# Utility of Serial Plasma Nucleosomes Concentrations for Monitoring Treatment Response and Disease Progression in Canines with Hematopoietic Malignancies

Heather Wilson-Robles, DVM
DACVIM (Oncology

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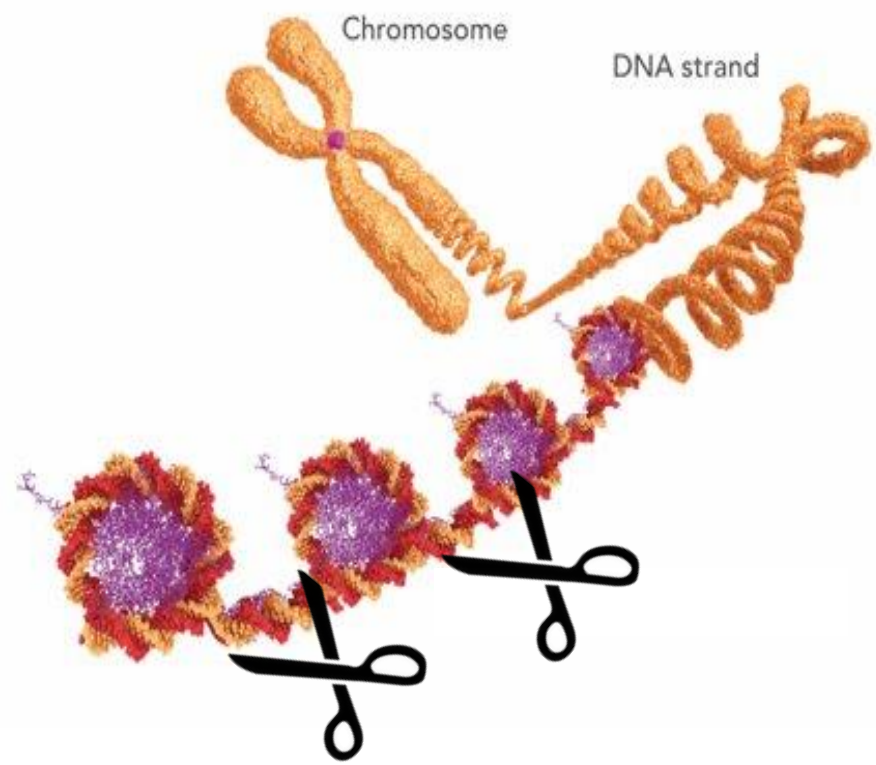
# The Basic Concept

- 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 [1], 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 [1], Petra Stieber, Joachim von Pawel, Hannelore Raith, Dorothea Nagel, Knut Feldmann, Dietrich Seidel

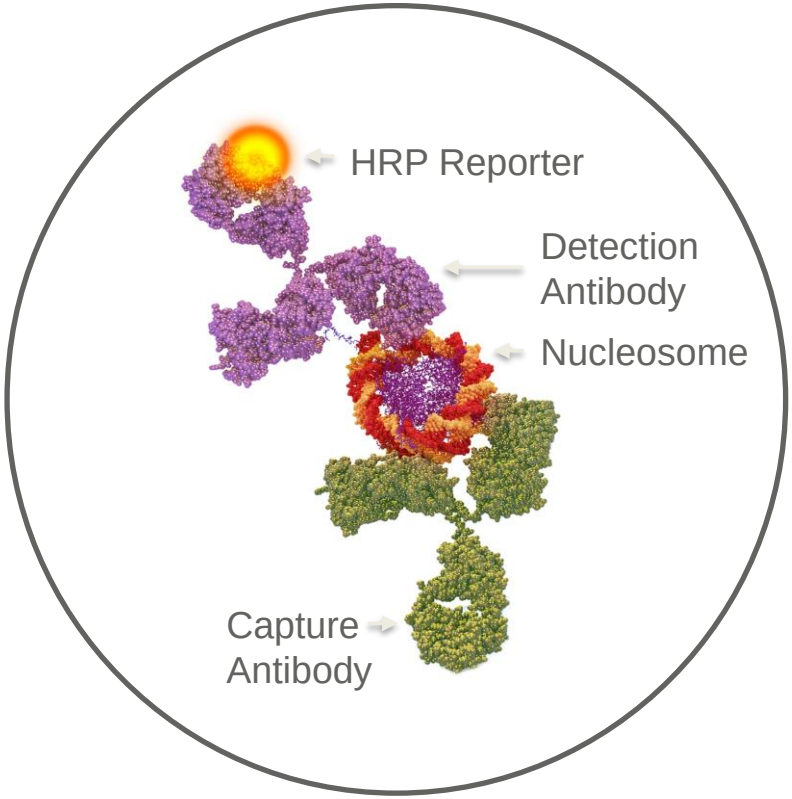
**Predictive and prognostic value of circulating nucleosomes and serum biomarkers in patients with metastasized colorectal cancer undergoing Selective Internal Radiation Therapy**

