Utility of Monitoring H3.1 Plasma Concentrations as a Surrogate of Treatment Response and Remission in Dogs with Cancer

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Introduction

Nucleosomes consist of small fragments of DNA wrapped around a core of histone octamers. These fragments form the structural framework of chromosomes[1,2]. During periods of increased cellular turnover and replication, such as in cancer, nucleosomes are released into the plasma and can be measured as components of cell-free DNA (cfDNA)[2]. Elevated levels of plasma nucleosomes have been observed in dogs with various cancers, including lymphoma, hemangiosarcoma, malignant melanoma, and histiocytic sarcoma[1,3-5]. Nucleosomes have also proven to be a useful monitoring tool for canine lymphoma and other lymphoid malignancies[6]. In this study, the authors aim to demonstrate the efficacy of this assay as a monitoring tool for non-lymphoid malignancies.

Materials and Methods

Pet dogs with a confirmed diagnosis of non-lymphoid malignancies, naive to treatment and presenting at a participating clinic, were eligible for enrollment in this study. Animals were enrolled with informed consent from the owner, and the study was approved by the TAMU-IACUC. Dogs were fasted for a minimum of 4 hours before blood samples were collected into EDTA tubes. Samples were collected at diagnosis, during follow-up chemotherapy, and at recheck visits, following their individual treatment plans. Samples were centrifuged within 45 minutes of collection, and the plasma was collected and stored at -80°C for batch processing. Samples were analyzed using the Nu.Q® H3.1 ELISA (Belgian Volition) in accordance with the manufacturer's protocol. The standard curve was linearized and fit to a 4-parameter logistic curve using GraphPad Software (v8, San Diego, CA).

Results

- 22 Cases included:
  - 12 HSA
  - 6 Carcinomas
  - 2 OSA
  - One MM and one Insulinoma
- A total of clinical 217 visits with 153 data points (**Figure 1**)
  - H3.1 levels are significantly higher at diagnosis than remission (p=0.02)
    - 3.6 fold decrease in H3.1 signal at remission (72.3% decrease)
  - H3.1 levels are significantly higher at relapse than remission (p=0.02)
    - 9 fold increase in H3.1 signal at relapse (88.9% increase)
  - H3.1 levels are not significantly different at diagnosis or relapse.

	Diagnosis	Remission	Relapse
# of values	21	22	7
Minimum	8.400	1.300	63.80
25th Percentile	20.95	6.675	65.50
Median	53.60	14.90	134.2
75th Percentile	220.9	32.20	227.0
Maximum	646.6	85.90	707.0
Mean	162.1	22.12	211.8
Std. Deviation	211.7	20.53	226.1
Std. Error of Mean	46.20	4.376	85.46
Lower 95% CI	65.73	13.02	2.678
Upper 95% CI	258.5	31.22	420.9

- Regarding remission status
  - 13/22 (59%) had elevated values at the time of diagnosis
  - 2 additional cases had elevations at PD (one for OS metastasis and the other for a new tumor (HSA) that developed during the follow up period). These cases did not have elevated Nu.Q® at diagnosis.
  - 4 dogs (with increased Nu.Q® at the time of diagnosis) had a CR and were on monitoring protocols when progression was noted at a mean of 52.2 days (range 27-85 days)
  - 13 dogs were in remission and did not progress during the follow up period. Their values remained low.

Individual Cases

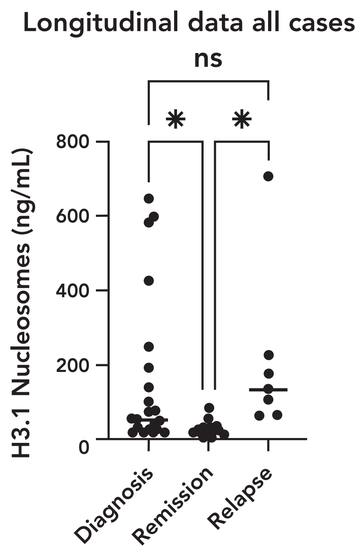

Figure 1: H3.1 plasma concentration in all dogs at diagnosis, remission and relapse. H3.1 plasma concentrations were significantly higher at diagnosis and relapse than remission (p= 0.02)

