

Health Study Reports



Submitted by
ANNE MARIE KUBACZ



RESEARCH PROGRESS REPORT SUMMARY

Grant 02946: Towards Curative Outcomes in Canine Hemangiosarcoma

Principal Investigator: Chand Khanna, DVM, PhD

Research Institution: Ethos Discovery

Grant Amount: \$348,559.00

Start Date: 11/1/2021 **End Date:** 10/31/2026

Progress Report: End-Year 3

Report Due: 10/31/2024

Original Project Description:

Canine hemangiosarcoma is the most aggressive cancer seen in all dogs, but disproportionately affects older, large breed dogs. Despite aggressive treatment with surgery and chemotherapy, more than 50% of dogs die due to metastatic spread of their cancer within 6 months and no significant advancements in the treatment of hemangiosarcoma have occurred in over 30 years. Several challenges have hindered improvements in outcomes including limitations in the capabilities of current imaging and/or blood-based markers to detect early relapse/metastasis, gaps in current understanding of the molecular biology of hemangiosarcoma, and a lack of effective therapies that effectively alter the aggressive metastatic behavior of this cancer. A nation-wide clinical trial for 400 dogs with splenic hemangiosarcoma seeks to deliver curative outcomes for dogs with this disease. With collaboration from AKC Canine Health Foundation, dog-owning families from outside the geographical enactment of 30 Ethos veterinary hospitals can join the trial to receive care, and to conduct genomic correlative studies with two internationally recognized scientific teams to gain critical new knowledge necessary to propel the field to this future goal of curative outcomes. Researchers will

utilize a patient-forward approach and leverage genomic insight into hemangiosarcoma to propose and answer the following questions:

- (1) are there molecular biomarkers that predict prognosis in dogs with this disease?;
- (2) can we define genomic subgroups of dogs with hemangiosarcoma who are most likely to benefit from specific anticancer drugs?;
- (3) can we define new therapeutic approaches to prevent the spread of this cancer?

This approach follows the road map used in transforming childhood leukemia from a fatal diagnosis to a commonly cured disease. By leveraging the strengths of a unique and multidisciplinary team of clinicians and scientists, this canine hemangiosarcoma study will lay the foundation for accelerating drug development and improving patient outcomes for dogs with this devastating disease.

Publications:

As of 10/31/2024, the following publications represent maturity of the preliminary data in support of the AKC-CHF grant:

1. Stewart SD, Ehrhart EJ, Davies R, Khanna C. Prospective observational study of dogs with splenic mass rupture suggests potentially lower risk of malignancy and more favourable perioperative outcomes. *Vet Comp Oncol*. 2020 Dec;18(4):811–817. doi: 10.1111/vco.12621. Epub 2020 Jun 15. PMID: 32458544. Our prospective analysis of a cohort of 41 dogs presenting with splenic tumor rupture demonstrated a higher prevalence of benign tumors (37.5%) in this cohort than predicted by antiquated retrospective studies in the field and revealed a near-certain survival following splenectomy in modern veterinary specialty practice (95% patients survived and discharged within 40 hours of hospitalization). Data from this publication provided foundational knowledge to influence our study design and power analysis for Ethos-PUSH. Importantly, this initial study created the necessary experience to de-risk and predict our successful launch of the Ethos-PUSH clinical trial.

2. Favaro PF, Stewart SD, McDonald BR, Cawley J, Contente-Cuomo T, Wong S, Hendricks WPD, Trent JM, Khanna C, Murtaza M. Feasibility of circulating tumor DNA analysis in dogs with naturally occurring malignant and benign splenic lesions. *Sci Rep*. 2022 Apr 15;12(1):6337. doi: 10.1038/s41598-022-09716-6. PMID: 35428782. This proof-of-concept study analyzed the utility and feasibility of using circulating tumor DNA (ctDNA) to support the diagnosis of benign versus malignant splenic tumor rupture and tumor burden monitoring in dogs with splenic hemangiosarcoma.