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

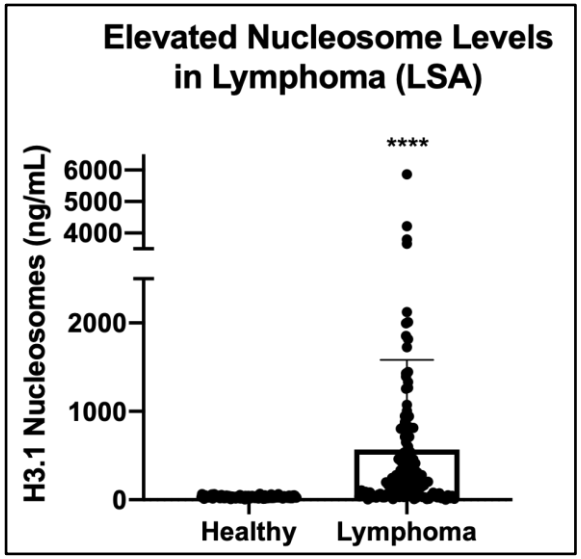
# Nu.Q® Technology

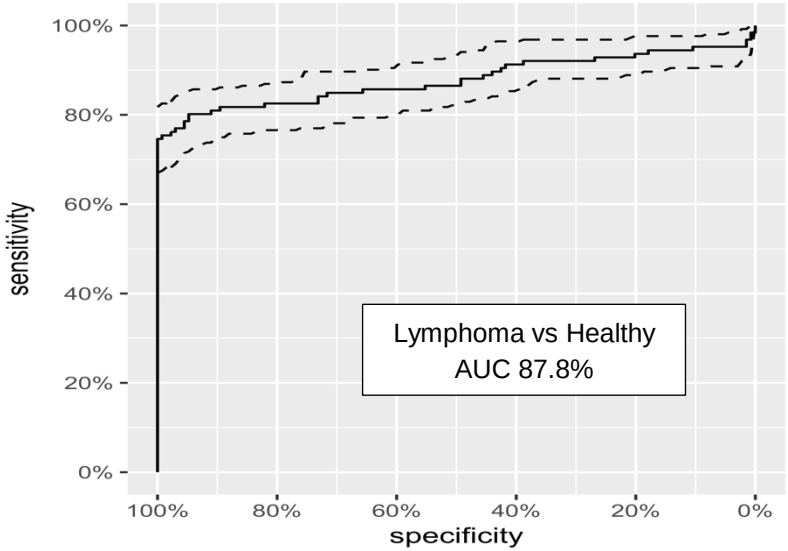
- **Proprietary epigenetic immunoassay platform**
- **Determine levels of circulating nucleosomes**
- **Profiles nucleosome epitopes**
  - Histone post translation modifications
  - Histone variants
  - DNA modifications

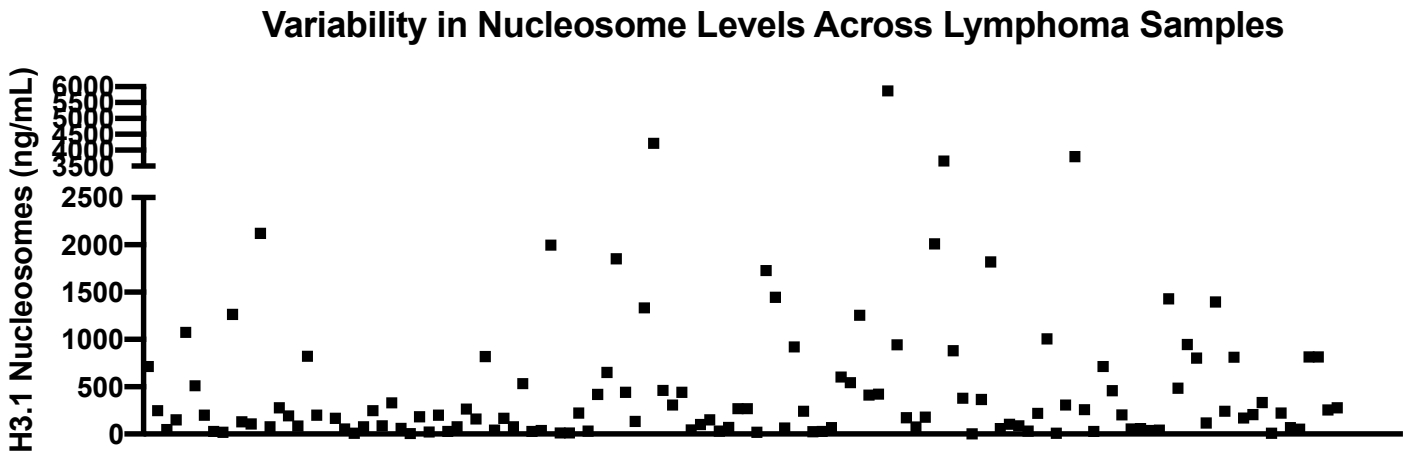
- **Flexibility of platform and diversity of modifications enables the development of disease specific panels**



