Overview of Mouse-Mouse Models

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Disclosures

- None

Overview

- Types of models
  - GEMMs
  - Chemically induced
  - Syngeneic grafts
- Approaches to understanding tumor immune responses
- Increasing the predictive value of mouse-mouse preclinical models

Genetically engineered mouse models (GEMMs)

- Combination of transgenic, knock-in, knock-out
- Germline vs restricted to tumor cell
- Stochastic vs generalized hyperplasia
- Characteristics
  - Long latency
  - Incomplete penetrance
  - Very few somatic mutations
  - Physiological mitotic rate and TME
  - Low rate of metastasis
  - Difficult to induce effective immune responses
  - Very high bar for therapies being tested

Genetically engineered mouse models (GEMMs)

- How to improve GEMMs for preclinical immune therapy testing
  - Increasing antigenicity
    - Mutator alleles
    - Chemical carcinogenesis
    - Model antigens
  - Enhanced immune backgrounds

Chemically-induced mouse models

- Induction of tumors by administration of chemical carcinogens
- MCA model (Schreiber)
- Characteristics
  - Fully penetrant
  - Variable latency (can be long)
  - Unclear histological cancer type (sometimes)
  - High number of somatic mutations
  - Can be very immunogenic
  - Often used as syngeneic grafts

Syngeneic mouse models

- Engraftment of mouse cancer lines that are not rejected upon grafting into an immune competent host
- B16, MC38, CT26
- Characteristics
  - Easy/cheap/fast to use
  - Typically subcutaneous injection of cells
  - Tumors can grow very quickly (2-3 weeks for B16)
  - Variable immunogenicity
  - Variable responses to immune therapies
  - Hard to compare with other syngeneic models

Syngeneic mouse models

- Characteristics
  - Driver genes are frequently not known
  - Contribution of endogenous retrovirus are not known
  - Mutation burden is frequently high
    - Makes comparison with low-mutation burden analog difficult
    - Makes identification of relevant antigens difficult

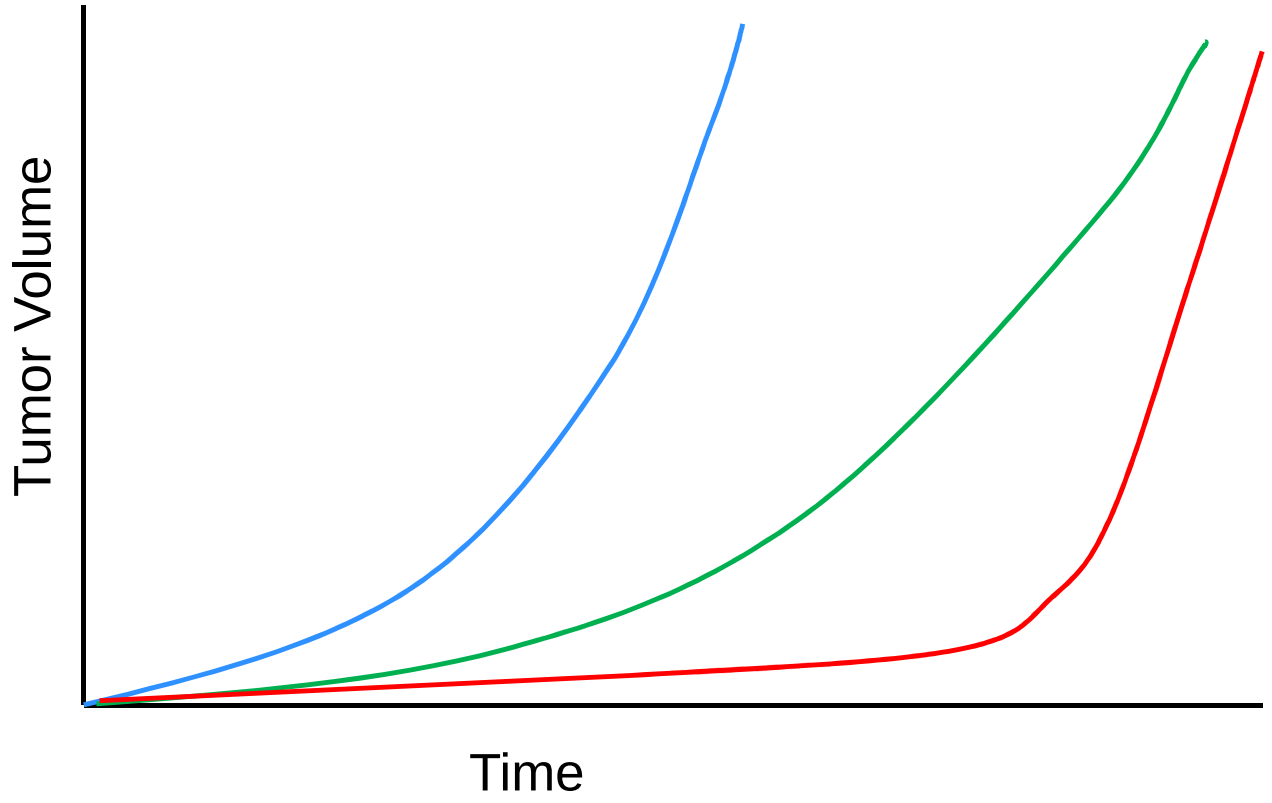
Syngeneic mouse models

- Ways to improve syngeneic models:
  - Use multiple lines driven by human-relevant genetic changes
  - Series of similar lines with variable mutation burden
  - Ability to evaluate antigen-specific responses
  - Advanced imaging available to follow immune responses sequentially
  - Evaluate anti-tumor responses at metastatic sites
  - Make lines from inbred cells using CRISPR

