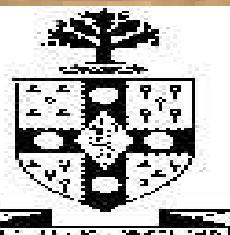


Primary Malignant Brain Tumor

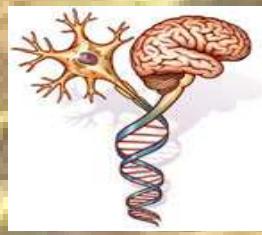
“An Untamed Tiger”

Abhijit Guha

Univ. of Toronto



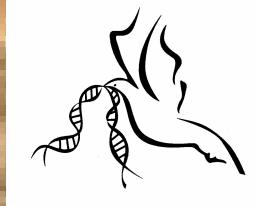
Univ. of
Toronto



Div. of Neurosurgery



Univ. Health
Network



Arthur & Sonia
Labatts Brain
Tumor Center



Prior Lab Members

Nelson Lau

Hao Ding

Matt Feldkamp

Collaborators

C. Stiles- PDGF, Signalling

* D. Gutmann- Transgenics, NF1

C. Kontos- Angiopoietin

D. Kaplan- Signalling/NF1

M. Moran- Proteomics

* B. Stanford- Gene Trapping

T. Pawson- Signalling

* M. Henkleman- Imaging

* A. Nagy- Transgenics

D. James- EGFR

* P. Shannon- Neuropathology



P. Dirks



A. Guha

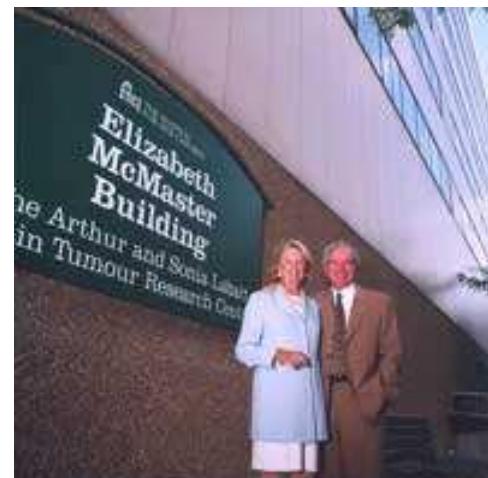


A. Huang



J. Rutka

Arthur & Sonia Labatts Brain Tumor Center



J. McGlade



M. Taylor



M. Bernstein



M. Fehlings



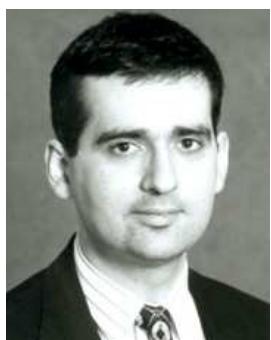
F. Gentilli



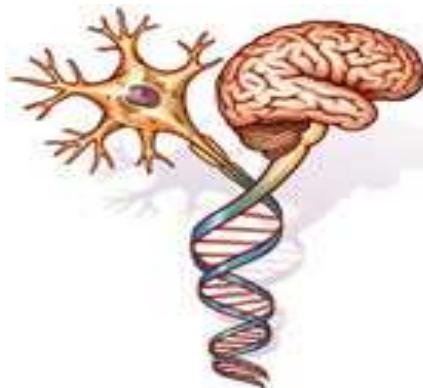
A. Guha



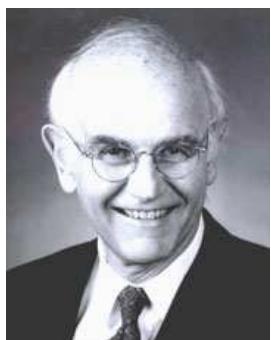
M. Hodaie



A. Lozano



**UNIV. of
TORONTO**



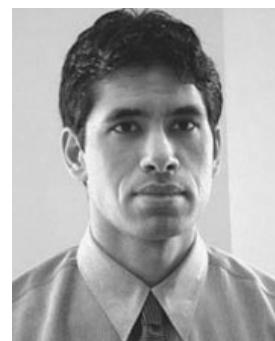
C. Tator



M. Tymianski



**UNIV. HEALTH
NETWORK**



T. Valiante



E. Masicotte



C. Wallace

DIVISION of NEUROSURGERY

1- Brief Review: Epidemiology- Clinical Presentation- Pathology

2- Current Knowledge of Molecular Pathogenesis of Gliomas

3- Major Obstacles in the Management of Gliomas:

- A: Current Management**
- B: Invasion**
- C: Angiogenesis**
- D: Resistance: Chemotherapy, Radiation**
- E: Tumor \longleftrightarrow Molecular Heterogeneity**

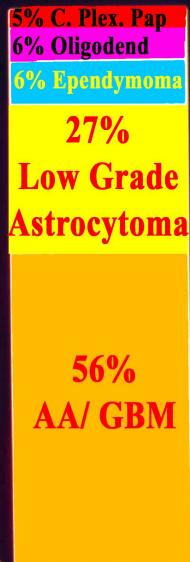
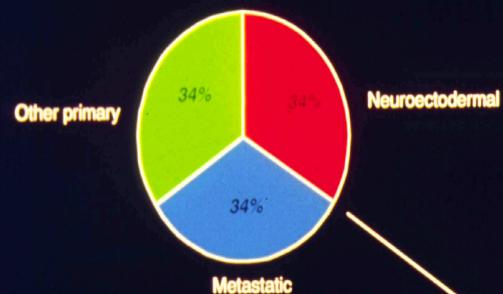
4- Novel Therapies:

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- A: Imaging \longleftrightarrow Biological/Molecular Correlates**
- B: “omics”: genomics/transcriptomics/proteomics**
- C: ??Cancer Stem Cells??**
- D: Genetically Engineered Murine (GEM) Glioma Models**

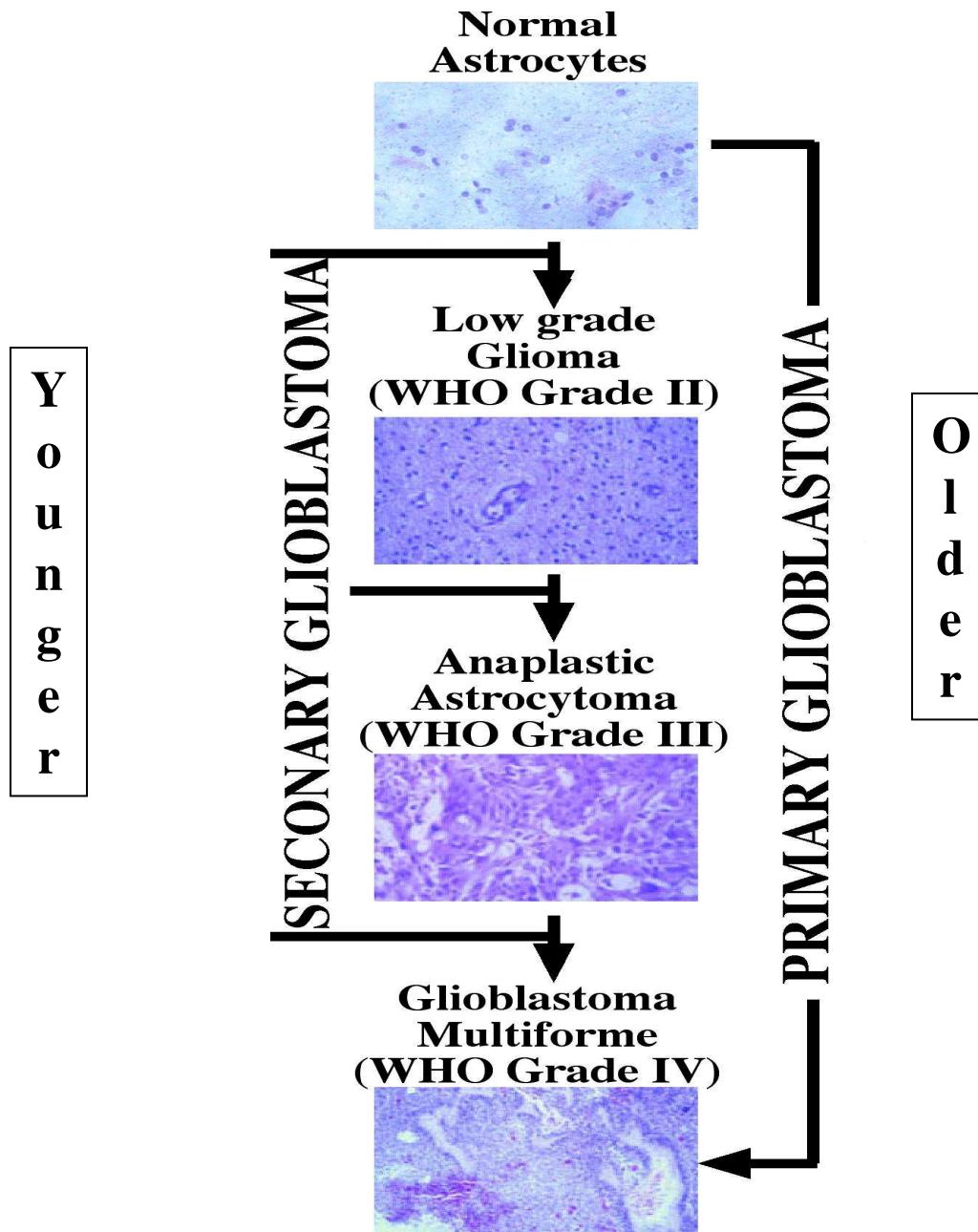
BRAIN TUMOURS (Zulch, 1996)



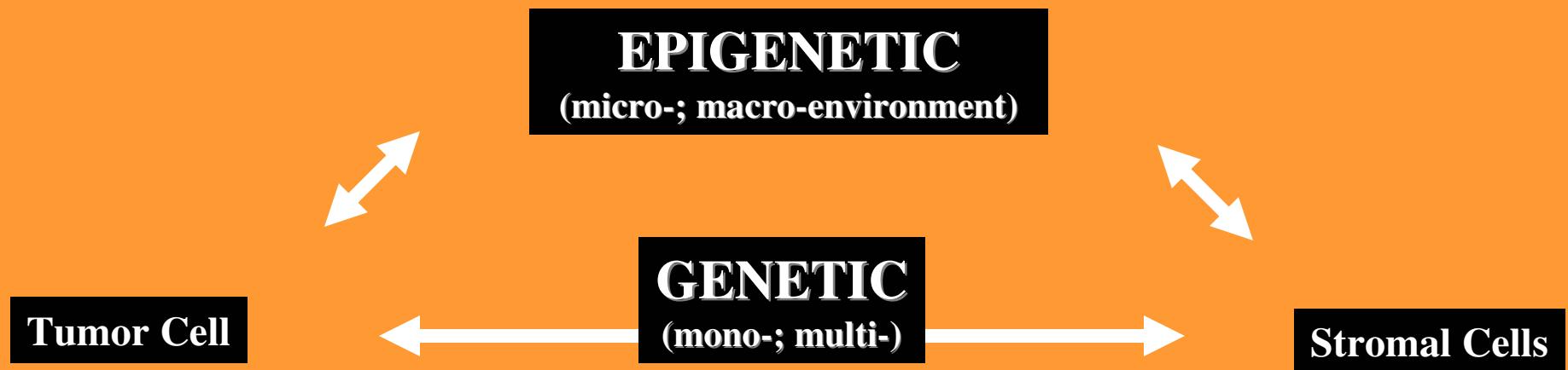
- * CANADA: 1500 cases/yr
- 4%: all cancer related deaths
- >95% spontaneous
- No environmental proven links
- Children: #1- Solid tumor,

- * Headache
- * Global Neuro Deficit
- * Focal Neuro Deficit
- * Seizures
- * Stroke

Adult ≠ Pediatric



Potential Benefits of Understanding the Molecular Genetics



- Understanding the molecular pathogenesis of gliomas: Pre-disposition & Sporadic
- Screening for “at risk” individuals
- Understanding “epigenetic” (tumor microenvironment) influences on glioma growth
- Development, characterization & utilization of GEMs
- Understanding ontogeny: CSC vs. Differentiated, Influence of genetic alterations
- Molecular classification to augment histopathological diagnosis
- Discovery, testing & monitoring response to conventional and biological Rx's

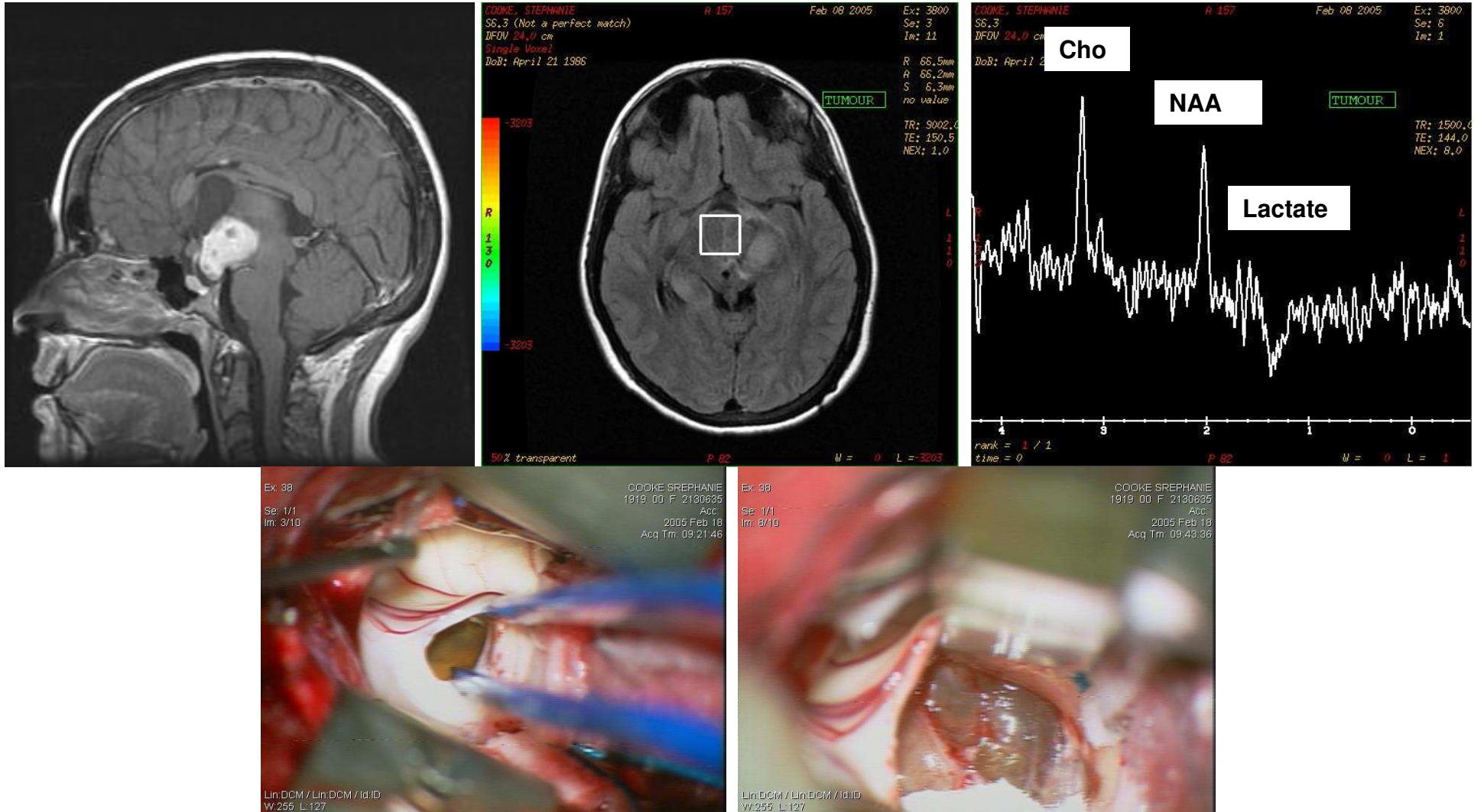
Familial Nervous System Tumor Pre-disposing Syndromes <5%

GEMs

<u>Syndrome</u>	<u>Gene</u>	<u>Chrom</u>	<u>Tumor Types</u>	<u>+/-</u>	<u>-/-</u>
NF1	Nf1	17q11	PNT, ONG, Pilocytic	▲ Astrocytoma	lethal*
NF2	Nf2	22q12	Schwannoma, Meningioma Astro, Ependymoma	Osteosarcoma	lethal*
Tuberous Sclerosis (TSc)	TSC1	9q34	SEGA		Eker rat
	TSC2	16p13			
Li-Fraumeni	TP53	17p13	Astro, PNET	Non CNS	Non CNS
Cowden	PTEN	10q23	Lhermitte-Duclos Dysplastic Ganglio	Non CNS	lethal*
Turcot	APC	5q21	Medulloblastoma	Non CNS	
	hMLH1	3p21	Glioblastoma		
	hPSM2	7p22			
Nev Bas Cell	PTCH	9q31	Medulloblastoma	Non CNS	lethal
Carc Syndr (Gorlin)				Medulloblastoma	

? Molecular Biology of Pre-disposition Syndromes ?

- **Pathogenesis of Sporadic tumors ~ Pre-disposition tumors**
- **Screen “at risk” pre-disposed individuals**
- **Develop GEMs based on identified genetic alteration to study:**
 1. role of implicated gene in development
 2. role of implicated gene in tumor development, ontogeny & progression
 3. interaction of implicated gene with other genetic alterations in tumor development
- **Risks of secondary carcinogenesis due to Rx (Rad, Chemo)**



Similarities

Increased Ras

Increased PI3K

Differences

Sporadic: Increased RTKs

NF1: Decreased Ras-GAP (Nf1-GAP)

(Lau, Gutmann, Guha- JNEN, 2000)

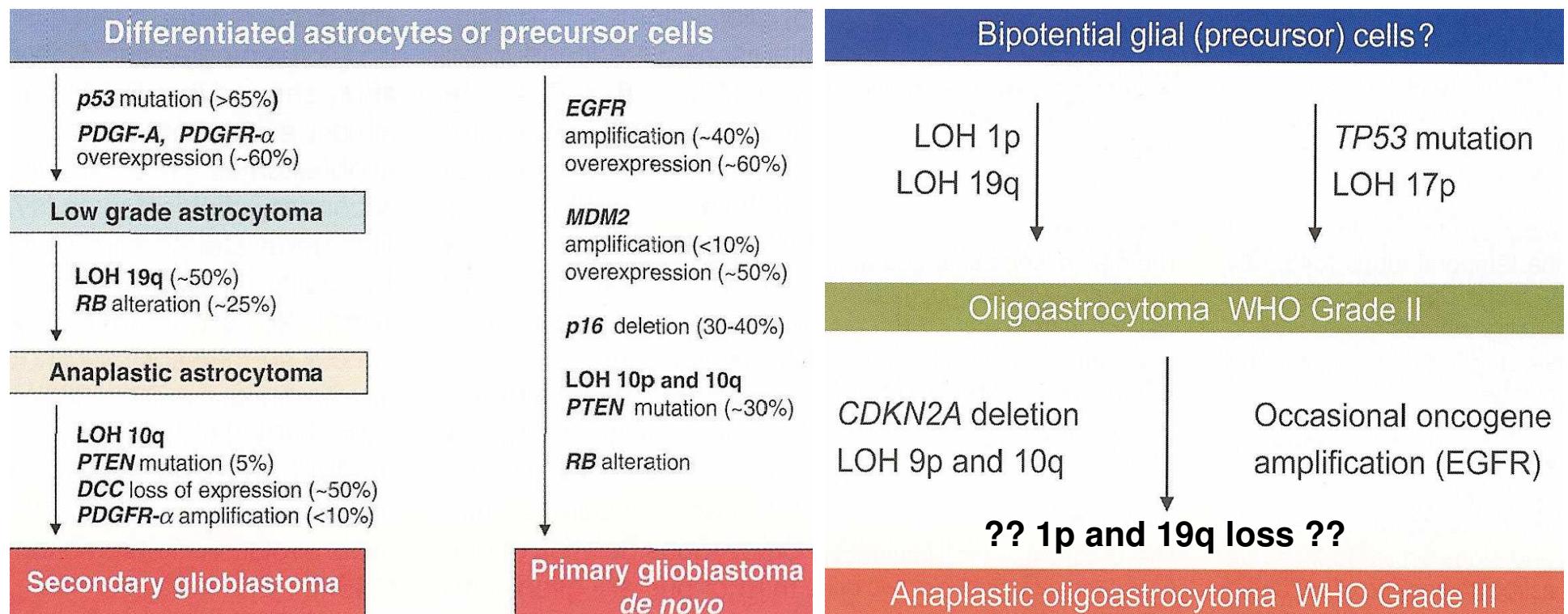
Critical genetic pathways in sporadic gliomas

