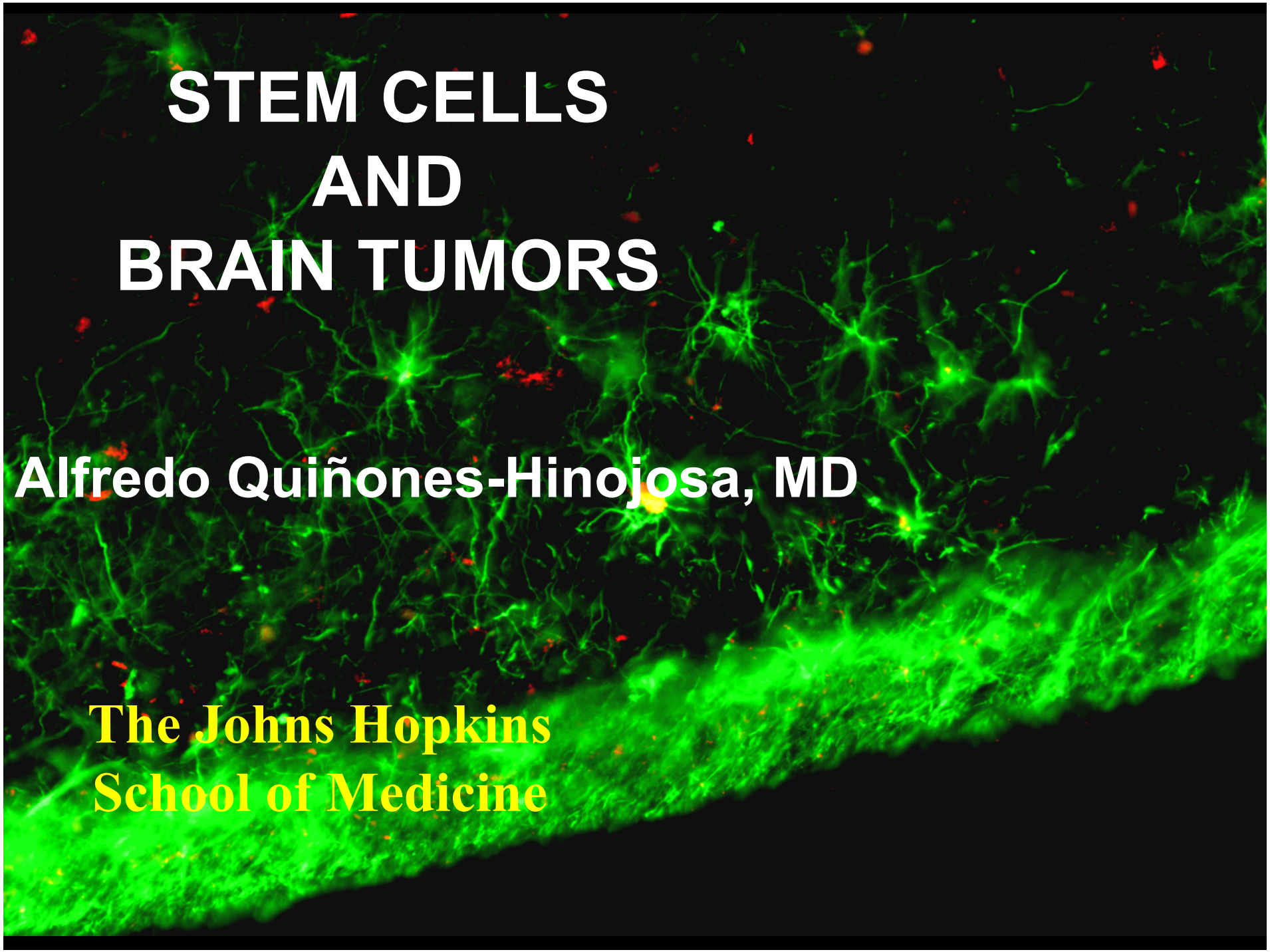
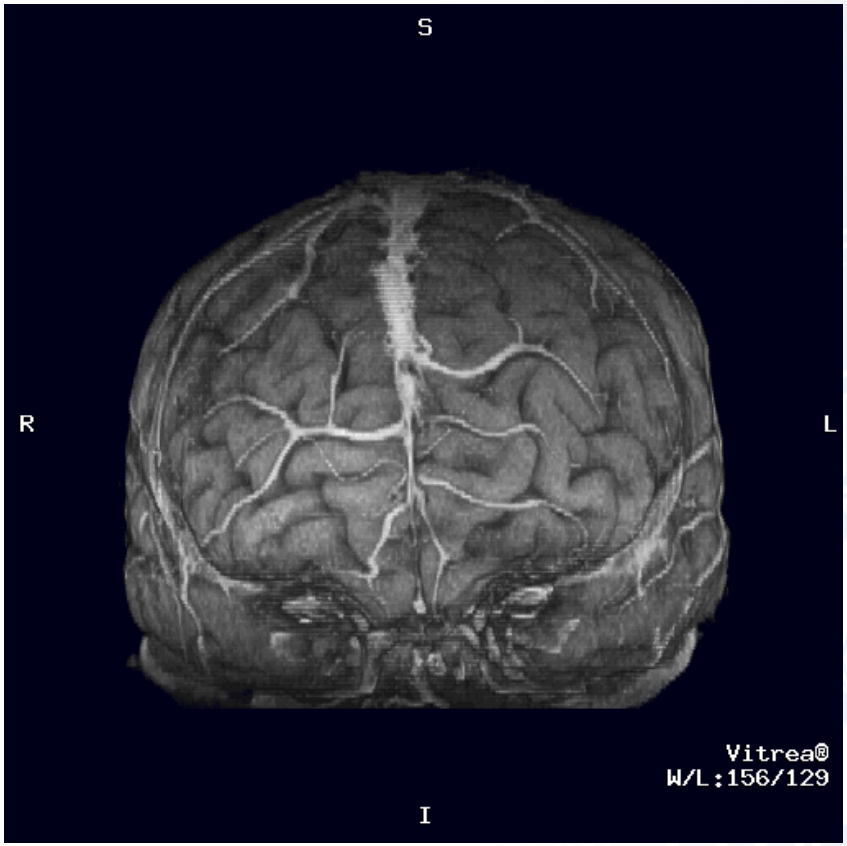

STEM CELLS
AND
BRAIN TUMORS
Alfredo Quiñones-Hinojosa, MD
The Johns Hopkins
School of Medicine



"harsh decree" that "everything may die, nothing may be regenerated" in the adult CNS

Outline

- **Controversy of Adult Neurogenesis**
- **Human Vs Rodent Stem Cell Niche**
- **Stem Cells in the Human Brain**
- **Stem Cells in Human Cancer**

HISTORY

The Controversy of Adult Neurogenesis

"…None of the methods used by these investigators are capable of distinguishing absolutely a multiplying neuroglia cell from a small mitotic neuron"

(Ramon y Cajal, 1913)

HISTORY

The Controversy of Adult Neurogenesis

1962 Joseph Altman: thymidine autoradiographic evidence for new neurons in the adult rat and cat

1977 Michael Kaplan: combined [^{3}H]-thymidine labeling and electron microscopy to confirm Altman's claims by showing mitotic neuronal precursors lining the lateral ventricles

HISTORY

The Controversy of Adult Neurogenesis

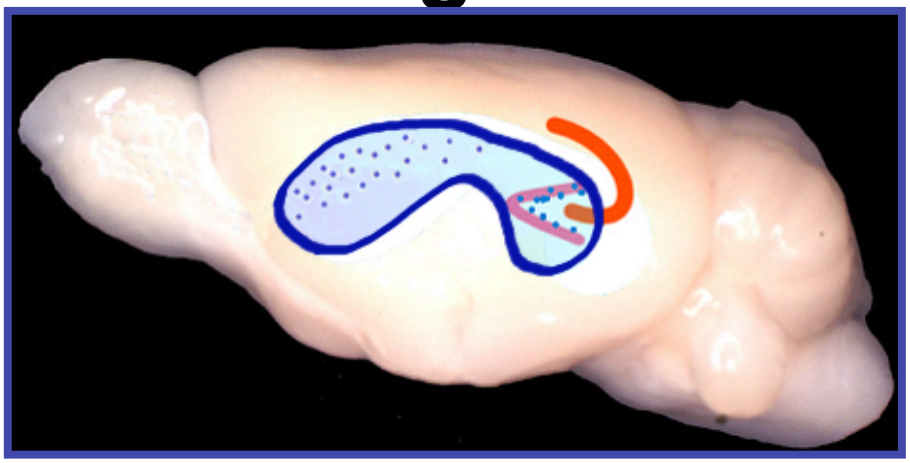
1980s Nottebohm Colleagues :

AVIAN BRAIN

1. production of new cells with thymidine labeling
2. new cells were neurons receiving synapses
3. neurons responded to sound with action potentials.

HISTORY

The Controversy of Adult Neurogenesis



Hippocampal Dentate Gyrus

Subventricular Zone (SVZ)

- **1997 Organization and Cytoarchitecture of Rodent SVZ**
- **1999 Adult Neural Stem Cells in Rodents are Astrocytes**

Outline

- **Controversy of Adult Neurogenesis**
- **Human Vs Rodent Stem Cell Niche**
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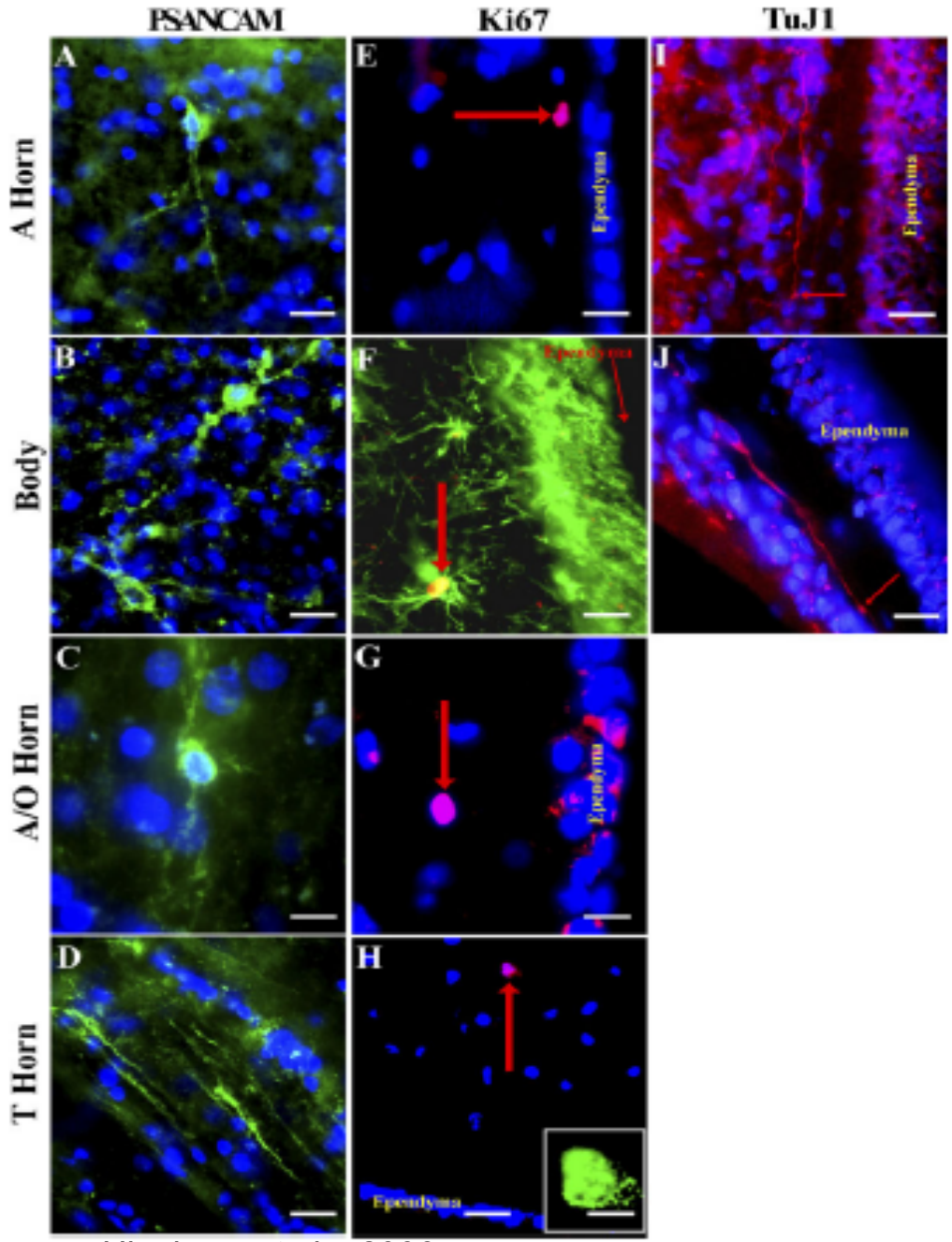
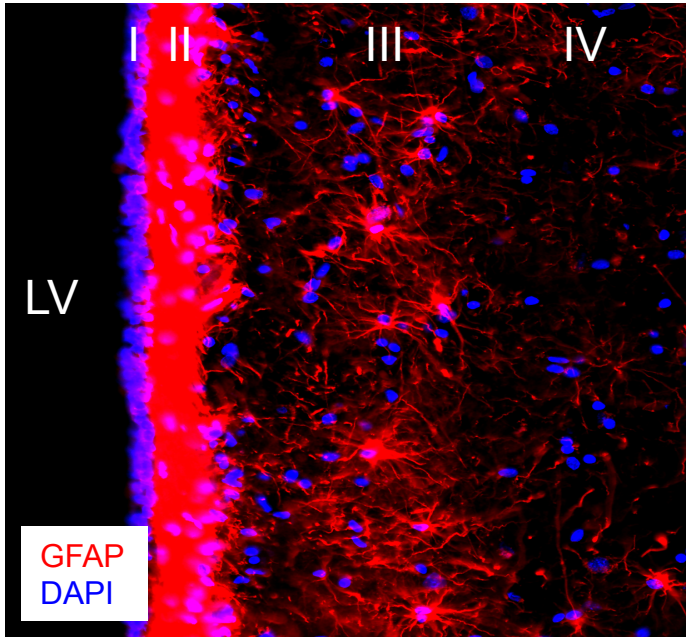


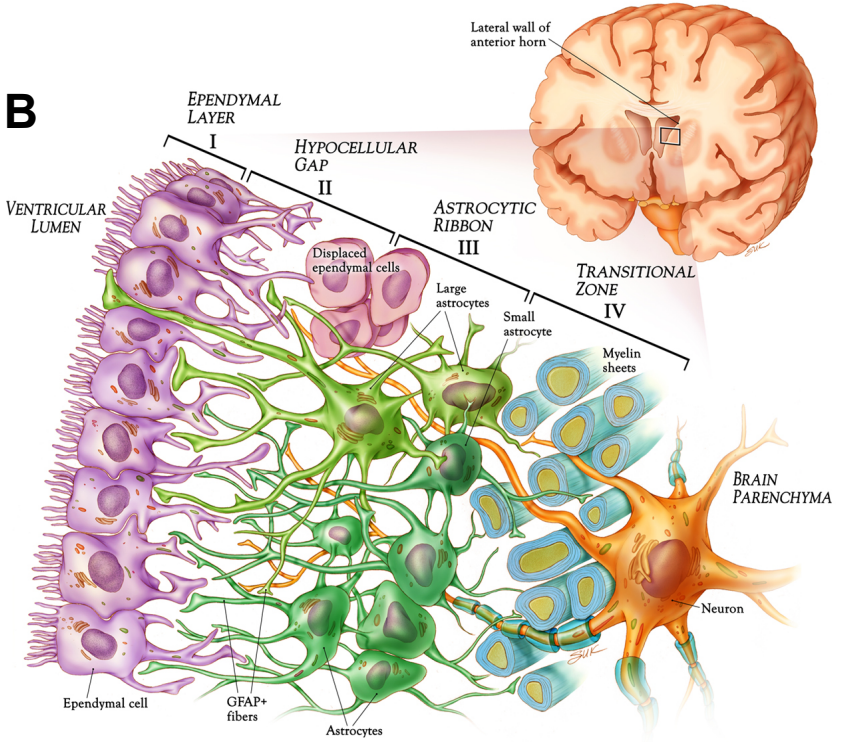
Quinones-Hinojosa, et al , 2006 Figure 5

