



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

DEPARTMENT  
OF VETERINARY  
MEDICAL SCIENCES

**DOTTORATO IN SCIENZE VETERINARIE  
XXXVIII CICLO  
AA 23-24**

**Studio dei paramenti morfologici e molecolari con valore diagnostico e prognostico nelle neoplasie degli animali domestici**

**Dottoranda: Elena Brigandì**

**Supervisore: Prof. Avallone Giancarlo**

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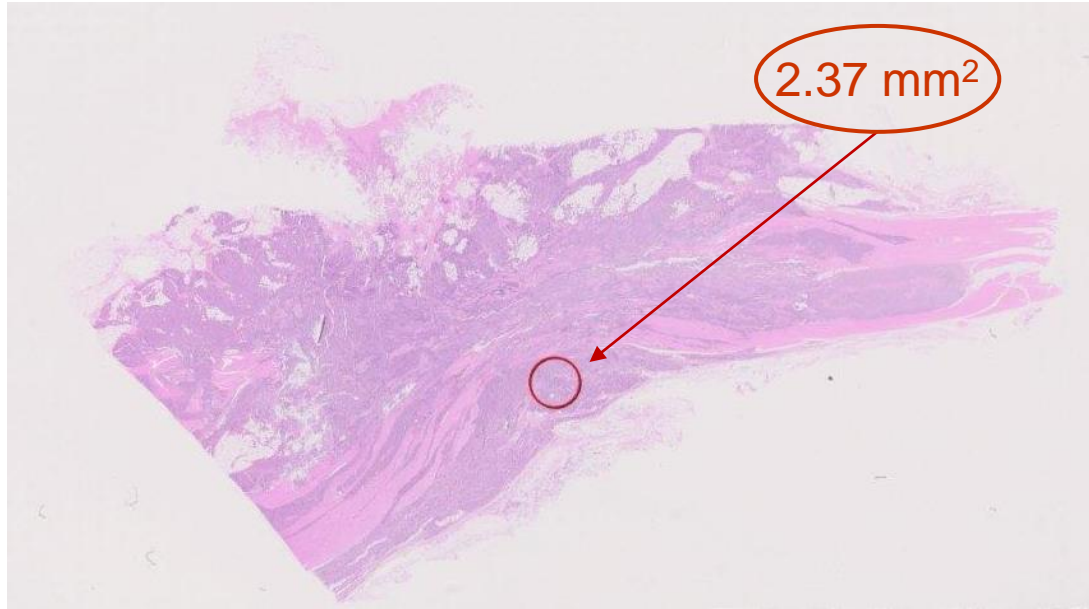


# 1) Descrizione dei modelli spaziali di proliferazione nelle neoplasie canine

Introduzione e Scopo

Materiali & Metodi

Risultati e Discussione



LE DIFFERENZE DI VALUTAZIONE TRA UN OSSERVATORE E L'ALTRO POSSONO ESSERE NUMEROSE



Iniziative di standardizzazione:

- Morfologia delle figure mitotiche
- Dimensione dell'area della conta mitotica
- Localizzazione dell'area della conta mitotica



Valutazione della **disposizione spaziale** delle **aree a più alta proliferazione** nei tumori canini in cui la conta mitotica è un fattore prognostico riconosciuto



Definizione di un **modello di distribuzione degli hotspot** sulla base della:

- distribuzione (**focale/multifocale, diffuso**)
- localizzazione (**centrale, periferica, sparsa**)