Cell cycle genes

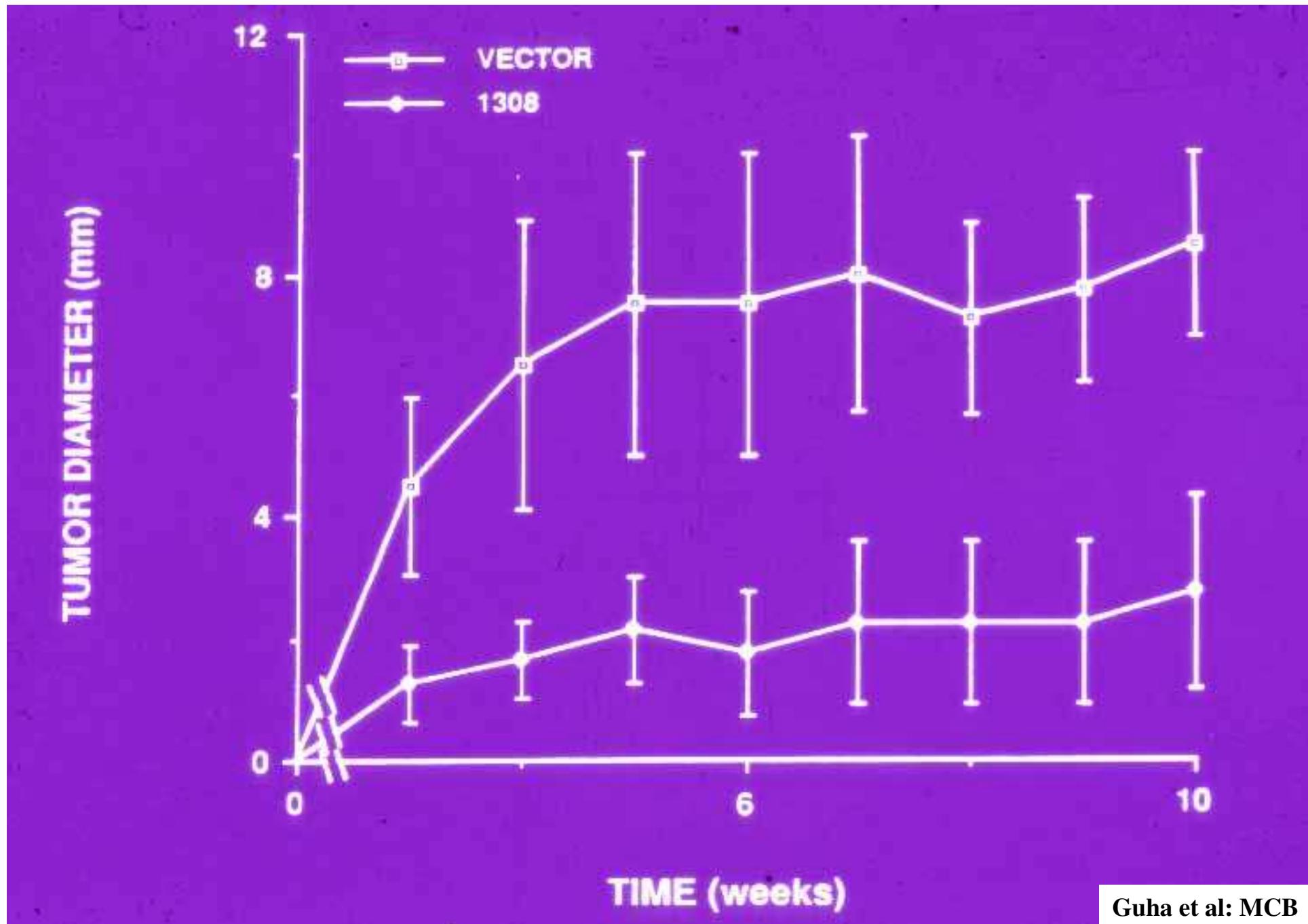
- Rb pathway- Rb, p16, CDK4
- p53 pathway- p53, MDM2, p19

Aberrant Signaling

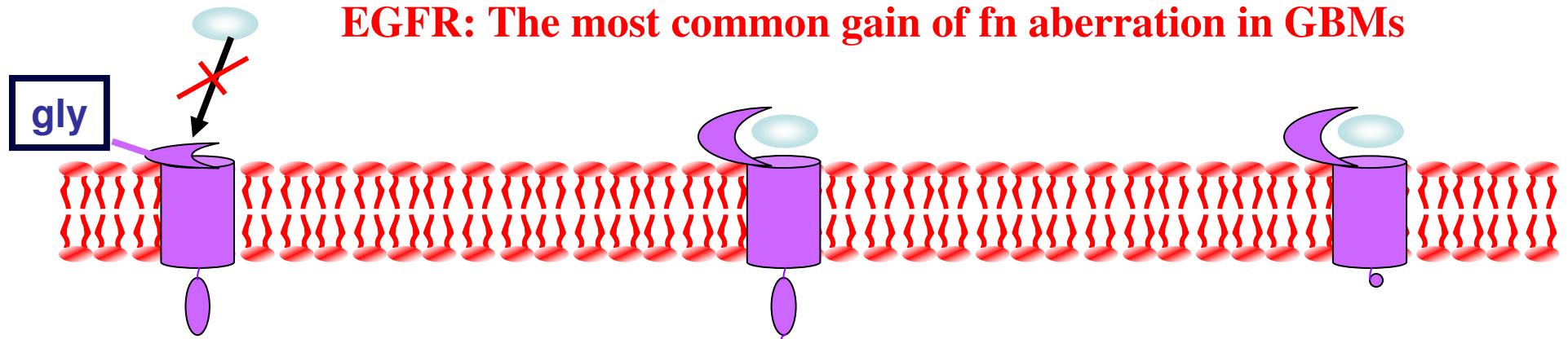
- GF & RTKs: EGFR, PDGFR, VEGFR etc.
- Signaling: G-proteins (Ras, Rac), PI3K, Jak-Stat



Inhibition of PDGF Autocrine/Paracrine Stimulation in Gliomas



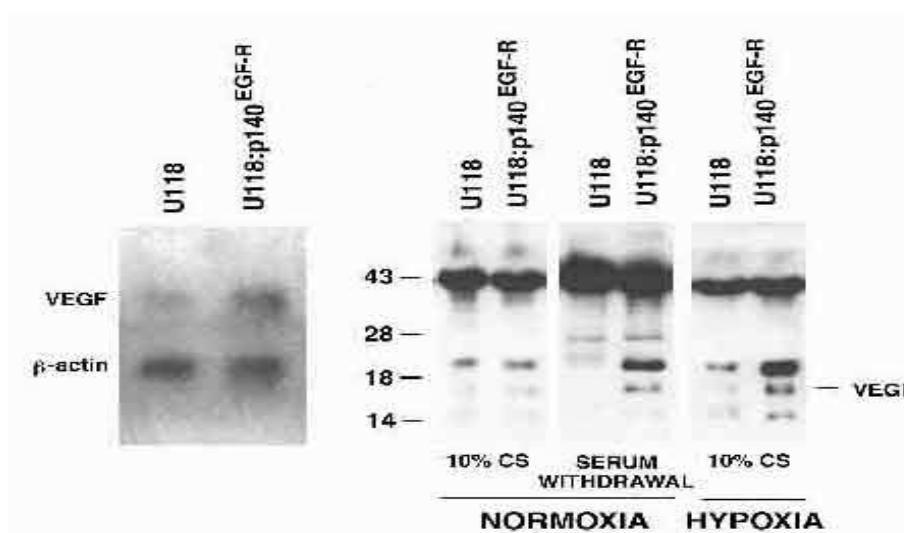
EGFR: The most common gain of fn aberration in GBMs



EGFRvIII
40% of Ampl

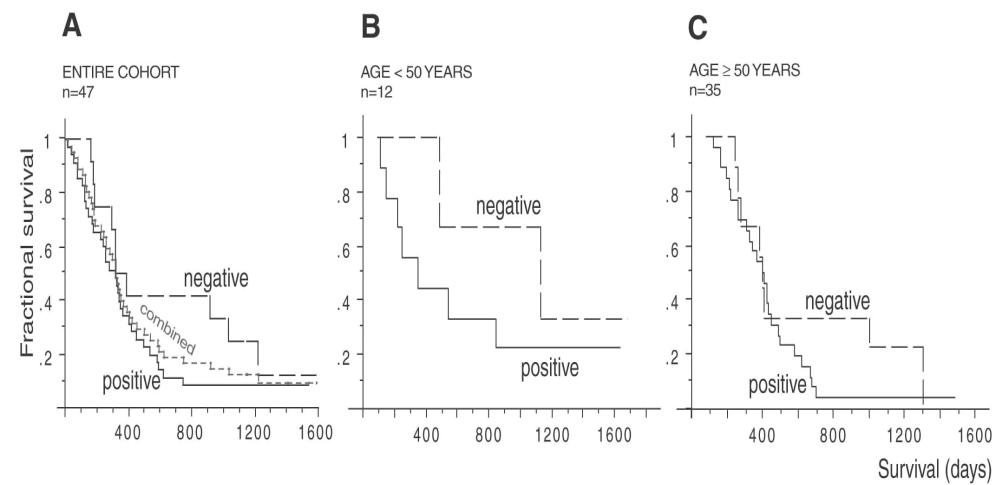
wt EGFR:
Ampl-50% of
GBMs

EGFR C958
15% of Ampl
(only in EGFRvIII)



EGFRvIII

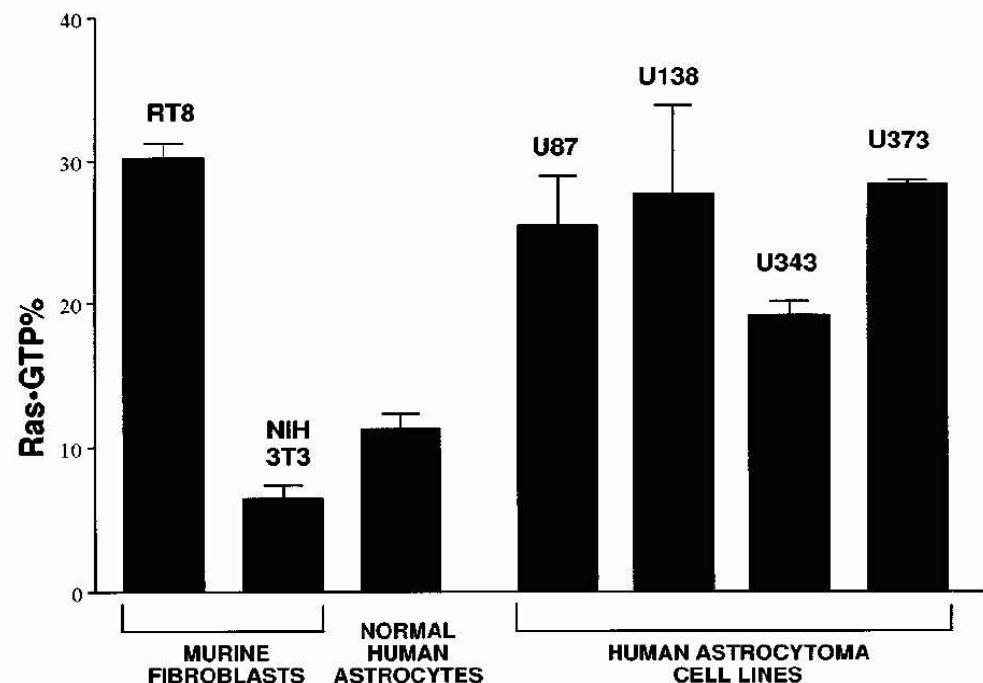
- Increased Angiogenesis (VEGF-Guha et al)
- Increased Invasion (MMP-J. Uhm)



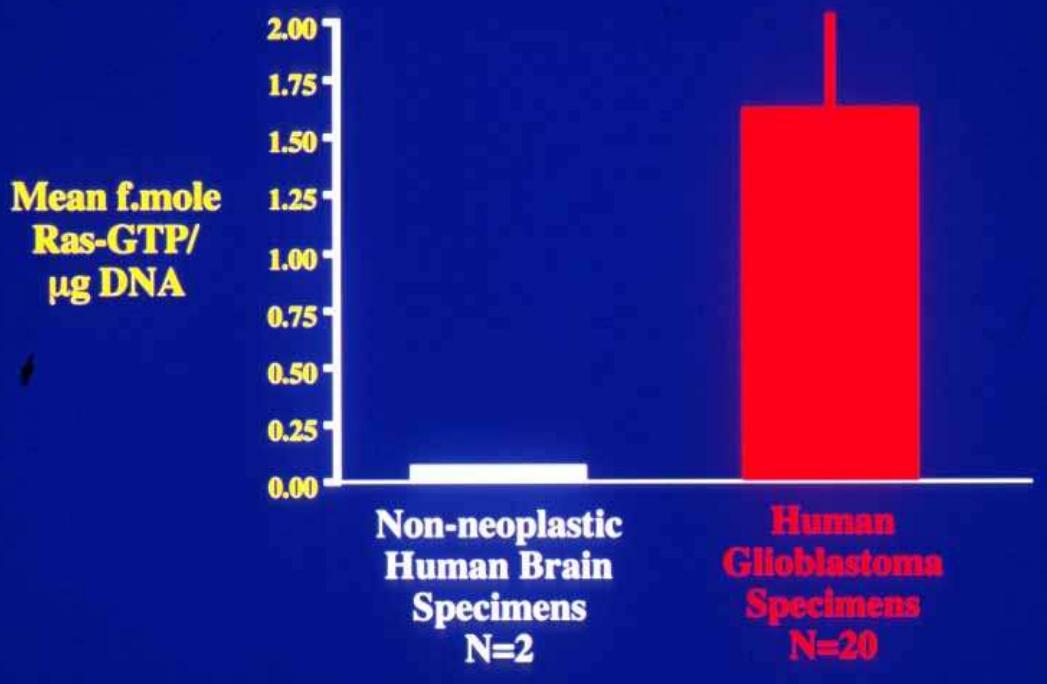
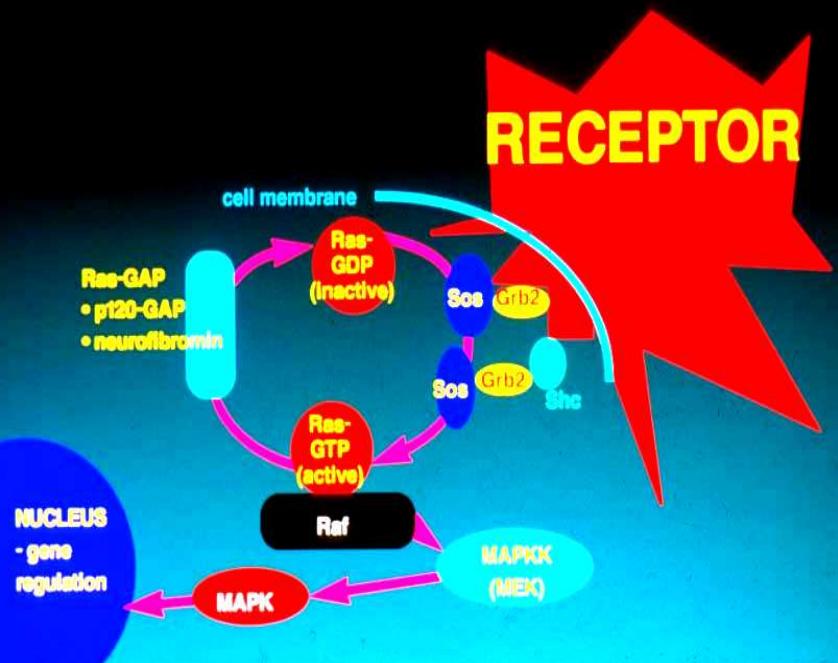
- Negative Prognosticator <50 yrs

Incidence of Ras mutations in human cancer

Glioblastoma multiforme	0 %
Breast	0-8
Ovary	0
Cervix	0
Gastric adenocarcinoma	0
Sarcomas	0
Bladder carcinoma	7-17
Lung adenocarcinoma	22-33
Colon adenocarcinoma	40-47
Thyroid follicular adenocarcinoma	53
Acute myeloid leukemias	19-70
Pancreas adenocarcinoma	84-93
Leukemias	
AML	19-70
ALL	0-18
CML	0-50
Lymphomas	0



RECEPTOR



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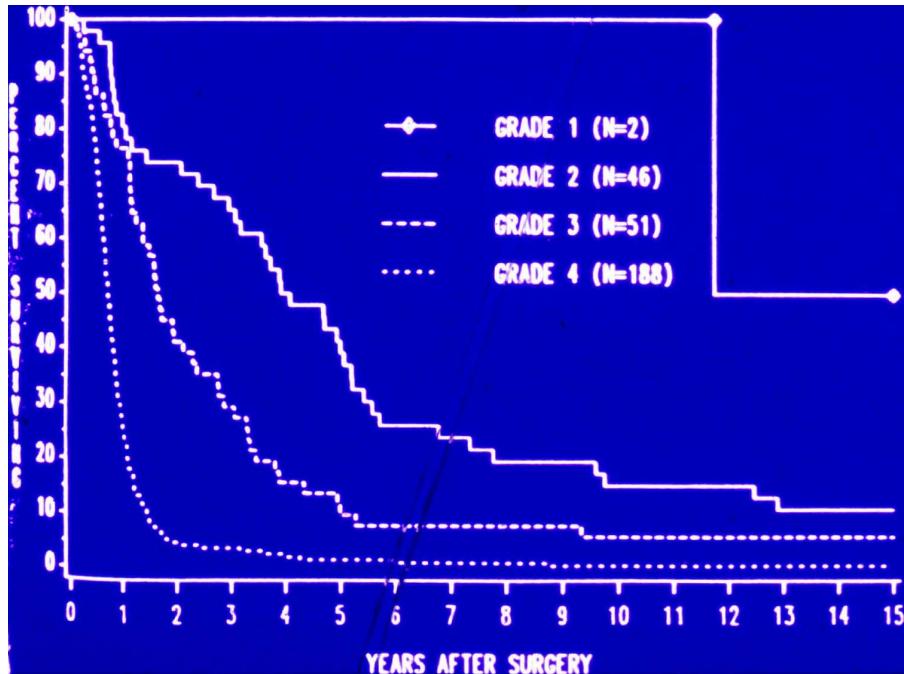
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Why Surgery

- Diagnosis +
- Debulk to relieve increased pressure +
- Neurological improvement +/-
- Prognosis +/-
- Total vs sub-total +/-
- Improve effect of additional Rx +/-