A

Human Brain SVZ

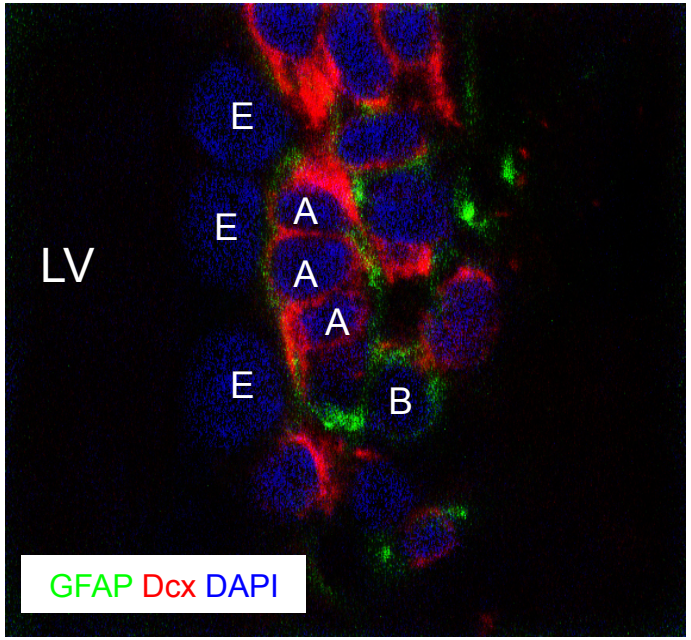


B

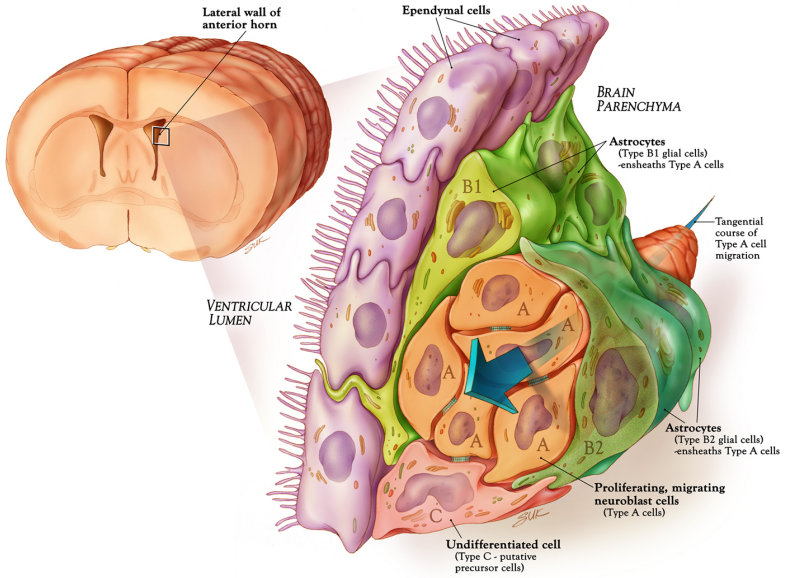


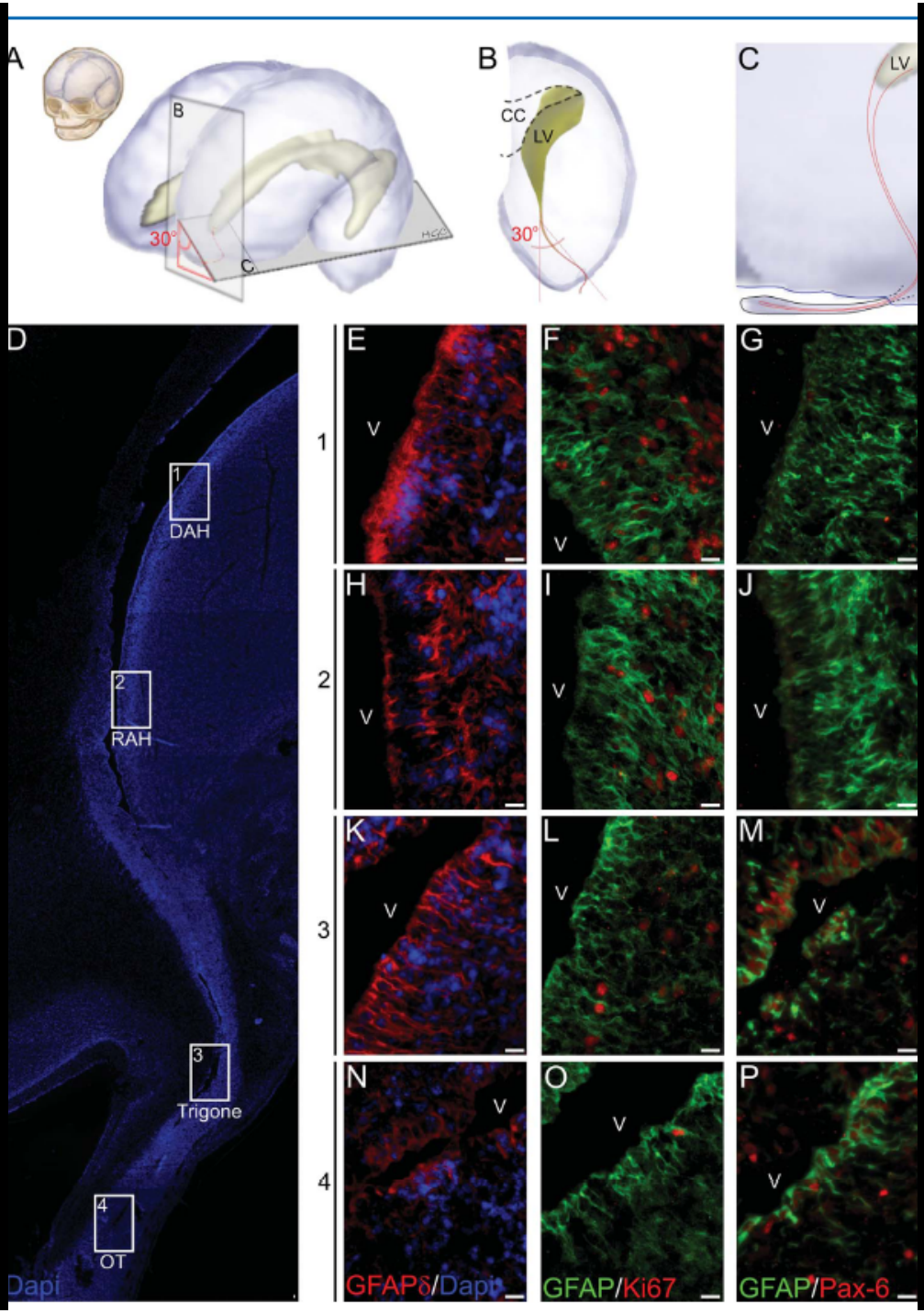
C

Rodent Brain SVZ

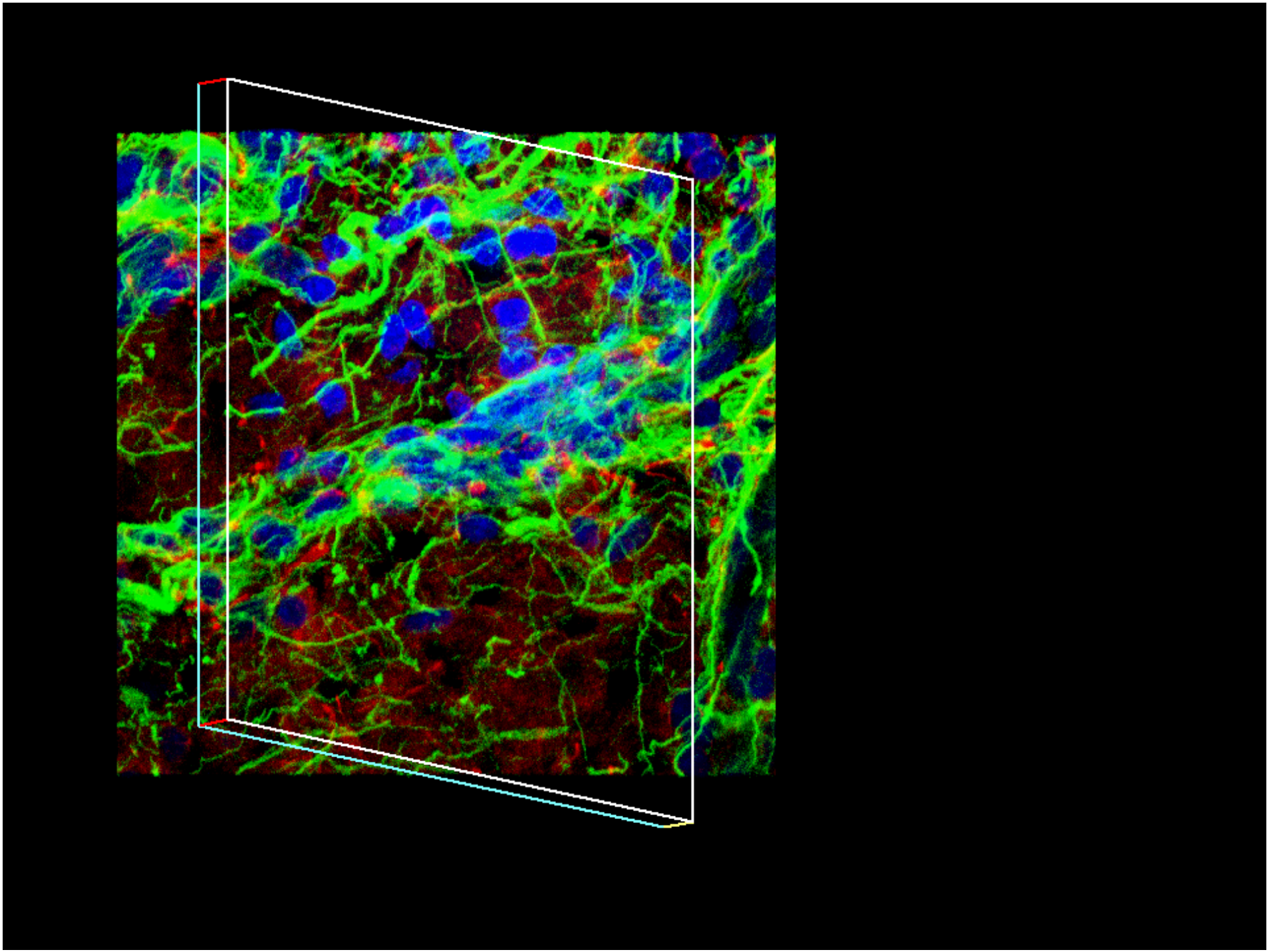


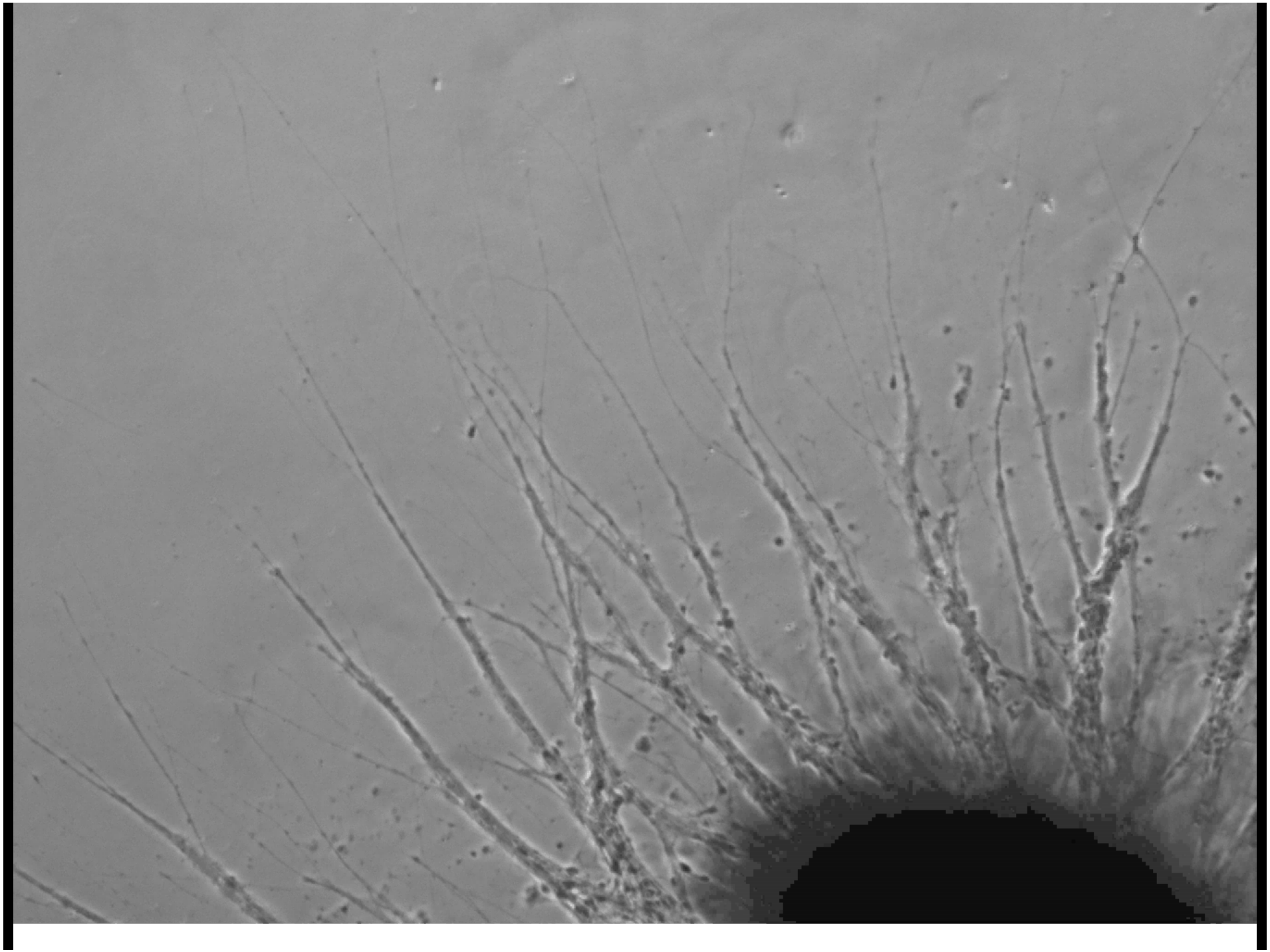
D





Guerrero-Cazares et al 2011





Outline

- **Controversy of Adult Neurogenesis**
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- **Stem Cells in the Human Brain**
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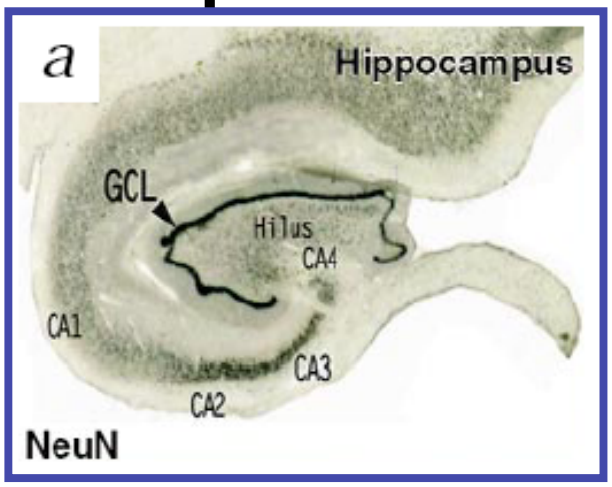
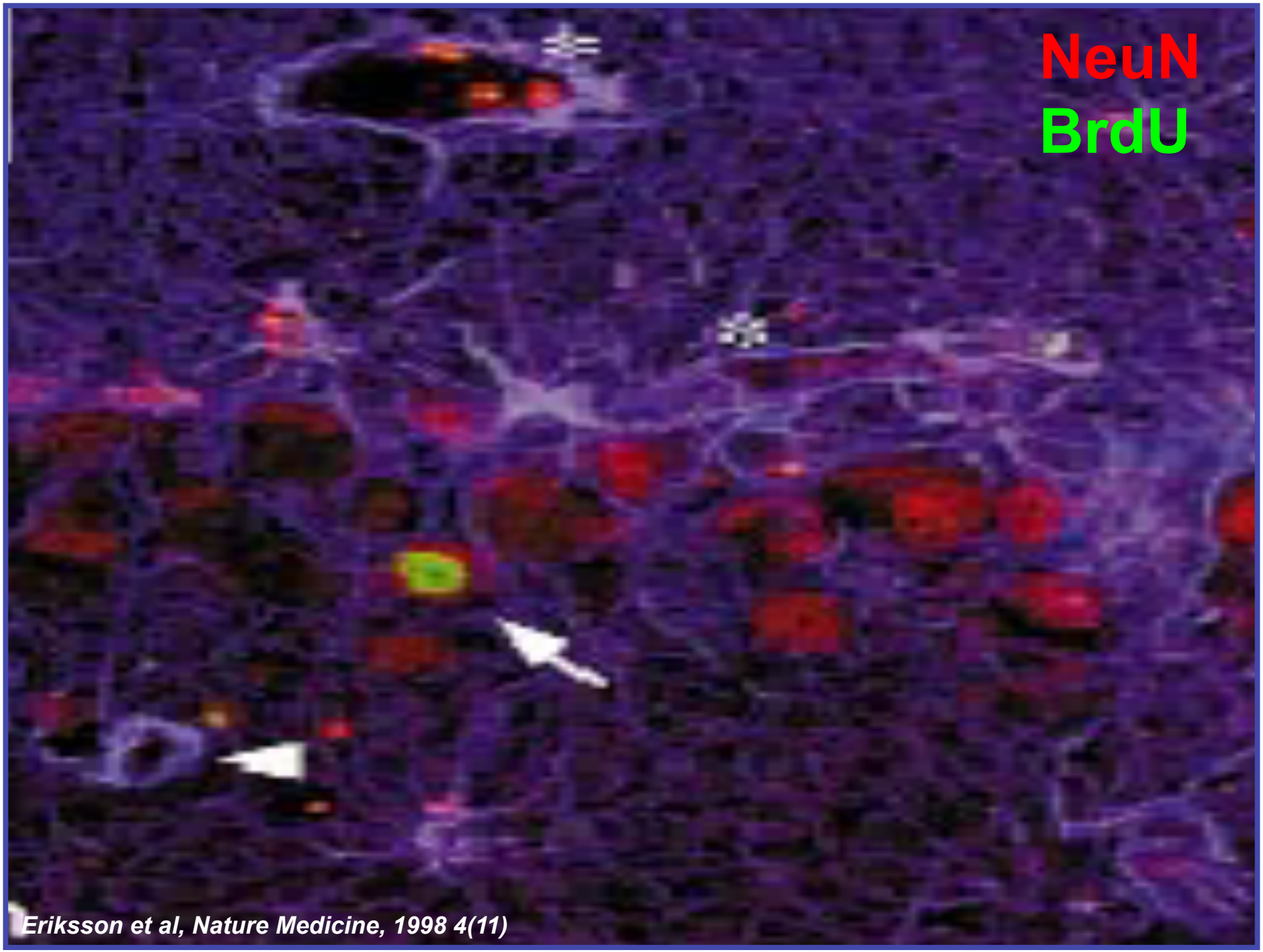
Adult Human Neural Stem Cells?

1994 Goldman et al: adult human neurogenesis from temporal horn SVZ *in vitro*

(*Kirschenbaum et al. 1994, Cereb Cortex*)

1998 Gage et al: *in vivo* neurogenesis in adult human hippocampus





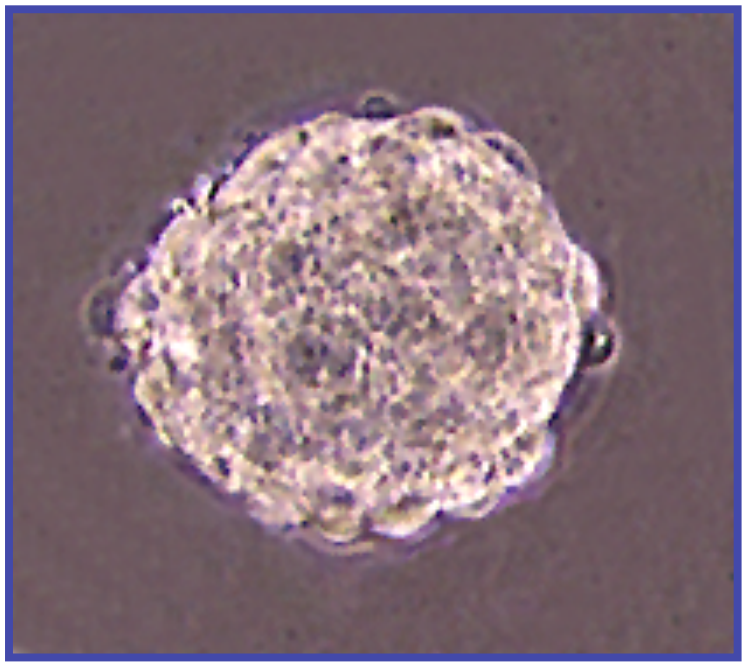
Eriksson et al, Nature Medicine, 1998 4(11)

The Adult Human SVZ Contains Neural Stem Cells

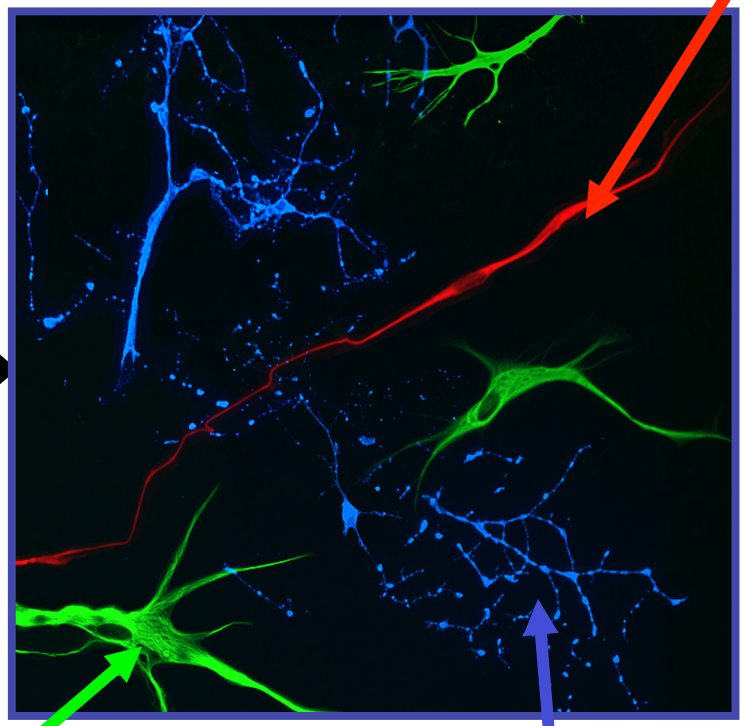
- **SVZ Specimens: 62.57 ± 7.46 neurospheres / well**
- **Purified SVZ Astrocytes: 109.29 ± 8.67 neurospheres / well**
- **Cortex & Striatum: no neurospheres**

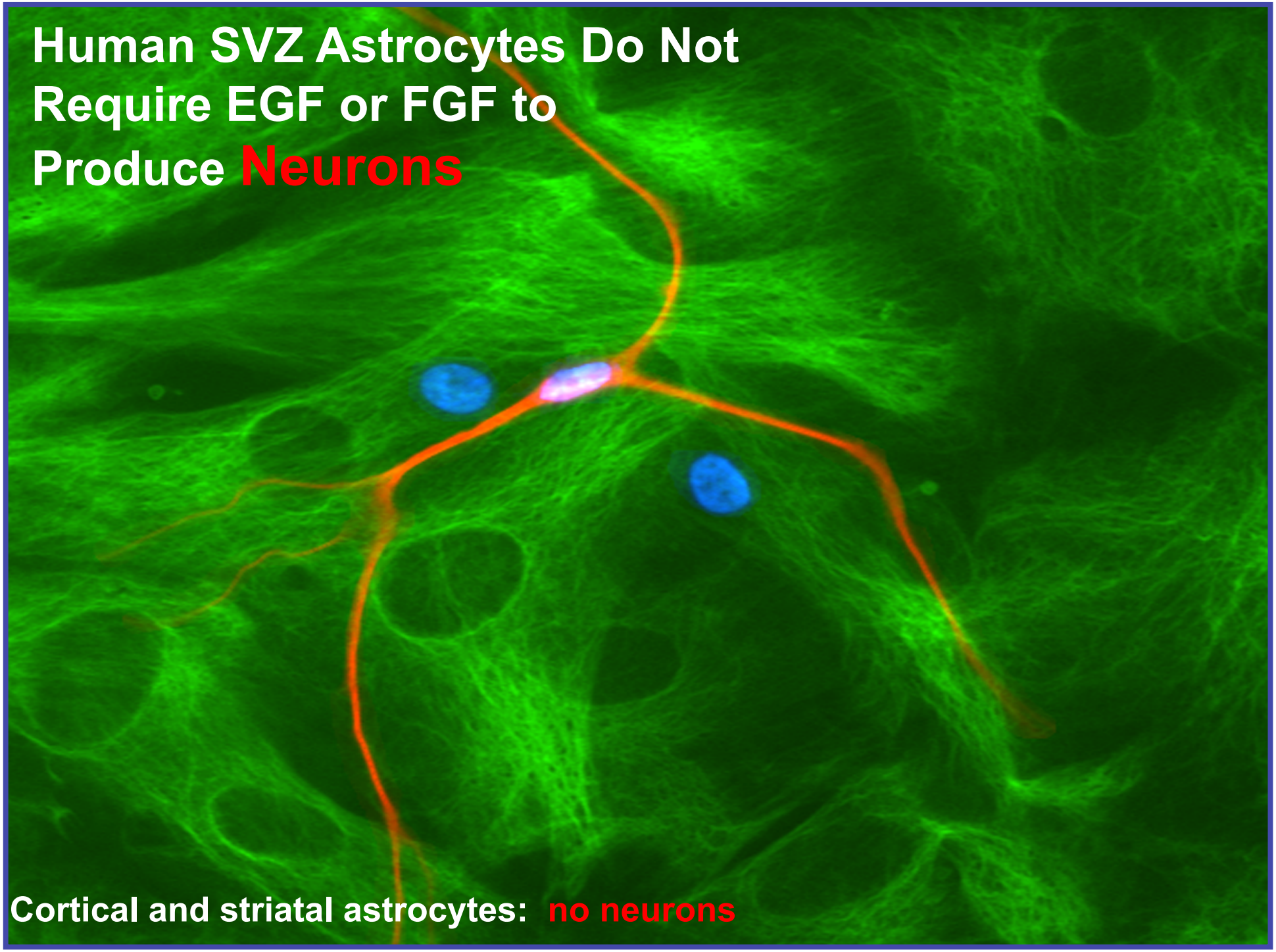
Sanai et el, Nature, 2004

The Adult Human SVZ Contains Neural Stem Cells



Sanai et el, Nature, 2004




Human SVZ Astrocytes Do Not
Require EGF or FGF to
Produce Neurons
Cortical and striatal astrocytes: no neurons

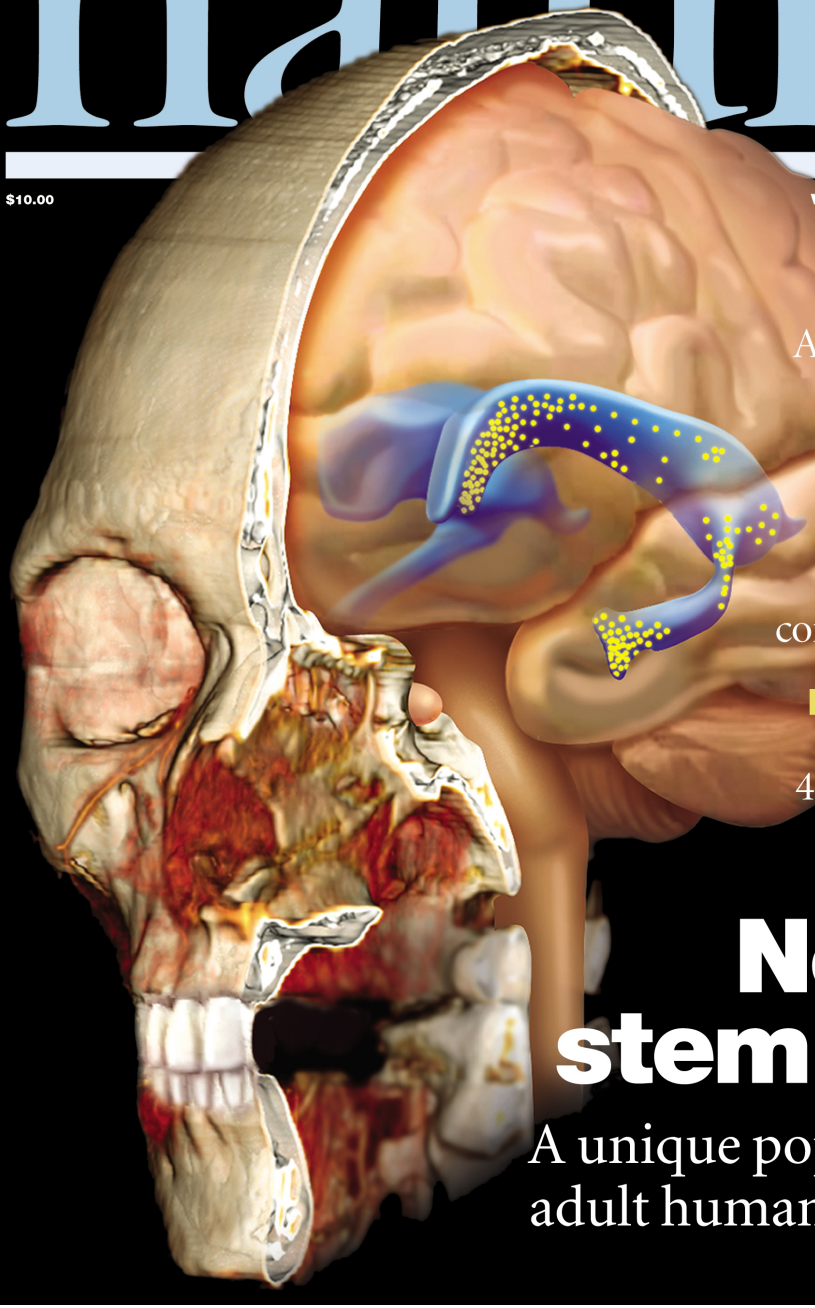
19 February 2004

International weekly journal of science

nature

$10.00

www.nature.com/nature



The Assisi earthquake
Aftershocks were fluid driven

My name is LUCA
Unveiling the last universal common ancestor

Homokaryosis
How to survive 400 million years without sex

Neural stem cells

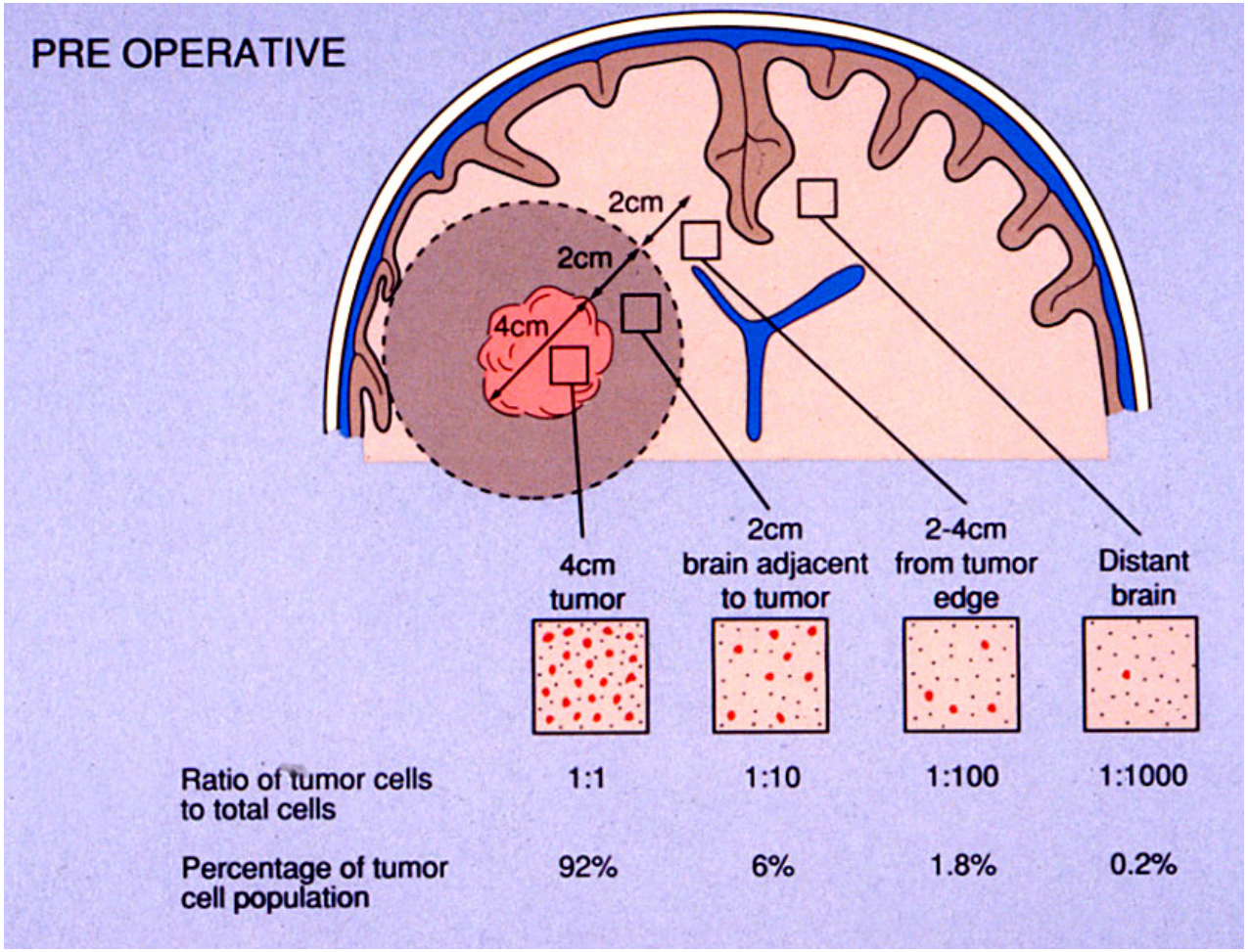
A unique population of adult human astrocytes

Outline

- **Controversy of Adult Neurogenesis**
- **Human Vs Rodent Stem Cell Niche**
- **Stem Cells in the Human Brain**
- **Stem Cells in Human Cancer**

Intraaxial tumors:

- LGG: JPA, Astrocytoma, oligodendroglioma
- HGG: Grade III Astrocytoma, Anaplastic Oligodendroglioma, GBM



At diagnosis 10^8 – 10^{10} cells

Malignant Glioma Epidemiology

- Approximately 20,500 people in the US are diagnosed with cancer of the brain and nervous system annually
  - About 12,740 patients die annually as a result of these malignant tumors

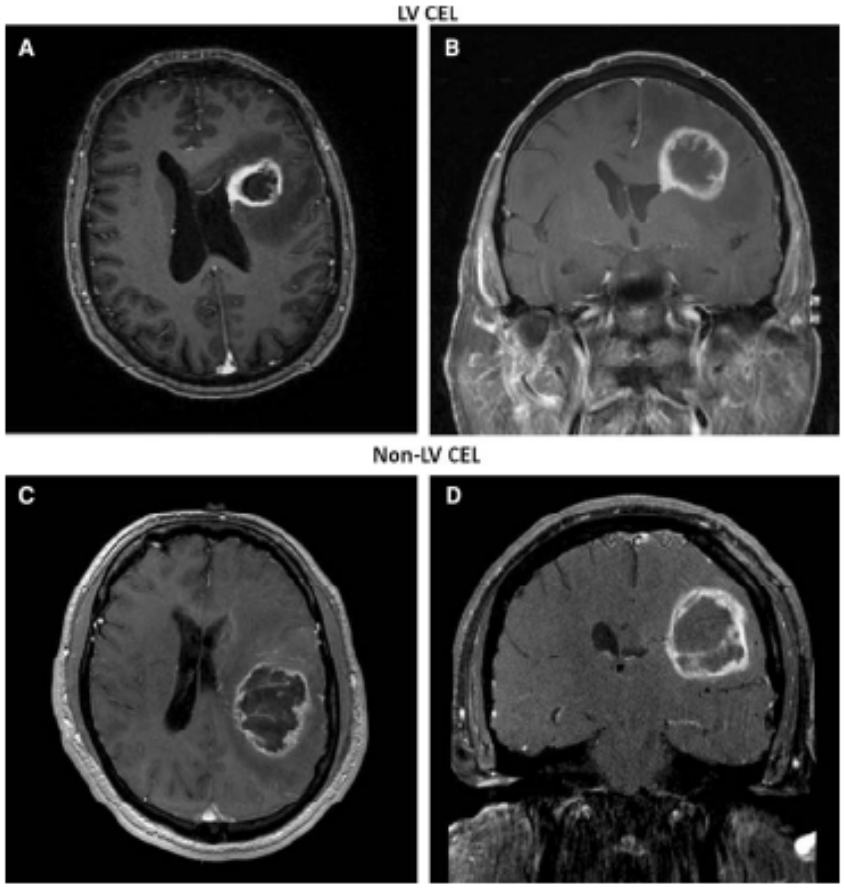
American Cancer Society. Cancer Facts and Figures 2007.

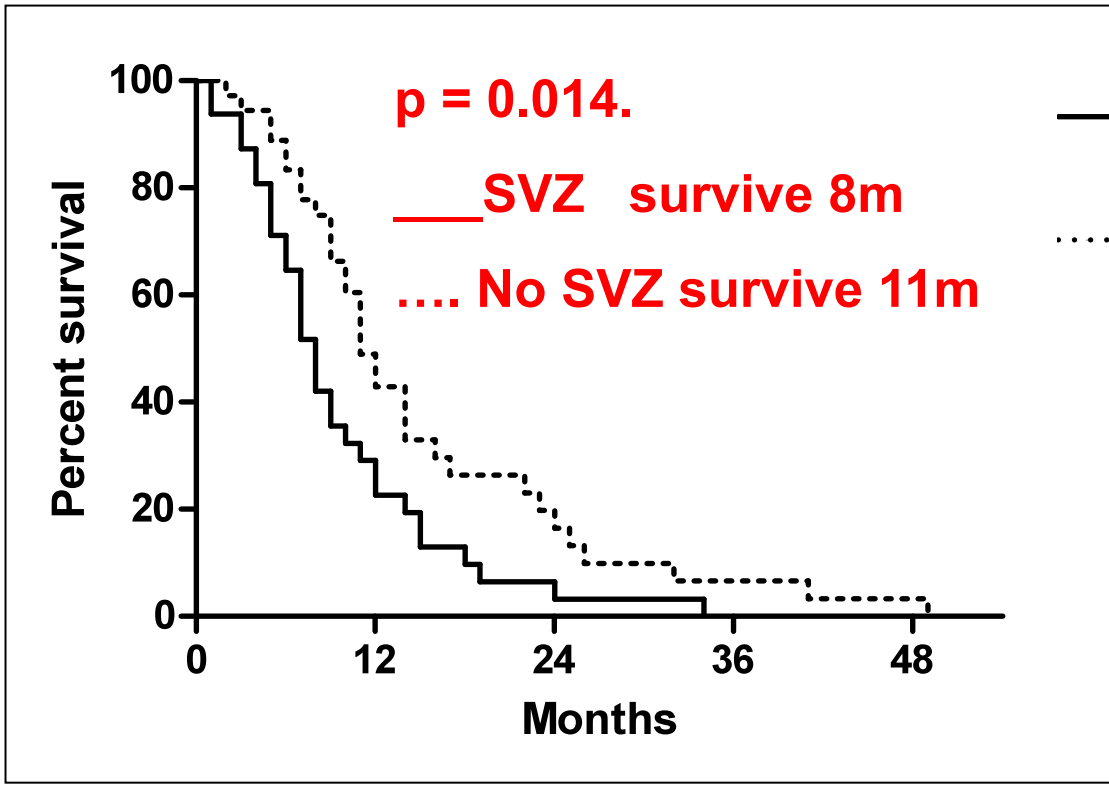
J Neurooncol (2008) 89:219–224
DOI 10.1007/s11060-008-9609-2

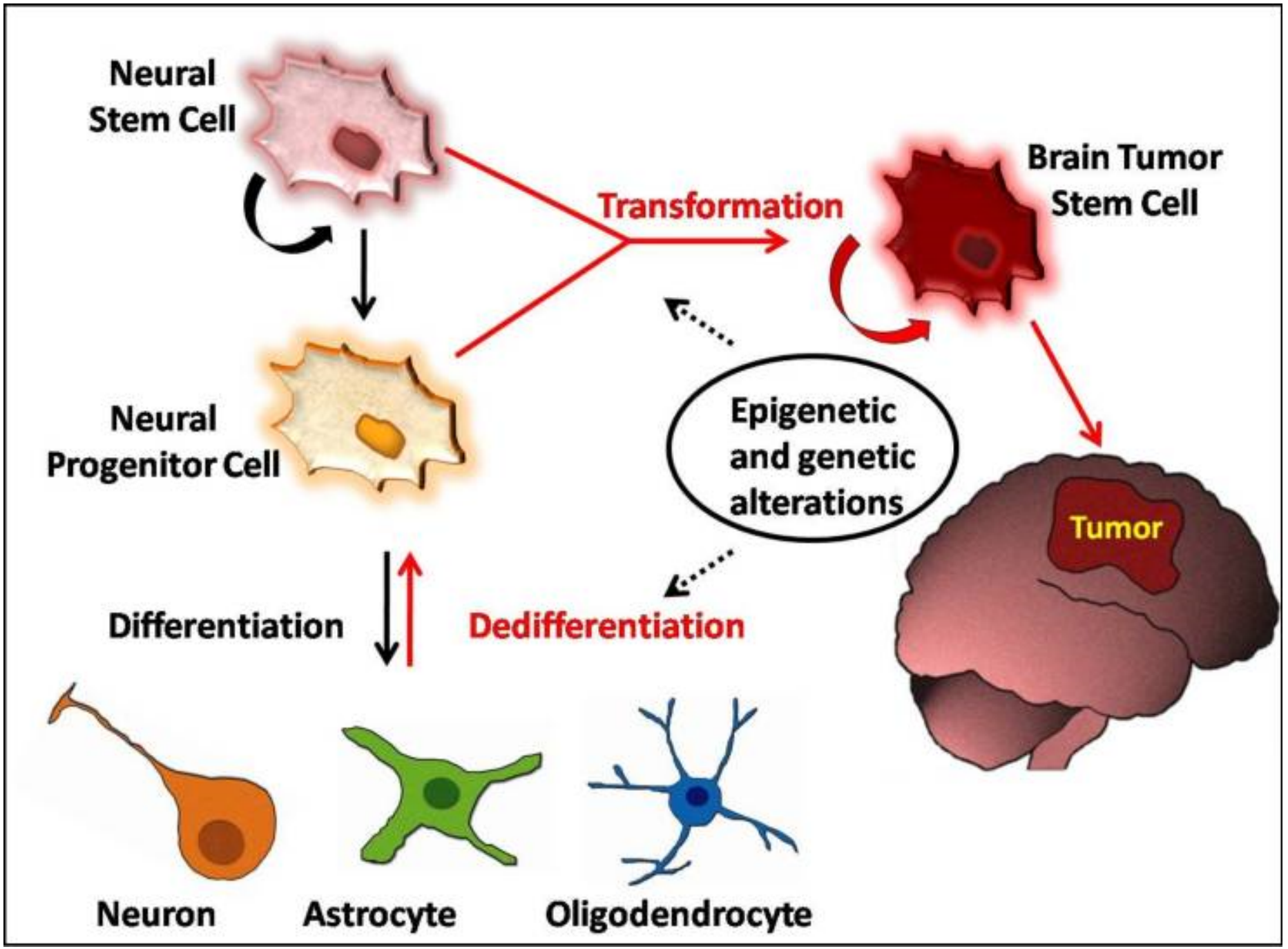
CLINICAL-PATIENT STUDIES

Relationship of glioblastoma multiforme to the lateral ventricles predicts survival following tumor resection

Kaisorn L. Chaichana · Matthew J. McGirt · James Frazier · Frank Attenello · Hugo Guerrero-Cazares · Alfredo Quinones-Hinojosa

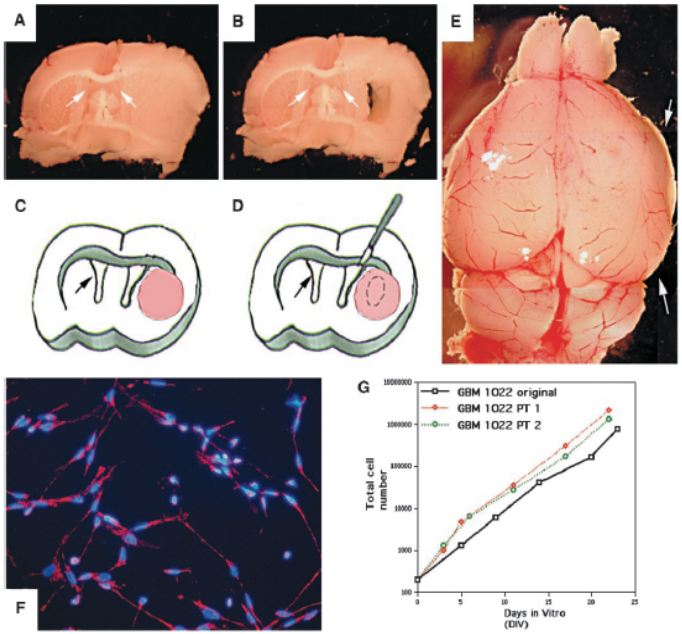






Achanta P, Sedora Roman NI, Quiñones-Hinojosa Anticancer Agents Med Chem. 2010

Brain Tumor Stem Cells



Galli et al.

