

VCA Welfare Foundation Research updates August 2012

The AKC Canine Health Foundation (CHF) has provided the following research updates on two of the grants the VCA Welfare Foundation is currently funding:

GRANT PROGRESS REPORT REVIEW

Grant: 01131: *Genetic Background and the Angiogenic Phenotype in Cancer*

Principal Investigator: **Research Institution:** **Start Date:** 1/1/2010

Dr. Jaime F Modiano, VMD PhD; University of Minnesota **End Date:** 12/31/2012

Report to Grant Sponsor from Investigator:

Data pertaining to aim 1 - establish unique gene expression signatures in HSA samples from each breed.

We have confirmed that there are unique, breed-related properties identifiable as intrinsic properties of isolated hemangiosarcoma cells. However, in the context of tumors as tissues (that is, when tumor cells are considered in their whole environment), these differences are no longer apparent, suggesting that at least in advanced stages of the disease when tumors are collected, the role of breed specific factors is reduced or absent. Our data show that hemangiosarcoma represents at least two and perhaps three molecularly distinguishable conditions. Various biochemical processes appear to contribute to this molecular stratification, including adipogenesis, inflammation, coagulation, and angiogenesis. These processes also may modulate the multipotency of hemangiosarcoma cells and their interactions with the local microenvironment. Indeed, hemangiosarcoma cells themselves may comprise only a modest proportion of the tumors, with the vast majority of the tumor consisting of "reprogrammed" stromal cells such as tumor-associated fibroblasts, myeloid and inflammatory cells, and endothelial cells. Our ongoing efforts include defining the role of these distinct metabolic signatures on hemangiosarcoma multipotency.

Data pertaining to aim 2 - how small molecule inhibitors that act directly and indirectly on angiogenic pathways affect HSA cells derived from dogs of each of these breeds.

We reported previously on our work evaluating the effect of endothelin receptor and VEGF receptor inhibitors and the potential to use VEGF, EGF, and urokinase (uPA) as targeting agents for hemangiosarcoma. We also have evaluated the role of IL-8 (as part of a separate AKC CHF-funded project). We have considered additional molecules that may be critical for hemangiosarcoma growth and development, and specifically CSF-1R, CD44, CXCR4, and CXCR7 among others. We have generated CSF-1R knockdown cells and CD44 knockdown cells on a hemangiosarcoma background and have generated the tools to knockdown CXCR4 and CXCR7. We also have obtained compounds to inhibit these molecules biochemically (neutralizing antibodies and small molecules). The main test we plan to use to assess the importance of these molecules is the serial sphere forming assay test, although we also will examine phenotypic and functional differences in the cells. We anticipate that formation of non-adherent spheres that retain multipotency will be the best surrogate to assess the possible role that these molecules play on intrinsic differentiation in vivo, although specific in vivo assays will be needed to establish microenvironment interactions.

Data pertaining to aim 3 - examine how attenuating vascular endothelial growth factor receptors affects pro-inflammatory environments generated by HSA cells.

Our data show that hemangiosarcoma cells are extremely resistant to >1,000 biologically active compounds (LOPAC library), with M concentrations required to achieve any meaningful cytotoxicity. The efforts from this aim are redirected to evaluating compounds that affect pathways enriched in hemangiosarcoma stem cells, principally NFkB.

Value added - we are fortunate to be able to enhance the objectives of this grant by creating synergy among multiple projects. Each project has a specific focus and is carefully managed to ensure there is no budgetary overlap. Yet, we have used these various and complementary, non-overlapping funding sources to develop a comprehensive program to study the biology of hemangiosarcoma. By leveraging

these funding sources together, we can accelerate discovery and impact, and build stronger collaborative relationships.

GRANT PROGRESS REPORT REVIEW

Grant: 01426: *c-Kit Mutation and Localization Status as Response Predictors in Canine Mast Cell Tumors Treated with Toceranib or Vinblastine: A Response-Adaptive Randomized Trial*

Principal Investigator: **Research Institution:** **Start Date:** 1/1/2011

Dr. Douglas H Thamm, VMD; Colorado State University **End Date:** 12/31/2012

Report to Grant Sponsor from Investigator:

While surgery remains the mainstay of treatment for canine mast cell tumors (MCT), surgery alone is not curative in some cases, and not possible in other cases. Medical therapy remains an important component of MCT therapy. New drugs that affect signaling through the KIT growth factor receptor are showing considerable promise for the treatment of canine MCT, and MCT with mutations in the KIT protein that make it constantly active may be more sensitive to KIT inhibitors. The drug combination vinblastine and prednisone has roughly the same effectiveness as KIT inhibition against canine MCT; however, the two treatments have not been compared head-to-head, and it is not clear whether vinblastine or KIT inhibitors are more appropriate for the treatment of MCT without KIT mutations. We have recently developed a rapid test, which can be performed on fine-needle aspirates, to determine whether MCT possess KIT mutations or not. We are investigating the predictive value of KIT mutation status using this rapid genotyping assay, as well as KIT staining on biopsy sections, in dogs with measurable MCT randomized to receive either vinblastine or the KIT inhibitor toceranib (Palladia). Randomization utilizes a novel adaptive statistical strategy that makes use of the KIT assay results.

To date, 28 dogs have been enrolled in this study at 4 participating study sites. Although the exact number of dogs required to complete the study is unknown owing to the adaptive study design we are using, we expect the number to be between 60 and 80. We anticipate our enrollment rate to increase owing to commencement of enrollment at 1 additional site.

The results of this study will clarify whether KIT mutation testing is a useful decision-making tool for the selection of the best possible medical therapy for dogs with MCT.

FMI about VCA WF-sponsored research, please email vcawf2000@gmail.com.