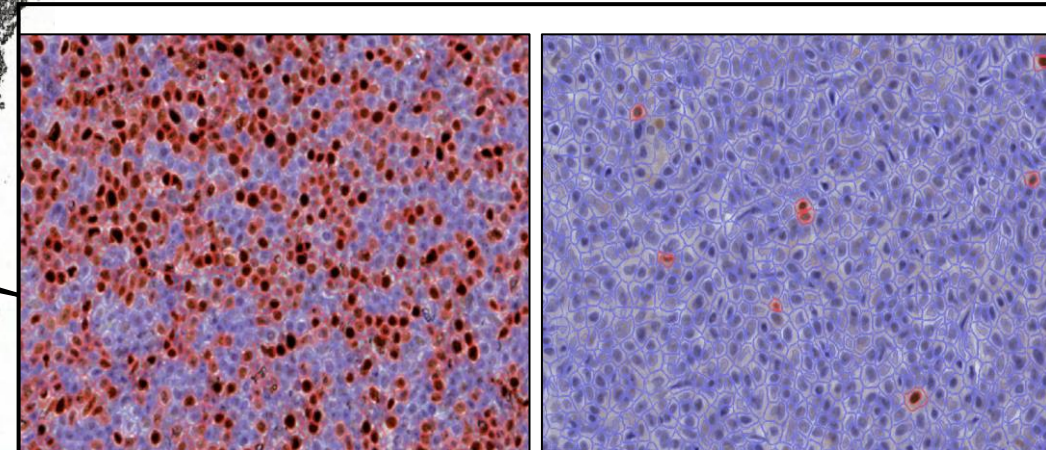
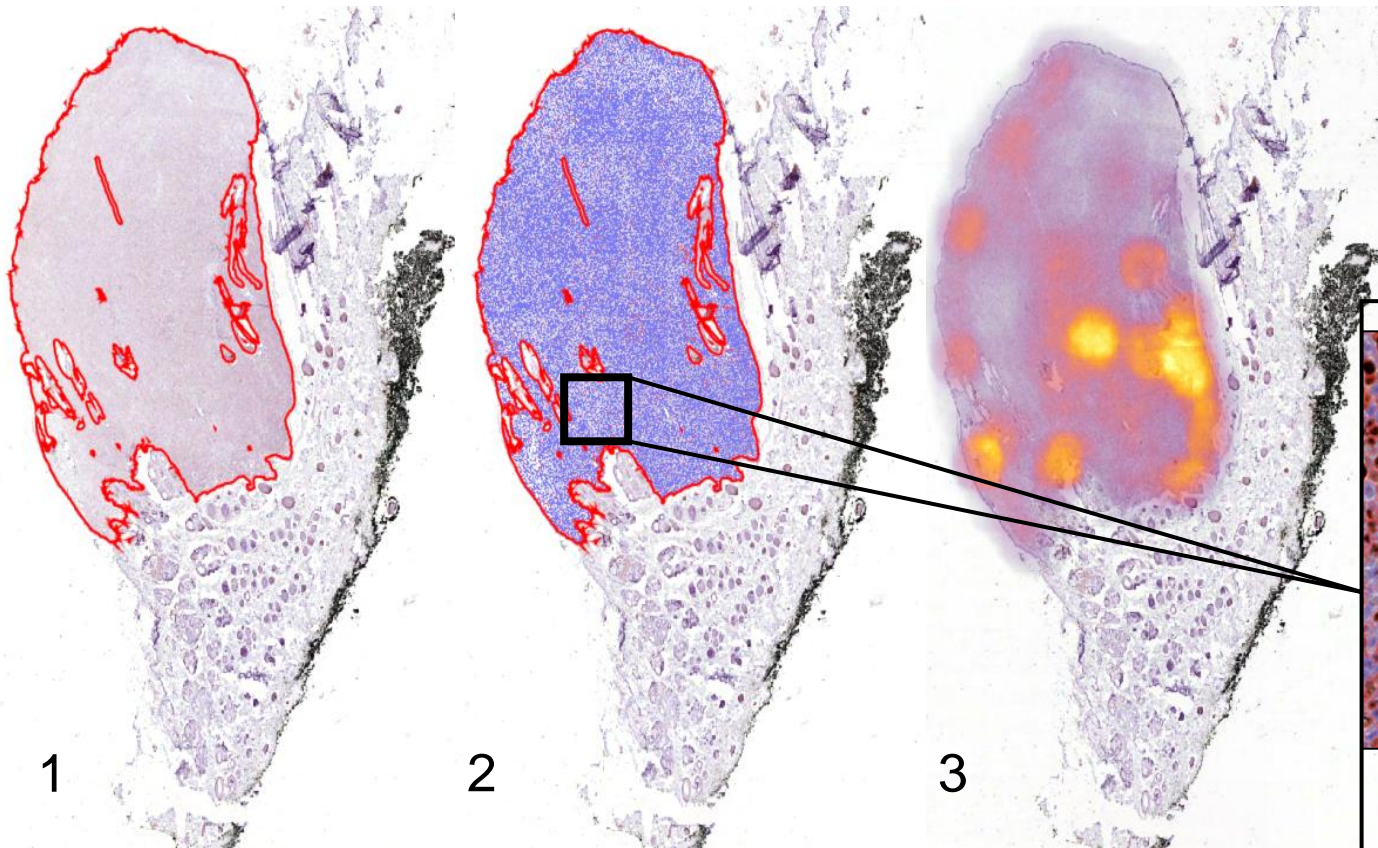
# 1) Descrizione dei modelli spaziali di proliferazione nelle neoplasie canine

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- ✓ 185 casi: melanomi, mastocitomi, carcinomi mammari e sarcomi dei tessuti molli
- ✓ Analisi immunohistochimica per Ki67
- ✓ Analisi d'immagine sulle scansioni con QuPath



Positive cell detection in due tumori con due diversi gradi di proliferazione

Analisi d'immagine con QuPath:

1) Selezione dell'area di interesse 2) Positive cell detection; 3) Density map

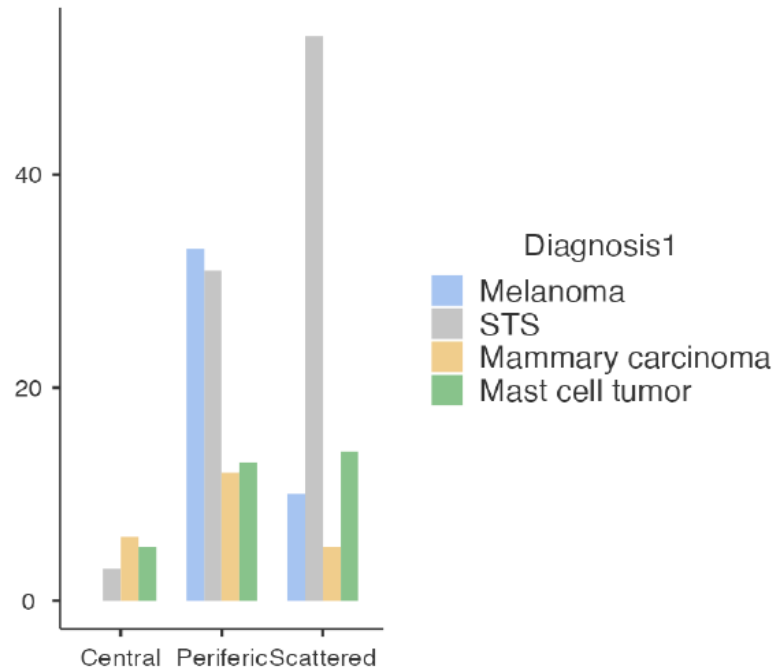


# 1) Descrizione dei modelli spaziali di proliferazione nelle neoplasie canine

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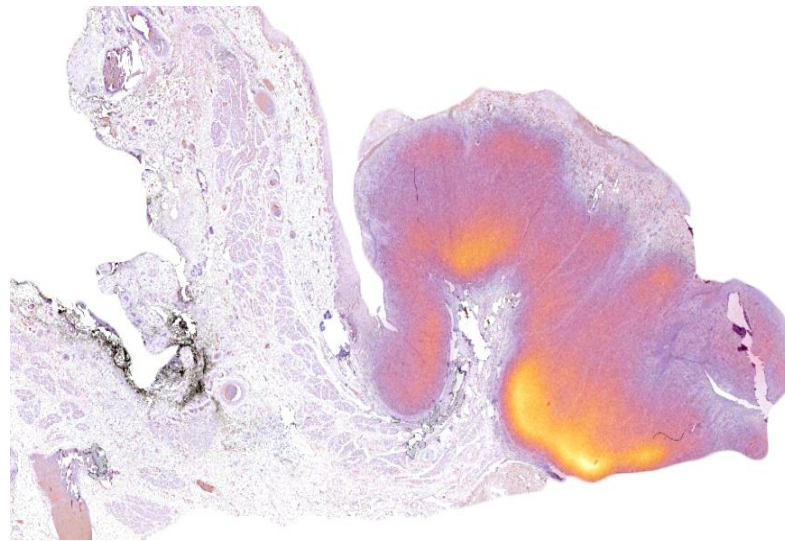
Risultati e Discussione