Concomitant Radiation + Chemotherapy

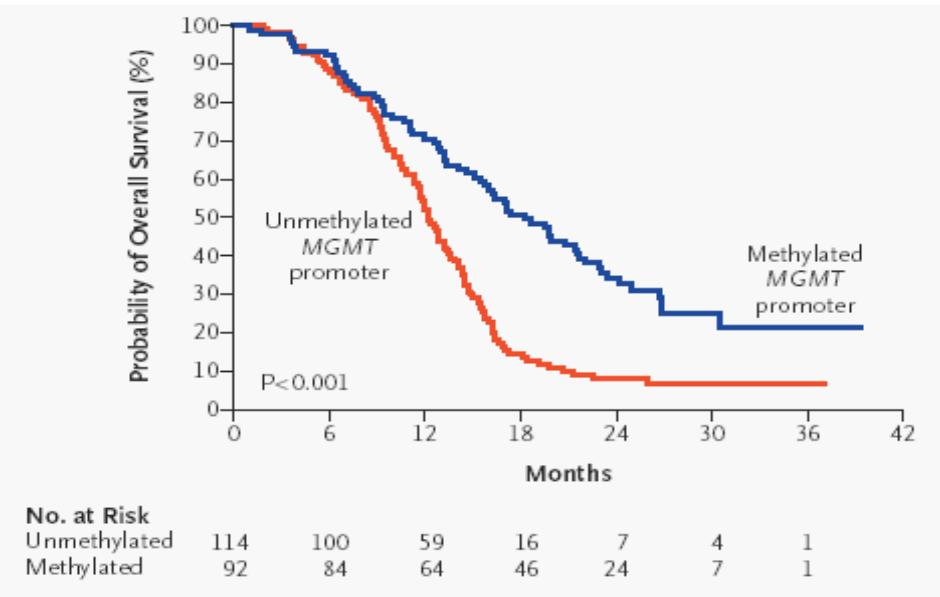
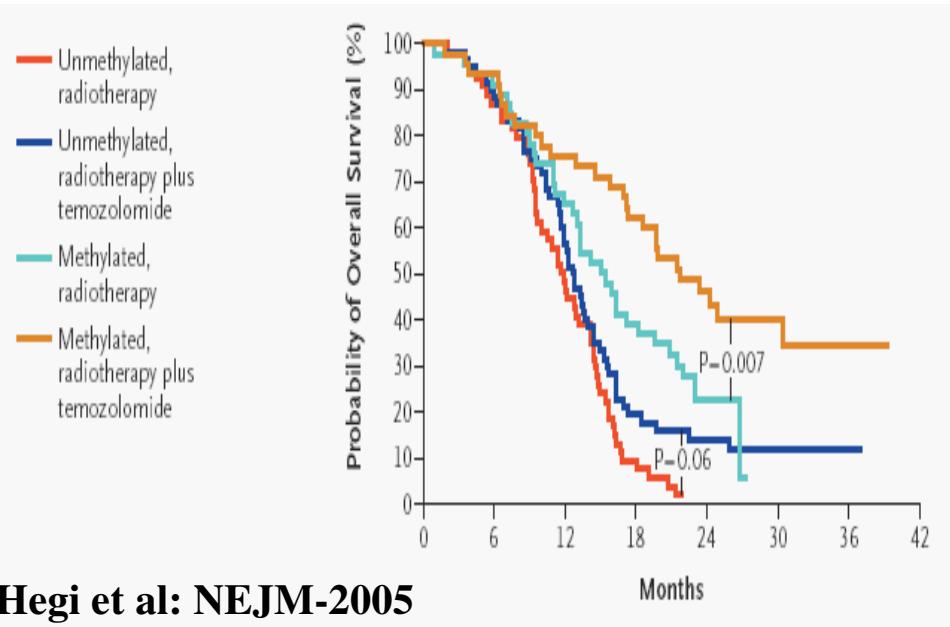
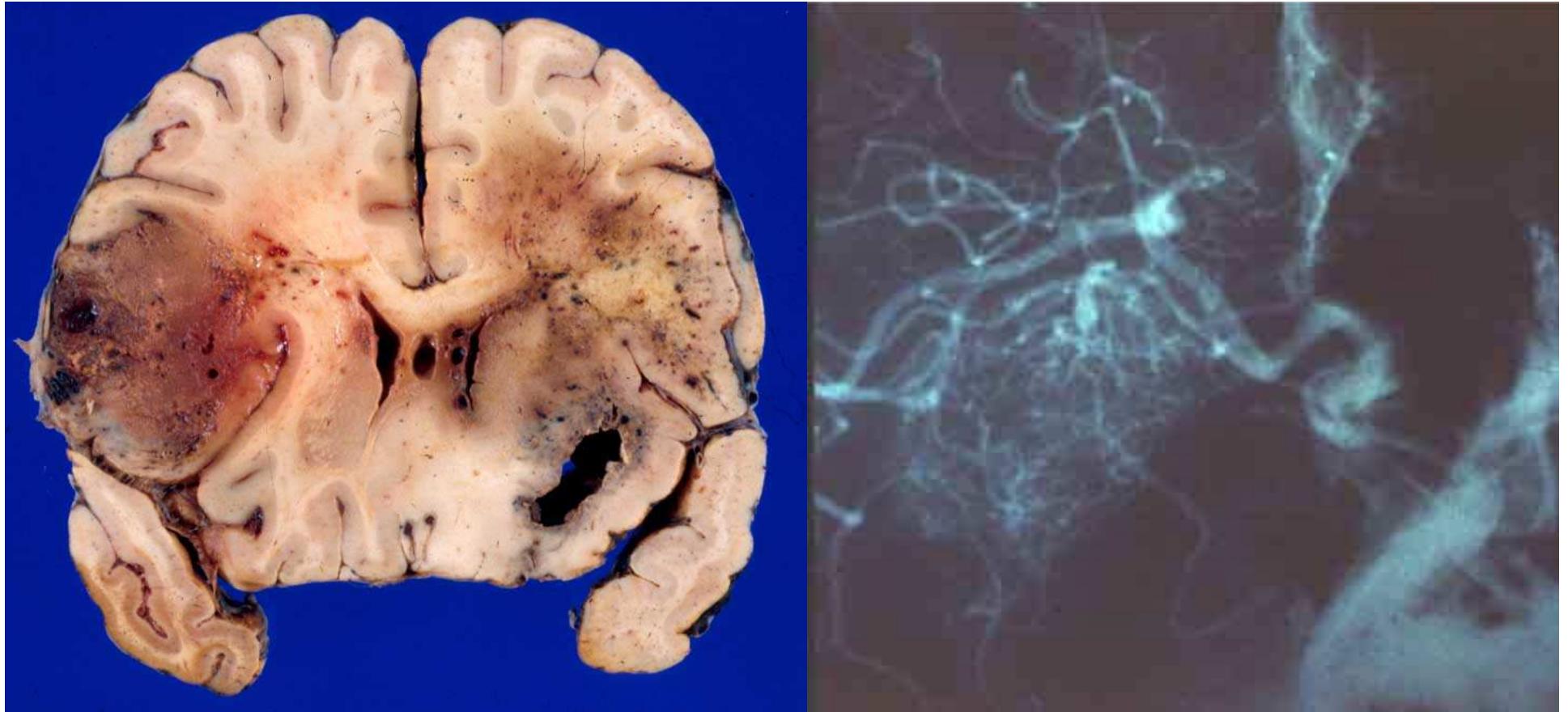


Figure 2. Kaplan-Meier Estimates of Overall Survival, According to *MGMT* Promoter Methylation Status.



Hegi et al: NEJM-2005



***Infiltrative, Multifocality**

*** Angiogenic, Edemogenic**

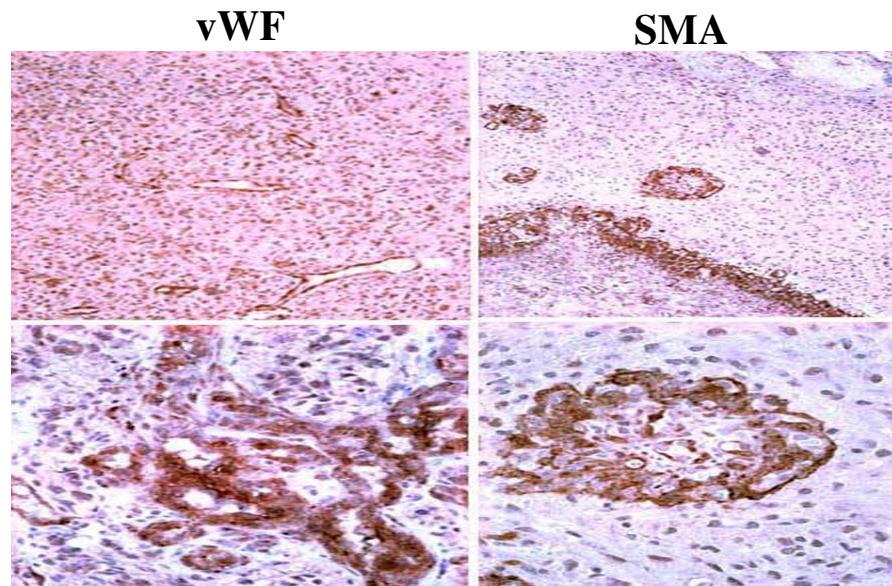
*** Neural toxicity, Eloquence**

*** Heterogeneity: Path/Molecular**

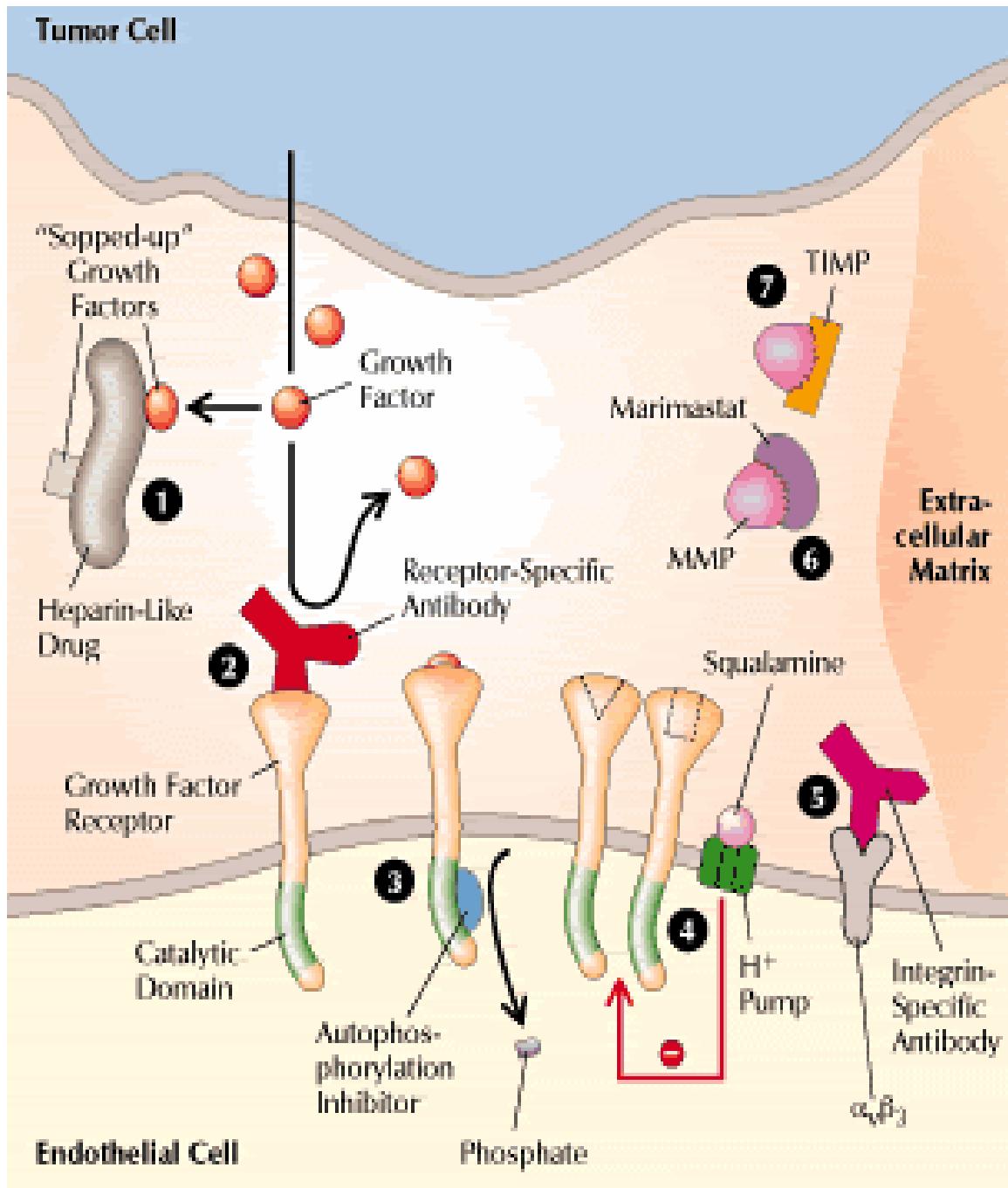
*** Resistance, BBB**

Pathology of GBM Vasculature

- **GBM- highly vascularized cancer; 40X increased angiogenesis**
- **GBM EC- 12.5% proliferation; t_{1/2} (days):1-10 c/w 80(lung); 8000 (brain)**
- **Abundant and abnormal vessels:**
 - Highly heterogeneous vascular morphology
 - Tortuous sinusoidal vessels of varying diameter
 - Low functional perfusion of tumor vessels: Only 50-70% of the capillaries are perfused compared to >95% of the normal cerebral capillaries, increased resistance in the microvasculature
 - Endothelial Hyperplasia, SMC, PC
 - Abnormal BBB- Edema
 - Dysautoregulation;Intratumoral Hemorrhage



Overview of Anti-Angiogenic Strategies



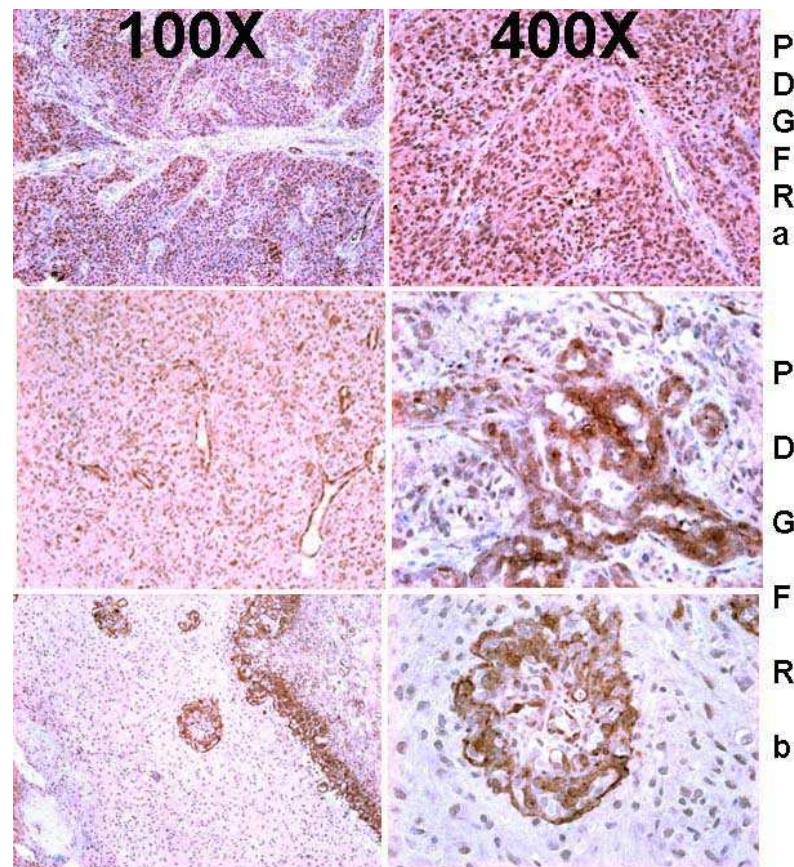
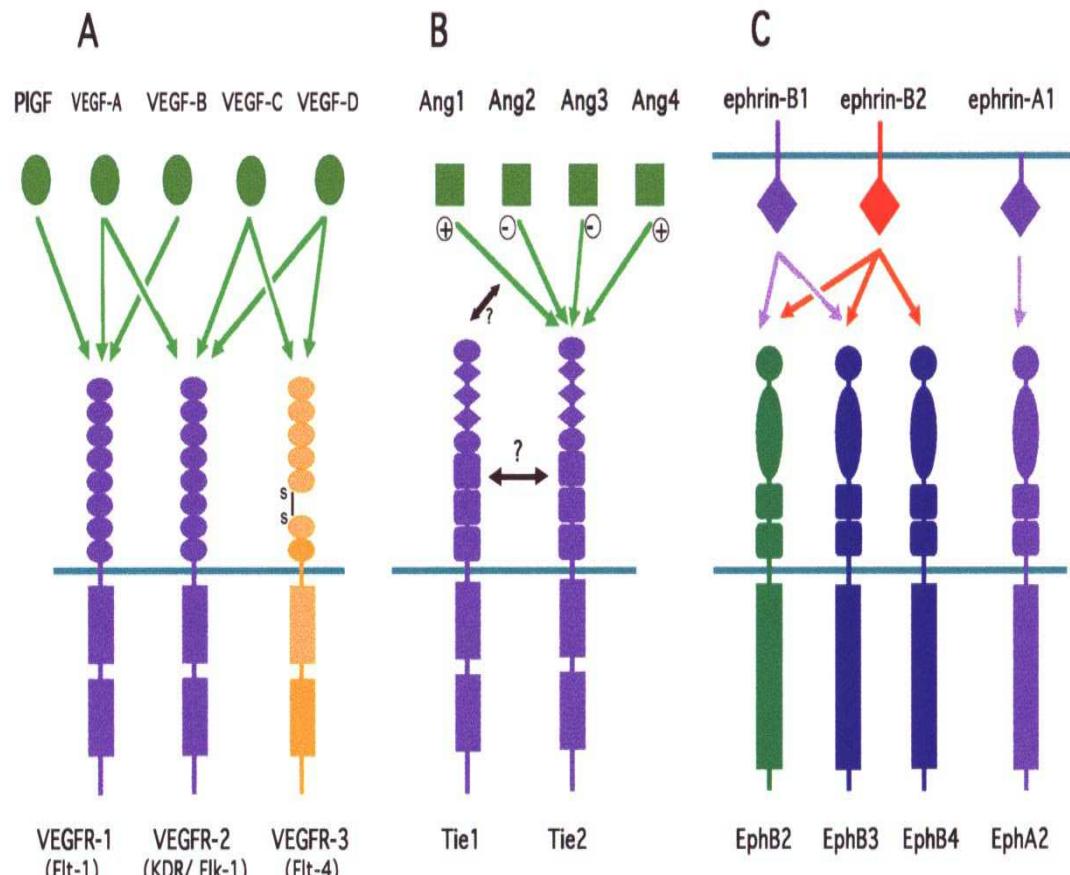
Theoretical Advantages

- 1: Minimal Side Effects-** Mainly downregulated in the healthy adult
- 2: Decreased Resistance-** Tumor angiogenesis is a physiological host mechanism...Long term Rx
- 3: Potentiation of Anti-tumorigenic Effect-** Each tumor capillary supplies hundreds of tumor cells
- 4: Easy Access of EC to Therapies-** Direct contact of the vasculature to the circulation ??? No concern of BBB???
- 5: Can Synergize with Radiation/Chemo-** By modulating tumor hypoxia, drug delivery etc.
- 6: GBMs are highly angiogenic**
but
no conclusive proof of benefit in majority of clinical trials

EC Specific

Mitogenic
Angiogenic Link

EC Non-specific



KEY: Ig-Like Loop EGF-Like FN3III-Like Cystein Rich Kinase

Anti-VEGF Strategies

Targeting VEGF

Neut Ab-VEGF
SolubleVEGFR
Ribozymes
Anti-sense

Targeting VEGFR and its Signaling

Neut Ab- Prevent dimerization
Tyrosine Kinase Inhibitors
p21-Ras, PI3K

SU11248

Split Kinase Inhibitor

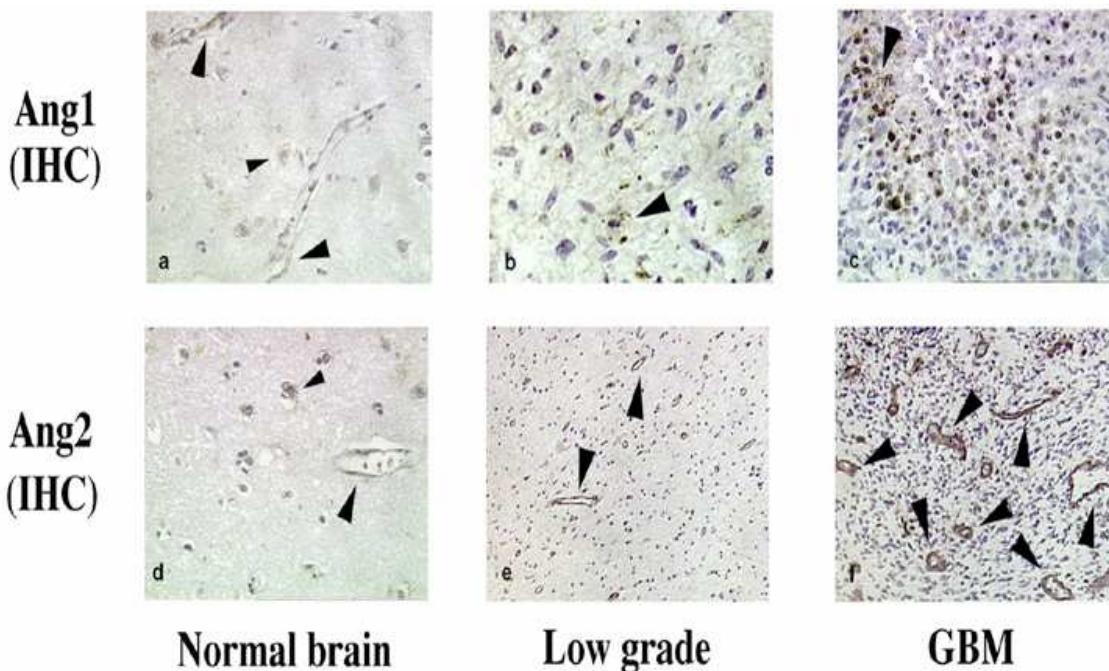
VEGFRs, PDGFRs, FGFRs

Targeting Regulators of VEGF

Regulators of HRE: HIF1a
Regulators of Signaling Pathways: p21-Ras, PI3K



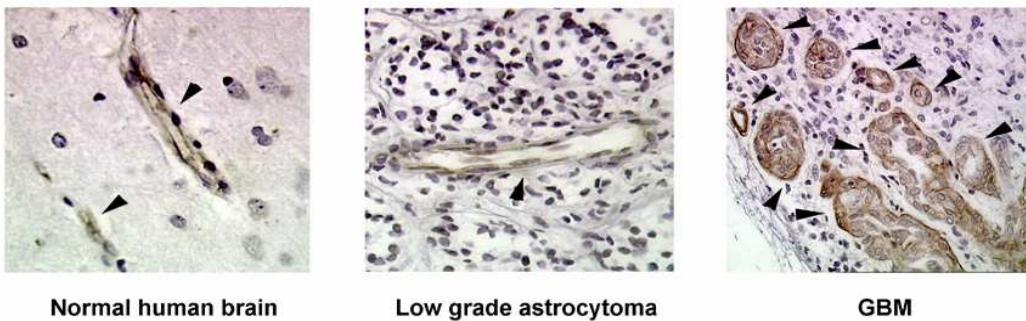
Expression of Ang's/Tie2 in Human Astrocytomas



