RESEARCH PROGRESS REPORT SUMMARY

Grant 01759: Disrupting the Differentiation of Cancer Stem Cells to Prevent the Spread of Hemangiosarcoma

Principal Investigator: Dr. Jaime F Modiano, VMD, PhD

Research Institution: University of Minnesota

Grant Amount: $233,914.00

Start Date: 1/1/2013 **End Date:** 12/31/2015

Progress Report: FINAL

Report Due: 12/31/2015 **Report Received:** 2/13/2016

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:

Hemangiosarcoma is a rapidly fatal disease. The lifetime risk is alarmingly high for some breeds like Golden Retrievers (~20% will die of this disease) and Portuguese Water Dogs (~15% will die of this disease). Furthermore, the risk of hemangiosarcoma is not limited to a single breed. In fact so many dogs are at risk to develop hemangiosarcoma that 40 Breed Clubs designated it as a research priority for 2012. Despite considerable efforts to find effective treatments, the outcome for dogs with hemangiosarcoma has changed very little over the past 30 years. We believe this is because our understanding of this disease is still rudimentary, but that is changing. Recent evidence suggests hemangiosarcoma conforms to the "cancer stem cell" model, where a defined subset of cells is responsible for initiating and maintaining the tumor. These cells are resistant to conventional therapies and they also are very adaptable, being able to survive in a variety of niches. In the case of hemangiosarcoma, the cancer stem cells also retain or acquire the potential to differentiate along several different lineages. For this project, we will use this property against the tumor by modulating factors that support the self-renewal of the stem cell compartment and by inducing their terminal differentiation along alternate pathways that have reduced malignant potential. We propose that disrupting the interactions between hemangiosarcoma cancer stem cells and their microenvironment will enhance the sensitivity of these cells to conventional and targeted therapies and improve the outcomes of dogs with this disease.

Grant Objectives:

1. Define the role of CXCL12 (stromal derived factor-1 or SDF-1) and interleukin-8 (IL-8) in maintaining hemangiosarcoma self renewal and multipotency in vitro

2. Determine the potential to direct hemangiosarcoma differentiation in vivo by genetic or pharmacologic alteration of CXCL12 and IL-8 chemokine signals

3. Determine the potential to delay metastasis of hemangiosarcoma in vivo by pharmacologic alteration of inflammation and peroxisome proliferator activated receptor (PPAR) agonists.

Publications:

Manuscripts

1. Gorden BH, Kim JH, Sarver AL, Frantz A, Breen M, Lindblad-Toh K, O'Brien TD, Sharkey LC, Modiano JF, Dickerson EB. (2014). Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. Am J Pathol, 184(4), 985-95. PMID: 24525151

2. Kim JH, Frantz AM, Anderson KL, Graef AJ, Scott MC, Robinson SR, Sharkey LC, O'Brien TD, Dickerson EB, Modiano JF. (2014). Interleukin-8 promotes canine hemangiosarcoma growth by regulating the tumor microenvironment. Exp Cell Res, 323(1), 155-164. PMID: 24582862

3. Rodriguez AM, Graef AJ, LeVine D, Cohen IR, Modiano JF, Kim JH. (2015). Association of sphingosine-1-phosphate (S1P)/S1P receptor-1 pathway with cell proliferation and survival in canine hemangiosarcoma. J Vet Intern Med, 29(4), 1088-97. PMID: 26118793

4. Im KS, Graef AJ, Breen M, Lindblad-Toh K, Modiano JF, Kim JH. (2015). Interactions between CXCR4 and CXCL12 promote cell migration and invasion in canine hemangiosarcoma. Vet Comp Oncol, 2015 Sep 3. doi: 10.1111/vco.12165. [Epub ahead of print]. PMID: 26337509

5. Kim JH, Graef AJ, Dickerson EB, Modiano JF. (2015). Pathobiology of hemangiosarcoma in dogs: research advances and future perspectives. Veterinary Sciences, 2, 388-405

Abstracts

1. Graef AJ, Kim JH, Sarver AL, Frantz AM, O'Brien TD, Sharkey LC, Dickerson EB, Modiano JF (2013). Gene expression profiling reveals a role of CXCR4/7 in canine hemangiosarcoma. Proceedings of the 2013 International Conference on Advances in Canine and Feline Genomics and Inherited Diseases

2. Kim JH, Sarver AL, Frantz AM, Scott MC, Graef AJ, Tonomura N, Elvers I, Thomas R, Lewellen M, Dickerson EB, Breen M, Lindblad-Toh K, Modiano JF (2013). Germ-line risk factors are associated with upregulation of genes mediating cell cycle arrest and stem cell activity in canine hemangiosarcoma. Proceedings of the 2013 International Conference on Advances in Canine and Feline Genomics and Inherited Diseases