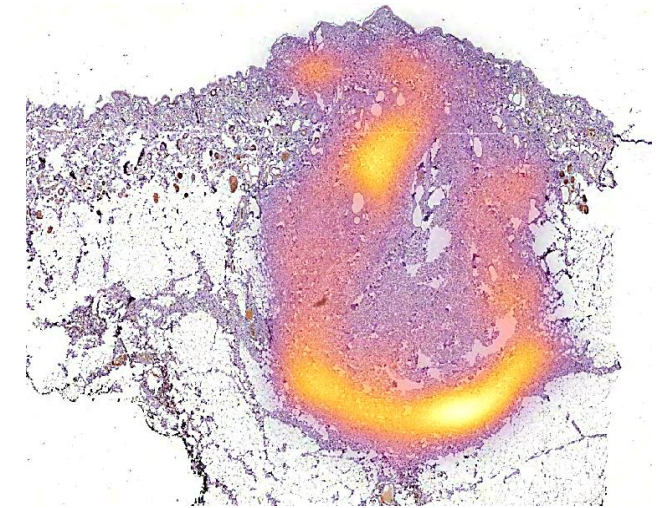
$\chi^2$  Tests

	Value	df	p
$\chi^2$	41.3	6	<.001
Test esatto di Fisher			<.001 <sup>a</sup>
N	185		

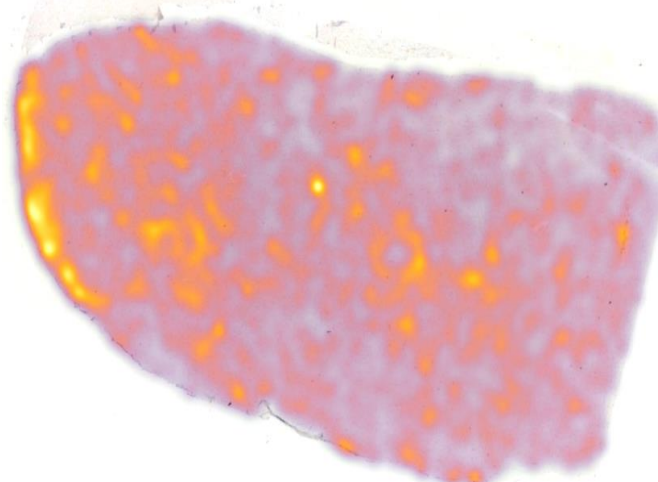
<sup>a</sup> Monte Carlo simulation



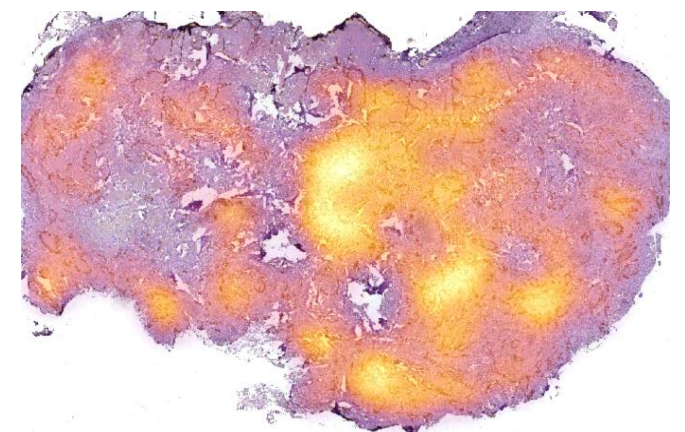
Modello periferico profondo in un melanoma cutaneo canino



Modello multifocale periferico in un mastocitoma canino



Modello multifocale disperso in un sarcoma dei tessuti molli canino (liposarcoma)



Modello multifocale centrale in un carcinoma mammario canino semplice



# 1) Descrizione dei modelli spaziali di proliferazione nelle neoplasie canine

## Description of spatial proliferation patterns in canine neoplasms

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### Background:

In many canine malignancies, mitotic count (MC) is a prognostic factor. Standardization initiatives have defined the morphology of mitotic figures and size of the area, while selection of the area where to perform the MC within the tumor still needs standardization. This study aimed to evaluate the spatial arrangement of the highest proliferative areas in canine tumors for which MC is a recognized prognostic factor, to define the best candidate areas for MC assessment.

### Materials and Methods:

Ki67 IHC was performed in canine melanomas, mast cell tumors (MCTs), mammary carcinomas (CMCs), and soft tissue sarcomas (STSs). Ki67 density maps were built on scanned slides using QuPath software. Analysis was performed starting with manual selection of the neoplastic tissue within the whole section and applying the positive cell detection tool (Analyze → Cell analysis → Positive cell detection). Based on the positive cell detection, a density map was created to define areas of higher proliferation (hotspots) (Figures 1-3). Each density map was visually analyzed by an experienced pathologist to categorize it into a specific group, classified as distribution (focal/multifocal or diffuse) and extension (central, peripheral, scattered).

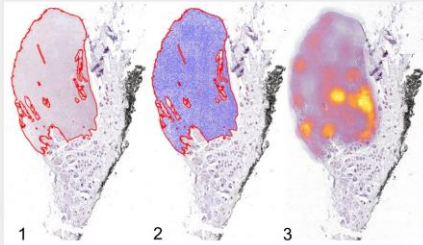
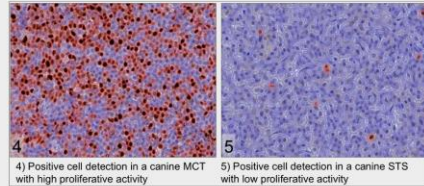
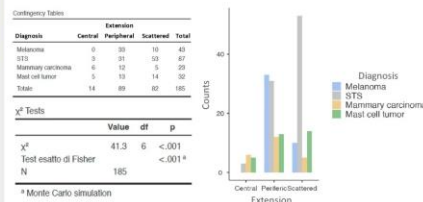


Image analysis with QuPath software:  
 1) Selection of area of interest; 2) Positive cell detection; 3) Density map



### Results:

A total of 185 cases were collected including: 43 melanomas, 32 MCTs, 23 CMCs, and 87 STSs (of which: 22 perivascular wall tumors, 15 myxosarcomas, 16 leiomyosarcomas, 22 liposarcomas and 12 fibrosarcomas). **Ki67-extension was peripheral in 89 cases, scattered in 82 and central in 14, correlating with diagnosis (p<0.001).** In melanomas and CMCs, the extension was mainly peripheral (77% and 52% respectively), in STSs scattered (61%), while in MCTs peripheral and scattered extensions were similar. Central extension was evident in 26% of CMCs, 16% of MCTs, 3% of STSs, and 0% of melanomas. Multifocality characterized 95% of the cases.