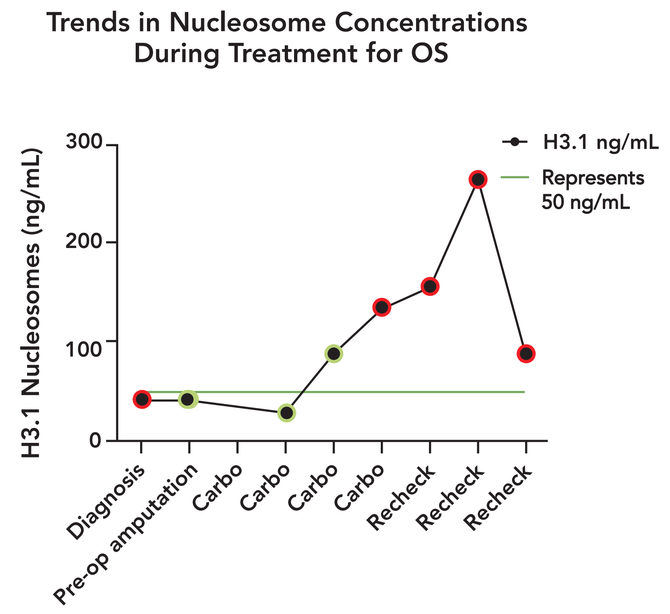

Figure 2: 9 y/o FS St. Bernard diagnosed with OS. Had a normal Nu.Q® value at diagnosis but as she developed metastatic disease her H3.1 plasma concentration increased. The first elevation was noted 85 days before metastatic lesions were seen on chest x-rays.

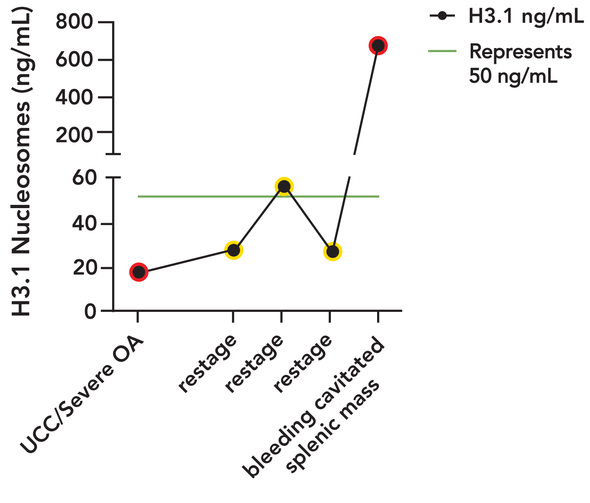

Figure 3: H3.1 trends in a 13 y/o MN Staffordshire Terrier diagnosed with UCC and treated with palliative RT. On the last visit he presented to the ER with a hemoabdomen and a bleeding splenic mass. He was euthanized and HSA was diagnosed on necropsy.

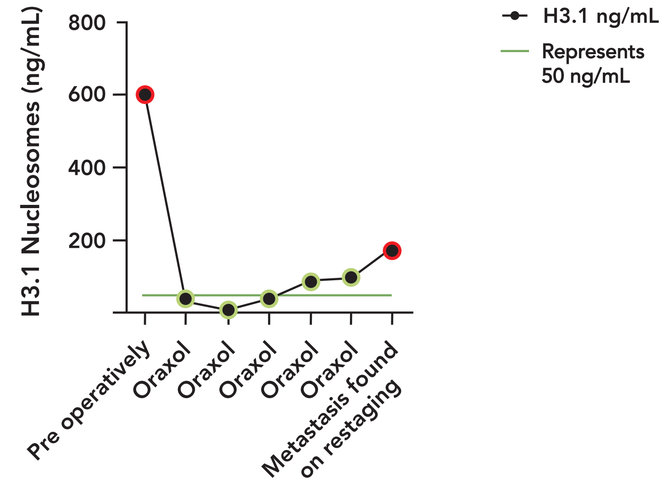

Figure 4: H3.1 plasma concentration trends in a 10 year old FS goldendoodle presented to the ER with a hemoabdomen, was diagnosed with stage II HSA, treated with splenectomy followed by Oraxol therapy.

However, she developed metastatic disease that was discovered during a regular restaging visit. H3.1 plasma concentrations begin to increase 2 visits (6 weeks) before metastasis was discovered.

