



GRANT PROGRESS REPORT SUMMARY

Grant: 01484: *Identification and Characterization of a Canine Derived Single Chain Antibody that Binds and Neutralizes Canine VEGF*

Principal Investigator: Dr. Nicola J Mason, BVetMed, PhD

Research Institution: University of Pennsylvania

Grant Amount: \$70,907.00

Start Date: 1/1/2011 **End Date:** 6/30/2012

Progress Report: Mid-Year 2

Report Due: 6/30/2012 **Report Received:** 11/18/2012

Recommended for Approval:

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Canine hemangiosarcoma (HSA) is a common and highly aggressive tumor of blood vessels that is oftentimes fatal. At diagnosis most dogs have evidence of metastatic disease and despite chemotherapy, survival times rarely exceed 6 months. Novel approaches to the treatment of this disease are needed. The use of targeting antibodies against vascular endothelial growth factor (VEGF), a protein that promotes tumor growth and spread, plus chemotherapy has prolonged disease free survival in several different tumor types in man. It is the aim of this proposal to isolate a canine derived antibody fragment that can specifically bind to and neutralize the tumor promoting effects of canine VEGF. It is hypothesized that a VEGF specific antibody fragment that is canine in origin may be used in the veterinary clinics to retard or prevent the development of metastases in dogs with HSA. We have generated diverse libraries of canine antibody fragments that we will screen against canine VEGF to select fragments that specifically recognize canine VEGF. We will isolate VEGF specific antibody fragments using previously described techniques that are routinely performed in our laboratory and test their ability to inhibit the tumor promoting effects of VEGF in vitro. This work will build on our previous studies supported by the CHF that describe the technology to generate canine antibody fragment libraries. This canine-derived, tumor-specific targeting approach is the first of its kind in the veterinary field and if successful this agent may also be used to treat many other tumor types in the dog.



Grant Objectives:

Objective 1: To isolate canine derived scFvs that bind specifically to canine VEGF

Objective 2: To isolate VEGF-specific scFvs that inhibit VEGF bioactivity.

Publications:

Report to Grant Sponsor from Investigator:

In this proposal we set out to utilize our canine-derived scFv phage display platform technology to isolate canine antibody fragments that bind and neutralize Vascular Endothelial Growth Factor (VEGF). VEGF is a protein growth factor that promotes the growth and survival of new blood vessels. Tumors require the growth of new blood vessels to supply them with oxygen and nutrients that allow the tumor to grow. VEGF is a major contributor to this angiogenesis (growth of new blood vessels).

Bevacizumab (Avastin) is a humanized antibody that recognizes and neutralizes human VEGF. It is currently approved for the treatment of a number of different cancers including metastatic colon cancer, and non-small cell lung cancer. FDA approval and on-going clinical trials attest to the potential of VEGF inhibition as a treatment for many different cancer types in humans, however, the therapeutic effects of VEGF neutralization on cancer growth and metastasis have not been evaluated in the dog. Expression of VEGF has been reported in a wide range of different tumor types in the dog including hemangiosarcoma, malignant melanoma, soft tissue sarcomas, mast cell tumors, nasal carcinomas, intracranial neoplasias, and simple mammary gland adenocarcinomas and inflammatory mammary carcinoma. Serum levels of VEGF are increased in dogs with osteosarcoma, malignant melanoma and HSA and in dogs with osteosarcoma and malignant melanoma, serum levels correlate with disease free interval and survival times respectively. Together these reports suggest that VEGF plays an important role in the progression of these tumor types in the dog and as such represents a potential therapeutic target. However, to date there are no canine-derived antibodies that bind and neutralize the angiogenic activity of canine VEGF.

During this project, we identified 3 canine-derived scFv or antibody fragments that bind to canine VEGF. Furthermore results from cell-based assays in vitro suggest that these antibody fragments might inhibit the growth stimulating effects of VEGF on healthy endothelial cells and on a canine hemangiosarcoma cell line in vitro. These results suggest that not only do these antibody fragments bind VEGF but they also might inhibit its biological activity, which would be essential for any therapeutic effect. We have since developed several sophisticated techniques including surface plasmon resonance (SPR) to more closely interrogate the ability of our isolated antibody fragments to neutralize the activity of VEGF (i.e. inhibit the ability of VEGF to interact with a key VEGF receptor). Disappointingly, the results of these experiments have shown that the most abundant antibody fragment that we isolated from our library does not



appear to affect the interaction of VEGF with its receptor suggesting that this clone would not inhibit the biological effects of VEGF on tumor blood vessels. However, we are now testing the remaining 2 isolated scFv using these advanced techniques to determine whether they can neutralize VEGF.

In summary, this work has 1) demonstrated that canine derived antibody fragments that bind to VEGF can be isolated from libraries using simple panning techniques; and 2) optimized sophisticated techniques (surface plasmon resonance) that we can now use to determine whether antibody fragments interact with molecules of interest and can inhibit particular molecular interactions in vitro prior to their translation into patients with diseases such as hemangiosarcoma. We will continue to utilize these techniques to evaluate the remaining 2 scFv. If we find that one or both of these scFv neutralize VEGF activity then we intend to identify commercial partners that will assist in generating canine monoclonal antibodies based on these scFv that could then enter clinical trials in patients with hemangiosarcoma or other tumor types that are associated with excess production of VEGF.