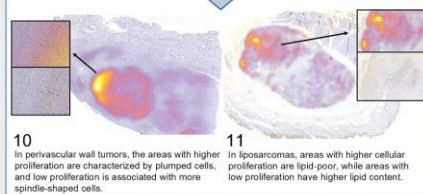
Ki67-extension was peripheral in 89 cases, scattered in 82 and central in 14, correlating with diagnosis (p<0.001).

### Conclusions:

Based on these findings, highest proliferative areas should be searched at the periphery in melanomas while counting should not be limited to the periphery in STSs and MCTs. Finally, it should be considered that in CMCs the highest proliferative area is frequently central. These preliminary results can represent a useful indication to standardize the best tumor site for MC in canine tumors.

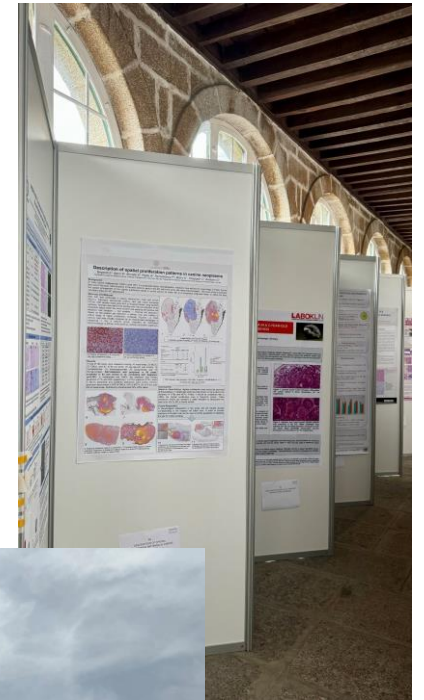
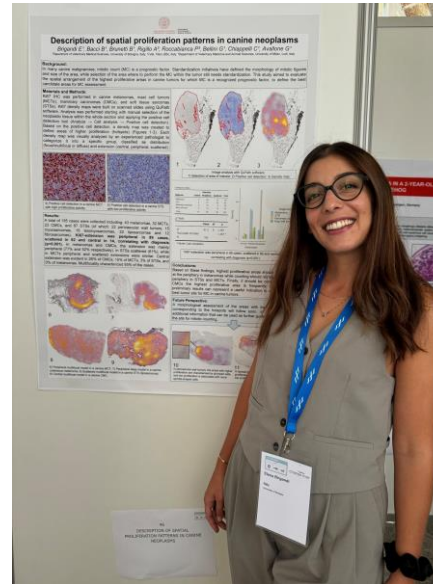
### Future Perspective:

A morphological assessment of the areas with the highest density corresponding to the hotspots will follow soon, in order to provide additional information that can be used as further guidelines for selecting the site for mitotic counting.



10 In perivascular wall tumors, the areas with higher proliferation are characterized by plumped cells, and low proliferation is associated with more spindle-shaped cells.  
 11 In liposarcomas, areas with higher proliferation are lipid-poor, while areas with low proliferation have higher lipid content.

5th Cutting Edge Pathology Congress  
 August 28th to 31st 2024  
 San Lorenzo de El Escorial (Madrid)



## 2) Studio del microambiente tumorale nei sarcomi dei tessuti molli del cane

### Introduzione e Scopo

### Materiali & Metodi

### Risultati e Discussione

SARCOMI DEI TESSUTI MOLLI (STS) =

- ✓ tumori eterogenei con origine mesenchimale
- ✓ in media, il 25%–50% di questi pazienti sviluppano malattia ricorrente e/o metastatica
- ✓ immunogenicità relativamente bassa



- **Caratterizzare la risposta immunitaria linfocitica nei STS canini**
- **Confrontare la risposta immunitaria** in relazione a:
  - ✓ Differenti istotipi
  - ✓ Grado istologico
- **Aumentare l'obiettività** nella valutazione dei linfociti infiltranti il tumore (TILs)