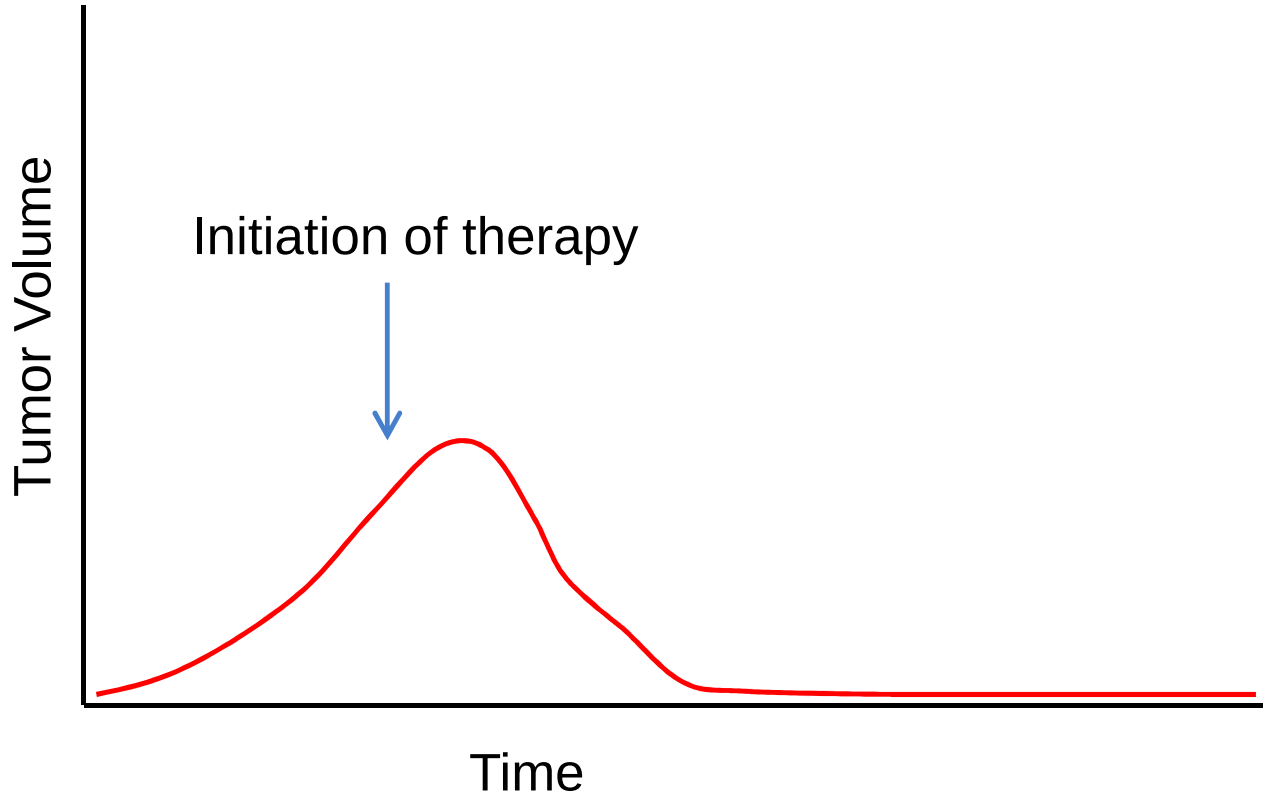
What is an effective immune response in a mouse model?

- Usually tumor volume plots are performed
- Significant separation of curves is often the endpoint
  - Would be progressive disease in humans
- Overall survival (to a tumor volume endpoint)
- Correlative studies are often performed
  - Quantitation of T-cell infiltrate by flow cytometry
  - Usually performed at the end of the experiment, when tumors are ~1 cm^3 in volume

What is an effective immune response in a mouse model?



What is an ideal immune response in a mouse model?



Yale University Mouse Melanoma (YUMM) lines

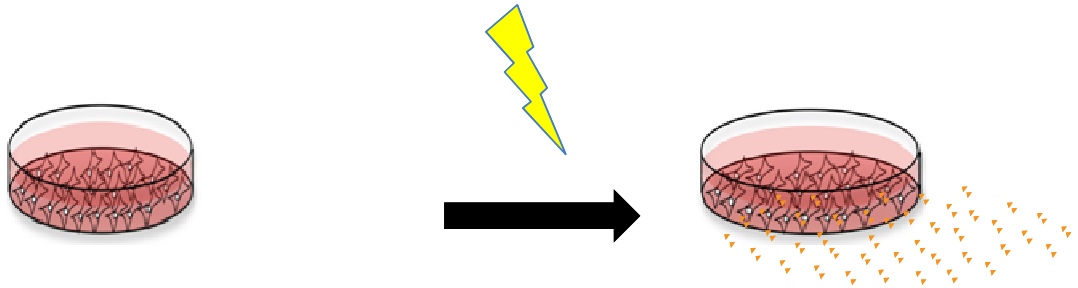
Syngeneic mouse melanoma lines

Name	Genotype	Status
YUMM1	*$Braf^{V600E}Pten^{-/-}$ $Cdkn2a^{-/-}$*	20 lines
YUMM2	*$Braf^{V600E}Pten^{-/-}$ $Bcat^{sta/+}$*	2 lines
YUMM3	*$Braf^{V600E}Cdkn2a^{-/-}$*	4 lines
YUMM4	*$Pten^{-/-}$ $Cdkn2a^{-/-}$*	4 lines
YUMM5	*$Braf^{V600E}$ $p53^{-/-}$*	4 lines
YUMM6	*$Braf^{V600E}Pten^{-/-}$*	1 line
YUMM7	*$Braf^{V600E}Bcat^{sta/+}Cdkn2a^{-/-}$*	In progress
YUMM8	*$Braf^{V600E}Lkb1^{-/-}Cdkn2a^{-/-}$*	In progress
YUMM9	*$Nras^{Q61R}$ $Cdkn2a^{-/-}$*	In progress
YUMM10	*$Nras^{Q61R}$ $p53^{-/-}$*	In progress

