

Novel drug approaches in veterinary cancer therapy

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INTRODUCTION

In the last six years there have been a number of developments in the field of medical therapy for veterinary cancer. These developments represent the first sorties into new medical territories. The resulting pharmaceutical agents or strategies are pioneer therapies which merit interest not only because they represent novel approaches to cancer therapy but also because they have yielded positive evidence of progress in disease control.

The purpose of this article is to introduce three new anti-neoplastic therapeutic strategies, to outline the differences between new and old and to provide clinical advice where appropriate. The therapeutic paradigms to be addressed are:

Metronomic chemotherapy;

Tyrosine kinase inhibitors; and,

Liposomal nanoparticles.

METRONOMIC CHEMOTHERAPY

The concept of metronomic, or 'low-dose' chemotherapy is based upon the observation that certain conventional cytotoxic agents, when administered at low doses, can exert an anti-angiogenic effect. This was first demonstrated in 2000 (Browder 2000) though the work that preceded this revelation can be traced back to 1971 when Judah Folkman proposed the critical importance of a pro-angiogenic phenotype to the development of cancer (Folkman 1971).

There are multiple proposed mechanisms of action of metronomic chemotherapy. The first is an anti-proliferative effect targeted to activated tumour blood vessel endothelial cell precursors. These are cells that are shed from the bone marrow and migrate to the site of a developing tumour under chemical instruction from the developing tumour itself. These endothelial precursor cells are not neoplastic but they become critical structural elements that permit the growth of the neoplastic cells, both in the primary tumour site and at distant metastatic sites. There is believed to be a direct chemical effect by the metronomic chemotherapy against the proliferation of these cells. The continuous low-dose administration schedule allows continuous control of these cells rather than permitting periods of rapid progressive growth, as typically occurs in cases receiving conventional doses of chemotherapy. Drug resistance that may be present in the

neoplastic cells is not expected to have an influence on the sensitivity of endothelial cell precursors to metronomic chemotherapy (Browder 2000).

The second mechanism of action is an increase in the production and release of endogenous angiogenesis inhibitors (Ng 2004). Angiogenesis is described as a process in balance. Under normal conditions of homeostasis proangiogenic and antiangiogenic factors are produced in equal amounts and a state of vascular dormancy is maintained. For a successful cancer to develop there must be an angiogenic switch which leads to an excess of proangiogenic factors and a drive to the development of new blood vessels. Multiple endogenous antiangiogenic factors have now been identified of which one in particular, thrombospondin-1, has been shown to induce endothelial cell apoptosis. Thrombospondin-1 is upregulated in response to low-dose cyclophosphamide with consequent endothelial cell apoptosis and tumour growth suppression (Hamano 2004).

Other suggested mechanisms of action include a normalisation of anti-tumour immunity and induction of a dormant tumour state (Pasquier 2010, Burton 2011). Ongoing research is directed at understanding the true mechanisms of action of metronomic chemotherapy; this information will allow the more intelligent design of improved drug products and treatment schedules. Most of the published work in metronomic chemotherapy reports research studies using laboratory rodents and tumour xenografts. In the veterinary arena there are five published studies reporting the results of metronomic chemotherapy in multiple dogs. The first two reported metronomic chemotherapy following splenectomy for splenic haemangiosarcoma (Lana 2007) and following resection of soft tissue sarcomas in dogs (Elmslie 2008). There are two papers reporting use of metronomic chemotherapy in an eclectic collection of tumour presentations in dogs, one reporting daily doses of lomustine (Tripp 2011) and one reporting daily doses of chlorambucil (Leach 2012). None of these papers provide compelling evidence of a superior effect of metronomic chemotherapy over what might be termed standard care. However, there is some evidence of efficacy at least. In the case of dogs with haemangiosarcoma, the survival of treated cases was comparable with historical controls treated by adjuvant doxorubicin chemotherapy (Lana 2007). Cases were few and superior outcomes have

been reported in other studies so one must be cautious about assuming that a true benefit existed in this study population. In the Elmslie study (2008) there was a marked statistically significant difference between the survival outcomes for cases that did and did not receive adjuvant metronomic chemotherapy. However, the improved outcome reported for patients receiving metronomic chemotherapy were comparable with outcomes reported for similar cases that have not received chemotherapy (Chase 2009, Stefanello 2009, McSparran 2009). In the study reporting metronomic lomustine therapy, there was too much inconsistency in the management of the enrolled patients to draw any conclusions about efficacy. The purpose of the study was to assess toxicity rather than treatment effect. However, an assumption of efficacy is made due to the prolonged survival reported for some patients (Tripp 2011). In my opinion, the chlorambucil studies were interesting but failed to provide sufficient evidence of efficacy. There was, however, good evidence of treatment tolerability. Further work will hopefully provide more compelling evidence of a treatment benefit (Leach 2012, Schrempp 2013).

