

# 2022 ACVIM FORUM ▶ HYBRID

## Evaluation of Plasma Nucleosome Concentrations in Healthy Dogs and Dogs with Various Common Cancers.

Heather Wilson-Robles, DVM, DACVIM (Oncology)

Friday, June 24, 2022 | 9:45-10:15 AM

**RECONNECT**  
to Community, Science + Medicine



# Speaker Disclosure

Evaluation of Plasma Nucleosome Concentrations in Healthy Dogs and Dogs with Various Common Cancers

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## **FINAL DISCLOSURE:**

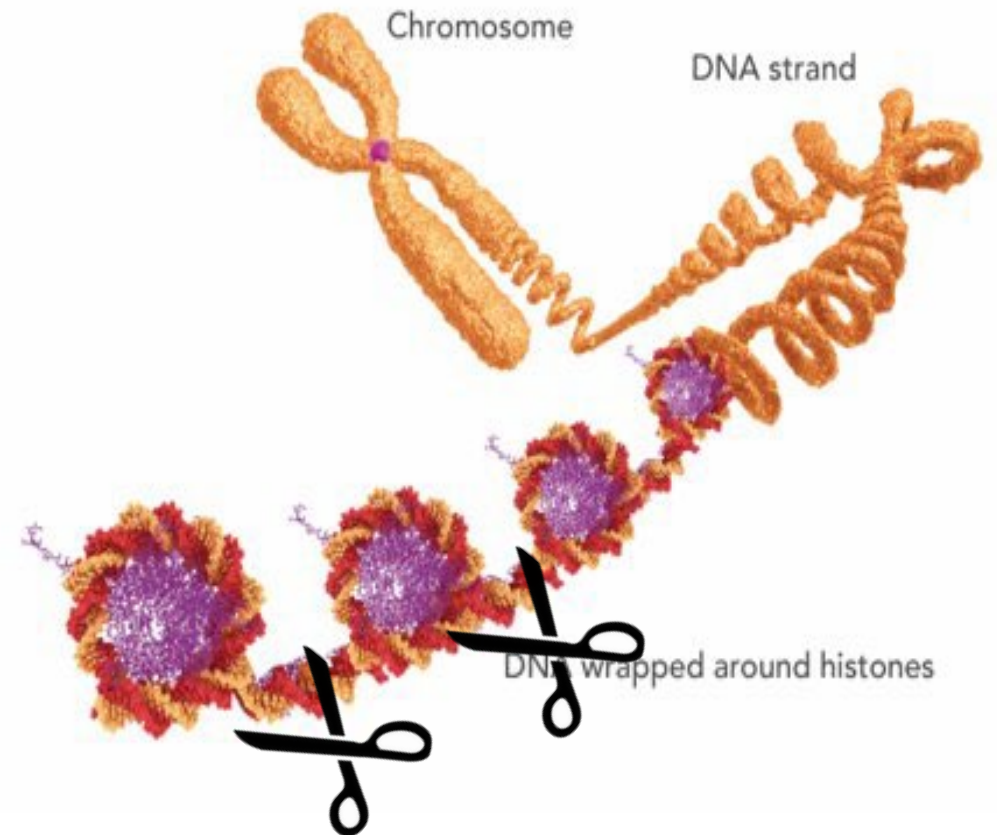
Grant/Research Support- Volition Veterinary Diagnostic Development

Grant/Research Support- Fred and Vola Palmer Chair in Comparative Oncology (Texas A&M University)

Consulting Engagement- Volition Veterinary Diagnostic Development

# The Basic Concept

- Cancer & cell death results in chromatin fragmentation and release of nucleosomes into the blood.
- Nucleosomes contain >200 possible modifications that regulate every fundamental cellular process.
- Measuring nucleosome levels and modifications in circulation have the potential to be both prognostic and diagnostic markers for disease.



# Circulating Nucleosomes in Cancer

## **Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNase, cytokeratin-18 fragments and survivin**

[Oliver J Stoetzer](#)<sup>1</sup>, [Debora M I Fersching](#), [Christoph Salat](#), [Oliver Steinkohl](#), [Christian J Gabka](#), [Ulrich Hamann](#), [Michael Braun](#), [Axel-Mario Feller](#), [Volker Heinemann](#), [Barbara Siegele](#), [Dorothea Nagel](#), [Stefan Holdenrieder](#)

## **Circulating nucleosomes predict the response to chemotherapy in patients with advanced non-small cell lung cancer**

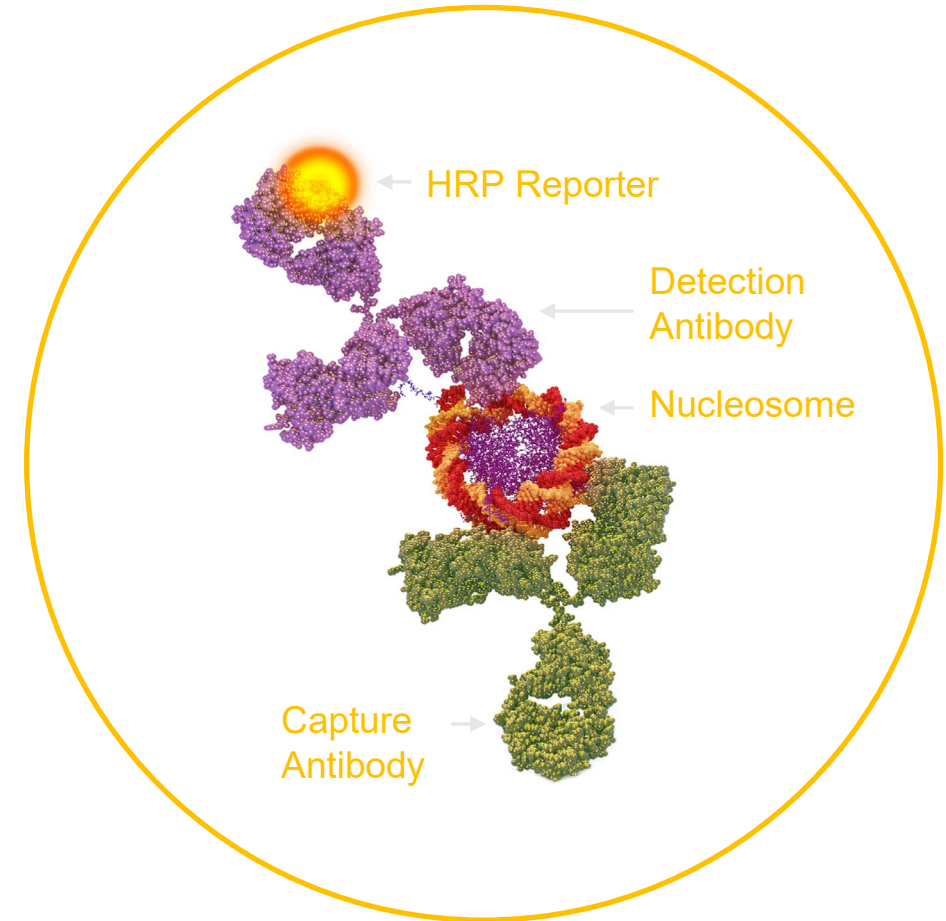
[Stefan Holdenrieder](#)<sup>1</sup>, [Petra Stieber](#), [Joachim von Pawel](#), [Hannelore Raith](#), [Dorothea Nagel](#), [Knut Feldmann](#), [Dietrich Seidel](#)

## **Predictive and prognostic value of circulating nucleosomes and serum biomarkers in patients with metastasized colorectal cancer undergoing Selective Internal Radiation Therapy**

