practice. Ifosfamide has been demonstrated to be more effective than cyclophosphamide for treatment of sarcomas and testicular tumors in human medicine, and it is therefore becoming more widely used in human and veterinary oncology.

Mechanism of Action: The mechanism of action is presumed to be the same as for cyclophosphamide. Subtle differences in the molecular pharmacology of the two drugs are possible because of the different locations of the two chloroethyl chains on ifosfamide.

Pharmacokinetics: Activation of the ifosfamide prodrug is by hydroxylation in the liver by cytochome p450. Ifosfamide becomes a strong electrophile by forming carbonium ions or transition complexes with target molecules in tissues. This results in the formation of covalent bonds through the alkylation of phosphate, amino, sulfhydryl, hydroxyl, carboxyl and imidazole groups. Its cytotoxic properties are due to its alkylating effects on DNA. Approximately 50% of the dose of active ifosfamide is excreted in the urine. The half-life in humans is schedule dependent; at high doses in human medicine the terminal half-life is 16 hours, while divided daily doses yield a terminal half-life of 7 hours. Ifsofamide has been used

in veterinary medicine at doses of 350-375 mg/m2 IV in dogs, along with a vigorous intravenous diuresis protocol using mesna as a urothelial protectant. Surprisingly, dose escalation studies have shown the dose appropriate for cats is 900 mg/m2 IV, with the diuresis protocol described hereafter. The diuresis protocol reported used mesna intravenously at 20% of the calculated mg dose of ifosfamide with 0.9% NaCl at a rate of 18.3 ml/kg/hour for 30 minutes prior to ifosfamide administration. Ifosfamide is given over 30 minutes, while intravenous saline diuresis is continued at the above infusion rate, with additional bolus doses of mesna as above at 2 and 5 hours. This protocol is repeated at 3-week intervals. The mechanism of resistance is presumed to be the same as for cyclophosphamide. Cross-resistance with cyclophosphamide is considered likely, although not absolute for all cases.

Toxicity: The primary toxicities of ifosfamide are myelosuppression and urothelial toxicity, but alopecia, nausea, vomiting and CNS effects have also been seen in human medicine. Ifosfamide can cause a Fanconi-like syndrome, as well as tubular and glomerular damage.

Indications: Ifosfamide is used in human medicine for any indications appropriate for cyclophosphamide use. Ifosfamide appears to be more active than cyclophosphamide for treatment of human testicular cancers and sarcomas. This drug has been noted to induce remissions in metastatic hemangiosarcoma and osteosarcoma patients.

Contraindications: Known hypersensitivity reactions. Severe leukopenia, thrombocytopenia and severe renal and/or hepatic impairment. Ifosfamide should not be used in cases with known hemorrhagic cystitis. The drug should not be administered to patients with known renal insufficiency.

Paclitaxel *(Taxol) was discovered in the late 1960's as part of a National Cancer Institute screening program involving 35,000 natural materials. It was initially approved for treatment of carcinomas of the ovary, breast, and lung in human medicine. The drug is commonly used to treat an expanding list of human cancers, and is finding more applications in veterinary oncology as well.*

Mechanism of Action: Paclitaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells that it is essential for mitotic and interphase cellular functions. Taxol binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells.

Pharmacokinetics: Paclitaxel is highly lipophilic and insoluble in water. It is therefore available only in a diluent of polyoxyethylated castor oil (Cremaphor EL). The diluent has direct cytotoxic and anaphylactogenic effects in and of itself, and contributes therefore to both the mechanism of action and significantly to the toxicity profile of the drug. The dose for dogs is reported to be 1.13 mg/kg, infused intravenously diluted to 0.6 mg/ml after extensive pretreatment with antihistamines and corticosteroids to prevent anaphylactic reaction. The dose in cats has been reported to be 5 mg/kg. Cats are much less sensitive to the anaphylactoid effects of paclitaxel. The pretreatment protocol involves 5 days of oral prednisolone at 1 mg/kg, oral diphenhydramine 1 mg/kg and oral famotidine 0.5 mg/kg once daily for 5 days as a preparatory regimen. After parenteral administration, paclitaxel is metabolized in the liver by the cytochrome P450 system. It is avidly bound to proteins and less than 25% of the administered dose is recovered in urine as unchanged drug. Paclitaxel has a wide volume of distribution and biphasic plasma clearance, with half-lives of 1-6 hours and 5-17 hours for initial and terminal

elimination phases, respectively. Hepatic metabolism is extensive, with high concentrations of the drug in bile.

