**Using ribosomal RNA disruption as a predictor of early relapse in canine lymphoma**

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**Introduction:**
      Canine lymphoma is one of the most prevalent types of cancer in dogs, responsible for approximately 9% of all canine cancer, and occurring at an incidence rate of 6-30 per 100,000 dogs. Factors such as age, sex, etc. are not useful in identifying those animals with particularly aggressive disease, or those animals which will respond well to chemotherapy. Canine patients with lymphoma of all subtypes generally undergo chemotherapy with a standard multi-agent sequential "CHOP" protocol consisting of multiple cycles of cyclophosphamide ($250 \text{ mg/m}^2$), doxorubicin ($30 \text{ mg/m}^2$), vincristine ($0.7 \text{ mg/m}^2$), and prednisone (0.5-2 mg/kg), with each drug given approximately weekly. Dogs usually receive four full cycles of CHOP chemotherapy, taking approximately 25 weeks (6 months) to administer.  Effectiveness of therapy is monitored by physical measurement of lymph nodes, and shrinkage is considered a sign of success; complete clinical remission is achieved when lymph nodes reach normal size.
      CHOP chemotherapy is highly effective in achieving initial clinical remission in approximately 80% of treated dogs, although approximately 20% of all treated dogs will not achieve clinical remission, for reasons that are not well understood. Of the 80% of dogs whose lymph nodes return to normal size after CHOP, (i.e. achieve compete remission), almost half will relapse and have disease progression within 8 months of starting chemotherapy, hence requiring a second cycle of chemotherapy. Thus, although CHOP chemotherapy is highly effective for many dogs, second line or 'rescue' therapy is required for those animals that do not achieve remission, and for dogs that relapse and develop resistance to the CHOP agents. Presently, there are no ways to distinguish dogs that will have a prolonged remission from those who will have a short (less than 8 months) remission, or from those that will not achieve remission at all.
      We have been exploring evaluation of predictive biomarkers for canine lymphoma with an Ontario company called Rna Diagnostics (RnaDx; see letter of collaboration). RnaDx has developed a *novel biomarker assay* known as the RNA Disruption Assay (RDA).  Their work suggests that RDA may reflect cancer cell necrosis, and hence, response to chemotherapy. In this study we intend to test whether this RDA biomarker assay will have similar predictive value for canine lymphoma patients undergoing CHOP chemotherapy.

**Objectives**:
      If we could identify those dogs with unsuccessful responses to CHOP earlier in the course of their therapy, we could switch them sooner to an alternative or second line/rescue approach, thus resulting in optimal therapy outcomes for more of our canine lymphoma patients. The objective of this study, therefore, is to correlate changes in RDA with unsuccessful CHOP chemotherapy, as defined by failure to achieve remission and/or relapse from remission within 8 months of initiating treatment

**Materials and Methods**

*Patient characteristics:* Dogs diagnosed with multicentric lymphoma (lymphosarcoma) who have not received prior treatment (including prednisone) for their disease will be eligible for this study. There are no breed, sex or age restrictions, but dogs must not have concurrent or prior neoplasia of any sort. Dogs will receive their full course of CHOP and will be monitored for at least 8 months after the start of therapy to categorize their response, as per standard of care for this disease (*i.e.* determination of clinical status [complete remission, relapse, *etc.*]).

*Sample collection:* Samples will be collected from each dog on day 0 (prior to receiving first chemotherapy), and at the beginning of the second, third and fourth rounds of CHOP chemotherapy (approximately days 0, 42, 84 and 147) for a total of 4 fine needle aspirate (FNA) biopsy samples per patient.

*Statistical analysis:* Associations between maximum RDA and clinical response ('failure of CHOP chemotherapy' [no remission and/or relapse < 8 months] versus 'no failure of CHOP chemotherapy') will be assessed using one-way ANOVA. We will also calculate probabilities, negative and positive predictive rates, false negative rates and confidence intervals for these values. Values obtained from this study will be used to inform design of future clinical trials for this predictive biomarker.

**Anticipated Results and Significance:** We anticipate that tumour samples from dogs that do not demonstrate disrupted RNA between the start of chemotherapy and subsequent CHOP cycles will represent dogs that ultimately fail to achieve remission and/or will relapse within 8 months. Lymphoma is one of the most prevalent cancers seen in dogs, and it is frequently treated with combination chemotherapy. While the standard of care CHOP protocol is highly effective at inducing initial remission in most dogs, approximately 60% of treated dogs will either fail to achieve initial remission, or will relapse within 8 months. Second line 'rescue' therapy can be successful, but at this time we have no way of identifying which dogs should abandon CHOP chemotherapy and switch to alternative rescue regimes, other than waiting for them to fail to achieve remission or to relapse early. *This study is **not** designed to make changes in treatment decisions for dogs with lymphoma.* However, if we find that RDA analysis is predictive of response, then future clinical trials will explore how best to use this biomarker to choose optimal treatment for all dogs with lymphoma. It is also possible that RDA analysis will be informative for treatment outcome in other types of companion animal cancer.

**Note:** Clients will cover cost of initial diagnosis, treatment visits, and CHOP protocol; there will be no cost to clients for FNA or sample analysis.

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