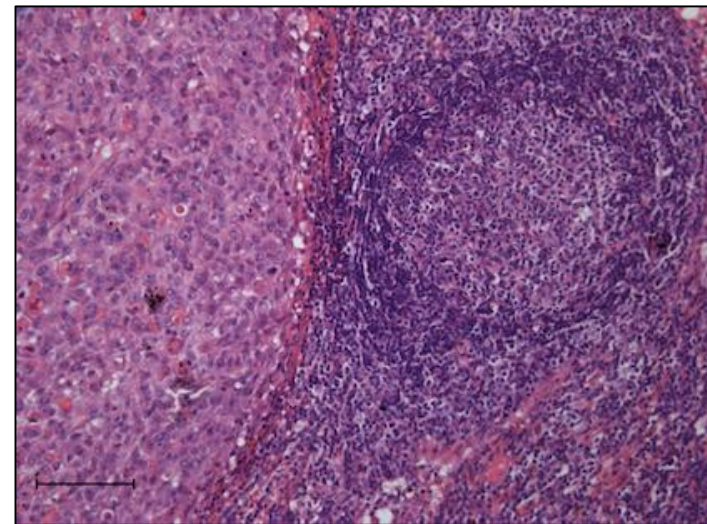


The impact of hypoxia on regulatory T cells in canine cancer and inflammation

BSAVA 2016

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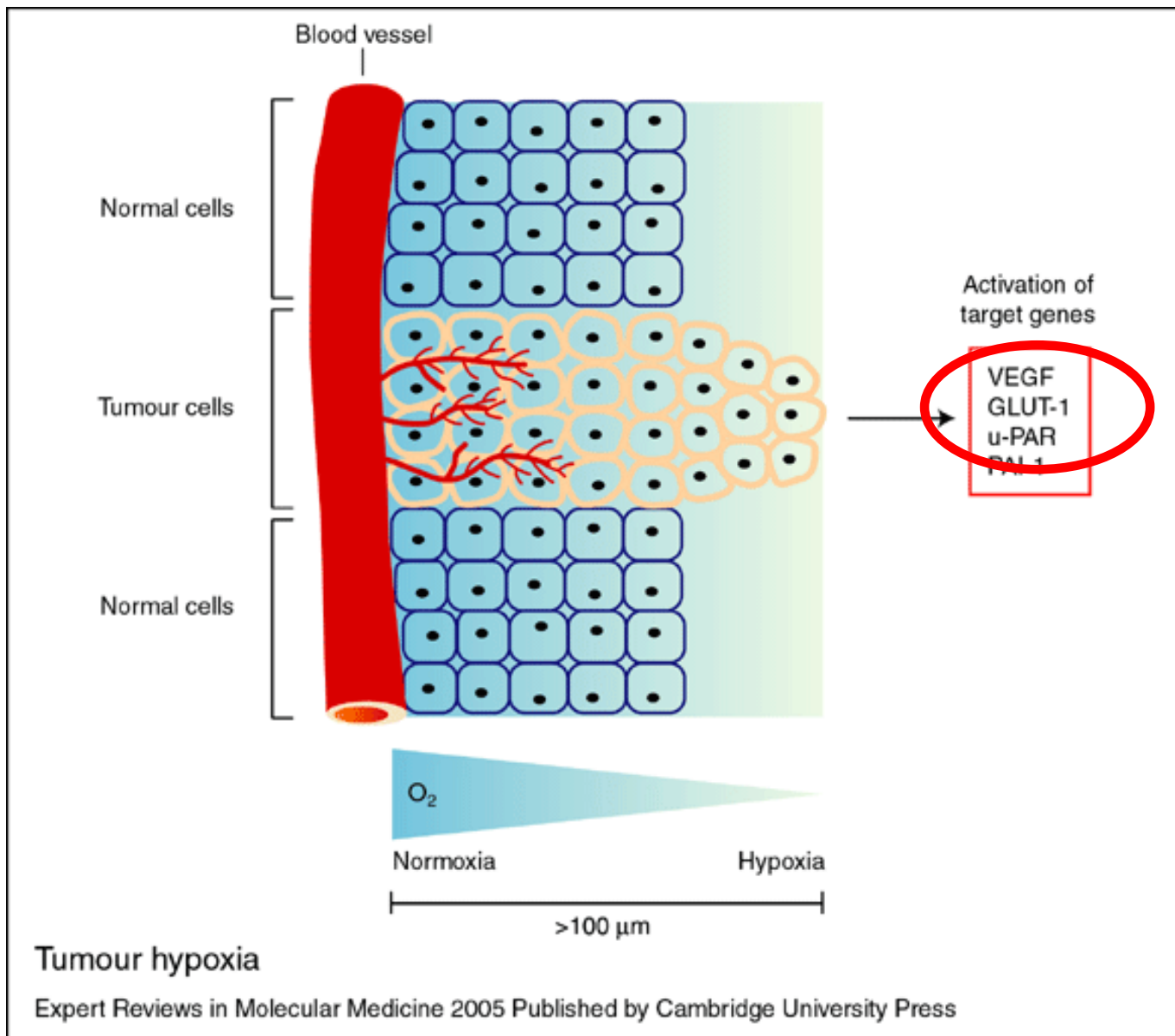
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Hypoxia

