In the search for less toxic and more effective cancer therapies, an important goal has been to develop agents that specifically target some characteristic unique to cancer cells and absent from normal cells. As a generalization, most chemotherapeutics are most active against rapidly dividing cells or cells in particular phases of the cell cycle and this gives a relative advantage against tumour cells compared to most normal cells. However, rapid turnover is not unique to cancer cells, and the effect on rapidly dividing normal populations – bone marrow, GI mucosa, reproduction and wound healing - results in the main side effects of chemotherapy.

The first step in the development of a targeted therapy is to identify a tumour-specific target, and the next step is to develop a treatment that exploits it. The search for a target has been underway for decades, and while a target that is absolutely unique to cancer still remains elusive, some promising advances have been made. Once a target is identified, possible approaches to exploiting it include immunotherapy directed against a tumour-specific antigen, and using an inhibitor to block a critical metabolic pathway. Two recent breakthroughs in veterinary oncology each use one of these approaches.

**Oncept Immunotherapy and Melanoma**

Immunotherapy seeks to harness aspects of the immune system in recognizing and attacking cancer cells; using either nonspecific immunomodulators such as cimetidine, TNF and IL-2, or liposome encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE); or a specific approach such as a monoclonal antibody (passive immunotherapy), or a vaccine (active immunotherapy) against a tumour-specific antigen. Attempts at cancer immunotherapy of various cancers in humans and other animals have met with mixed success.

One cancer for which immunotherapy is increasingly being considered an important component of therapy is melanoma, with agents including interleukins, interferons, other cytokines, adoptive immunotherapy, and vaccines all under investigation. Recent advances include better understanding of the value of immune checkpoint proteins such as CTLA4, PD1 and CD40 and using monoclonal antibodies directed against these; and increased understanding of dendritic cells and their use in cancer vaccine development. However significant challenges remain to clinically effective melanoma vaccination in humans, including dysfunctional host immune responses and tumour cell immune evasion.

**Canine Melanoma**

In dogs, oral melanoma is the most common oral malignancy. Unlike cutaneous melanomas, which are often benign, oral melanomas are usually malignant. Aggressive local growth and distant metastasis are common, but recent studies suggest not uniform. Some studies have found a WHO staging scheme prognostic, with significantly longer survival after surgery.
alone for dogs with stage I (<2cm diameter) tumours (median = 17 months) than for dogs with stage II or III (primary tumour >2 cm, or any bone invasion, or lymph node metastases) disease (median = 5.4 months). One study found that dogs with melanoma of the lip lived a median of 25 months (1-year survival = 57.4%; 2-year survival = 34.3%) which was significantly longer than for dogs with melanoma at other oral sites (7 months). Shorter average survival times are seen for dogs with tumours that are larger, have high numbers of mitotic figures per 10 high-power fields (mitotic index), have marked nuclear atypia and more inflammation or necrosis: these characteristics are added into a numerical score with >10 being considered malignant.

While approximately 85% of cutaneous melanomas are benign, subungual (nail bed) melanoma is a notable exception and is often also highly malignant. As for oral melanomas, pathology is important in determining the likelihood of malignant behaviour in cutaneous melanoma. One study made a distinction between the behaviour seen for melanomas of the feet and the lips, from those seen elsewhere in the skin and oral cavity, respectively. Dogs with melanoma of the feet or lips had a median survival of 22 months with a 30% death rate due to melanoma; which was better than for dogs with oral melanoma, but worse than for dogs with cutaneous lesions. In one study, the median survival after surgery for dogs with digital melanoma was 12 months, with 13% alive 2 years after surgery.