3. Cawley JR, Stewart SD, Mochel JP, Veluvolu S, Khanna C, Fenger JM. Pharmacokinetic Exposures Associated With Oral Administration of Sorafenib in Dogs With Spontaneous Tumors. *Front Vet Sci*. 2022 May 19;9:888483. doi: 10.3389/fvets.2022.888483. PMID: 35664857; PMCID: PMC9162170. Data generated from this walk-in trial defines the tolerability and pharmacokinetic-based dosing of Sorafenib in cancer-bearing dogs. We now propose appropriate dosing of Sorafenib as part of the Ethos-PUSH treatment arm for dogs with splenic hemangiosarcoma.

4. Wong S, Ehrhart EJ, Stewart S, Zismann V, Cawley J, Halperin R, Briones N, Richter K, Sivaprakasam K, Perdigones N, Contente-Cuomo T, Facista S, Trent JM, Murtaza M, Khanna C, Hendricks WPD. Genomic landscapes of canine splenic angiosarcoma (hemangiosarcoma) contain extensive heterogeneity within and between patients. *PLoS One*. 2022 Jul 22;17(7):e0264986. doi: 10.1371/journal.pone.0264986. PMID: 35867969; PMCID: PMC9307279. An analysis of the genomic heterogeneity of canine hemangiosarcoma using the genomic platform (Vidium Animal

Health SearchLight DNATM Assay) being employed in Ethos-PUSH described our perspective that genomic subgroups that exist within hemangiosarcoma and has generated new questions about potential therapeutic arms that may be aligned with these genomic subgroups. These questions surrounding therapeutic-genomic biomarker matches are a central correlative of our proposed patient-forward approach to cancer drug development in Ethos-PUSH. This manuscript describes sources of geographic heterogeneity within hemangiosarcoma and proposes strategic approaches to address this heterogeneity through multiple geographic sampling of hemangiosarcoma lesions for genomic analysis that are being utilized in Ethos-PUSH.

5. Miller M, Stewart S, Wendelburg K, Lew F, Berg J, et al. (2022) Survivorship Endpoints in Pet Dogs with Naturally Occurring Cancer as Models for Human Cancer. *J Oncol Res Ther* 7: 10148. DOI: <https://doi.org/10.29011/2574-710X.10148>. We have completed a broad review of reported clinical outcomes for dogs with localized splenic HSA following splenectomy and adjuvant doxorubicin chemotherapy. We have identified three high quality published clinical trials of dogs with these clinical characteristics to create a synthesis/aggregation of foundational data regarding outcomes for these patients, including the rate of early relapse and the current rate of Exceptional Drug Responders (proposed as progression-free survival ≥ 12 months). This analysis of outcomes seeks to move the field of veterinary oncology beyond its current focus on reporting median outcomes alone. Furthermore, these aggregated data have helped support the modeling and power analysis for clinical and correlative endpoints of the current study.

Publications from this ongoing project are expected following the maturity of the clinical cohort 6 months (landmark analysis of PFS) and 12 months (identification of EDRs) post-splenectomy. The following manuscripts (published or in preparation) describe new data generated through the Ethos-PUSH trial in support of the AKC-CHF grant. Importantly, the preparation and submission of this work has directly supported the clinical residency training and professional advancement of several Ethos Veterinary Health House Office and clinician-scientists:

6. Ramirez AR, Stewart SD, Khanna C. Ultrasonographic liver nodules are more often benign lesions in dogs with hemoperitoneum secondary to splenic tumor rupture. *J Am Vet Med Assoc*. 2024 Aug 7;262(11):1499–1503. doi: 10.2460/javma.24.04.0254. PMID: 39111340. Here we evaluate the reliability of preoperative abdominal ultrasonography, a commonly used staging tool for dogs with hemoperitoneum due

to presumed splenic tumor rupture, in detecting metastatic lesions in the liver. In comparison to intraoperative observation and liver biopsy histology, our data demonstrate a poor association between the identification of liver lesions on preoperative ultrasonography and the presence of metastatic disease on liver biopsy. Furthermore, up to 22% of liver lesions observed during operation were missed on preoperative ultrasonography. This study challenges conventional perceptions around the approach to staging in dogs with hemoperitoneum and advocate for a reevaluation of the staging approach with more comprehensive modalities like whole-body CT.