3. Kim JH, Anderson KL, Frantz AM, Graef AJ, Dickerson EB, Modiano JF (2013). IL-8 and Slug regulate cancer cell self-renewal and microenvironment interactions in hemangiosarcoma. Proceedings of the 2013 Veterinary Cancer Society Meeting

4. Graef AJ, Kim JH, Modiano JF. Inhibition of CXCR4 in canine hemangiosarcoma. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN

5. Kim JH, Rodriguez A, Graef AJ, Modiano JF. Sphingosine 1-phosphate signaling pathway in canine hemangiosarcoma (2013). Proceedings Masonic Cancer Center Research Symposium (also supported by CHF A-2081)

6. Witschen P, Graef AJ, Im KS, Modiano JF, Kim JH. (2014). Role of CD44 signaling in canine hemangiosarcoma. Proceedings of the 2014 Merial NIH National Veterinary Scholars Symposium

7. Im KS, Graef AJ, Kim JH, Modiano JF. CXCL12 binding to CXCR4 regulates canine hemangiosarcoma cell migration and invasion. Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 1, 2014

8. Kim JH, Sarver A, Yoon U, Graef AJ, Im KS, Breen M, Lindblad-Toh K, Modiano JF. Discovery and pathogenic significance of chromosome translocations in canine hemangiosarcoma. Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 1, 2014

9. Witschen P, Graef AJ, Im KS, Modiano JF, Kim JH. Role of CD44 signaling in canine hemangiosarcoma. Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 1, 2014

10. Graef AJ, Kim HK, Im KS, Modiano JF. CXCR4/CXCL12 regulates inflammatory response to promote progression of canine hemangiosarcoma. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN October 20, 2014

11. Rodriguez A, Graef AJ, Im KS, LeVine D, Modiano JF, Kim JH. S1P/S1P1 pathway promotes cell proliferation and survival in canine hemangiosarcoma. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN October 20, 2014

12. Kim JH, Sarver A, Graef AJ, Modiano JF. Discovery and pathogenic significance of chromosome translocations in canine hemangiosarcoma. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN October 20, 2014

13. Im KS, Graef AJ, Kim JH, Modiano JF. CXCL12-CXCR7 axis regulates maintenance of canine hemangiosarcoma progenitor cells. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN October 20, 2014

14. Witschen P, Graef AJ, Im KS, Modiano JF, Kim JH. Role of CD44 signaling in canine hemangiosarcoma. Proceedings Masonic Cancer Center Research Symposium, Minneapolis, MN October 20, 2014

15. Kim JH, Sarver A, Yoon U, Graef AJ, Im KS, Breen M, Lindblad-Toh K, Modiano JF. (2015). Discovery and pathogenic significance of chromosome translocations in canine hemangiosarcoma. 2015 MSI Research Exhibition, University of Minnesota

16. Modiano JF. Spontaneous Animal Models: Unsung Heroes, Proceedings 2015 ACVIM Forum

17. Im KS, Graef AJ, Kim JH, Modiano JF. (2015). CXCR4 and its ligand CXCL12 regulate canine hemangiosarcoma cell migration and invasion. Proceedings of the Annual Meeting of the American College of Veterinary Pathology

Report to Grant Sponsor from Investigator:

We completed progress to achieve the aims. Our results confirm and extend the notions that interactions between the tumor and its local environment regulate hemangiosarcoma progression. Yet, variability in cells within tumors can reduce the predictability of hemangiosarcoma behavior, and possibly contribute to therapy resistance. For example, hemangiosarcomas respond to the degradation of their supporting matrix by recruiting inflammatory cells and blood vessels. But the magnitude of this effect is variable among different hemangiosarcomas, which requires us to consider that these tumors might adapt efficiently to very different microenvironments. The hemangiosarcoma microenvironment also

tends to be rich in a molecule called CXCL12, which is used as a means of communication between the tumor cells and the normal supporting cells. Only some of the tumor cells have the receptors that transmit the signals from CXCL12. These cells help to support the tumor, and also can be efficient mediators of metastasis. But in their absence, other mechanisms might perform these functions. Attenuating inflammation and modulating the metabolic activity of the cells shows modest effects on hemangiosarcoma cell growth, but neither approach is completely effective to eliminate the tumor. This suggests that blocking specific pathways might have positive therapeutic effects in selected patients, but managing this disease will require combining strategies that lower the capacity of cells to simply switch their behavior to use alternate pathways to survive and thrive.