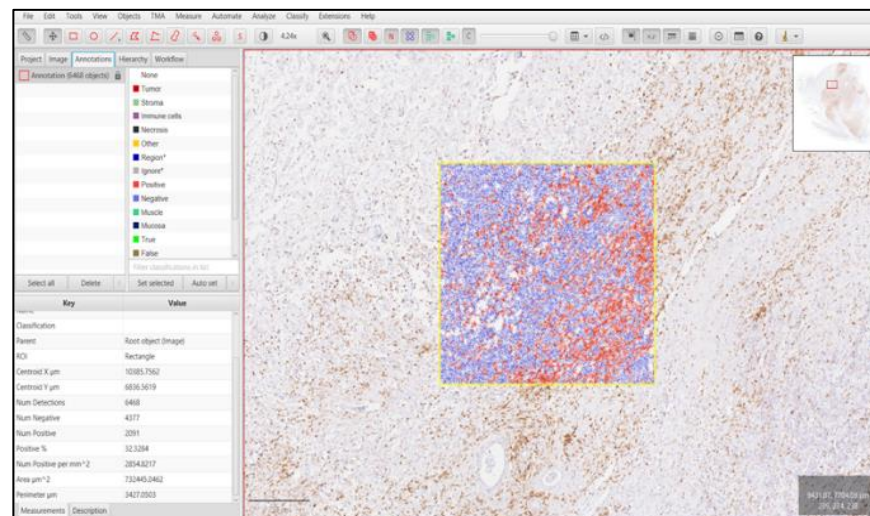
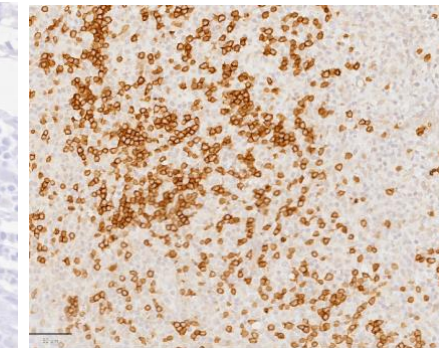
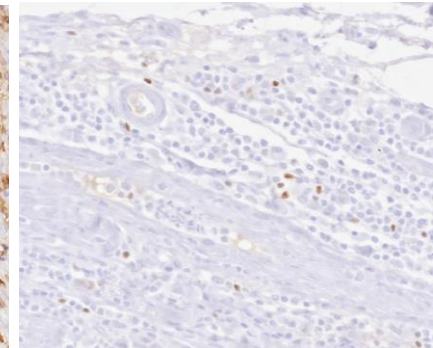
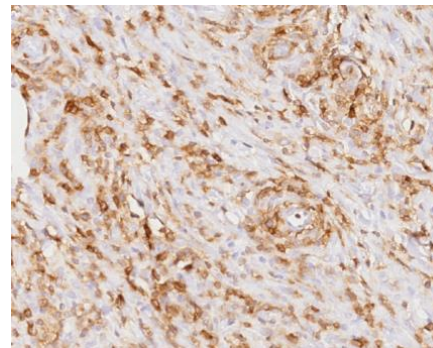
# 2) Studio del microambiente tumorale nei sarcomi dei tessuti molli del cane

Introduzione e Scopo

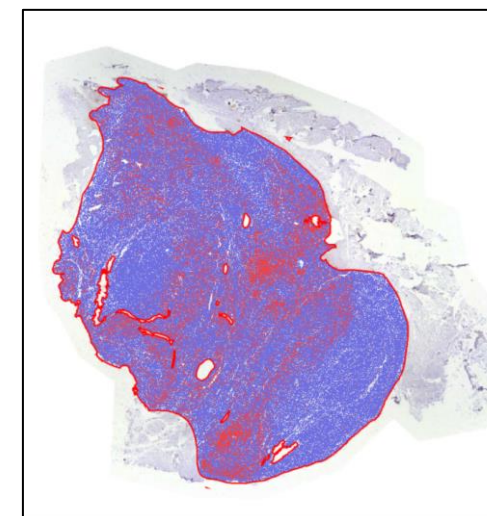
Materiali & Metodi

Risultati e Discussione

- ✓ **87 STS, 5 istotipi** differenti: **tumori perivascolari, mixosarcomi, leiomiosarcomi, liposarcomi e fibrosarcomi**
- ✓ Analisi immunoistochimica per **CD20** (linfociti B), **CD3** (linfociti T totali) e **FOXP3** (linfociti T regolatori)
- ✓ Analisi d'immagine sulle scansioni con QuPath



Positive cell detection (CD3 IHC - PWT)



Densità (cellule/mm<sup>2</sup>):