Surgery remains the mainstay of local treatment for melanoma. Most dogs with oral tumours are euthanized because of progression or recurrence of local disease, but if surgery is aggressive from the outset, it may prolong survival as well as provide palliation. Likewise for subungual tumours, digital amputation is warranted. Radiation therapy has been shown to improve local control of intraoral melanoma if complete surgery is not anatomically possible. If local control can be achieved, the primary problem becomes the risk of metastasis. When metastatic disease occurs in patients with digital melanoma, it usually occurs within 6 months (median 5.3 months) of treatment. The growth rate of melanoma metastases may vary, and it is probably this variation, rather than the time at which metastasis occurs, that determines survival time if local disease is controlled. Some dogs may tolerate pulmonary metastatic disease with very little apparent impact on their quality of life for some months. The best chemotherapeutic responses reported for melanoma are for platinum drugs. One chemotherapy study reported responses (PR and CR combined) in 7 of 25 dogs (28%) with unresectable oral melanoma, including one dog with a complete response lasting nearly 3 years. Overall median response duration was 5.5 months. Similar response rates have been seen in established metastatic disease. As chemotherapy is always more likely to be successful when disease is microscopic, the outcome in the adjuvant setting would be expected to be somewhat more favourable. A combination of radiation therapy and chemotherapy used as an adjunct to incomplete surgical excision was reported in 39 dogs with incompletely resected oral melanoma. Only six dogs (15%) had local recurrence (within a median time of 4.6 months) and 20 dogs (51%) developed metastatic disease within a median time of 10.2 months. Median survival time for all 39 dogs was 12 months.

### Xenogeneic Murine Tyrosinase DNA Vaccination (Oncept) For Dogs with Melanoma

In early 2010 the USDA gave full licensure to a xenogeneic human tyrosinase DNA vaccine to treat dogs with melanoma, following a period of conditional licensure starting in 2007 during which the vaccine was available to veterinary oncologists in the US for trials. The vaccine is now available in the US as Oncept, produced by Merial, for use by veterinary specialists practicing oncology. The vaccine is given as an initial series of 4 treatments, using a specifically designed needle-less transdermal injector, 2 weeks apart, and then a booster every 6 months.

DNA vaccination is used because DNA is relatively inexpensive and easy to purify in large quantity. The gene is cloned into a bacterial expression plasmid with a constitutively active
promoter. The plasmid is introduced into the skin or muscle where dendritic cells present the transcribed and translated antigen in the proper context of major histocompatibility complex and co stimulatory molecules. The plasmid also contains immunostimulatory sequences that may act as an adjuvant in the immune response.

The Oncept vaccine encodes the melanosomal glycoprotein tyrosinase, which is essential in melanin synthesis. In mice, immunization with xenogeneic human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T-cells against melanoma cells, but immunization with mouse tyrosinase-related DNA did not, showing that xenogeneic DNA vaccination could break tolerance against a self tumour differentiation antigen.\(^\text{10}\)

The first developmental studies of the vaccine in 9 dogs with stage 2, 3, or 4 dogs treated at 3 different dose levels of human tyrosinase plasmid DNA. That study reported that toxicity was limited to mild local reactions at injection sites. One dog with pulmonary metastases (stage IV disease) had a complete clinical response for 11 months, and 2 other dogs with stage IV disease had survivals of 14 months and over 19 months in the face of significant bulky metastatic disease. Two dogs with locally controlled stage II/III disease had survivals of between 16 and 17 months with no evidence of melanoma on necropsy. Four other dogs were euthanized because of progression of the primary tumour. The Kaplan-Meier median survival time for all nine dogs was 13 months.\(^\text{10}\) In a subsequent study, a large number of dogs were vaccinated at varying dose levels of human or mouse tyrosinase or GP75, with or without additional of human GM-CSF. Overall survival time for 33 dogs with loco-regionally controlled stage II-III melanoma across the xenogeneic vaccine studies was nearly 19 months. This was better than historic controls. In addition, dogs vaccinated with human tyrosinase were shown to have 2- to 5-fold increases in circulating antibodies to human tyrosinase.\(^\text{11}\) A later data analysis showed that dogs which received any melanoma vaccine had a median survival time of $>31$ months (median not reached) for stages I and II, $>54$ months (median not reached) for stage III, and just under 8 months for stage IV.\(^\text{12}\)

A study of 58 dogs with digital melanoma demonstrated overall median survival time of nearly 16 months for dogs treated with xenogeneic DNA vaccine following complete loco-regional control, with a 1-year survival rate of 63%. Median survival time for dogs with metastasis was 3.5 months versus nearly 18 months for dogs without metastasis ($P<.0001$).\(^\text{13}\)

Oncept is labeled for use in the treatment of dogs with stage II or stage III oral melanoma and for which local disease control has been achieved. This means that the primary tumour may be of any extent, as long as it is locally controlled using surgery and / or radiation; and that if lymph node metastases are present, the involved nodes have also been removed or irradiated. Dogs with distant metastases are excluded from the label indication. Therefore the goal of vaccination would be prevent local recurrence and delay or prevent the onset of metastases.

