Navigating the Tumor Microenvironment Using Flow Cytometry

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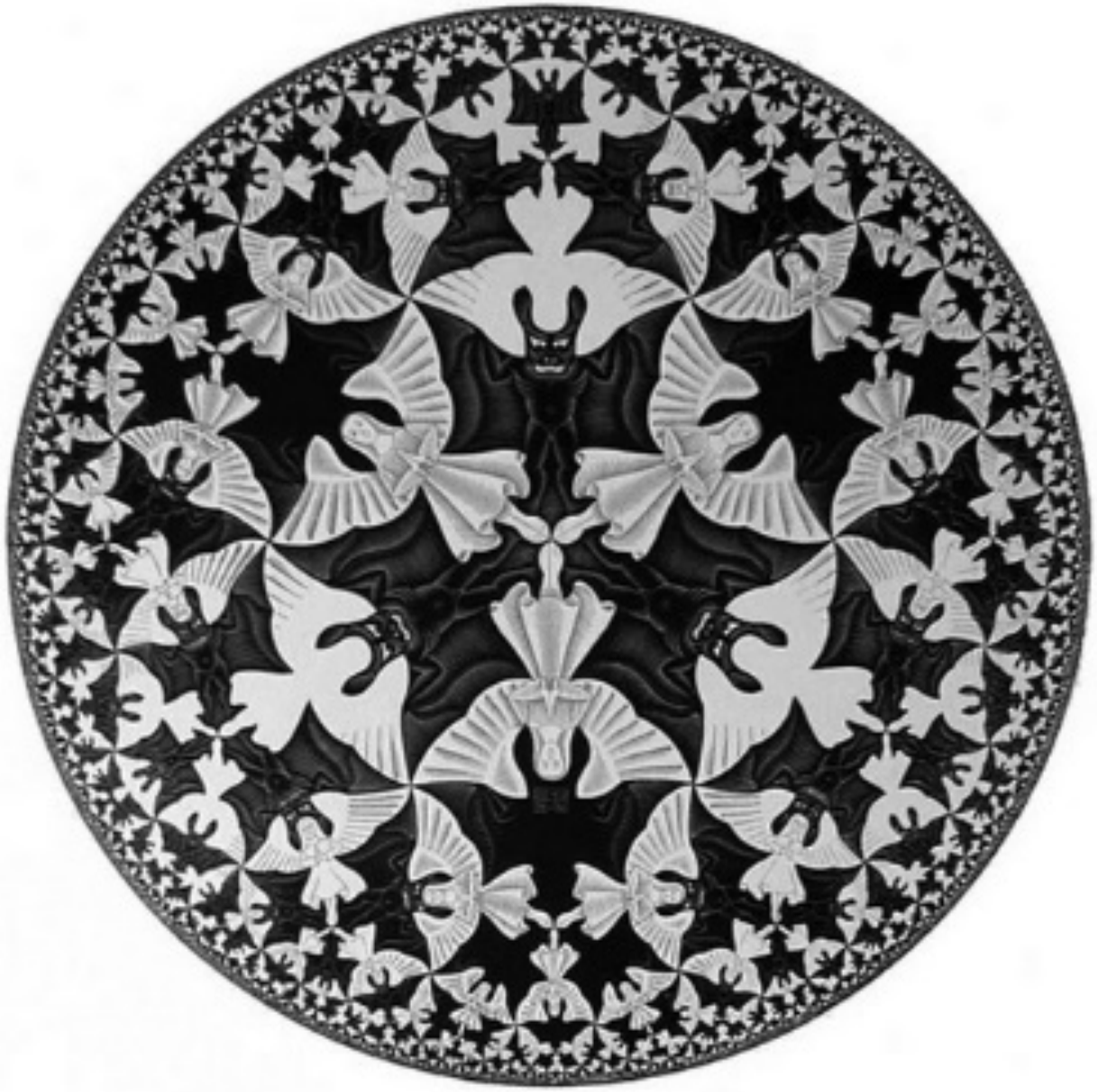
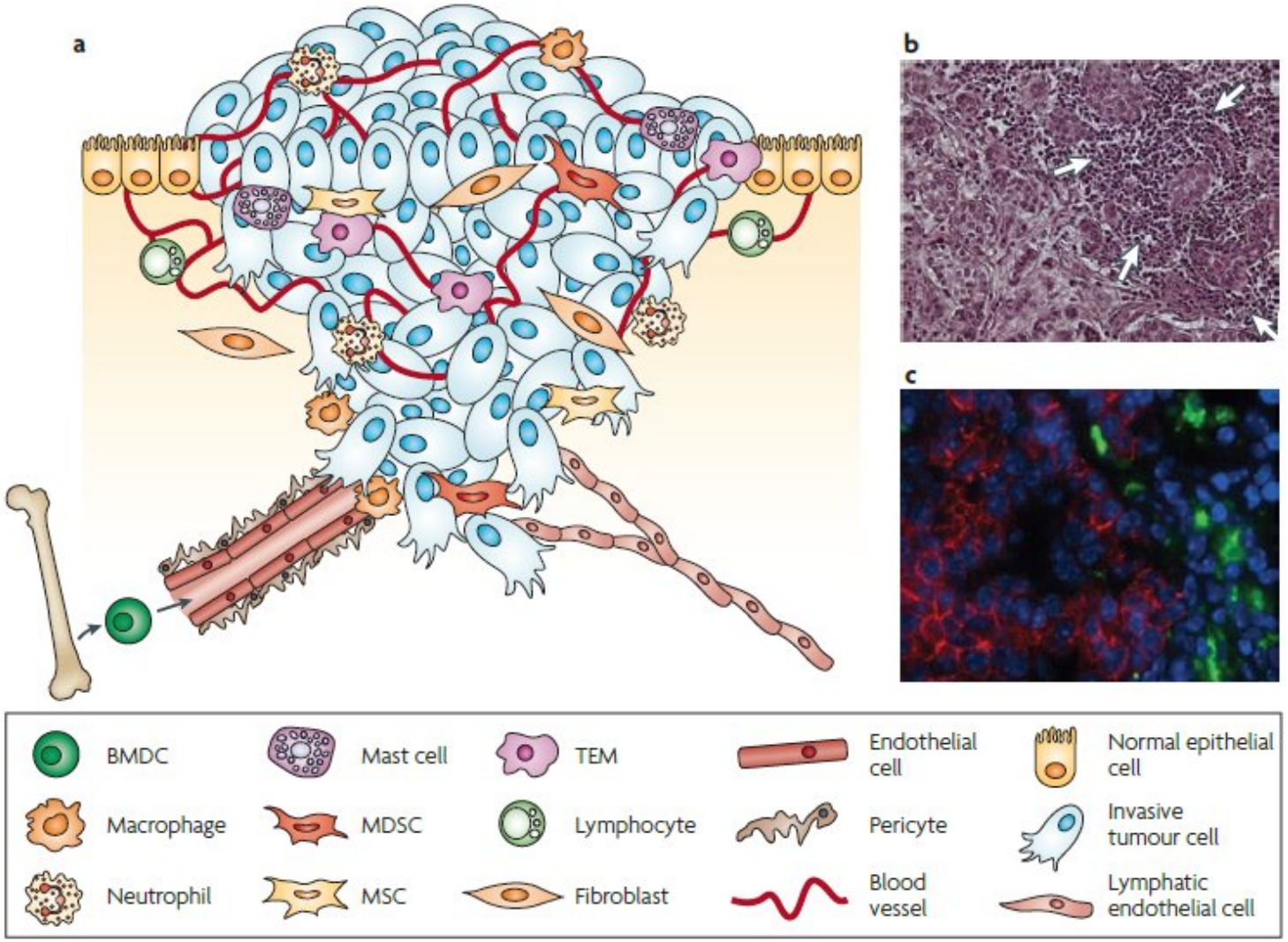
What is the Tumor “Microenvironment”?



What is the Tumor “Microenvironment”?



Joyce 2009 Nature Rev Cancer

What is the Tumor “Microenvironment”?