- **Definition** –low O₂ levels such that cellular biology is compromised
- **Hallmark** of solid tumours
- Linked to **therapeutic failure** in neoplasia treatment protocols
- Confers a **worse prognosis** to cancer treatment
- **Glut1** widely used as a biomarker of hypoxia

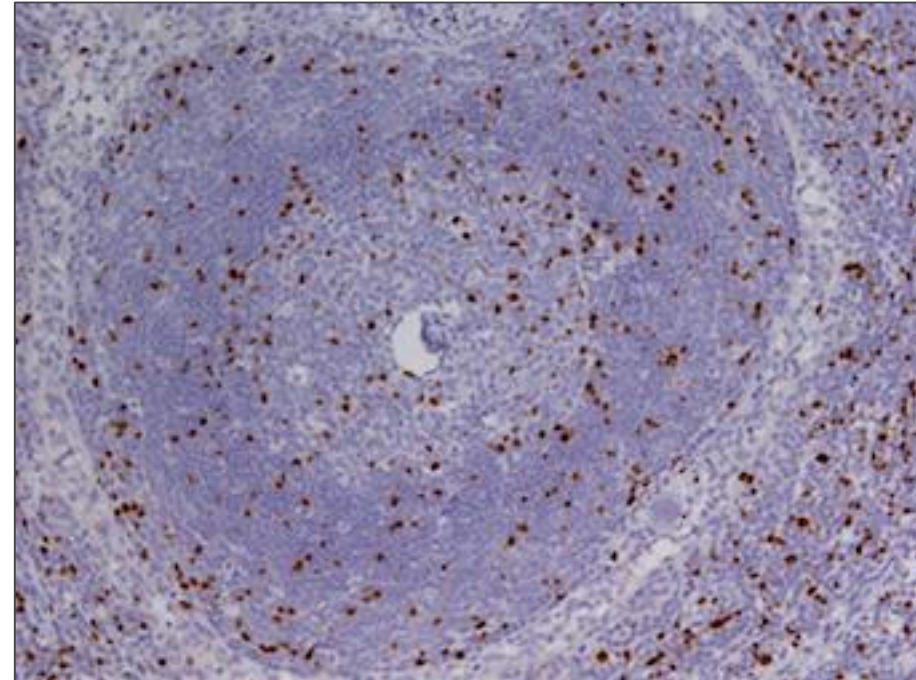
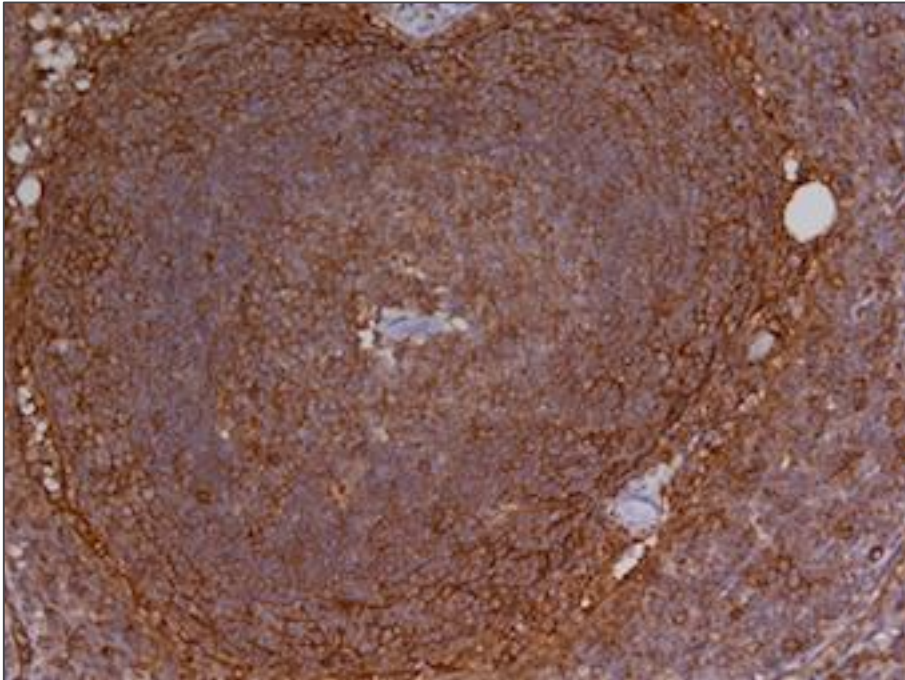


