The Brain of a Younger Host is More Permissive for Breast Cancer Brain Metastasis

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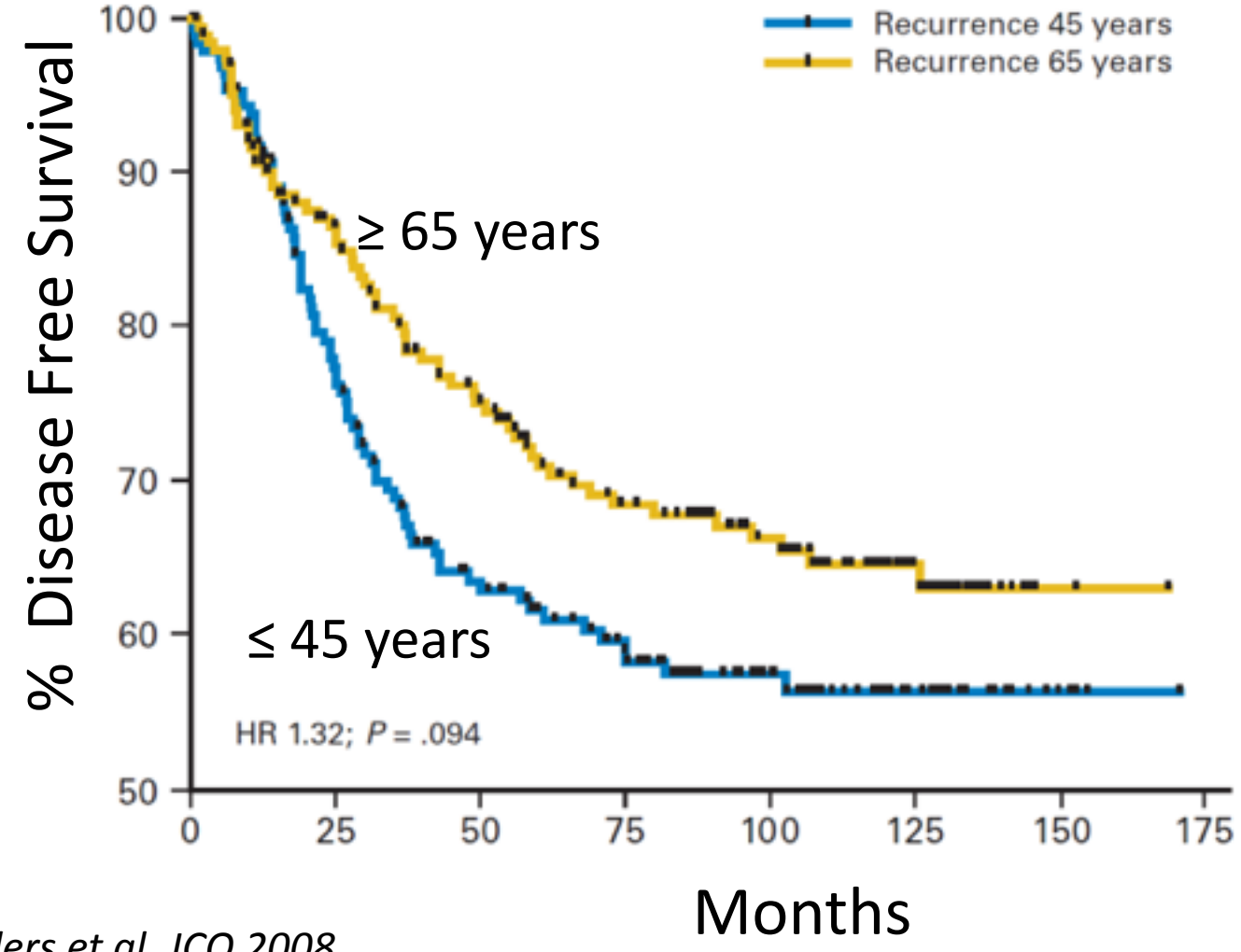
Young women with breast cancer often have a poorer prognosis



Young breast cancer patients have increased risk of brain metastasis

Evans et al. Clin. Oncol, 2004
Hung et al. PLoS One, 2014

Anders et al. JCO 2008

Why is breast cancer in younger women more aggressive?