[Yvonne Nadine Fahmueller](#),<sup>1</sup> [Dorothea Nagel](#),<sup>1</sup> [Ralf-Thorsten Hoffmann](#),<sup>2,4</sup> [Klaus Tatsch](#),<sup>3,5</sup> [Tobias Jakobs](#),<sup>2,6</sup> [Petra Stieber](#),<sup>1</sup> and [Stefan Holdenrieder](#)<sup>1,7</sup>

# H3.1 ELISA Assay

- **Proprietary epigenetic immunoassay platform**
- **Determine levels of circulating nucleosomes**
- **Profiles nucleosome epitopes**
  - Histone post translation modifications
  - Histone variants
  - DNA modifications
- **Flexibility of platform and diversity of modifications enables the development of disease specific panels**





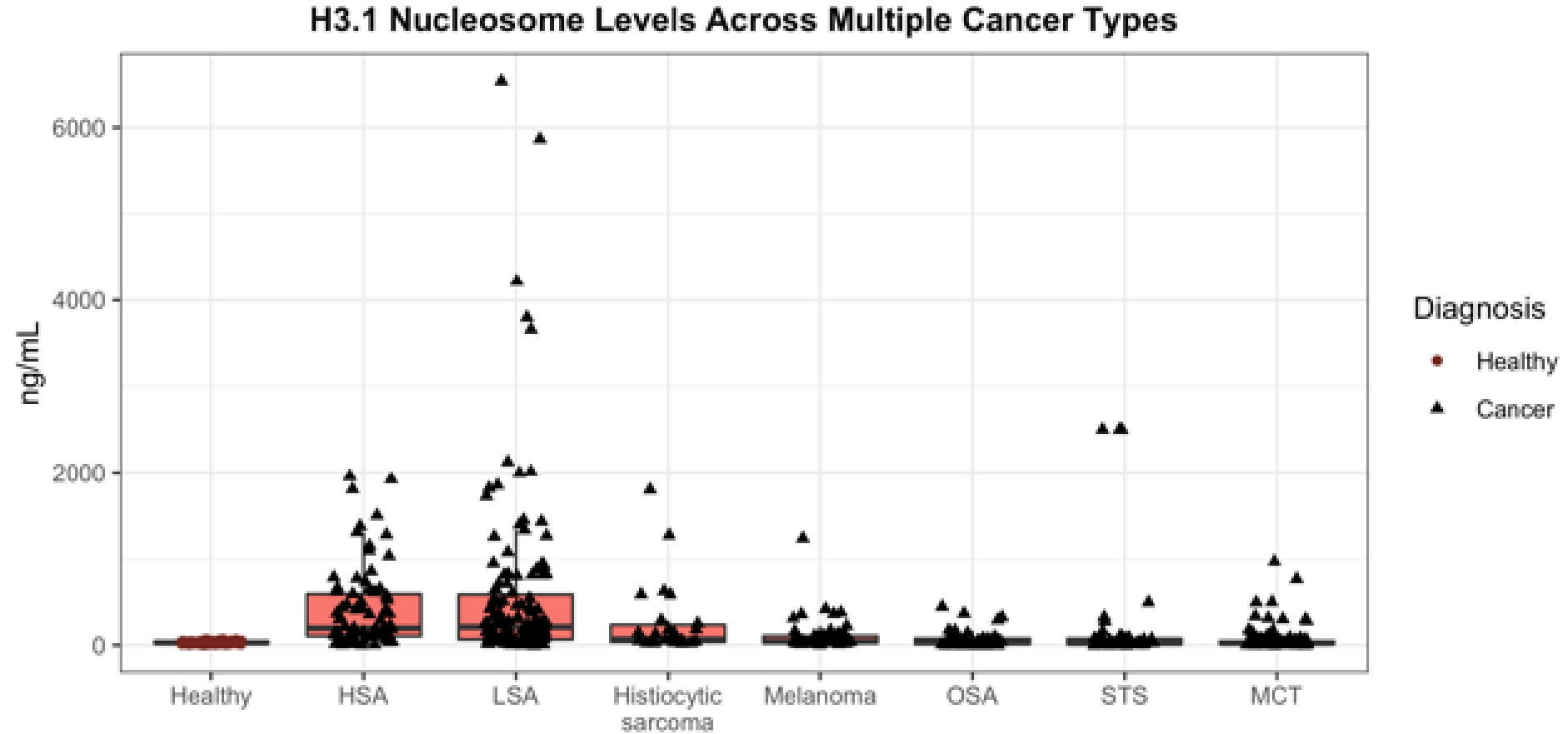


## Materials and Methods

# Sample Collection and Case Recruitment

- Samples were either collected at the Texas A&M Small Animal Teaching Hospital (AUPs CA 2019-0211 and 2017-0350) or from the NCI Division of Cancer Treatment and Diagnosis Biorepository.
- A total of 134 healthy animals were recruited for this study.
- A total of 528 canine cancer samples were either prospectively collected at TAMU or purchased from the NCI DCTD.
- Plasma was isolated within 1 hour of collection and stored at -80C until samples could be run in batches.
- Animals were all fasted a minimum of 4 hours before collection.
- All samples were run on the Nu.Q<sup>®</sup> H3.1 ELISA assay (Belgian Volition, SRL, Isnes, Belgium) according to the manufacturers protocol.
- Statistical analysis was performed using Receiver Operator Characteristic (ROC) curves, Wilcoxon rank sum tests and Kruskal-Wallis test for repeat measures with a Dunn's multiple comparison test using Graph pad prism v.9 and R v. 3.4.3.

