



VETERINARY ONCOLOGY CONSULTANTS

helping veterinarians treat pets with cancer

Newsletter

November 2007

Greetings colleagues!

We hope you're all well. It's been an exciting time for us and we have lots of news to share! We have 2 new clinical trials to announce that will be performed at the Animal Referral Hospital (ARH) in Strathfield.

The first is a chemotherapy dose intensification trial for dogs with lymphoma. This is a first-line therapy trial looking at using bone marrow stimulatory cytokines to increase the dose of chemotherapy that dogs can receive without increased adverse effects, and then looking to show that the increased dose will improve remission and survival times, and cure rates! This is building on a previous study we performed at Tufts where we showed that bone marrow transplantation could increase the dose of chemotherapy that dogs can receive safely, and those dogs did have improved outcomes relative to standard dose chemotherapy – the cure rate was nearly 40%. Now we are hoping to show that this simpler form of haematologic support can confer similar benefits. Dogs will be treated at the ARH and need to see us there to be evaluated for study inclusion before receiving any treatment for lymphoma. The cost for the investigational part of the treatment is generously subsidized by the ARH to keep the cost to the client of the trial protocol approximately equivalent to that for standard dose chemotherapy.

We are also very excited to announce a new collaboration with EnGeneIC Pty Ltd. EnGeneIC has developed a chemotherapy drug delivery vehicle called "minicells" or EDV. These are submicroscopic membrane pockets derived from bacterial cells but there are no bacterial cells in the final preparation and there is no risk of infection from the minicells. The minicells can be loaded with chemotherapy drugs and targeted specifically to cancer cells using antibodies. When they are injected, the antibody-targeted minicells deliver the chemotherapy drugs directly to the cancer cells rather than to the rest of the body. Thus, the total dose of drug given to the

patient is very low, but the effect on the cancer cells can be very potent. Minicells have been used to deliver chemotherapy to dogs previously without significant adverse effects. This study seeks to determine if this novel technology can be used to overcome the difficulties in treat brain tumours with chemotherapy caused by the blood-brain barrier excluding treatment drugs. This is a significant

problem in humans as well as in dogs. Dogs with clinical and MRI findings characteristic of glioblastoma multiforme (GBM), and no major intercurrent disease, will be accepted into the study. Dogs will receive all of their testing and

treatment at the Animal Referral Hospital and must be evaluated for study entry prior to receiving any therapy for their brain tumour. Treatment will involve injection of chemotherapy-loaded minicells up to weekly, with follow up imaging as indicated. Testing (including imaging) to determine if dogs are eligible is at the clients' expense, however the cost of all treatment and follow up testing including imaging will be generously funded by EnGeneIC.

We envision that other minicell trials will follow in the coming months for animals with other cancers. In particular, dogs with chemoresistant lymphoma may be eligible shortly. Please enquire if you have a potential candidate.

Also, our collaborative study looking at therapy for Tasmanian Devils affected with Devil Facial Tumour Disease looks like it will be in progress soon. We are looking very forward to hopefully helping affected animals towards recovery in the near future.

Finally, big news for veterinary oncology in Australia: Animal Cancer Care in Brisbane will be opening their doors for radiation therapy in the coming days. Congratulations Rod, Valerie, and Team!

Best regards, *Tony and Angela*

Veterinary Oncology Consultants' mission is to assist other veterinarians in providing the highest possible quality of life for pets with cancer and their human families, by making evidence- and compassion-based recommendations for their care and providing educational materials.

Summary: Clinical Trials

- Now:** *Brain tumours in dogs
*Untreated lymphoma in dogs
Soon: *Chemoresistant lymphoma in dogs

New Literature

For a long time some veterinary oncologists have promoted the idea of high grade 2 and low grade 2 MCT with little objective data. Studies that look at cellular proliferation (Ki-67 and PCNA) as well as mutations in the gene *c-kit* have failed to provide consistently useful data. This publication gives some hard criteria for us to start to separate out not only grade 2 but also grade 3 MCT. Like all systems it is probably not the whole answer, but it is a practical step forward.

Mitotic index is predictive for survival in canine cutaneous mast cell tumours. Romansk, E et al. Vet. Pathol. 2007; 44:335-341.

Mitotic index (MI) is a strong predictor of overall survival for dogs with cutaneous MCTs. Medical records from 148 dogs with MCTs were reviewed for tumour grade, local recurrence, metastatic disease, date of death, and outcome.

The histologic region of the tumour with the highest mitotic activity was evalu-

ated, and the MI value was defined as the number of mitotic figures/10 high-power (400x) fields.

The MI correlated directly with tumour grade ($P < .0001$).