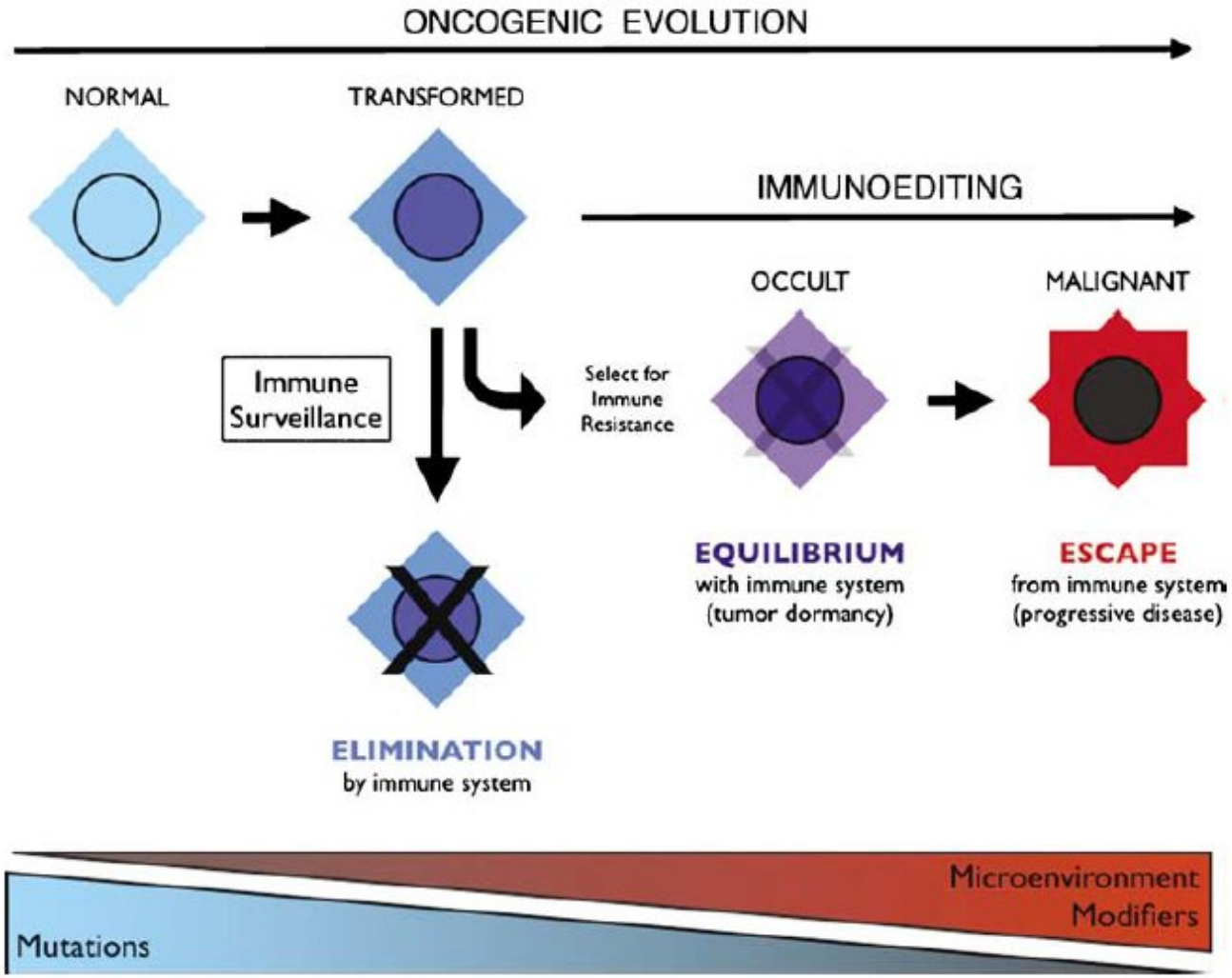
The tumor microenvironment is comprised of many different normal “host” cells including macrophages, granulocytes, lymphocytes, endothelial cells and mesenchymal cells

In some solid tumors, the cancer cells are the minority population!

The microenvironment plays multiple roles in tumor progression: Invasion, Metastasis, Immune Escape, Drug Resistance

The microenvironment can be a major prognostic indicator

The Tumor Microenvironment Evolves…



Prendergast 2008 Oncogene

Glioblastoma Multiforme (GBM)

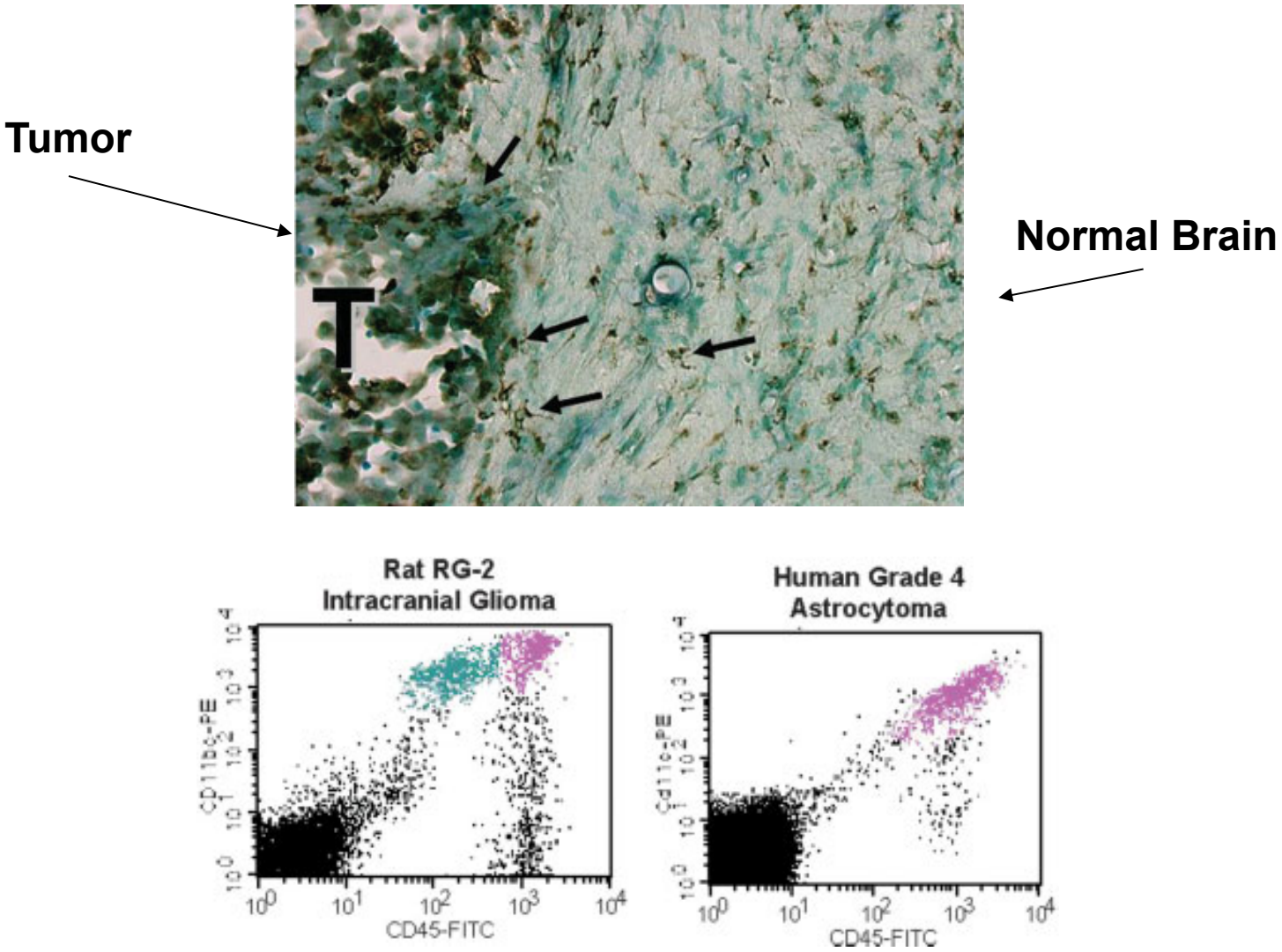
- Arise from astrocytes (support cells in the brain)

- The most aggressive form of adult human brain tumor (Median survival is less than 12 months)

- GBM is characterized by highly invasive nature and ill-defined borders

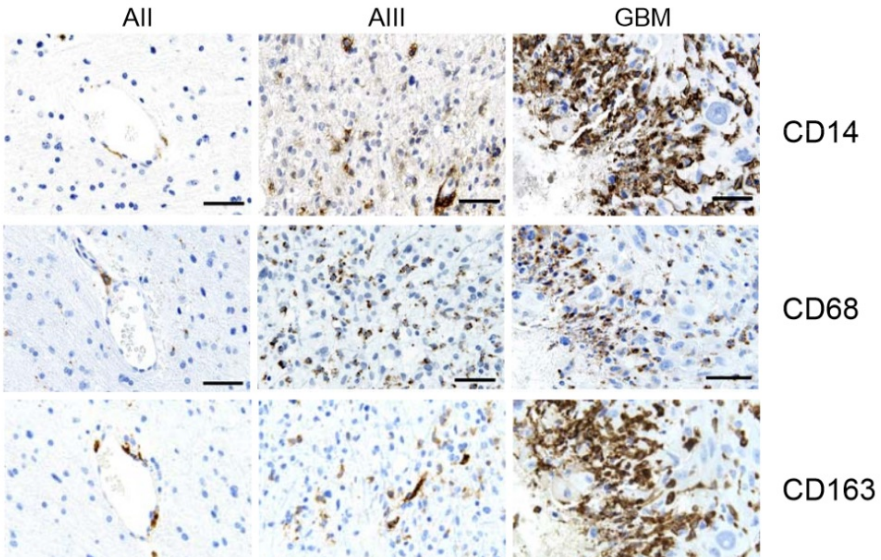
- GBM is highly resistant to conventional and immunotherapy

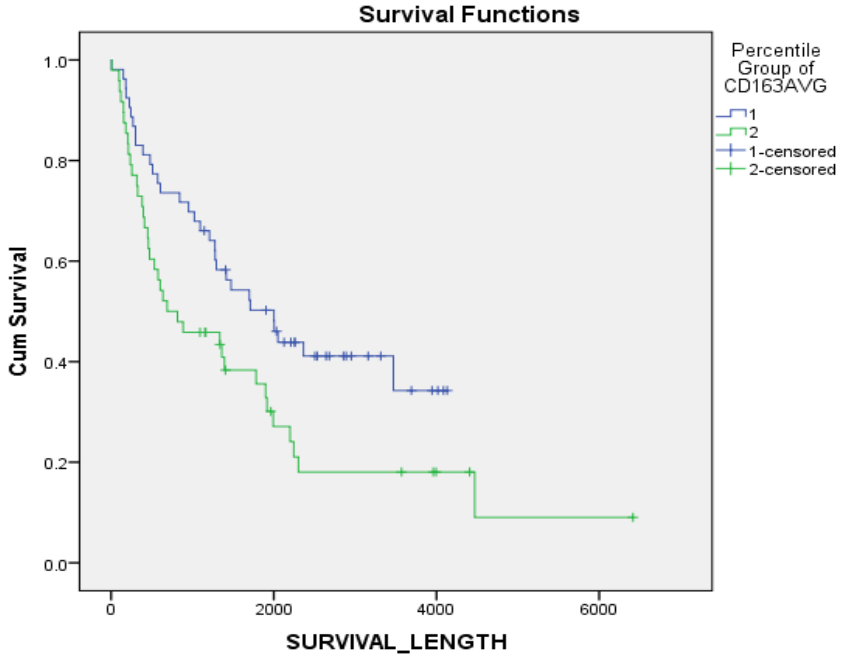
Glioma Tumors are Heavily Infiltrated with Microglia