Docetaxel is an semisynthetic analog of paclitaxel that has been used in Europe and is reportedly less anaphylactogenic than the original compound. Docetaxel has been administered at 30 mg/M2 IV.

A new formulation of paclitaxel in under development in Europe with an indication for veterinary application. This agent is solubilized without the use of cremophore, and thus should be much less toxic. The dose of this investigational agent is 175 mg/m2 IV over 15-30 minutes every 21 days. Studies of this agent are ongoing but very promising response rates have been noted in the pilot studies.

Toxicity: Dose limiting toxicities include myelosuppression, hypersensitivity reactions, arrhythmias, and neuropathy in humans. The hypersensitivity reaction requires pretreatment prophylaxis with corticosteroids, diphenhydramine and an H2-receptor antagonist. Hypersensitivity reactions seen include head shaking, pruritus, edema, erythema, hypotension, bronchospasm, and bradycardia. Anorexia, nausea and vomiting can be seen. Hypersensitivity reactions may be dependent on rate of administration, and slow infusion is recommended.

Indications: Therapeutic indications in veterinary medicine are still being explored, but it appears that paclitaxel may be useful for treatment of a variety of carcinomas in dogs and cats, including mammary and pulmonary carcinomas.

Contraindications: Known severe hypersensitivity reactions, myelosuppression, and significant liver dysfunction are contraindications.

Gemcitabine ***(2,2'-difluorodeoxycytidine) was synthesized as an analogue of cytarabine. It was the first drug licensed based not sole on antitumor efficacy but also on improved quality of life scores in the pivotal study of human patients with pancreatic carcinoma. The drug was licensed for human use in the late 1990's.***

Mechanism of Action: Gemcitabine is incorporated into the replicating DNA chain in place of the normal deoxycytidine base, causing chain termination. It also increases its own intracellular concentration by a positive feedback loop of activation. The drug requires intracellular phosphorylation by deoxycytidine kinase in order to achieve the activated triphosphate form. The drug blocks ribonucleotide reductase function, which depletes the normal cytidine base. This results in greater incorporation of the gemcitabine in the DNA as fewer molecules of the normal base are available to compete for insertion. Resistance appears to be due to reduced nucleoside transport into the cells, and also by decreased activity of the enzyme deoxycytadine kinase.

Pharmacokinetics: Gemcitabine is administered by intravenous bolus or short (30 minute) infusion. The drug appears to be tolerated by both dogs and cats, and doses of 300-400 mg/m2 as a 20 minute infusion have been reported for weekly administration as a single agent. Dose and schedule optimization studies are ongoing and the reader is referred to the current veterinary literature or consultation with a veterinary oncologist for current dosing recommendations. The drug has a short plasma half-life and is cleared by a two-compartment model. Longer infusions result in longer half-life and significantly greater myelosuppression due to increased exposure of normal marrow cells passing through S-phase of the cell cycle. Gemcitabine is metabolized almost exclusively in the kidneys. This drug is synergistic in combination with carboplatin, where we have employed a fixed doublet dose of 2 mg/kg gemicitabine over 20 minutes, followed by a four hour delay, then 10 mg/kg carboplatin (dose

not to exceed 300 mg/m2) on a 3 week cycle, with gemicitabine given alone on week 2 followed by a rest week.
Toxicity: Myelosuppression is dose limiting. Nausea and vomiting are rare and generally mild.
Indications: The drug is used in human medicine for treatment of pancreatic carcinoma, and has also been used in combination with other drugs for treatment of other gastrointestinal, genitourinary, and respiratory carcinomas. The drug appears to be synergistic with platinum agents and is a very potent radiosensitizer.
Contraindications: Significant hepatic or renal impairment, known hypersensitivity.

Vinorelbine **is a semi-synthetic vinca alkaloid. The drug was invented in France in 1980 and licenced for treatment of lung cancer in people in 1991.**
Mechanism of Action: Inhibition of microtubule assembly occurs as a result of binding to tubulin subunits. Vinorelbine arrests cells in the G2/M phase of the cell cycle.
Pharmacokinetics: vinorelbine is administered by intravenous bolus. An oral formulation of the drug have been available in Europe since 2004 as well. The drug appears to be tolerated by both dogs and cats, and doses of 15 mg/m2 IV as a 5 minute infusion has been reported for weekly administration as a single agent. The drug has a plasma half-life and is of approximately 24 hours. Vinorelbine is metabolized in the liver and excreted largely into the bile, with a small amount of renal excretion also. We have used the drug as a weekly injection on a "3 week on, one week rest" schedule.
Toxicity: Myelosuppression is dose limiting. Nausea and vomiting are rare and generally mild. Extravascular injection results in perivascular injury and the drug induces phlebitis. Peripheral neuropathy has been reported in human patients.
Indications: The drug is used in human medicine for treatment of carcinomas, particularly of the lung, breast and prostate. We have seen responses in pulmonary carcinoma, metastatic mammary tumor, and also long term control of pleural effusion in thoracic mesothelioma.
Contraindications: Hepatobiliary dysfunction, known hypersensitivity.