Cancer Res, 2004

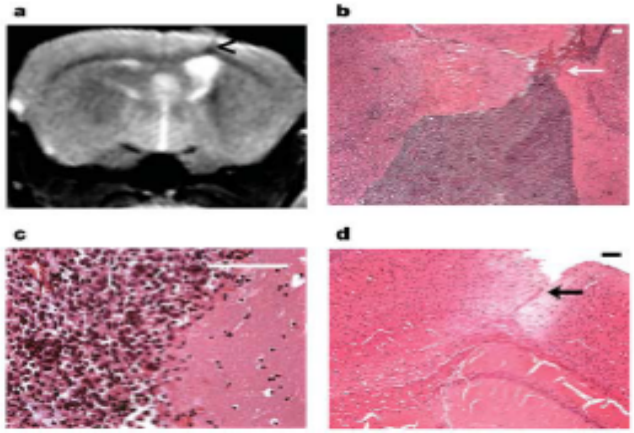
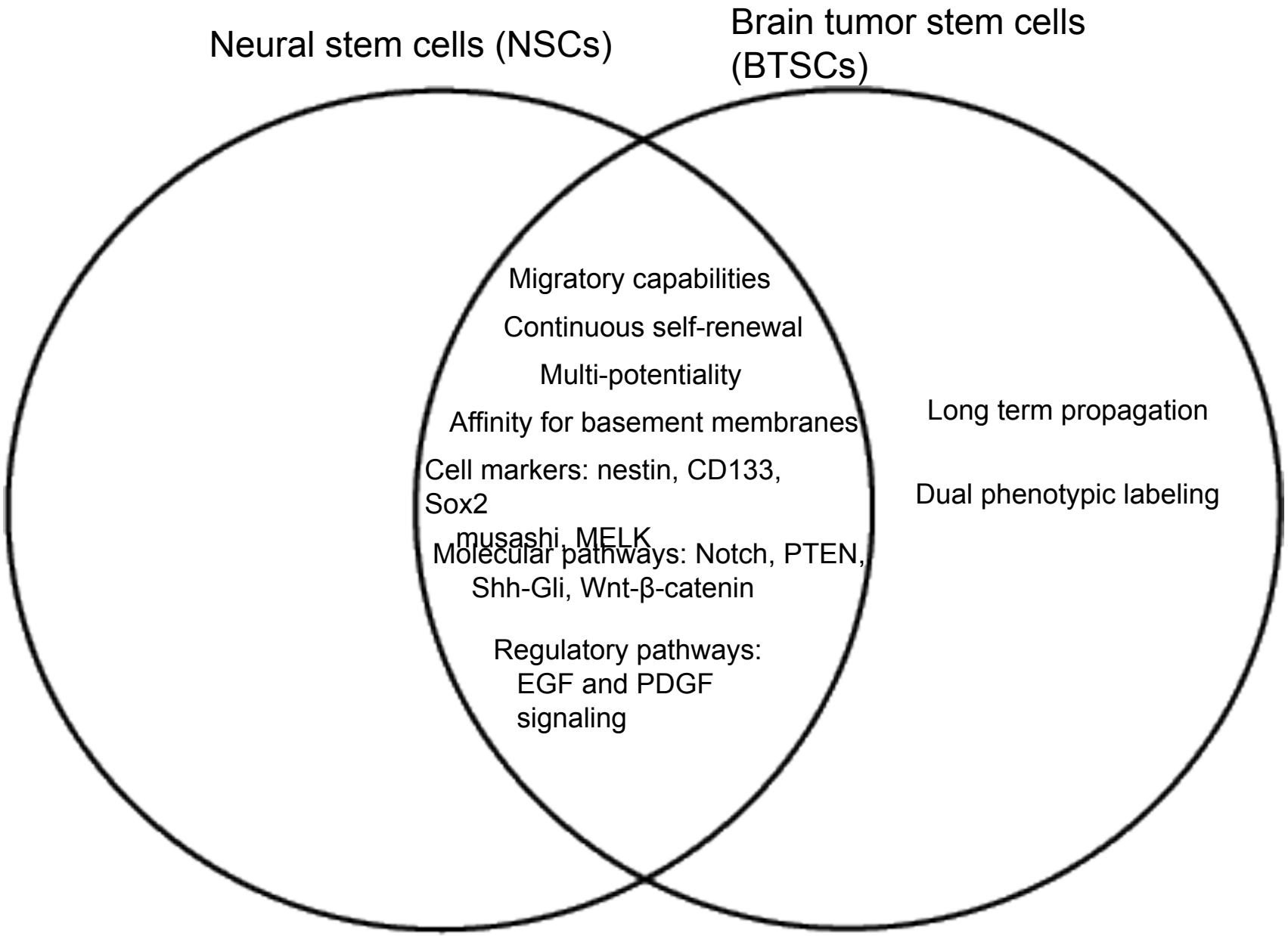


Figure 1 $CD133^+$ tumour cells initiate tumours upon intracranial transplantation into the adult NOD-SCID mouse forebrain. a, Magnetic resonance imaging (MRI) scan of a mouse injected with 1,000 $CD133^+$ medulloblastoma cells shows an enhancing mass under the injection tract (arrowheads) 14 weeks post-injection. b, c, Low (b) and high (c) magnification histological sections of the xenograft show a highly cellular mass below the injection site (white arrow in b). d, Histological section of mouse brain injected with $CD133^-$ medulloblastoma cells shows the injection tract (black arrow), but no tumour formation. Scale bar on all panels represents 100 microns.

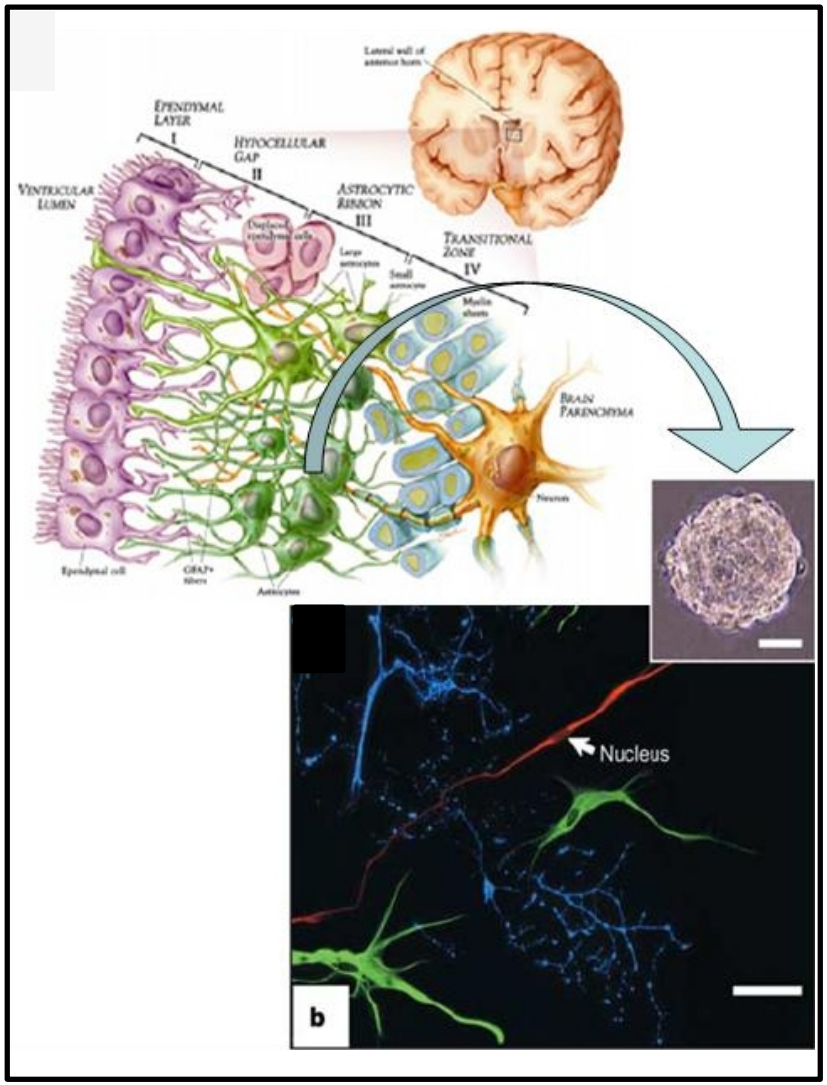
Singh et al.

Nature, 2004



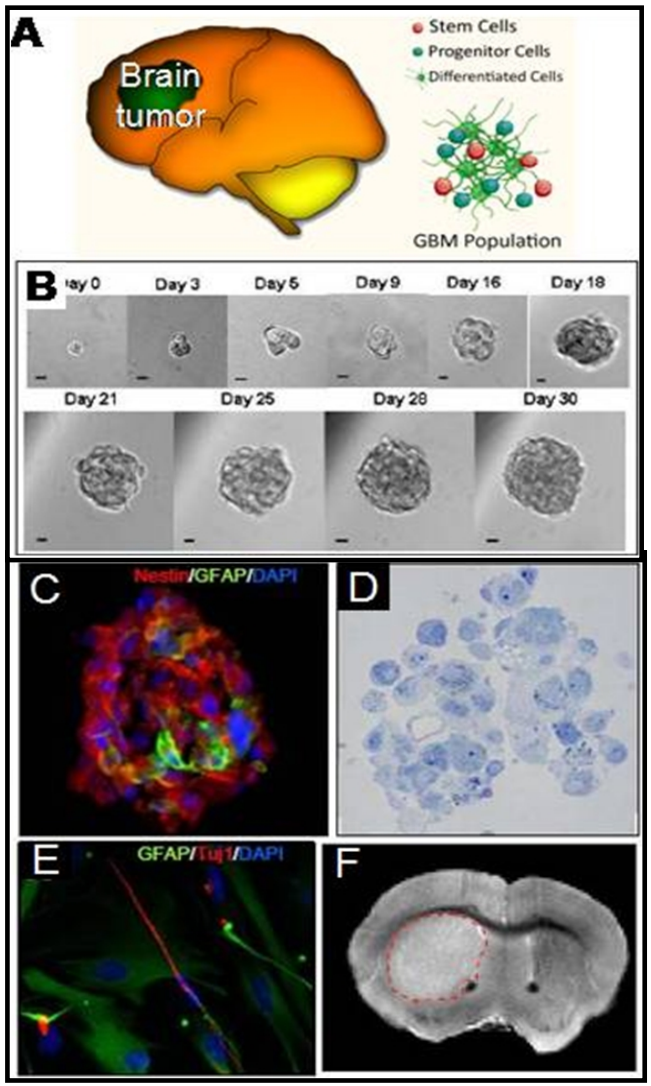
Quinones and Chaichana, Exp Neurol 2007

Brain Tumor Stem Cells: Characteristics



Quinones-Hinojosa A, et al., 2006
Quinones and Chaichana, Exp Neurol 2007
Sanai N, *Nature* 427: 740-744, 2004.

Brain Tumor Stem Cells: Characteristics

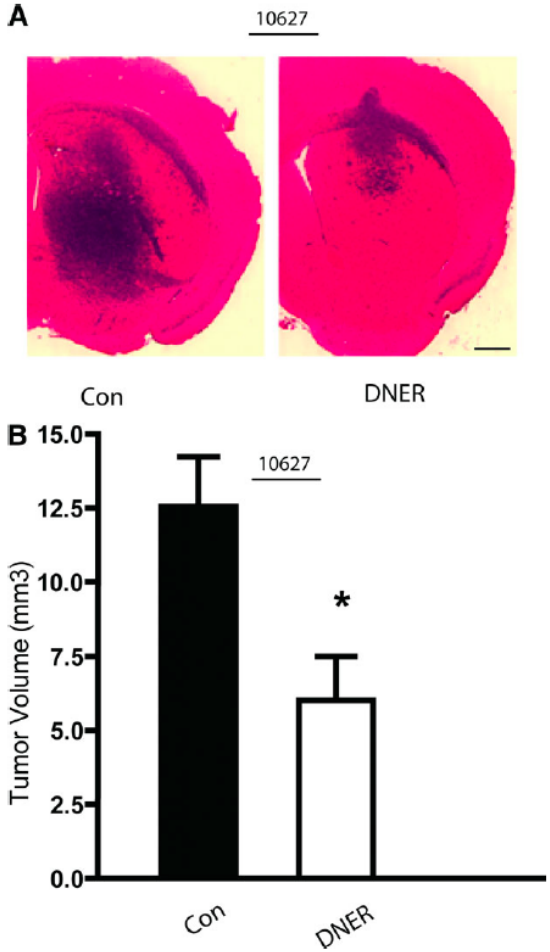


Quinones-Hinojosa A, et al., 2006
Quinones and Chaichana, Exp Neurol 2007
Sanai N, *Nature* 427: 740-744, 2004.

CANCER STEM CELLS

DNER, an Epigenetically Modulated Gene, Regulates Glioblastoma-Derived Neurosphere Cell Differentiation and Tumor Propagation

PENG SUN,[a] SHULI XIA,[a] BACHCHU LAL,[a] CHARLES G. EBERHART,[b] ALFREDO QUINONES-HINOJOSA,[c] JAREK MACIACZYK,[e] WILLIAM MATSUI,[d] FRANCESCO DIMECO,[f] SARA M. PICCIRILLO,[g] ANGELO L. VESCOVI,[g] JOHN LATERRA[a]



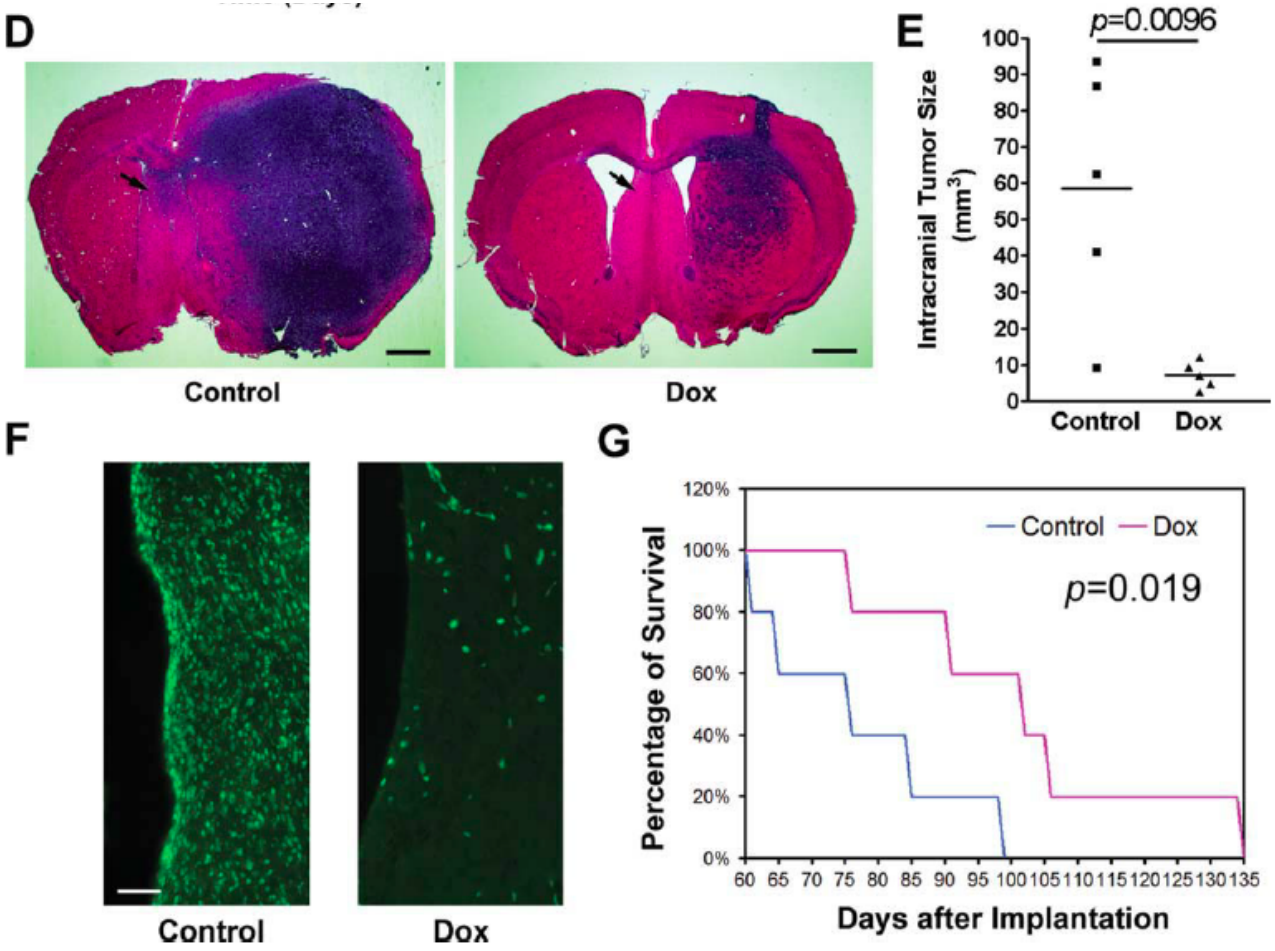
DNER, Delta/Notch-like epidermal growth factor-related receptor

derived from GBM neurospheres.

STEM CELLS *EXPRESS* April 9, 2009.

Krüppel-Like Family of Transcription Factor 9, a Differentiation-Associated Transcription Factor, Suppresses Notch1 Signaling and Inhibits Glioblastoma-Initiating Stem Cells

MINGYAO YING,[a,b] YINGYING SANG,[a] YUNQING LI,[a,b] HUGO GUERRERO-CAZARES,[c] ALFREDO QUINONES-HINOJOSA,[c] ANGELO L. VESCOVI,[d] CHARLES G. EBERHART,[e] SHULI XIA,[a,b] JOHN LATERRA[a,b,f,g]



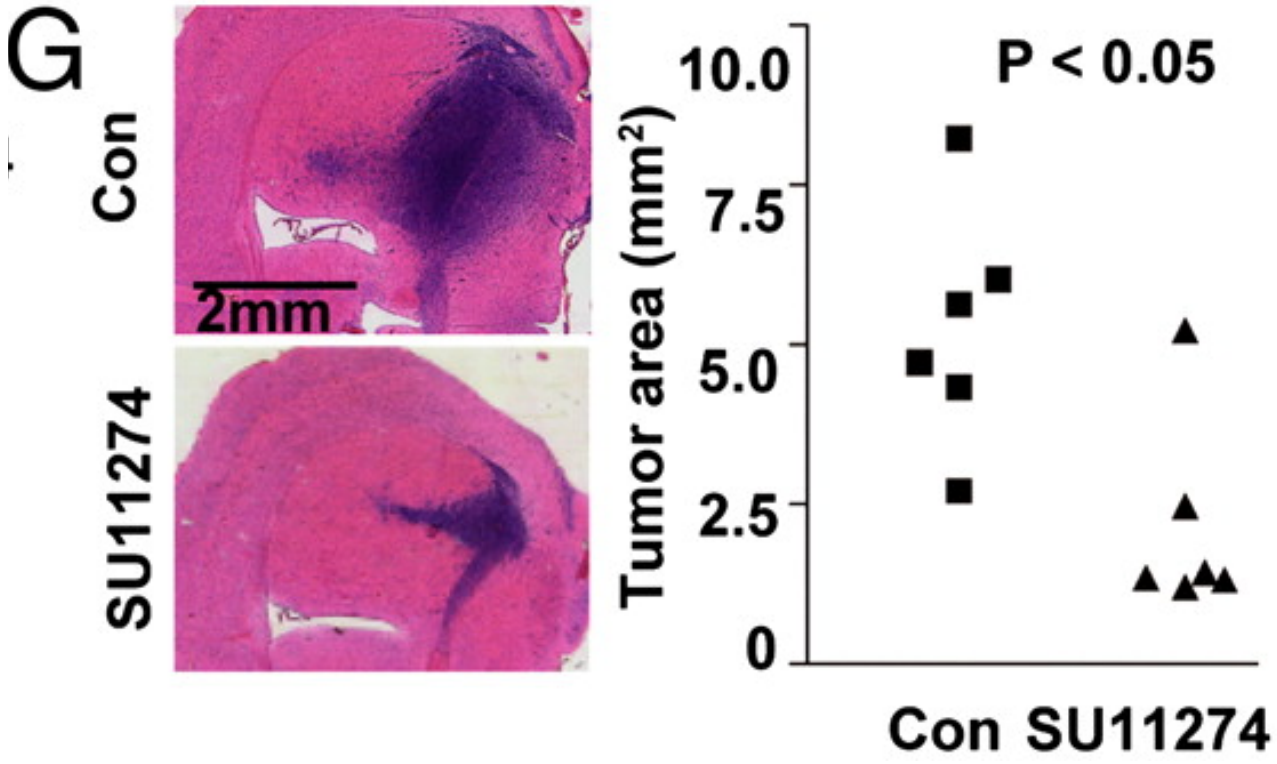
STEM CELLS 2011;29:20–31

c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype

Yunqing Li[a,b,1], Angela Li[a], Martin Glas[c,d], Bachchu Lal[a,b], Mingyao Ying[a,b], Yingying Sang[a], Shuli Xia[a,b], Daniel Trageser[c], Hugo Guerrero-Cázares[e], Charles G. Eberhart[f], Alfredo Quiñones-Hinojosa[e,g], Bjorn Scheffler[c], and John Laterra[a,b,g,h,1]

[a]Hugo W. Moser Research Institute at Kennedy Krieger, Baltimore, MD 21205; Departments of [b]Neurology, [e]Neurosurgery, [f]Pathology, [g]Oncology, and [h]Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21287; and [c]Institute of Reconstructive Neurobiology and [d]Division of Clinical Neurooncology, Department of Neurology, University of Bonn Medical Center, D-53105 Bonn, Germany

Edited by George F. Vande Woude, Van Andel Research Institute, Grand Rapids, MI, and approved May 12, 2011 (received for review November 10, 2010)



Conclusions

- **There is Adult Neurogenesis in the mammalian brain**
- **The stem cell niche, specifically the SVZ, is different between the Human and the Rodent**
- **Stem Cells in the Human Brain do exist**
- **Stem Cells in Human Cancer**

- Whether neral stem cells give riste to tumors is not known
- What is known is of a population of cells within tumors that behave like stem cells
- The future is bright and we will continue with the quest to find the etiology of brain tumors

Acknowledgements:

HHMI 1997 & 1998

NIH (1F32NS047011) 2003-2004

UCSF Neurosurgery and Stem Cell Program
MS Berger

Alvarez-Buylla

Spain
Prof Verdugo
Mexico
O Gonzalez-Perez

Hopkins
Neurosurgery
Neurooncology
ICE
Sidney Kimmel Cancer Center

Current Support

NIH

Johns Hopkins Clinician Scientist Award
Howard Hughes Medical Institute
American Assoc Neurological Surgeons
American College of Surgeons
American Society of Clinical Oncology
Brain Tumor Founders Collaborative
Children's Cancer Foundation
Robert Wood Johnson