7. Elise Ruffoni, Jacob Cawley, Samuel Stewart, Chand Khanna, Chris Thomson, Garrett Tougas, Joelle Fenger, Heather Wilson-Robles. A prospective observational study on 326 canines with ruptured splenic masses suggest benign lesions are more common than previously reported. (Manuscript in preparation). We have described the clinical characteristics of the first 326 dogs presenting with spontaneous hemoperitoneum enrolled into our PUSH study. In stark contrast to knowledge provided by antiquated retrospective studies that led many of us to believe that it was very unlikely (as low as a 4% chance) that an older large breed dog with splenic tumor rupture would have a benign splenic tumor, our prospective clinical trial demonstrates that the chance of splenic tumor rupture being benign is much higher. Communication around this data should recognize the new knowledge generated from prospective studies (including, but also beyond our studies) and should change communication with owners to reduce the risk of misinformed euthanasia in dogs presenting for hemoabdomen secondary to splenic tumor rupture.

8. Jordan Ziegler*, Jacob Cawley, Saya Press, Stephanie Istvan, Samuel Stewart, Chand Khanna, Heather Wilson-Robles, Joelle Fenger. Tolerability assessment of orally administered paclitaxel (Oraxol) with encequidar in dogs with spontaneous malignancy (Manuscript in preparation). Data generated from this walk-in trial defines the tolerability and recommended dose schedule for Phase 2 clinical trials for oral paclitaxel (Oraxol) with encequidar in cancer-bearing dogs. We now propose appropriate dosing of Oraxol with encequidar as part of the Ethos-PUSH treatment arm for dogs with splenic hemangiosarcoma.

9. Patrick Khalife*, Jacob Cawley, Samuel Stewart, Garrett Tougas, Heather Wilson-Robles, David Bruyette, Chand Khanna, Joelle Fenger. Tolerability evaluation of combination verdinexor (Laverdia®-CA1) and doxorubicin in tumor-bearing dogs (Manuscript in preparation). The results from this phase 1 tolerability study of combination verdinexor (Laverdia®-CA1) and doxorubicin in dogs with spontaneous tumors describe the adverse event profile and recommended dosing schedule for this combination therapy for future Phase 2 clinical trials. Based on these tolerability data, we will con-

sider this novel drug combination as a future treatment arm for our subsequent, planned segment of Ethos-PUSH (Segment 2) for dogs with localized splenic hemangiosarcoma.

10. Claire Quinn*, Samuel Stewart, Heather Wilson-Robles, Chand Khanna. Prevalence of cardiac events or lesions in canine patients with stage II splenic hemangiosarcoma (Manuscript in preparation). Canine hemangiosarcoma has a high proclivity for metastatic spread; however, the prevalence of cardiac (right atrial) involvement in dogs with splenic hemangiosarcoma remains unclear. In contrast to outdated retrospective studies that describe a relatively high rate (25%) of concurrent splenic and cardiac hemangiosarcoma, data from our prospective clinical trial demonstrate that the prevalence of cardiac events or lesions in dogs with stage II splenic hemangiosarcoma is much lower.

11. Brooke Fourthman*, Samuel Stewart, Heather Wilson-Robles, Chand Khanna. Prevalence of recurrent bleeding in dogs with benign splenic lesions (Manuscript in preparation). Knowledge generated from the Ethos-PUSH trial indicate that 38% of dogs presenting with a non-traumatic hemoabdomen secondary to a ruptured splenic mass will have nonmalignant histopathologic findings however, a subset of these patients will nonetheless suffer from premature death due to a rebleeding event despite their benign histology. Recent retrospective studies suggest that up to 17% of dogs with nonmalignant splenic histology experience adverse outcomes and die prematurely. This manuscript describes the clinical characteristics and incidence of recurrent bleeding events in dogs prospectively enrolled in the Ethos PUSH study that have benign splenic histology. Clinicians should start using evidence-based information about this at-risk group of dogs in their veterinary practice today.

**Ethos Veterinary Health House Officer*

Presentations:

The following national presentations include the preliminary data in support of Ethos-PUSH, the resultant study and design rationale, and preliminary data surrounding therapeutics that are now part of Ethos-PUSH that pre-date the launch of the Ethos-PUSH. Similar regional and local scientific presentations are not included here.