# Circulating Nucleosomes in Dogs with Lymphoma (previously published)




Dolan, C, Wilson-Robles, H et al.
Characterizing Circulating Nucleosomes in the plasma of dogs with lymphoma
BMC Vet Res 17, 276 (2021)

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# Materials and Methods

- 25 dogs with hematopoietic malignancies were recruited for this study.
- Blood samples were drawn at diagnosis and at chemotherapy appointments and rechecks thereafter.
- Samples were centrifuged at 3000g for 10 min and the plasma was immediately removed.
- Samples were frozen at -80C to be run later in batches.
- Plasma was provided to the Texas A&M GI laboratory to determine C-Reactive Protein levels.
- Thymidine kinase-1 Canine ELISA kit

# Materials and Methods

- Medical records were available for review in all 25 cases.
- All dogs with multicentric lymphoma were staged with the following tests:
  - CBC, Chemistry, UA
  - Thoracic radiographs
  - Abdominal Ultrasound
  - Bone marrow aspirate
  - Flow cytometry

# Materials and Methods

- One dog with cutaneous lymphoma was staged with a skin biopsy, CBC, Chemistry, UA, thoracic radiographs and abdominal ultrasound.
- One dog with AML staged with CBC, Chemistry, AUS, CXR and Flow cytometry.
- One dog with Multiple Myeloma staged with CBC, Chemistry, Thoracic and Abdominal CT.

# Materials and Methods

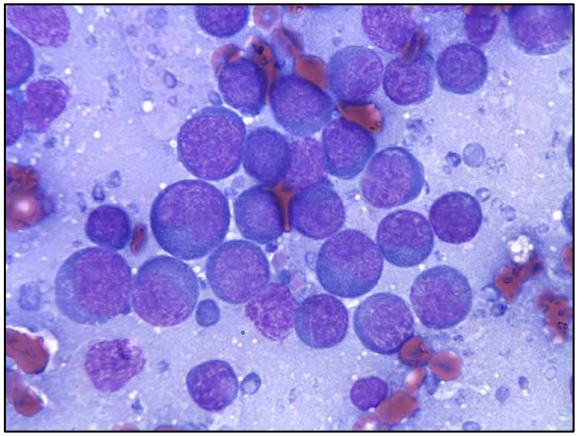
- Signalment, stage, phenotype and disease response information was recorded for each patient and matched to corresponding H3.1, CRP and TK1 plasma concentrations.
- CRP and TK1 analysis was not complete at the time of presentation for all cases.

# Results

- Signalment Summary:
  - 25 cases meeting criteria for enrollment
  - 15 MN, 9 FS
  - Median age 7 years (range 5-14 years)
  - Median weight 26.8 kg (range 4.9- 78 kg)
  - 5 mixed breed dogs, 4 Labrador retrievers, 2 miniature schnauzers, 2 Australian cattle dogs

# Results

- Disease Summary:
  - 15 dogs with B cell LSA
    - 4 stage IIIa and 1 IIIb multicentric LSA
    - 9 stage IVa and 1 Ivb multicentric LSA
  - 6 dogs with T cell lymphoma
    - 2 indolent T zone LSA (IIIa)
    - 2 stage IVa, 1 Ivb visceral lymphoma
    - 1 stage Va cutaneous lymphoma
  - 2 unknown phenotype
    - Both Vb



# Results

- 20/25 dogs had elevated nucleosome concentrations at diagnosis.
  - 2 T zone lymphomas within healthy range
  - 3 B cell lymphomas within healthy range
- Plasma H3.1 concentration at diagnosis:
  - 224.99 ng/mL median
  - 331.18 ng/mL (mean)
  - Range 78.2- >842.2 ng/mL

# Results

- Plasma H3.1 concentration at first CR
  - 32.49 ng/mL (median)
  - 80.37 ng/mL (mean)
  - Range: 0-561.6 ng/mL
- Lowest plasma H3.1 concentration during treatment.
  - 11.1 ng/mL (median)
  - 16.8 ng/mL (mean)
  - Range: 0-53.8 ng/mL