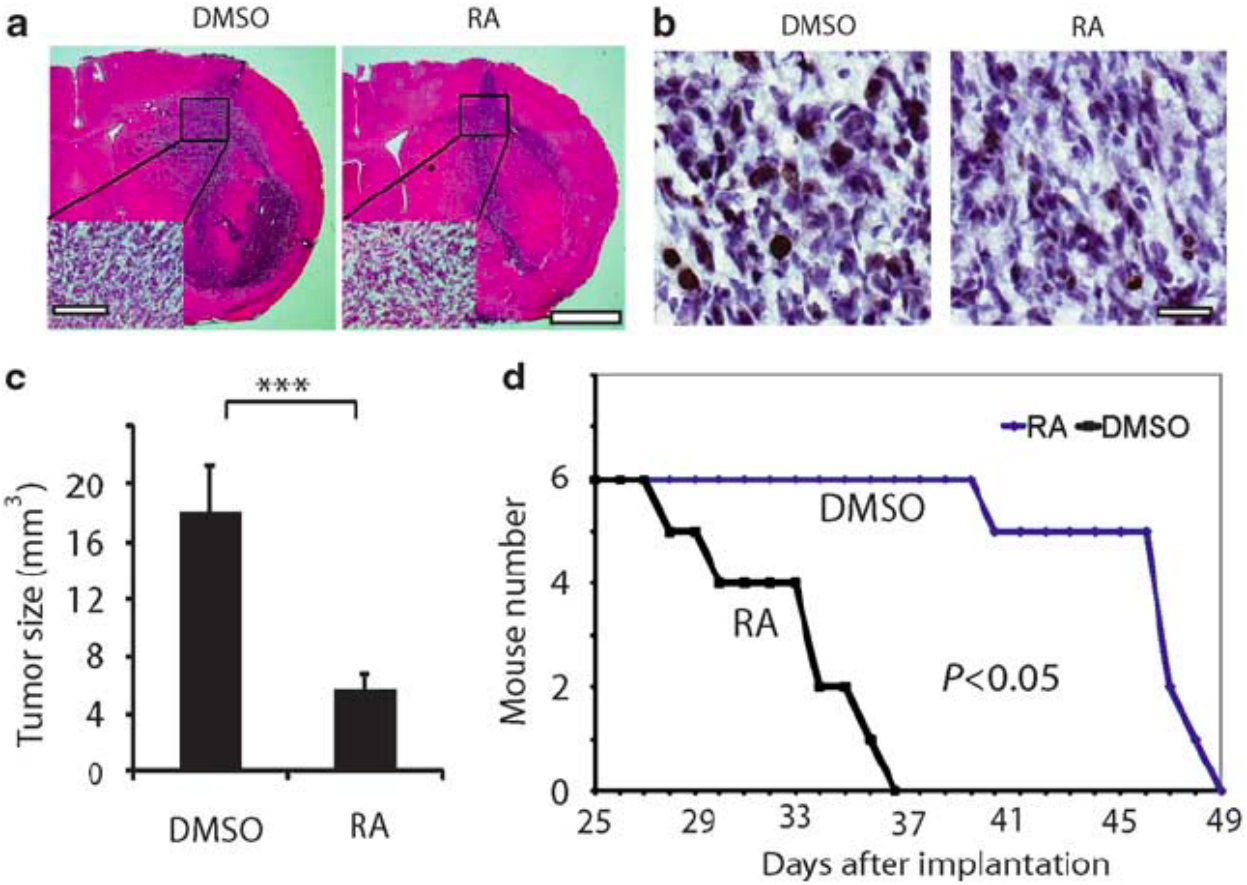
Oncogene (2011) 1–14

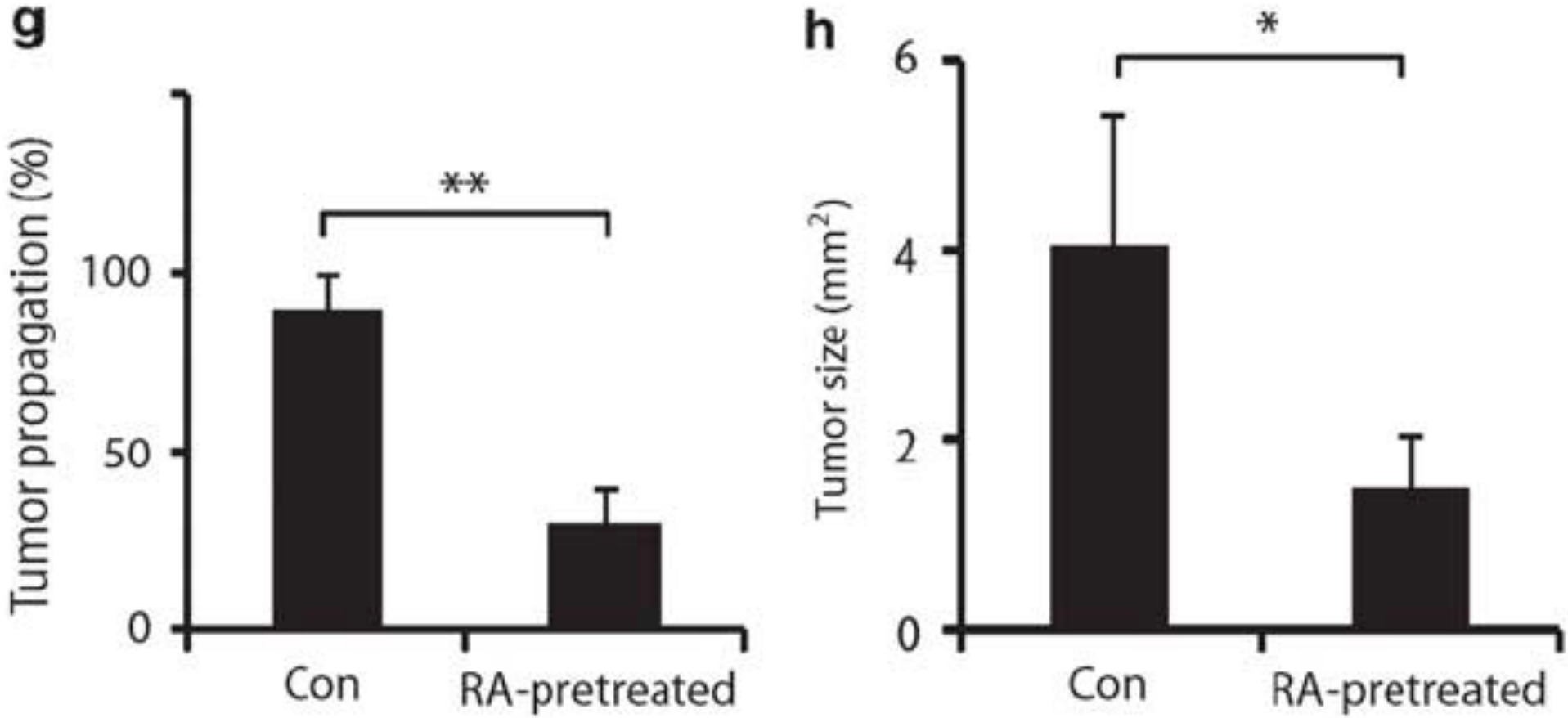
www.nature.com/onc

ORIGINAL ARTICLE

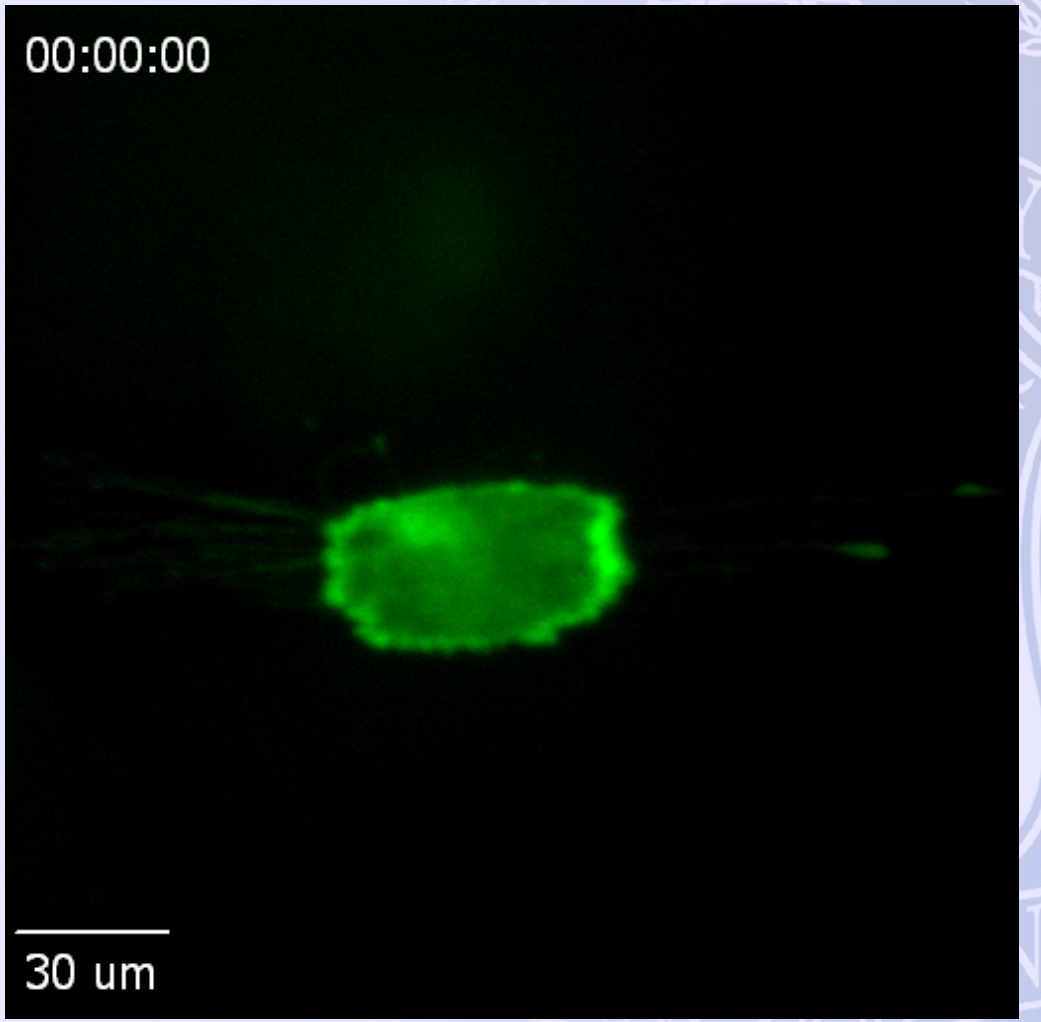
Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition

M Ying[1,2,6], S Wang[1,6], Y Sang[1], P Sun[1,2], B Lal[1,2], CR Goodwin[1], H Guerrero-Cazares[3,4], A Quinones-Hinojosa[3,4], J Laterra[1,2,3,5] and S Xia[1,2]

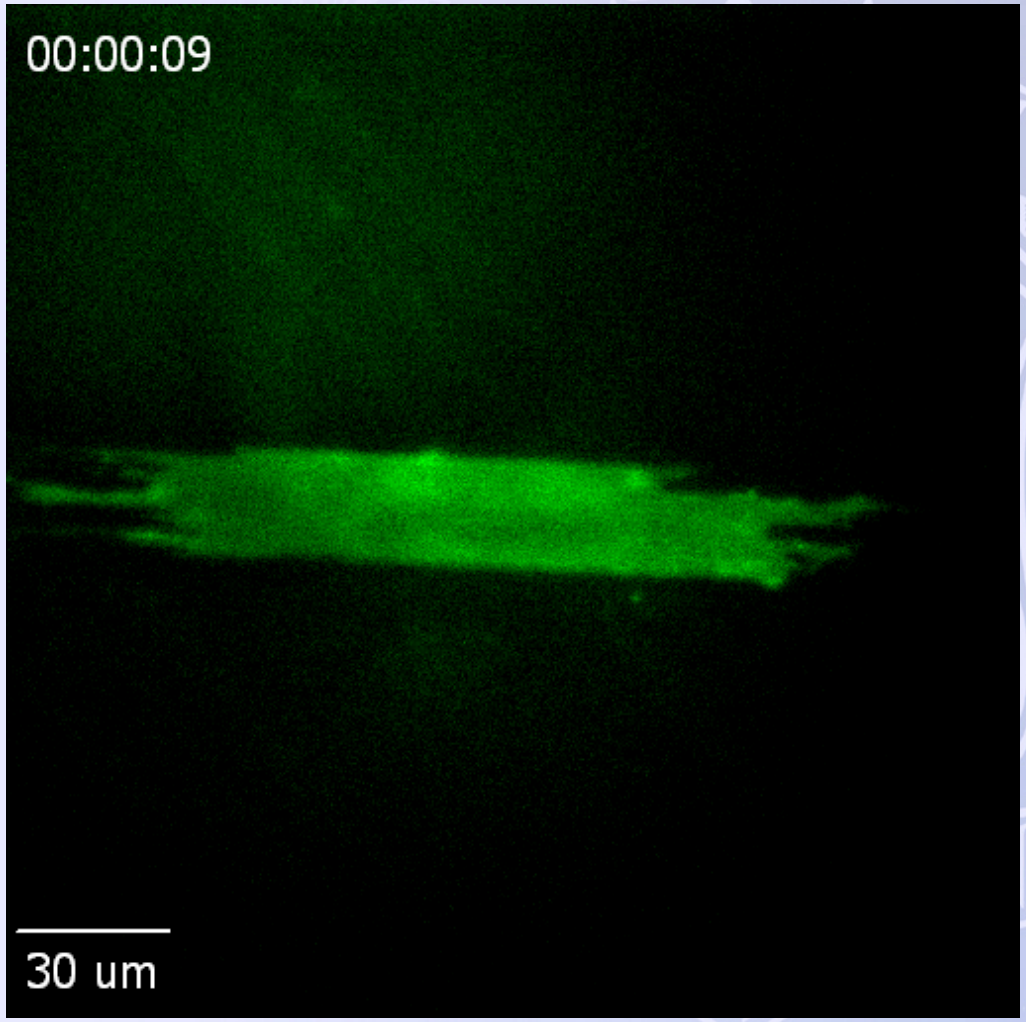



g
Tumor propagation (%)
100
50
0
**
Con
RA-pretreated
h
Tumor size (mm2)
6
4
2
0
*
Con
RA-pretreated

GFP-labeled GBM cell migration on a nanopatterned a surface



GFP-labeled GBM cell migration on a nanopatterned a surface

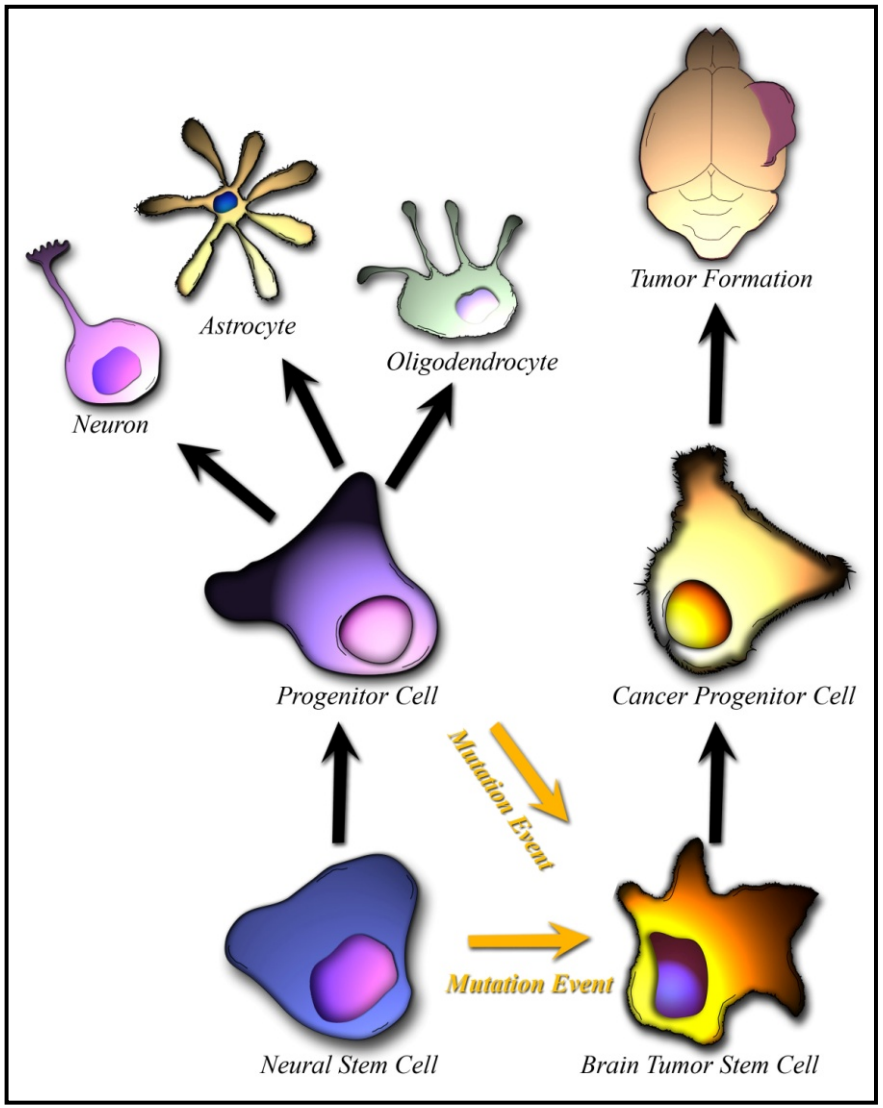


Malignant Gliomas Arise From Brain Tumor Stem Cells

- Share characteristics with normal Neural Stem Cells (NSCs):
  - Self-renewal
  - Proliferation
  - Differentiation

- Brain Tumor Stem Cells (BTSCs):
  - Initiate tumor formation, maintain tumor growth
  - Migrate long distances in brain parenchyma resulting in local tumor recurrence
  - Are highly radio/ chemoresistant

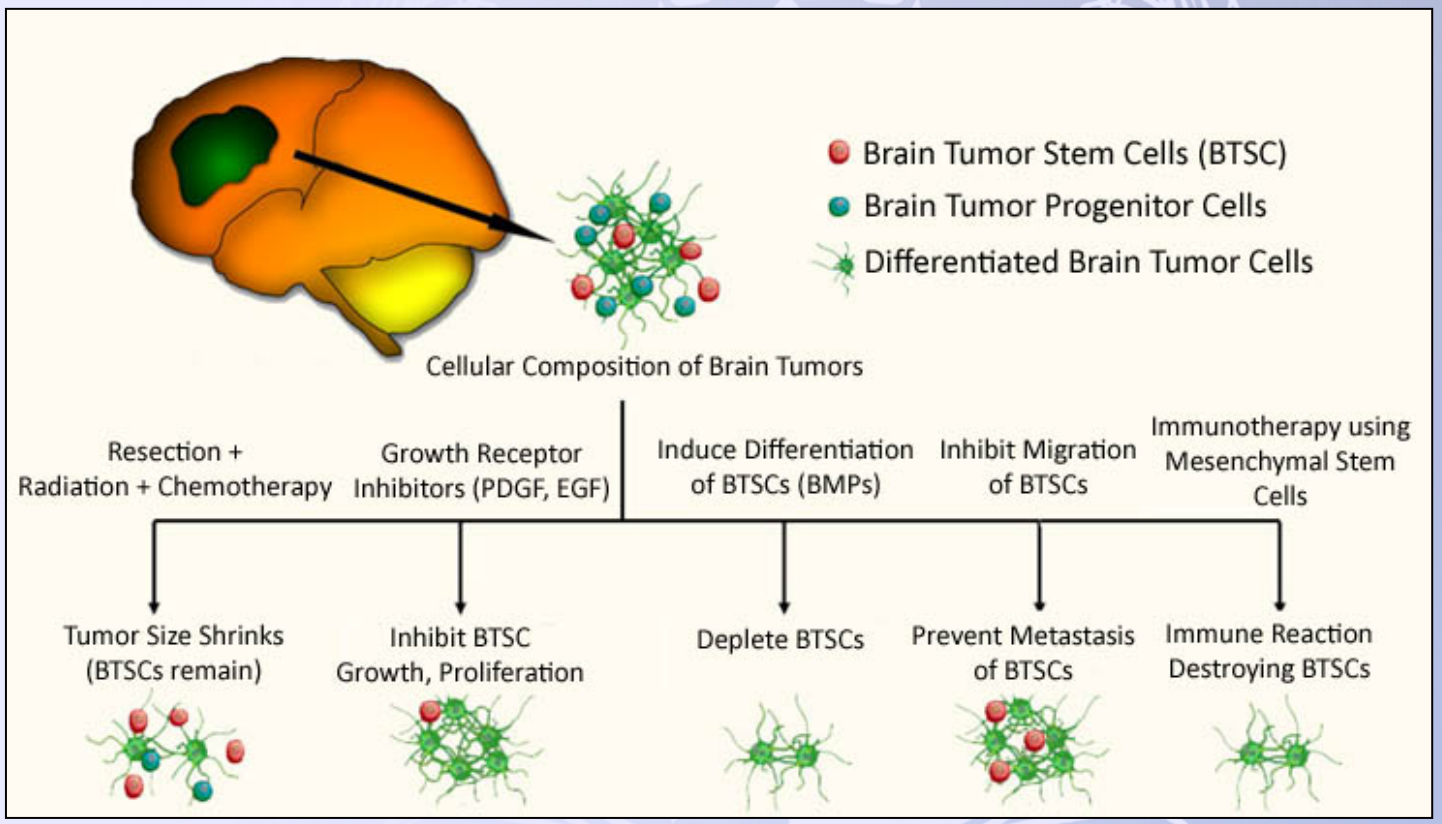
Malignant Gliomas Arise From Brain Tumor Stem Cells



Zaidi HA, Kosztowski TA, DeMico F, Quinones-Hinojosa A *Journal of Neuro-Oncology*, 2009

Current Therapies Fail To Destroy Brain Tumor Stem Cells

- Current therapies for brain tumors fail to target BTSC population
- Progression and local recurrence of tumor due to presence of BTSC
- Novel therapies which specifically target BTSCs will have best chance at preventing tumor recurrence

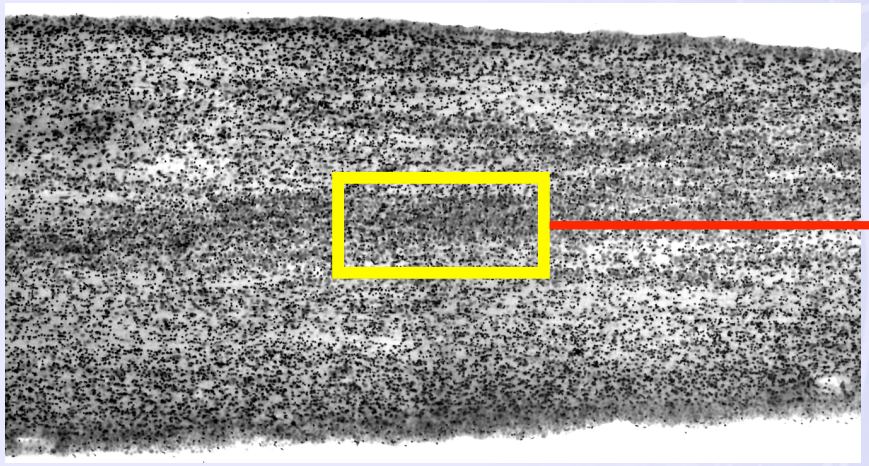


Adapted from:

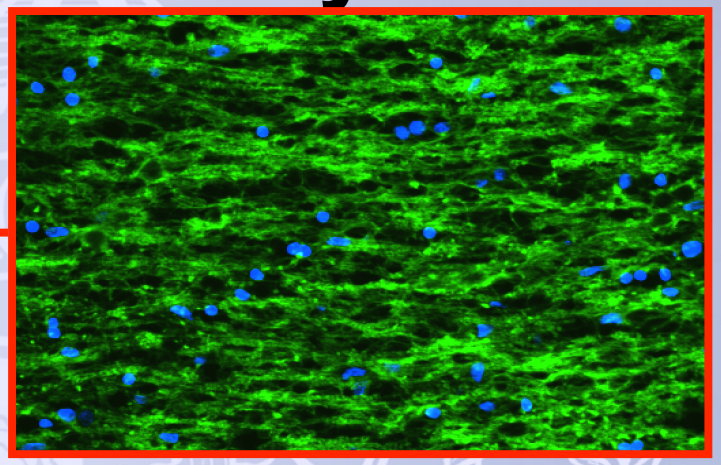
Zaidi HA, DeMico F, Quinones-Hinojosa A. "Brain Tumor Stem Cells," *Youman's Textbook of Neurological Surgery*, 2009

Zaidi HA, Kosztowski TA, Quinones-Hinojosa A. "Brain Tumor Stem Cells Evade Traditional Therapies and Necessitate the Development of Novel Treatment Modalities," *Neurosurgery*, 2009.

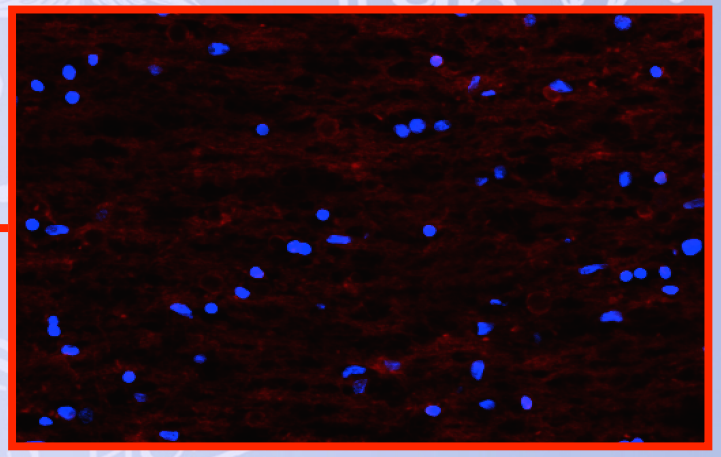
Progenitor Migration in the Human Brain?: The Human Olfactory Tract



GFAP



TuJ1



Sanai et al, Nature 2004

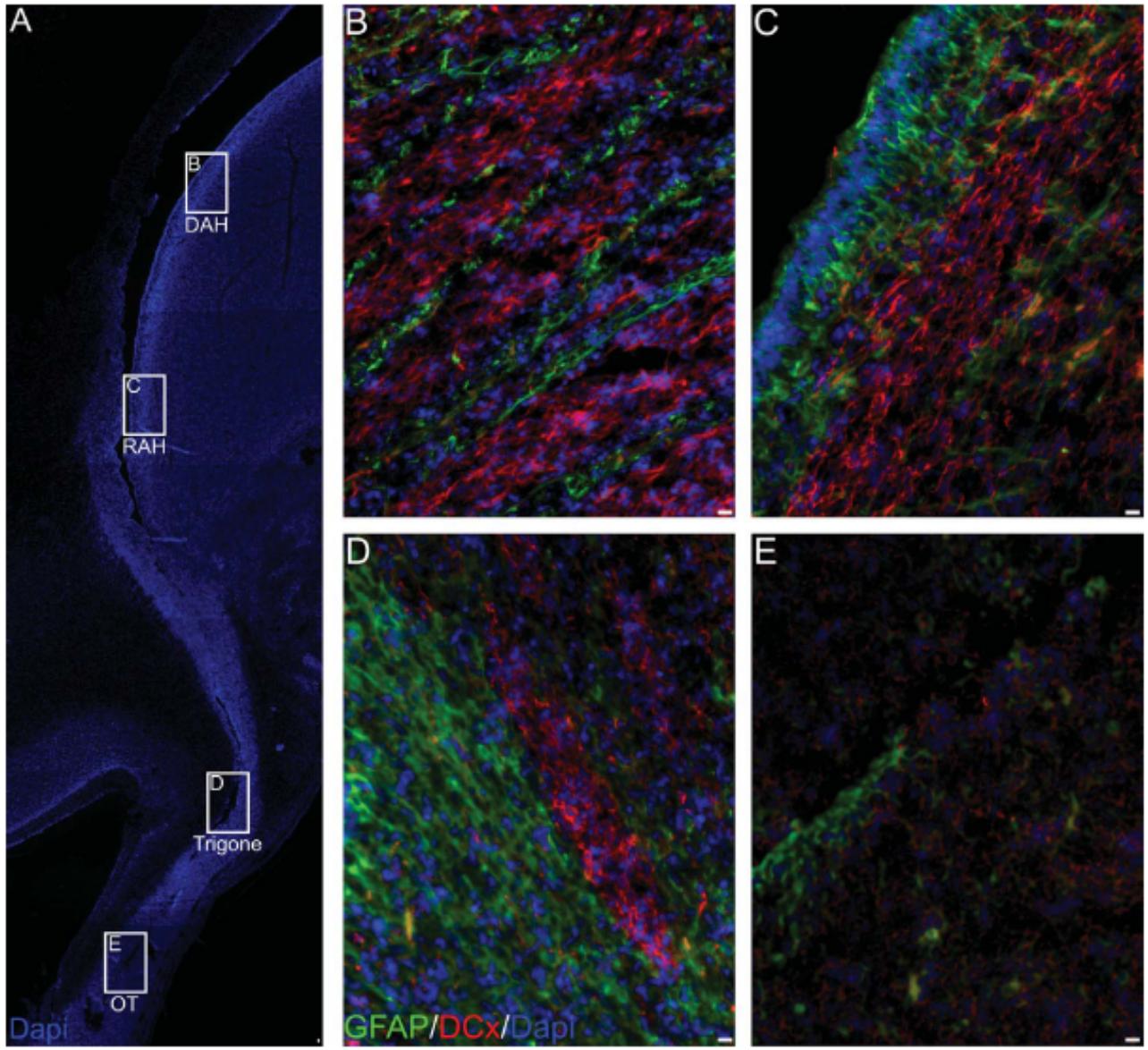
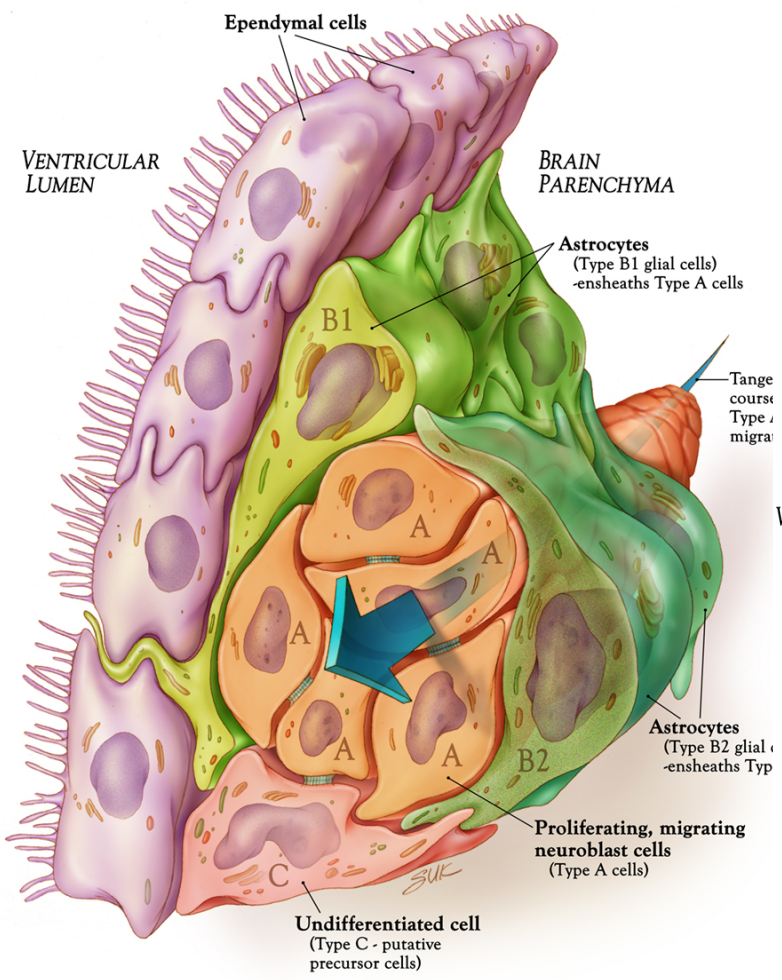
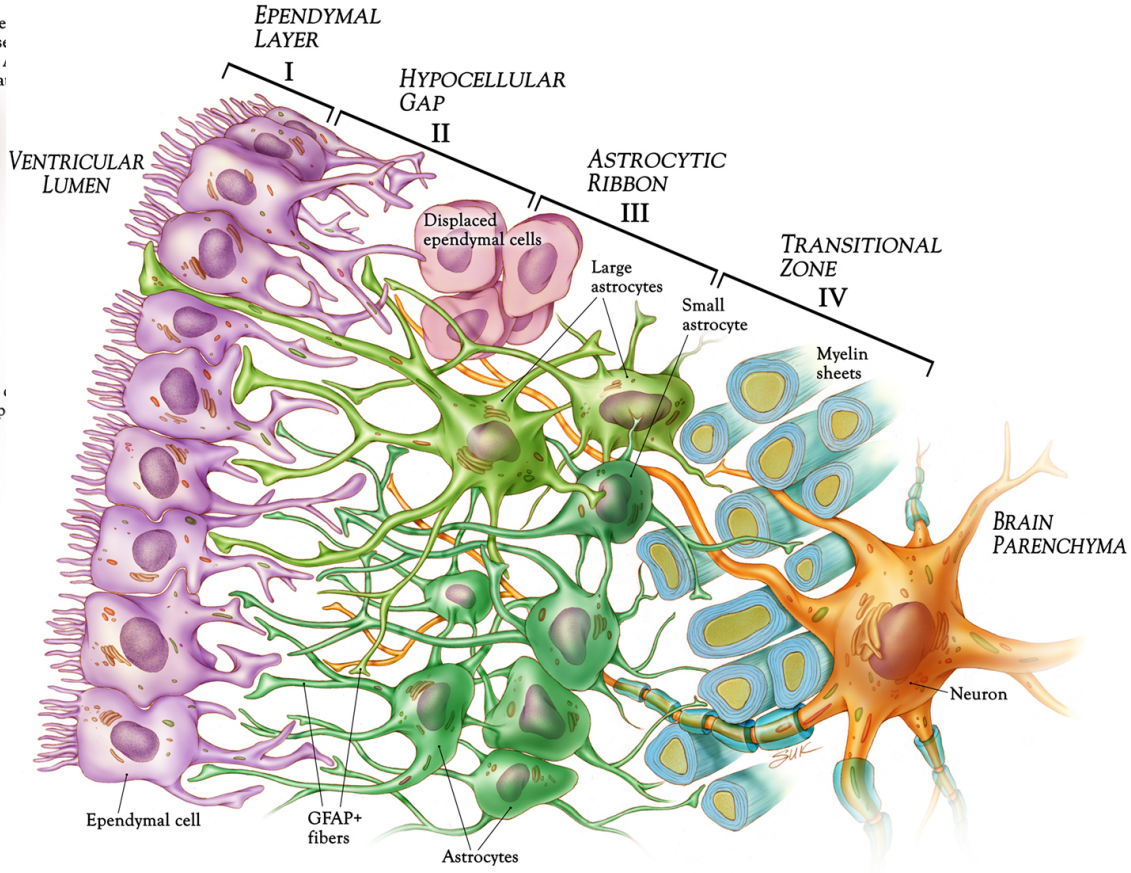