# Results

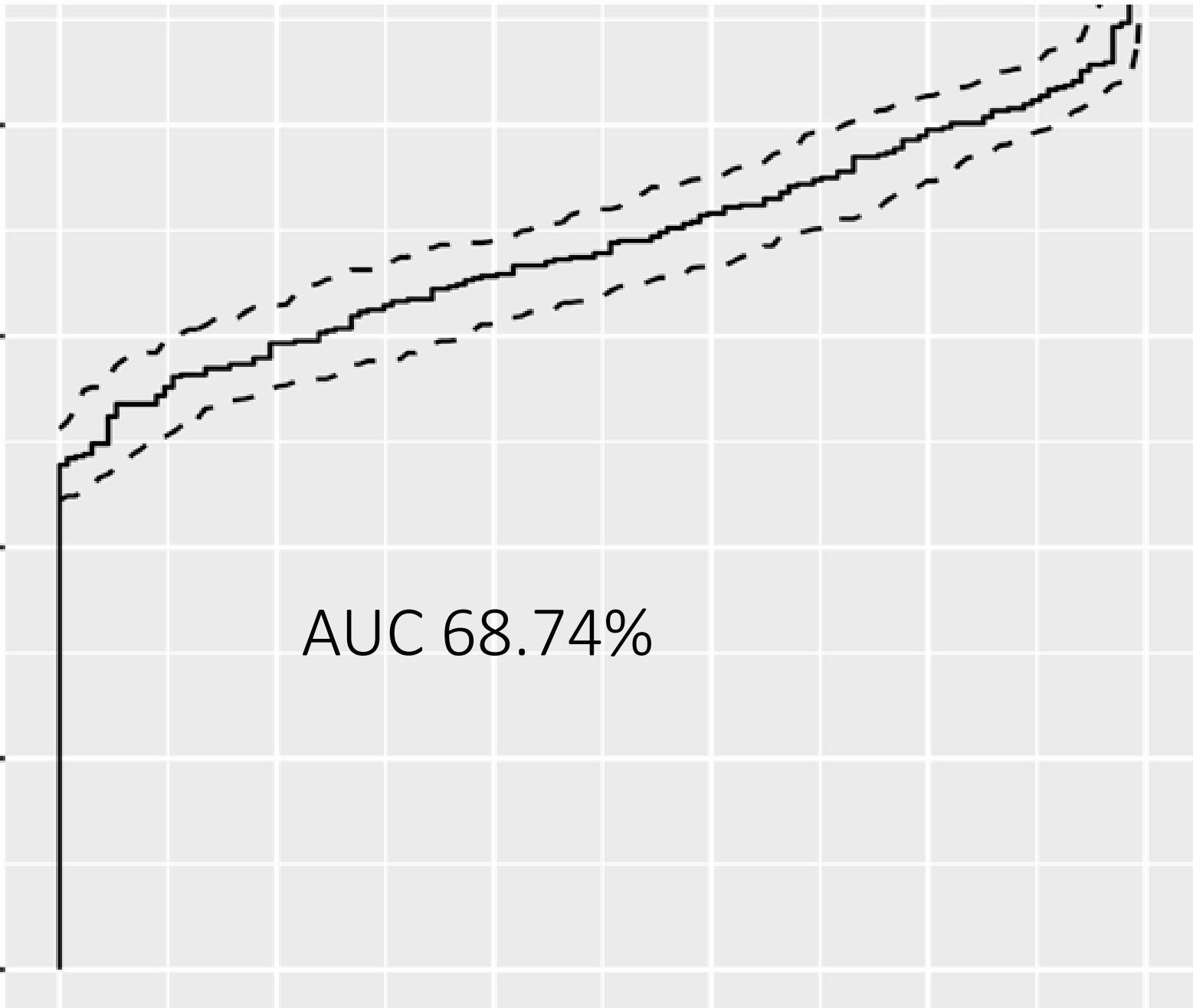




sensitivity

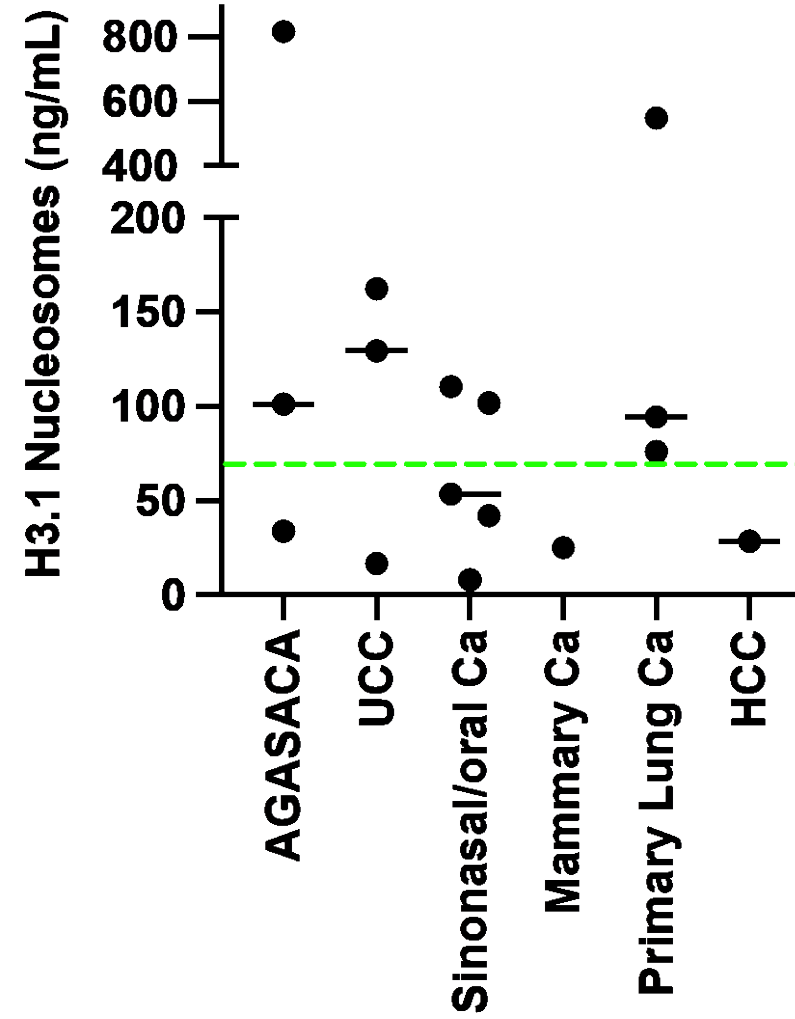
80%  
60%  
40%  
20%  
0%

AUC 68.74%

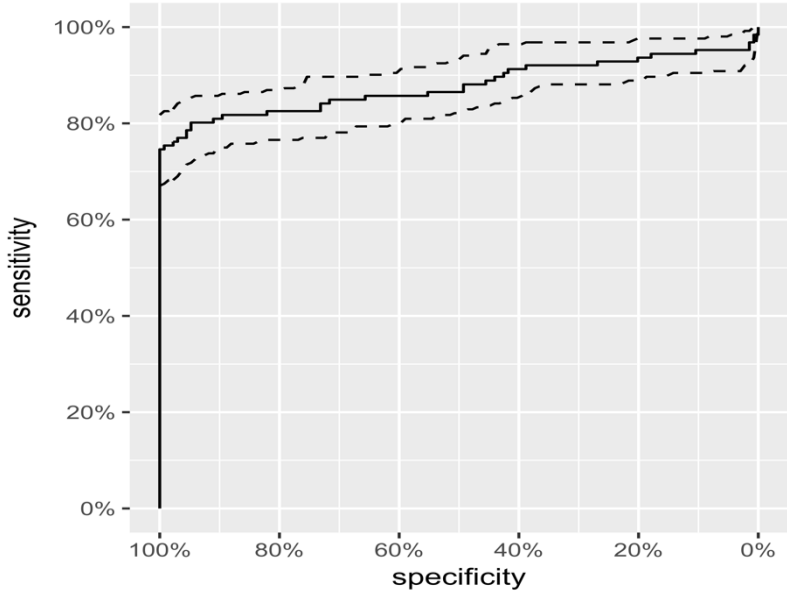
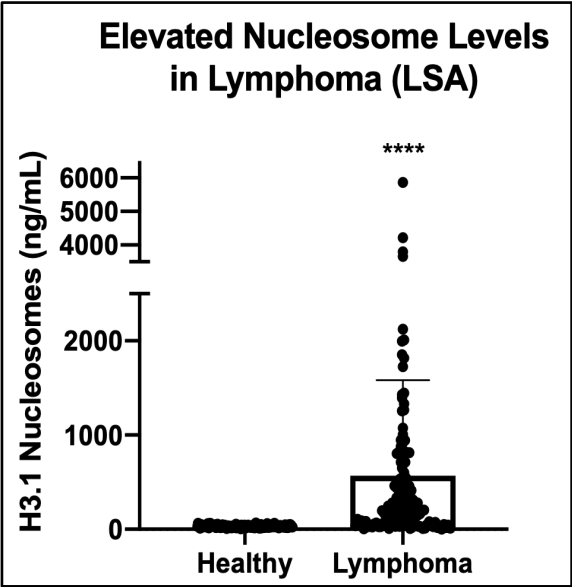