As noted above, occasional responses have been seen in macroscopic melanoma. However responses in bulky disease are rare and Oncept is not recommended as a treatment for macroscopic disease.

The use of Oncept in cats has only been reported anecdotally to date.

Table 1. Summary of median survival times of dogs with malignant melanoma treated differently.
<table>
<thead>
<tr>
<th>Oral</th>
<th>Surgery alone</th>
<th>Surgery + RT</th>
<th>Surgery +/- RT</th>
<th>Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>17 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II-III</td>
<td>5.4 months</td>
<td>12 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lip</td>
<td>25 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not lip</td>
<td>7 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital - all</td>
<td>12 months</td>
<td></td>
<td>16 months</td>
<td></td>
</tr>
<tr>
<td>+mets (stage IV)</td>
<td></td>
<td>3.5 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-mets (stage II-III)</td>
<td></td>
<td>18 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All malignant – stage II-III</td>
<td></td>
<td></td>
<td>19 months</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td></td>
<td>&gt;31 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td>&gt;31 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td></td>
<td>&gt;54 months</td>
<td></td>
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</tr>
<tr>
<td>Stage IV</td>
<td></td>
<td>8 months</td>
<td></td>
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</tbody>
</table>

**Palladia**

**Tyrosine Kinases**

Cancer cells rely on proteins that regulate signal transduction, cell survival and cell proliferation; and several key members of this regulatory family that have been identified in various cancers are tyrosine kinases (TKs). Several inhibitors of these proteins (tyrosine kinase inhibitors, TKIs) have now been approved for cancer treatment and have shown significant clinical efficacy.

Tyrosine kinases bind ATP to add phosphate groups to key tyrosine residues on themselves (autophosphorylation) and on other proteins, leading to intracellular signaling and alterations in gene transcription which affect cell proliferation, differentiation and survival. TKs expressed on the cell surface (receptor tyrosine kinases, RTKs) bind growth factors that regulate their activation. These RTKs include Kit, Met and EGFR, all of which are known to be dysregulated in various forms of cancer. Some, such as VEGFR, PDGFR, and FGFR are also important in promoting angiogenesis. TKs are often abnormally activated in malignant tumours through mutation, overexpression, and the generation of fusion proteins from chromosomal translocation. The best-characterized example is the Bcr-Abl fusion in chronic myelogenous leukemia leading to chronic activation of the TK Abl, a key step in the malignant transformation in this disease. Another example, Kit, is normally expressed on haematopoietic stem cells, melanocytes, and mast cells. Dysregulation occurs in human systemic mastocytosis, acute myelogenous leukemia, gastrointestinal stromal tumours (GISTs), and other cancers. Kit mutations have been documented in up to 30% of canine mast cell tumours (MCTs) as well as in canine GISTs and in feline MCTs.

**Tyrosine Kinase Inhibitors**

Various strategies have been explored to exploit this target, but the most successful is the group of drugs called small molecule tyrosine kinase inhibitors (TKIs). These drugs block the ATP binding site of kinases, acting as competitive inhibitors, so the kinase is not able to phosphorylate itself or initiate downstream signaling.

The prototype drug in this group and the first to be used clinically is imatinib (Gleevec / Glivec). Imatinib was designed specifically to target the constitutively active Bcr-Abl fusion protein in CML and is extremely successful clinically. For individuals in the chronic phase of CML, imatinib provides a molecular response for the majority of patients. Imatinib also blocks ATP binding of Kit, providing an overall response rate of 55% plus another 25% - 30% stable disease in GIST (compared to approximately 5% with previous
chemotherapy). There are now more than 8 TKIs FDA approved in the US for use alone or in combination for CML, GIST, ALL, non small cell lung cancer, pancreatic cancer, breast cancer, and renal cell carcinoma. More recently TKIs are being investigated for their role in nonmalignant proliferative diseases such as atherosclerosis, rheumatoid disorders, and pulmonary fibrosis.