- Linfociti B
- Linfociti T
- Linfociti T-reg



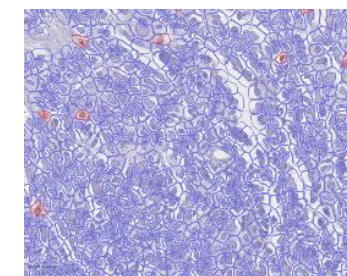
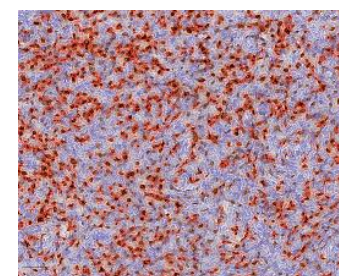
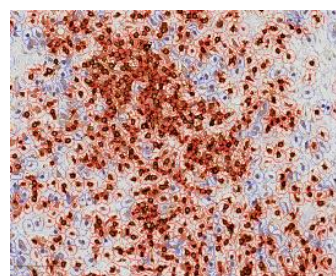
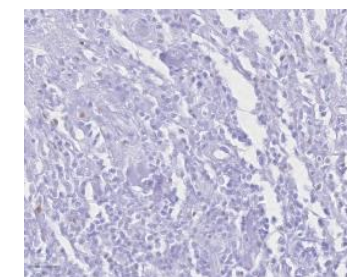
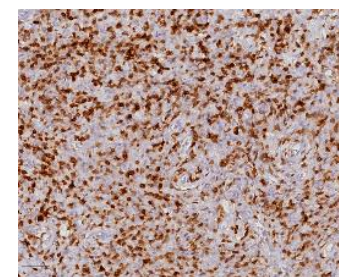
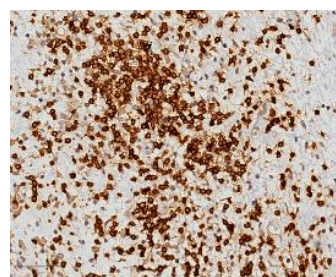
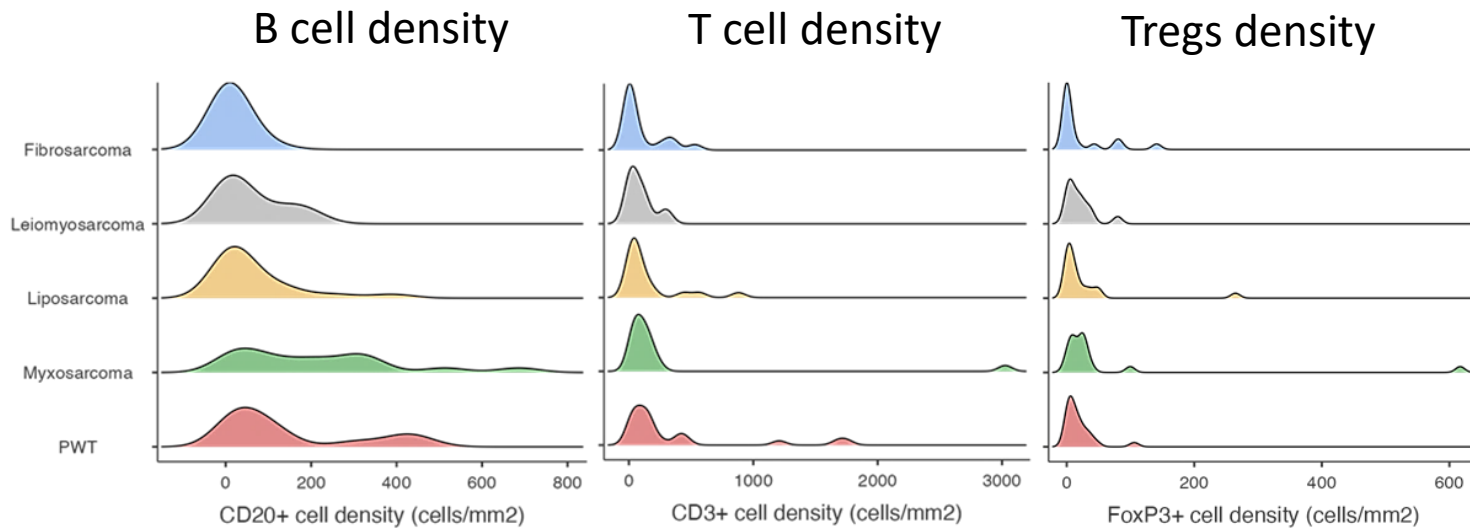
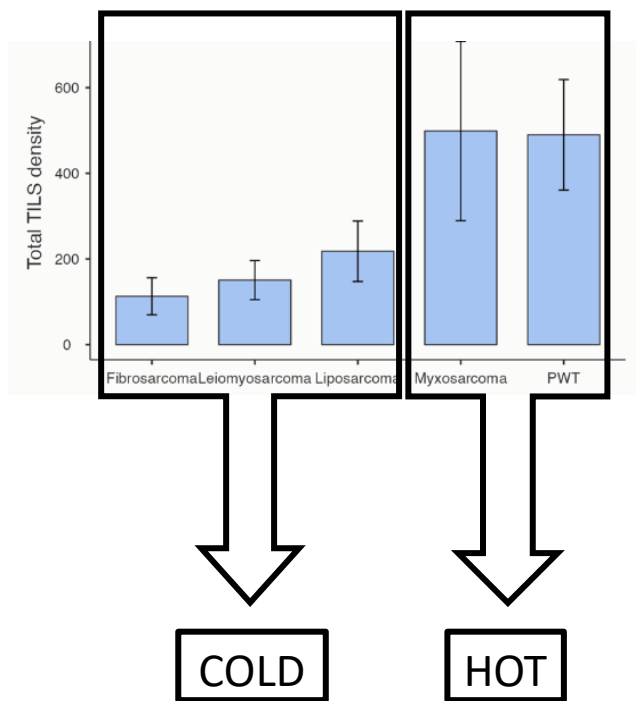
- Densità totale dei TILs
- % T-reg
- Rapporto B/T

# 2) Studio del microambiente tumorale nei sarcomi dei tessuti molli del cane

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ISTOTIPO TUMORALE ↔ Densità totale dei TILS  
 Tipo linfocitario  
 % Tregs  
 B/T