Ang1 expressed by Tumor Cells
GBM>LGA>NB

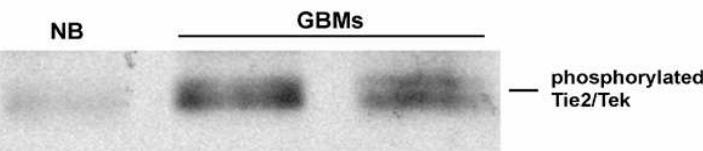
Ang2 expressed by EC
GBM>LGA>NB

A. Immunohistochemistry



Tie2 expressed by EC
GBM>LGA>NB

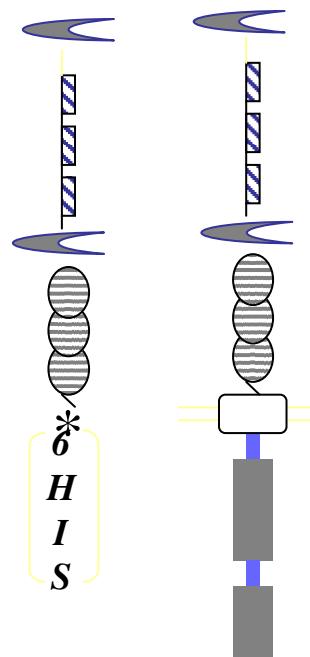
B. Western Blot



Zadeh, Ding'00

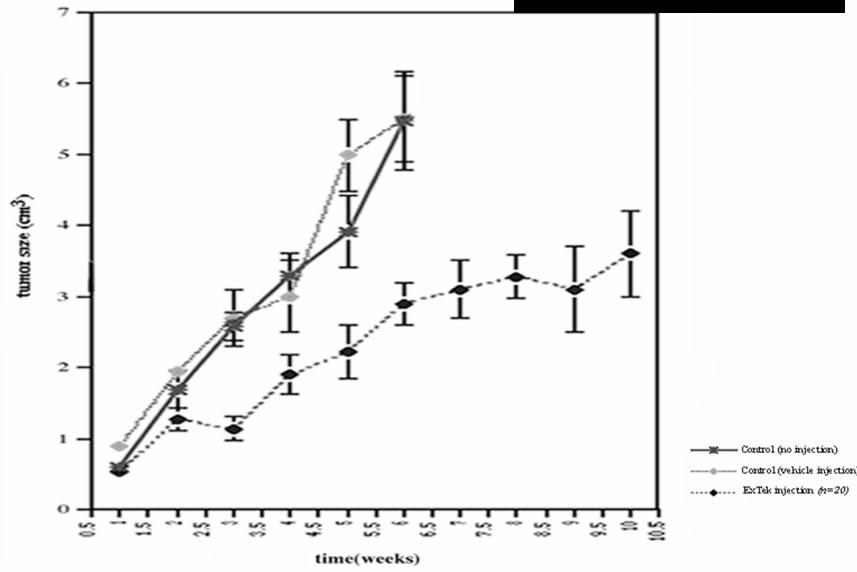
Robust Tie2 phosphorylation

Effect of ExTek on GBM Xenograft Growth/Angiogenesis



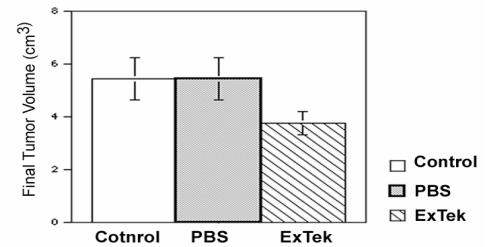
A.

Subcutaneous

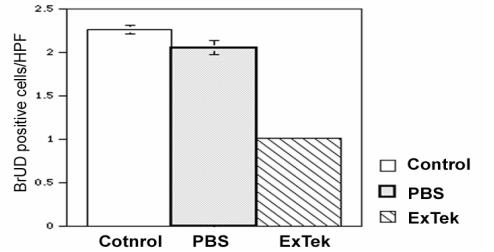


B.

Final Tumor Volume

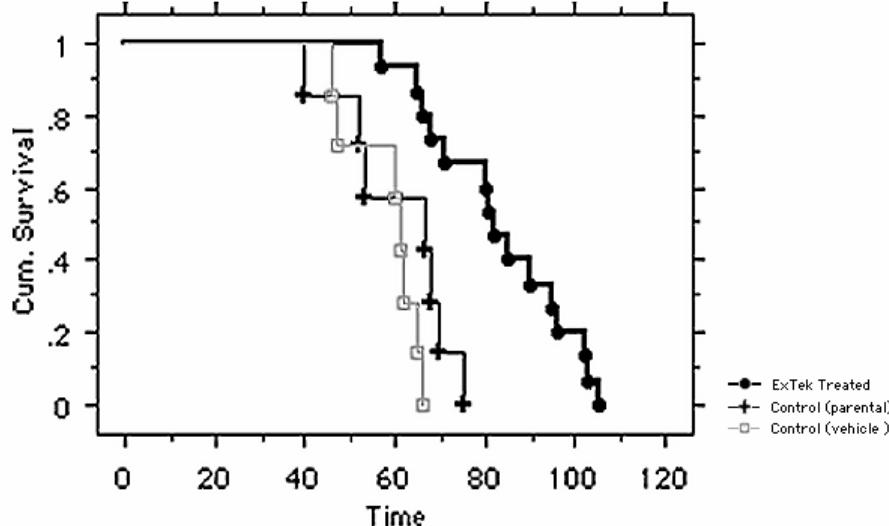


C. Tumor Proliferation



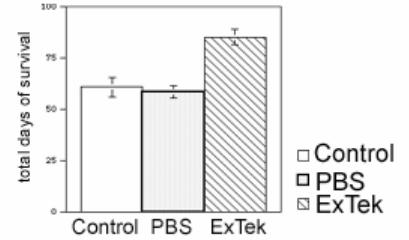
A.

Intracranial

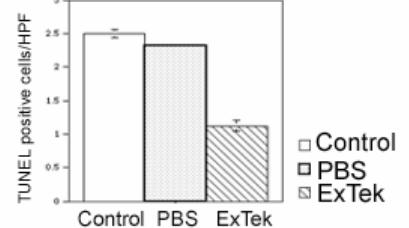


B.

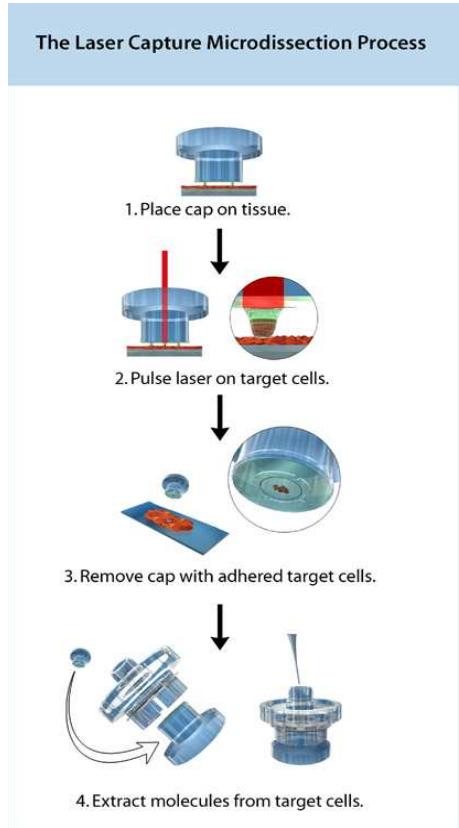
Total Survival



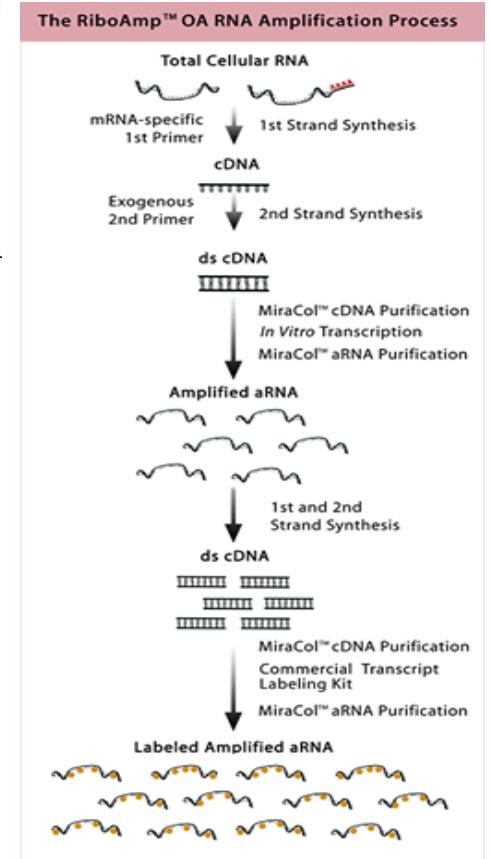
C. TUNEL stain



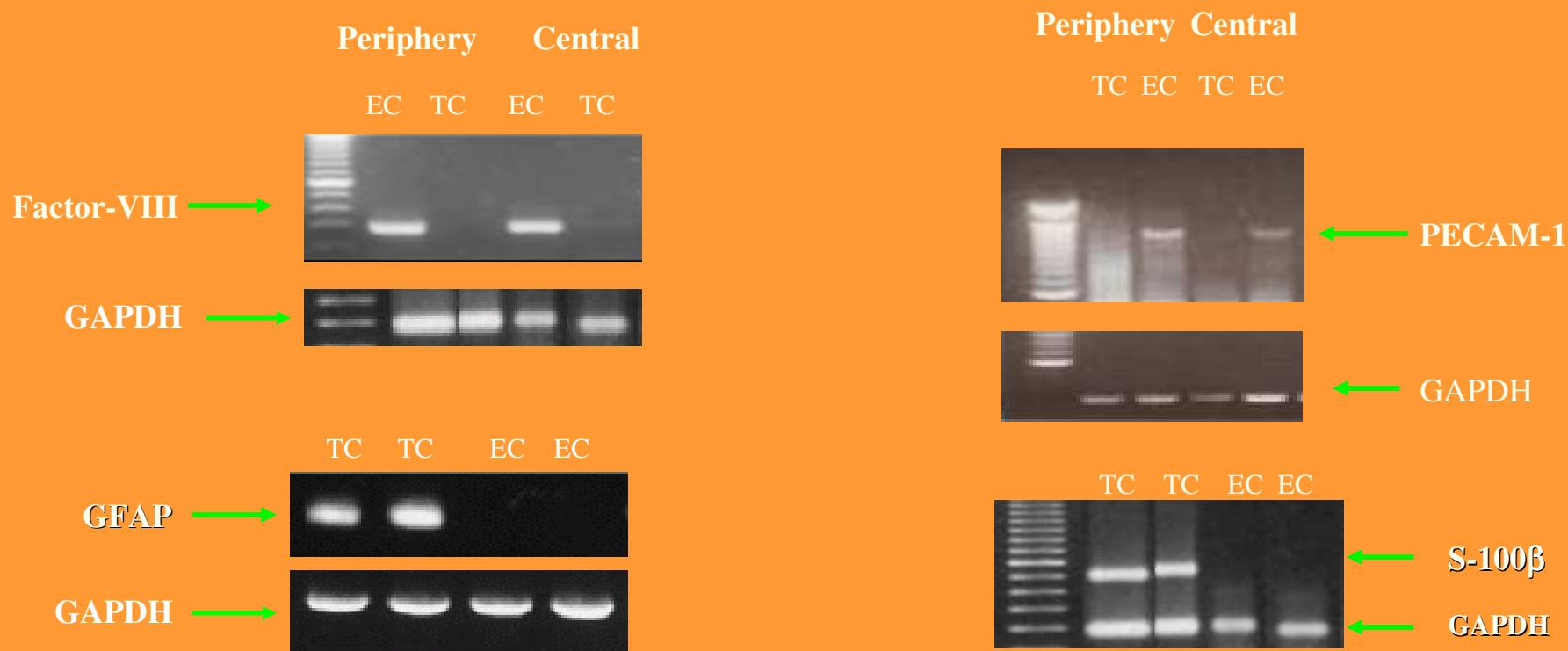
TUMOR HETEROGENEITY



Progression	Normal Brain	Infiltrating Astrocytoma (WHO grade II)	Anaplastic Astrocytoma (WHO grade III)	Glioblastoma Multiforme (WHO grade IV)
Biology		Infiltration	Proliferation/expansion	Hypoxia/necrosis
Genetic events		p53 loss	p16, p14ARF loss PDGFR amplification	EGFR amplification chromosome 10 losses (PTEN)
Angiogenic events		VEGF ↑	PDGFR ↑ Angiogenesis	VEGF ↑↑ Microvascular hyperplasia



LCM isolated Tumor vs. EC specificity analysed by reverse-transcriptase PCR using Factor VIII & PECAM-1 (EC) plus GFAP & S-100 β (Astrocytoma Cells)



Quantification of mRNA expression of VEGF and its receptors

No. of Sample used = 16

Primer	Fold Increase	Cell type	Average C _T from three independent experiments.	
			Infiltrating Zone	Peri-necrotic Zone
VEGF-R2/ Flk-1	5.1	Endothelial Cells	28.05 ± 0.09	17.31 ± 0.15
VEGF-R1/Flt-1	4.5		33.09 ± 0.10	21.52 ± 0.12
GAPDH	-		32.11 ± 0.02	31.72 ± 0.07
VEGF	6.2	Tumor cells	29.51 ± 0.13	16.35 ± 0.06
GAPDH	-		34.56 ± 0.07	33.89 ± 0.03