Figure 5. Doublecortin (DCx)-positive structures in the anterior SVZ. **A:** Reconstruction of the entire connection between the anterior horn and the olfactory tract. DCx+ cells were found at every region with different organization. At the DAH (**B**), RAH (**C**), and olfactory trigone (**D**) DCx+ cells were found aligned to GFAP-positive cells. At the OT, no GFAP+ cells were observed in alignment with the less abundant DCx+ cells. Scale bars = 10 μm.

Rodent Brain SVZ



Adult Human Brain SVZ



Sanai et al,2004
Quinones et al, JCN 2006
Quinones and Chaichana, Exp Neurol 2007

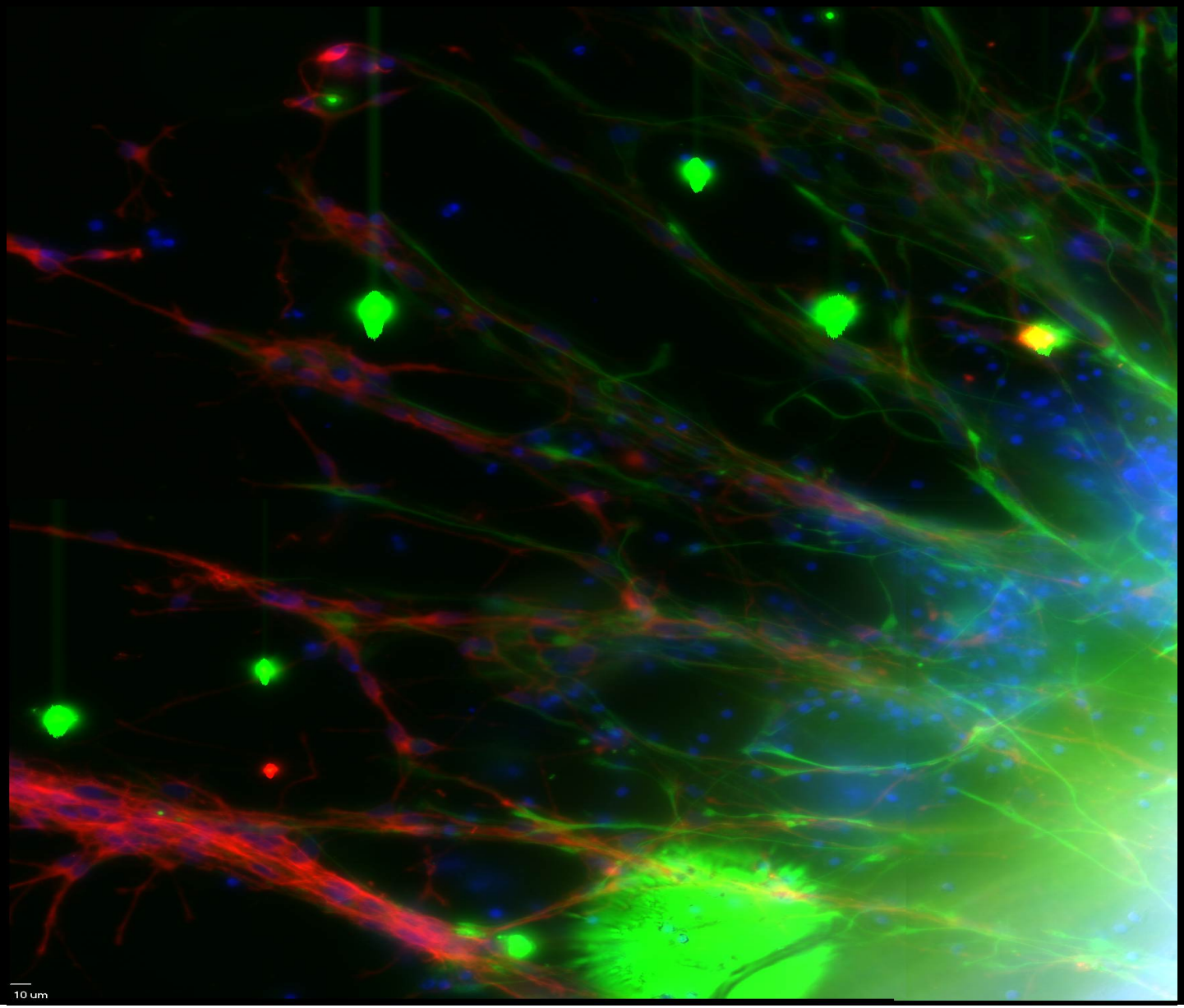
Cell-based Therapy for Malignant Gliomas

Sources of Neural Stem Cells for treatment

- Adult CNS
- Embryonic CNS
- Induced Pluripotent Stem Cells
  - Transduction of "stem cell factors":
    - » Sox2, Musashi-1, OCT4, Nanog
    - » Hair follicles

Garzon-Muvdi, T., Quinones-Hinojosa, A. ILAR J. 2009

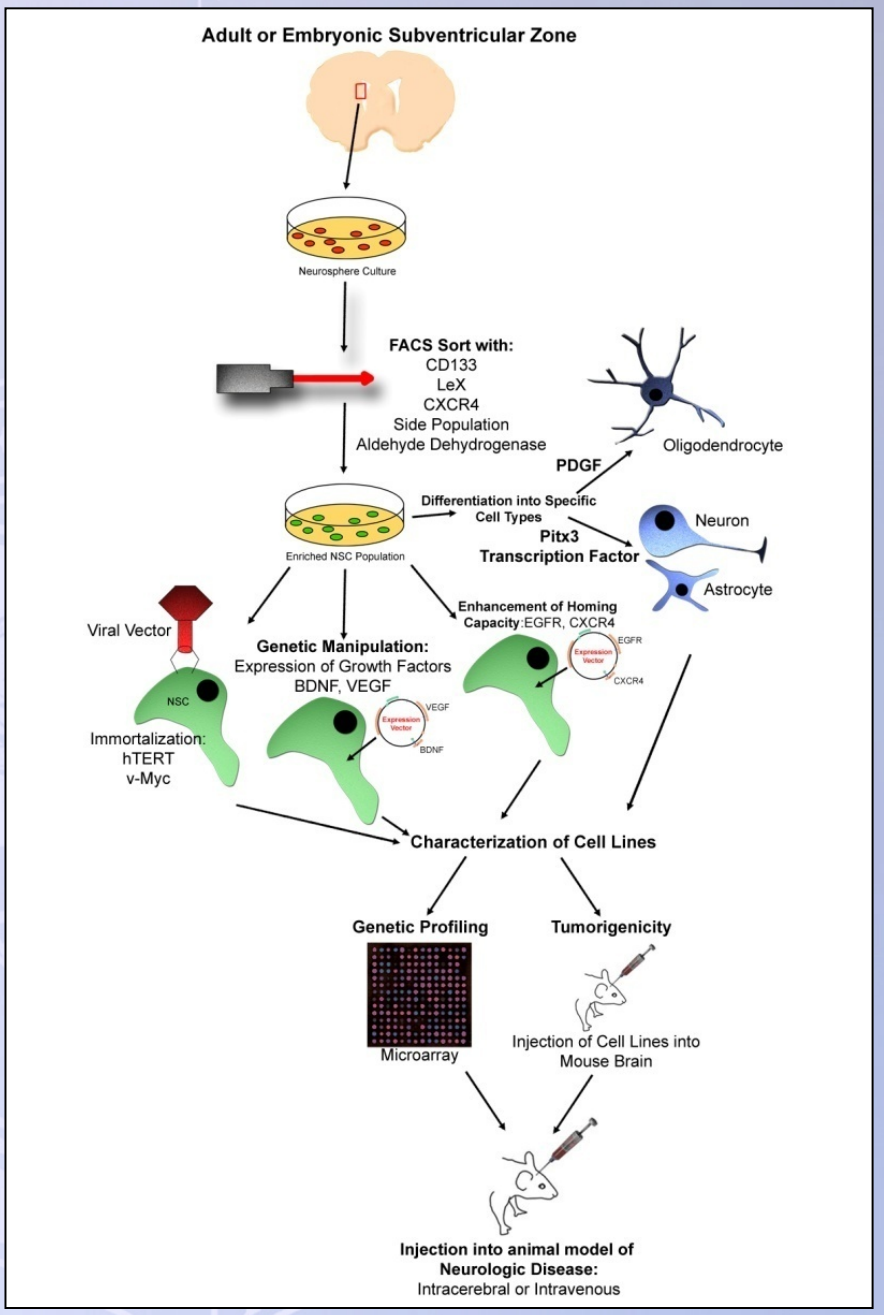
Ex Vivo Human Migration



Modification of NSCs for CNS disease treatment

- Culture
- Identification and sorting
- Genetic modifications
- Characterization

- Trials in animal models (preclinical)



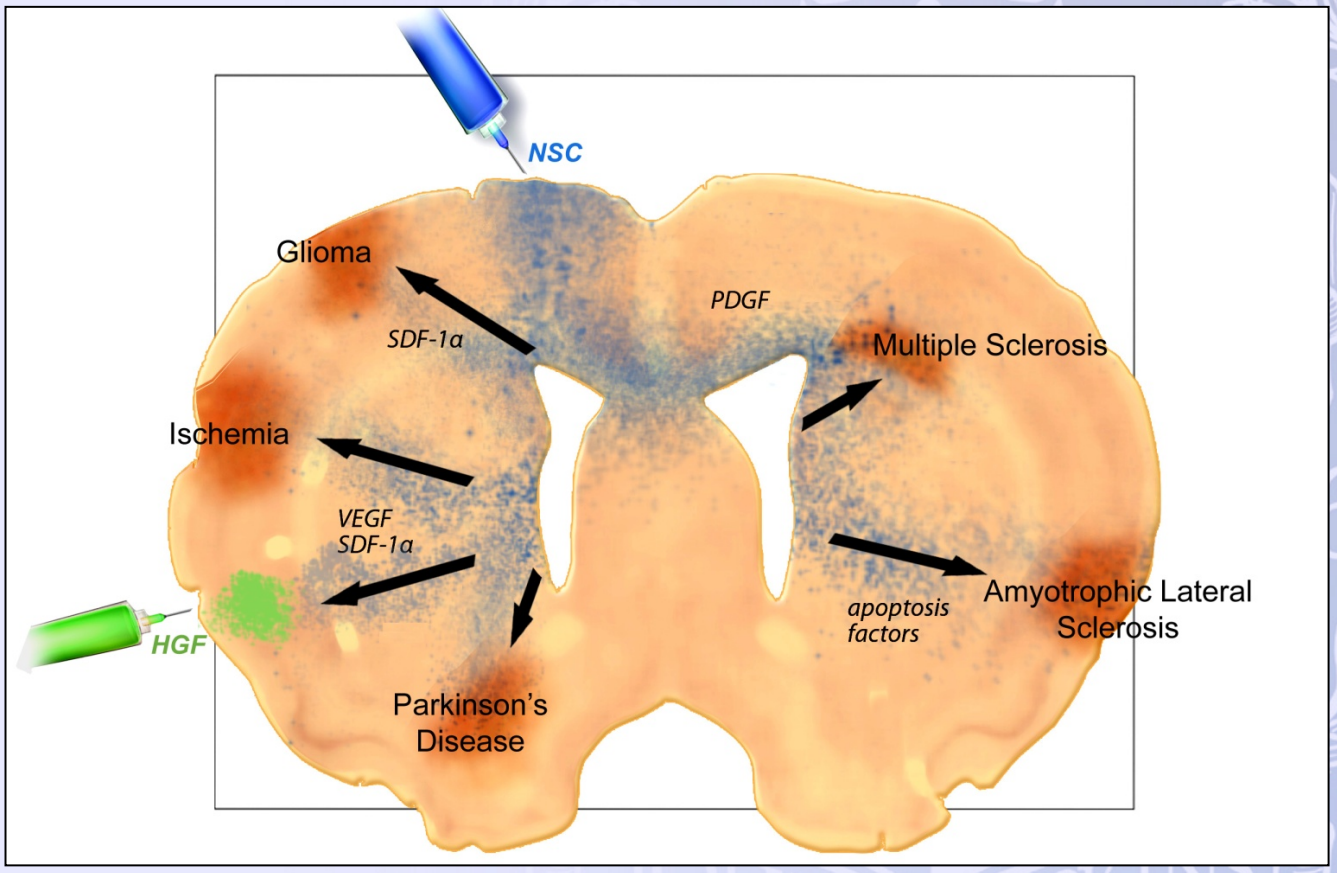
Garzon-Muvdi, T., Quinones-Hinojosa, A. ILAR J. 2009

Neural Stem Cells for Regenerative Therapy

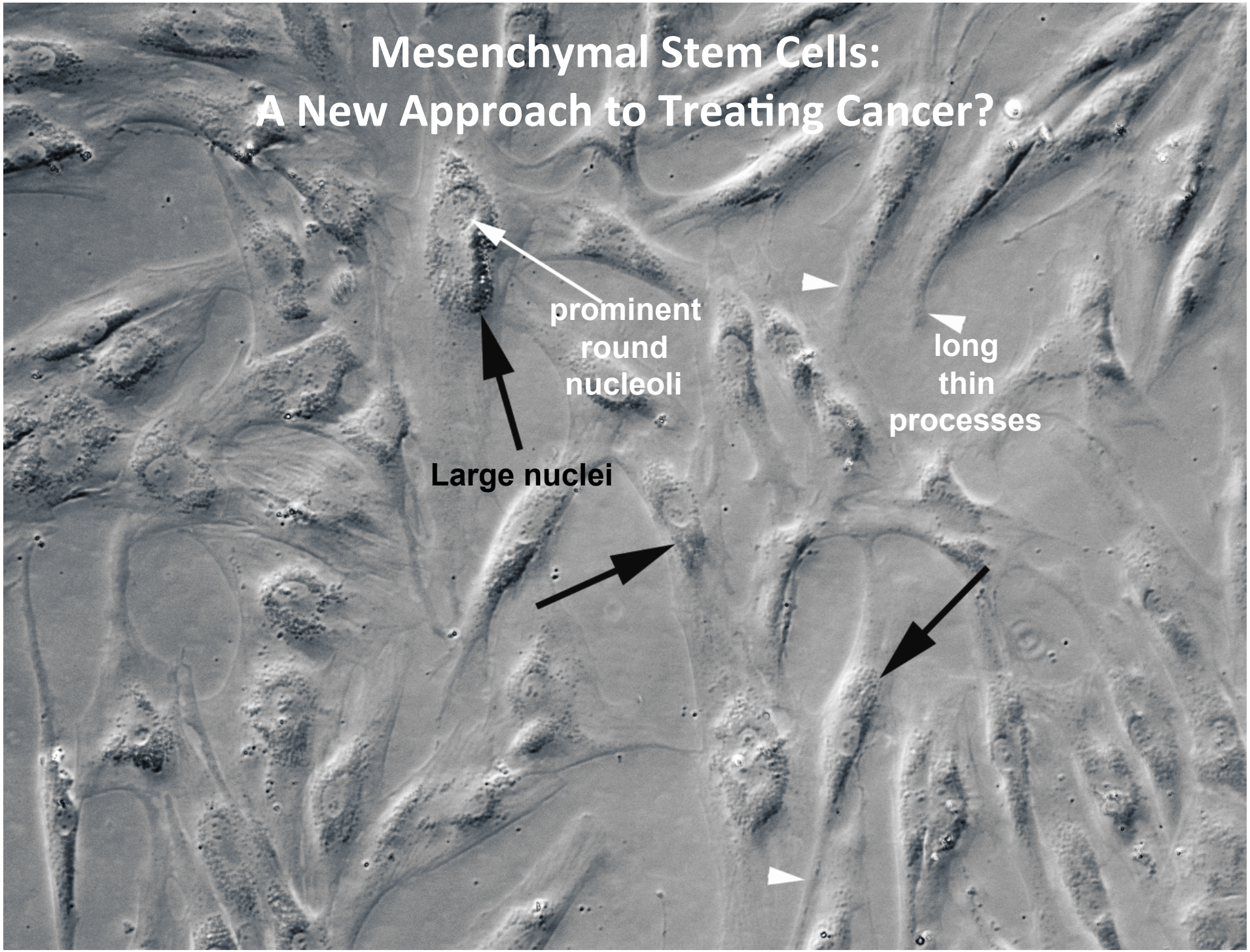
- Endogenous or exogenous NSCs
- Migration can be directed with humoral factors:
  - Hepatocyte growth factor
  - Vascular endothelial growth factor
  - Stromal derived growth factor
- Tropism to:
  - Multiple Sclerosis lesions
  - Ischemic stroke
  - Glioma – NSCs share this characteristic with Mesenchymal Stem Cells

Garzon-Muvdi, T., Quinones-Hinojosa, A. ILAR J. 2009

NSCs for the treatment of CNS diseases

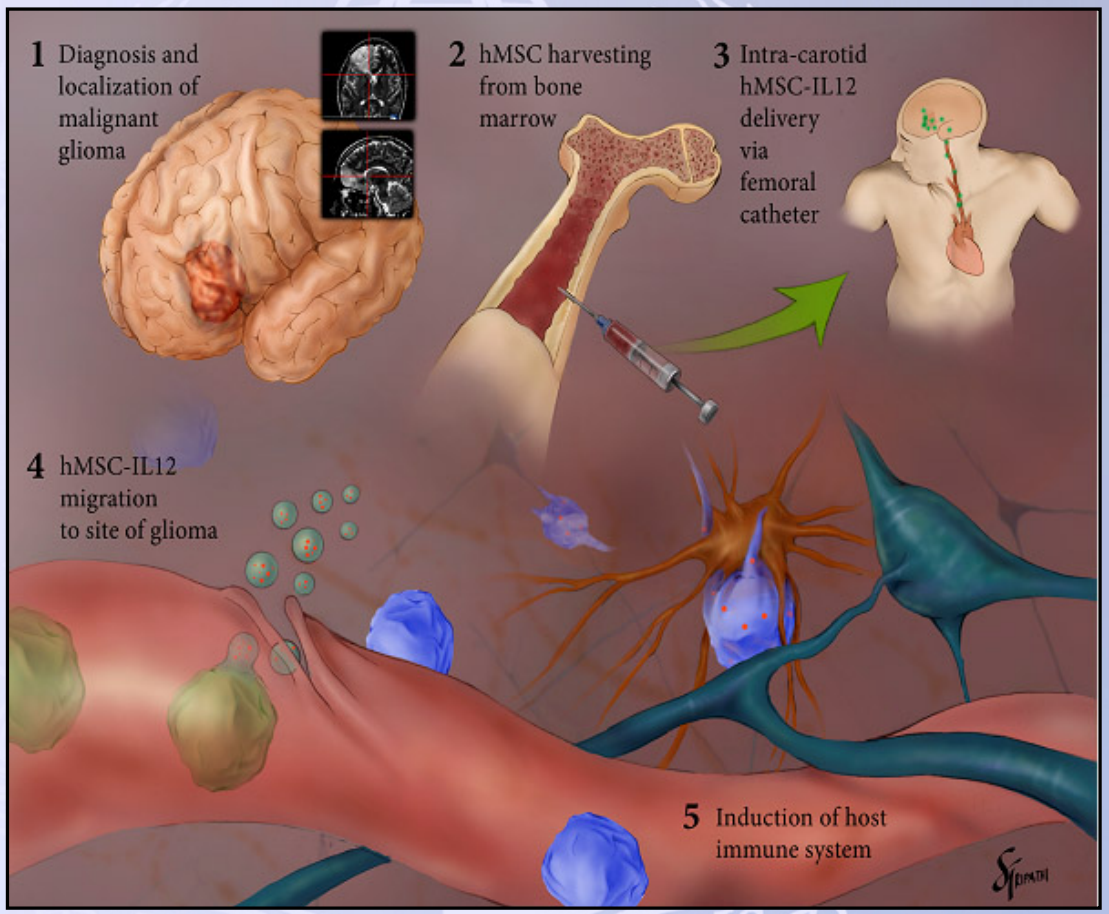


Garzon-Muvdi, T., Quinones-Hinojosa, A. ILAR J. 2009


Mesenchymal Stem Cells:
A New Approach to Treating Cancer?
prominent
round
nucleoli
long
thin
processes
Large nuclei

Mesenchymal Stem Cells As Delivery Vehicles for Glioma Therapy

- MSCs have natural affinity to migrate towards gliomas, BTSCs
- Bone Marrow Derived MSCs have been shown to be used as delivery vehicles for various anti-tumoral agents (i.e. HSV-Thymidine kinase, IL-2, IL-18, IL-23, TRAIL)
- Several Difficulties of Bone Marrow-MSC which their limit clinical use:
  1. Invasive Surgery
  2. Short Life Span ex vivo
  3. Small Extraction Yield
- Adipose tissue represent a new source of Mesenchymal Stem Cells ideal for large scale clinical use:
  1. Safely accessible, small incision
  2. 100% harvest efficiency
  3. Long lifespan



Adapted from:

Kosztowski TA, Zaidi HA, Quinones-Hinojosa A. "Application of Neural and Mesenchymal Stem Cells in the Treatment of Gliomas," *Exp Rev AntiCa Ther* 9(5) 2009.

Zaidi HA, Momin E, Quinones-Hinojosa A. "Mesenchymal stem cells for neural transplantation—Applications for Immunotherapy," *Current Immunology Reviews*, 2009

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ARTICLE

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Mesoporous Silica-Coated Hollow Manganese Oxide Nanoparticles as Positive T_1 Contrast Agents for Labeling and MRI Tracking of Adipose-Derived Mesenchymal Stem Cells

Taeho Kim,[†,‡,§] Eric Momin,[∥] Jonghoon Choi,[†,‡] Kristy Yuan,[∥] Hasan Zaidi,[∥] Jaeyun Kim,[†,‡,§] Mihyun Park,[§] Nohyun Lee,[§] Michael T. McMahon,[†,⊥] Alfredo Quinones-Hinojosa,[∥] Jeff W. M. Bulte,[†,‡,¶,#] Taeghwan Hyeon,[*,§] and Assaf A. Gilad[*,†,‡,⊥]

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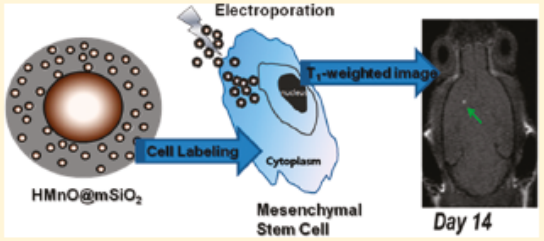
Supporting Information

ABSTRACT: Mesoporous silica-coated hollow manganese oxide coated ($HMnO@mSiO_2$) nanoparticles were developed as a novel T_1 magnetic resonance imaging (MRI) contrast agent. We hypothesized that the mesoporous structure of the nanoparticle shell enables optimal access of water molecules to the magnetic core, and consequently, an effective longitudinal (R_1) relaxation enhancement of water protons, which value was measured to be 0.99 ($mM^{-1}s^{-1}$) at 11.7 T. Adipose-derived mesenchymal stem cells (MSCs) were efficiently labeled using electroporation, with much shorter T_1 values as compared to direct incubation without electroporation, which was also evidenced by signal enhancement on T_1-weighted MR images in vitro. Intracranial grafting of $HMnO@mSiO_2$-labeled MSCs enabled serial MR monitoring of cell transplants over 14 days. These novel nanoparticles may extend the arsenal of currently available nanoparticle MR contrast agents by providing positive contrast on T_1-weighted images at high magnetic field strengths.



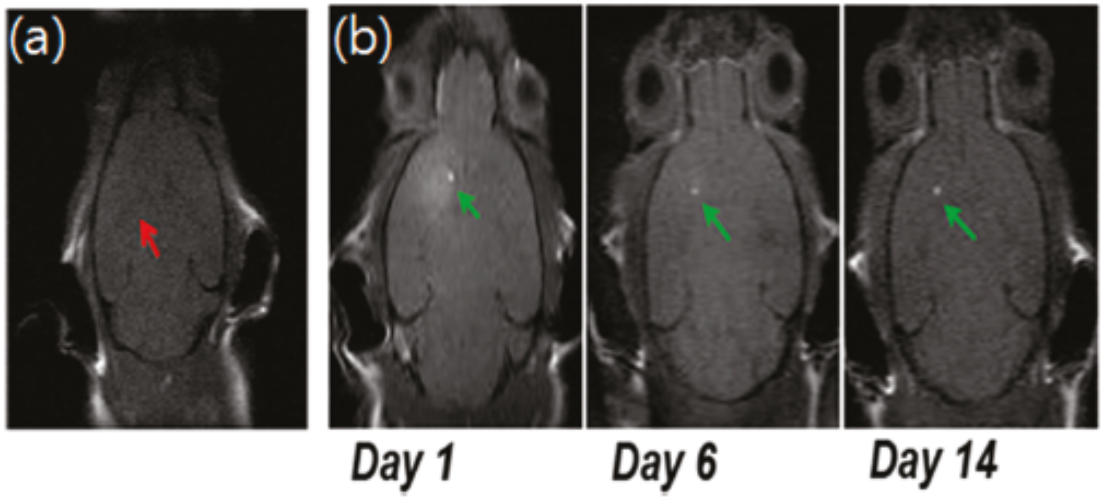


Figure 4. In vivo MRI of transplanted MSCs. (a) No hyperintense signal (red arrow) was detected in mouse transplanted with unlabeled MSCs. (b) Hyperintense signals (green arrows) were detected in mouse transplanted with HMnO@mSiO_2-labeled MSCs and were still visible 14 days after injection.