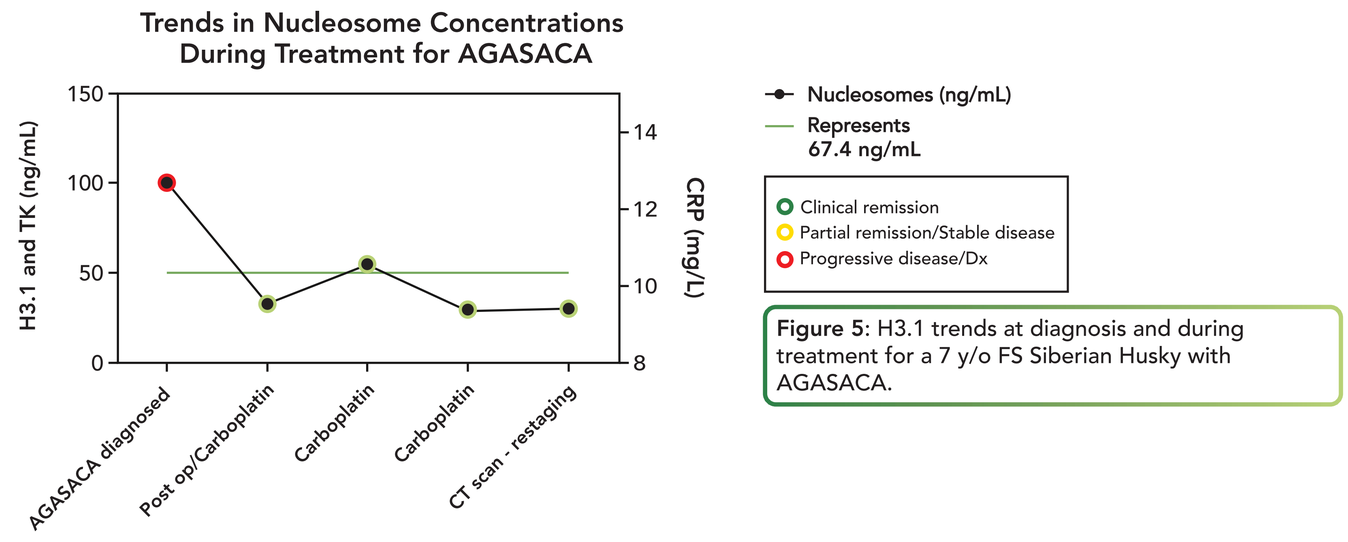

Figure 5: H3.1 trends at diagnosis and during treatment for a 7 y/o FS Siberian Husky with AGASACA.

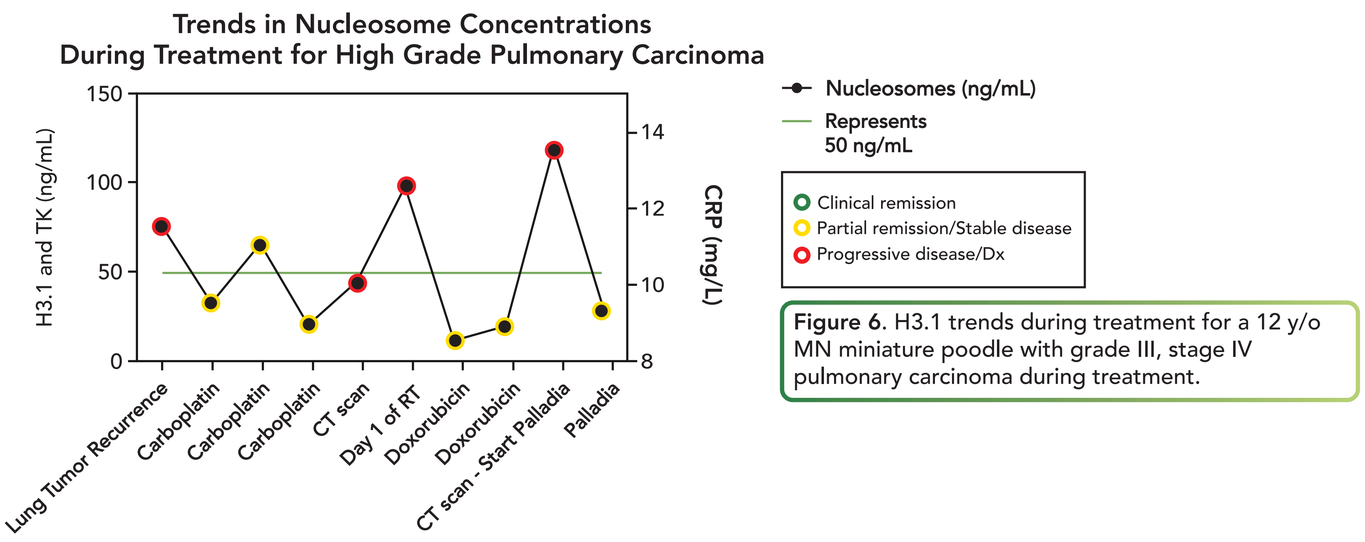

Figure 6. H3.1 trends during treatment for a 12 y/o MN miniature poodle with grade III, stage IV pulmonary carcinoma during treatment.

References

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