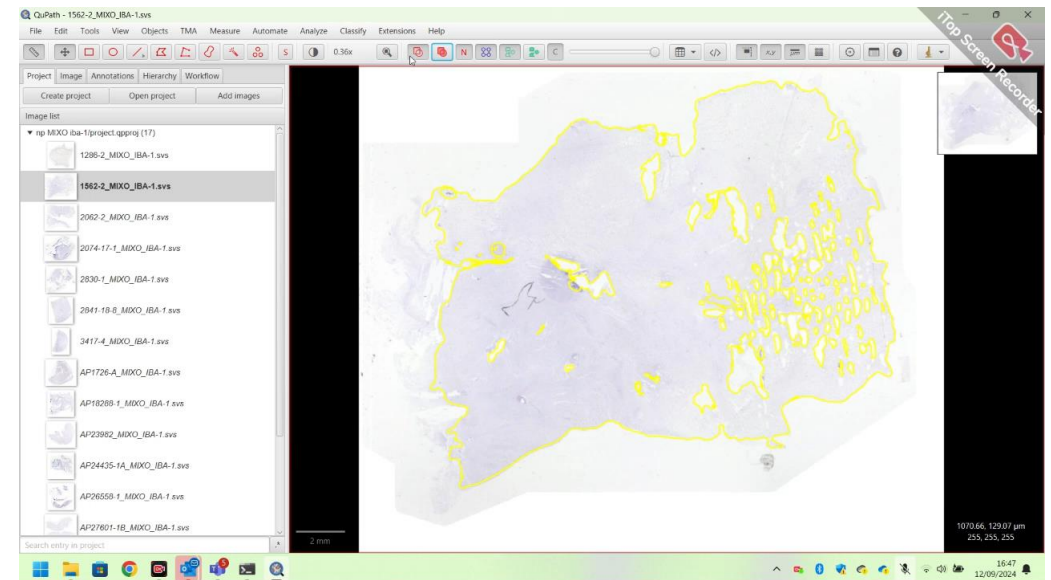
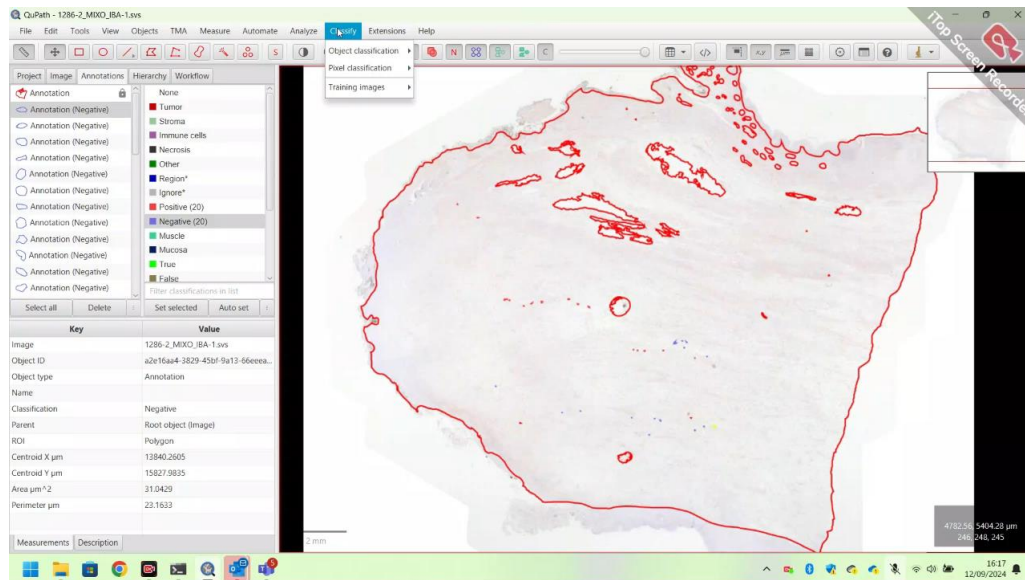
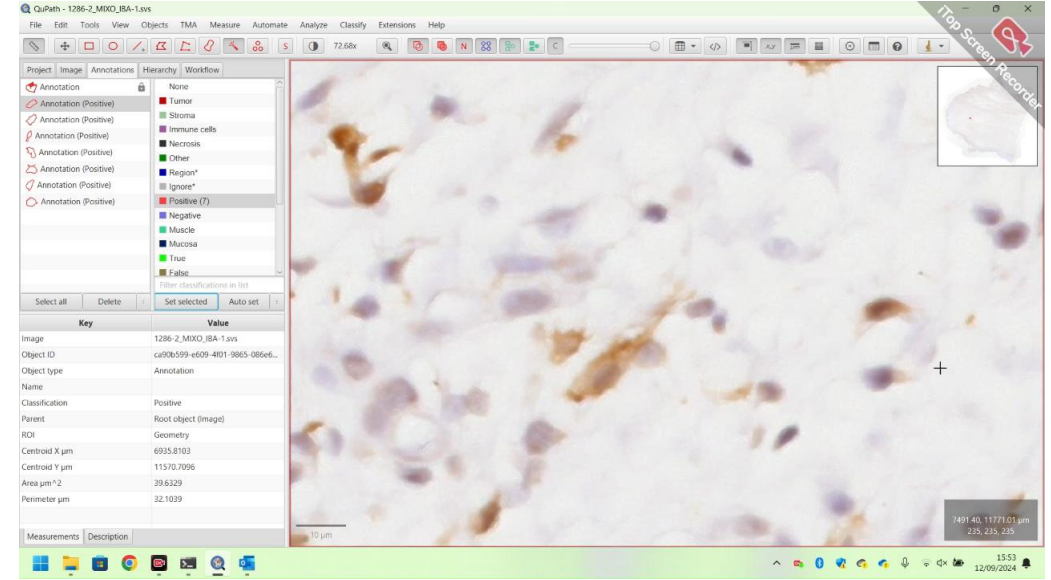
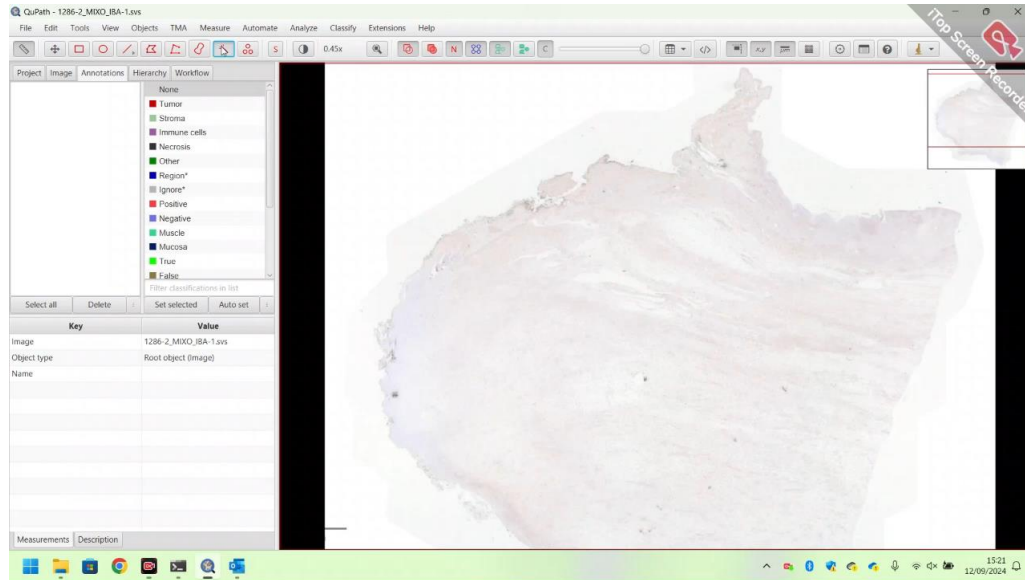
Mixosarcomi: ↑ CD20+ ↑ FoxP3+ ↑ B/T = pro-tumorale

Tumori Perivascolari: ↑ CD3+ ↓ FoxP3+ = anti-tumorale

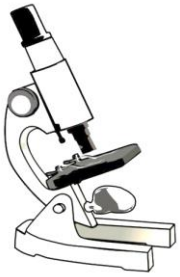


# 2) Studio del microambiente tumorale nei sarcomi dei tessuti molli del cane

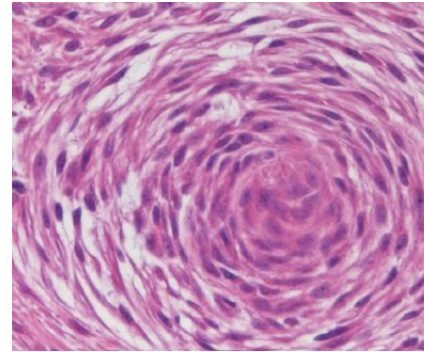
Analisi della popolazione macrofagica tramite l'analisi immunohistochimica di **IBA-1** e CD204