Tumor

At diagnosis

- More lymph node involvement
- More vascularized
- Higher grade

Molecular subtypes

- More likely to be hormone receptor negative

Mutations

- Higher prevalence of GATA3 mutations (TCGA database)

Multivariate analyses: After accounting for these characteristics, younger age is still independently associated with poorer outcome

Even **within** molecular subtypes, younger age is still associated with poorer outcome

Overall, **less** somatic mutations in tumors from younger patients

Why is breast cancer in younger women more aggressive?

Tumor

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Host

Hypothesis

Factors specific to the young host promote breast cancer progression and metastasis to the brain

Ultimate goal…Age-tailored therapies

The brain of a younger mouse is more permissive for breast cancer brain metastasis

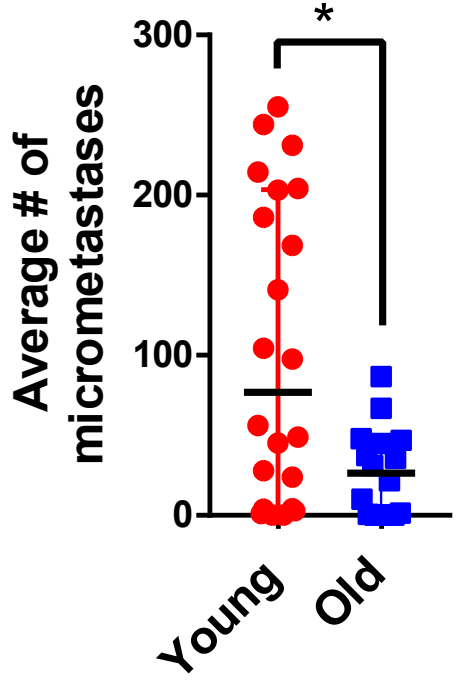
MDA-MB-231-BR (immunocompromised)

Young (2 mo.)
Old (13 mo.)

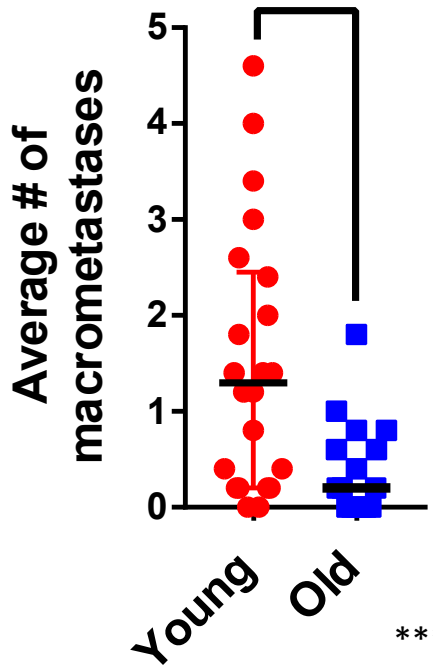
nude

Day 30
# of Brain Metastases?

Micro-metastases

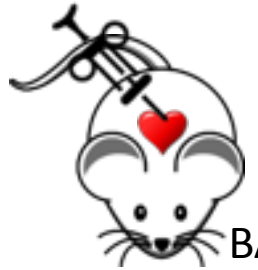


Macro-metastases



4T1-BR (immunocompetent)

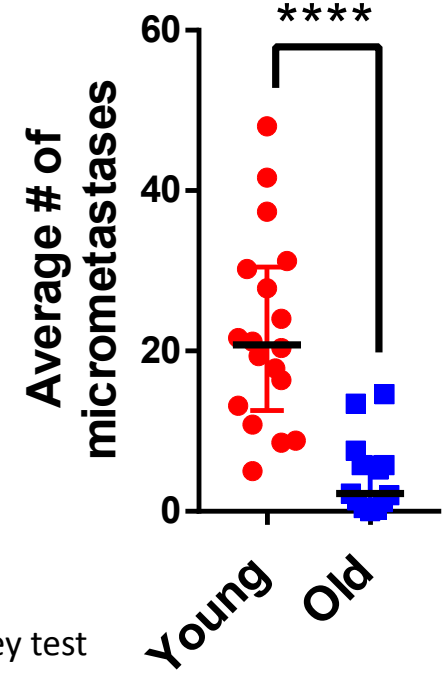
Young (6 mo.)
Old (18 mo.)