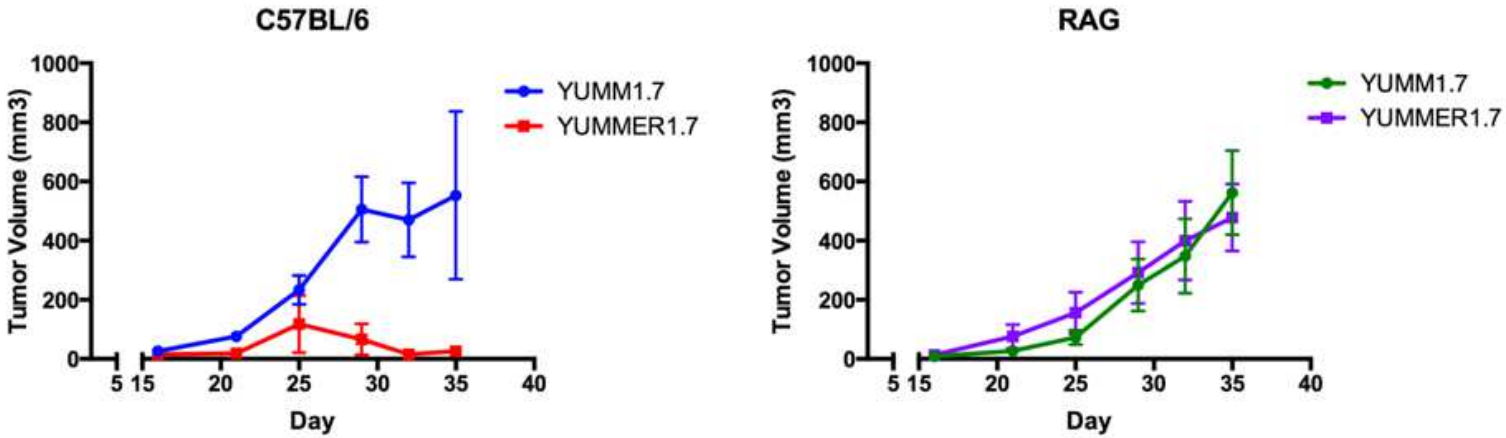
Meeth et al., PCMR, 2016

YUMM*ER* 1.7 Cell Line

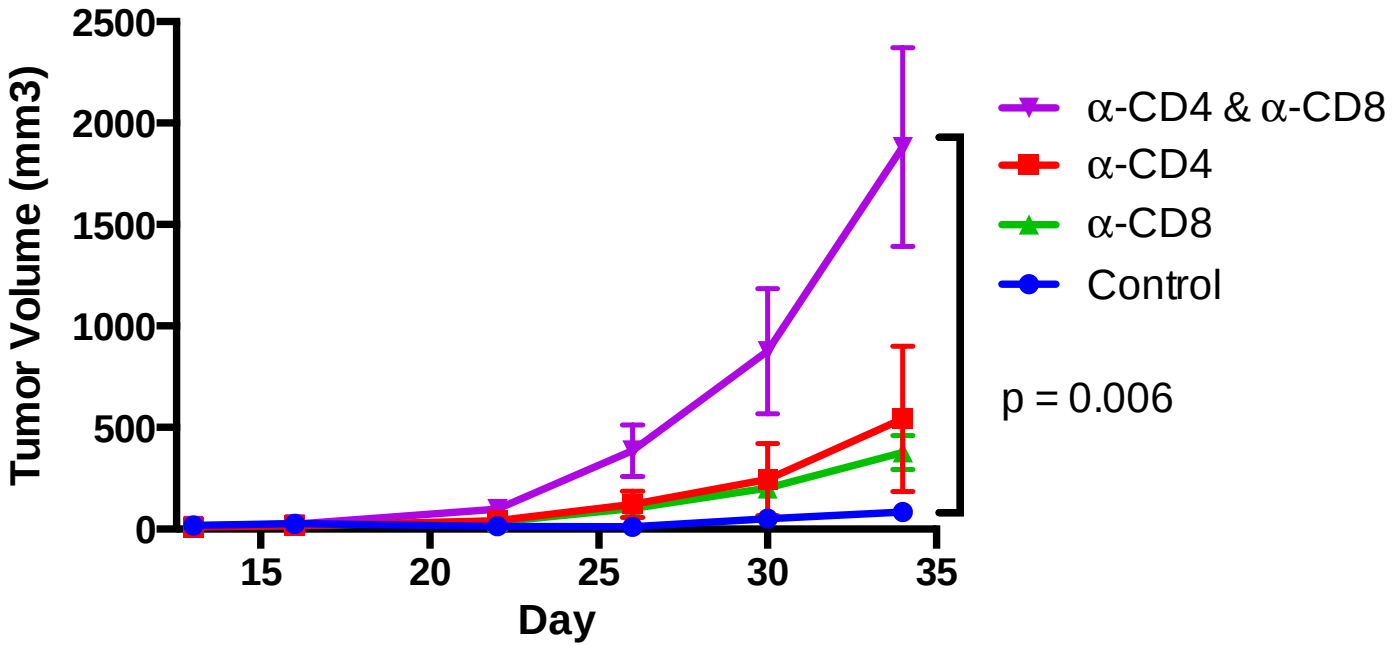
- Parental cell line (YUMM1.7) exposed to 3 rounds of high-dose UVB radiation and clonally selected



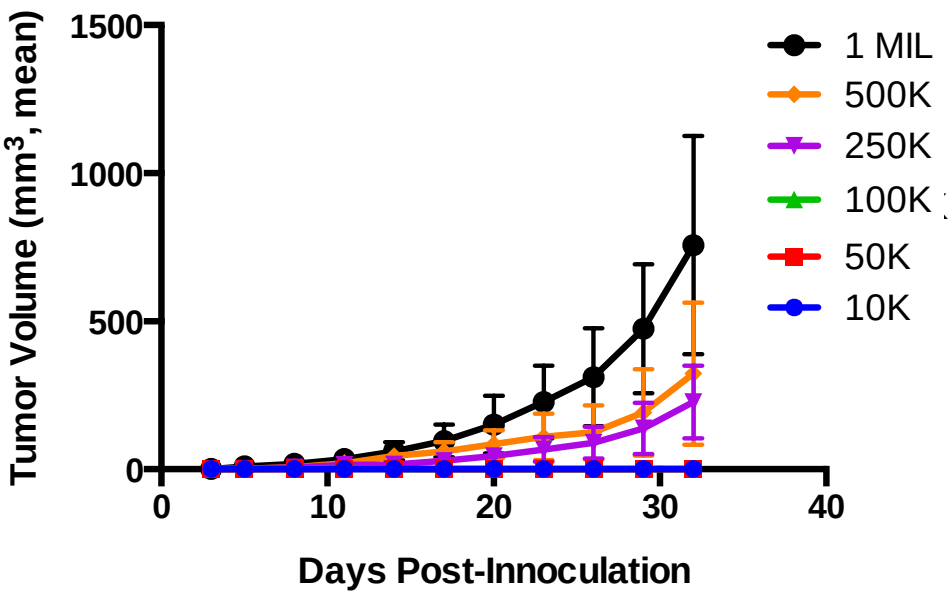
YUMMER regresses in wt C57BL/6 background