Conclusions

- BTSCs may originate GBM
- NSCs and A-MSCs as delivery vehicles
- NSCs can be modified for treatment of other CNS diseases

Contributing Lab Members

Hasan Zaidi
HHMI Fellow

Kristy Yuan
HHMI Fellow

Eric Momin
Doris Duke Fellow

Project Aims

- Aim 1
  - Establish cell line from fat tissue which exhibits MSC-like properties
- Aim 2 (*in vitro*)
  - Examine whether Adipose Derived MSCs (A-MSCs) migrate selectively towards gliomas *in vitro*
  - Examine whether A-MSCs contribute to tumor growth *in vitro*
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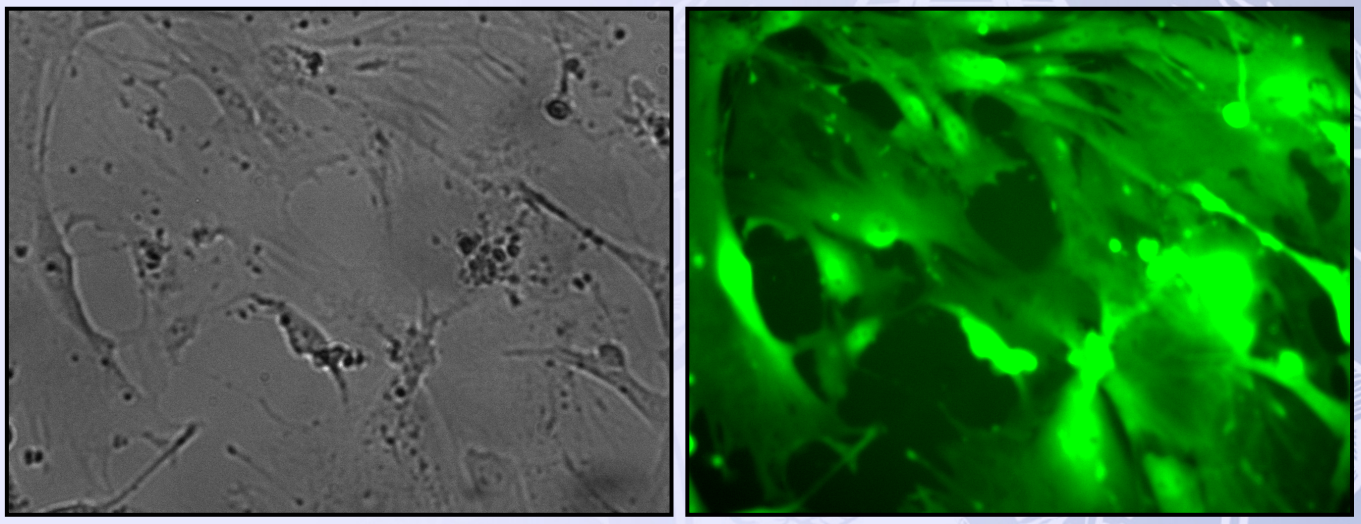
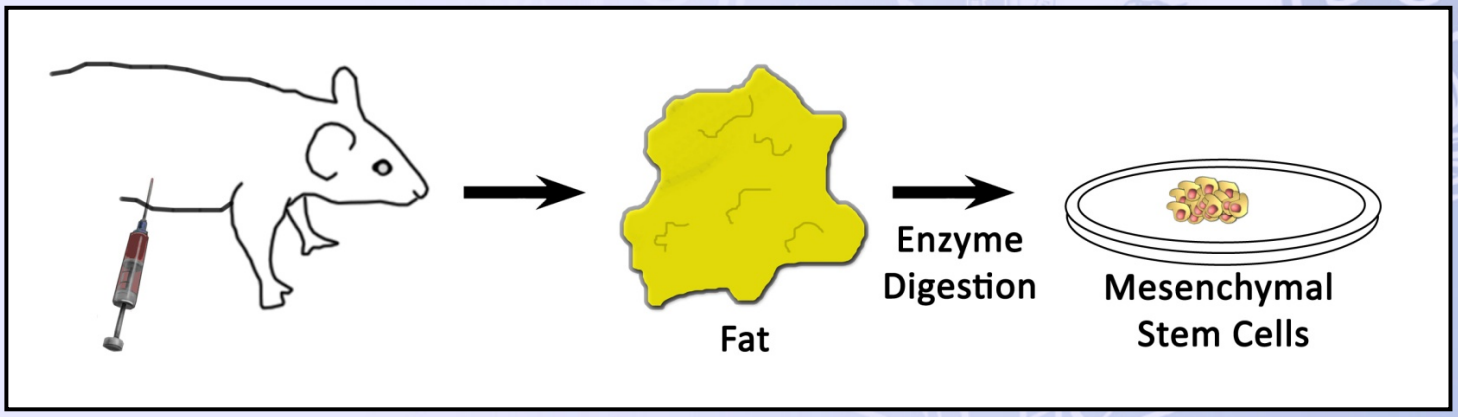
Adipose Derived Mesenchymal Stem Cells harvested from Rosa26-eGFP-DTA mice express GFP



(Left) BF view of AMSCs harvested from fat of Rosa26-eGFP-DTA mice with (right) identical view of cells under fluorescence.



Fat was extracted from transgenic animals which constitutively express GFP in all cells

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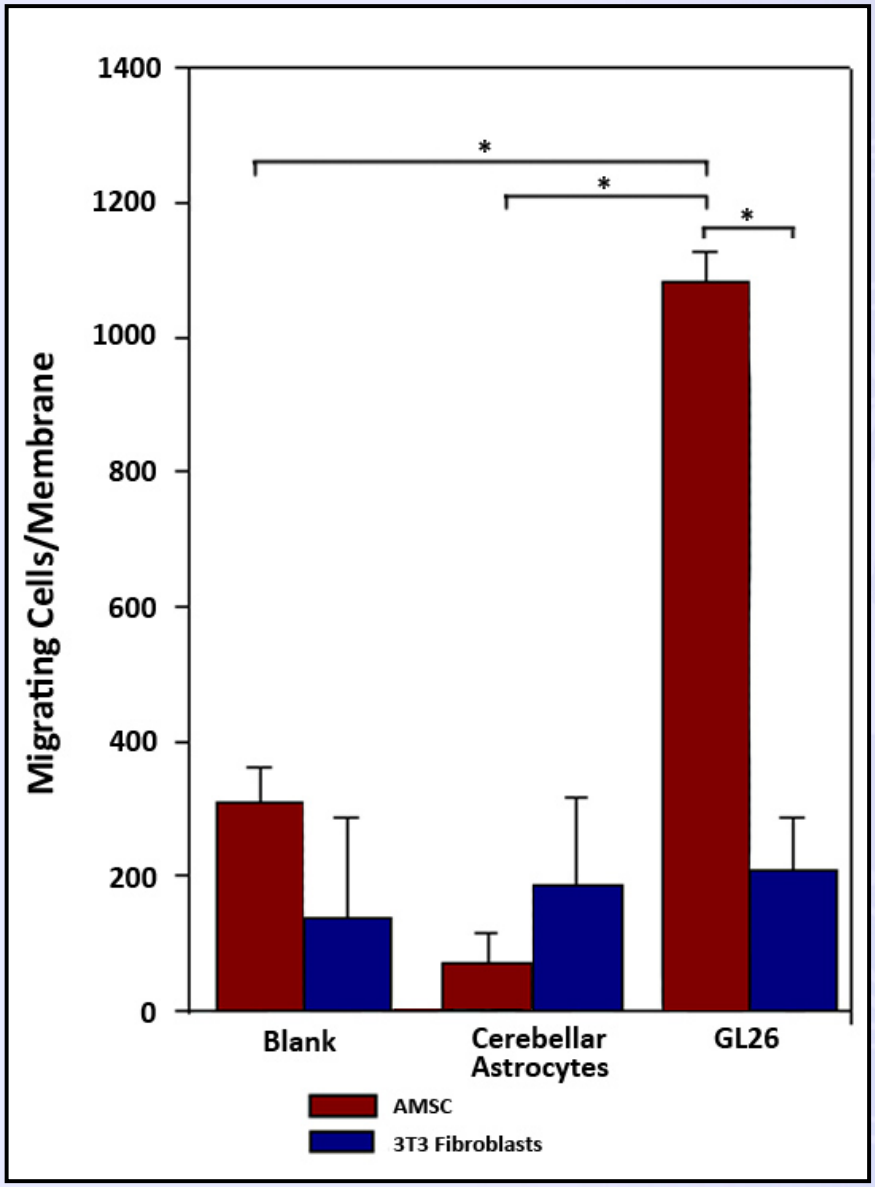
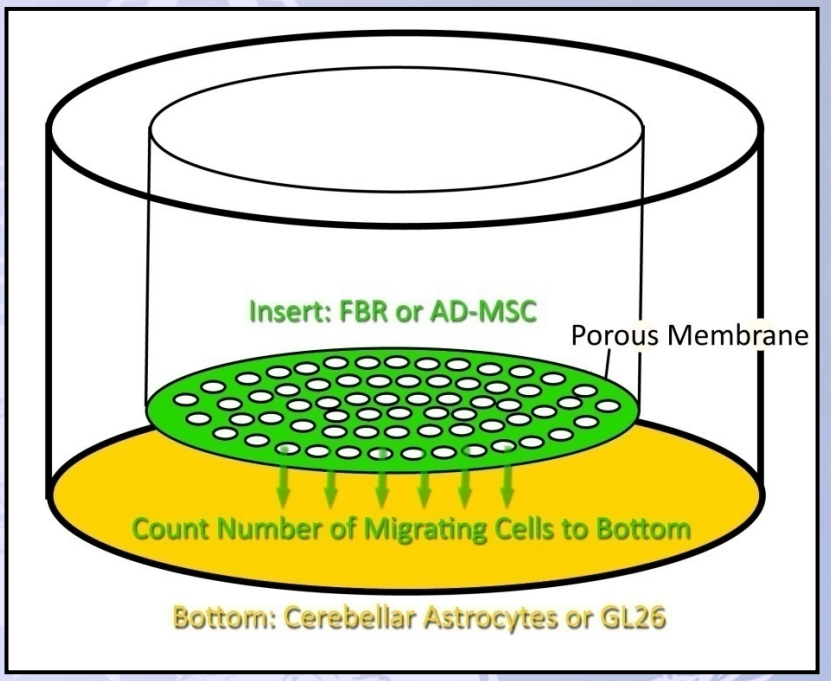
AMSCs display selective tropism for GL26 glioma *in vitro*



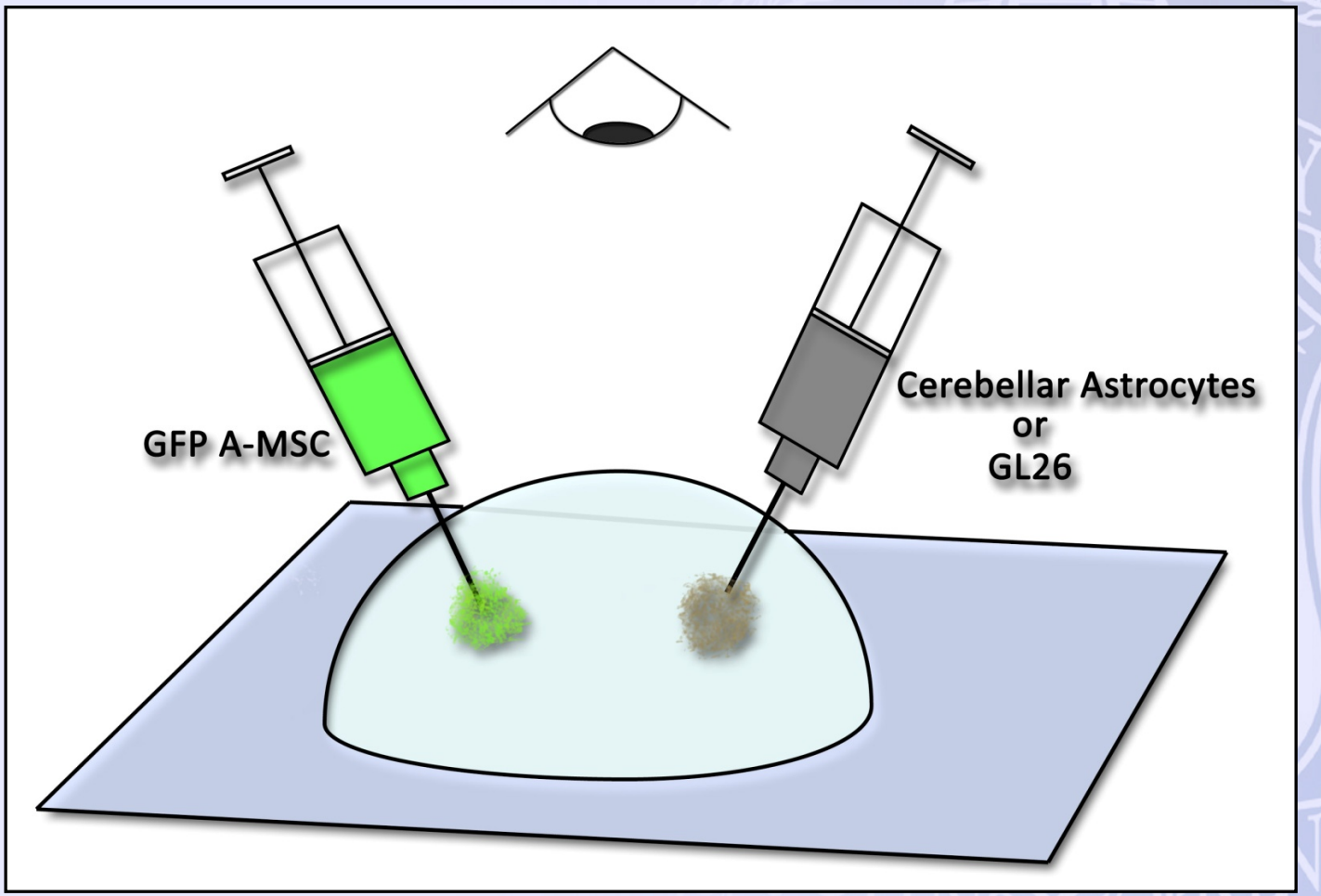
Boyden Chamber Assay



AMSCs or A-MSCs were plated in insert and allowed to migrate towards either blank media, Cerebellar Astrocytes, or GL26 for 48hrs

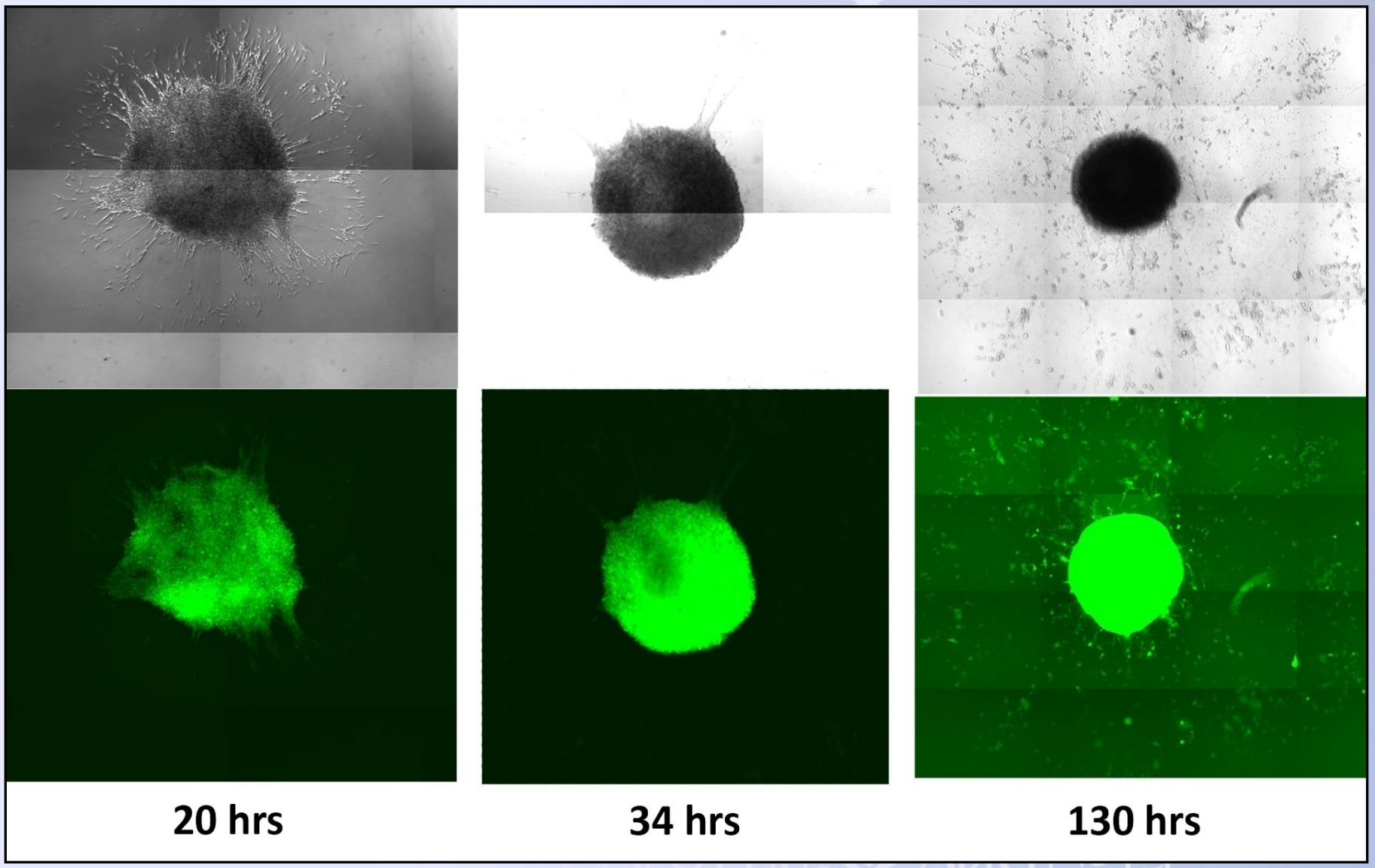
Compared to Fibroblasts, A-MSCs preferentially migrate towards GL26 tumor cells as opposed to blank media or Cerebellar Astrocyte control. *p-value <0.001

Matrigel Assay



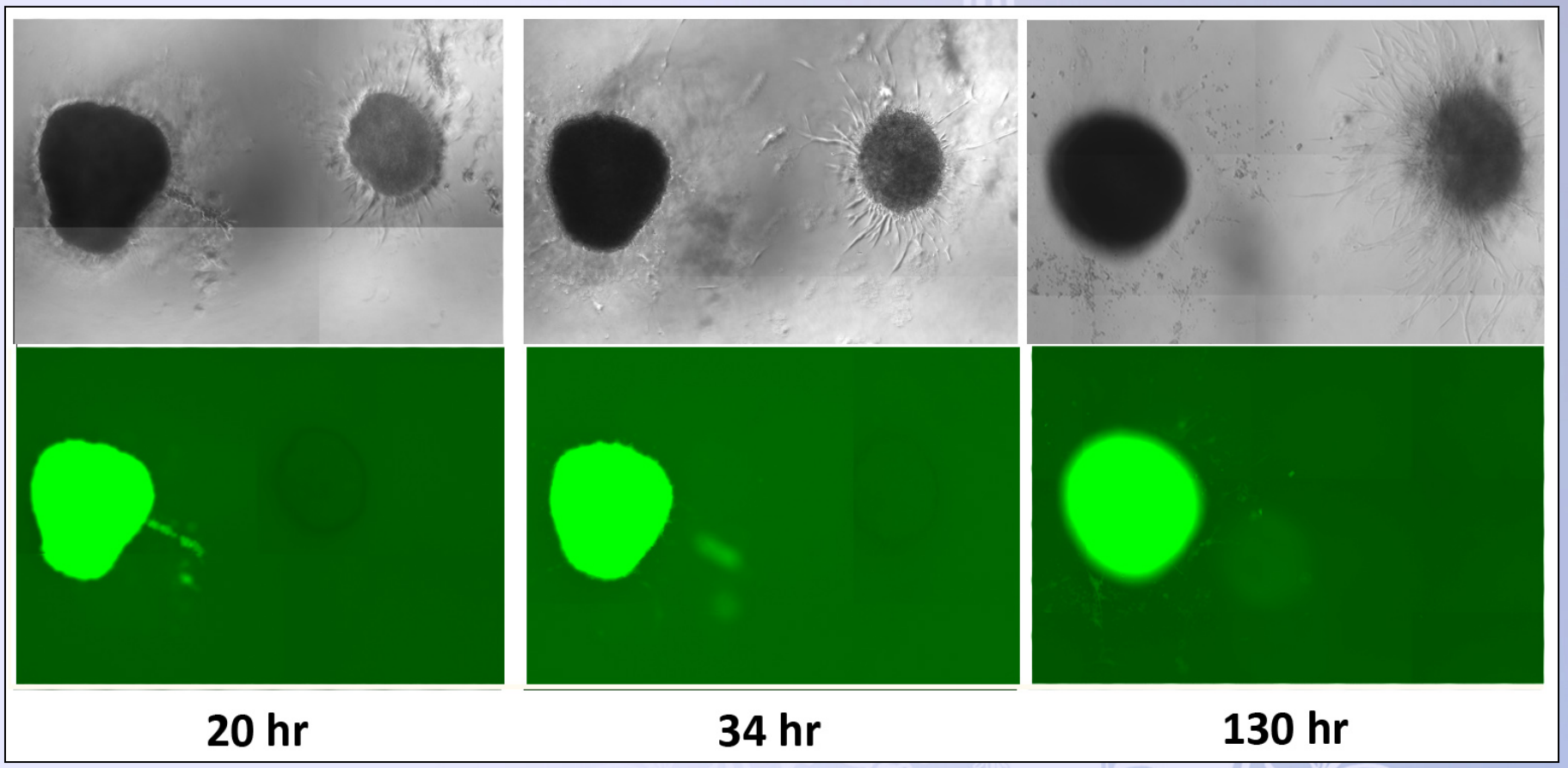
- Protenacious Material
- Mimics Extracellular Enviornment
- Allows one to observe the migration of cells overtime

AMSCs selectively accumulate at sites of malignant cells



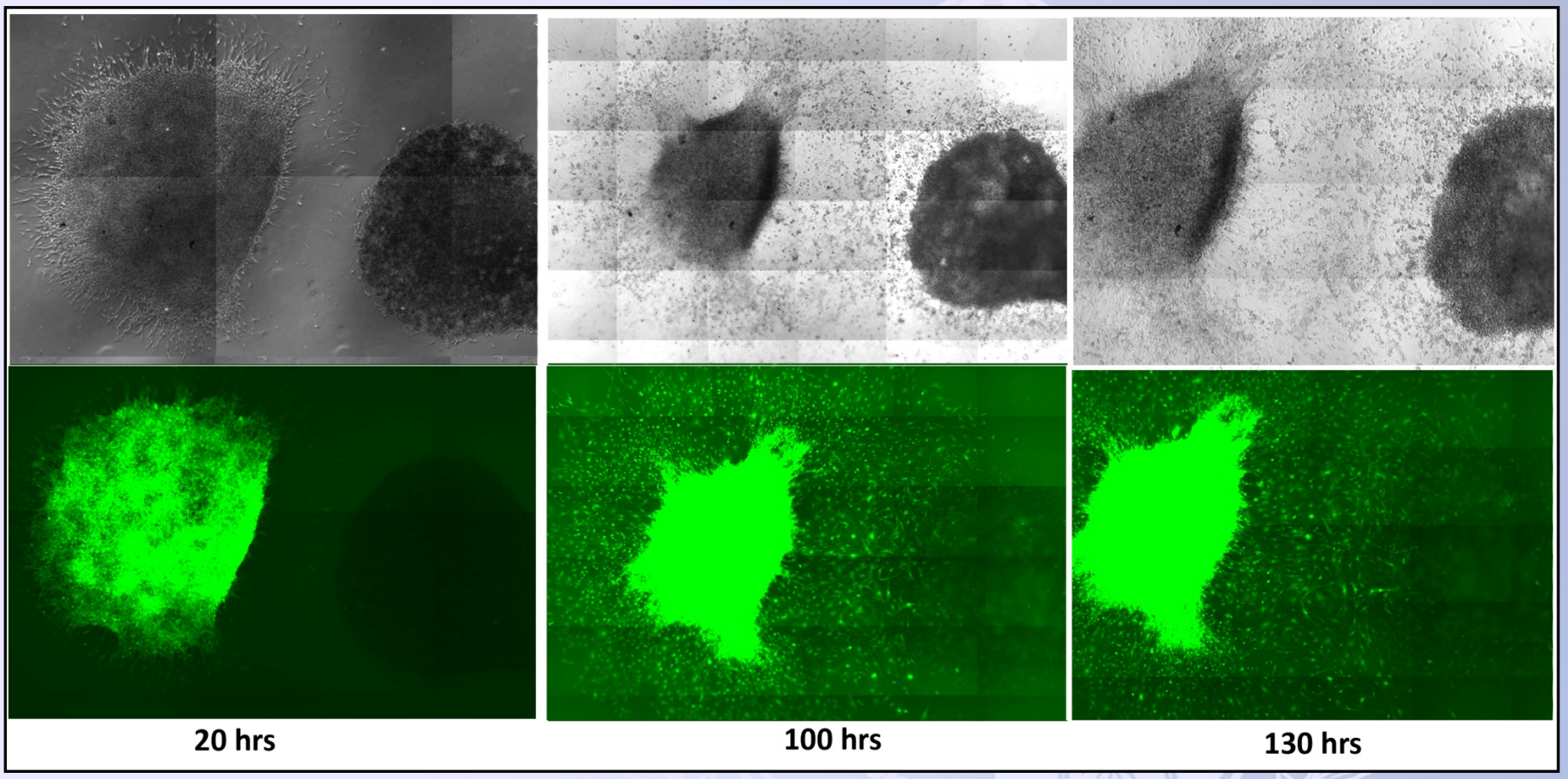
A-MSCs were injected in matrigel and followed for 7d. A-MSCs migrate centrifugally from the spot in which they were placed.

AMSCs selectively accumulate at sites of malignant cells



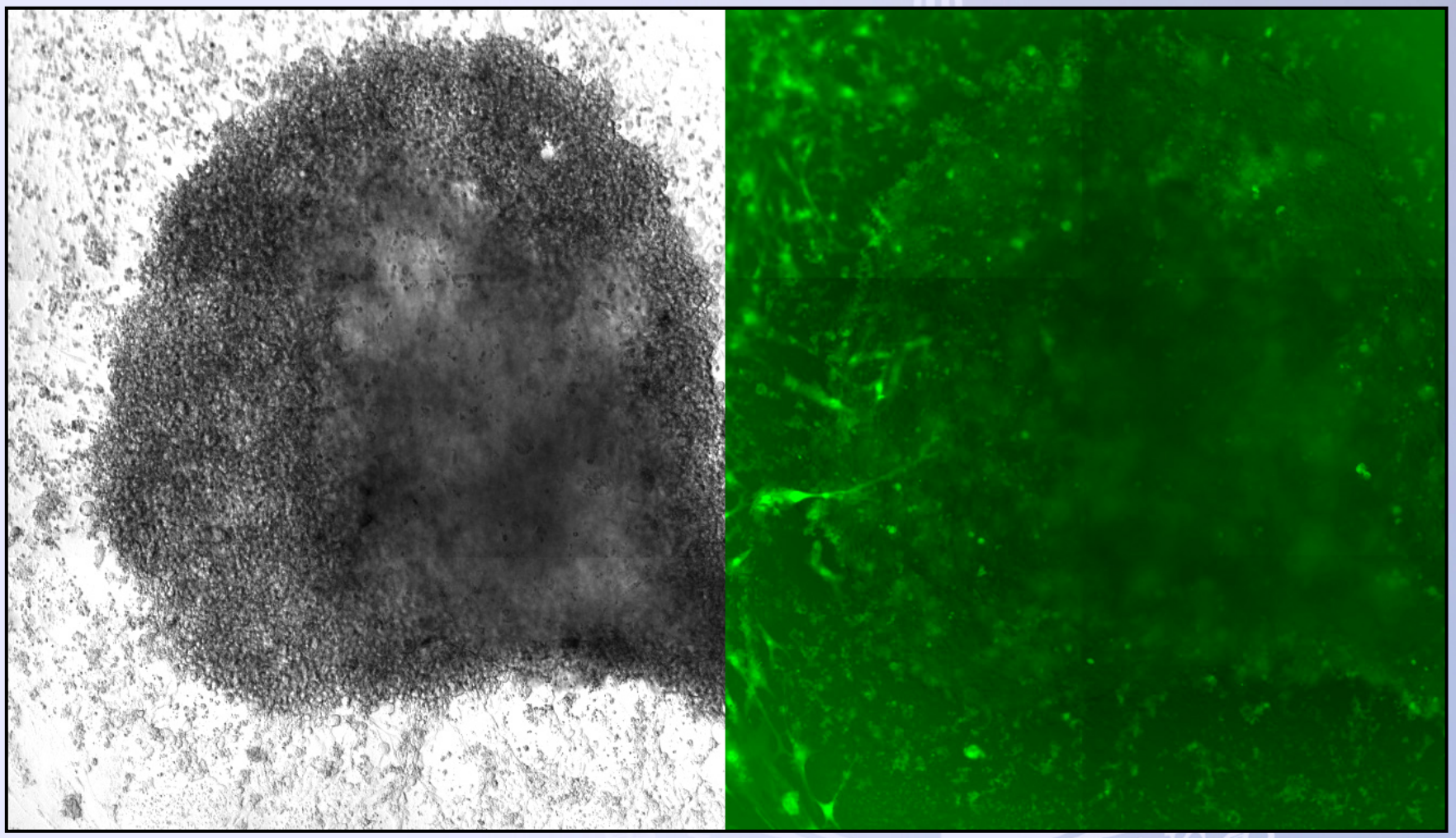
When placed in matrigel with Cerebellar Astrocytes (*unlabeled, right*), A-MSCs (*GFP Labeled, left*) do not exhibit extensive migration towards normal brain cells.