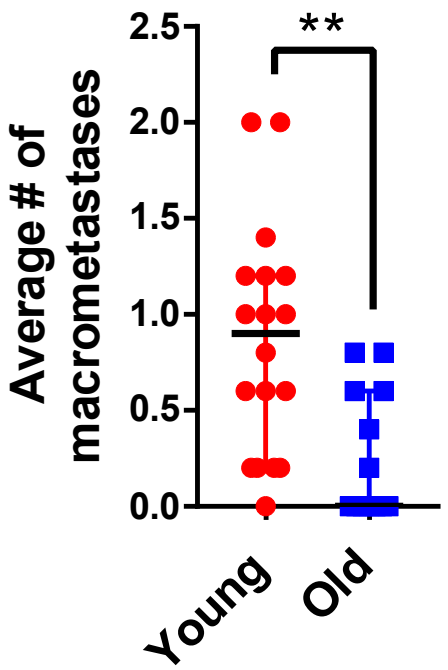
BALB/C

Day 13
# of Brain Metastases?

Micro-metastases



Macro-metastases



* $p < 0.05$, ** $p < 0.01$,
**** $p < 0.0001$; Mann-Whitney test

The effect of age on breast cancer metastasis is specific to the brain

Cell line	Species	Metastatic Site	Effect of Age?
MDA-MB-231BR	Human	Brain	**Yes (Higher in young)**
4T1-BR	Mouse	Brain	**Yes (Higher in young)**
MDA-MB-231	Human	Lung	**None**
6DT1	Mouse	Lung	**None**
HRM1	Mouse	Lung	**None**
MVT1	Mouse	Lung	**None**
E0771	Mouse	Lung	**None**

Mechanism

Hormones? No

Proliferation? No

Astrocytic neuroinflammatory response? No

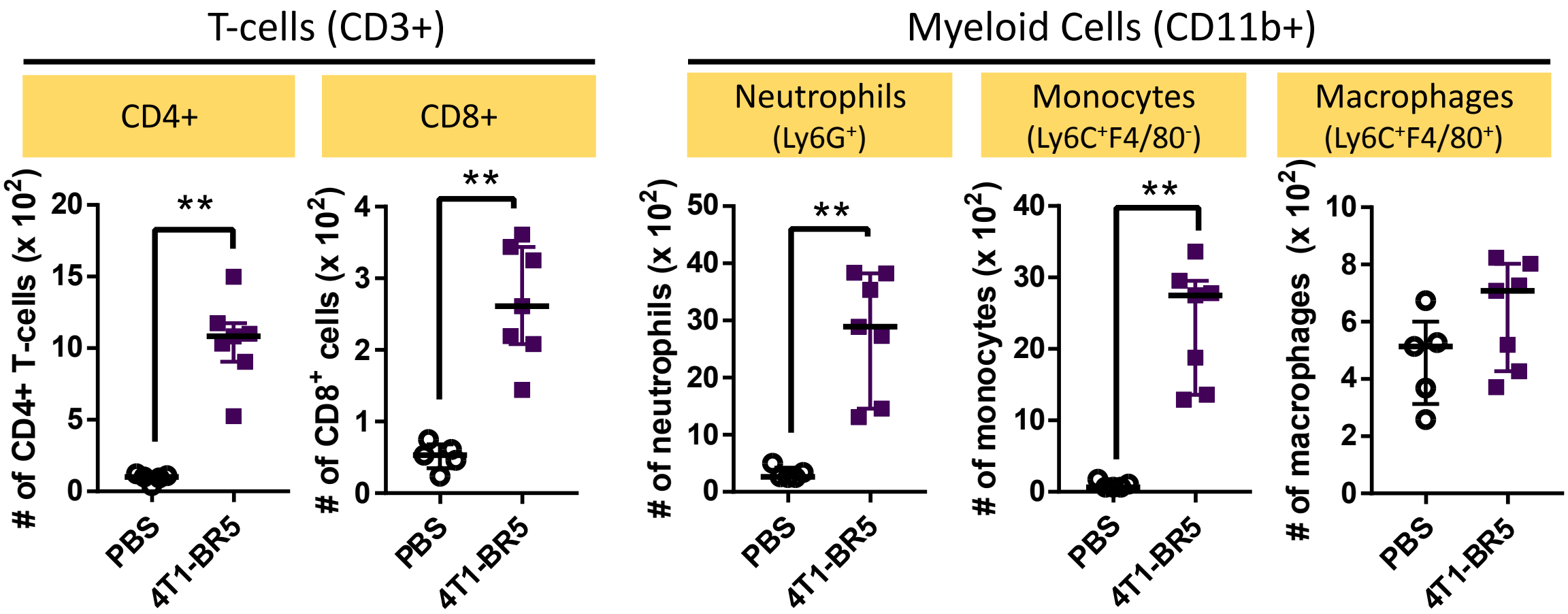
Immune response?