Watters et al J Neurosci Res (2005)

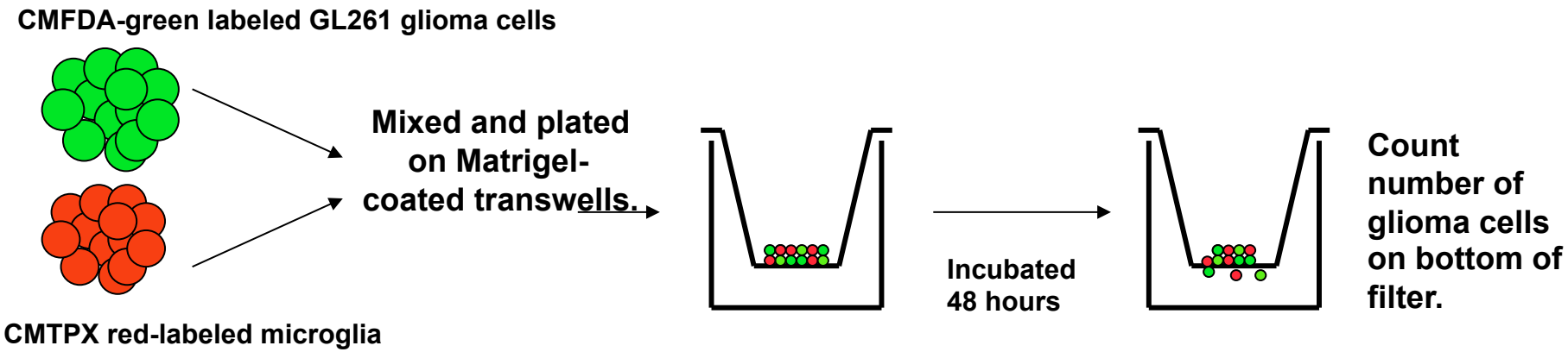
Gliomas with high microglia/macrophage density have worse prognosis





Prosniak et al, CCR 2013

In vitro invasion assay for GBM



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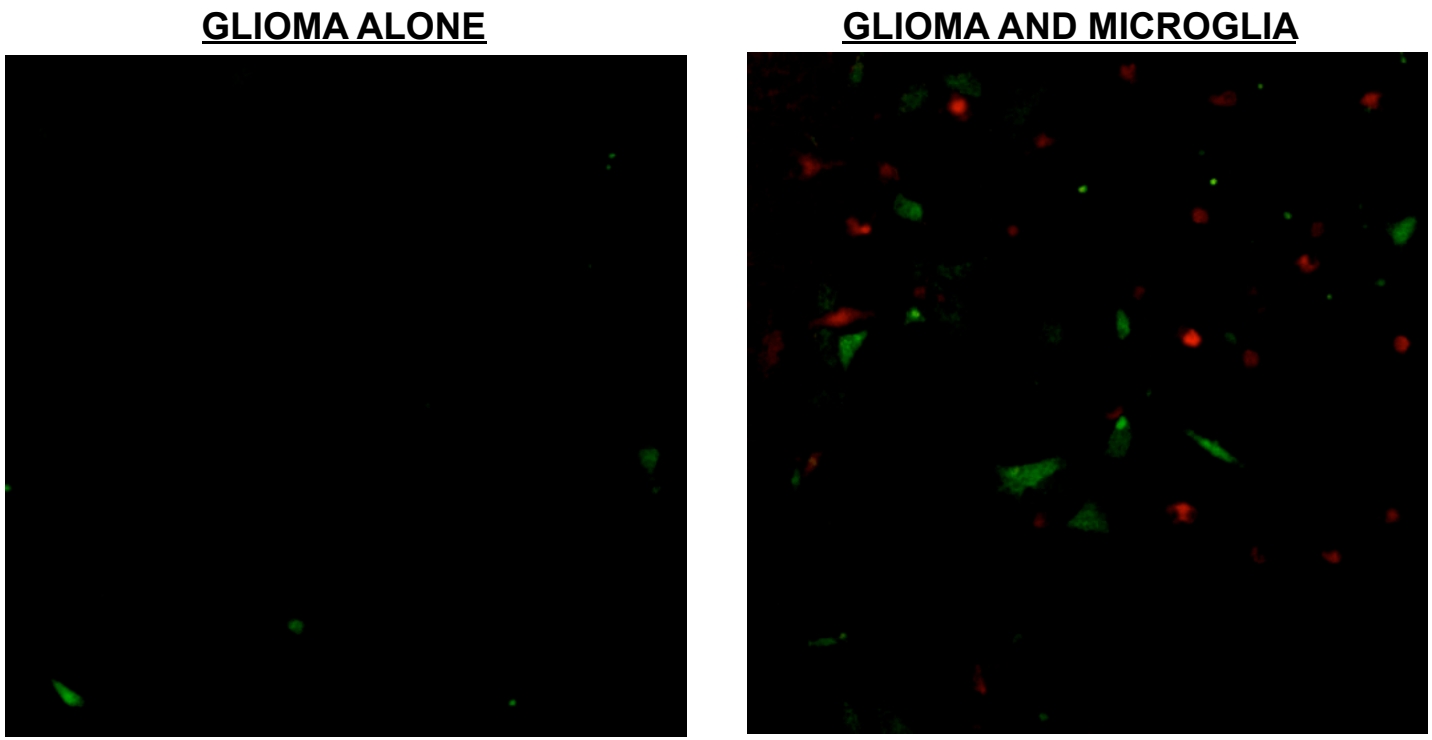
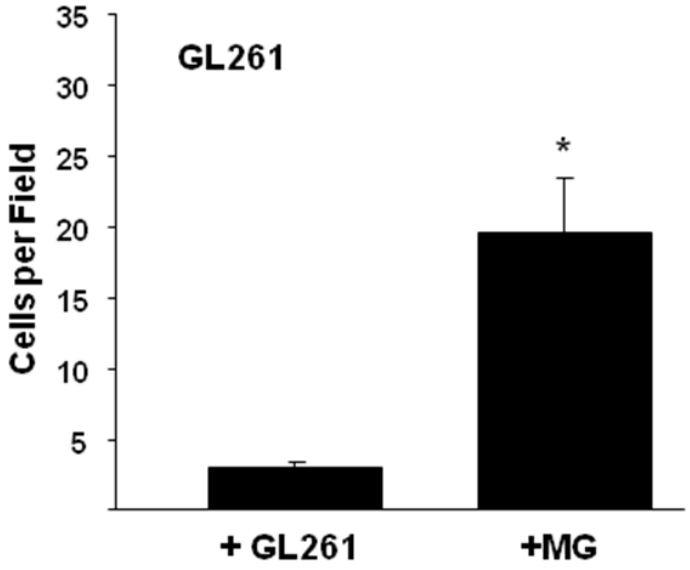
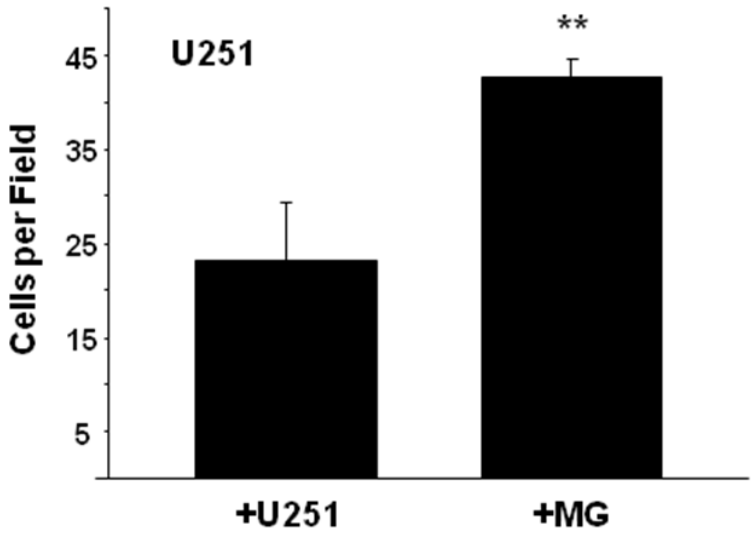