Depletion of either CD4 or CD8 T cells increases growth significantly



Regression of YUMMER is titratable



Injection Number	Palpable Tumors by Day 32
10,000	0/5
50,000	0/5
100,000	0/5
250,000	**5/5**
500,000	**5/5**
1 Million	**5/5**

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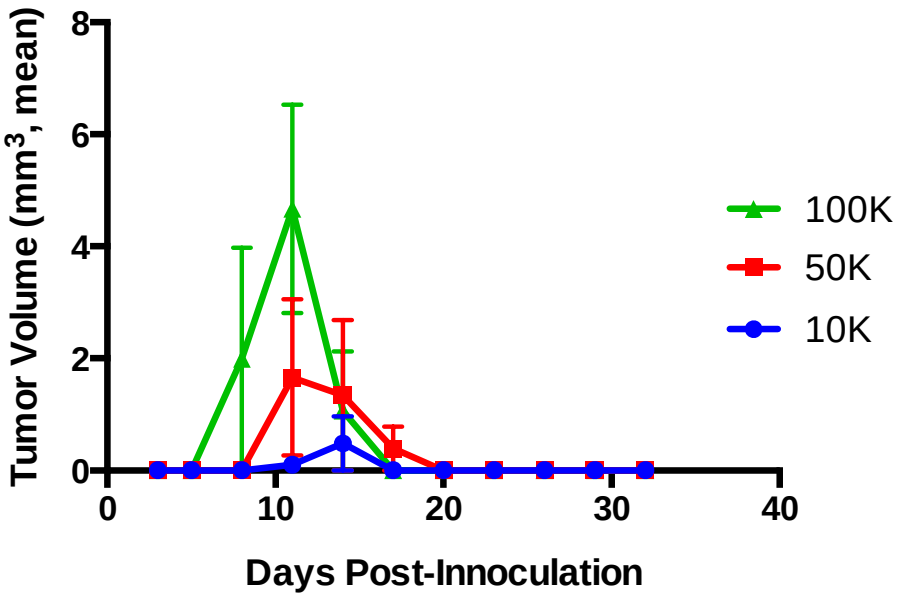

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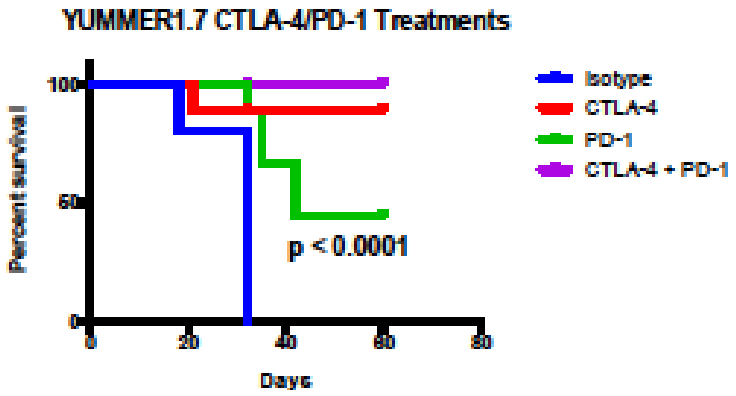
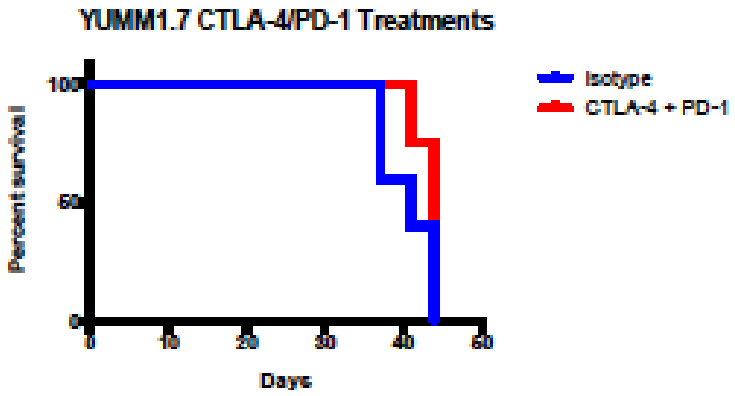
The YUMMER model is very responsive to checkpoint inhibition