## Miscellaneous Cancer Cases

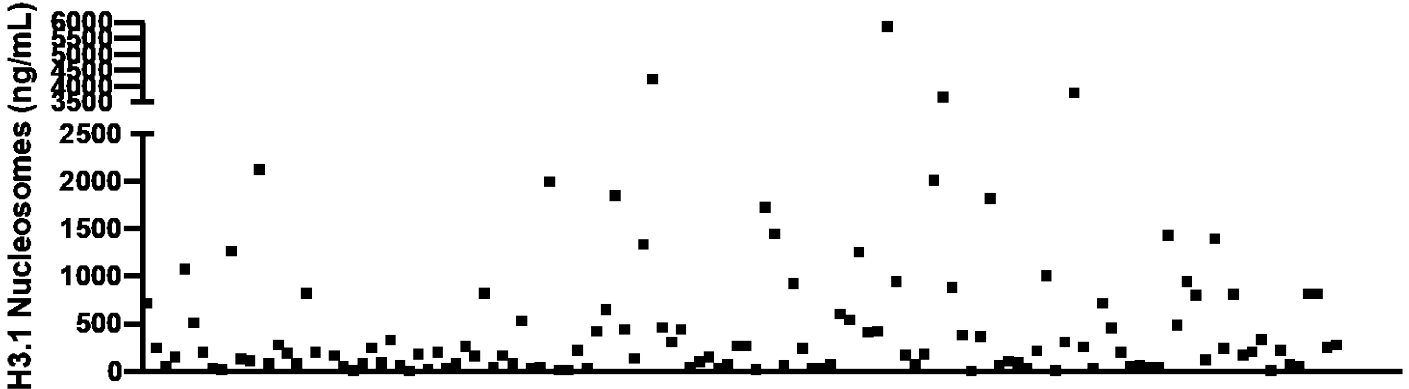
Additional  
Cases



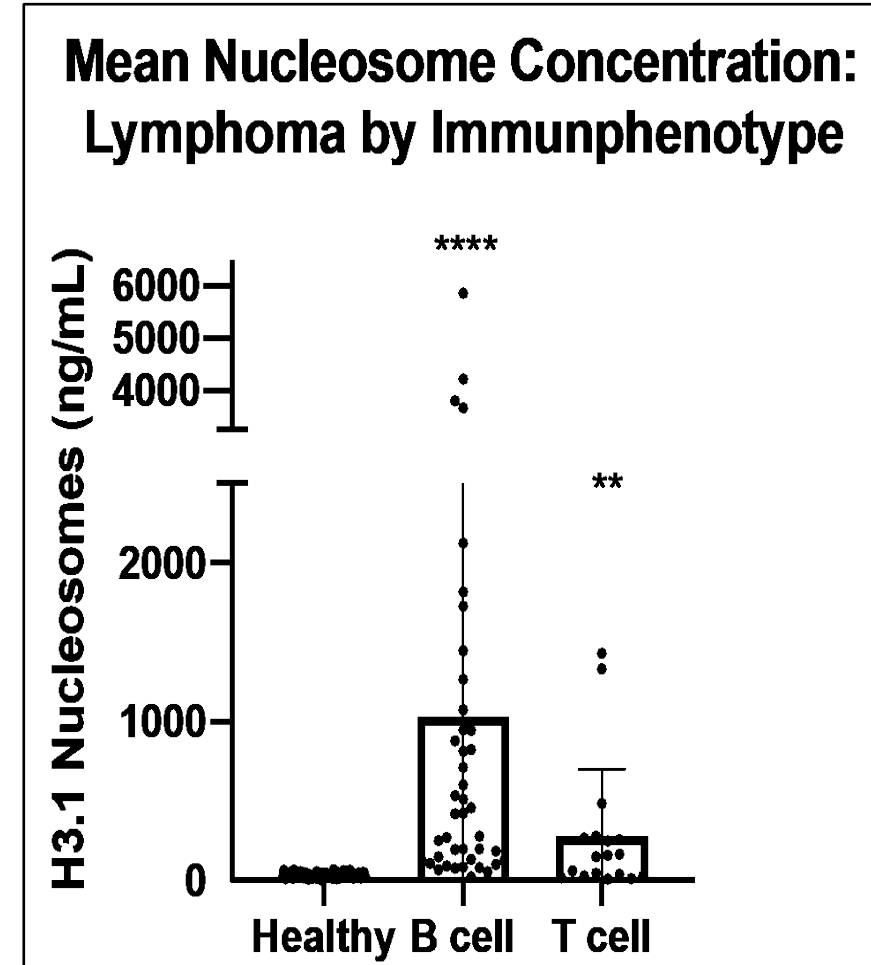
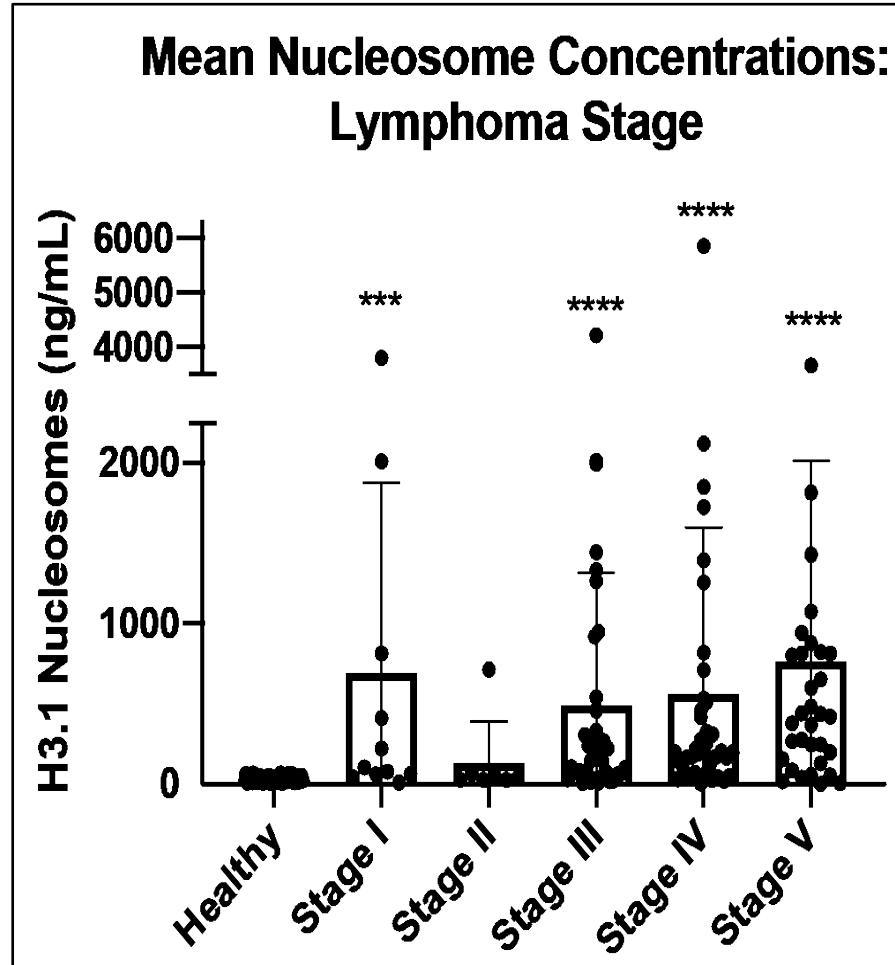
# Circulating Nucleosomes in Dogs with Lymphoma