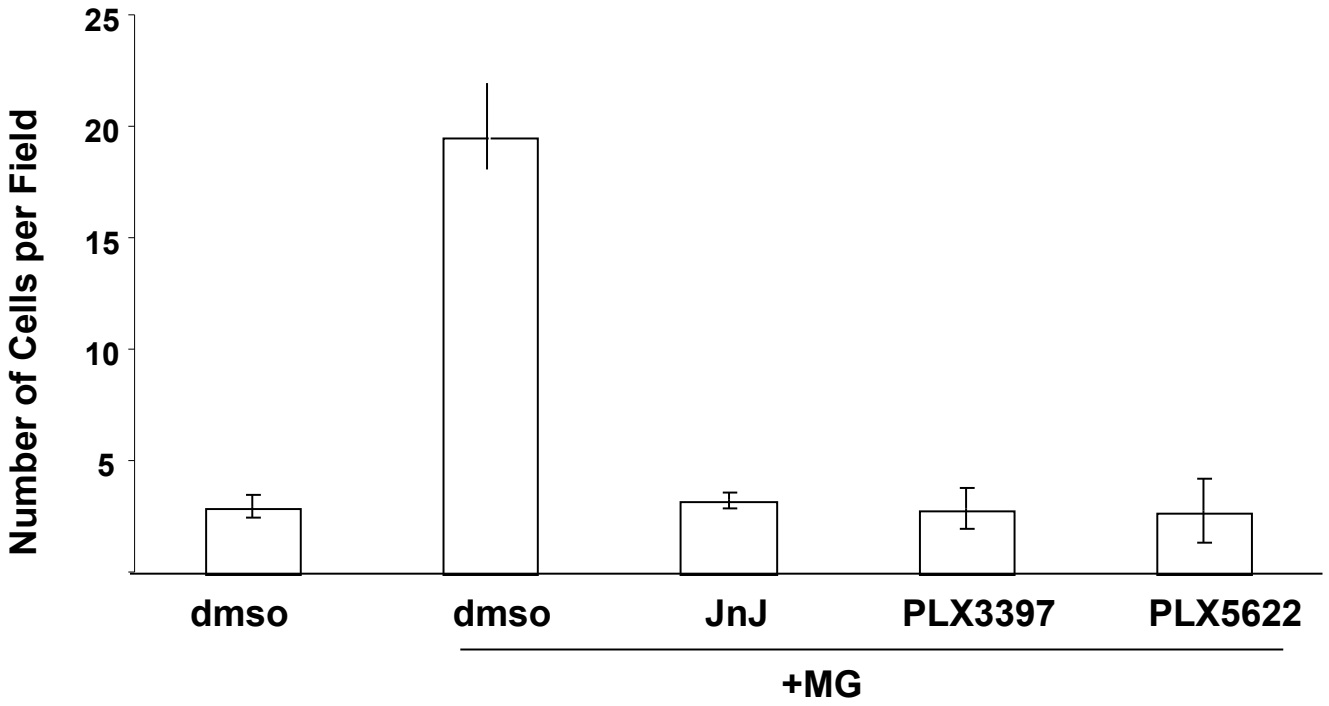
Microglia/macrophages stimulates glioma invasion





Coniglio et al 2012 Mol Med

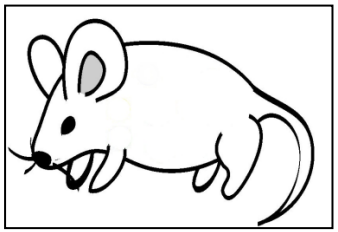
Microglia stimulation of glioma invasion is CSF-1R dependent



Coniglio et al 2012 Mol Med

In vivo testing of the role of CSF-1R in invasion

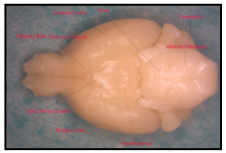
Day 1: Intracranially inject GL261 cells into cortex



Control

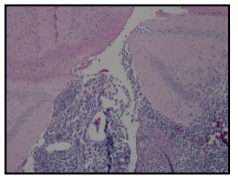
PLX3397

Day 2: Separate mice into two groups: One fed with control chow, the other with Chow containing PLX3397.



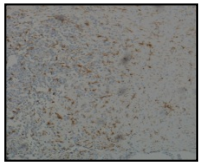
Day 14: Harvest brains, fix and submit for histological analysis and IHC.