Temozolomide ***is the oral formulation of the active form of the imidazole carboxamide alkylating agent dacarbazine (DTIC). This drug was licensed for use in the United States in 1999 for treatment of anaplastic astrocytoma and glioblastoma multiforme. Since its original licensure, the drug has found expanded utility in the treatment of refractory lymphoma and sarcomas in veterinary oncology.***
Mechanism of Action: Temozolomide inhibits DNA and RNA synthesis by creation of 06-methyguanine adducts in DNA. Thus, this drug is cell cycle phase nonspecific.
Pharmacokinetics: Dacarbazine is a prodrug and is biotransformed by liver microsomal enzymes into MTIC. Temozolamide does not require hepatic enzyme activation and therefore has a more predictable pharmacologic profile than dacarbazine. The drug has minimal binding to plasma proteins and tissues, and the half life of the active compound is 1.8 hours. The drug is spontaneously degraded in the body to inactive compound, so elimination is not a concern for this agent. The drug depletes the DNA repair enzyme 0-6 alkylguanyl alkyltransferase, which means that over the course of 5 days of drug administration every 21 days, the pills at the end of the 5 day administration cycle have more profound effect than those administered initially. As the agent causes DNA injury, the repair enzyme is depleted to late injuries are more damaging to the cancer cells as well as to normal replicating cells in the marrow.

Toxicity: Myelosuppression and gastrointestinal signs can be seen in association with temozolomide use. The drug is best absorbed on an empty stomach, but it is a gastric irritant. Thus it is recommended that the patient be fasted for at least one hour prior to drug administration, then fed in 20-30 minutes. Metaclopromide is helpful as an antiemetic. If the capsules are chewed by the dog, the drug can act as a mucosal irritant to care must be taken when pilling dogs. In cats, we have observed apparent cardiac adverse effects and pleural effusion in 20% of cats treated. We are currently not recommending this drug for use in cats until further study to predict the risk of significant toxicity is completed.
Indications: Temozolomide has been used to treat malignant melanoma and lymphomas in the dog, and has shown some effect in combination with doxorubicin and vincristine to treat hemangiosarcoma.
Contraindications: Known hypersensitivity to the drug and myelosuppression prior to administration are contraindications.

Small Molecular Inhibitor Therapy – While a variety of pathways have been established as anticancer treatment targets, signaling molecules in the receptor tyrosine kinase class seem to be the closest to providing a window to clinical applicability. The promiscuous RTKI imatinib mesylate (Gleevec) has been used in cats and dogs with mast cell disease with some success. The dose in cats is 10 mg/kg daily, but remission in visceral mast cell disease may take several weeks to be detectable. In the dog, the dose used has ranged from 5 mg/kg to 10 mg/kg daily, with significant hepatobiliary toxicity noted in some instances. Until the underlying mechanism of this potentially lethal toxicity has been identified, the drug should be used with extreme care and clear client informed consent of risk in canine patients.

Masitinib
History: Masitinib is the first drug to be specifically licensed for the treatment of canine mast cell neoplasia. It has been available for clinical use in Europe since 2008 and is currently under review by the FDA for use in the United States. It is manufactured by a small biopharmaceutical company called AB Sciences.
Chemistry: Masitinib is a 2-amino-4-aryl-thiazole with a molecular weight of 498.66 Daltons, and belongs to the family of tyrosine kinase inhibitors, a rapidly expanding class of antitumor agents.
Mechanism of Action: The primary target for Masitinib is the tyrosine kinase receptor c-kit, whose ligand is stem cell factor (SCF). Activation of this receptor is pivotal in regulating the growth, differentiation, adhesion, motility and cell death of mast cells. It has been shown that 30% of canine malignant mast cells express a mutated isoform of c-kit that leads to constitutive activation of the receptor in the absence of ligand binding. This in turn leads to dysregulation of key physiological processes, ultimately allowing uncontrolled cellular growth and resulting in more aggressive tumor biology. Masitinib acts by potently inhibiting the phosphorylation of various isoforms of c-kit including both wild and mutant types (most common being at exons 8, 9 or 11), blocking the intracellular signals driving tumor progression. It also has affinity for additional receptors such as platelet derived growth factor (PDGF) and the cytoplasmic kinases Lyn, Fyn and Lck. Inhibition of these pathways may further limit activation of mast cells.
Pharmacokinetics: Masitinib is labeled for oral use and achieves peak concentration approximately 1 to 2 hours after administration in both dogs and cats. The oral bioavailability is reported to be 83%, with wide distribution throughout the body and rapid elimination (only