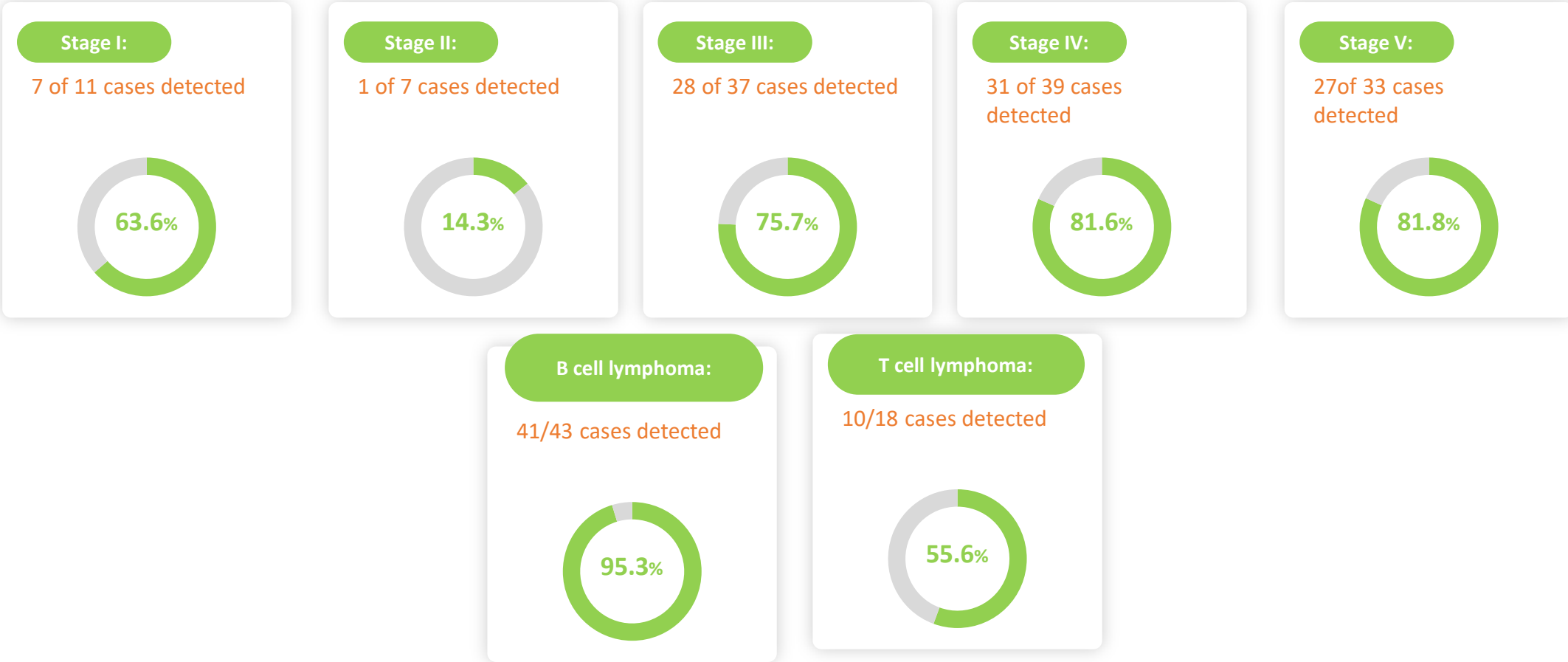
### Variability in Nucleosome Levels Across Lymphoma Samples



# Lymphoma

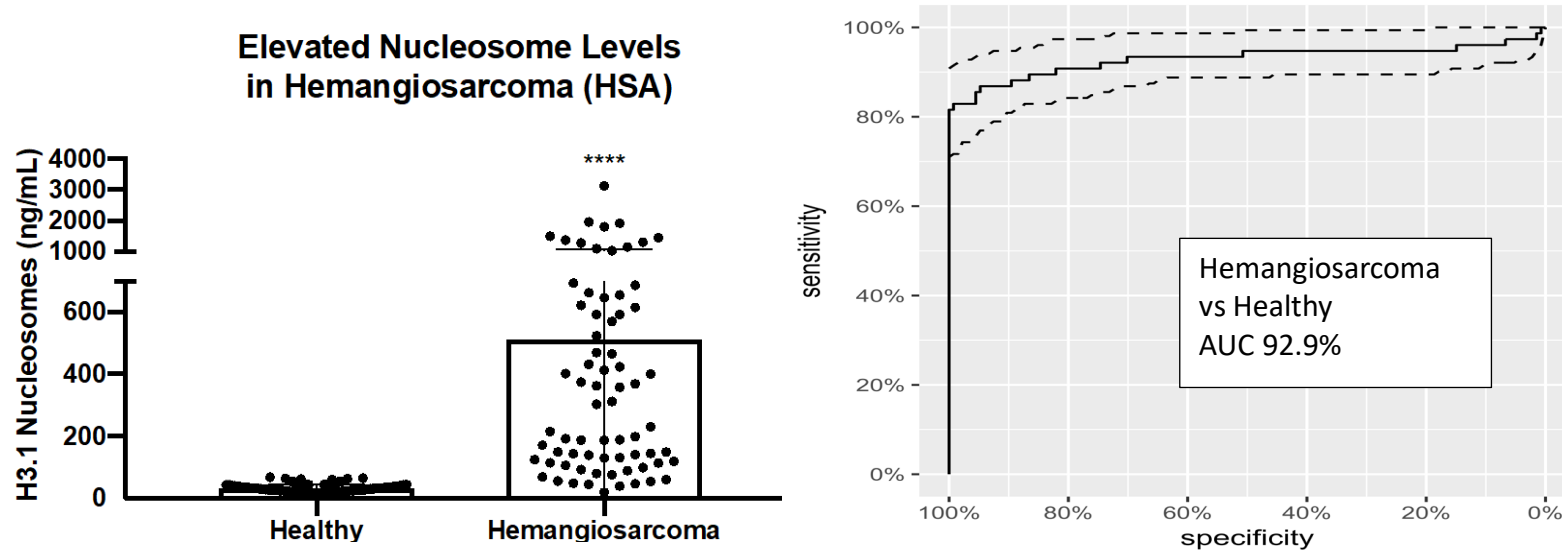


# Diagnosis by Lymphoma Stage

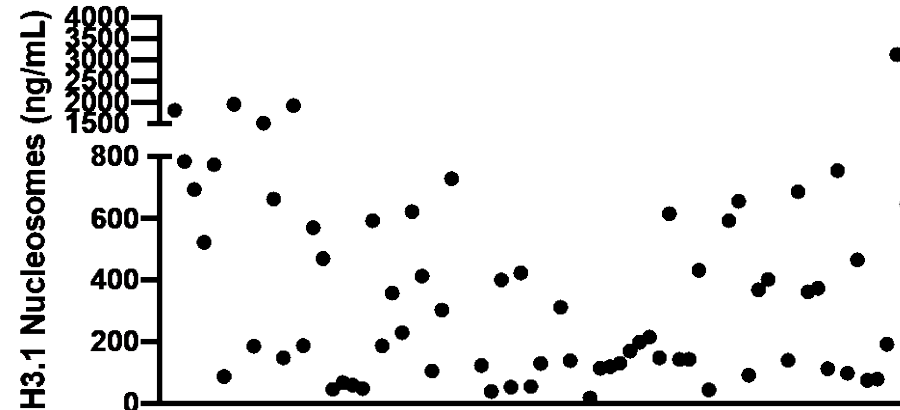




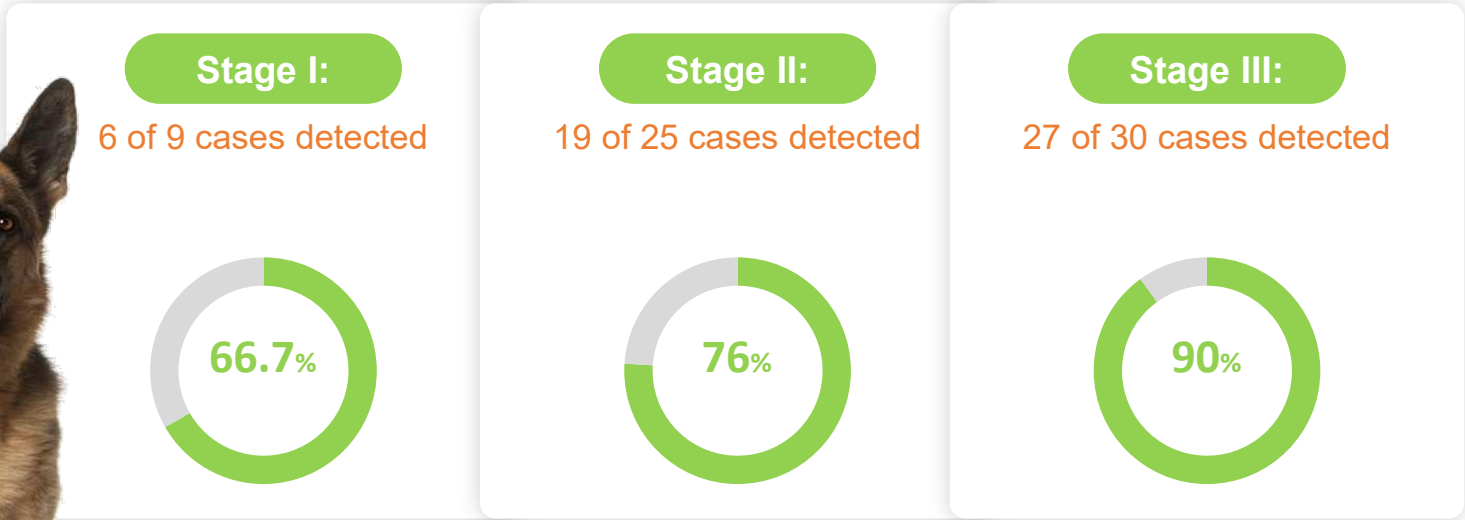
# Circulating Nucleosomes in Dogs with Hemangiosarcoma