Other microenvironment components?

Brains with metastases have elevated # of peripheral immune cells

Multi-parameter flow cytometry of brain immune cells in young mice

○ Healthy (PBS) ■ Tumor-bearing (4T1-BR)



Collaboration with Dr. Dorian McGavern, NINDS, NIH

Median ± interquartile range; ** $p < 0.01$, Mann-Whitney Test

No difference in # of microglial cells between brains with and without metastases

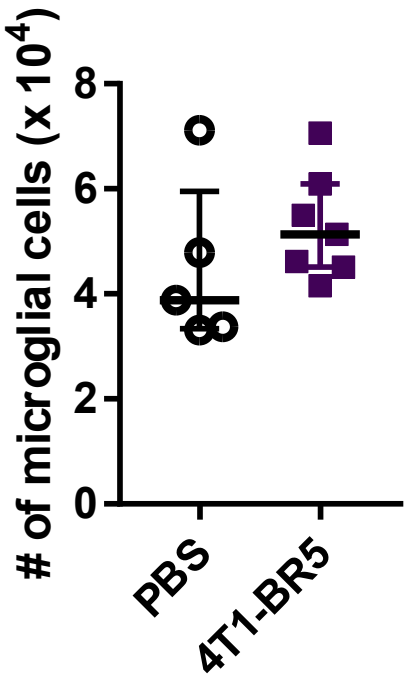
Multi-parameter flow cytometry of brain immune cells in young mice

○ Healthy (PBS) ■ Tumor-bearing (4T1-BR)

Microglia

- Phagocytic cells
- Have highly ramified processes
- Major function: immune surveillance
- Long lived cells

Microglia



Old mice have a subset of microglial cells that are characterized by high side-scatter profile