EC : VEGFR1/Flt-1 is regulated by hypoxia
VEGRR2/Flk-1 is regulated by hypoxia

TC : VEGF is regulated by hypoxia

GAPDH: Control with no change by hypoxia

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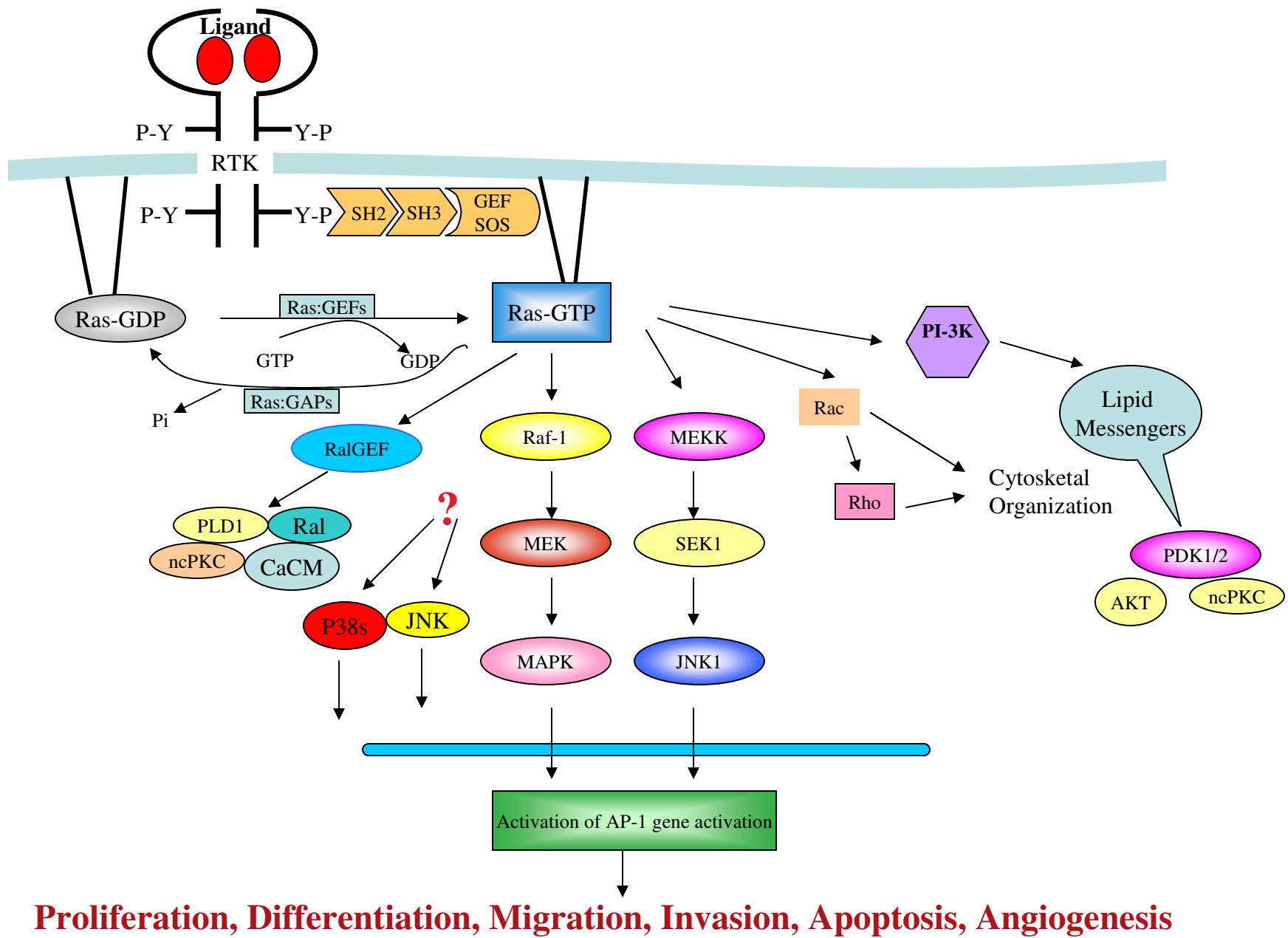
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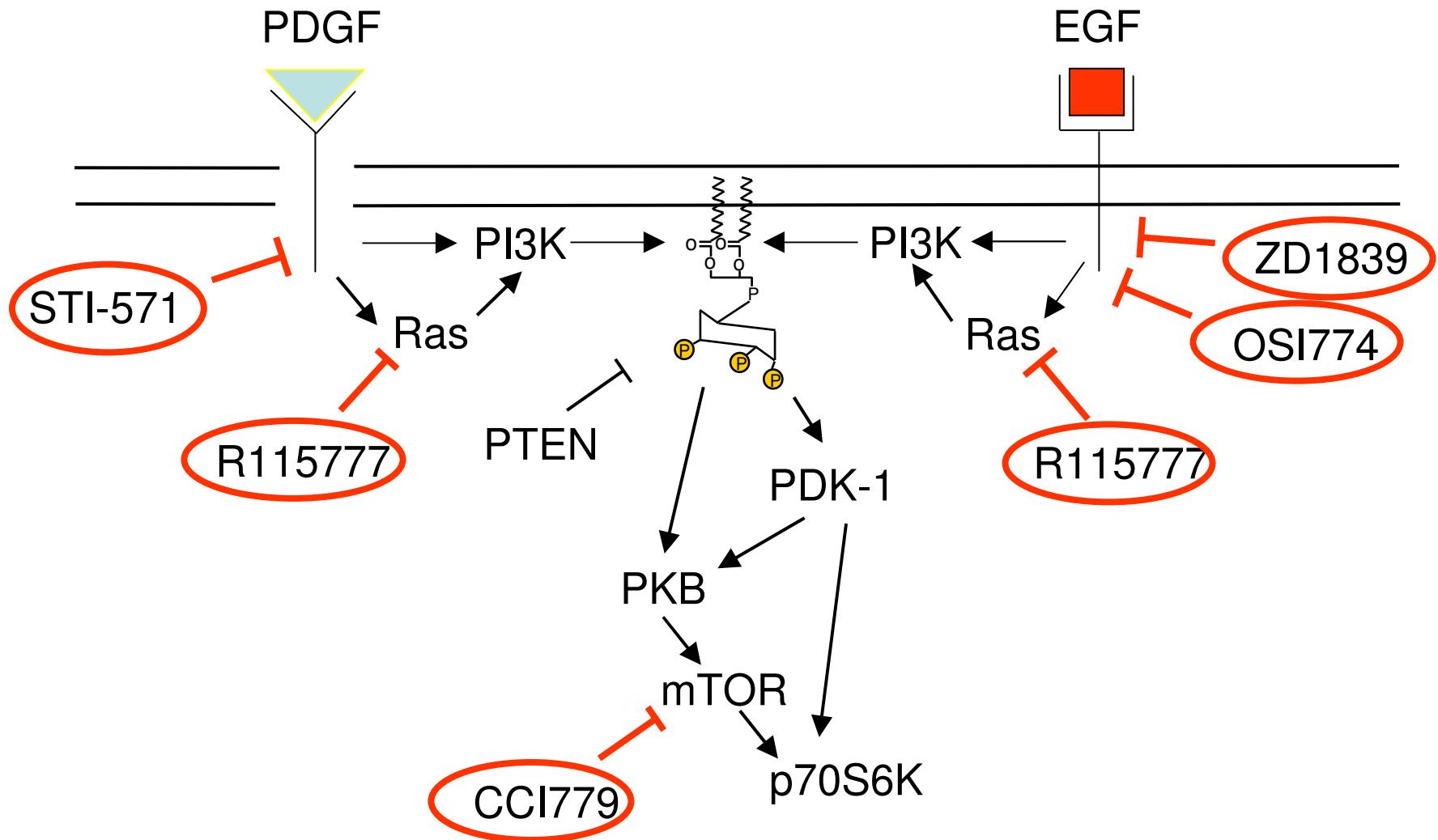
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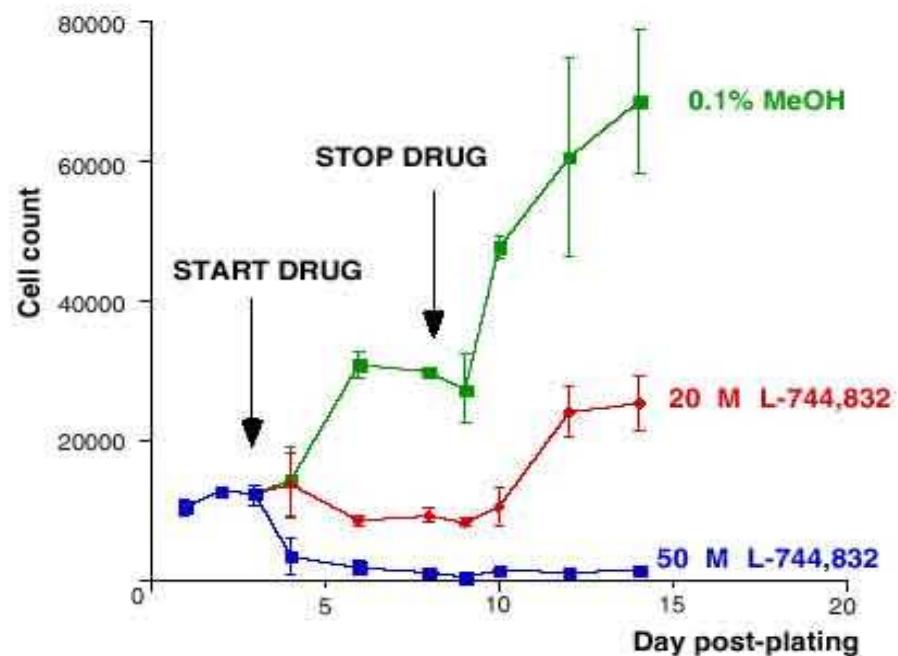
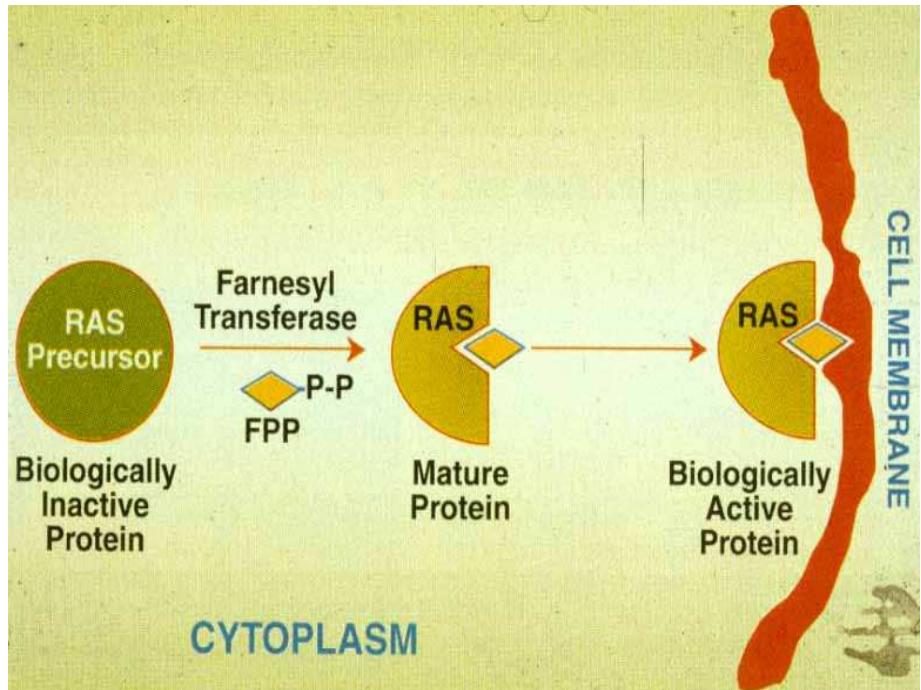
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Growth Factor and Signaling Pathways

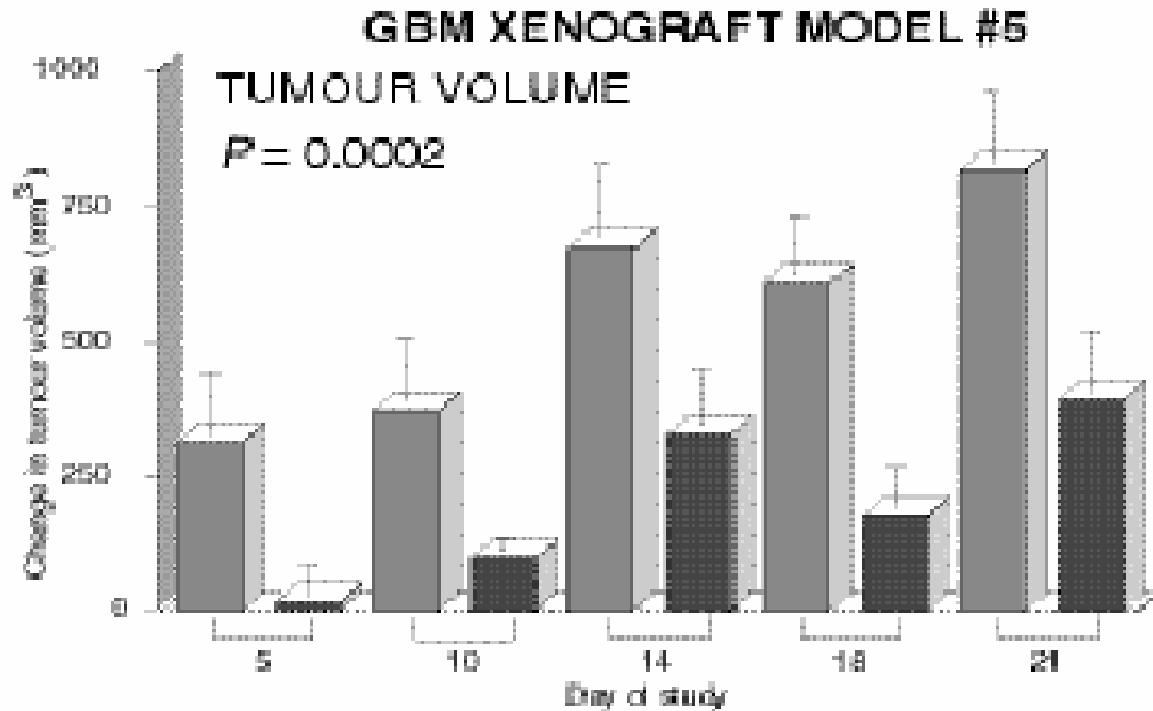


Variety of Targets Against Signaling Complexes



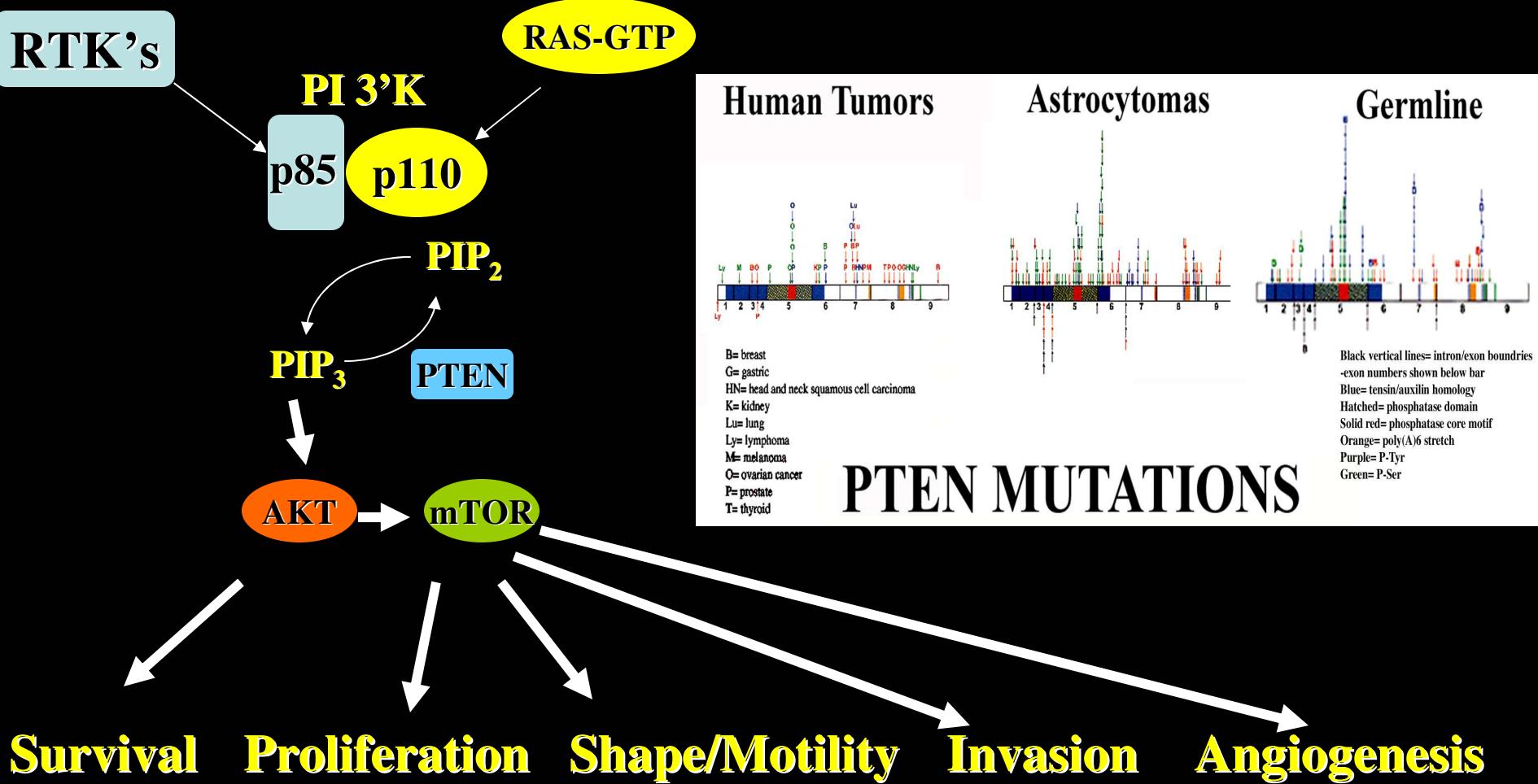


**SCH:66336
(50 mg/kg bid)**



Feldkamp, Guha:
Oncogene'00, CRes'02

PI3' Kinase Signaling in Astrocytomas



Horizon + Future: SMI against Akt, mTOR and its effectors

Angiogenesis inhibitors in clinical trials

Drug	Mechanism	Trial
Marimastat Bay 12-9566 AG3340	Synthetic MMP Inhibitor Synthetic MMPI Synthetic MMPI	Phase III for cancer of breast, lung(NSCL), lung, glioblastoma Phase III for carcinoma of lung, ovary, pancreas Phase III for lung (NSCLC), prostate
CGS 27023A AE-941 (Neovastat) BMS-275291 Penicillamine	Synthetic MMPI Naturally occurring MMPI Synthetic MMPI Urokinase inhibitor	Phase I/II Phase III for colon and NSCLC potentially: glioblastoma Phase I Phase II for glioblastoma
TNP-470 (fumagillin derivative) Squalamine Combretastatin	Inhibits EC growth Inhibits Na-H exchanger, NIHE3 EC apoptosis	Phase I/II lymphomas, acute leukemias, phase II for advanced, adult solid tumors Phase III for lung (NSCLC); phase III for prostate cancer, on hold in brain tumors Phase I; phase II
Endostatin Angiostatin Penicillamine	Inhibits EC proliferation Blocks VEGF & EC proliferation Blocks EC migration/proliferation	Phase I solid tumors Phase I/II/III solid tumors Phase II for glioblastoma
Farnesyl Transferase Inhibitor (-L-778,123 -SCH66336 -R115777) Anti-VEGF Antibody Thalidomide SU5416 SU6668	Blocks EC migration/proliferation Monoclonal antibody Blocks activity of bFGF, VEGF Blocks VEGF receptor, (Flk-1) Blocks VEGF, bFGF PDGF receptor	Phase II glioblastoma Phase II/III for lung, breast, renal prostate, colorectal Phase II for Kaposi's sarcoma, glioblastoma , prostate, lung, breast Phase I/II for Kaposi's sarcoma; phase I/II for metastatic, colorectal Phase III for advanced cancer
PTK787/ZK22584 Interferon-alpha Suramin Vitaxin EMD121974	Blocks VEGF receptor signaling Inhibition of bFGF & VEGF Blocks PDGF receptor binding Antibody to α_1, β_3 integrin on EC Small molecule inhibitor of integrin present on EC	Phase I for glioblastoma and Kaposi's sarcoma, phase I/II for vHL Phase II/III Phase II for glioblastoma Phase II for leiomyosarcoma Phase I/II for Kaposi's sarcoma, brain tumors ,
Penicillamine	Sulphydryl group binds copper; clears copper through urine	Phase II for glioblastoma
Tetrathiomolybdate CAI ABT-627	Thiol groups tightly bind copper Inhibitor of calcium influx Endothelin receptor antagonist	Phase I/II trial for advanced metastatic cancer, multiple tumor types Phase II/III for ovarian, non-small cell lung, renal cell Phase I, refractory prostate and other malignancies; phaseII glioblastoma , prostate
Interleukin-12	Induction of interferon-gamma Down-regulation of IL-10	Phase I trials for ovarian, renal cell, melanoma, gastrointestinal Phase I/II for Kaposi's sarcoma Inhibits production of MMPs
IM862	Induction of IP-10 Blocks VEGF & bFGF	Phase III for Kaposi's sarcoma
PNU-145156E	Blocks angiogenesis induced byTat protein	Phase I/II trial for solid tumors

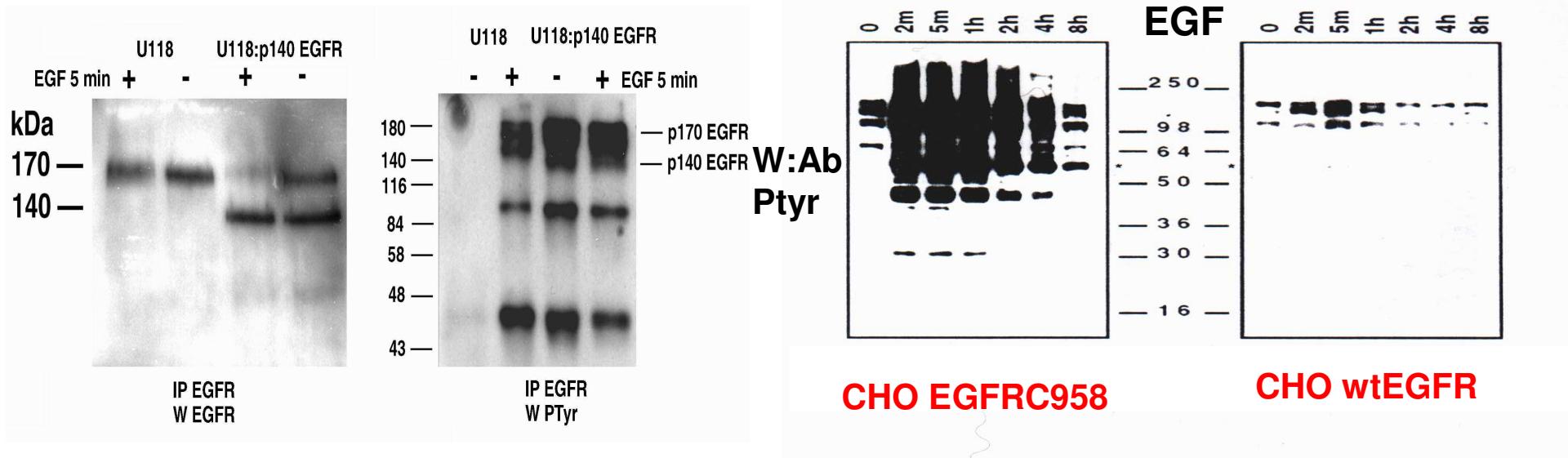
Table 1 Targeted agents and their current status in clinical testing

Drug	Target	Disease	Clinical trial status
Imatinib (Gleevec)	Abl	CML	Approved
	Kit	GIST	
	PDGFR	HES	
		CMMML	
		DFSP	
Gefitinib (Iressa)	EGFR	Lung cancer	Approved
Bevacizumab (Avastin)	VEGF ligand	Colon cancer	Approved
CCI-779 RAD-001	mTOR	Various cancers	Phase I, II, III
BMS-354825	Abl	CML	Phase I
	KIT	GIST	
PKC-412	FLT3	AML	Phase I/II
MLN-518			
CEP-701			
BAY 43-9006	VEGFR	Kidney cancer	Phase I/II
	RAF	Melanoma	
SU-011248	VEGFR	Kidney cancer	Phase I/II

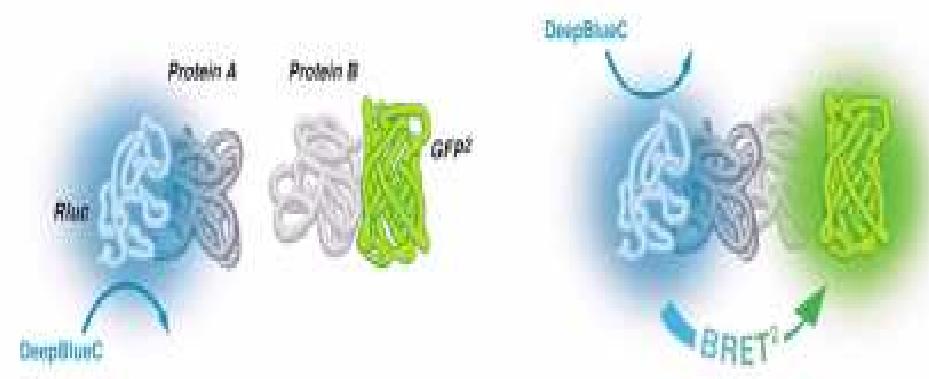
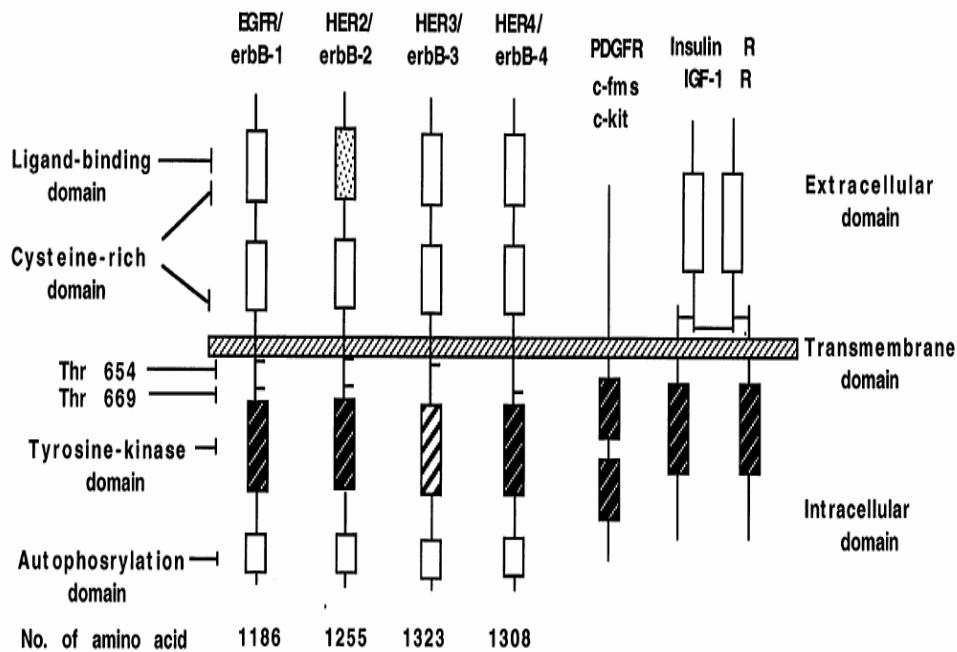
AML, acute myeloid leukaemia; HES, hypereosinophilic syndrome; CMMML, chronic myelomonocytic leukaemia; DFSP, dermatofibrosarcoma protuberans.