## Variability in Nucleosome Levels Across Hemangiosarcoma Samples



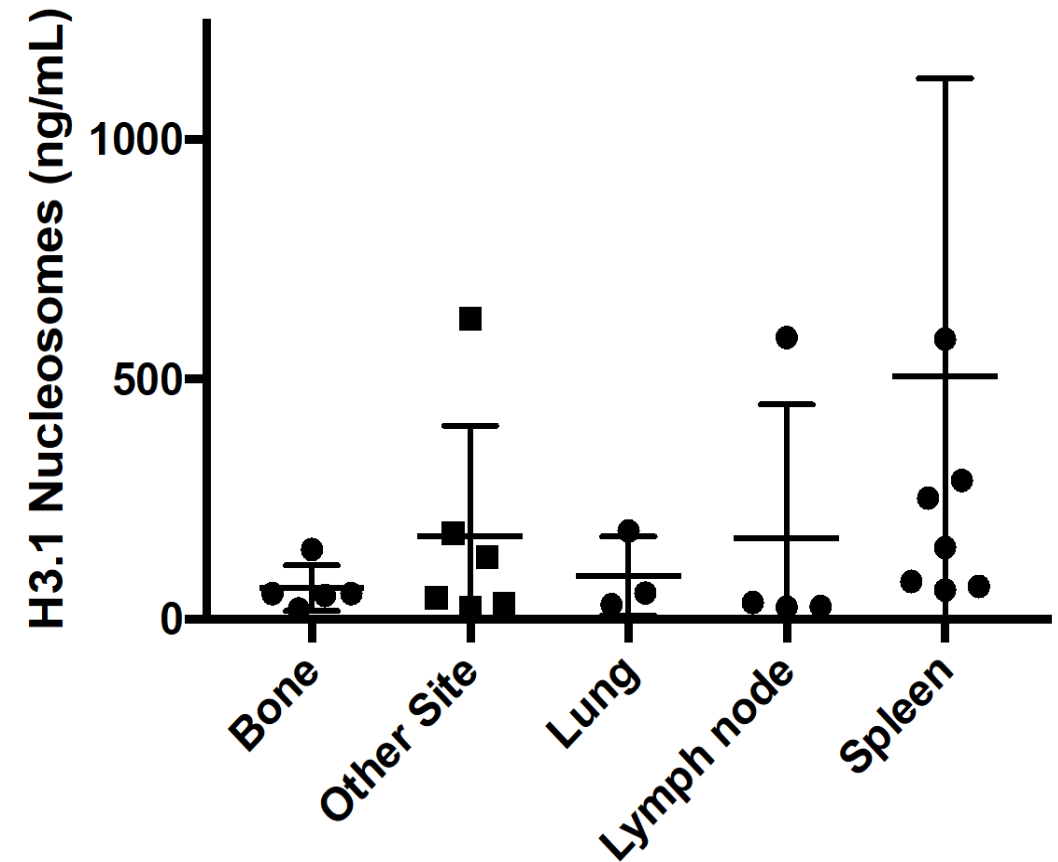
# Diagnosis by Stage in HSA



# Histiocytic Sarcoma

- 26 cases total
- H3.1 Concentration
  - Median 69.6 ng/mL
  - Mean 261.22 ng/mL
  - Range 21.8-1800
- Location
  - Visceral (n=21)
  - Bone (n=5)
- Versus healthy
  - $p < 0.0001$

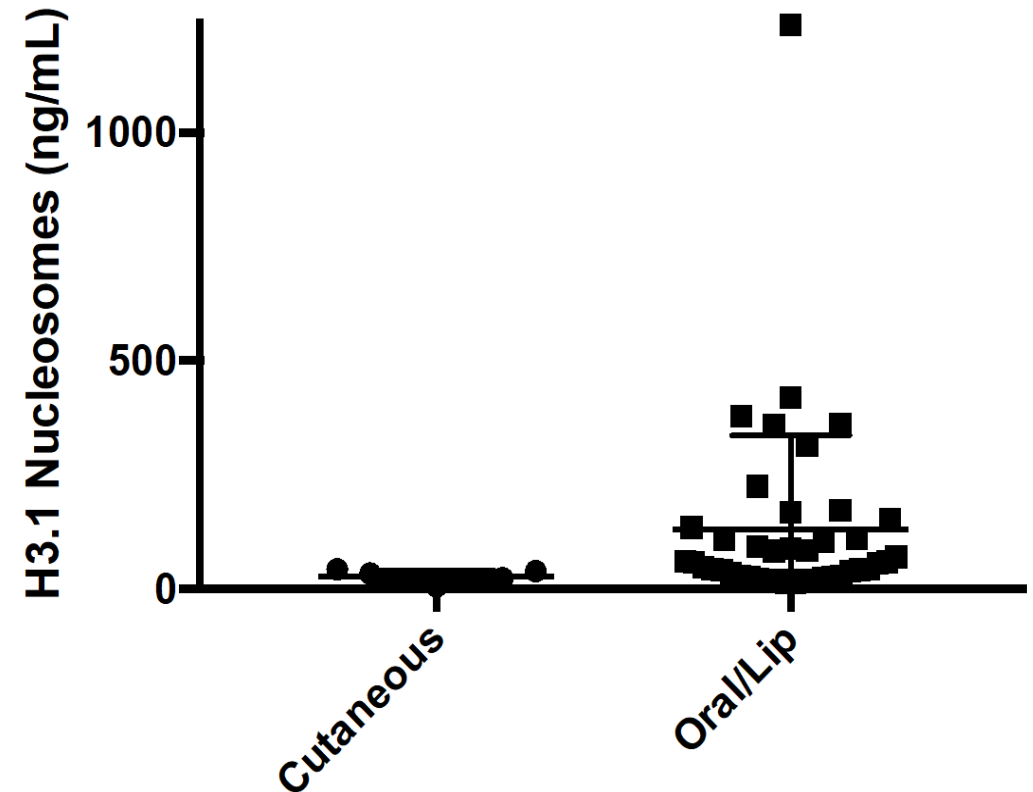
## Plasma Nucleosome Concentrations Histiocytic Sarcoma



# Oral Melanoma

- 49 cases in total
  - 7 haired skin
  - 42 oral tumors
- H3.1 Concentration
  - Median for cutaneous tumors – 24.8 ng/mL
  - Median for oral tumors – 60.0 ng/mL
- Tumor Size
  - Median for cutaneous tumors- 3 cm
  - Median for oral tumors- 4 cm
- Versus Healthy
  - $p= 0.000025$