Regulatory T cells

- Normally maintain **immune tolerance**
- Implicated in **suppressing** the anti-tumour inflammatory immune response
- **FoxP3** widely used as marker for Tregs

Glut1 and FoxP3⁺ cells...

ARE THEY LINKED?



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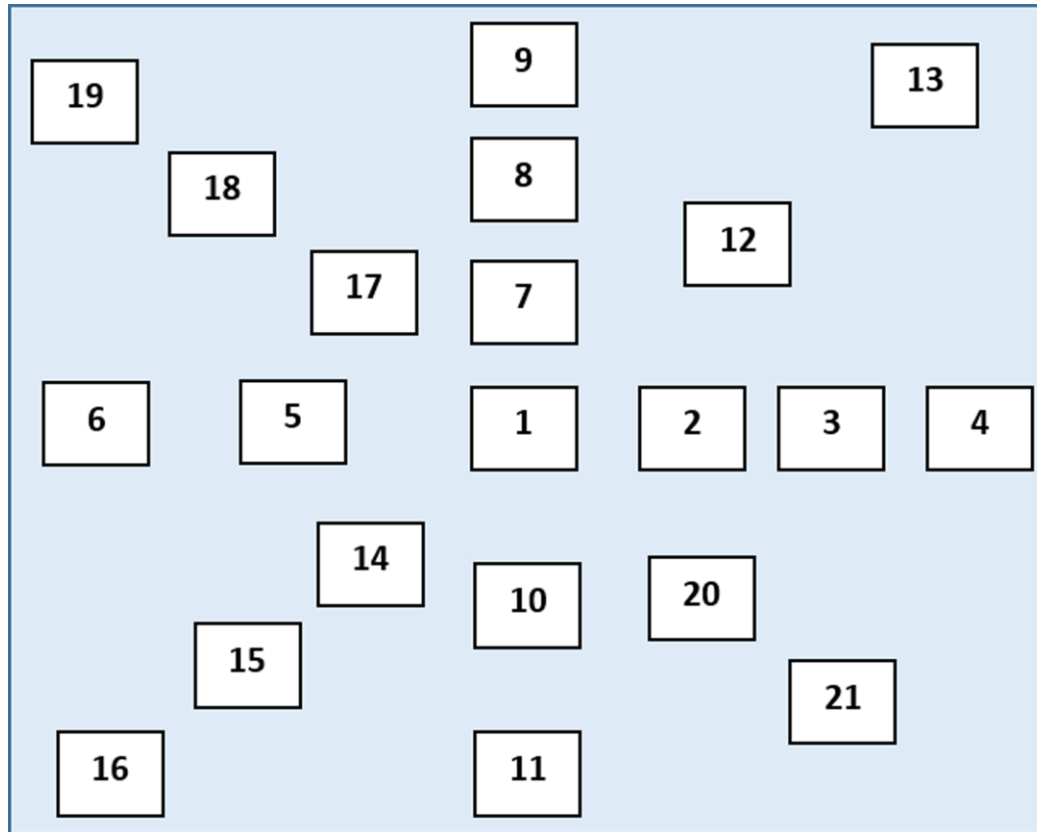
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Immunohistochemistry