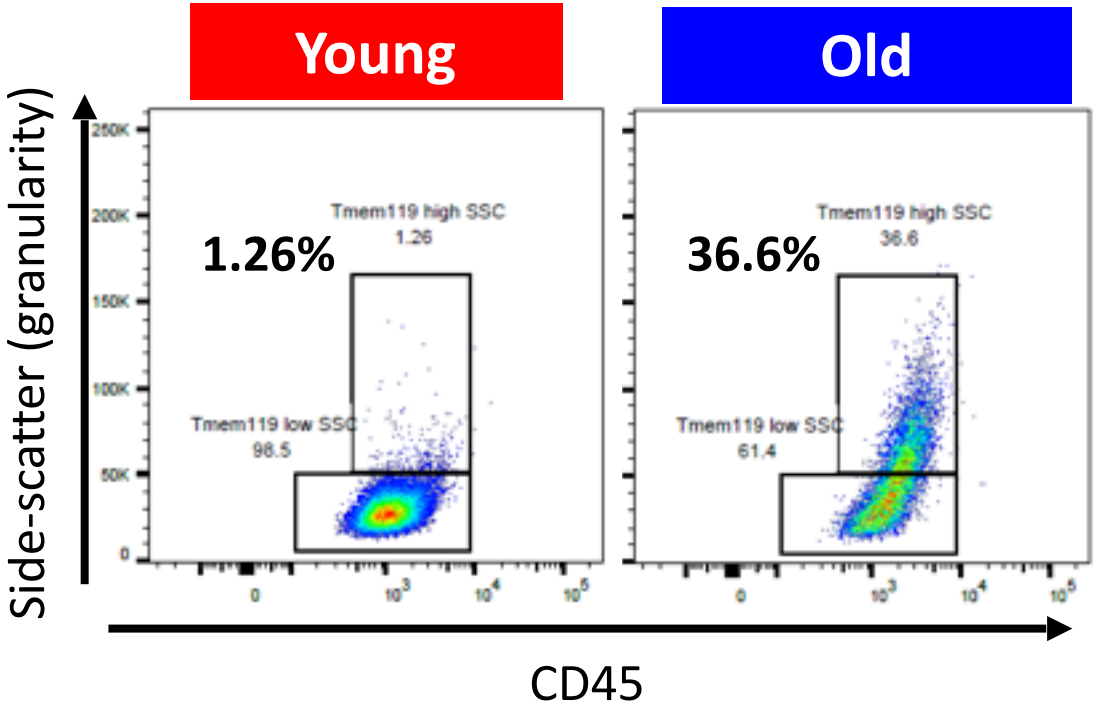
Age-associated microglia
have altered morphology and function:

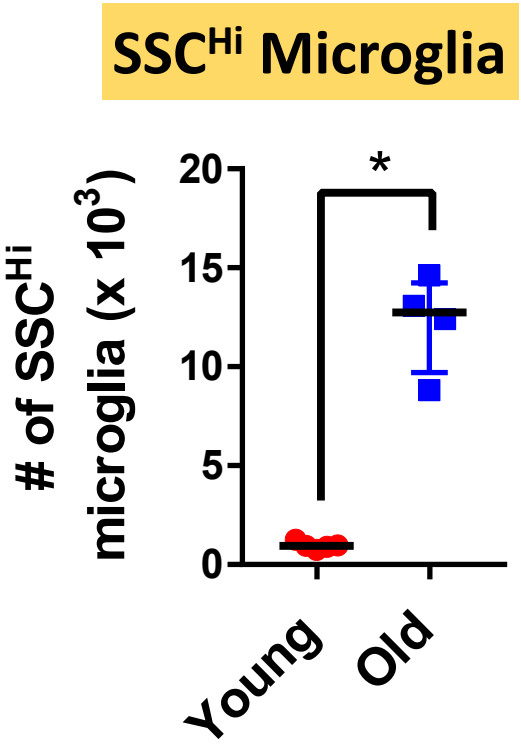
- De-ramified
- More granular
- Highly autofluorescent
- Elevated expression of pro-inflammatory genes (M1-like)

Also present in neurodegenerative diseases
(*e.g.* Alzheimer's)

Ritzel et al. Neurobiolo. Aging (2015), Norden and Godbout., Neuropathol Appl Neurobiol. (2013), Raj et al. Front. Mol. Neurosci. (2017)

$CD45^{+}CD11b^{+}Tmem119^{+}$ microglia:





Median ± interquartile range; * $p < 0.05$, Mann-Whitney Test

Summary

- Young mouse brains were more permissive for breast cancer brain metastasis *(consistent with epidemiological data)*
- Effect of age was organ-specific (no effect in the lungs)
- Brains with metastases are inflamed
  (elevated # of T-cells and myeloid cells)
- Aged brains have a subset of microglia with a high side-scatter profile
  (demonstrated by others to be in a pro-inflammatory state)

Ongoing work and Future Directions

- Compare immune infiltration patterns between young and old mice with brain metastases
- Perform immune depletion studies to test whether loss of specific immune subsets (especially microglia) will abolish the age effect
- Characterize the proteome and transcriptome of brains from young and old mice with and without brain metastases

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