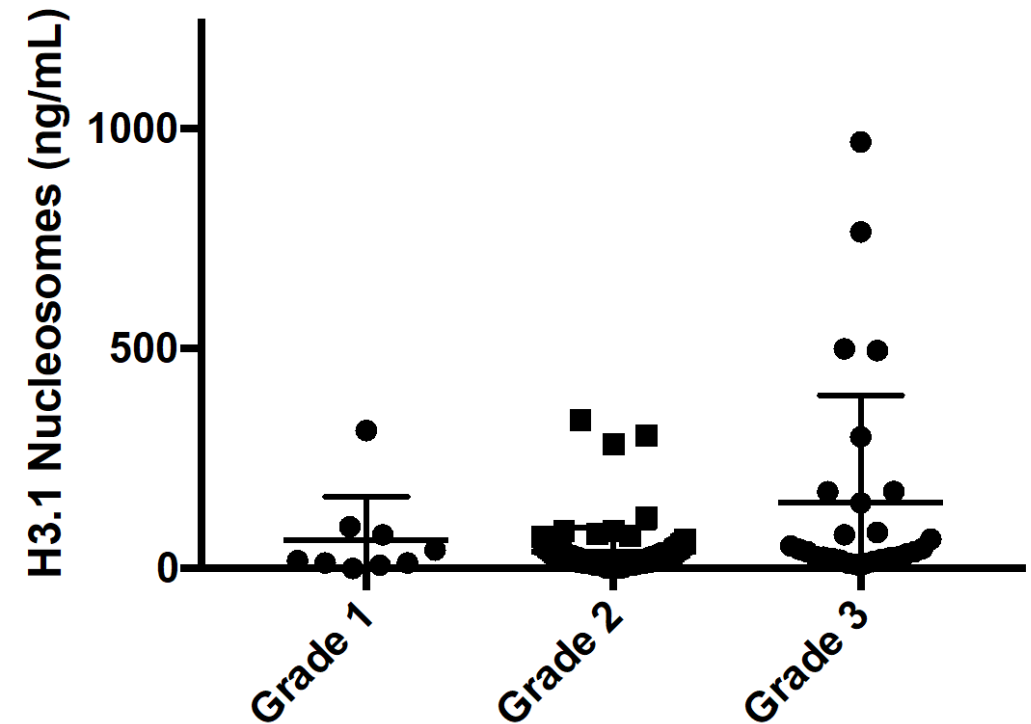
## Plasma Nucleosome Concentrations Melanoma



# Mast Cell Tumors

- 126 cases in total
- Tumor grade
  - 9 grade 1
  - 87 grade 2
  - 26 grade 3
- H3.1 concentrations
  - Low grade median 21.68 ng/mL; mean 41.9 ng/mL
  - High grade median 38.2 ng/mL; mean 149.7 ng/mL
  - $p = 0.005$
- When compared to healthy dogs
  - $p > 0.99$  (low grade)
  - $p = 0.005$  (high grade)

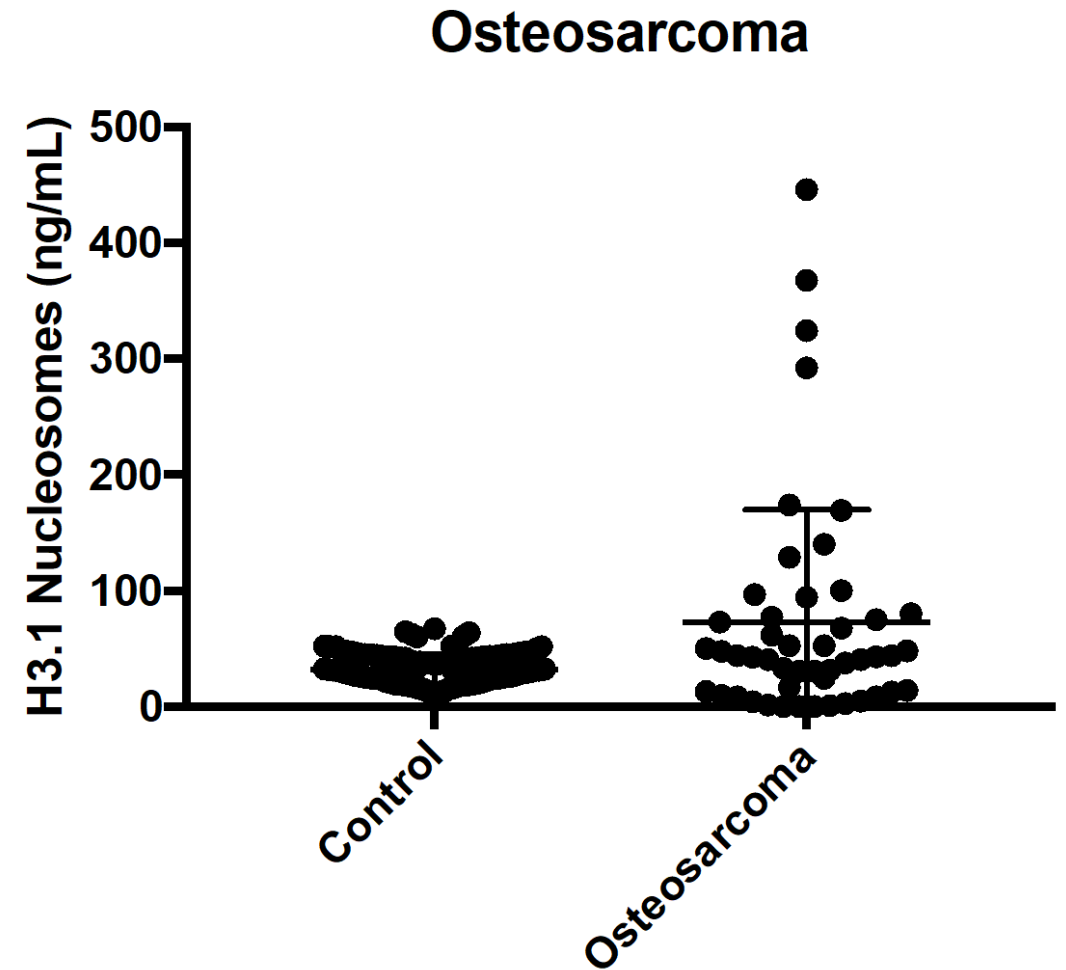
## Plasma Nucleosome Concentrations Mast Cell Tumors