- **Post mortem/biopsy samples** taken including...
 - Epithelial (n=21), mesenchymal (n=14) and round cell (n=22) tumours
 - Metastatic (n=10) and tumour-draining (n=7) lymph nodes
 - Inflammatory lymph nodes (n=12)
- Standard **immunohistochemistry** protocol to label them for Glut1 and FoxP3

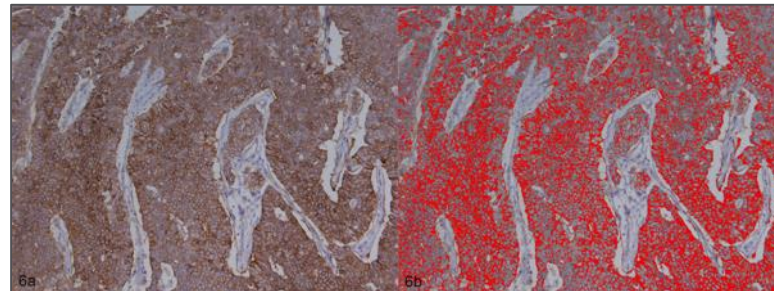
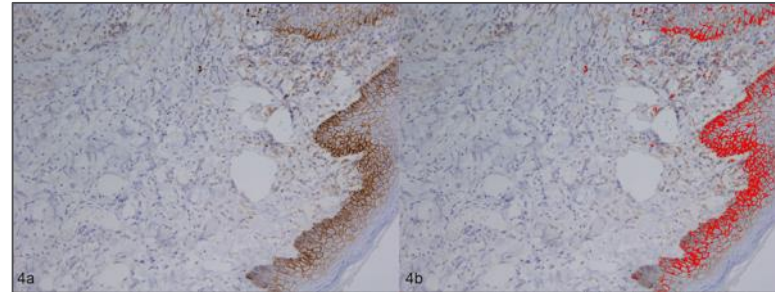
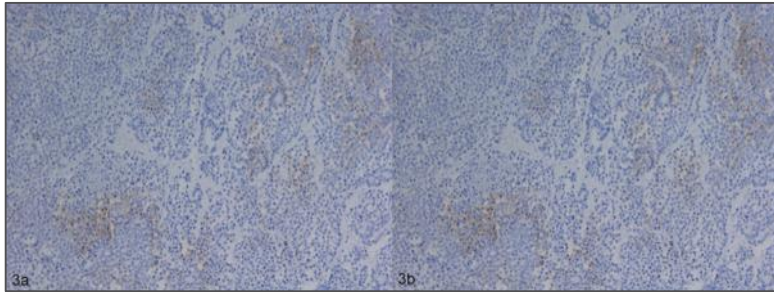
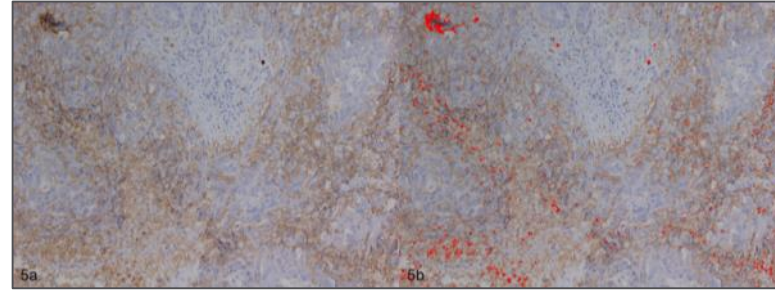
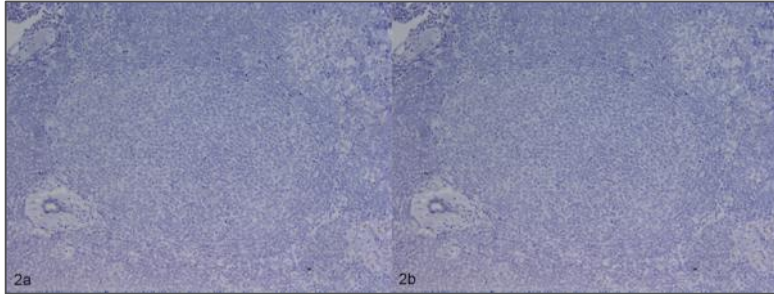
Image analysis



Glut1 scoring – percentage, intensity and immunoreactivity

- Glut1 scores taken through...
 - % cells labelling positively for Glut1
 - designated **strong intensity** % cover of stained areas
- Overall **immunoreactivity** score