Sawyer:Nature'04

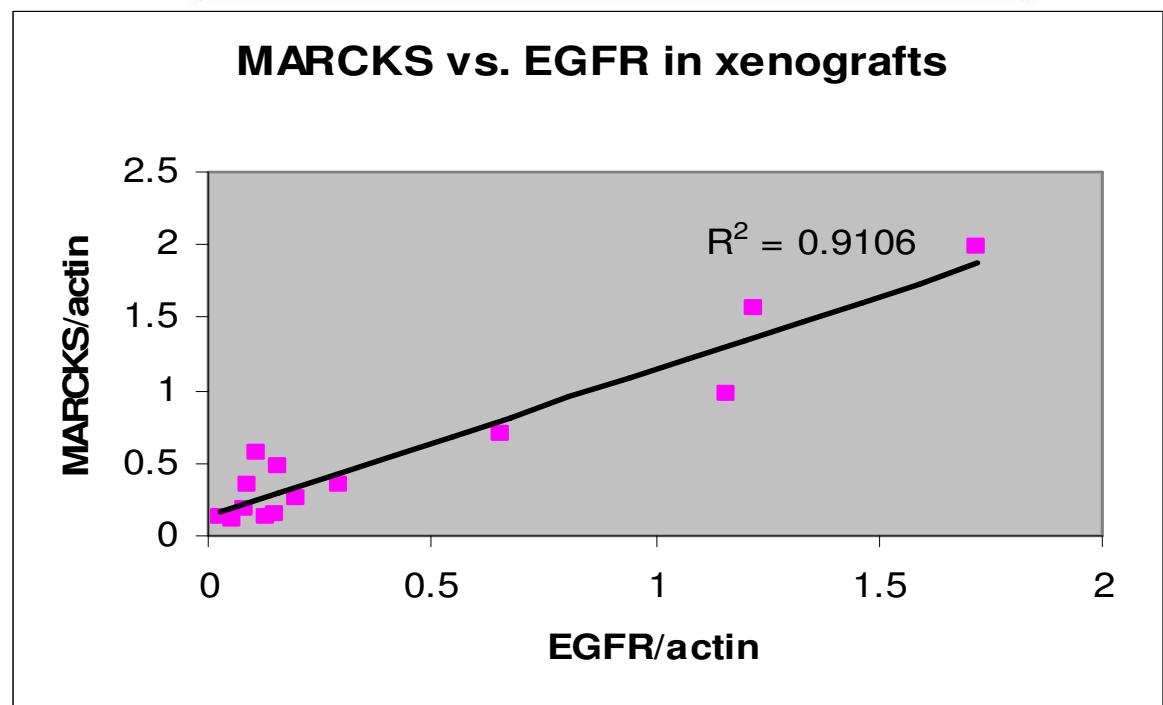
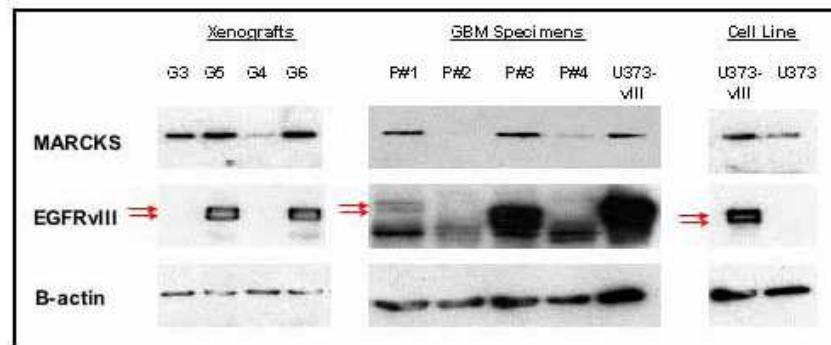
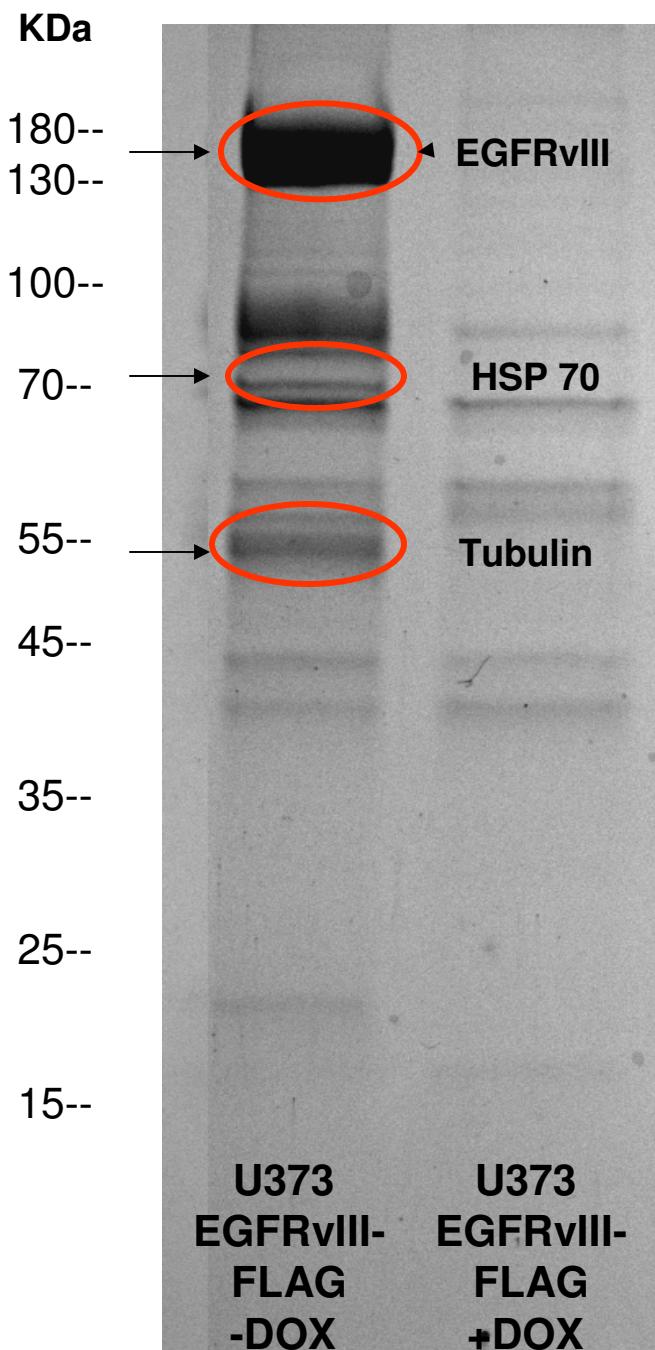
Signaling Differences



EGFR subfamily



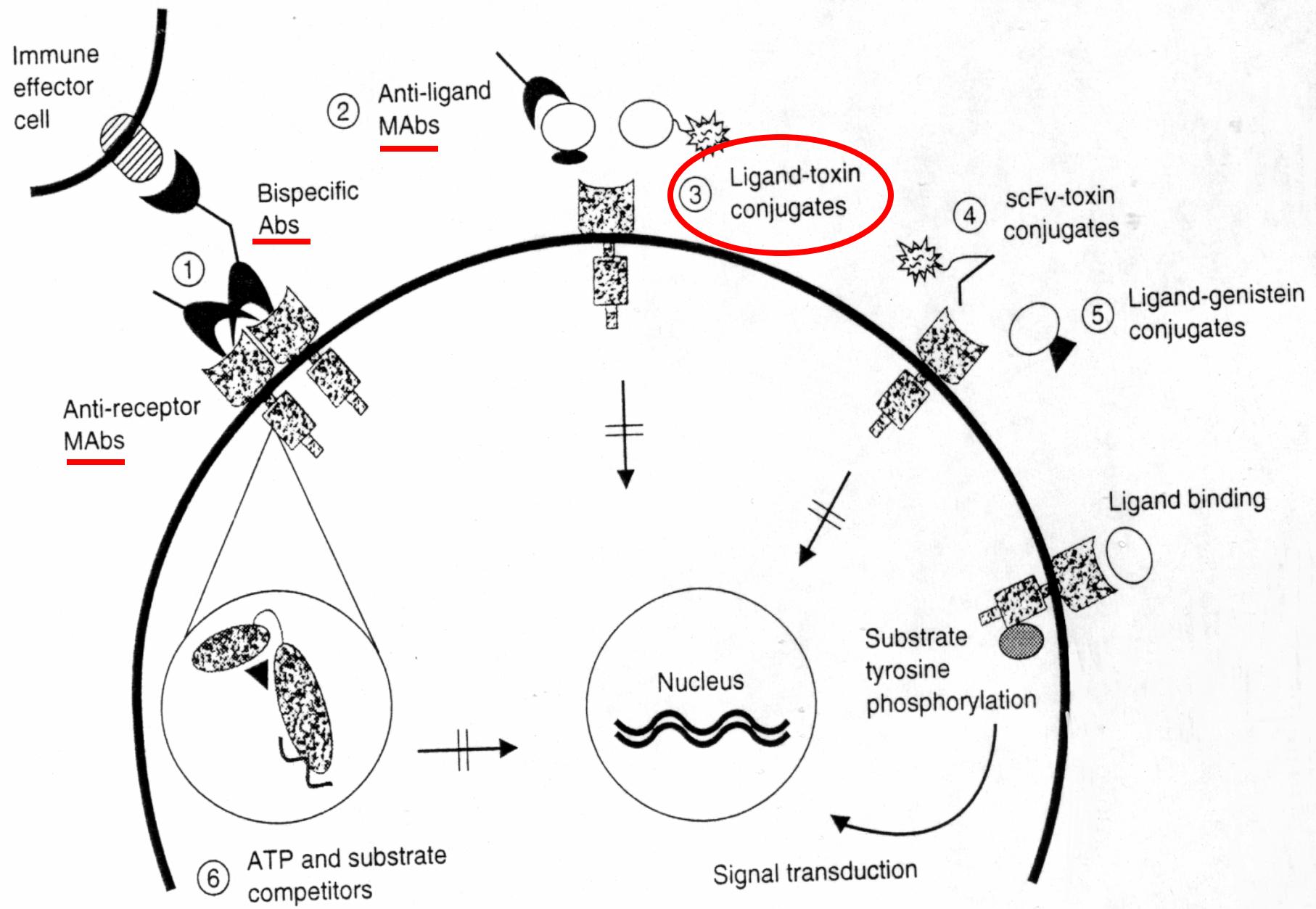
MS to Study Interacting & Differential Expressed Proteins (ICAT)



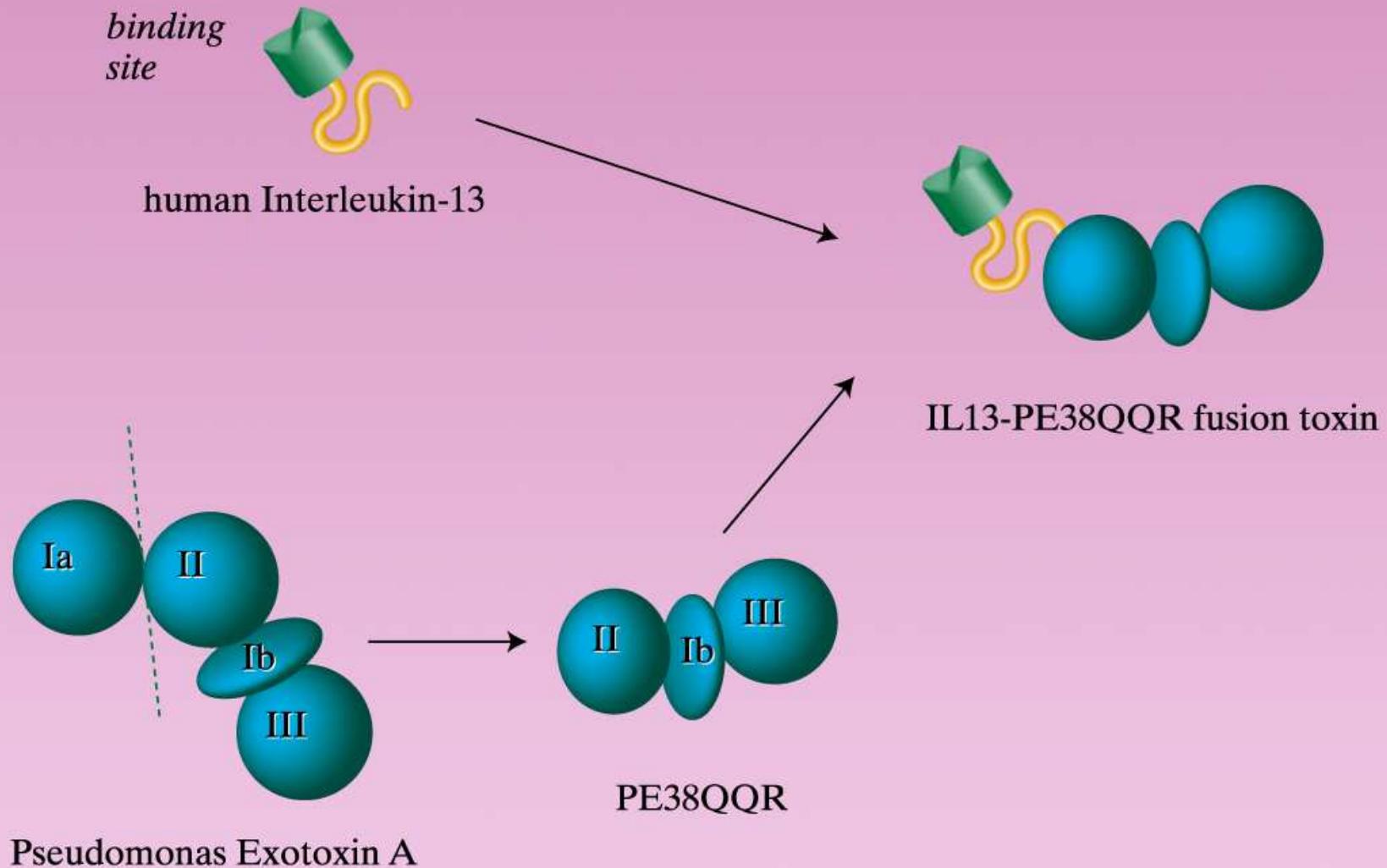
MARCKS: Proteomics derived differentially expressed protein in GBMs with EGFR amplification +/- EGFRvIII

?? Target for GBMs with EGFR aberrations??

Non-SMI Strategies



Development of IL13-PE38QQR



Cancer Cell

Normal Cell

IL13-PE38QQR

IL13
Receptor

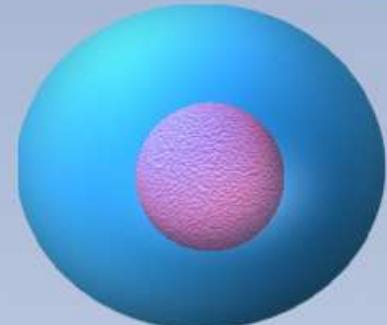
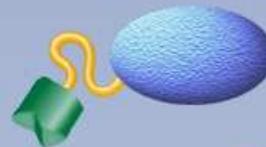
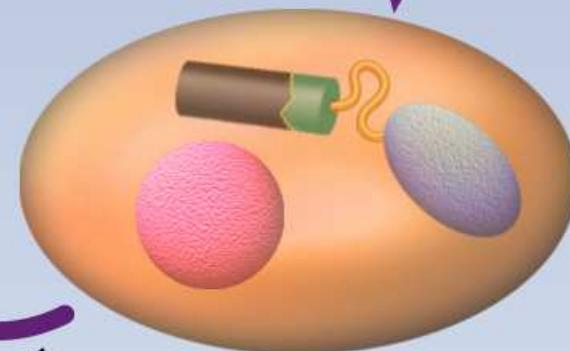
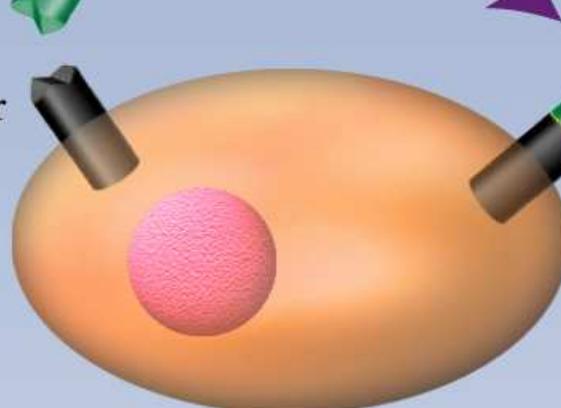
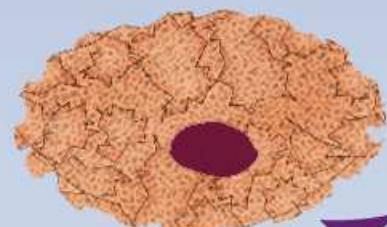
Binding