AMSCs selectively accumulate at sites of malignant cells



When placed with GL26 Murine Glioma (*unlabeled, right*), A-MSCs (*GFP labeled, left*) migrate in large numbers towards tumor cells and accumulate at tumor

AMSCs selectively accumulate at sites of malignant cells



Closer view of GL26 in previous image shows GFP labeled A-MSCs infiltrating GL26 spot in matrigel

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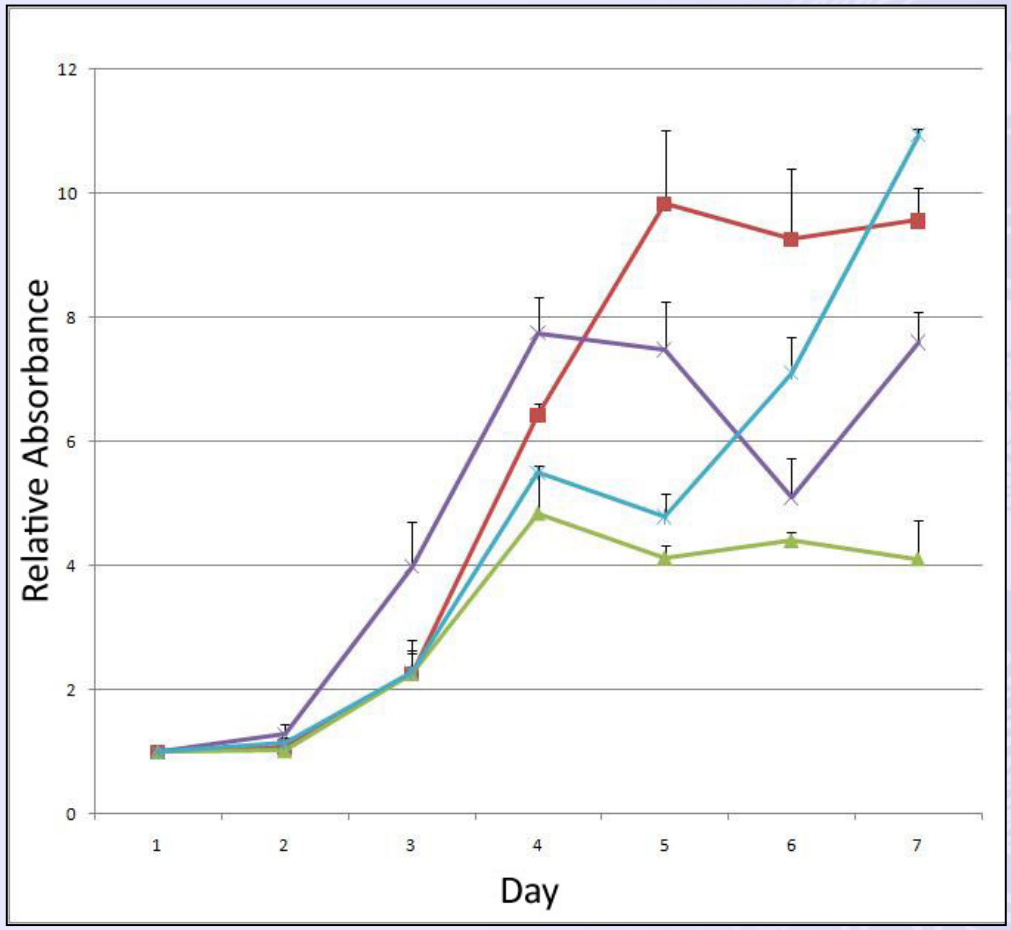
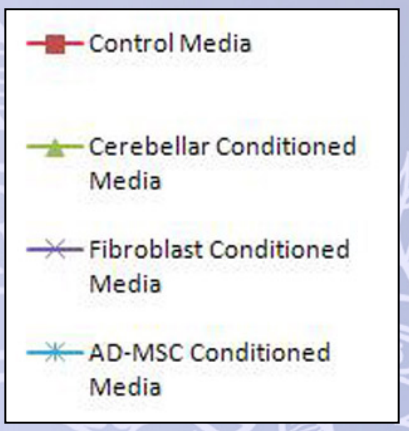
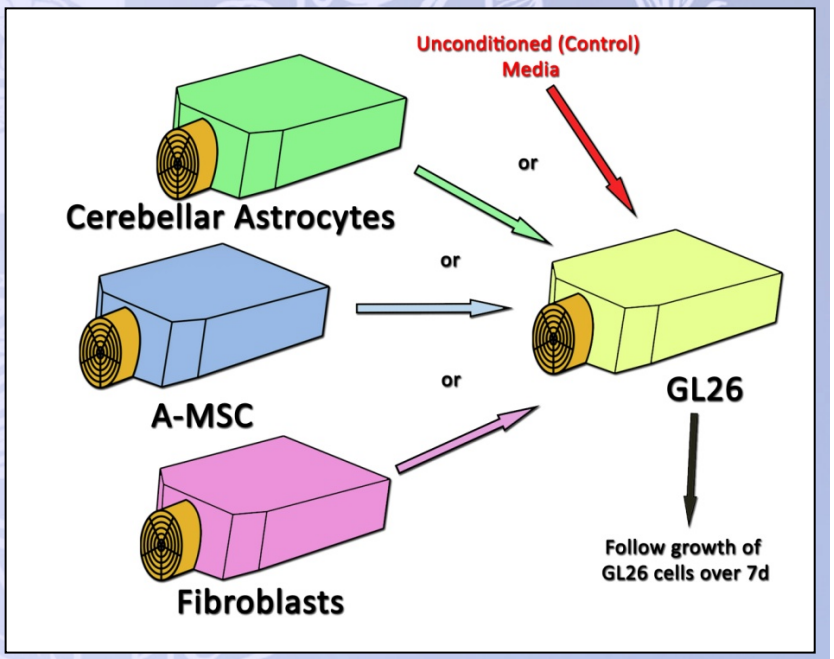
Effect of Conditioned Media on the Growth of GL26

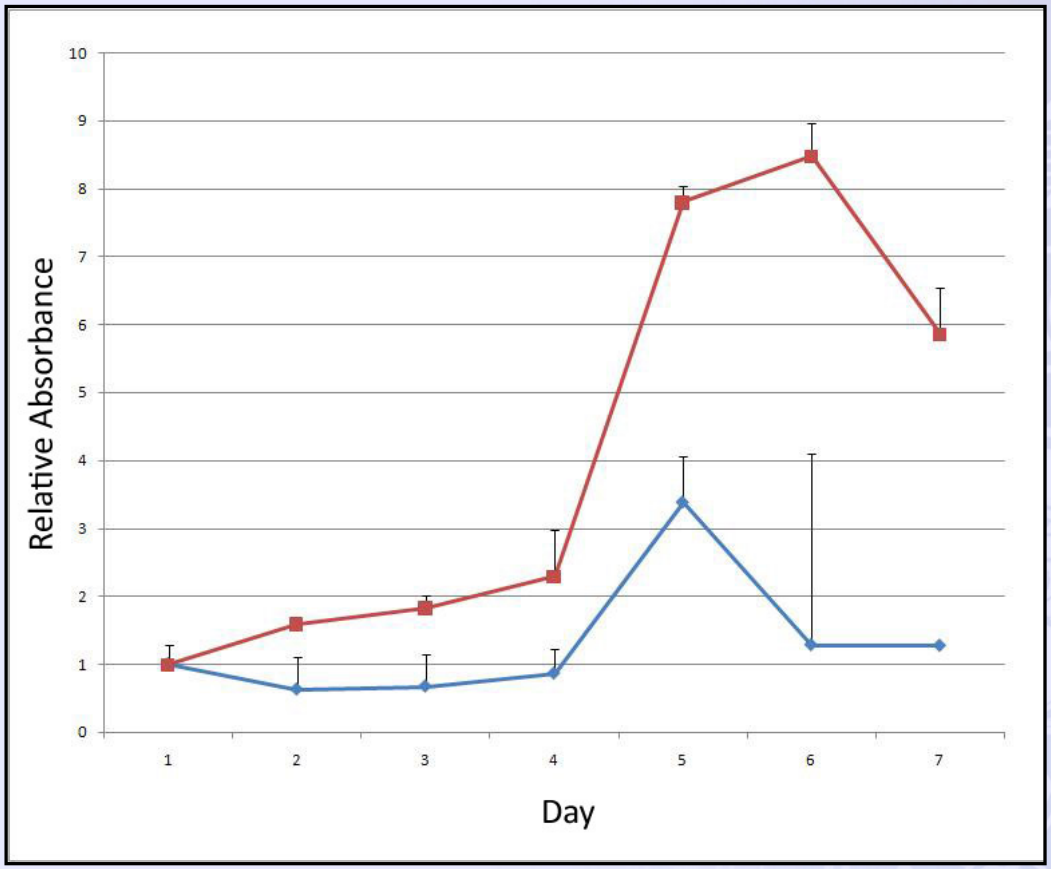


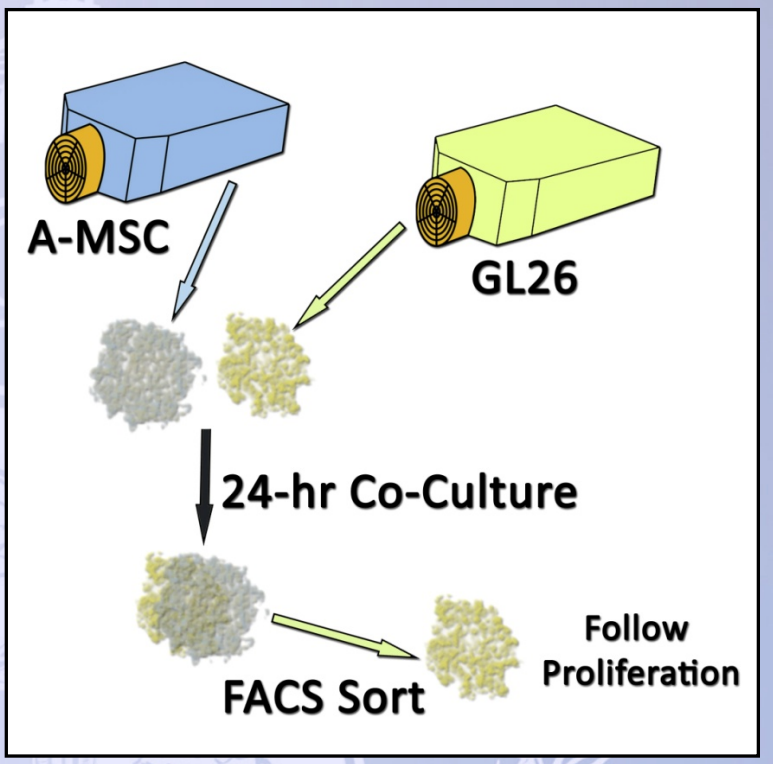


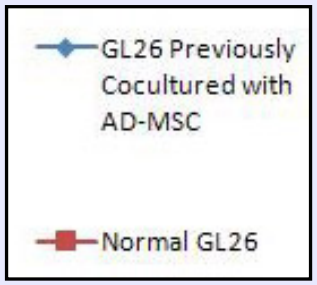


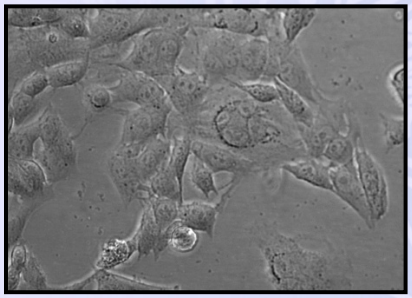
GL26 cells were exposed to conditioned media from A-MSCs for 7days did not exhibit enhanced proliferation rate.

Effect of Direct Cell Contact of A-MSC on Growth of GL26

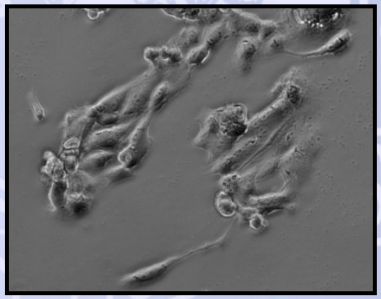








Normal GL26



GL26 Previously
Co-Cultured with A-MSC

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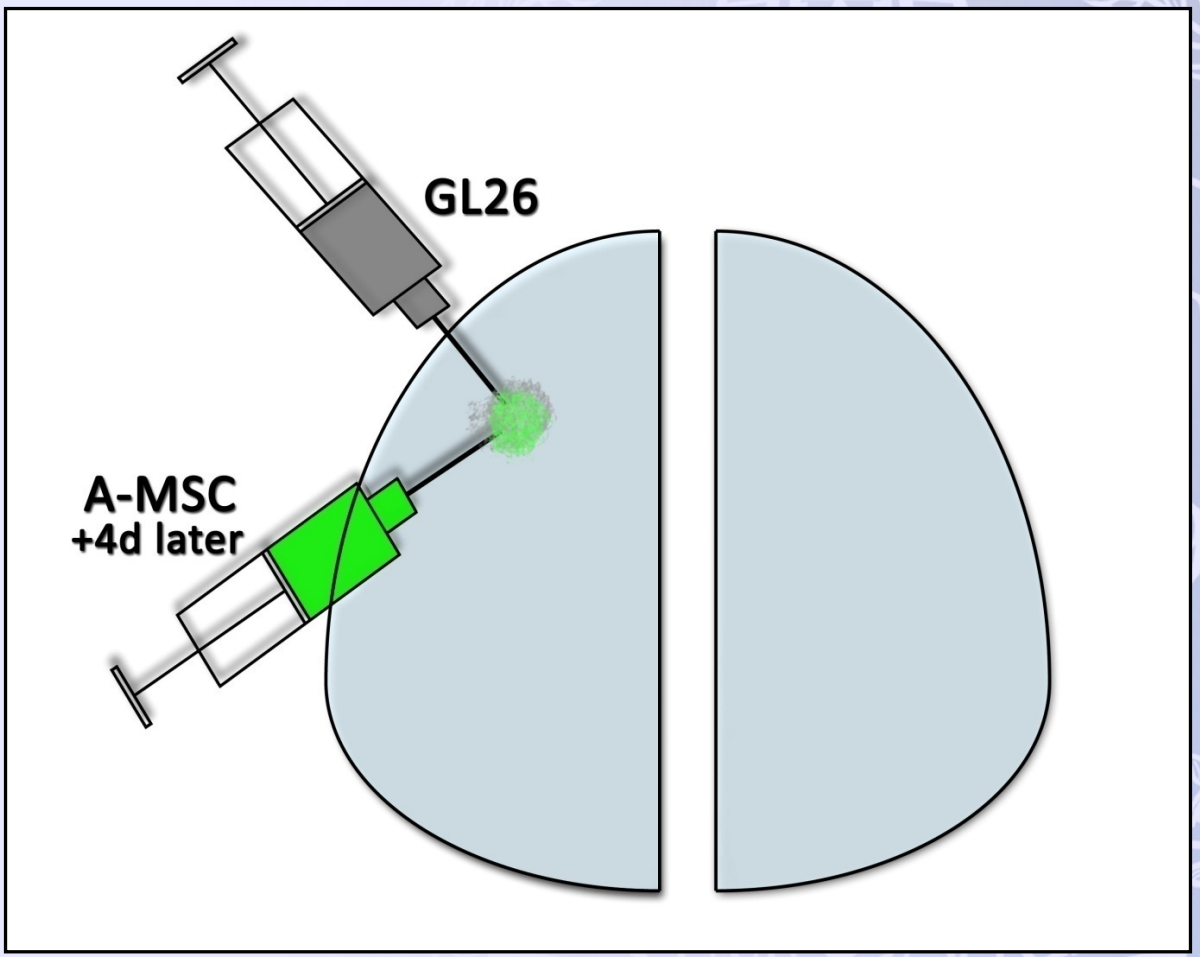
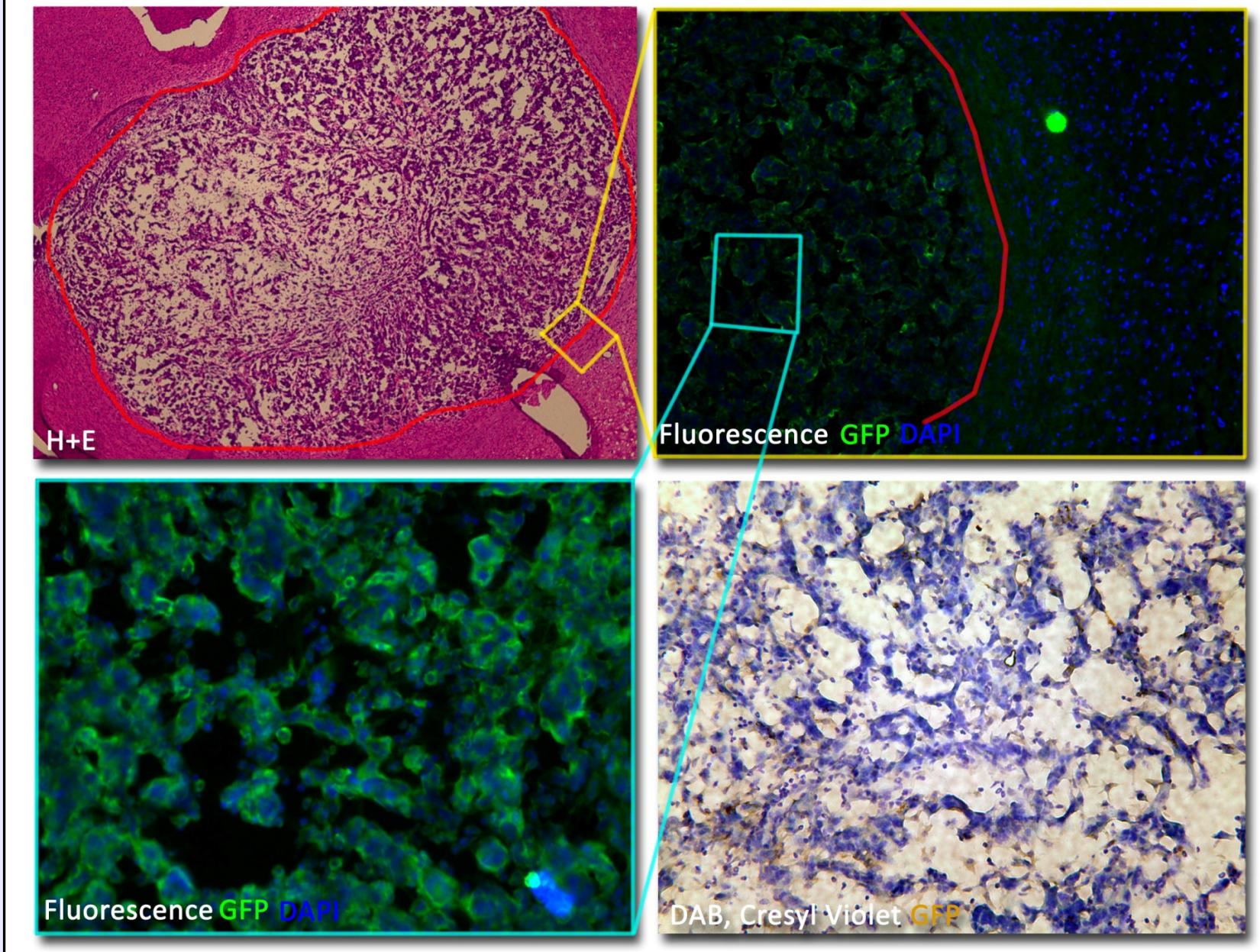
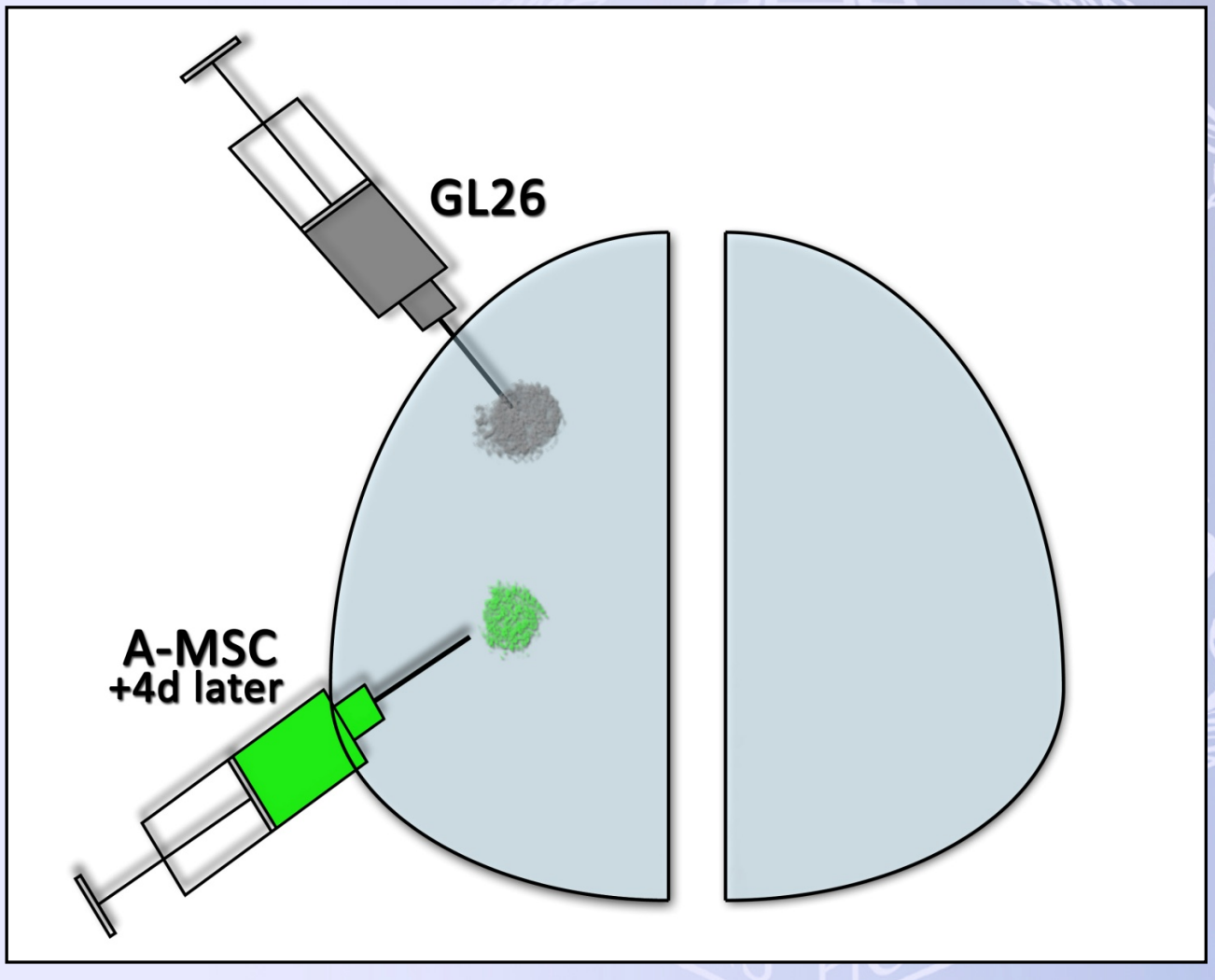
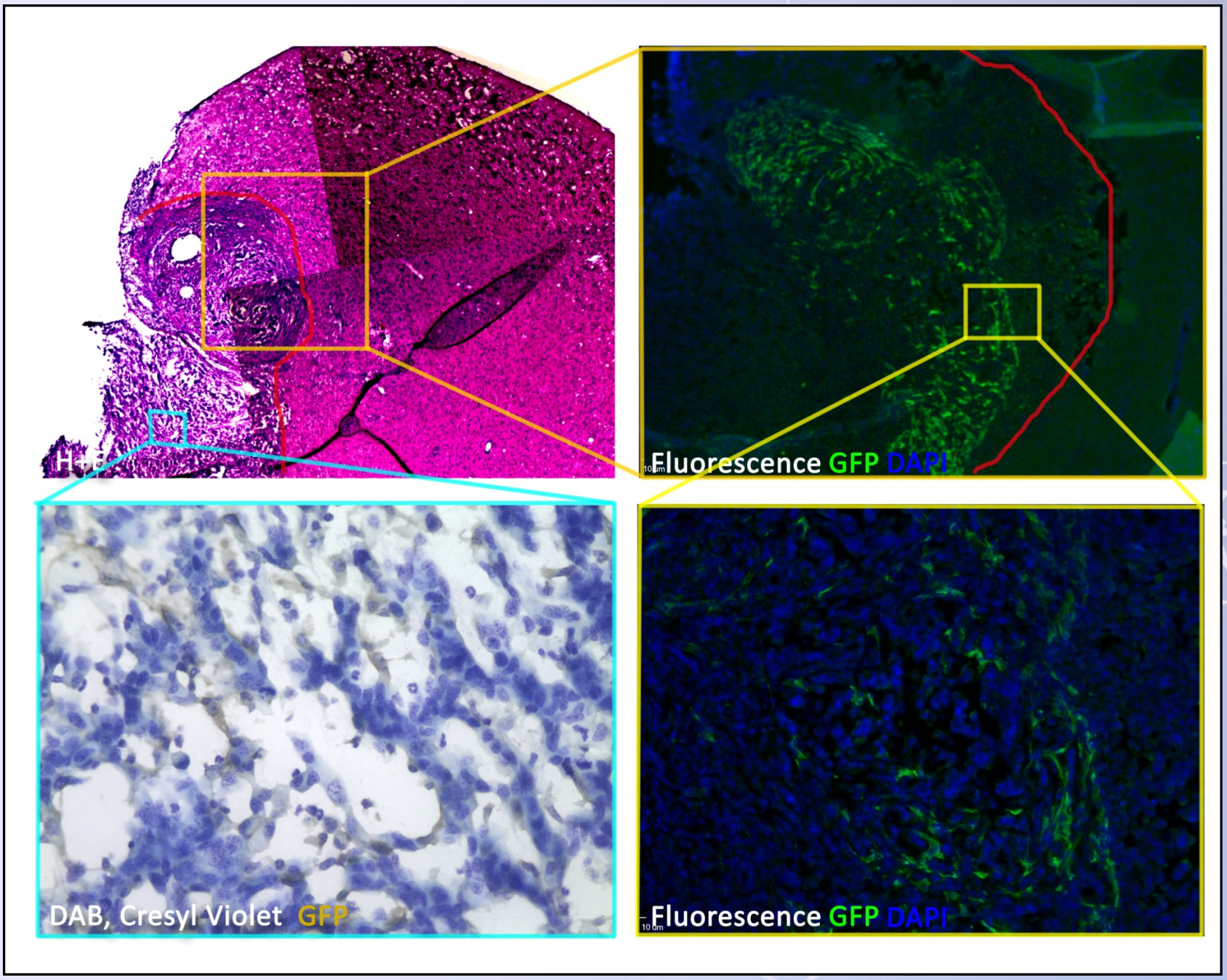
Intratumoral Injection of AMSCs