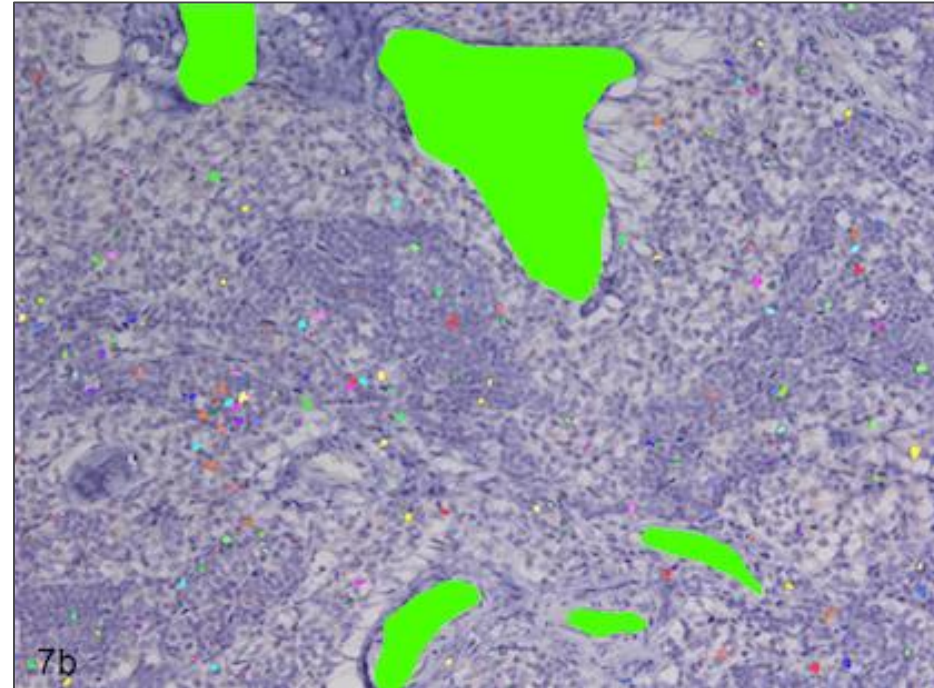
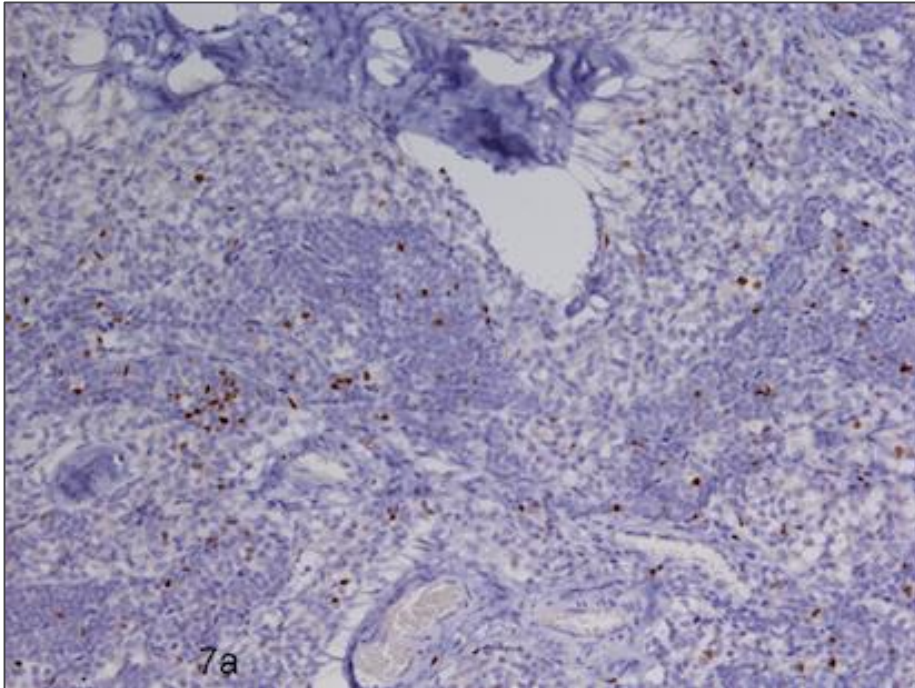
Glut1 labelling



FoxP3⁺ cell density

- Both **software** and **manual counting** used
- **Regions of exclusion** selected and edited
- Appropriate counting method applied.