Treatments used include:

Drug Combination	Indication	Reference
Cyclophosphamide 12.5-25mg/m ² SID alternating with Etoposide 50mg/m ² SID every three weeks Piroxicam 0.3mg/kg SID	Splenic haemangiosarcoma in dogs post-splenectomy	Lana 2007
Cyclophosphamide 10mg/m ² SID Piroxicam 0.3mg/kg SID	Incompletely resected soft tissue sarcoma in dogs	Elmslie 2008
Lomustine 2.84mg/m ² SID	Assorted neoplastic complaints in dogs	Tripp 2011
Chlorambucil 4mg/m ² SID	Assorted neoplastic complaints in dogs	Leach 2012
Chlorambucil 4mg/m ² SID	Transitional cell carcinoma of the urinary bladder in dogs	Schrempp 2013

Although the classic acute adverse effects associated with conventional cytotoxic chemotherapy are avoided by the use of metronomic chemotherapy protocols, they are not risk free. Mild to moderate gastrointestinal complications are seen in up to 25% of cases. Cyclophosphamide-based treatment still induces a risk of sterile haemorrhagic cystitis. Protracted metronomic lomustine was associated with potentially life-threatening thrombocytopenia. Currently, metronomic chemotherapy remains an area of active research. In the veterinary arena, there is still an insufficient body of evidence for explicit treatment recommendations. It is important to note that none of the treatment strategies reported incorporate drugs licensed for administration to dogs or cats.

TYROSINE KINASE INHIBITORS

Historically medicine has been practised by empirical methods: using the fundamental scientific principles of observation, rationalisation, hypothesis and challenge, products with medicinal properties have been administered and refined and in parallel with this so has our understanding of the diseases we are treating. Most medications are derived from natural products or are synthetic compounds that have been developed to replicate the biochemical effects of a previous product with known medicinal properties.

In 1991 work commenced to design a molecule for the treatment of chronic myelogenous leukaemia (CML) in humans. The molecule was not derived from a previously employed but inferior product; instead it was derived by completely new computer design methods. CML typically occurs through a single chromosomal translocation that results in a mutated and thus perpetually activated growth factor receptor. The three dimensional structure of this mutated receptor was defined and then a molecule was designed to fit into and obstruct the active site of the mutant protein in an exquisitely specific manner so to minimise off-target interactions and side effects (Figure 1). The molecule, STI571, then renamed imatinib (Gleevec, Novartis) was granted FDA approval in 2001 and it has revolutionised the management of its target disease (Druker 2001).

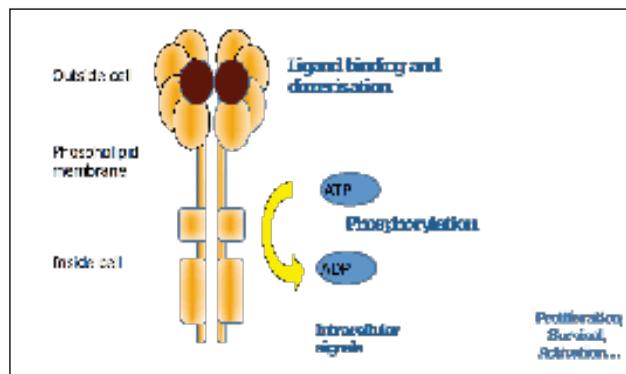
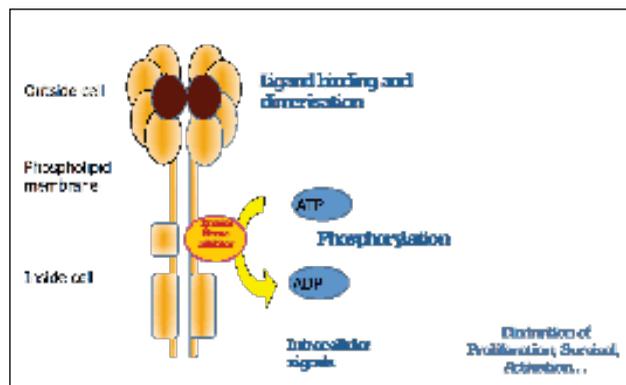


Figure 1. Diagram illustrating mode of action of tyrosine kinase inhibitor drugs. Part i: A mutation in the receptor molecule leads to constitutive activation of a growth or survival pathway.



Part ii: In the presence of a selective inhibitor molecule, the constitutively activated molecule is inhibited; nuclear transduction of growth or survival signals does not proceed.

The mutated growth factor receptor is a member of a 'superfamily' of growth factor receptors that biochemists refer to as tyrosine kinase receptors making reference to their method of activation and signal transduction. Gleevec was the first of nearly 30 tyrosine kinase inhibitors (TKIs) now licensed for medical use, two of which are licensed for administration to dogs for the treatment of cutaneous mast cell neoplasia.