H&E

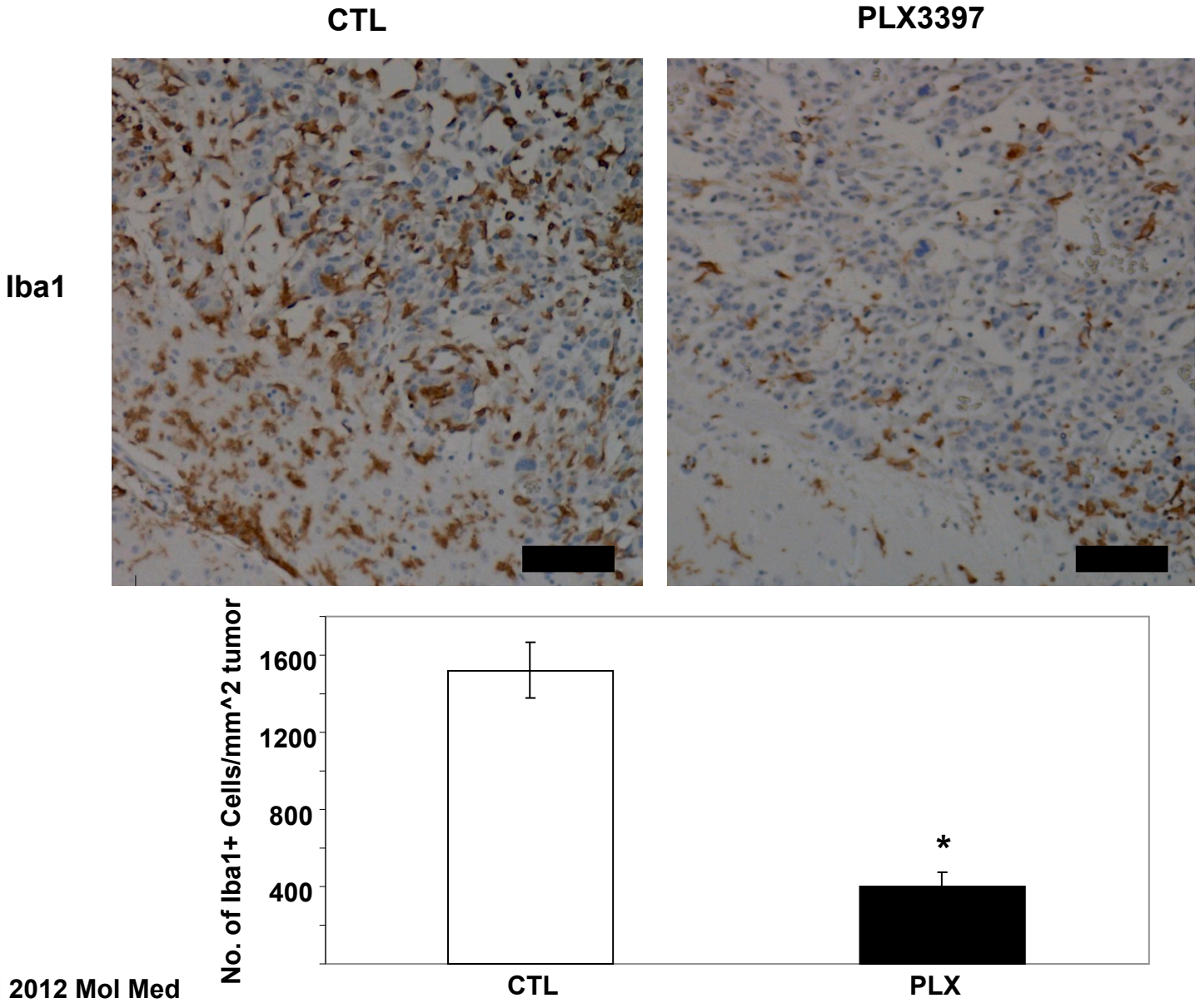


Ki67

Iba1

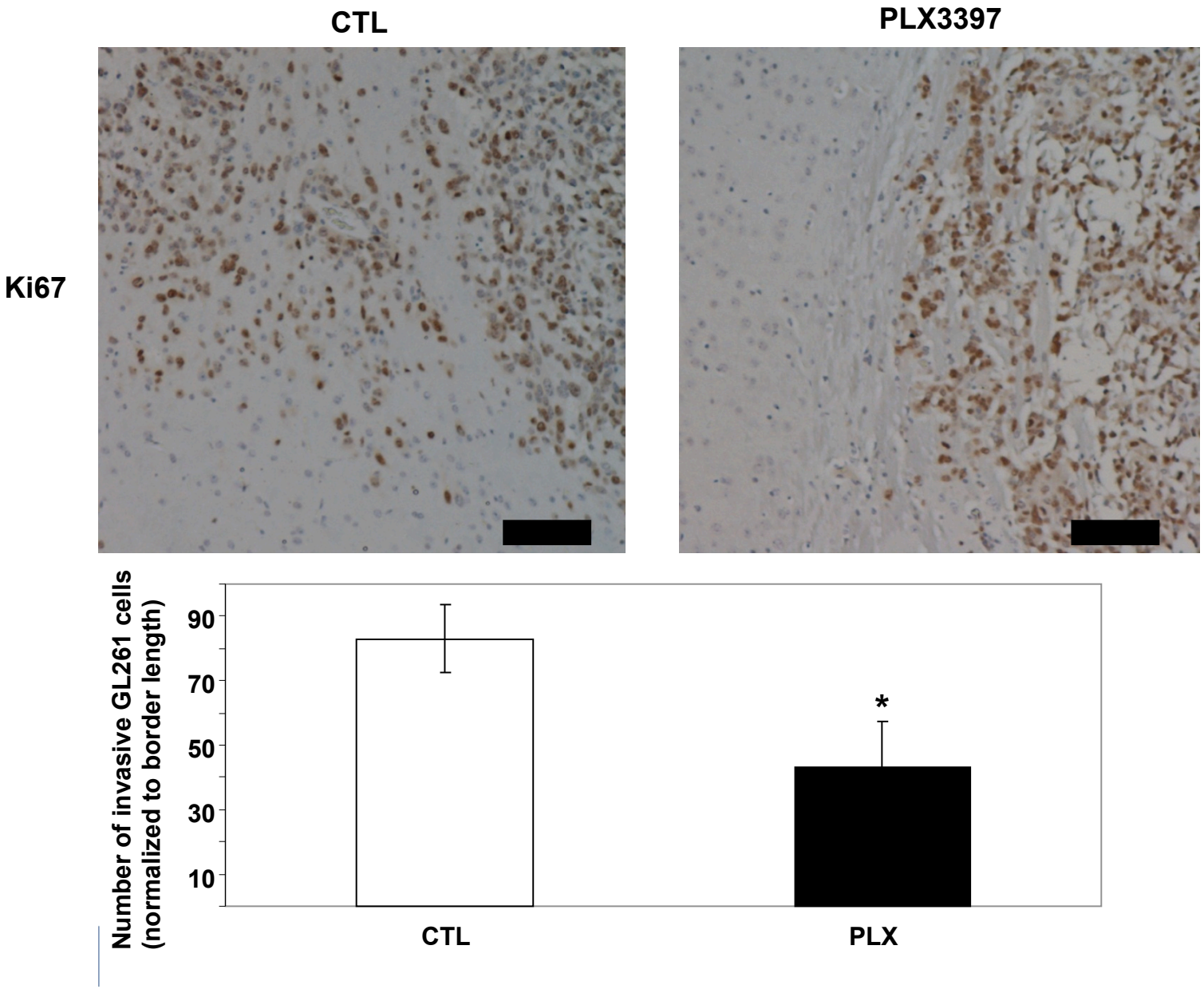


CSF1R inhibition reduces glioma associated microglia



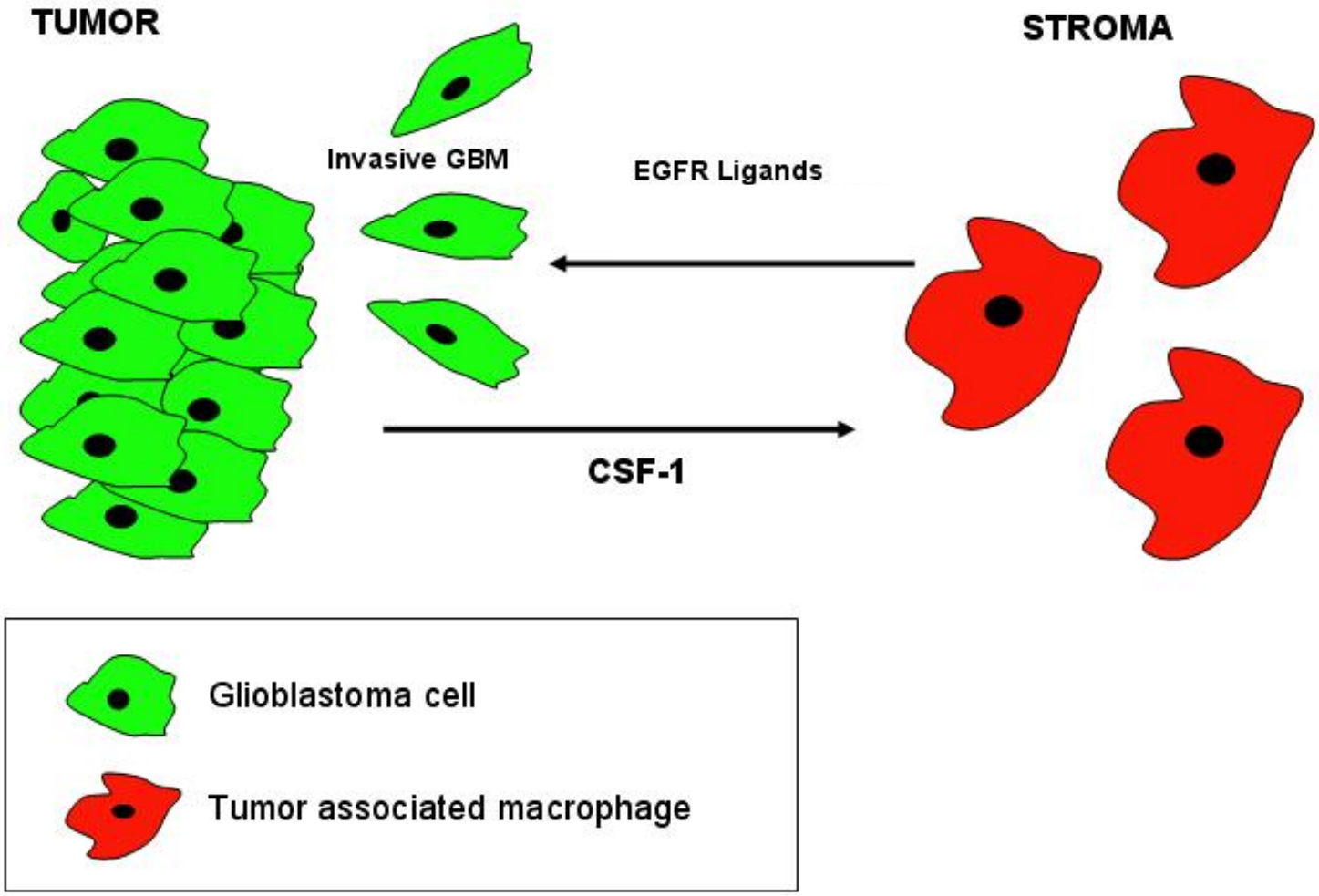
Coniglio et al 2012 Mol Med

CSF1R inhibition reduces glioma invasion



Coniglio et al 2012 Mol Med

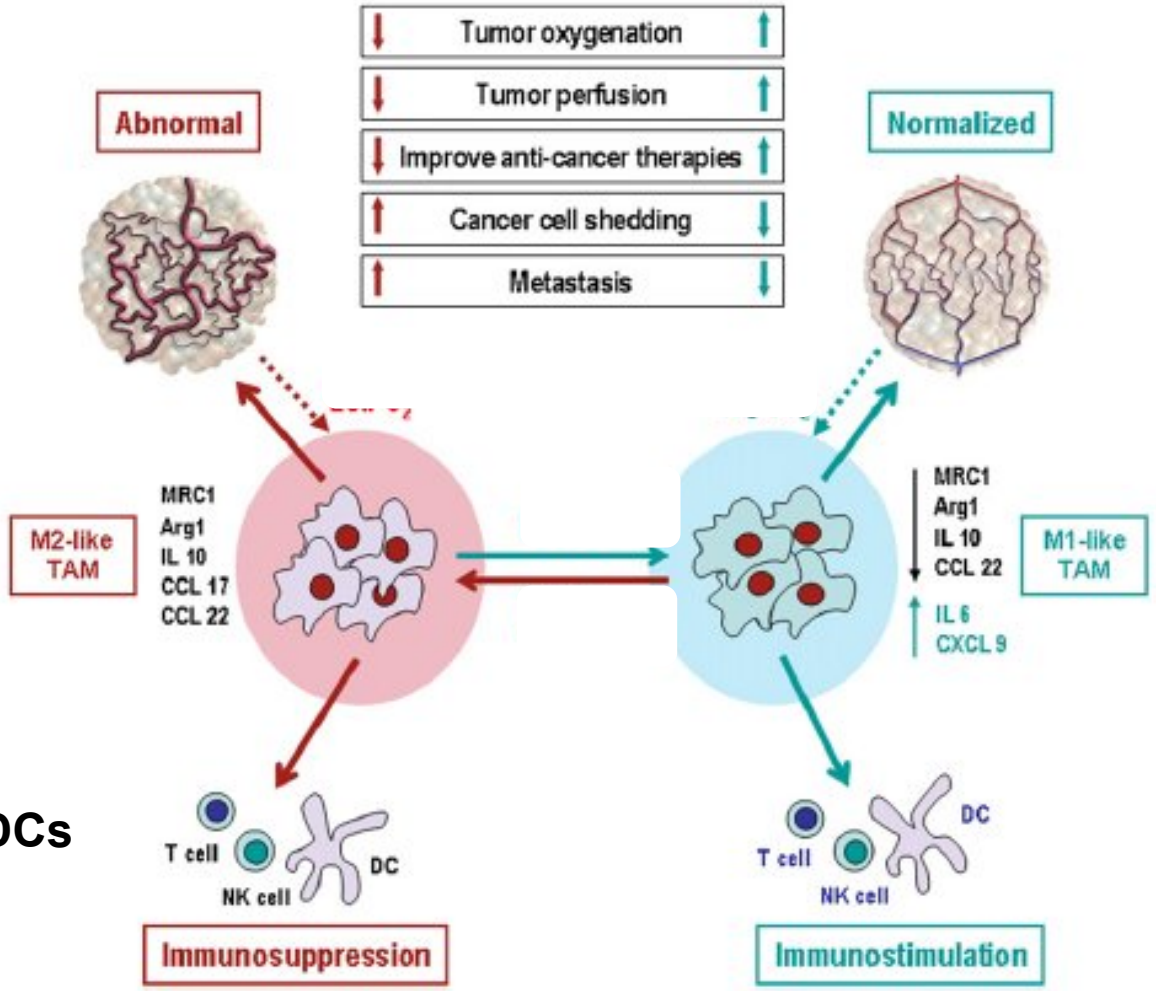
Microglia-stimulation of glioma is dependent on CSF-1R signaling



Macrophage Polarity Dictates Microenvironment

“M2”

“M1”

