GRANT PROGRESS REPORT REVIEW

Grant: 01139: *Immune Targeting of Canine Hemangiosarcoma Using a Canine Derived Single Chain Antibody Approach*

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

Research Institution: University of Pennsylvania - School of Veterinary Medicine

Grant Amount: $123,125.40

Start Date: 1/1/2009 **End Date:** 12/31/2010

Progress Report: 18 month

Report Due: 6/30/2010 **Report Received:** 7/27/2010

Recommended for Approval: Approved

(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:

Background: Canine hemangiosarcoma is a common and highly aggressive tumor of blood vessels that is often fatal. At diagnosis most dogs have evidence of metastatic disease and despite chemotherapy, survival times rarely exceed 6 months. New approaches to the treatment of this disease are needed. The use of monoclonal antibodies and antibody fragments to directly target different tumors has shown promise in clinical trials in man.

Objective: This project aims to use a new canine synthetic antibody system to target the tumor and deliver cytotoxic agents directly to both primary and metastatic lesions. Using advanced molecular techniques, the researchers intend to review antibody responses that dogs with hemangiosarcoma may make against their own tumors and use these as a template to generate canine antibody fragments that specifically recognize tumor particles. Tumor-specific antibody fragments will be linked to an exotoxin and evaluated for their ability to kill canine hemangiosarcoma cells in vitro. This allows for the direct delivery of cytotoxic agents to the tumor, which decreases side effects and increases therapeutic value. This work aims to develop the first canine-derived, tumor-specific targeting approach for the treatment of HSA and to provide proof-of-principal for this approach that can then be used to therapeutically target many other tumor types in this species in vivo.

Grant Objectives:
Objective 1: To generate phage display combinatorial antibody libraries from dogs with HSA.

Objective 2: To identify tumor-specific scFv of canine origin that bind specifically to canine HSA cell lines.

Objective 3: To generate a canine scFv based immunotoxin and evaluate its specificity and cytotoxicity against canine HSA in vitro.

Publications:
- Braganza, A., Wallace, K., Pell, L., Parrish, C.R., Siegel, D.L., Mason, N.J., 2010, Generation and validation of canine single chain variable fragment phage display libraries. Veterinary Immunology and Immunopathology In Press, Accepted Manuscript.(Epub) DOI: 10.1016/j.vetimm.2010.07.026

Report to Grant Sponsor from Investigator:
Canine hemangiosarcoma 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. Our work supported by the Canine Health Foundation and its associated breed clubs aims to generate a platform technology for generating canine derived antibody fragments that can specifically target tumor cells. Such antibody fragments can be linked to toxic agents and used to deliver these drugs directly to a cancer cell allowing for increased drug delivery and reduced toxic side effects. No such targeting system is currently available for use in the dog although similar targeting approaches are used commonly and effectively in the human cancer clinic. The work performed during the first year of this two-year proposal has lead to our ability to generate libraries of synthetic, canine antibody fragments. Each fragment is specific for a particular molecule. Such molecules may be those expressed on the surface of cancer cells, molecules associated with tumor growth factors or molecules expressed on the surface of infectious agents. Indeed, in theory, any molecule may be recognized by one or more antibody fragments contained within our canine antibody fragment libraries. Having generated these libraries we are now able to use simple panning techniques to isolate fragments that specific bind to molecules of interest.

In order to provide proof-of-principle that antibody fragments that target specific molecules exist within the libraries that we have generated, we have utilized canine parvovirus (CPV) molecules to select CPV specific antibody fragments from antibody libraries. This approach was successful and we have now isolated an antibody fragment of canine origin that specifically targets and binds to canine parvovirus. This finding provides proof-of-principle that these libraries contain a diverse array of antibody fragments that can be selected based on their ability to bind to certain target molecules. We are now performing further screening studies to determine whether the selected CPV-specific antibody fragment is capable of targeting and neutralizing CPV, a finding that would possibly provide us with a much needed therapeutic agent to treat dogs with clinical parvoviral disease. While this work was intended to provide proof that generated antibody libraries contain antibody fragments that target specific molecules, it also clearly provides an insight into the potential of this technology to impact the treatment of multiple disease processes including infectious disease.

We have now generated several different canine antibody fragment libraries from dogs with hemangiosarcoma and are now starting to screen these libraries to identify and isolate antibody fragments that specifically target hemangiosarcoma cells. In addition we are screening our antibody fragment libraries for fragments that can bind to and neutralize Vascular Endothelial Growth Factor (VEGF). This growth factor plays an important role in ensuring that new blood vessels are generated in response to the presence of the tumor. New blood vessels support tumor growth and agents that inhibit growth factors like VEGF are important in the treatment of many different malignancies. A human antibody known as Avastin that targets VEGF is currently used to treat patients with advanced colonic adenocarcinoma.

It is important to note that since the antibody fragments we have generated are replicas of canine antibody fragments they should elicit minimal immune responses when used in vivo. As such, these antibody fragments should be able to be administered multiple times if necessary, without loosing their potency. The results of our work to date have been compiled in a manuscript that is nearly complete and will be submitted shortly to the Journal of Immunological Methods. In the second and final year of this grant support, we aim to vigorously screen antibody fragment libraries generated from 10 dogs with hemangiosarcoma for fragments that specifically bind to hemangiosarcoma cells. Once we have identified such fragments we will link them to a cytotoxic agent and determine their ability to specifically kill malignant cells in vitro, prior to testing these agents in canine patients with hemangiosarcoma.

In summary, our work has lead to the development of the first canine-derived, antigen-specific targeting approach that may be used for the treatment of many different cancer types including HSA. Furthermore, we have identified potential agents that might be used to bind and potentially neutralize canine VEGF. We are exceptionally enthusiastic about this novel technology and wish to thank the CHF and its supporting breed clubs that have made and continue to make this work possible.