trace amounts detectable 24 hours post-dose). Masitinib is highly bound to plasma proteins (approximately 93%). Excretion is predominately through the feces, with the parent compound accounting for nearly 50% of the material excreted. Several metabolites are detected including a carboxylic acid derivative in the urine and N-desmethylated form in the feces that retain biologic activity.

Toxicity: Gastrointestinal signs including vomiting, diarrhea and loss of appetite are noted commonly, but are typically mild and self-limiting. A protein loss syndrome has been observed in dogs receiving Masitinib. In some cases toxicity can be dose-limiting, with recommendations to discontinue therapy if severe hypoalbuminemia (<0.75) noted. Although rare, hemolytic anemia has been documented and also warrants discontinuation of therapy. Additional known toxicities include elevation of liver transaminases and/or neutropenia. In most cases Masitinib seems to be well tolerated. Drugs that interact with the same CYP450 isoenzymes (2C9, 2D6 and 3A4) as Masinitib should be avoided if possible.

Indications: Masitinib is indicated for use in the treatment of non-resectable local and metastatic canine mast cell neoplasia, particularly those tumors carrying a mutation of c-Kit. It is also in clinical trialing for treatment of canine atopic dermatitis and human inflammatory diseases including rheumatoid arthritis, mastocytosis, asthma and psoriasis. Several additional clinical trials are being proposed to evaluate efficacy in canine T-cell lymphoma, canine melanoma and canine hemangiosarcoma.

Contraindications: Currently the use of Masitinib in combination with other myelosuppressive agents, including traditional cytotoxic chemotherapies has been unexplored, and should be approached with caution. Masitinib should be avoided in patients with history of renal disease or known episodes of proteinuria. It should not be used in pregnant bitches or dogs intended for breeding.

Toceranib

History: Developed by Pfizer to target the tyrosine kinase receptors c-Kit, VEGF and PDGF, Toceranib is the first drug of its class to be licensed for use in veterinary medicine, and the first dog-specific anticancer drug licensed in the U.S. in general. It is expected to be commercially available in the United States in 2010 and is already available for use under the guidance of veterinary oncologists at several institutions.

Chemistry: Toceranib (5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-*N-(2-pyrrolidin-1-ylethyl)-1H-pyrrole-3-carboxamide),* also known as SU11654, has a molecular weight of 494.46 Daltons and belongs to the class of tyrosine kinase receptor inhibitors.

Mechanism of Action: Similar to Masitinib, Toceranib exerts its effect by interfering with the phosphorylation of various tyrosine kinase receptors, including c-Kit, VEGF-R, PDGF-R, and FLT3. It blocks phosphorylation by directly interfering with ATP kinase and the intracellular domain of the receptor kinase molecule. As with Toceranib this leads to cessation of intracellular signaling for growth and differentiation driving the disease progression. Cell death occurs via cell cycle arrest and apoptosis. As with some other tyrosine kinase receptors it is reported to have anti-angiogenic properties.

Pharmacokinetics: Toceranib is licensed as a tablet and is widely distributed, reaching maximum serum concentration in about 4-6 hours after oral administration. Oral bioavailability is 77% and the compound is highly protein bound in serum (91-93%). The elimination half-life after oral administration is about 16 hours, with linear pharmacokinetics up to a 5 mg/kg

dosage. Excretion occurs through the vomit, feces and urine

Toxicity: Gastrointestinal toxicity including diarrhea, vomiting, weight loss and anorexia are possible. Diarrhea is observed most commonly (about 46% of patients) and can be dose-limiting and fatal if untreated. Myelosuppression, with neutropenia most commonly seen, is generally mild with no reports of Grade 3 or 4 toxicity during clinical trialing. Rare toxicity includes elevation of ALT, hypoalbuminemia and musculoskeletal disease. In addition, although not a direct toxicity of the drug, patients can experience severe signs related to degranulation of mast cell tumors during treatment.