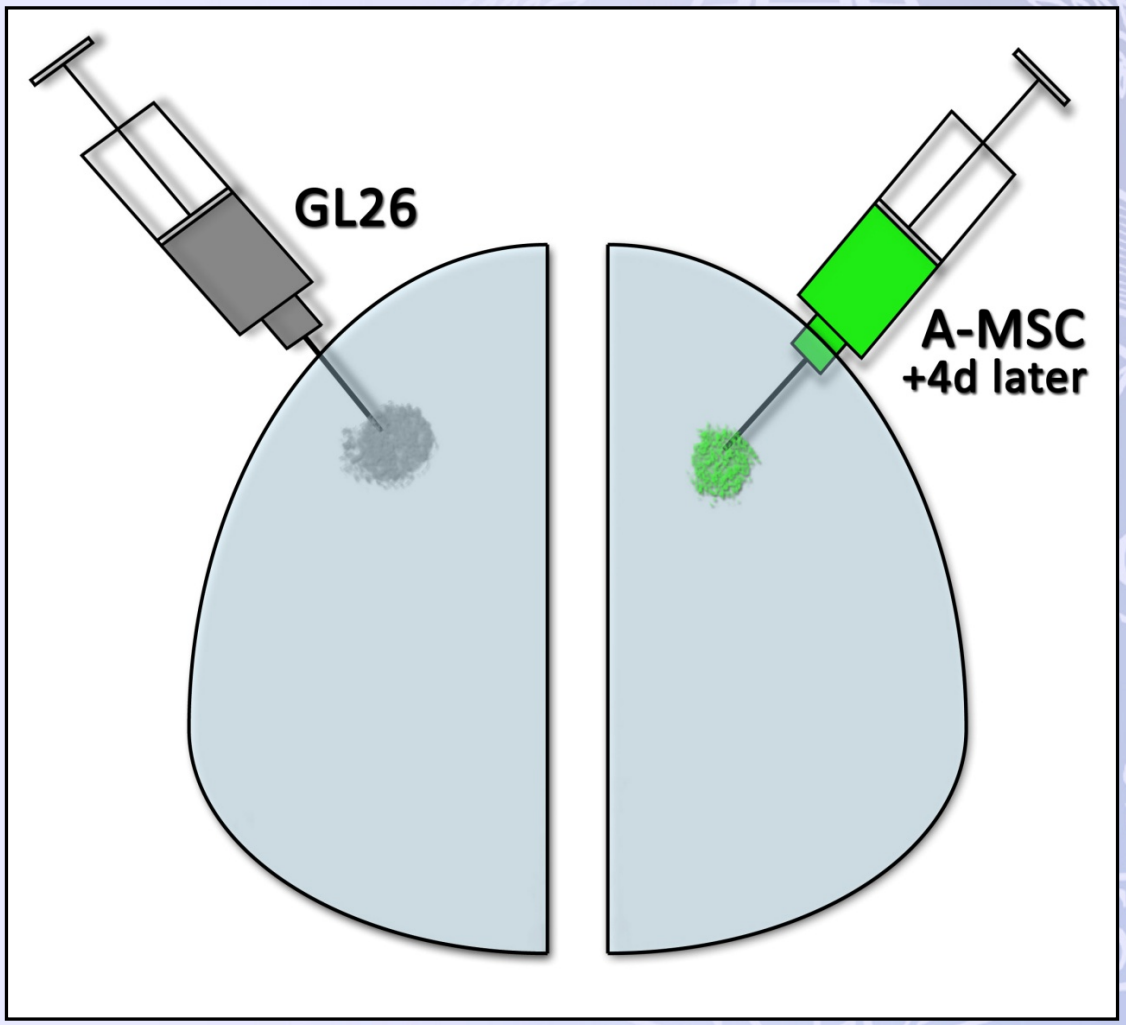

H+E
Fluorescence GFP DAPI
Fluorescence GFP DAPI
DAB, Cresyl Violet GFP

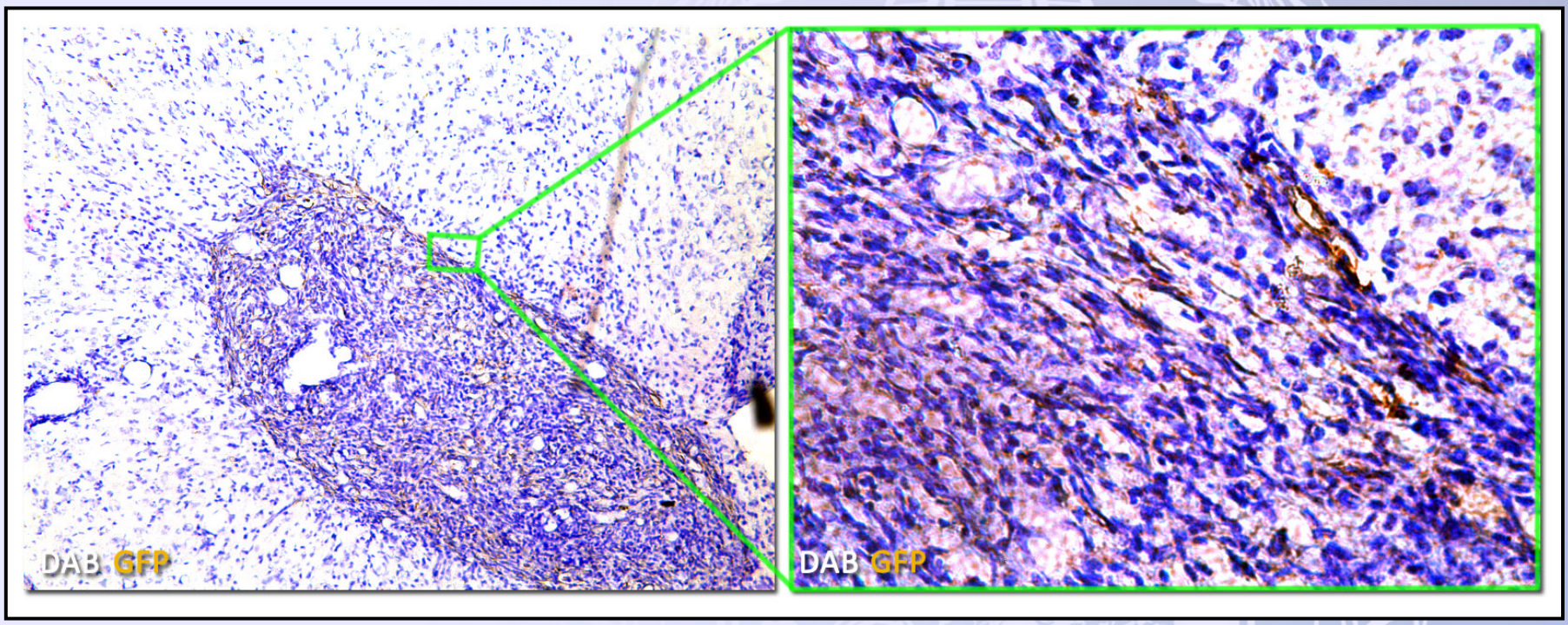
Ipsilateral Injection of AMSCs




H+E
Fluorescence GFP DAPI
DAB, Cresyl Violet GFP
Fluorescence GFP DAPI

Contralateral Injection of AMSCs




DAB GFP
DAB GFP

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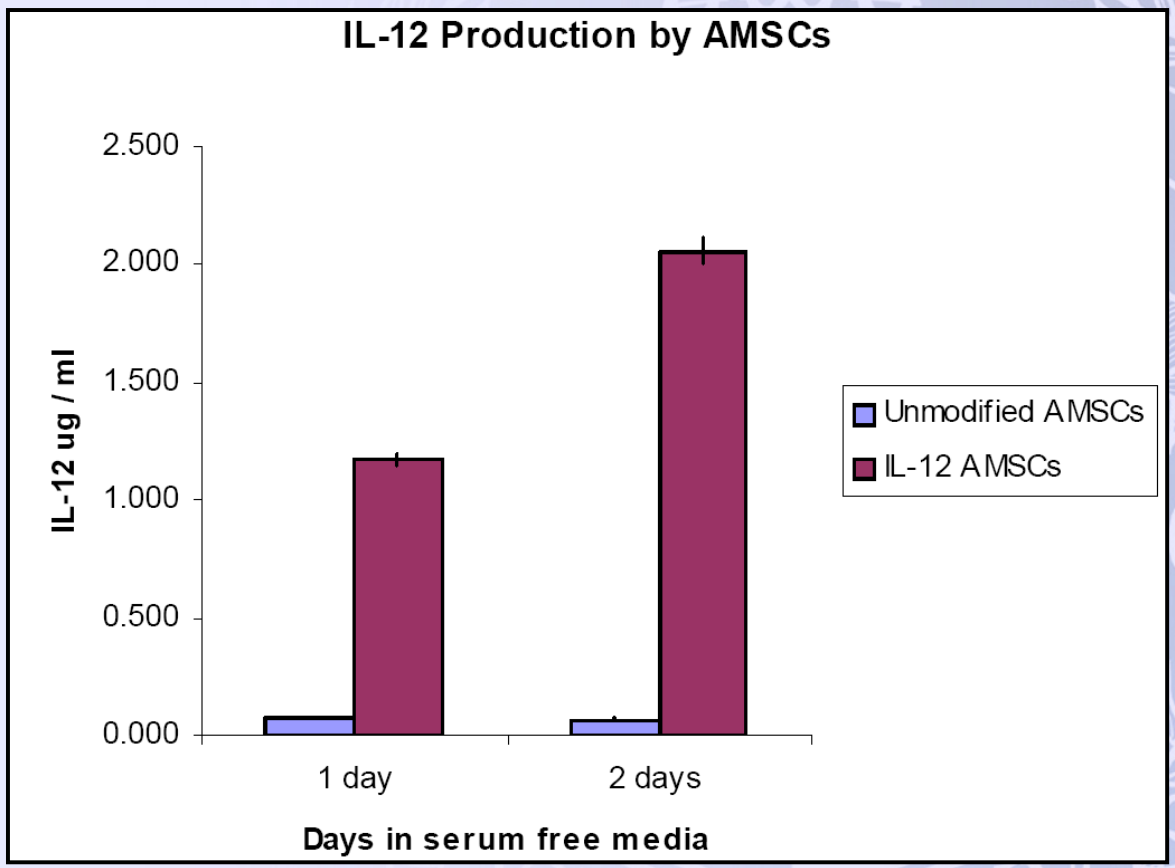
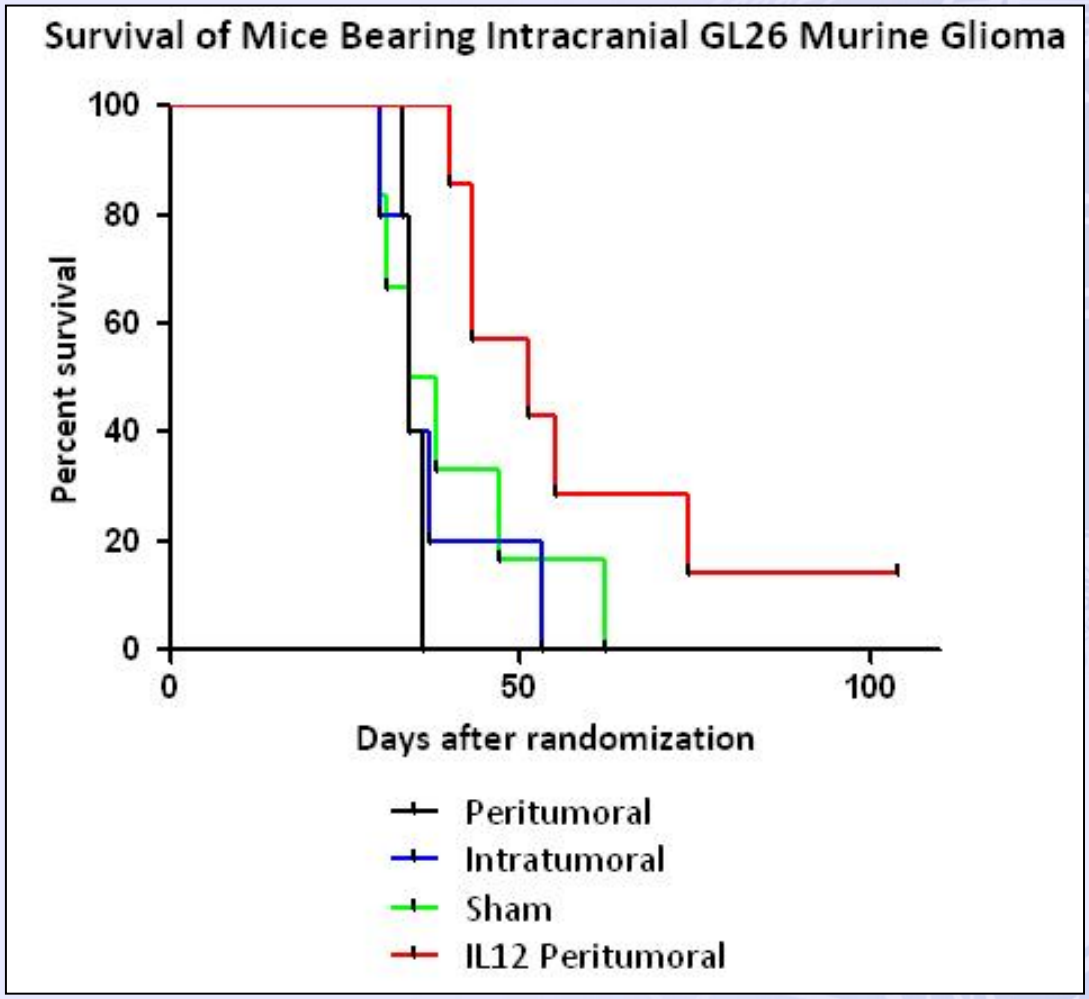
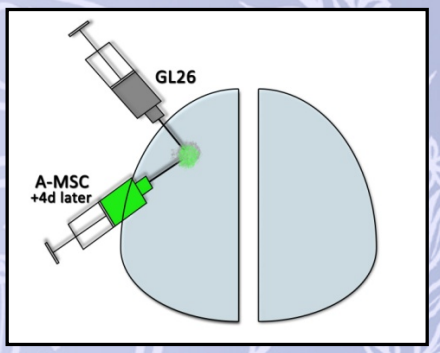
  - Examine whether A-MSCs contribute to tumor growth *in vitro*
- Aim 3 (*in vivo*)
  - Examine whether A-MSCs exhibit migration towards intracranial gliomas *in vivo*
  - Examine whether A-MSCs can carry a therapeutic gene (Interleukin-12) to intracranial gliomas and confer a survival advantage

A-MSCs Effectively Incorporate Interleukin-12 Transgene

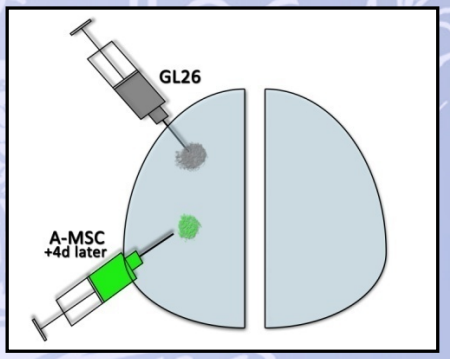


A-MSCs Effectively Carry Interleukin-12 (IL-12) to Intracranial Glioma and Confer a Survival Advantage





Intratumoral



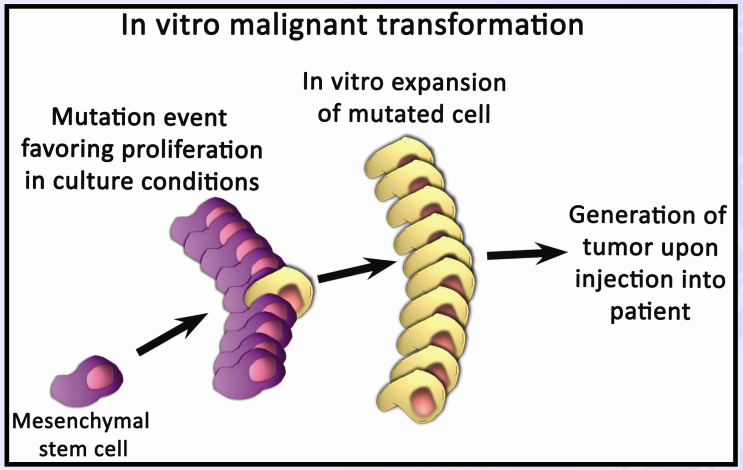
Peritumoral

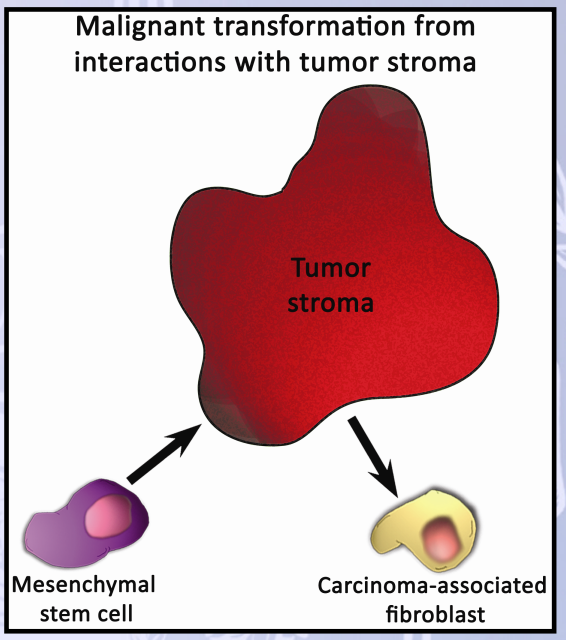
N=6

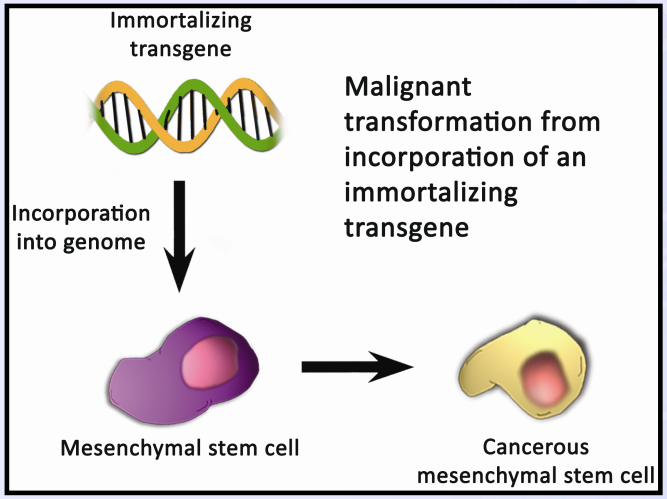
Conclusions

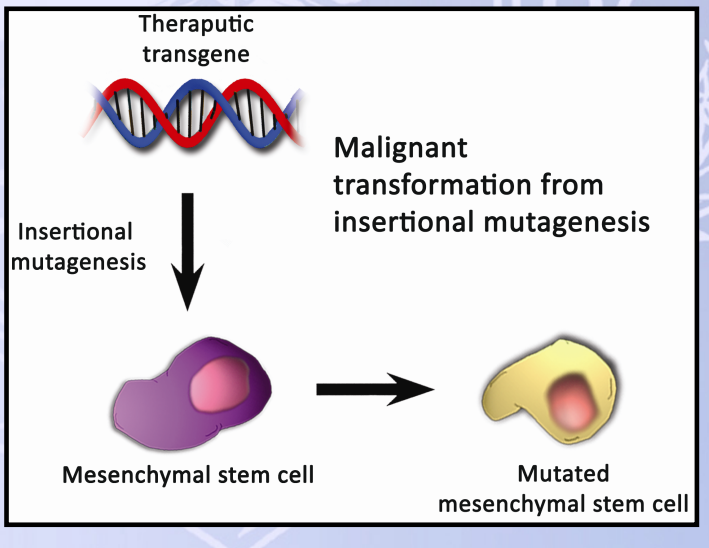
1. A-MSCs can be easily collected from fat tissue
2. A-MSCs migrate in large quantities to gliomas *in vitro* and *in vivo*
3. A-MSCs do not enhance the growth of gliomas *in vitro* or decrease survivability of mice *in vivo*
4. When genetically modified to produce a theraputic agent (i.e. IL-12), A-MSCs confer a survival advantage to mice bearing intracranial gliomas

Future Directions: Are MSCs dangerous?





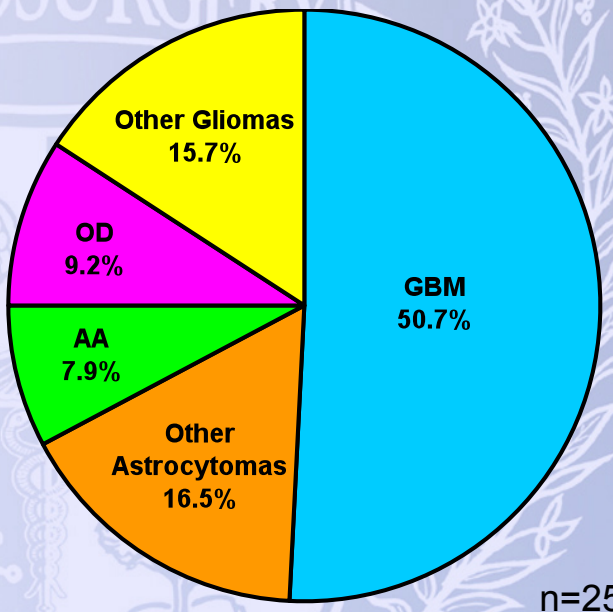




Malignant Glioma Epidemiology

- Approximately 20,500 people in the US are diagnosed with cancer of the brain and nervous system annually
  - About 12,740 patients die annually as a result of these malignant tumors
- Approximately 7.4 cases of primary malignant tumors of the CNS are diagnosed per 100,000 people per year

Distribution of Primary Brain and CNS Gliomas*



n=25,539

*Adapted from CBTRUS. Statistical Report. 2005.
OD, oligodendroglioma; AA, anaplastic astrocytoma.

CBTRUS. Statistical Report. 2005.
American Cancer Society. Detailed guide: brain/CNS tumors in adults. 2007.
American Cancer Society. Cancer Facts and Figures 2007.

Human SVZ Astrocytes Do Not Require EGF or FGF

Astrocytes isolated from SVZ, cortex, and striatum

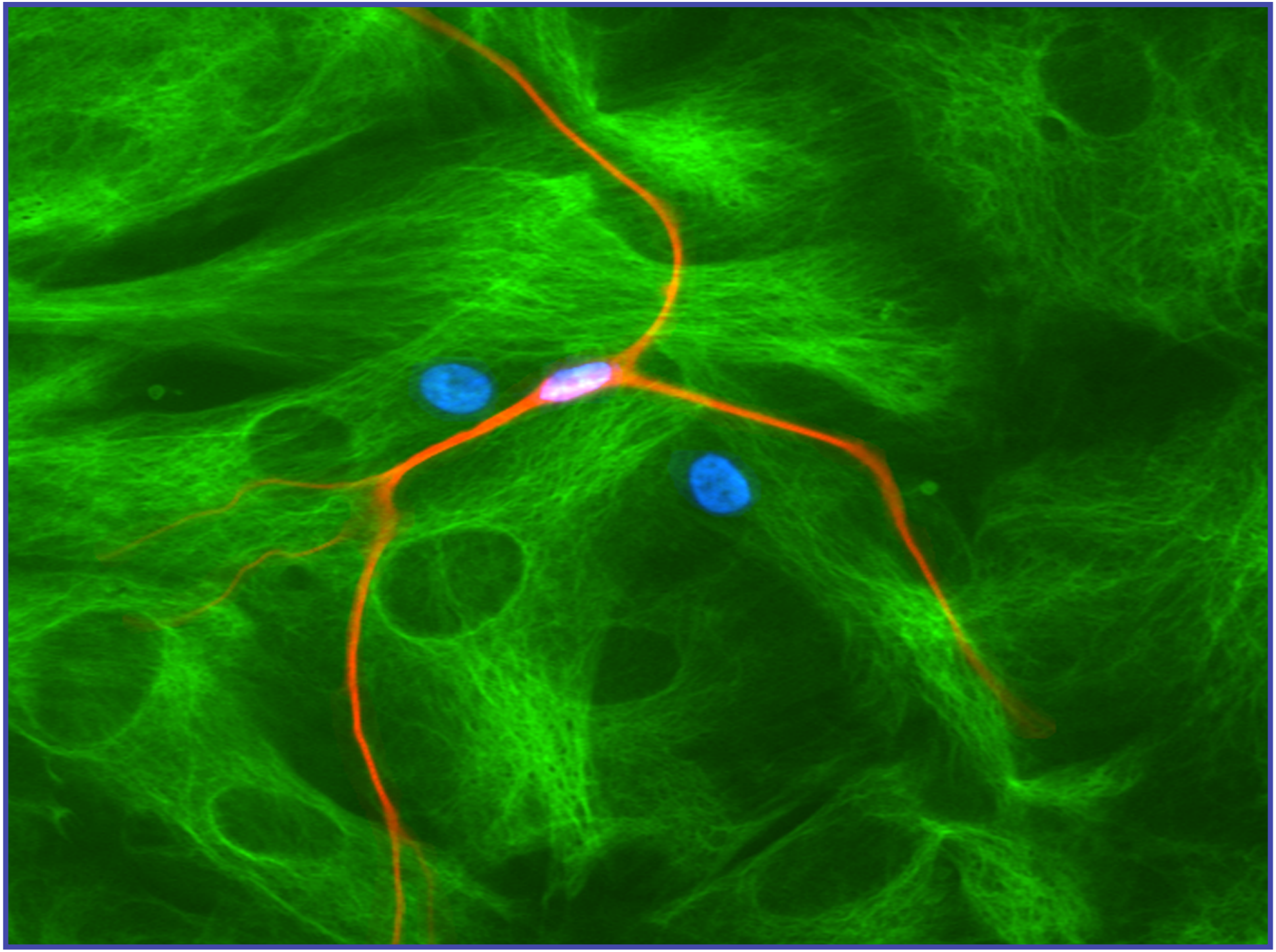
⬇ **DAPI**

Astrocytes clonally cultured on a cortical astrocyte Monolayer (no EGF/FGF)

⬇

✓ **5 of 64 SVZ astrocyte colonies contained new neurons**

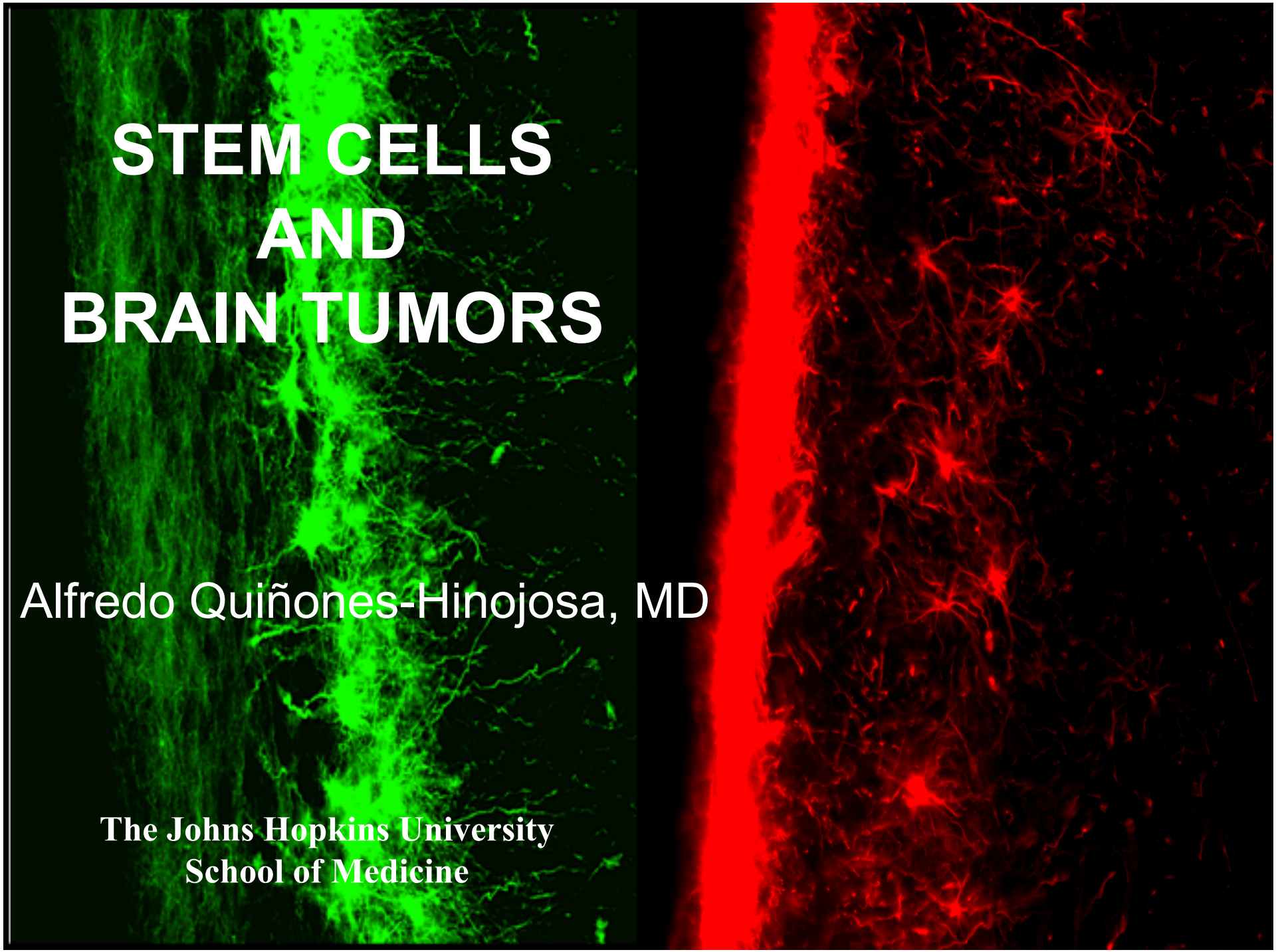
X **Cortical and striatal astrocytes: no neurons**



STEM CELLS AND BRAIN TUMORS

Alfredo Quiñones-Hinojosa, MD

The Johns Hopkins University
School of Medicine



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