2021:

1. Khanna C. Delivering Hope for Canine Hemangiosarcoma. September 6-9, 2021. 2021 Western Veterinary Conference, Las Vegas, Nevada, USA (in person).
2. Cawley J. Improved Perspectives Around Canine Hemoperitoneum & The Future of Hemangiosarcoma Diagnostics & Therapeutics. September 6-9, 2021. 2021 Western Veterinary Conference, Las Vegas, Nevada, USA (in person)

3. Khanna C. Delivering Genomic and Precision Medicine to Veterinary Oncology. October 30- November 2, 2021. American College of Veterinary Pathologists 2021 Annual Meeting (virtual)
4. Khanna C. Improving the Delivery of Precision Medicine in Veterinary Oncology. November 4-7, 2021. Veterinary Cancer Society 2021 Annual Conference, Boise, Idaho, USA (virtual)
5. Stewart S*, Ehrhart E.J., Davies R, Khanna C. Two prospective trials demonstrate lower risk for malignancy in canine hemoperitoneum secondary to ruptured splenic tumors. November 4-7, 2021. Veterinary Cancer Society 2021 Annual Conference, Boise, Idaho, USA (*Primary presenter, virtual)
6. Cawley J, Stewart S, Khanna C, Fenger J*. Establishment of Tolerability and Pharmacokinetic Parameters for Ethos-PUSH (Ethos-Precision Medicine Umbrella Study for Hemangiosarcoma) Anticancer Agents in Cancer-Bearing Dogs. November 4-7, 2021. Veterinary Cancer Society 2021 Annual Conference, Boise, Idaho, USA (*Primary presenter, virtual)
7. Cawley J*, Stewart S, Fenger J, Lovrenert K, Bartels C, Ehrhart E.J., Khanna C, Scacheri P. Sequencing the epigenome of splenic tumours may uncover new insight into the biology and therapy of canine splenic hemangiosarcoma. November 4-7, 2021. Veterinary Cancer Society 2021 Annual Conference, Boise, Idaho, USA (*Primary presenter, virtual)

2022:

8. Cawley J, Stewart S, Khanna C, Fenger J*. Establishment of Tolerability and Pharmacokinetic Parameters for Ethos-PUSH (Ethos-Precision Medicine Umbrella Study for Hemangiosarcoma) Anticancer Agents in Cancer-Bearing Dogs. April 9-12, 2022. Veterinary Cancer Society 2022 Mid-Year Conference, Puerto Vallarta, Jalisco, MEX (*Primary presenter, in person)
9. Cawley J*, Stewart S, Fenger J, Wong S, Hendricks W, Khanna C, Scacheri P. New Insights into Hemangiosarcoma Biology Suggest Rap1 Signaling Pathway as Therapeutic Target. April 9-12, 2022. Veterinary Cancer Society 2022 Mid-Year Conference, Puerto Vallarta, Jalisco, MEX (*Primary Presenter, virtual)
10. Khanna C. Obstacles and Opportunities in the Delivery of Genomic Medicine. April 9-12, 2022. Veterinary Cancer Society 2022 Mid-Year Conference, Puerto Vallarta, Jalisco, MEX (in person)
11. Khanna C. Delivering Patient Forward Selectivized Medicine to Canine Hemangiosarcoma. June 21-25, 2022. 12. Stewart S. Two Prospective Trials Demon-

strate Lower Risk for Malignancy in Canine Hemoperitoneum. June 21-25, 2022. 2022 American College of Veterinary Internal Medicine Forum, Austin, Texas, US (in person).

13. Fenger J. Tolerability and Pharmacokinetic Parameters for Anticancer Agents in Dogs. June 21- 25, 2022. 2022 American College of Veterinary Internal Medicine Forum, Austin, Texas, US (in person).

2023:

14. Fenger J*, Cawley J, Stewart SD, Bruyette D, Khanna C. Tolerability evaluation of combination verdinexor (LAVERDIA-CA1) and doxorubicin in tumor-bearing dogs. June 16, 2023. 2023 American College of Veterinary Internal Medicine Forum, Philadelphia, PA, US (*primary presenter, in person).
15. Fenger J. Targeted Therapies: Clinical Applications, Combinations and Clinicians' Experience. August 25, 2023. 2023 American College of Veterinary Internal Medicine Advanced Continuing Education, Novel Oncology Therapeutics: Targeting Hope Live Virtual Course.