FoxP3⁺ cell density



Statistical Analysis

- Generalised estimating equation (**GEE**) with a **ordinal log-link** function – Glut1 vs. origin, histotypes, malignancy
- **GEE** with a **negative binomial log-link** function – FoxP3⁺ vs. several factors
- Glut1 score vs. FoxP3⁺ cells within tumour types and LN categories

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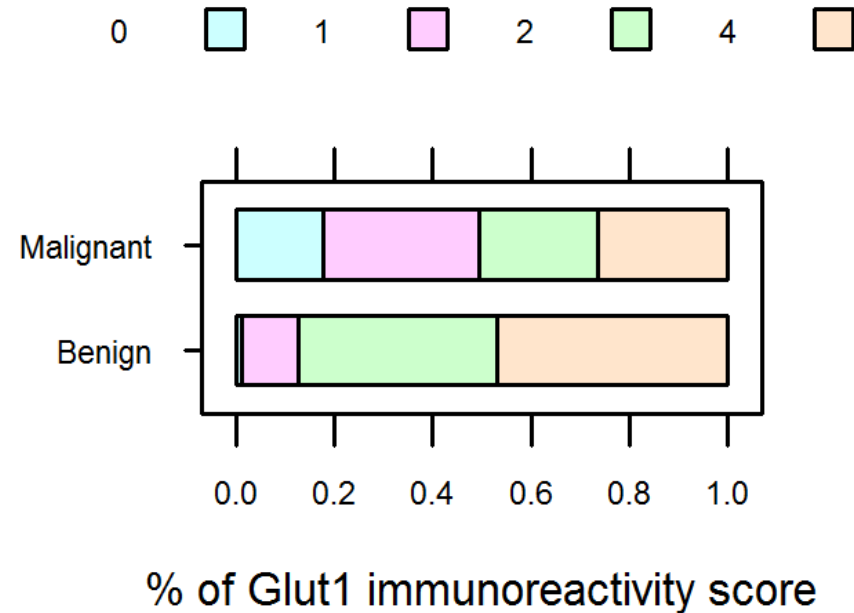
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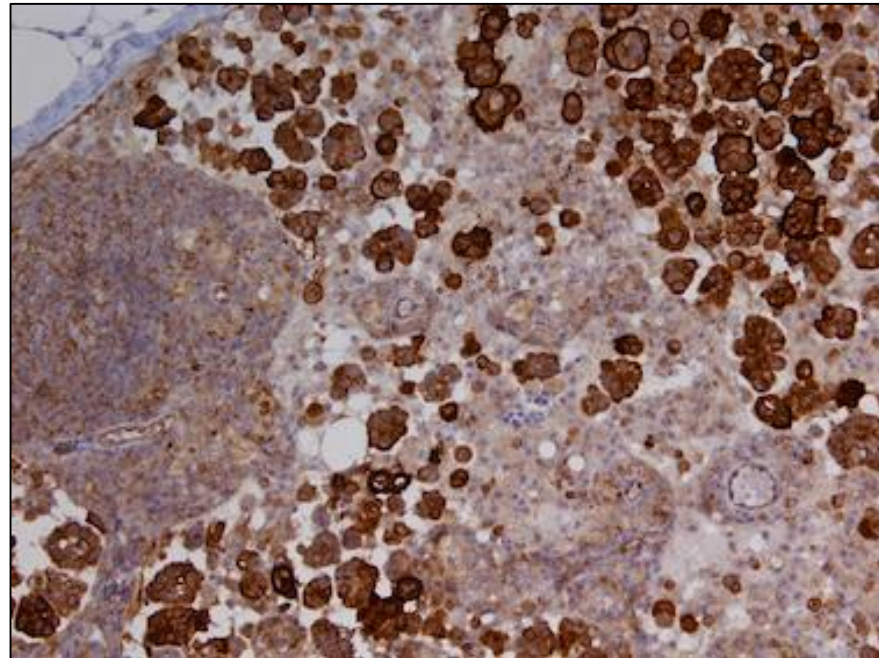
Glut1 expression

- Malignant < benign ($p < 0.001$)