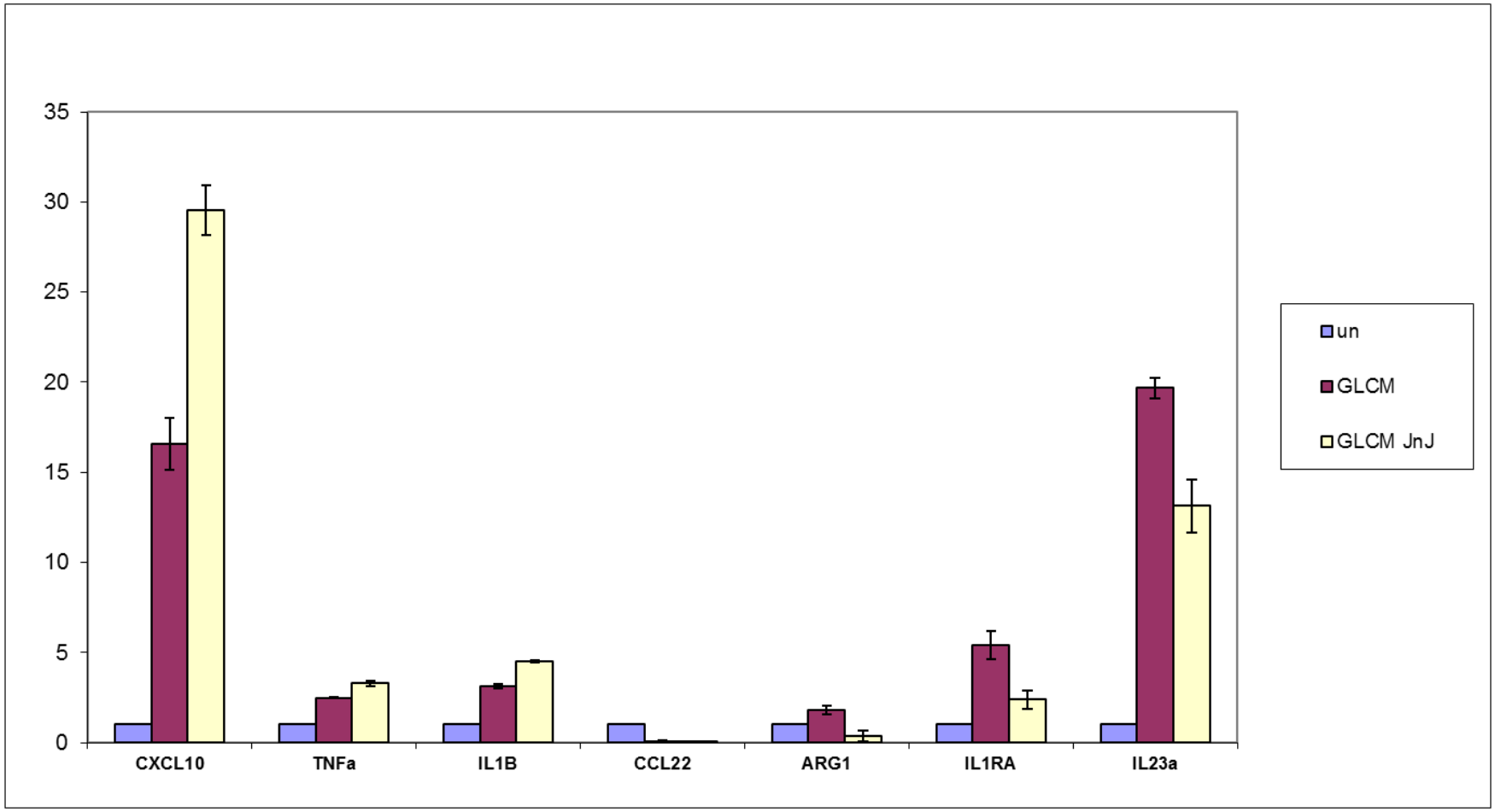


TREGs
MDSCs
Tolerogenic DCs

CD8+ CTLs
NKs
DC

Adapted from Huang 2011 Cancer Cell

CSF-1R is able to regulate microglia polarity in vitro



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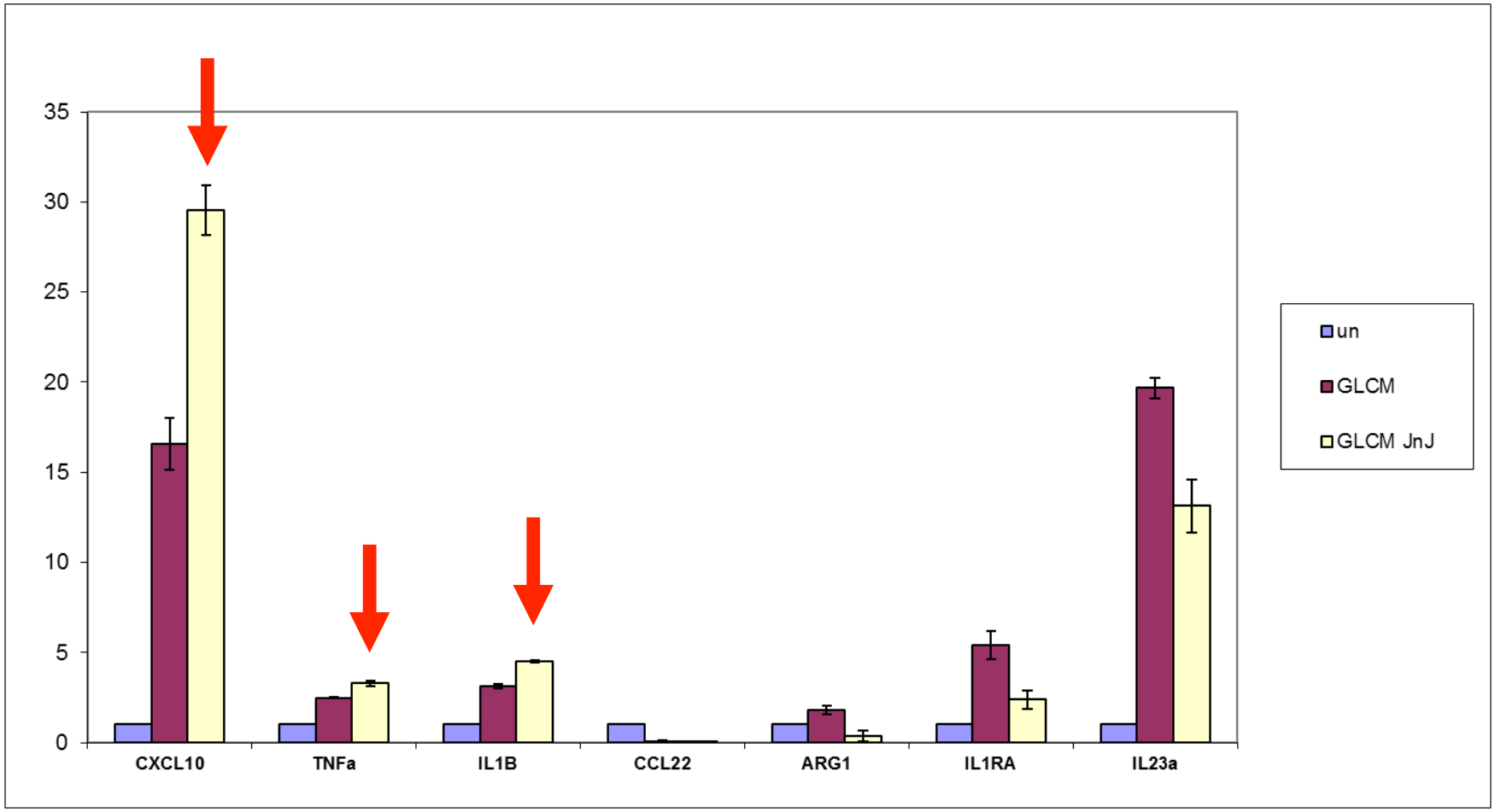