Indications: Toceranib is licensed for the treatment of Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement in dogs. A phase 1 clinical trial also demonstrated limited efficacy against canine soft tissue sarcoma, mammary carcinoma and multiple myeloma. Given its activity against several tyrosine kinase receptors, its usefulness is likely to extend beyond the treatment of mast cell neoplasia, but awaits further study.

Contraindications: Toceranib should not be used in dogs that are pregnant or lactating, or intended for breeding. It is not recommended for patients with visceral mast cell disease and should not be used in patients with preexisting diarrhea or gastrointestinal bleeding. As with Masinitib, its use in combination with traditional cytotoxic drugs should be approached with caution.

Concurrent medications are recommended to be used with toceranib due to significant risk of gastrointestinal toxicity. The current recommendations from Dr. Cheryl London, the key developer of this drug in veterinary medicine, is to first try to treat dogs that have minimal residual tumor burden if at all possible, due to the risk for significant degranulation effect with toceranib. Also, it is recommended that the starting dose be 2.5 mg/kg given on an every other day basis, or even on a MWF basis, rather than the 3.25 mg/kg dose given daily as per the manufacturer's recommendations. The lower dose has similar biologic activity to what is seen with the higher dose, but the toxicity profile is much more tolerable. Additionally, Dr. London recommends premedication for at least 5 days, but out to 2 weeks, with corticosteroids, antihistamines of H1 and H2 class, and potentially even a proton pump blocker (omeprazole). Prednisone is recommended to be given on the off day of toceranib administration. Loperamide is the currently recommended agent for managing diarrhea induced by toceranib, and it should be administered on days of toceranib administration as needed for diarrhea. This is because gastrointestinal toxicity associated with toceranib has been linked to perforating gastric ulcers and even death of some patients. It is further recommended that clients be advised of the GI toxicity risk, and thus be on the alert for any level of GI signs, including anorexia as a minimum, and also vomiting and diarrhea. In face of GI signs drug administration should be halted (a drug holiday) and gastroprotectants should be used until all signs resolve. At that time, toceranib therapy can be reinstituted with a dose reduction and/or increased interval between doses. These modifications have resulted in a much more favorable toxicity profile for the drug.

Antitumor Vaccines-

Long-term survival of dogs with advanced malignant melanoma was achieved after DNA vaccination with xenogeneic human tyrosinase antigen, available under limited license in the

United States from Merial. This vaccine is administered with a needleless injector system into the muscle of the dog, where the human tyrosinase gene is expressed and causes an immune response against the foreign species' protein. The therapeutic vaccines are administered every 2 weeks for 4 treatments, then every 6 months thereafter as booster doses. In the phase one trial for the vaccine, median survival for 9 dogs treated was 389 days, with complete responses reported and greater that 588-day survival for one dog with bulky non-resectable disease. Dogs that demonstrated antigen specific antibody responses were more likely to have positive responses clinically. Subsequent clinical trials have documented that dogs with local and regional disease control (with effective surgery and radiation therapy) with residual microscopic or presumed microscopic disease have greater that 2-year median survival time. Dogs with metastatic or non-resectable gross disease have typically fared much worse than those with microscopic (or non-existent) residual disease. New modifications on the xenogeneic vaccine strategy are being investigated, with a reported median disease free interval of 389, 153, and 224 days for dogs with Stage II- IV tumors, treated with the human tyrosinase DNA, a murine GP75 DNA vaccine, or a murine tyrosinase vaccine, respectively.

Gene therapy has also been employed experimentally in dogs with malignant melanoma. Intratumoral administration of DNA encoding bacterial superantigen of staphylococcal enterotoxin B combined with either genes for GM-CSF or IL-2 resulted in an overall response rate of 46% in 26 dogs treated. Intratumoral infiltration with CD4+ and CD8+ T cells and macrophages was noted, as well as high levels of antitumor cytotoxic T lymphocyte activity in peripheral blood. Dendritic cell vaccines have also been attempted, using ex-vivo differentiated bone marrow or peripheral blood derived dendritic cells have been created by exposing blood or bone marrow mononuclear cells to differentiation conditions in cell culture. These dendritic cells are then loaded with antigens (cell extracts, nucleosome fragments from apoptotic bodies created in primary tumor culture) then used as active specific tumor vaccine therapy against melanoma.