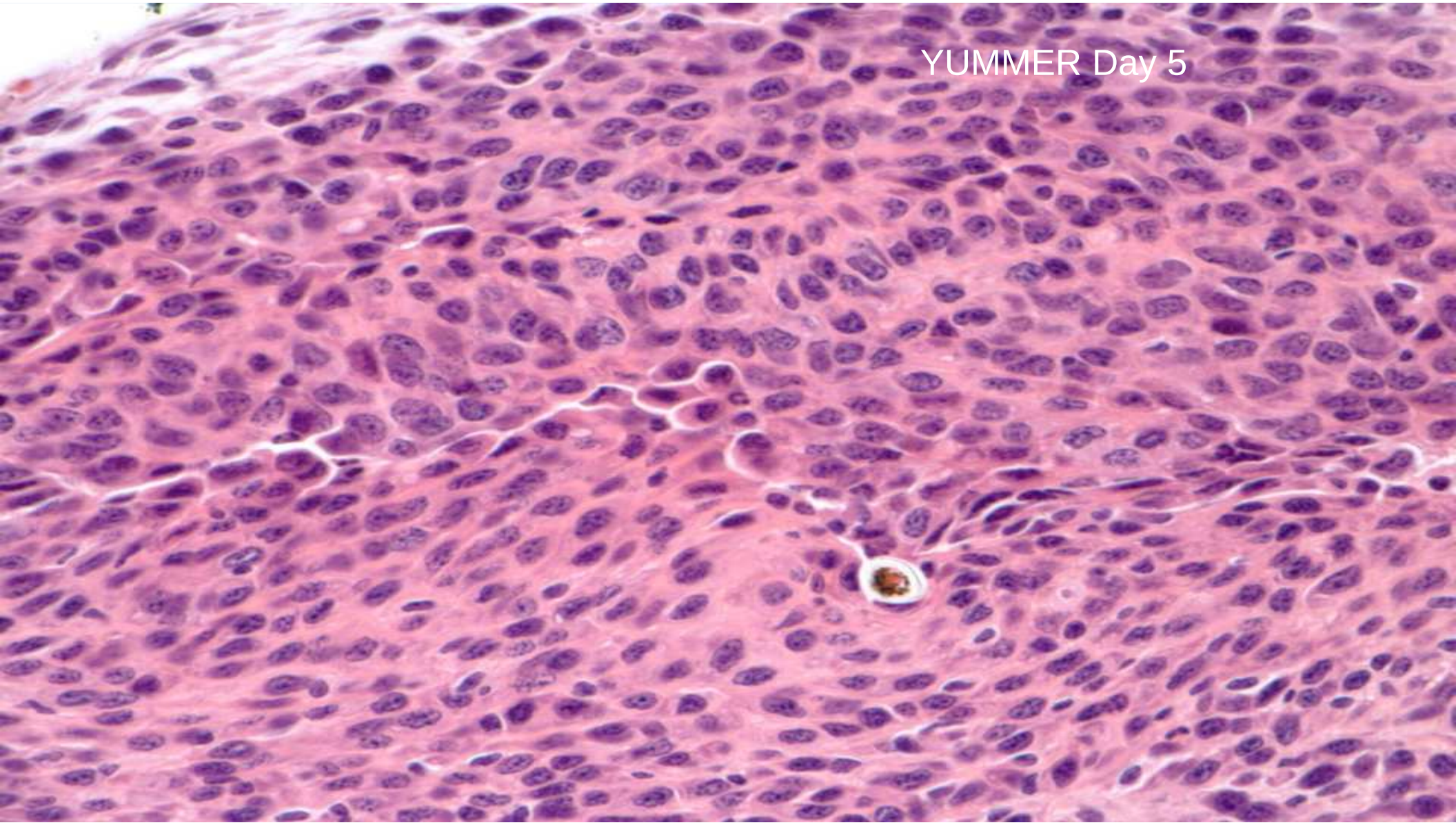


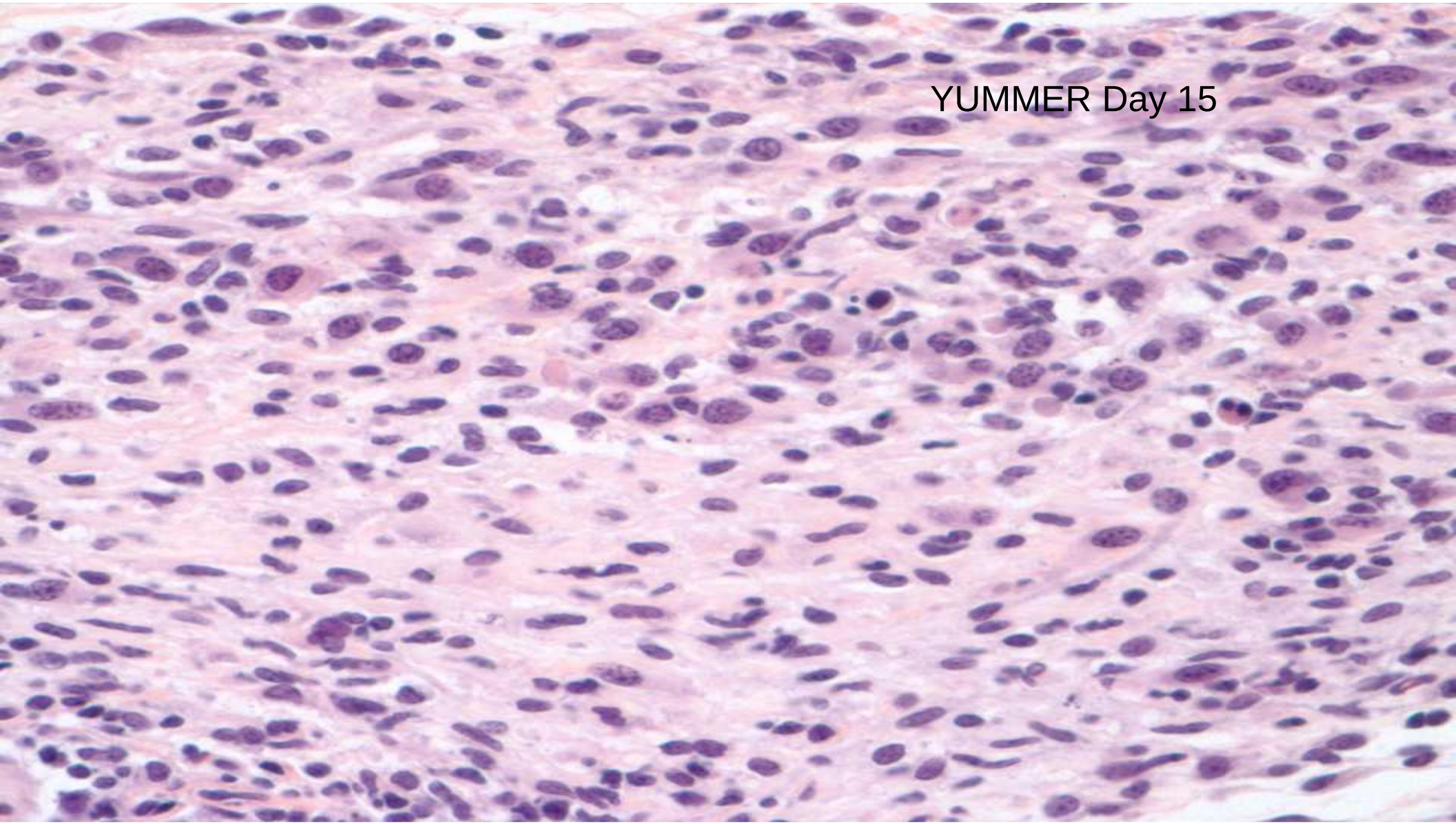


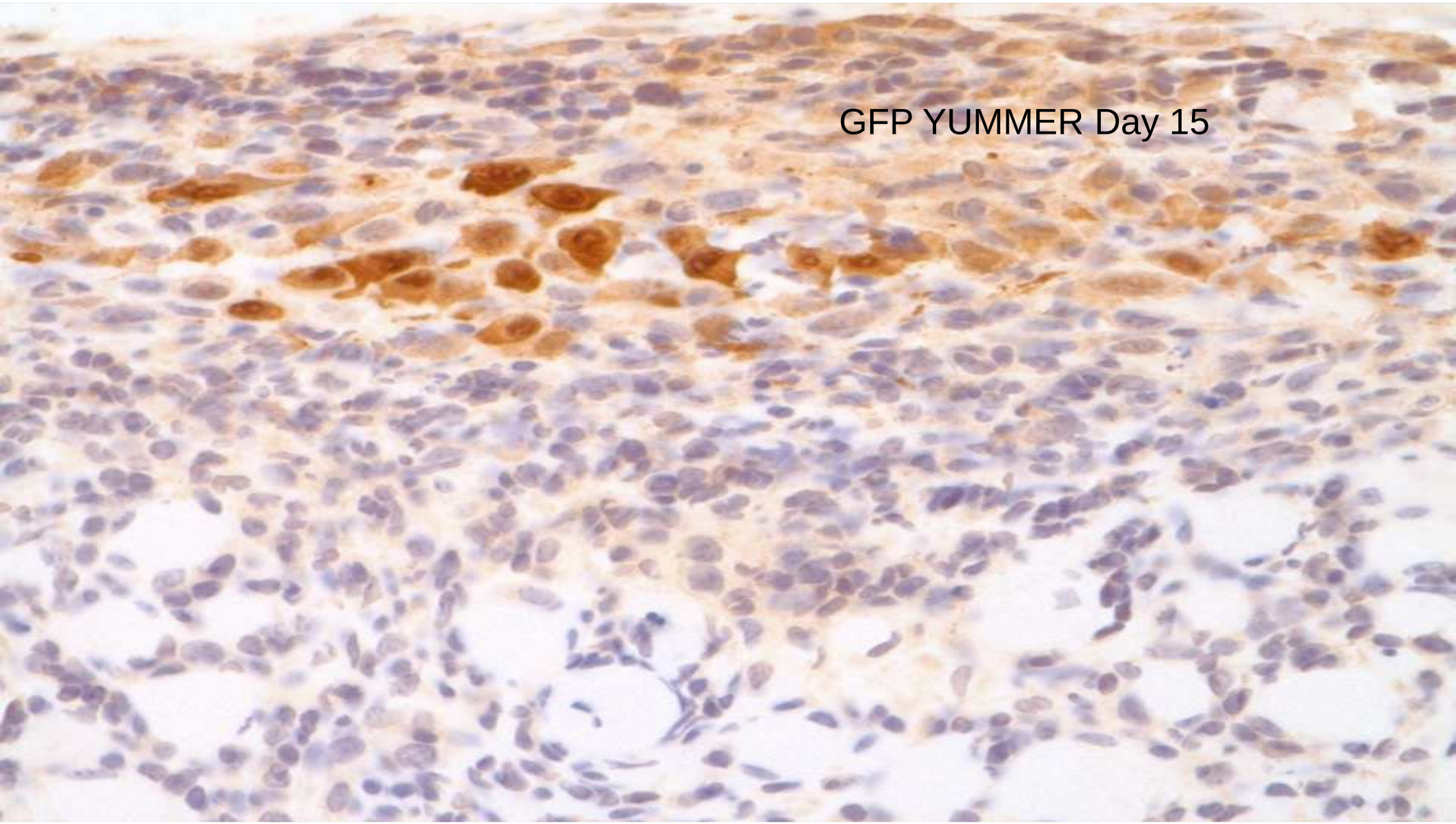
Characteristics of the anti-melanoma tumor immune response

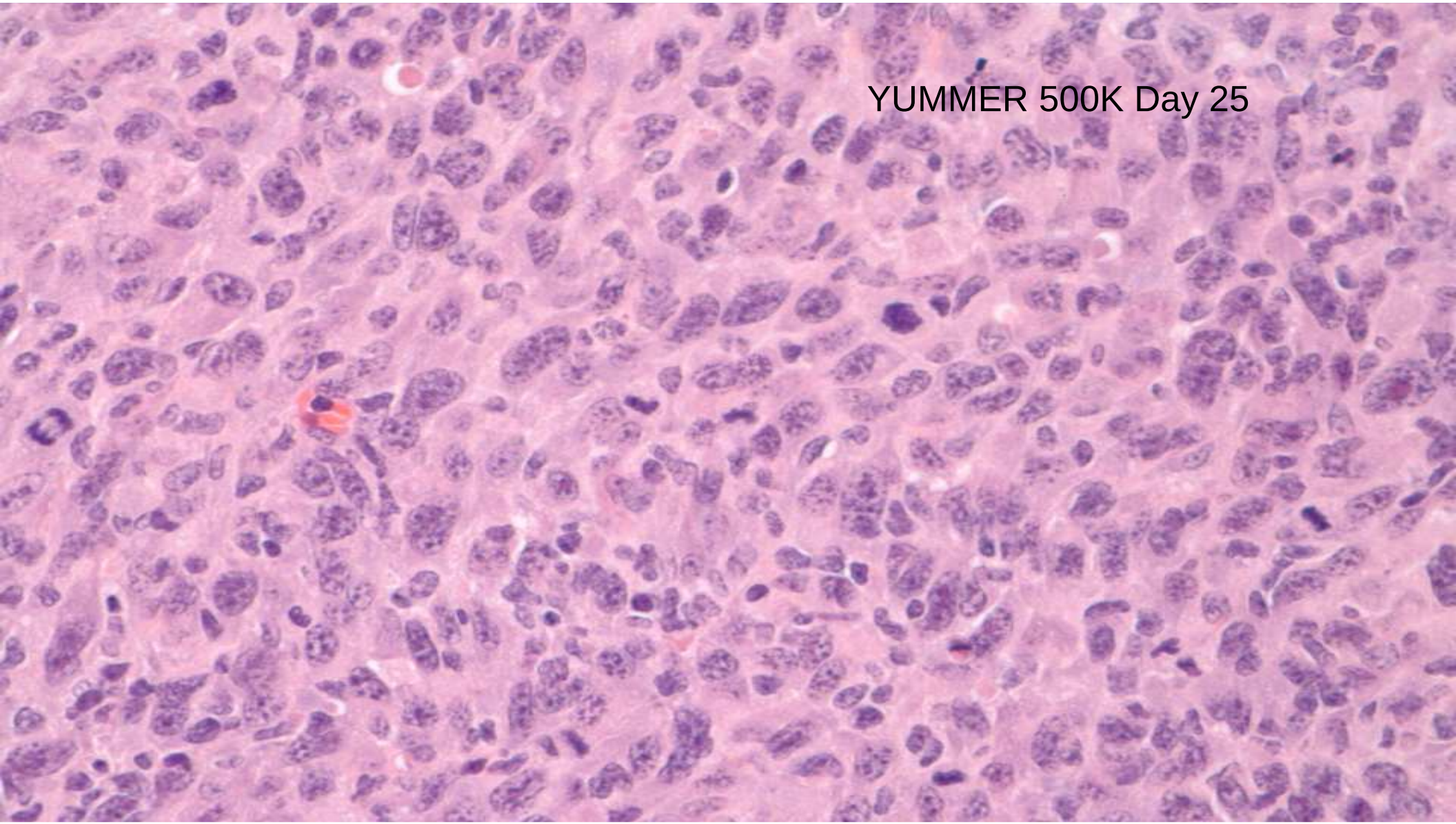
- Early myeloid infiltration
- Characteristic onset of T-cell infiltration (Day 7)
- Distinct onset of immune-mediated cell killing (Day 8)
- Tumor regression vs escape (by Day 15-18)
- Plenty of T-cells in escaping tumors, most are at tumor-infiltrating edge