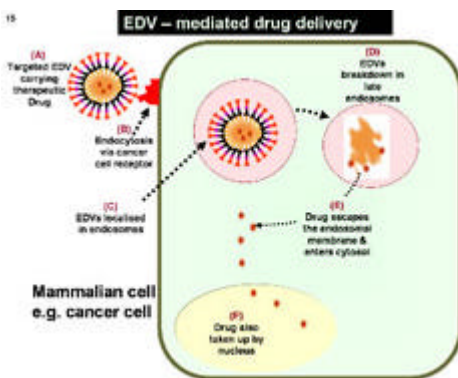
The median survival time for dogs with an MI =5 was significantly longer (70 months) than for those with an MI >5 (2 months), re-

gardless of grade ($P < .001$).

For grade II tumours with an MI =5, the median survival time (MST) was 70 months, compared with 5 months for those with an MI >5 ($P < .001$).

For grade III tumours with an MI =5, the MST was not reached, compared with less than 2 months for those with an MI >5 ($P < .001$).

Tell me more about these "Minicells" . . . *



EnGeneIC's proprietary drug delivery vector, called an EDV (EnGeneIC Delivery Vehicle), is uniquely differentiated from all currently known vectors and drug targeting systems. EDVs are anucleate bacterial minicells which can also be loaded with a variety of conventional and molecularly targeted chemotherapy drugs and targeted to receptors on cancer cells. Once delivered inside the cell via endocytosis the EDVs are broken down in the late endosomes and the chemotherapeutic drugs released into the cytoplasm. The drugs have been shown to remain bioactive and to be cytotoxic to the targeted cells.

Typical doses of drugs delivered via EDVs are around 1000 times less than the dose of the free drug required for equivalent tumour response. Accordingly EDV-delivered drugs show minimal toxic side effects and hence provide an *enormous increase in therapeutic index* in comparison to drug alone.

EDVs are expected to be safe drug delivery vehicles for:

- Targeted delivery of cytotoxics to effect tumour regression in a wide variety of cancers.
- Potential tailor made therapy with

the possibility of using cocktails of different drugs.

EnGeneIC has also developed proprietary scale-up manufacturing technology allowing the production of clinically meaningful doses of EDVs at high purity and low cost.

Principal characteristic of EDVs are:

- 400 nm diameter nanocell.
- Non-living, derived from a biological source i.e. no potential to replicate or be virulent *in-vivo*.
- Anucleate i.e. does not carry any DNA from the parent biological source and therefore unlike viral and bacterial vectors, it cannot revert to virulence through mutations in the endogenous genome.
- Surrounded by a rigid & stable biological membrane. Therefore, unlike liposomes, the EDVs do not fall apart or leak in the extracellular environment and release the payload to non-target cells, i.e. minimal to no toxicity.
- Readily packaged with a wide range of anti-cancer drugs and plasmid DNAs encoding RNAi sequences, prodrug genes etc. This is unlike liposomal and synthetic nanoparticle/polymer vectors where extensive chemical modifications are required to package hydrophobic drugs.
- Readily accommodate payloads ranging from 1 million to 10 million drug molecules per EDV.
- Can be specifically targeted to the desired cancer cells *in vivo* by a bispecific antibody where one arm carries anti-EDV specificity and the other arm has specificity to a tumor cell-surface receptor of choice. This is a potential hurdle with liposomes since attachment of additional

components to the liposomal surface decreases stability of the particles. Targeted EDVs have been shown to be stable and therapeutically effective *in vivo*.

A series of *in vivo* mouse xenograft studies where EDVs have been targeted intravenously and intra-tumorally to Her2 in breast and ovarian cancer xenografts, to EGFR in colon and breast cancer xenografts and to CD33 in leukemia xenografts have shown proof-of-concept of targeted drug delivery using EDVs.

In a previous pilot study done with Dr. Rod Straw, 2 dogs with Stage 4, T-cell lymphoma were treated with canine CD3-targeted, Dox-packaged EDVs. Results were achieved with 2,000-fold less doxorubicin via the EDVs compared to conventional chemotherapy.

*image and information are courtesy of EnGeneIC Pty Ltd

Oncotip : Chemo Flow Chart

Do you have trouble keeping track of what chemotherapy treatments your patients have received, when and how much, what leg was used for intravenous injection, cumulative doses of drugs, whether dose reductions have been made, when they were made and for what reason? It can become confusing at the least, and potentially lethal if a sensitivity to a certain drug ends up buried in the record.

VOC is pleased to offer our Chemotherapy Flow Sheet as a free download from our Website. It is a sheet we have been refining for the last 20 years and we think it offers a good compromise between being succinct and still allowing critical information to be easily accessible. Please let us know if you have suggestions for improvements though; we are always looking for ways to progress!