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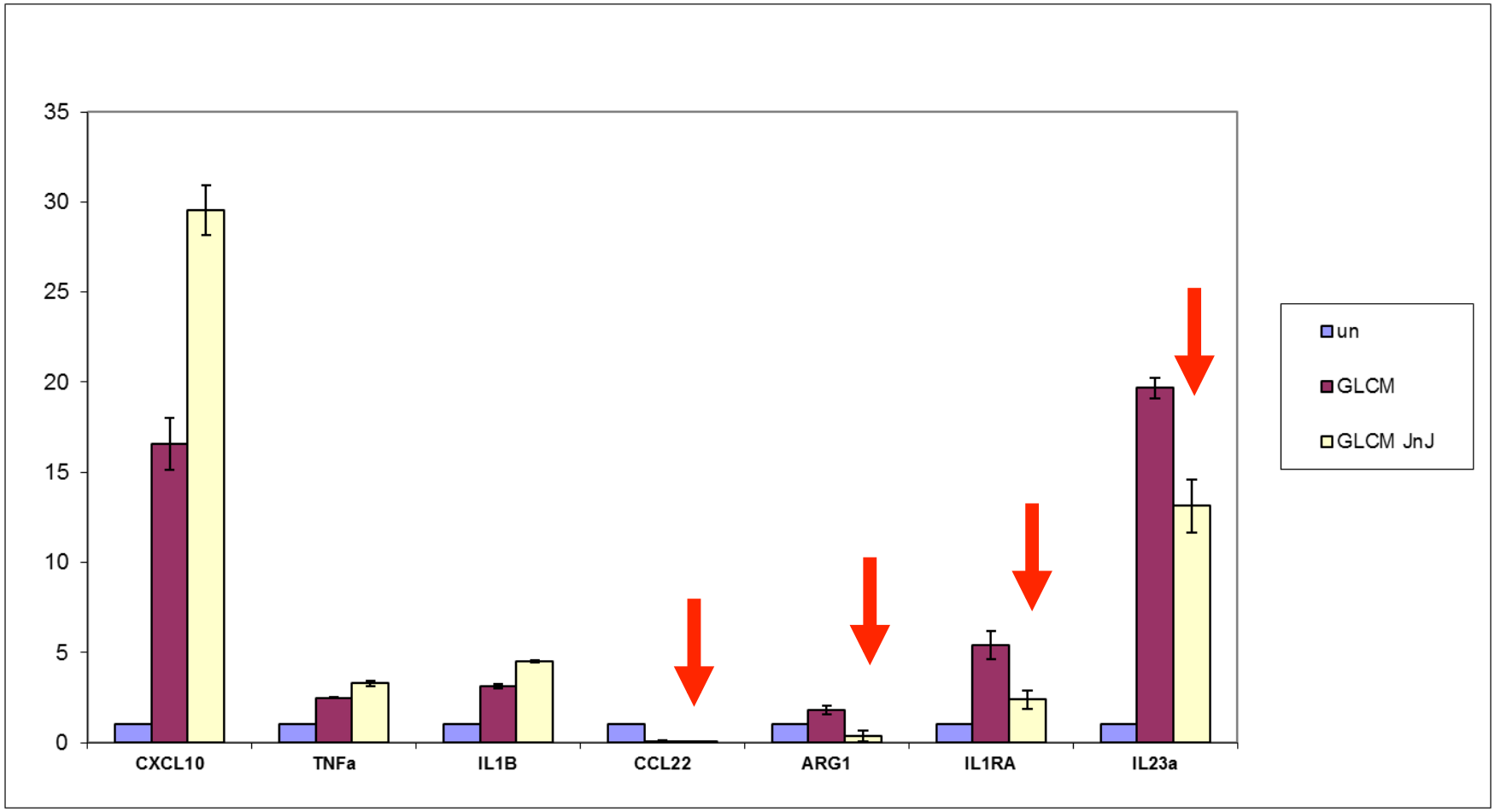

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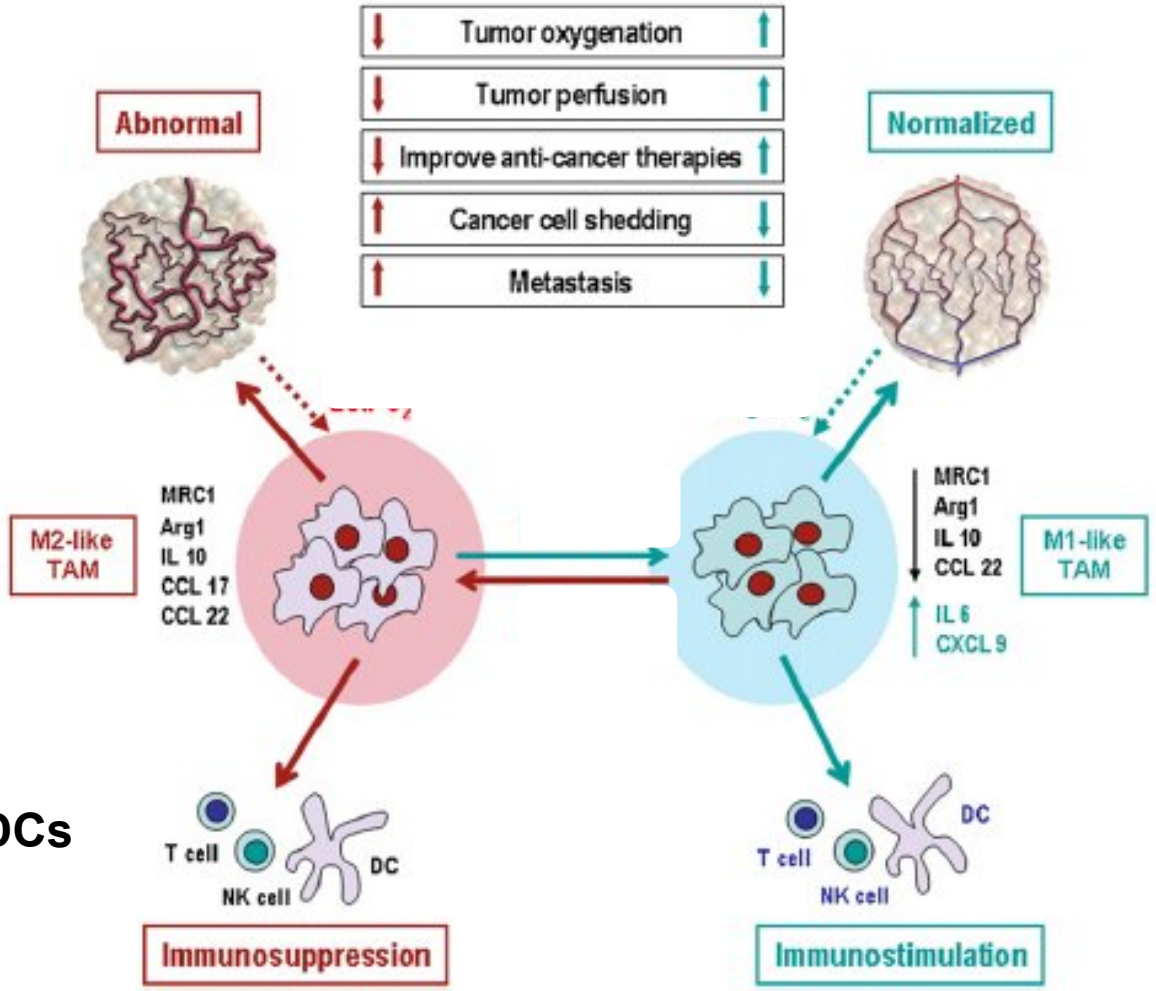
M1



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Macrophage Polarity Dictates Microenvironment

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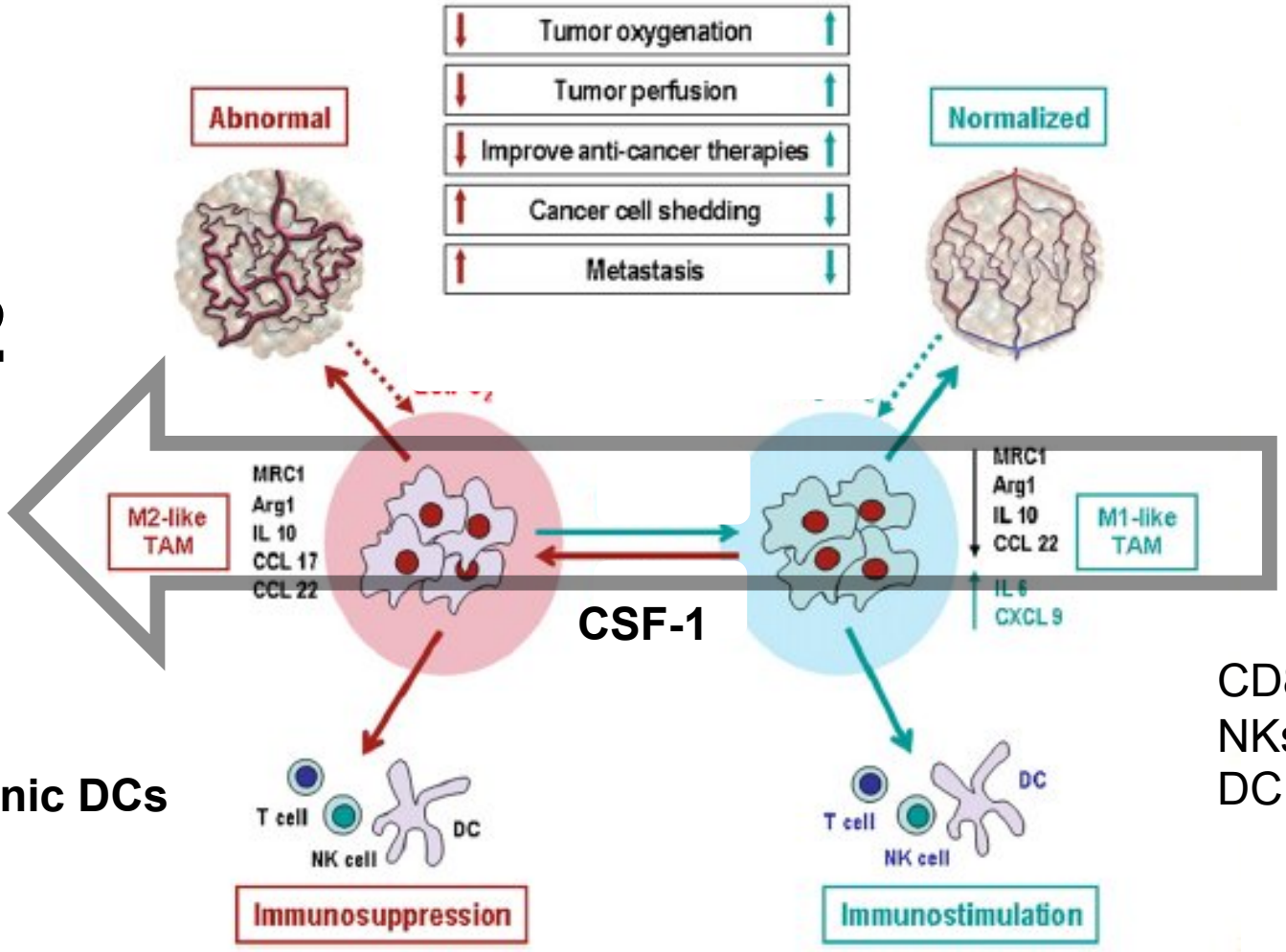

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Strategy:

We will administer CSF-1R inhibitors, chemokine inhibitors and NP particles to animals with GBM tumor and monitor microenvironment composition using FACS

FACS has multiple and unique advantages:

-Subgroups generally defined by 2 or more markers

-We can profile much of the immune cell composition of the microenvironment with available markers

Profiling the GBM microenvironment with FACS

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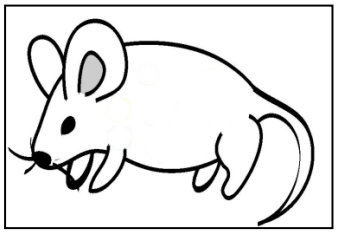

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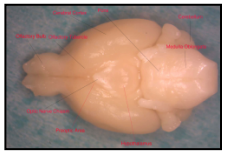


Day 2: Separate mice into two groups:
One fed with control chow, the other with
Chow containing PLX3397.