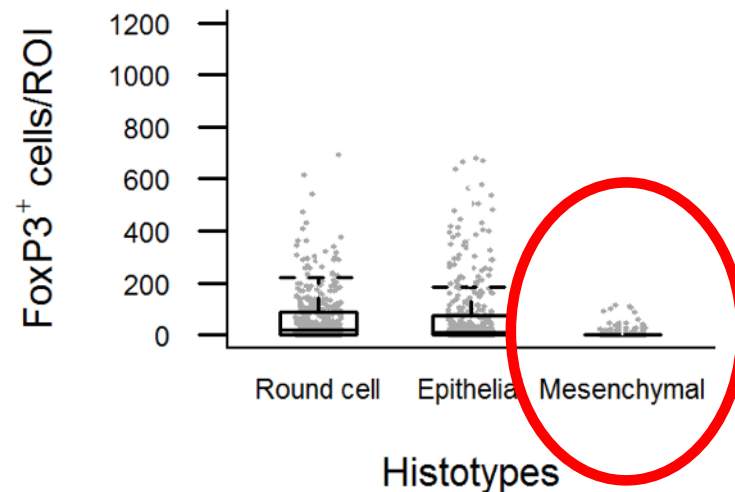
Glut1 expression

- Malignant < benign ($p < 0.001$)
- **Epithelial > other histotypes ($p = 0.022$)**



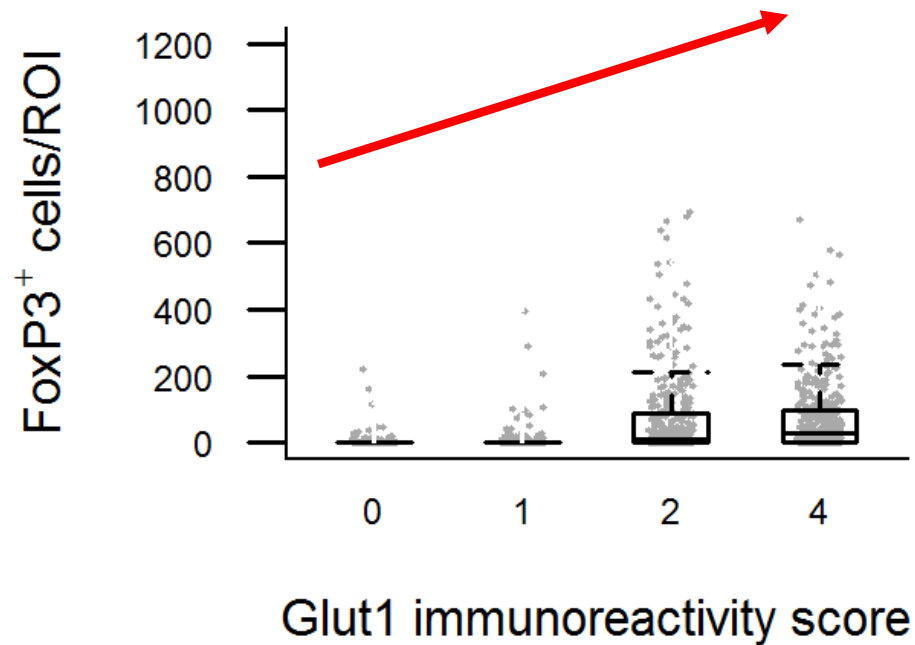
FoxP3⁺ cells

- **Large** variation in numbers between samples
- Distribution patterns varied widely
- Mesenchymal < epithelial and round
- **No difference** between benign and malignant samples