Internalization

Cell Death

without
Receptor

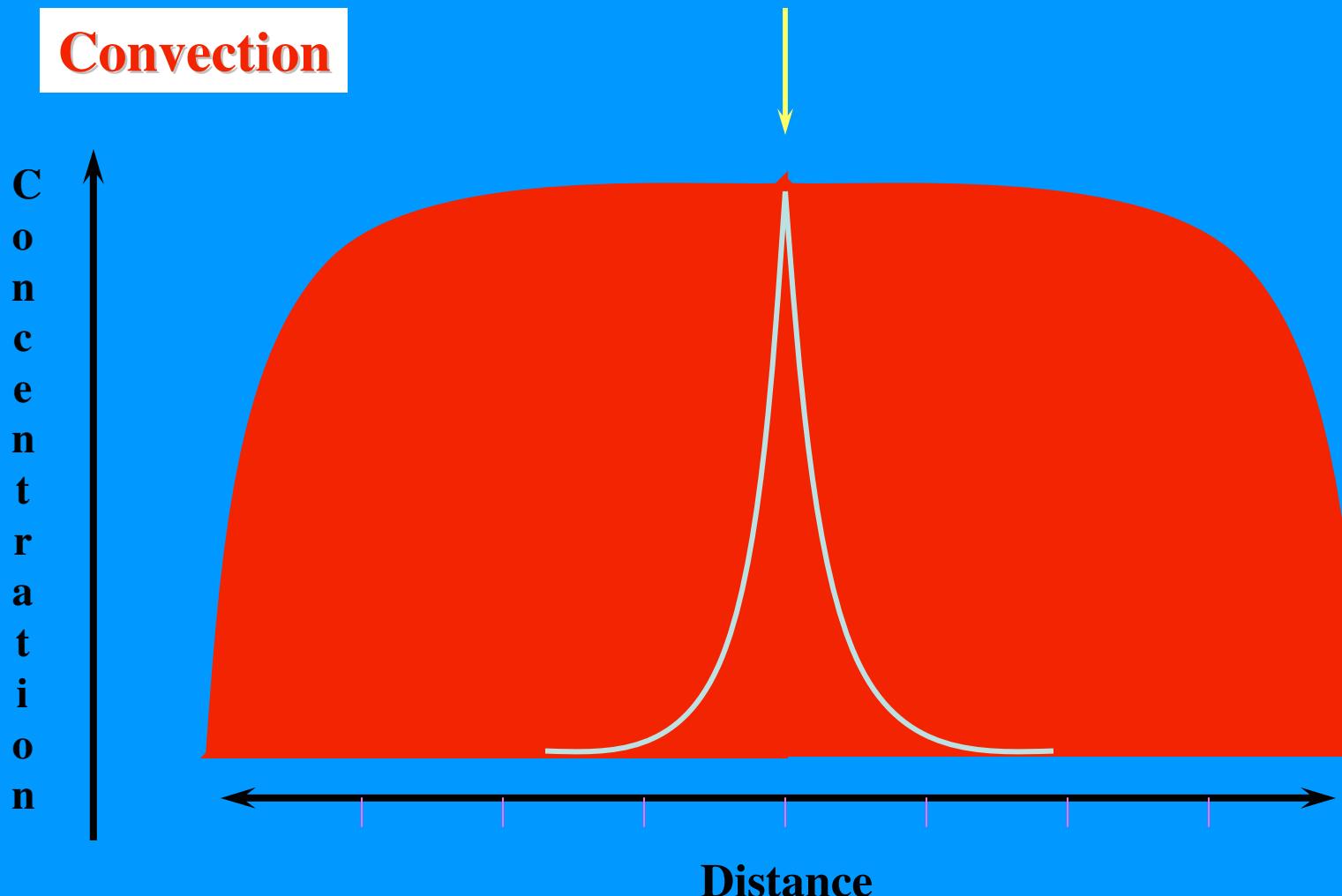
No Internalization
No Cell Death



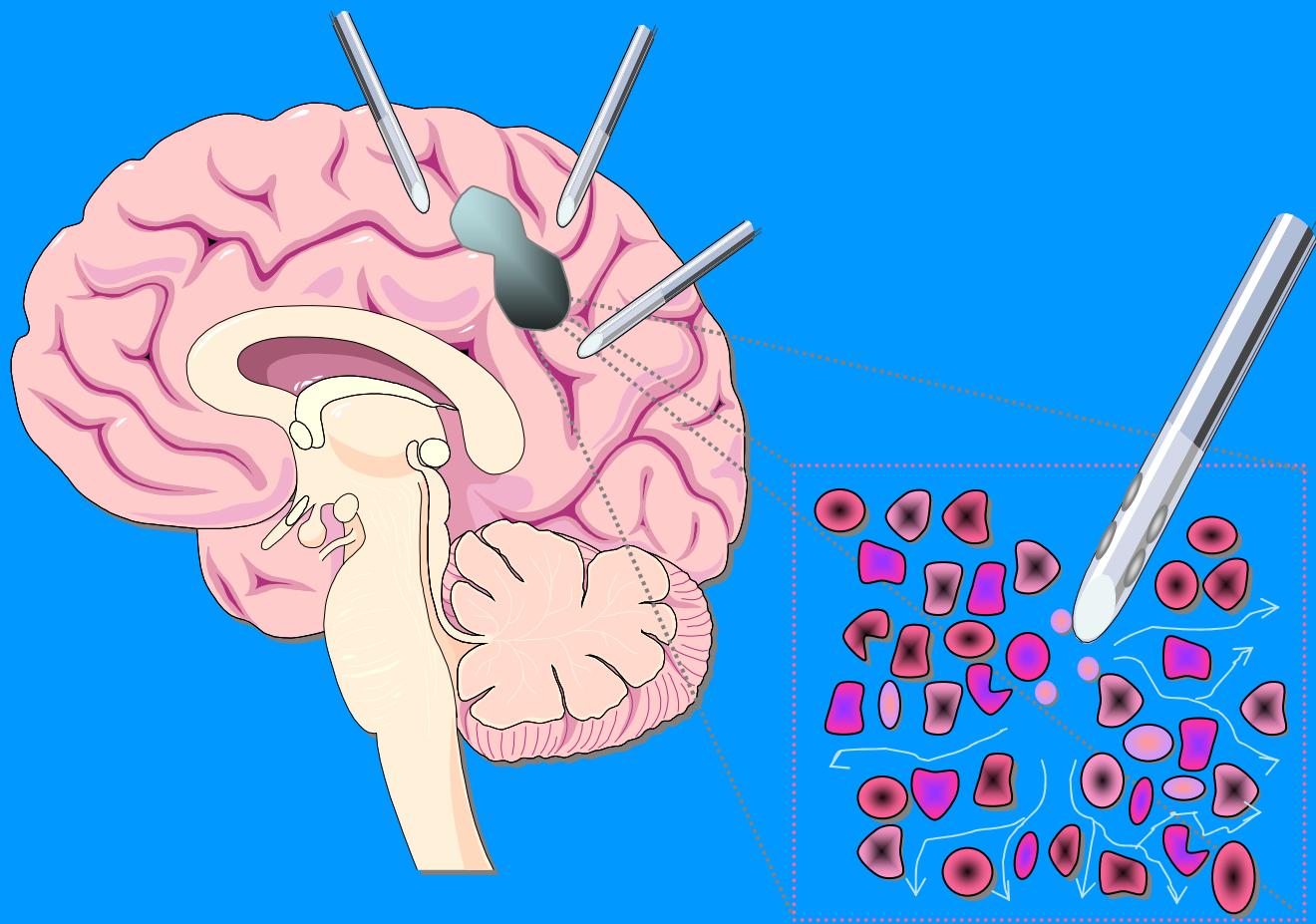
Intracerebral Chemotherapy

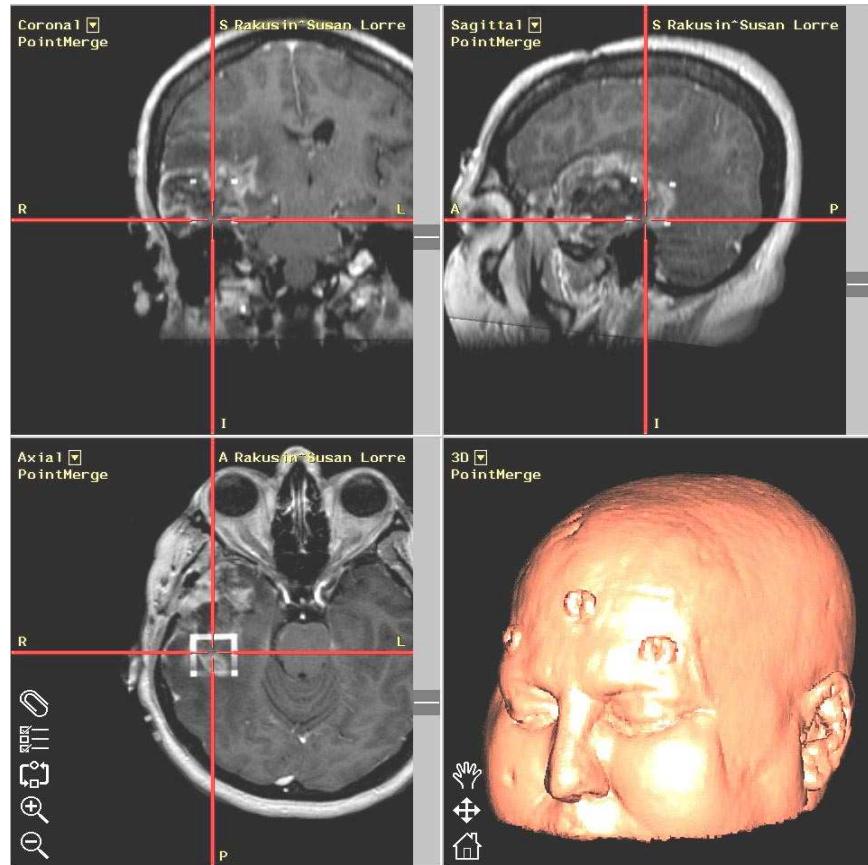
Diffusion

Convection



Convection Enhanced Delivery Basic Principles





Superior

Post

Ant



Inferior

Post

Ant

Catheter Placement: Stealth Stereotaxy



X- Brief Review: Epidemiology- Clinical Presentation- Pathology

X- Current Knowledge of Molecular Pathogenesis of Gliomas

X- Major Obstacles in the Management of Gliomas:

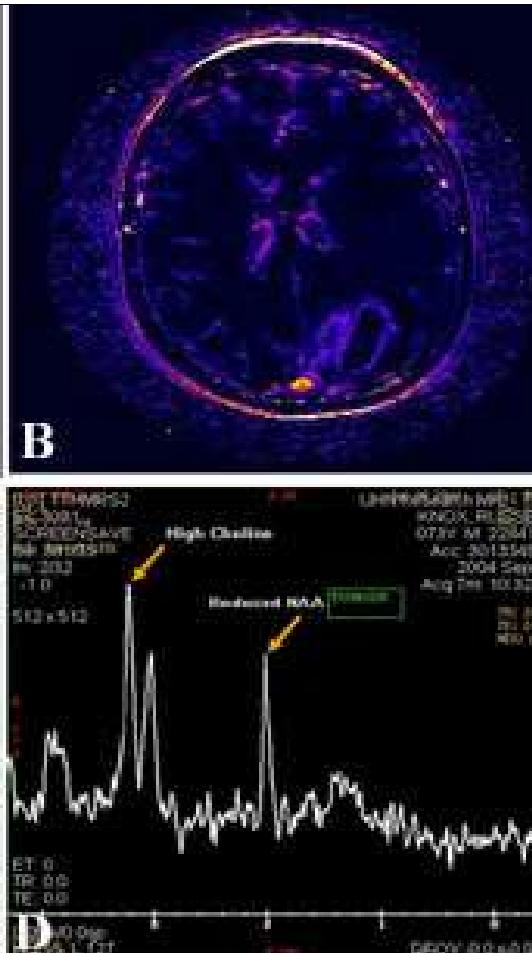
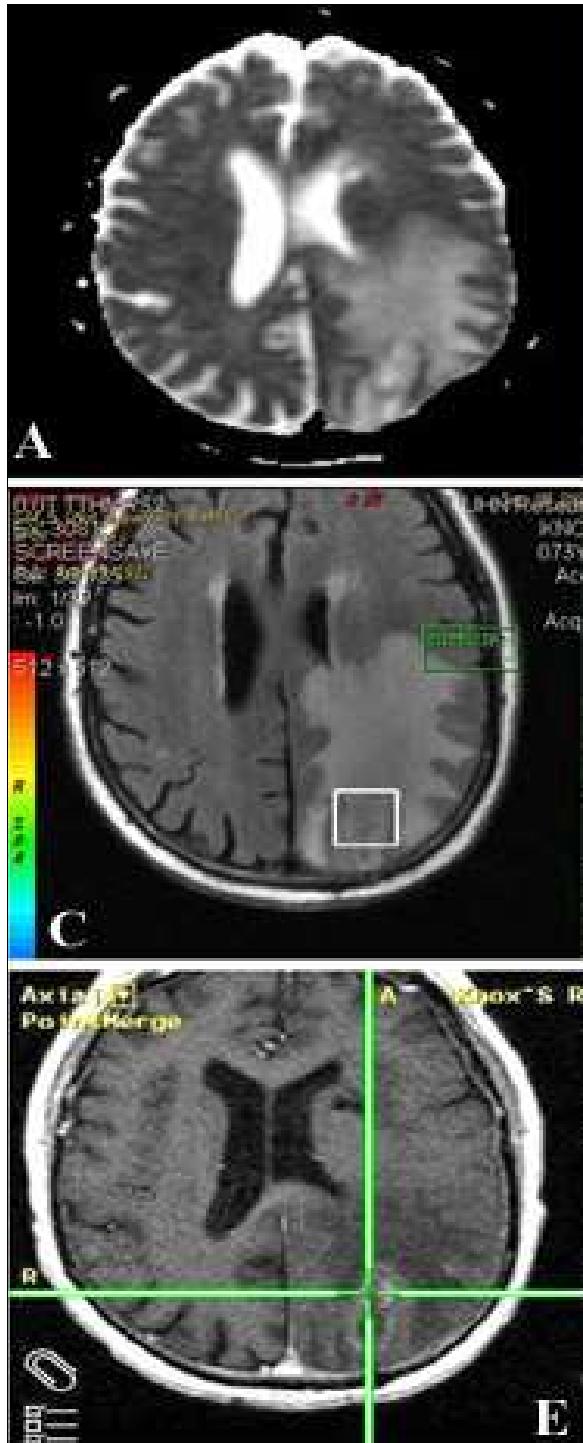
- A: Current Management**
- B: Invasion**
- C: Angiogenesis**
- D: Resistance: Chemotherapy, Radiation**
- E: Tumor \longleftrightarrow Molecular Heterogeneity**

X- Novel Therapies:

- A: Small Molecule Targeted Rx.**
- B: Immune Based: Immunotoxin Rx.**
- C: Photodynamic Rx.**
- D: Viral Rx.**
- E: Gene Rx.**

5- Research Frontiers:

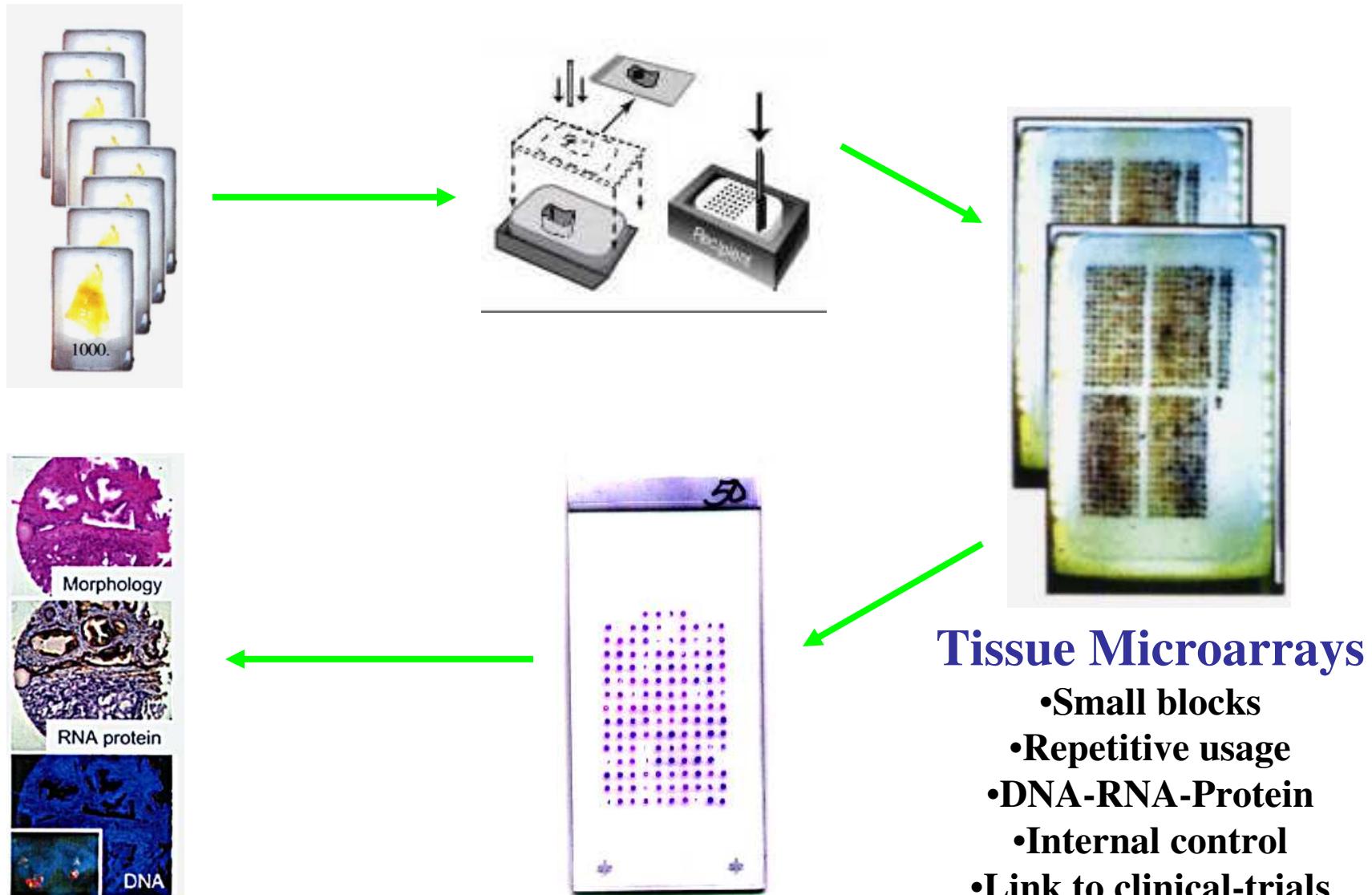
- A: Imaging \longleftrightarrow Biological/Molecular Correlates**
- B: “omics”: genomics/transcriptomics/proteomics**
- C: ??Cancer Stem Cells??**
- D: Genetically Engineered Murine (GEM) Glioma Models**



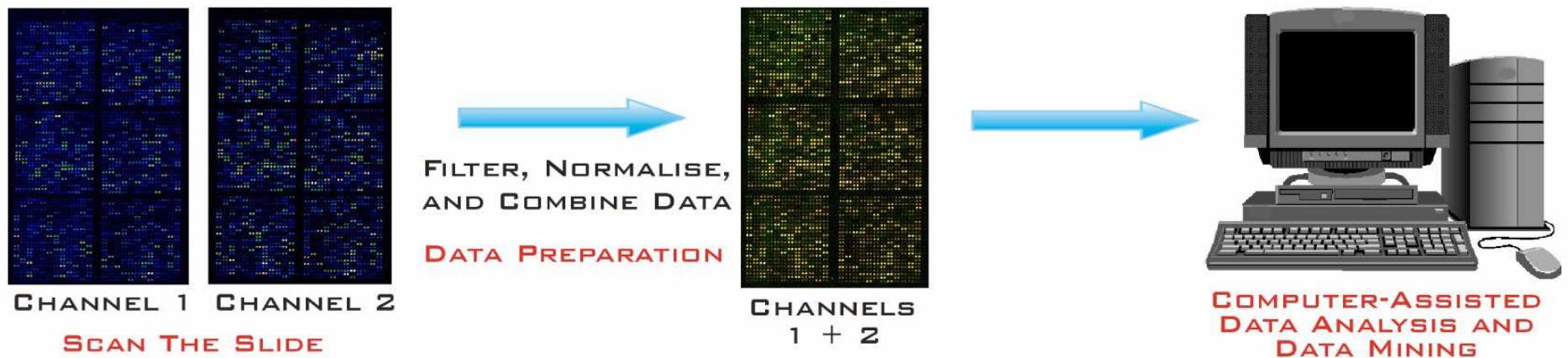
Horizon & Future

- Better understand the influence of epigenetic tumor microenvironment influences on the changing genetic profile of the tumor and stroma
- How it correlates to non-invasive biological imaging
- How it changes with treatment
- Able to switch our biological targeted Rx based on non-invasive and invasive molecular analysis of evolving tumor

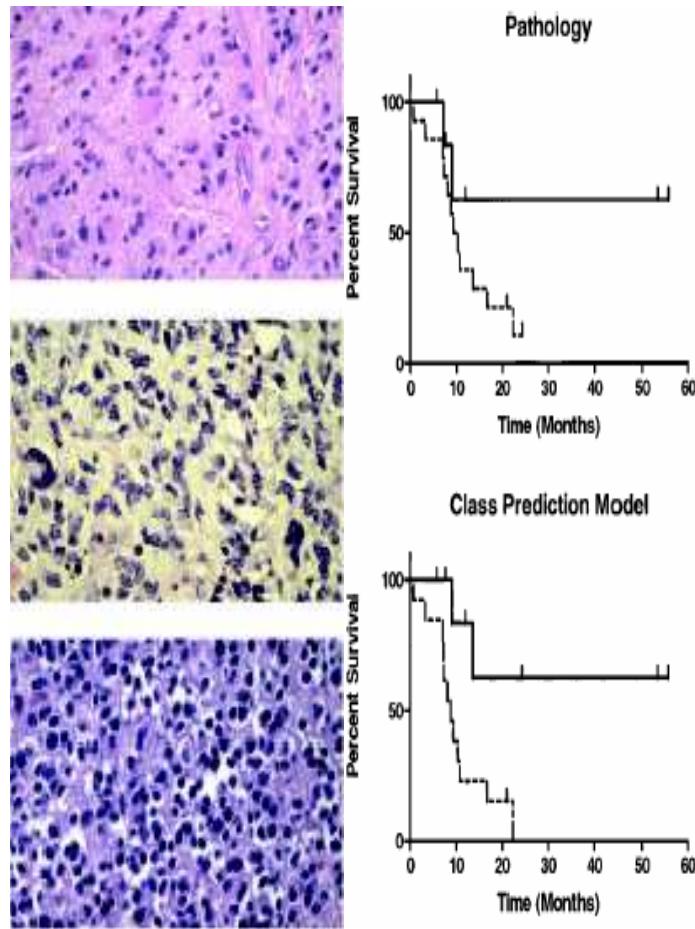
Canadian National Virtual Tissue Bank Linked with NCIC and CBTC Clinical Trials