Extending the CML model, a comparable mutation has been demonstrated in the cell surface growth factor receptor, c-kit, in a number of canine cutaneous mast cell tumours (London 1999). Early work indicated that approximately 8-12% of all mast cell tumours exhibited the mutated growth factor receptor but subsequent work has demonstrated that there is a very strong association between c-kit mutation and higher grade tumours (Zemke 2002).

Masitinib (Masivet, AB Science) is a highly selective inhibitor of mutated c-kit. It was the first TKI to reach the veterinary market. Licensed in Europe in June 2009 it has achieved extraordinary results in a selected population of canine cutaneous mast cell tumour patients (Vos, personal communication). The second veterinary TKI, also licensed for treatment of canine cutaneous mast cell tumours, is toceranib (Palladia, Pfizer).

In the Palladia and Masivet clinical trials, approximately 65% of mutated cases responded. In the Masivet trial 20% of non-mutated cases demonstrated a response compared to 39% in the Palladia trial. The reason for the difference in response among non-mutated cases is likely to be attributable to the broad spectrum of action of Palladia: in addition to its action against c-kit, there is also inhibition of a number of other tyrosine kinase receptors including Vascular Endothelial Growth Factor Receptor (VEGFR), resulting in an anti-angiogenic effect as well as growth inhibition through c-kit antagonism.

In addition to the efficacy shown against the tumours for which these agents were intended, there are reports of efficacy in other tumour types. Toceranib, perhaps due to its broader spectrum of target molecules, has a wider range of targets reported, including anal sac gland carcinoma, thyroid carcinoma and squamous cell carcinoma of the head and neck (London 2012).

SIDE EFFECTS

It is an important point to make that a new understanding of side effects is required as these agents work in a new way. One of the side effects of Masivet use, the so-called 'protein-losing syndrome' is a completely new phenomenon that will undoubtedly take time and coordinated investigation to thoroughly explore and get to grips with.

Masivet

Some 12.5% of cases receiving Masivet exhibited grade 3 or 4 side effects; these were 5% gastrointestinal, 5% protein-loss and/or renal and 2.5% haemolytic anaemia. Grade 3 can be loosely described as severe; grade 4 as life-threatening. The mechanism of gastrointestinal

signs is through interference with the c-kit receptor in the intestinal pacemaker cells, the intestinal cells of Cajal. A high proportion of cases exhibited low grade vomiting or diarrhoea but 94% of these spontaneously recovered without requiring treatment interruption or specific therapy. 7.5% developed protein-losing syndrome. It is now clear that the mechanism of the protein-losing syndrome is an effect on capillary fenestration diameter permitting egress of slightly larger molecules including albumin resulting in albumin loss from the circulation into the tissue fluid and into the glomerulus. Protein-losing syndrome is entirely reversible. Haemolytic anaemia remains poorly characterised. The average delay from initiation of therapy to detection of anaemia is 84 days. At the moment the advice must be to withdraw the therapy; failure to do so could result in death of the patient. Anaemia of chronic inflammatory disease is also seen and should not be confused with life-threatening haemolytic anaemia.

Palladia

Some 34.5% of cases with cutaneous mast cell tumours receiving Palladia exhibited grade 3 or 4 side effects; these were mostly gastrointestinal. Experience with the drug has identified other adverse events that were not frequently reported as significant findings in the first clinical trial including spontaneous haemorrhage, marked lethargy and gastrointestinal ulceration. The explanation for the high incidence of severe gastrointestinal side effects is the action of toceranib on blood vessels to the mast cell tumour. Damage to the vascular supply results in necrosis of neoplastic mast cells and degranulation effects. Sudden release of histamine, eotaxin and other inflammatory mediators results in gastric hyperacidity, gastric ulceration, hypotension, renal hypoperfusion, potentially DIC and death. Some key opinion leaders only administer this agent at a reduced dose and only after six weeks of pre-treatment with vinblastine and prednisolone in an attempt to abrogate the risk of these degranulation associated effects. Interestingly, it is my experience that the side effects experienced by dogs with non-mast cell neoplasia appear to be less severe.

In the event that toxic effects are noted, treatment should be withdrawn. Exact duration of the period of withdrawal is dependent on the severity of the manifestation of toxicity.

TREATMENT SUCCESS

Just over 20% of cases receiving masitinib in the published clinical trial remained alive without disease progression at the conclusion of the study two years after commencement of therapy (Figure 2). An interesting observation was also made: contrary to received wisdom when treating cancer with conventional chemotherapy wherein failure to induce tumour shrinkage is regarded as a treatment failure, it was noted that cases which had not developed progressive disease at 12 weeks after commencement of therapy remained in a form of stable disease for an extended period.