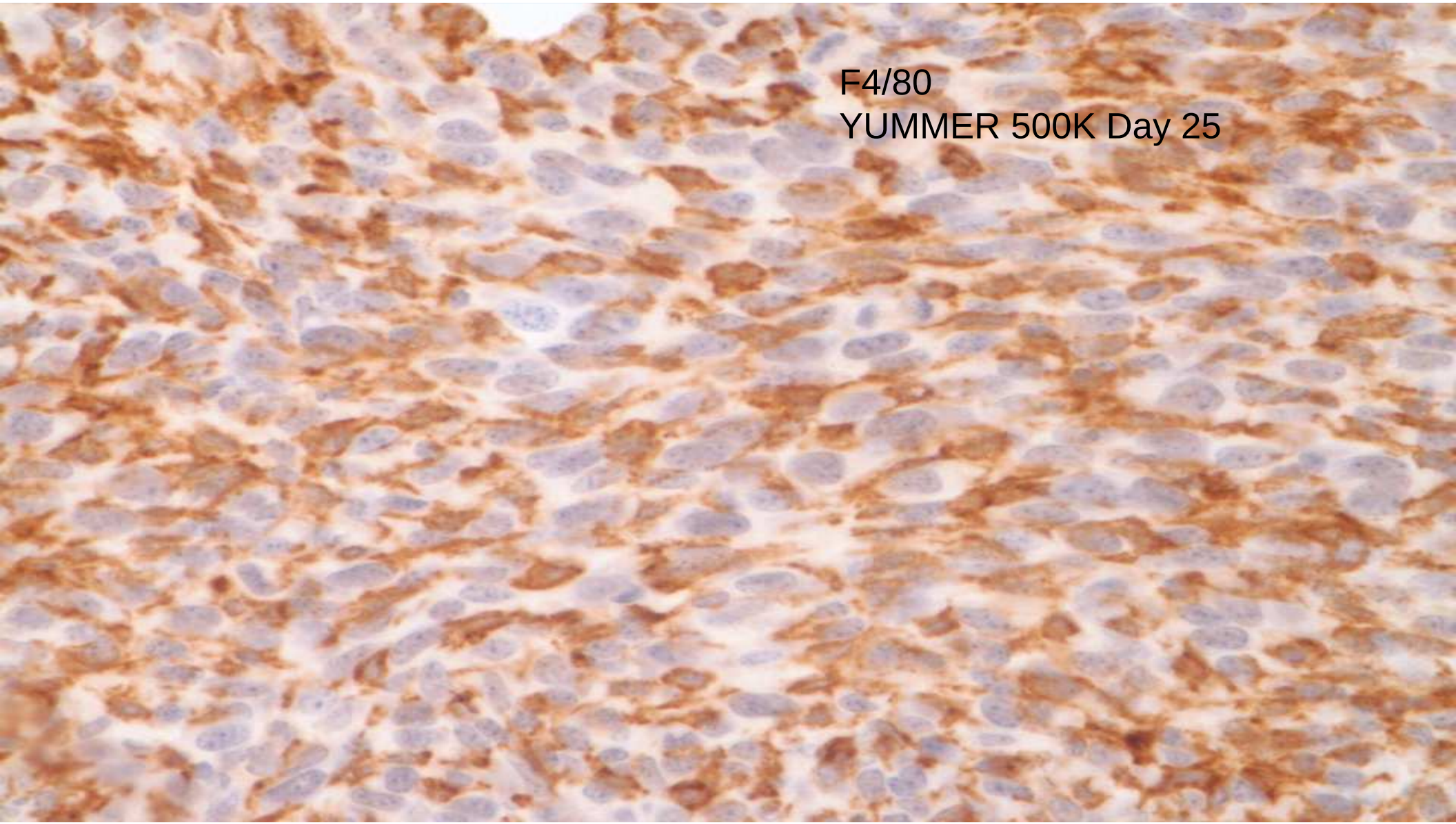
- The above features are not accurately represented by flow cytometry alone
- Quantitative pathology approaches