2024:

16. Insights from Prospective Studies in Canine Hemangiosarcoma. Friday, June 7, 2024. 2024 American College of Veterinary Internal Medicine Forum, Minneapolis, Minnesota, US. Preliminary findings of our Ethos-PUSH trial were presented at the 2024 ACVIM Forum. This two-hour Hemangiosarcoma Session featured scientific presentations on the Ethos-PUSH trial presented by Drs. Chand Khanna, Joelle Fenger, Heather Wilson-Robles, and Jacob Cawley followed by a clinician- scientist panel discussion.
17. Khalife, P. Tolerability evaluation of combination doxorubicin and verdinexor (LAVERDIA-CA1) in tumor bearing dogs: A phase I dose-finding study. October 17-19, 2024. Veterinary Cancer Society 2024 Annual Conference, Orlando, FL, US (Resident Oral Scientific Presentation).
18. Ruffoni, E. A prospective observational study of 316 canines with ruptured splenic masses suggests a higher percentage of benign splenic lesions. October 17-19, 2024. Veterinary Cancer Society 2024 Annual Conference, Orlando, FL, US (Oral Scientific Presentation).
19. Cawley, J. Understanding the clinical impact of recurrent bleeding in canine splenic hemangiosarcoma. October 17-19, 2024. Veterinary Cancer Society 2024 Annual Conference, Orlando, FL, US (Poster Presentation)
20. Bleeding Splens and Finding a Cure: Ethos PUSH.

Friday, October 25, 2024. 2024 American College of Veterinary Surgeons Surgery Summit, Phoenix, Arizona, US. This 4.5-hour seminar shared our progress and preliminary findings from high-level evidence on perioperative management of spontaneous hemoperitoneum, pre-surgical diagnostics, surgical outcomes (including time to surgery, enhanced recovery opportunities), secondary hemoabdomens, and accelerating drug development in the context of rigorous prospective clinical trials. Featured presentations by Drs. Chris Thomson, Chand Khanna, Samuel Stewart, and Jacob Cawley included:

- a. Chand Khanna, Ethos PUSH: Finding a cure for hemangiosarcoma
- b. Sam Stewart: Evidence based decisions on perioperative management of spontaneous
- c. Chris Thomson: Prospective outcomes of dogs undergoing splenectomy for hemoabdomen
- d. Jacob Cawley, A bleeding phenotype? Genomics, epigenomics, early re-bleeders and benign bleeders
- e. Chand Khanna: Novel therapeutic approaches for HSA

Report to Grant Sponsor from Investigator:

The fact that hemangiosarcoma is one of the most aggressive and frustrating cancers encountered by veterinary oncologists is undisputed. It is a cancer that can develop in any part of the body, has a high propensity to spread to other organs, and is not effectively treated with existing therapies in most dogs. This cancer carries an additional traumatic burden for the dog-owning community resulting from the frequent emergency presentation of this disease related to acute blood

loss from the rupture of a previously unrecognized primary tumor. Ethos Discovery has now recruited over 400 dogs to a 5-year, randomized prospective clinical trial that seeks to deliver curative outcomes to dogs with what is arguably the most aggressive canine cancer. Our approach to deliver this ambitious outcome is squarely focused on the conduct of high evidence, prospective clinical research through Ethos PUSH (Precision Medicine Umbrella Study for Hemangiosarcoma). To gain critical new knowledge necessary to propel the field to this future goal of curative outcomes, we will utilize a patient-forward approach and leverage genomic insight into hemangiosarcoma to propose and answer the following questions:

- (1) are there molecular biomarkers that predict prognosis in dogs with hemangiosarcoma;
- (2) can we define genomic subgroups of dogs with hemangiosarcoma who are most likely to benefit from specific anticancer drugs;
- (3) can we define new therapeutic approaches to prevent the spread of this cancer. Our approach follows the road map used in transforming childhood leukemia from a fatal diagnosis to a commonly cured disease.

Furthermore, the continued success of this project is reliant upon the high functioning, multi-institutional and interdisciplinary translational science team led through Ethos Discovery. This scientific team has coordinated the collection of biological samples from multiple Ethos Veterinary Health and non-Ethos hospitals throughout the US to allow the analysis of high quality biospecimens at the research centers in Phoenix (Vidium Animal Health) and Cleveland (Case Western Reserve University).