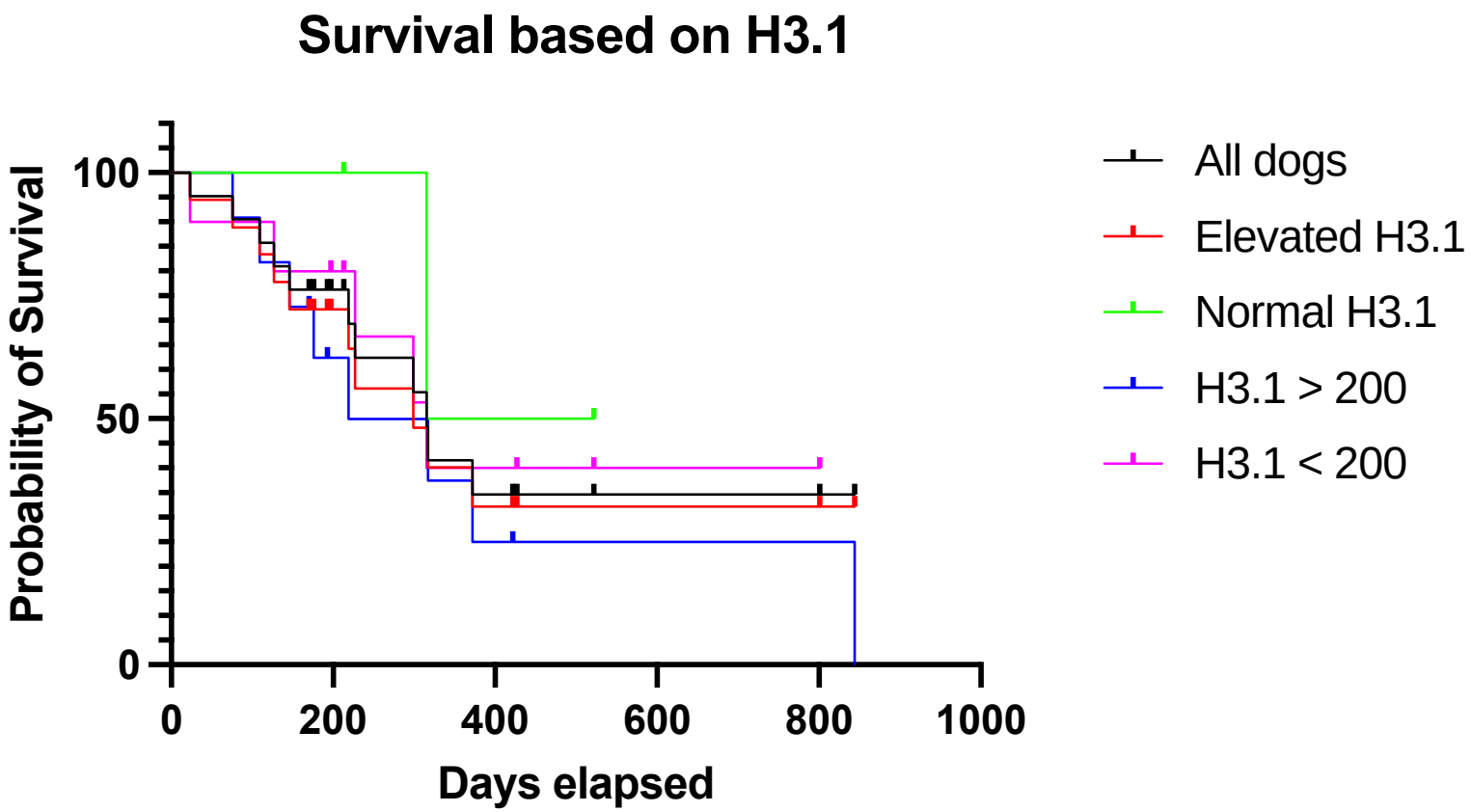
# Results

- 20/20 dogs had nucleosome concentrations drop into the healthy range during treatment.
  - Median time: 14.5 days
  - Mean 33.6 days
  - Range: 4-210 days
- Time to clinical remission or best response:
  - Median 24.5 days
  - Mean 29.95
  - Range: 6-85 days

# Results

- Median percent change from highest to lowest plasma H3.1 concentration:
  - Median percent change: 95.6%
  - Range: 75.2-100%
- Progression Free Survival
  - All dogs- (range 23-844) days
  - Elevated H3.1 (n=20)- (range 23-844) days
  - Normal H3.1 (n=3)- (range 213-522) days