Figure 2. Successful management of an ulcerated high-grade mast cell tumour of the perianal skin and soft tissues using a tyrosine kinase inhibitor. In only thirteen days the original mass which measured 79mm in diameter was reduced to a thin slip of granulation tissue dorsal to the anal orifice.

Long-term data regarding the response of mast cell tumours to toceranib are not yet available but it is perfectly feasible that a similar pattern of durable response might be seen.

CASE SELECTION

Candidates for TKI therapy are those which cannot be cured by surgery, exhibit high grade behaviour or have systemic metastases. It is important to consider whether someone else might be able to achieve complete resection of a tumour that you consider unresectable before committing to a course of medical therapy that may fail in an inappropriately selected case.

Patients with severe systemic disease require very careful management. Hepatopathy is a contraindication to masitinib therapy; it is rational to assume that the same applies to toceranib. These agents are metabolised by the hepatic microsomal oxidase system which has reduced capacity in hepatic disease states. This will lead to an inadvertent overdose of therapy and potentially disastrous consequences.

Neutropenia has been described with masitinib and toceranib. In both cases this is not something that is

expected to develop quickly and it will resolve on treatment withdrawal.

LIPOSOMAL NANOPARTICLES

Cytotoxic chemotherapy has been a part of the medical treatment for cancer since the 1950s. There are a number of problems associated with drug delivery to the target tumour which have taxed drug developers since that time. One of these problems is that of selective toxicity. If high concentrations of active drug could be delivered to the target whilst not being delivered to the rest of the body, a marked improvement in therapeutic window would be seen with the resulting capacity to introduce far greater drug doses to the tumour target. For a number of decades now medical scientists have worked to generate suitable carrier molecules which can safely and efficiently deliver drug molecules to the target tissue. Liposomes are 'nano-sized' amphipathic molecules which can be used to solubilise a range of medicinal compounds to achieve improved pharmacological properties (Figure 3).

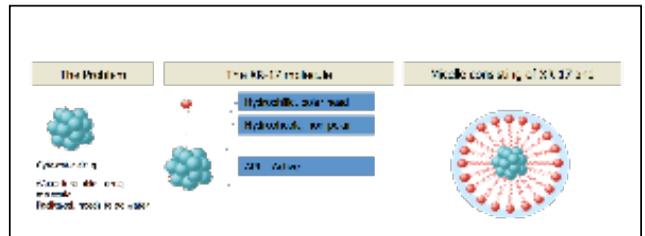


Figure 3. Liposomes can be used to safely solubilise drug molecules for intravenous administration.

In 2012, a study was published reporting a multicentre study examining the role of a novel formulation of the cytotoxic agent, paclitaxel, in their liposomal nanoparticle, XR-17 (Vail 2012). Paclitaxel is a potent cytotoxic agent widely used in human oncology in the treatment of a range of cancers. It is insoluble in aqueous solution and therefore has been formulated in Cremophor EL, a solubilisation agent which causes severe acute inflammatory reactions when administered to dogs. In its standard form, paclitaxel induces significant complications which require anti-histamine pre-medication to avoid potentially lethal anaphylactic complications. In one study, 64% of cases demonstrated acute inflammatory toxicities (Poirier 2004). Paclitaxel therefore cannot be administered intravenously in this form to dogs. XR-17 is a derivate of Vitamin A, with low toxicity, that is easily eliminated by the body. Using the XR-17 excipient, paclitaxel can be safely administered to dogs without acute inflammatory side effects on treatment administration, with early clinical trials demonstrating efficacy in a range of veterinary cancer patients (von Euler pers comm).

The clinical trial identified a statistically significant improvement in outcome using the liposomal nanoparticle-dissolved paclitaxel compared to control dogs that received oral lomustine in a randomised, blinded study. Whilst this is an exciting development in the field of canine mast cell tumours, there are far greater implications as the

XR-17 vehicle has been shown to be a functional molecule permitting enhanced drug delivery to the tumour with proven efficacy and safety. This will undoubtedly now serve as a model for further drug development opportunities. Liposomes lend themselves to modification by further targeting molecules. Tumours and tissues exhibit a series of markers which act as identity 'flags' that can be utilised to direct mobile particles to a specific target. These 'homing signals' would permit yet more refinement in the selective targeting of target tissues.

Liposomal compounds have already been developed which achieve targeting effects and altered drug release characteristics by virtue of their size and stability. The infinite possibilities for product modification for improved or even individualised tumour targeting, will make liposomal nanoparticle medicine an area of continuing active research and great optimism.

CONCLUSION

We are very fortunate to bear witness to a time in clinical veterinary practice when the science of drug design is truly making an impact on the lives and welfare of animals under our care. We stand at the brink of a new era in medicine; it seems reasonable to predict that the pace of change is only going to accelerate in the coming years.

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