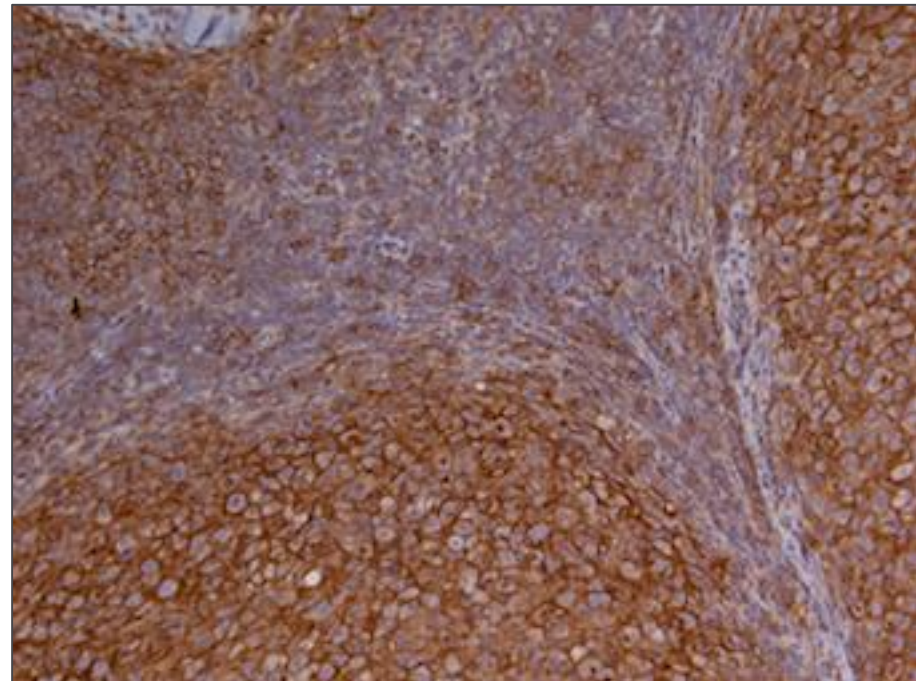
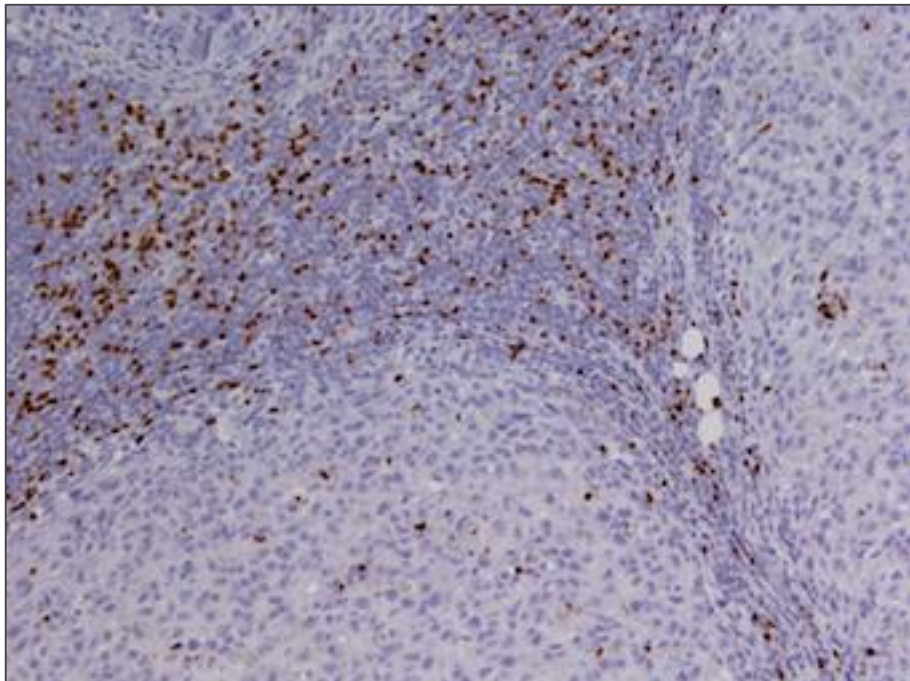
Glut1 vs. FoxP3⁺ cells

- Higher Glut1 expression correlated with more FoxP3⁺ cells



Why is there a relationship?

- Glut1 unlikely to be root for Treg accumulation
- **Hypoxia** may be the key



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Summary

- **Hypoxia** and **regulatory T cells** key in cancer
- **Immunohistochemistry** and **rigorous** image analysis methodology applied
- **Less Glut1 labelling** in malignant tumours vs. benign
- Increased Glut1 expression **correlated** with more FoxP3⁺ cells
- **Hypoxia may be driving the accumulation of regulatory T cells**

What could this mean for practice?




Source - <http://barkpost.com/wp-content/uploads/2015/06/happy-dog-running-by-500px.jpg>

Thanks

- Prof. Oliver Garden
- Dr. Henny Martineau
- Luca Fortuna
- Dr. Ruby Chang
- Dr. Andrew Hibbert
- Dr. Ester Hammond



A histological slide showing a dense population of cells with brown staining, likely indicating a specific marker or protein expression. The background is a light blue/purple color, typical of hematoxylin counterstain. The brown staining is distributed throughout the field, with some areas showing higher density.

Questions?