# Osteosarcoma

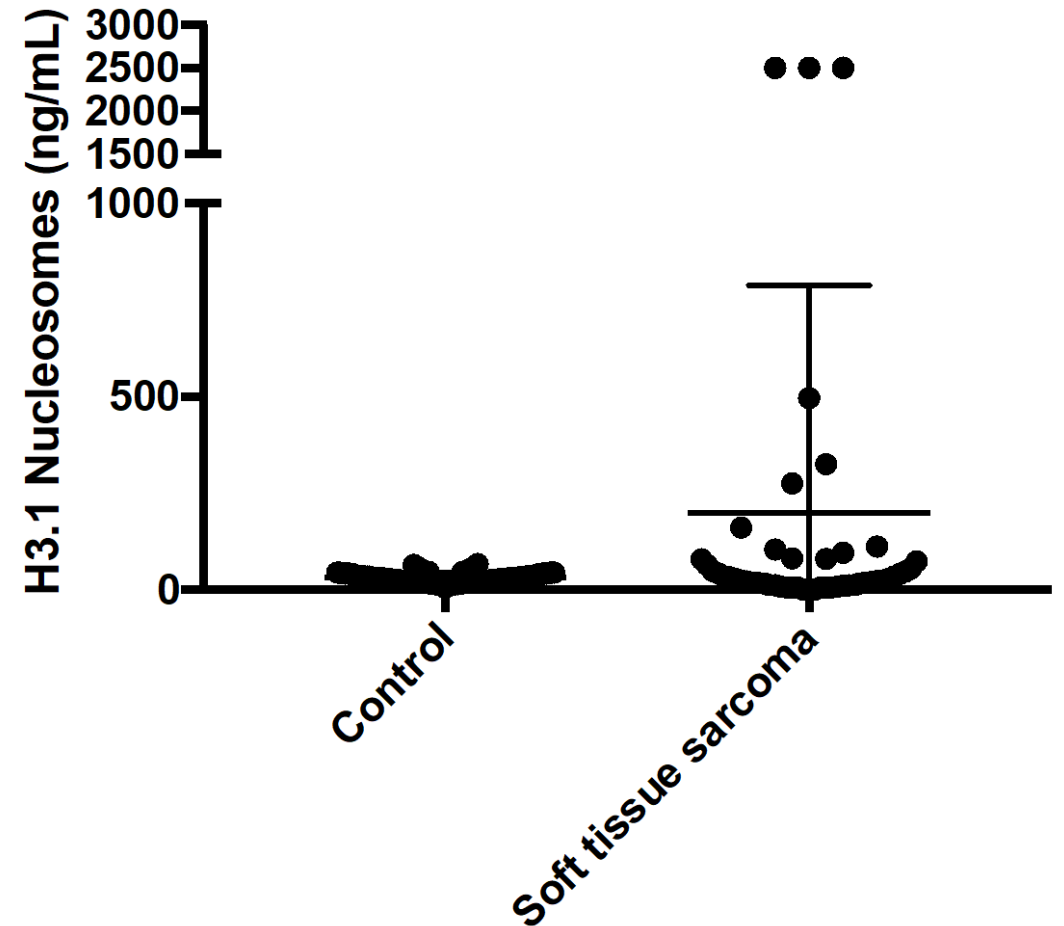
- 49 total cases
  - 17 elevated
  - Sensitivity 60.7% at 97% specificity
- H3.1 Concentration
  - Median 43.2 ng/mL; mean 72.7 ng/mL
  - Range 0.1-446 ng/mL
- Versus healthy
  - $p= 0.035$



# Soft Tissue Sarcoma

- 51 total cases
  - 15 elevated
  - Sensitivity 48.19% with a specificity of 97%
- H3.1 Concentration
  - Median 25.09 ng/mL; mean 200.07 ng/mL
  - Range 0.1-2500 ng/mL
- Versus Healthy
  - $p = 0.704$

## Soft Tissue Sarcomas



In this case series, plasma nucleosome concentrations were able to identify 174 of 229 (76%) systemic cancers (lymphoma, hemangiosarcoma and Histiocytic sarcoma).




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Overall, in 7 of the most common cancers, plasma nucleosome concentrations were able to identify 49.8% of all cancers tested.



Article

# The PATHFINDER Study: Assessment of the Implementation of an Investigational Multi-Cancer Early Detection Test into Clinical Practice

Lincoln D. Nadauld <sup>1,\*</sup>, Charles H. McDonnell III <sup>2</sup>, Tomasz M. Beer <sup>3</sup>, Minetta C. Liu <sup>4</sup>, Eric A. Klein <sup>5</sup>, Andrew Hudnut <sup>2</sup>, Richard A. Whittington <sup>6</sup>, Bruce Taylor <sup>6</sup>, Geoffrey R. Oxnard <sup>7</sup>, Jafi Lipson <sup>8</sup> , Margarita Lopatin <sup>9</sup>, Rita Shaknovich <sup>9</sup>, Karen C. Chung <sup>9</sup> , Eric T. Fung <sup>9</sup>, Deborah Schrag <sup>7</sup> and Catherine R. Marinac <sup>7</sup> 

## Discussion

- Similar findings to the PATHFINDER study (Galleri test by GRAIL)
  - Positive Predictive Value 49% in their study of over 50 different cancers in humans.

## Discussion

- Also similar to the OncoK9 test
  - Able to identify 54.7% of cancers they tested.
  - 85.4% of lymphoma, hemangiosarcoma and osteosarcoma cases.

# PLOS ONE

RESEARCH ARTICLE

Clinical validation of a next-generation sequencing-based multi-cancer early detection “liquid biopsy” blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study

Andi Flory<sup>1,2,3</sup>, Kristina M. Kruglyak<sup>1</sup>, John A. Tynan<sup>1</sup>, Lisa M. McLennan<sup>1</sup>, Jill M. Rafalko<sup>1\*</sup>, Patrick Christian Fiaux<sup>1</sup>, Gilberto E. Hernandez<sup>1</sup>, Francesco Marass<sup>1</sup>, Prachi Nakashe<sup>1</sup>, Carlos A. Ruiz-Perez<sup>1</sup>, Donna M. Fath<sup>1</sup>, Thuy Jennings<sup>1</sup>, Rita Motalli-Pepio<sup>1</sup>, Kate Wotrang<sup>1</sup>, Angela L. McCleary-Wheeler<sup>1,4</sup>, Susan Lana<sup>5</sup>, Brenda Phillips<sup>2</sup>, Brian K. Flesner<sup>4,6</sup>, Nicole F. Leibman<sup>7</sup>, Tracy LaDue<sup>8</sup>, Chelsea D. Tripp<sup>9</sup>, Brenda L. Coomber<sup>10</sup>, J. Paul Woods<sup>11</sup>, Mairin Miller<sup>3</sup>, Sean W. Aiken<sup>2</sup>, Amber Wolf-Ringwall<sup>12</sup>, Antonella Borgatti<sup>12</sup>, Kathleen Kraska<sup>2</sup>, Christopher B. Thomson<sup>3</sup>, Alane Kosanovich Cahalane<sup>13</sup>, Rebecca L. Murray<sup>9</sup>, William C. Kisseberth<sup>14</sup>, Maria A. Camps-Palau<sup>7</sup>, Franck Floch<sup>15,16</sup>, Claire Beaudu-Lange<sup>17</sup>, Aurélie Klajer-Peres<sup>18</sup>, Olivier Keravel<sup>18</sup>, Luc-André Fribourg-Blanc<sup>19</sup>, Pascale Chicha Mazetier<sup>20</sup>, Angelo Marco<sup>21</sup>, Molly B. McLeod<sup>22</sup>, Erin Portillo<sup>23</sup>, Terry S. Clark<sup>24</sup>, Scott Judd<sup>25</sup>, C. Kirk Feinberg<sup>21</sup>, Marie Benitez<sup>21</sup>, Candace Runyan<sup>26</sup>, Lindsey Hackett<sup>27</sup>, Scott Lafey<sup>28</sup>, Danielle Richardson<sup>11</sup>, Sarah Vineyard<sup>29</sup>, Mary Tefend Campbell<sup>30</sup>, Niles Dharajiya<sup>31,33</sup>, Taylor J. Jensen<sup>32,33</sup>, Dirk van den Boom<sup>33</sup>, Luis A. Diaz, Jr.<sup>33,34</sup>, Daniel S. Grosu<sup>1</sup>, Arthur Polk<sup>1</sup>, Kalle Marsal<sup>1</sup>, Susan Cho Hicks<sup>1</sup>, Katherine M. Lytle<sup>1</sup>, Lauren Holtvoigt<sup>1</sup>, Jason Chibuk<sup>1</sup>, Ilya Chorny<sup>1</sup>, Dana W. Y. Tsui<sup>1</sup>



## Summary

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Elevated plasma nucleosome concentrations may be a useful tool for the early detection of cancer in geriatric pet dogs.

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This test should not be used in lieu of traditional diagnostics but as a companion test.

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The ELISA platform lends itself to being a low-cost test that requires a small sample with a quick turnaround time.

# Questions?

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