# Results

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# Results

- Correlation Statistics
  - PFS vs % H3.1 Decrease
    - p 0.0976
    - r -0.38, 95% CI (-0.7-0.07), $R^2$ 0.15
  - PFS vs Days to Clinical Remission
    - p 0.78
    - r -0.06, 95% CI (-0.4942 – 0.38), $R^2$ 0.004
  - PFS vs Days to Normal Plasma H3.1 Concentration
    - p 0.54
    - r 0.15, 95% CI (-0.32-0.55), $R^2$ 0.02

# Cases: B cell lymphoma



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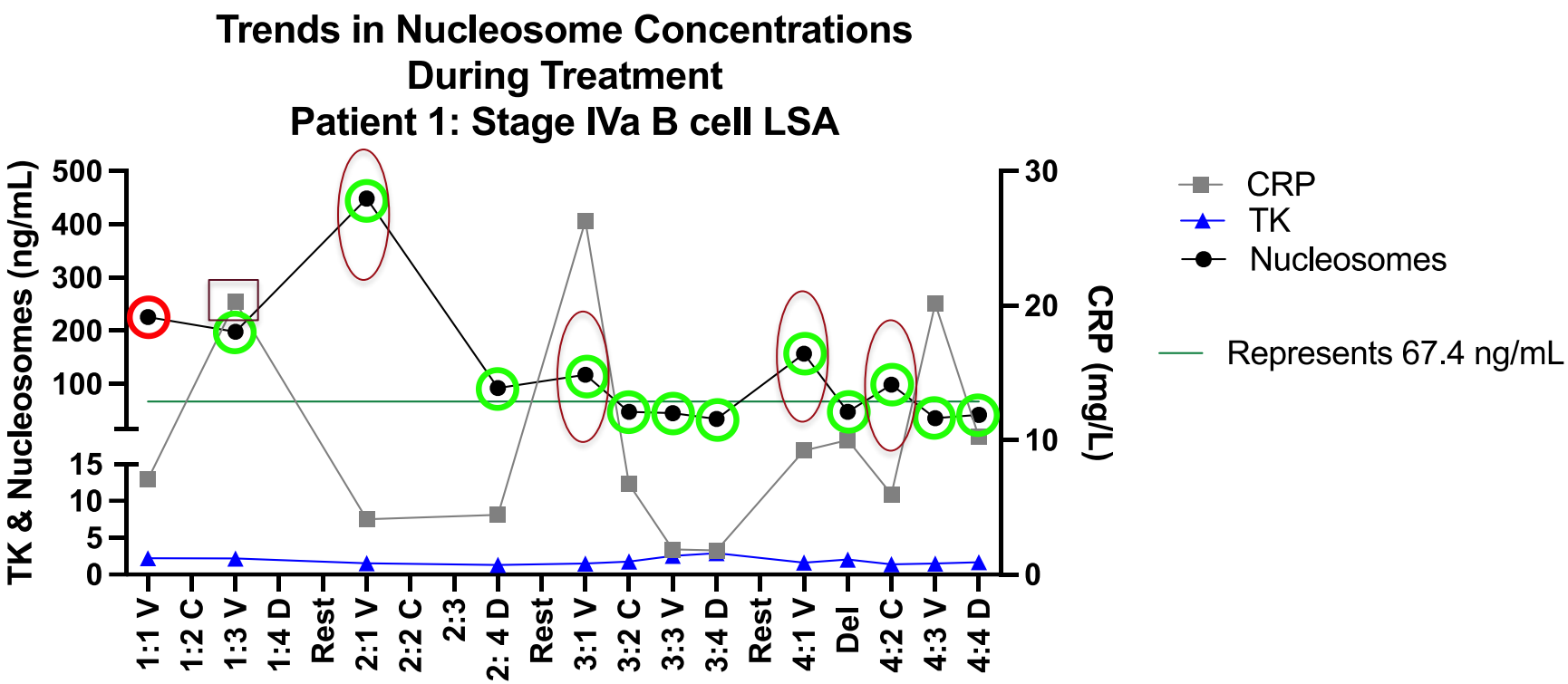
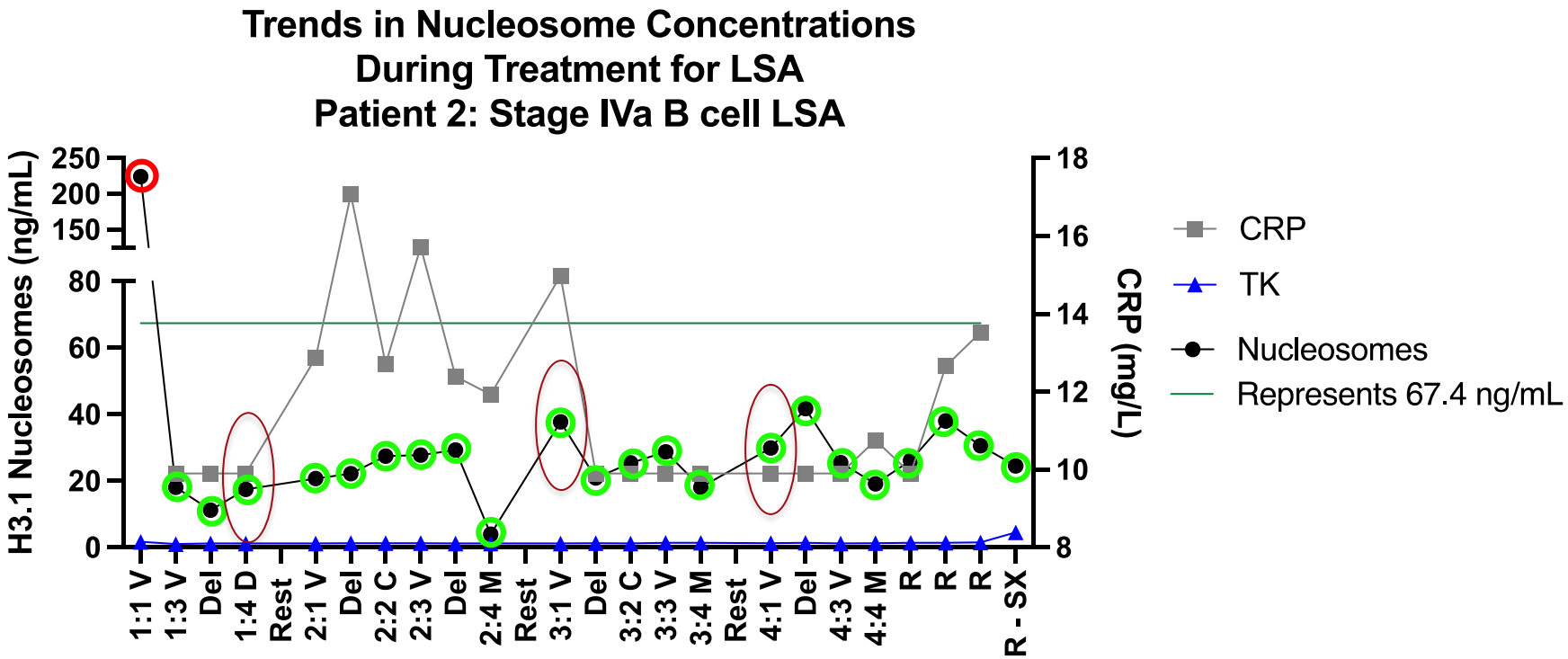
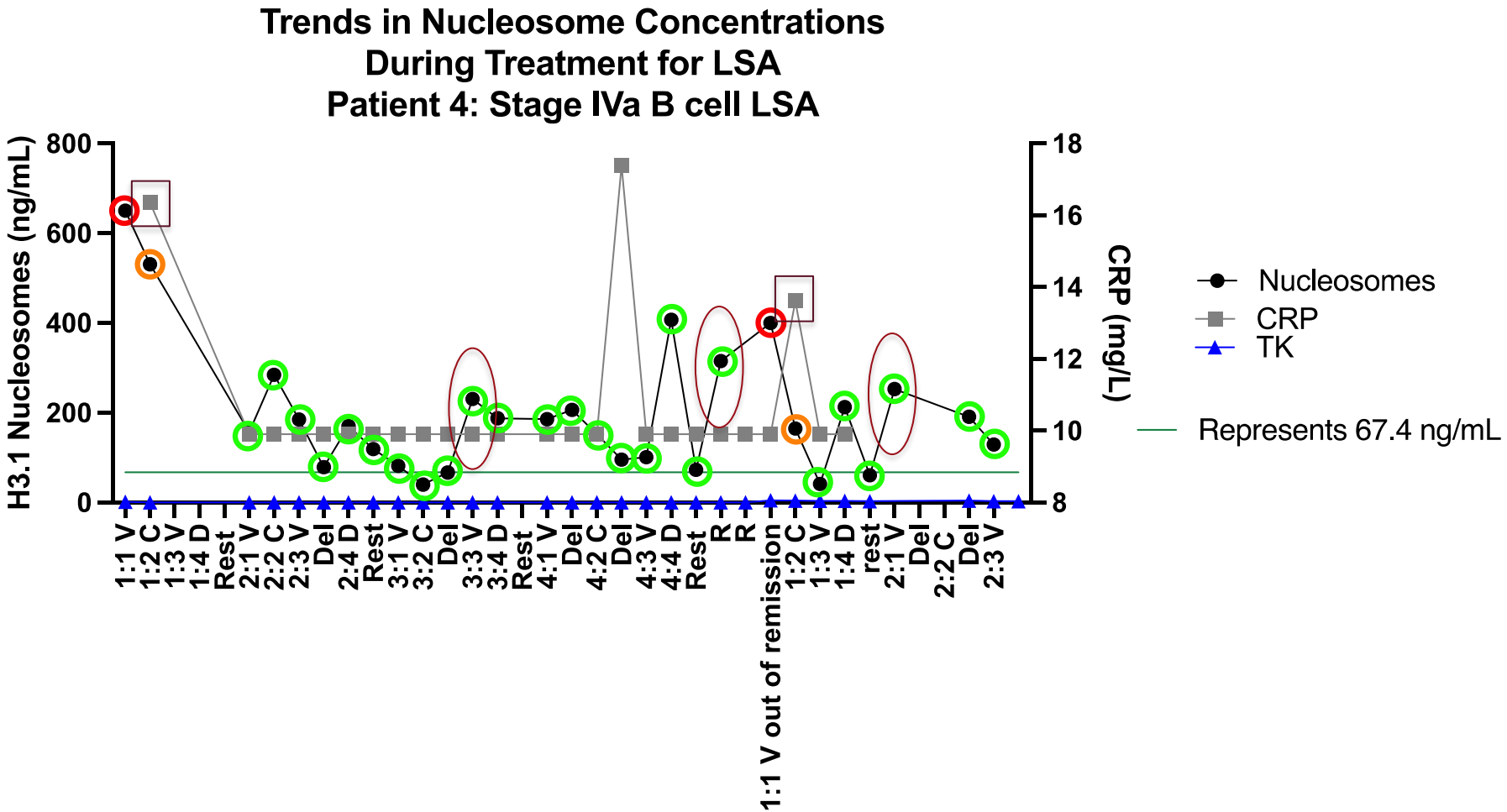
# Results