### 3) Studio dei livelli di espressione di EMA nei tumori delle guaine nervose e perivascolari (NST) (PWT)

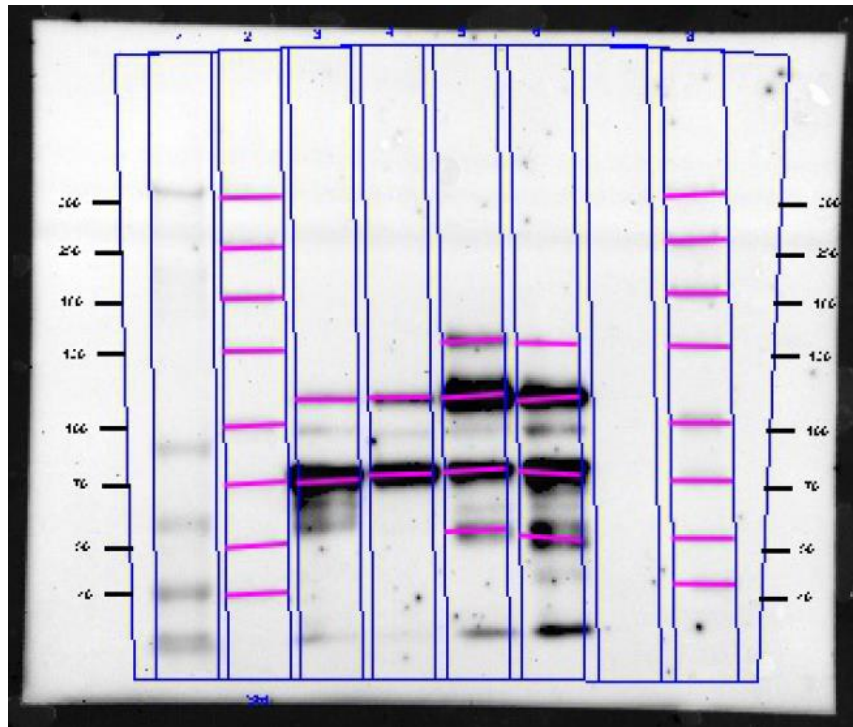


NST vs PWT: oggetto di dibattito

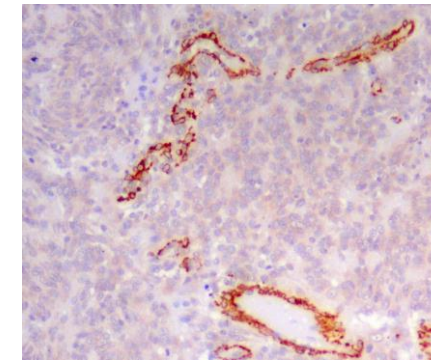


UOMO ► PWT e Perineuriomi ► EMA+

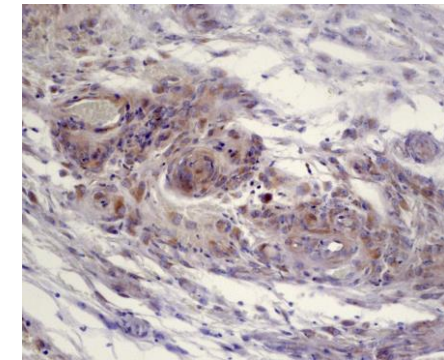
CANE ► PWT ► EMA?



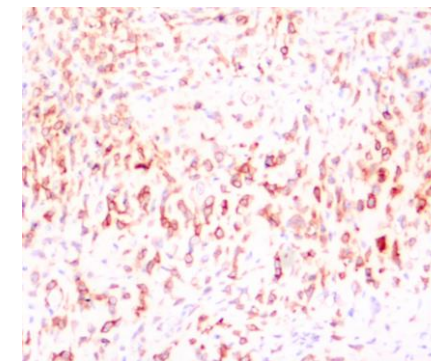
L'anticorpo **EMA** è stato validato tramite **Western Blot** e l'analisi immunocistochimica per EMA, actina, calponina e NGFR è in corso su una casistica preliminare di PWT e NST.



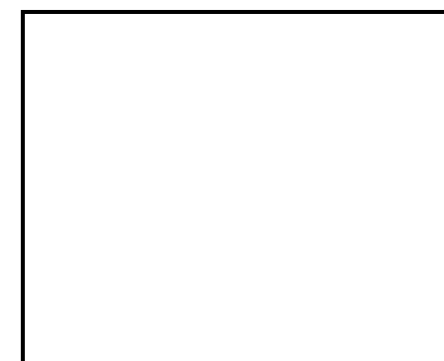
PWT alfa-actina+



PWT calponina+



NST NGFR+



EMA



# 4) Studio di proteomica su sarcomi dei tessuti molli del cane

Periodo all'estero presso "The Institute of Cancer Research", London (UK) da Gennaio 2025 a Maggio 2025



**ICR** The Institute of Cancer Research

nature communications



Article

<https://doi.org/10.1038/s41467-023-39486-2>

## The proteomic landscape of soft tissue sarcomas

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Check for updates

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Jyoti S. Choudhary<sup>4</sup>, Ian Judson<sup>3</sup>, Cyril Fisher<sup>7</sup>, Eugene F. Schuster<sup>8,9</sup>,  
Anguraj Sadanandam<sup>1</sup>, Tom W. Chen<sup>10,11</sup>, Daniel Williamson<sup>5</sup>, Khin Thway<sup>1,3</sup>,  
Robin L. Jones<sup>2,3</sup>, Maggie C. U. Cheang<sup>2</sup> & Paul H. Huang<sup>1</sup>✉

Il progetto si concentrerà sull'analisi proteomica di diversi istotipi di sarcomi dei tessuti molli canini, con l'obiettivo di scoprire biomarcatori proteici chiave.





A microscopic image of tissue, likely a histological section, showing a dense population of cells. The cells are stained with hematoxylin and eosin (H&E), resulting in a purple-blue color for the nuclei and a pinkish-red color for the cytoplasm and extracellular matrix. There are numerous small, dark brown spots scattered throughout the tissue, which could be indicative of certain cellular components or artifacts. A white rectangular box is overlaid on the center of the image, containing the text "Grazie a tutti per l'attenzione!".

***Grazie a tutti per l'attenzione!***