In dogs, imatinib is associated with idiosyncratic hepatotoxicity in some dogs, although this is not as common as initially thought. It has been provided responses in dogs with MCTs, including systemic mast cell disease. In cats, a phase I clinical trial in 9 cats with a variety of tumours showed no evidence of hematologic or hepatic toxicity and only mild GI toxicity, and a complete response in 1 cat with systemic mastocytosis. The major drawback to Gleevec use in veterinary medicine is its extremely high cost (~$3800 COST for 30-day supply for a 30 kg dog!).

Two TKIs have undergone registrational studies in dogs with mast cell tumours.

Masitinib (Masivet / Kinavet; AB Science) primarily targets Kit. A placebo-controlled randomized double blind phase III clinical trial in 202 dogs with non-metastatic grade 2 or 3 MCTs showed a 12-month complete response (CR) rate of 11.1% (vs. 2.9% with placebo (not significantly different)), and “controlled disease” (overall response rate (ORR) plus stable disease (SD)) of 23.1% (vs. 5.9% with placebo (significantly different)). Dogs with Kit mutations had 27.3% CR (vs. 0% with placebo (not significant), and 31.8% “controlled disease” (vs. 0% with placebo (not significant). The study did report significantly prolonged time to progression (TTP) of 118 days (vs. 75 days with placebo), which was greater in dogs with Kit mutations (230 days vs. 42 days with placebo). A more recent study reported improved survival in dogs with nonresectable MCTs compared with results for the placebo, with 59 of 95 (62.1%) and 9 of 25 (36.0%) dogs alive at 12 months and 33 of 83 (39.8%) and 3 of 20 (15.0%) dogs alive at 24 months, respectively; and median overall survival times of 20.5 months and 10.7 months. A recent study evaluated safety of Masitinib in healthy cats and found proteinuria in 10% of cats and neutropenia in 15% of cats, as well as an increase in serum creatinine concentration and adverse gastrointestinal effects in some cats; however masitinib was considered tolerated in the majority of cats. Masivet is approved in Europe and the UK for dogs with recurrent or non-resectable grade 2 or 3 MCT regardless of c-kit mutation status, and is being launched as Kinavet in the US, however the drug is not currently available in Australia.

Toceranib (Palladia; Pfizer Animal Health) is similar to sunitinib, in that it is active against members of the split-kinase RTK family (VEGFR, PDGFR and Kit), and has both anti-angiogenic and anti-tumour activity. In a placebo-controlled randomized study in dogs with non-resectable grade 2 and 3 MCTs, 86 Palladia treated dogs had an overall response rate (ORR, complete response (CR) plus partial response (PR)) of 37.2% (7 CR, 25 PR) compared to 7.9% (5 PR) with placebo (n=63). Following placebo escape, the total ORR for all 145 dogs receiving Palladia was 42.8% (21 CR, 41 PR); dogs with Kit mutations had an ORR of 82% (vs. 55% in dogs without mutations), and dogs without lymph node metastasis had higher response rate (67%, vs. 46% in dogs with metastasis). The pharmacokinetics of Palladia in dogs has been reported recently.

Since its commercial release in the US, Palladia has been used with some efficacy in a wide variety of diseases in both dogs and cats. There are multiple abstract reports of Palladia use in a variety of indications in the 2010 Veterinary Cancer Society Annual Conference Proceedings, and overall use of Palladia by US-based specialists is best summarized in the abstract by Johannes et al.
There have been several small reports of Palladia use in cats. In general Palladia is found to be well tolerated in cats, with the most recent report showing a limited adverse event profile and some efficacy in a variety of tumours.  

As targeted therapy, it would be hoped that the toxicity of TKIs would be minimal; however they should best be viewed similarly to other chemotherapeutics in terms of prevention and management of side effects. Both toceranib and masitinib can be associated with gastrointestinal side effects that can be significant, and each has specific side effects. Management requires good supportive care and the judicious use of treatment breaks or adjustments to the dose or schedule, as with other chemotherapy drugs.

Conclusion
The advent of effective tumour vaccine strategies and TKIs in veterinary oncology has generated a huge amount of excitement and anticipation. Although no single agent can be expected to replace all other treatments; the addition of a new, well documented active agent is always most welcome and will allow some patients to be treated that otherwise would not be able to respond to therapy. As they become commercially available in Australia over the coming years, these new agents will undoubtedly find their place in therapy of veterinary cancer patients and are likely to become a valuable component of treatment for many animals.

References