Trends in Nucleosome Concentrations
During Treatment
Patient 1: Stage IVa B cell LSA
TK & Nucleosomes (ng/mL)
500
400
300
200
100
15
10
5
0
CRP (mg/L)
30
20
10
0
1:1 V
1:2 C
1:3 V
1:4 D
Rest
2:1 V
2:2 C
2:3
2: 4 D
Rest
3:1 V
3:2 C
3:3 V
3:4 D
Rest
4:1 V
Del
4:2 C
4:3 V
4:4 D
CRP
TK
Nucleosomes
Represents 67.4 ng/mL

# Results


Trends in Nucleosome Concentrations
During Treatment for LSA
Patient 2: Stage IVa B cell LSA
H3.1 Nucleosomes (ng/mL)
250
200
150
80
60
40
20
0
CRP (mg/L)
18
16
14
12
10
8
1:1 V
1:3 V
Del
1:4 D
Rest
2:1 V
Del
2:2 C
2:3 V
Del
2:4 M
Rest
3:1 V
Del
3:2 C
3:3 V
3:4 M
Rest
4:1 V
Del
4:3 V
4:4 M
R
R
R
R - SX
CRP
TK
Nucleosomes
Represents 67.4 ng/mL

# Results


Trends in Nucleosome Concentrations
During Treatment for LSA
Patient 4: Stage IVa B cell LSA
H3.1 Nucleosomes (ng/mL)
800
600
400
200
0
CRP (mg/L)
18
16
14
12
10
8
Nucleosomes
CRP
TK
Represents 67.4 ng/mL
1:1 V
1:2 C
1:3 V
1:4 D
Rest
2:1 V
2:2 C
2:3 V
Del
2:4 D
Rest
3:1 V
3:2 C
Del
3:3 V
3:4 D
Rest
4:1 V
Del
4:2 C
Del
4:3 V
4:4 D
Rest
R
R
1:1 V out of remission
1:2 C
1:3 V
1:4 D
rest
2:1 V
Del
2:2 C
Del
2:3 V