Day 14:
Harvest tumor.
Dissociate into individual cells

Profiling the GBM microenvironment with FACS

Gr1+/Cd11b+

MDSC

CD4/CD25+

TREGs

CD11c/MHCII

DCs

CD11b/CD86+

M1 Macrophage

CD11b/CD206+

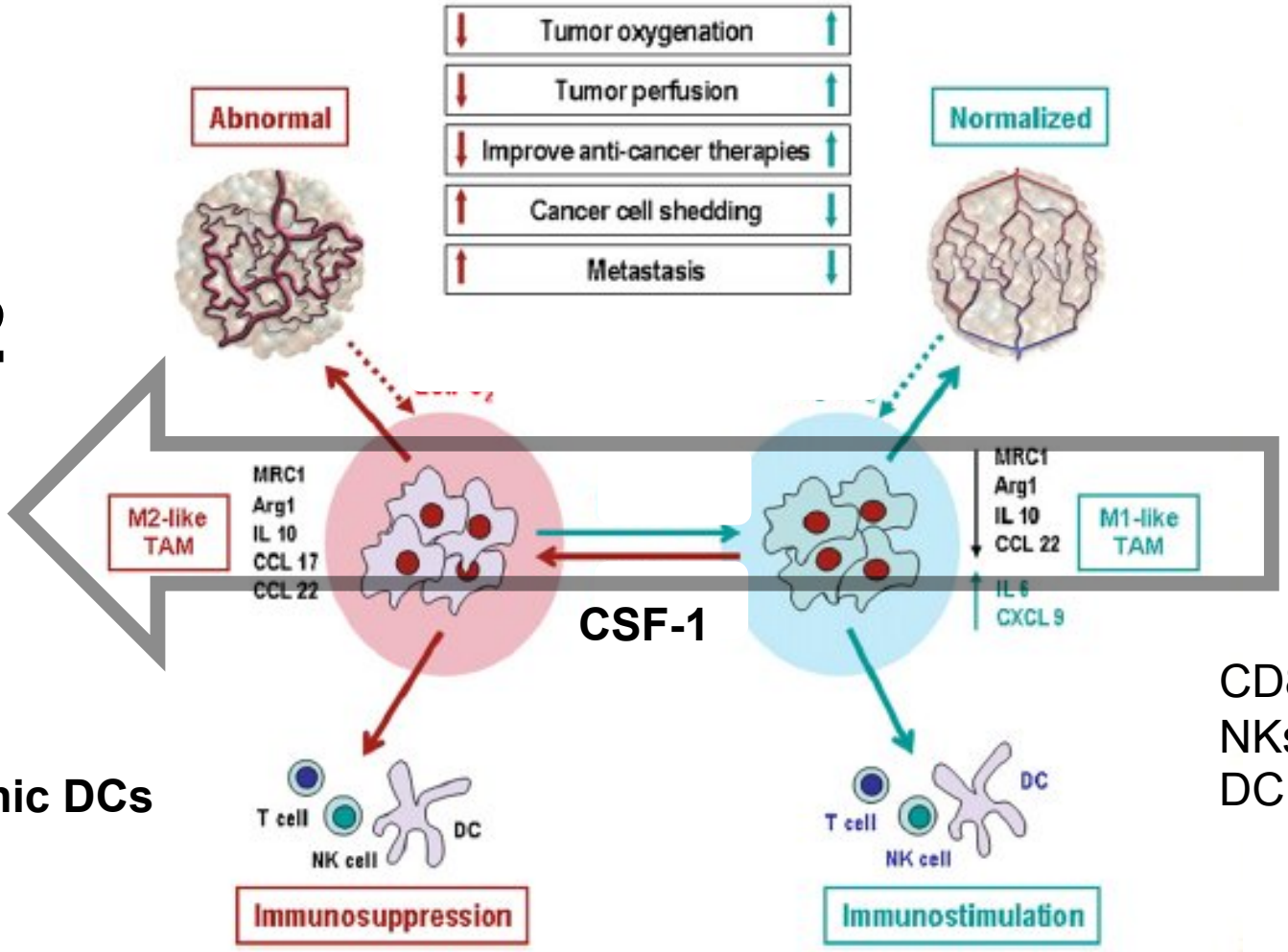
M2 Macrophage

Analyze populations using antibodies against multiple markers and multicolor FACS

Does inhibition of CSF-1R result in a repolarization of the microenvironment towards M1?

M2

M1



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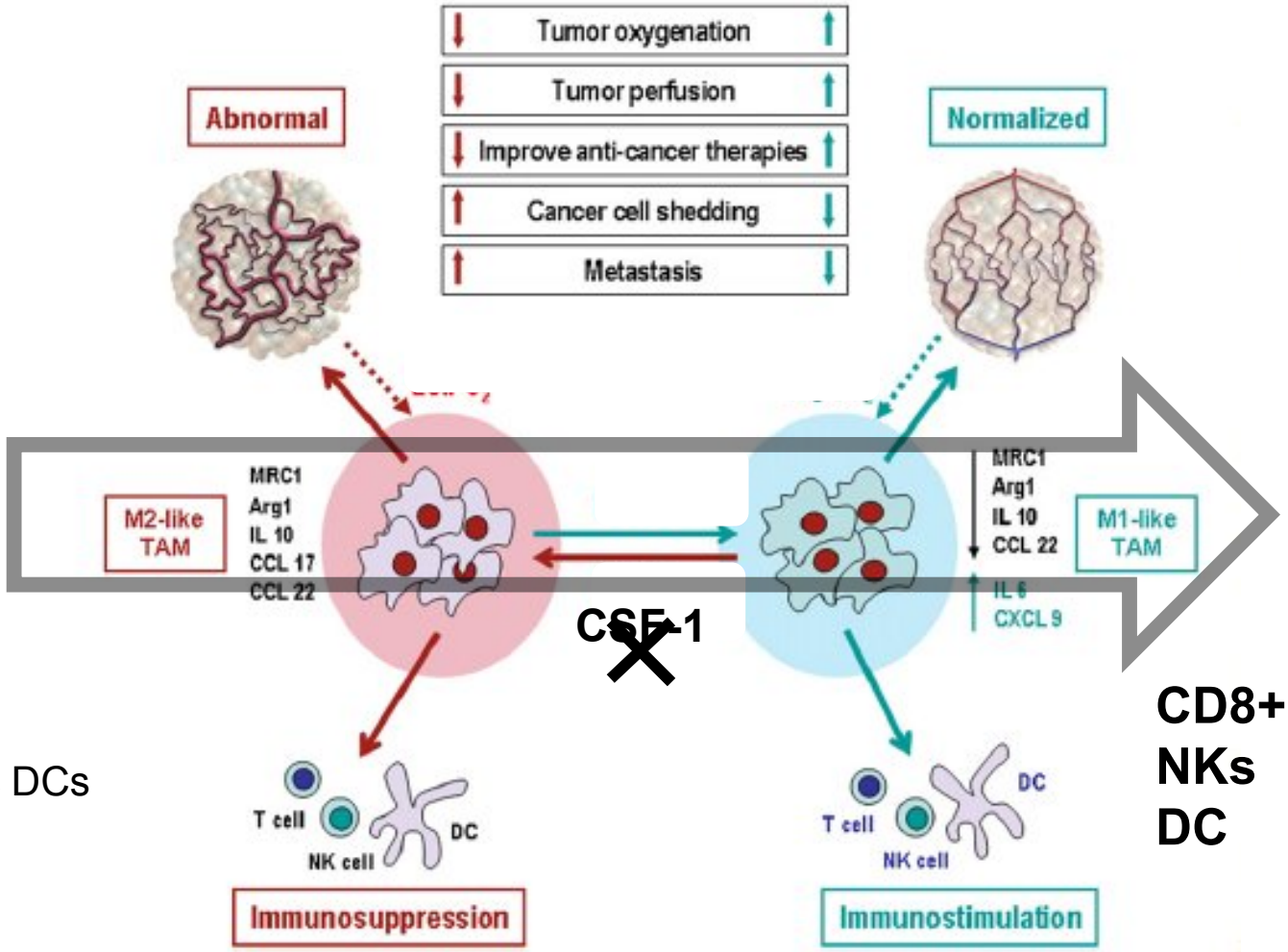

Adapted from Huang 2011 Cancer Cell

Conclusions

- **Microglia/macrophages comprise up to 1/3 of glioma tumor mass and is directly correlated with advanced disease**

- **Microglia/macrophages strongly stimulates glioma invasion and this is dependent on CSF-1R.**

- **Blockade of CSF-1R shift macrophage polarity markers toward a more M1 phenotype in vitro**

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Dr. James Merritt

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