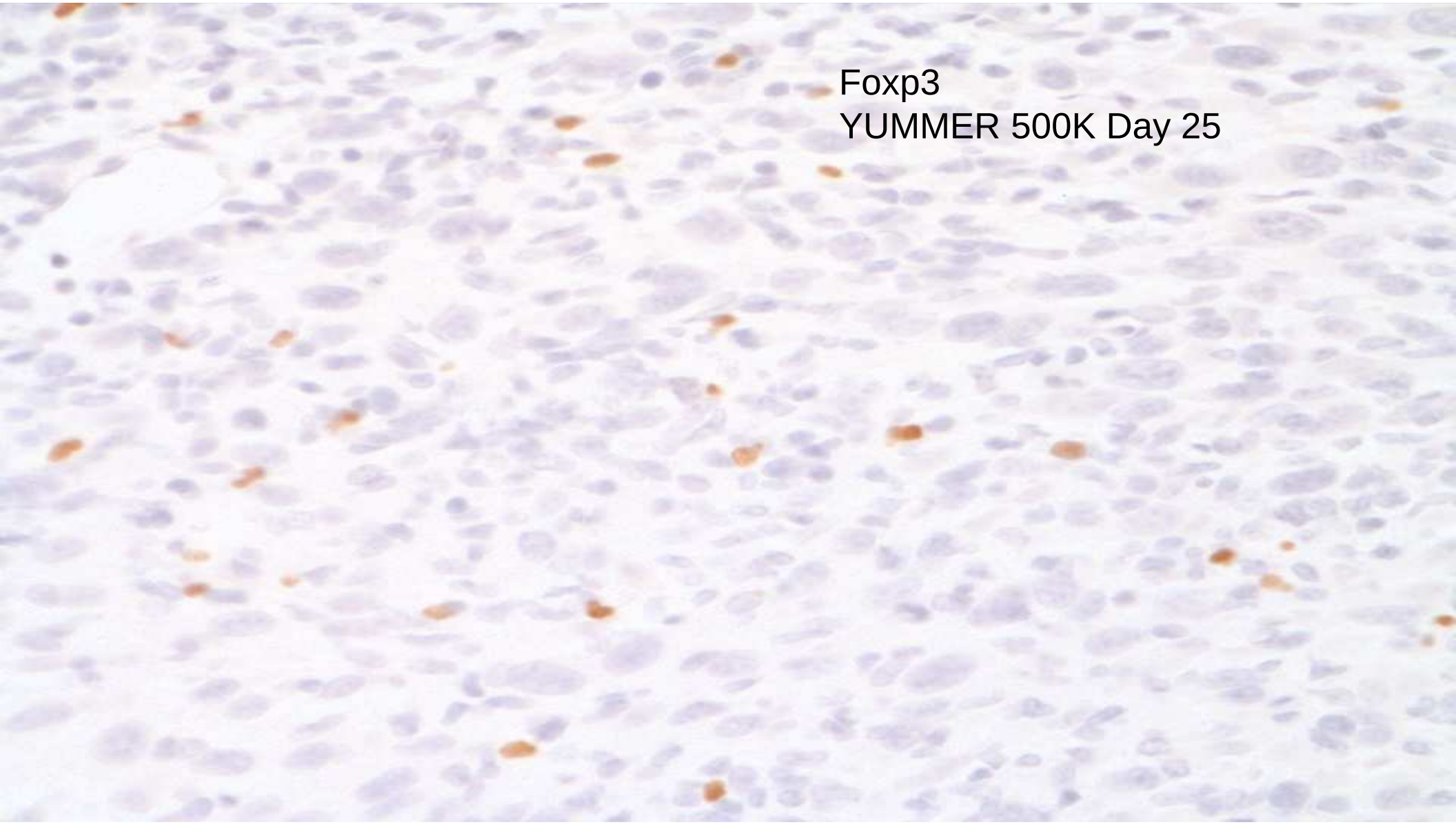

YUMMER Day 5


YUMMER Day 15

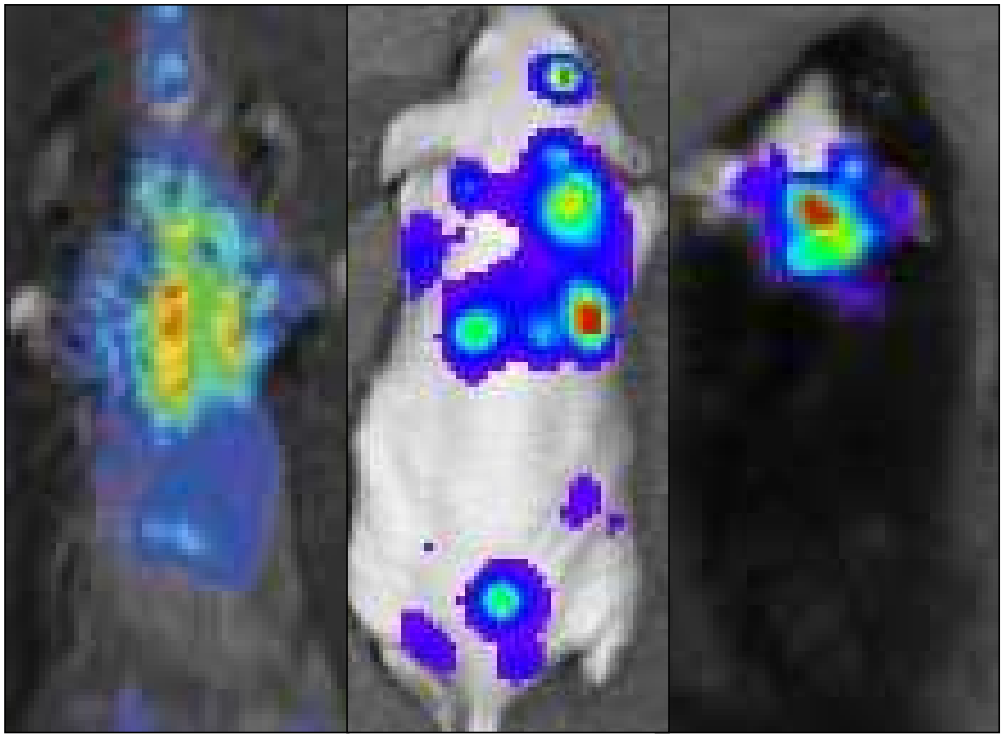

GFP YUMMER Day 15

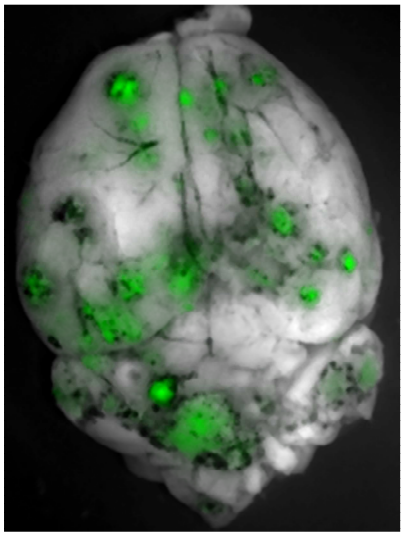

YUMMER 500K Day 25


F4/80
YUMMER 500K Day 25


Foxp3
YUMMER 500K Day 25

Left Ventricle Injection Models





Future Plans

- Syngeneic models
  - Variable mutation burden
  - Identification of class I and class II antigens
  - Functional evaluation of responses
  - Improved imaging
    - 2-photon
    - Light sheet
    - Cell line and host reporters
  - Single cell quantitative pathology
  - CRISPR screens
  - Combination therapies

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