# Cases: T cell lymphoma



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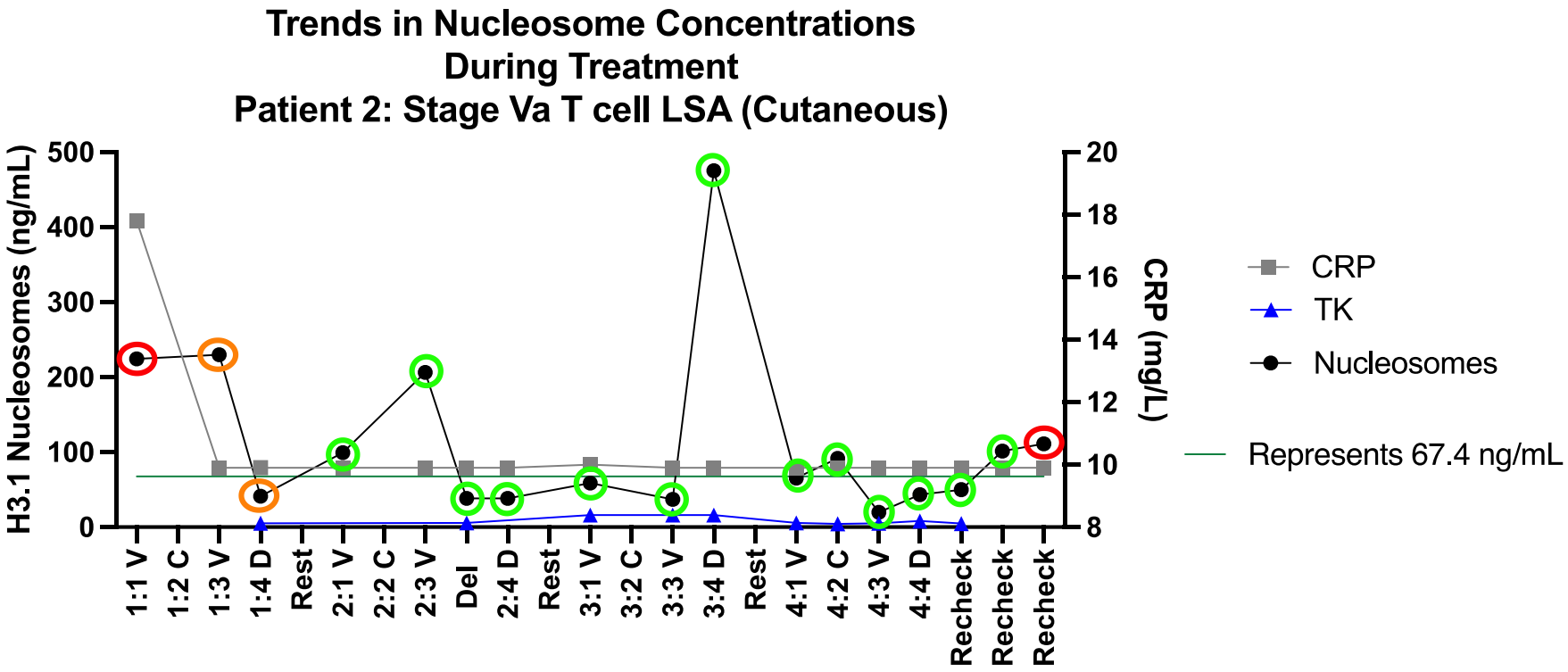
# Results


Trends in Nucleosome Concentrations
During Treatment
Patient 2: Stage Va T cell LSA (Cutaneous)
H3.1 Nucleosomes (ng/mL)
500
400
300
200
100
0
CRP (mg/L)
20
18
16
14
12
10
8
1:1 V
1:2 C
1:3 V
1:4 D
Rest
2:1 V
2:2 C
2:3 V
Del
2:4 D
Rest
3:1 V
3:2 C
3:3 V
3:4 D
Rest
4:1 V
4:2 C
4:3 V
4:4 D
Recheck
Recheck
Recheck
CRP
TK
Nucleosomes
Represents 67.4 ng/mL

# Conclusions

- Circulating nucleosome concentrations are elevated in many lymphoma cases (20/25 (80%) in this cohort).
- Circulating nucleosome concentrations change from week to week and appear to mirror disease response.
- There is no correlation between CRP and TK1 concentrations and circulating nucleosome concentrations.

# Conclusions

- No significant survival benefit for LSA cases based on H3.1 concentrations in this cohort.
  - Dogs with normal H3.1 had low numbers (n=3)
  - Larger cohort needed
- No correlation between PFS and:
  - % decrease in H3.1 concentration
  - Days to clinical remission
  - Days to normal H3. 1 concentration

# Future Directions

- Currently enrolling dogs with various chemotherapy or radiation responsive diseases to evaluate the utility of circulating nucleosomes as a surrogate for treatment response.
- Investigating the role circulating nucleosomes can play in determining MRD.
- Investigating prognostic significance of changes in nucleosome concentrations during treatment.

# Questions???

Email me at hwilson@cvm.tamu.edu