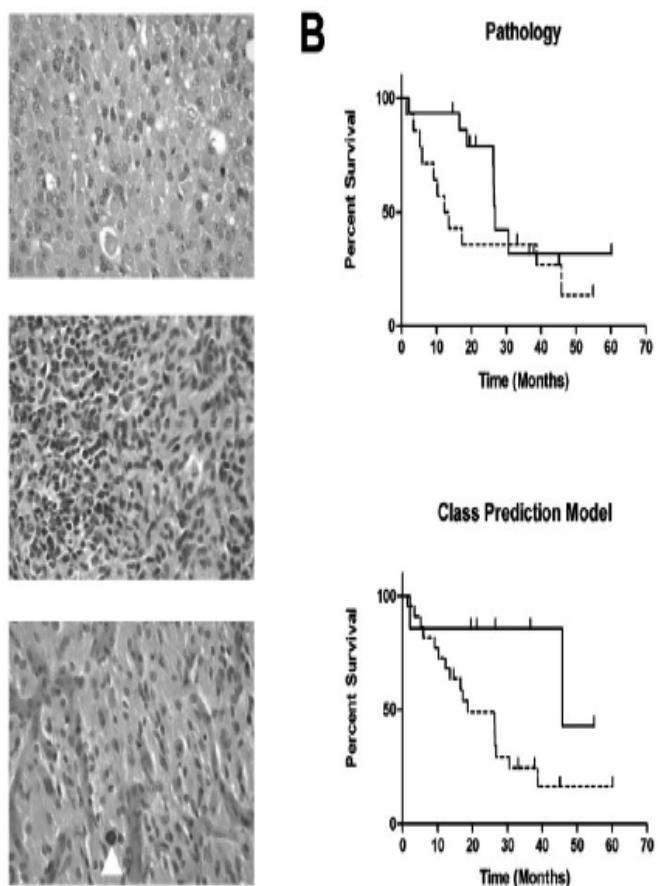
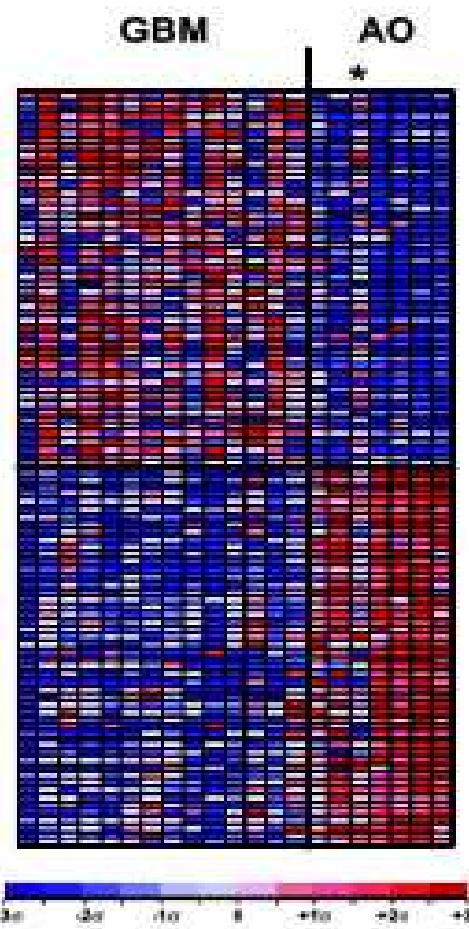
Data Processing and Analysis



Gene Expression-based Classification of Malignant Gliomas Correlates Better with Survival than Histological Classification (Nutt et al: Can Res'03)



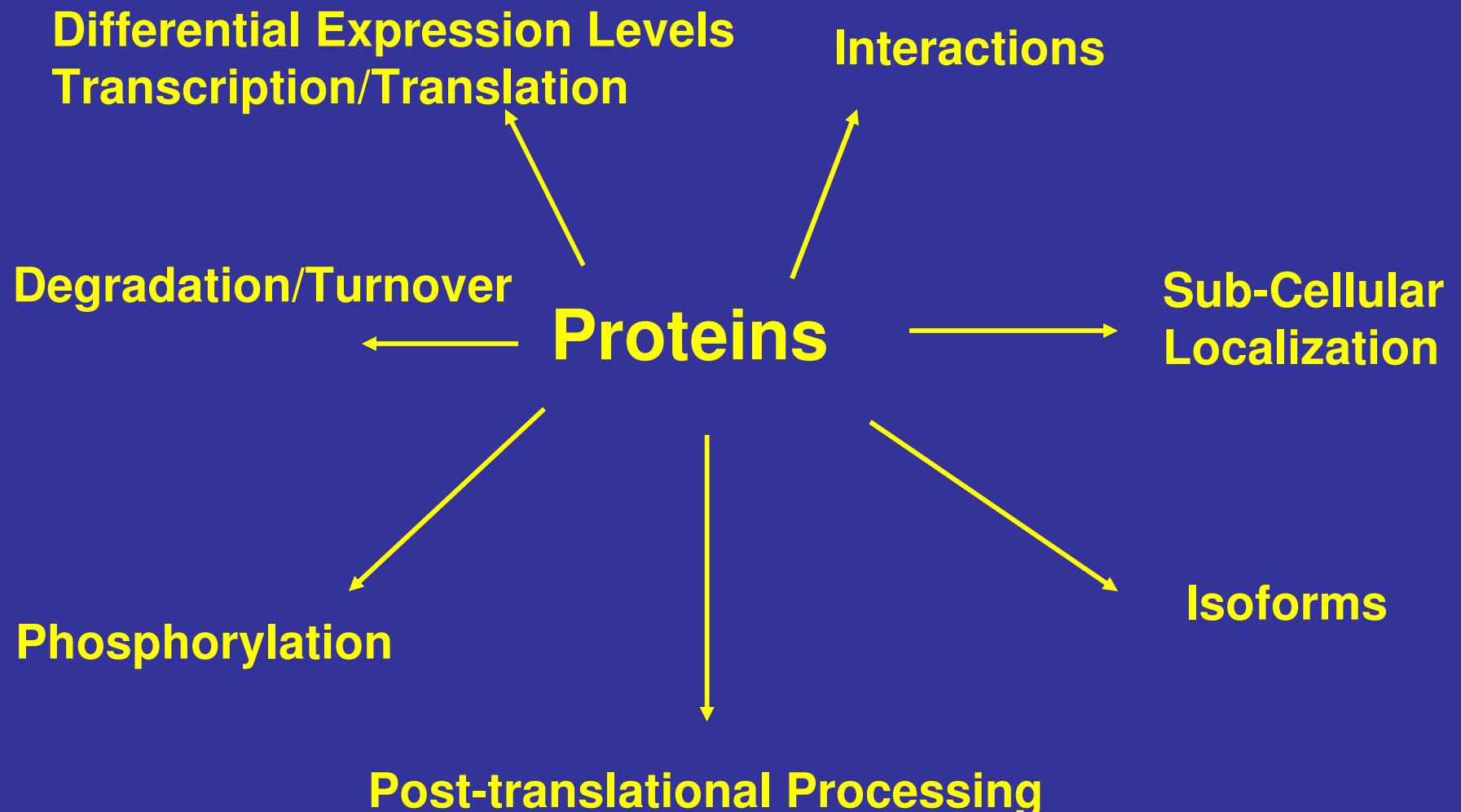
Classical GBM & AO



Non-classical GBM & AO

* Augmented Classical + Non-Classical Histological Diagnosis*

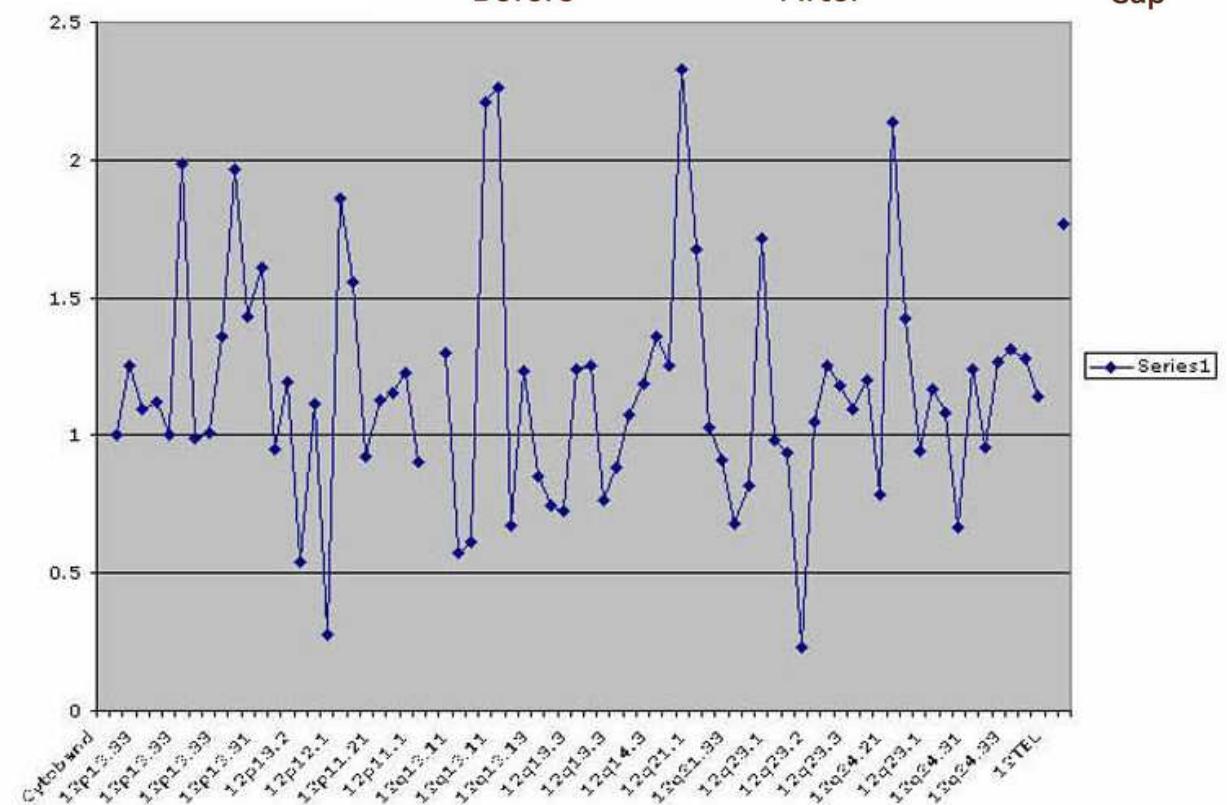
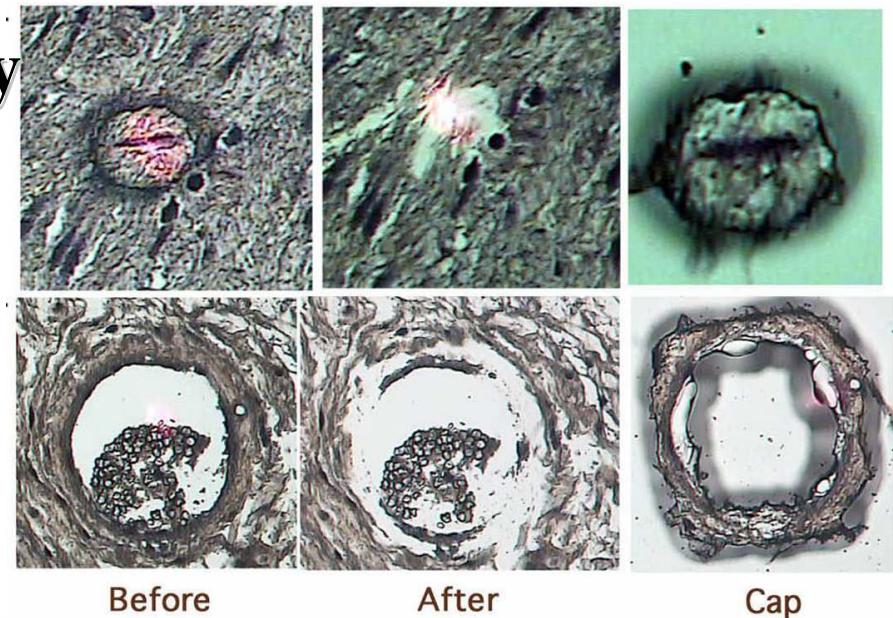
Proteomics: The study of Proteins



Molecular Tumor Heterogeneity

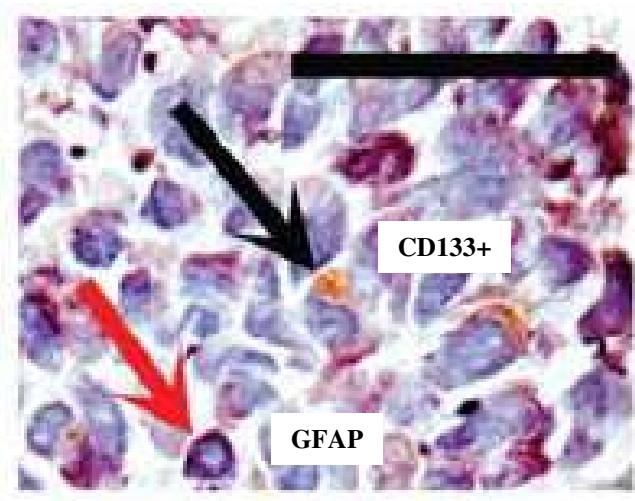
LCM Coupled:

1. Micro-genomics-DNA
2. Micro-array-RNA
3. Micro-proteomics-Protein

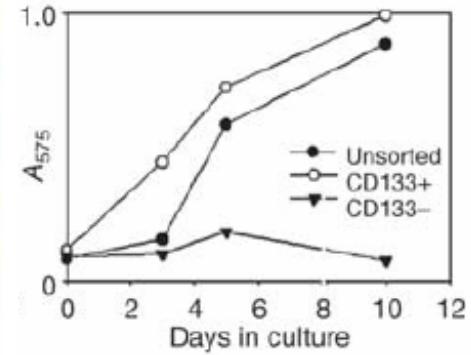


Molecular Prognosticators in Neuro-oncology

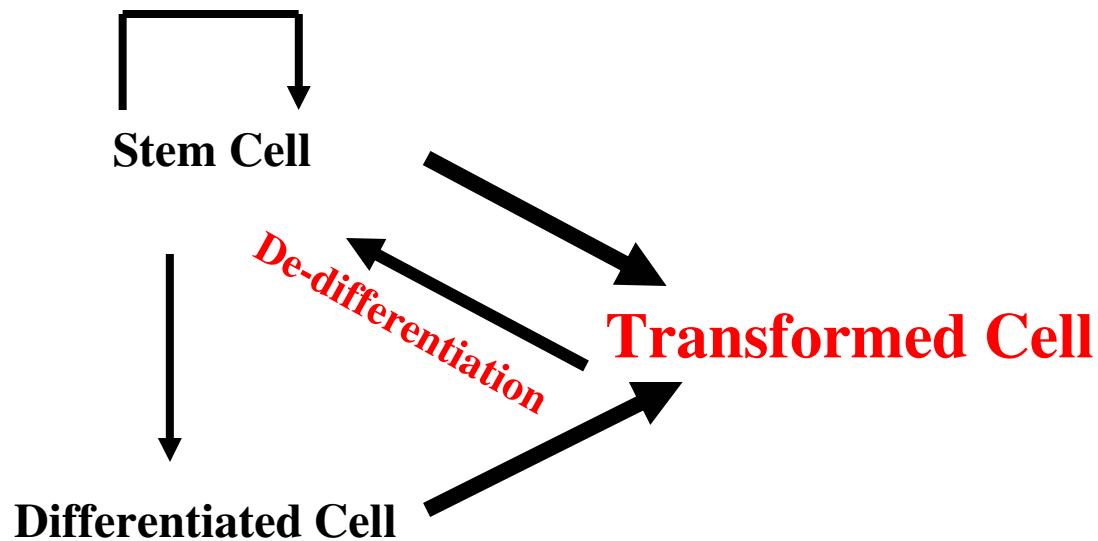
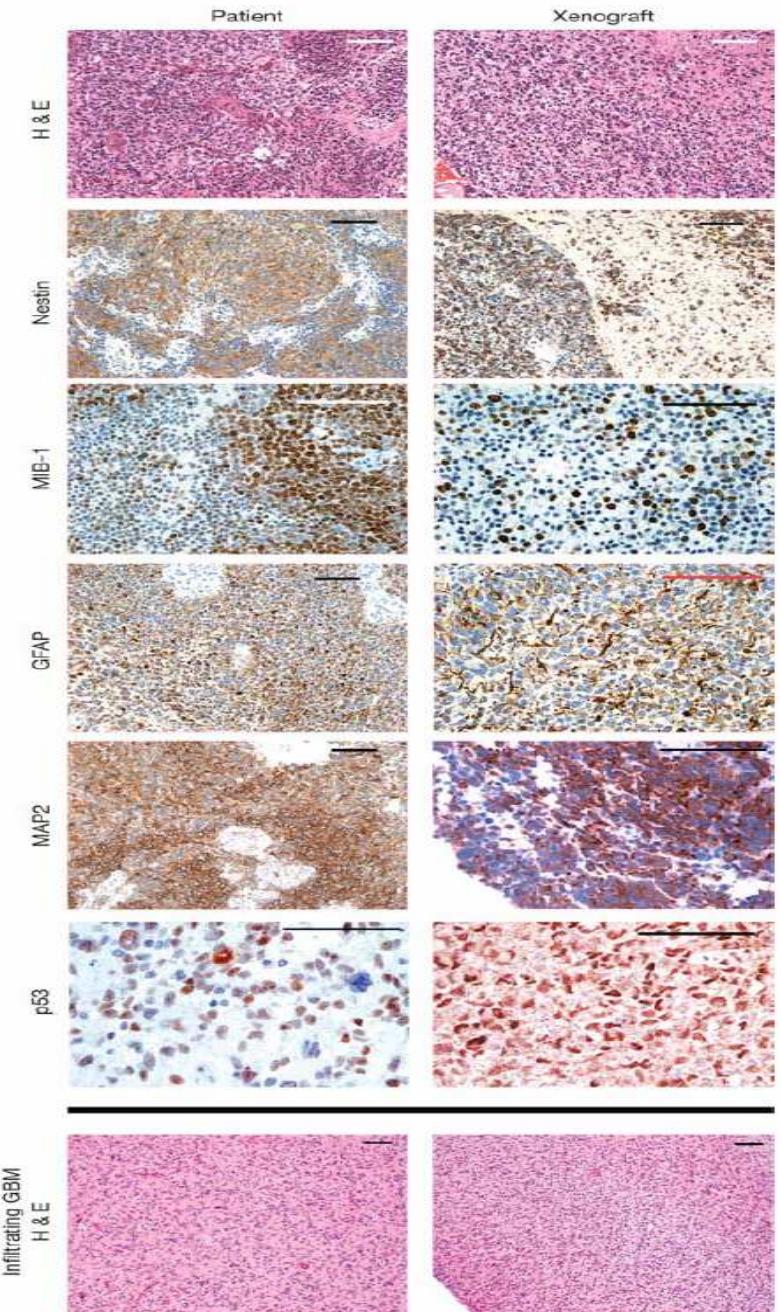
- Oligodendrogioma
 - LOH @ 1p, 19q
- Gliomas
 - EGFR ampl/mutations
 - PTEN mutations/loss of expression
 - MGMT Methylation
- Medulloblastoma
 - i(17q); LOH @ 10q & 9p; MYCC ampl.
- Neuroblastoma
 - -1p36; MYCN ampl.; LOH @ 11q & 14q; +17q
- Ependymoma
 - Aberration chr. 22, LOH @ 11q



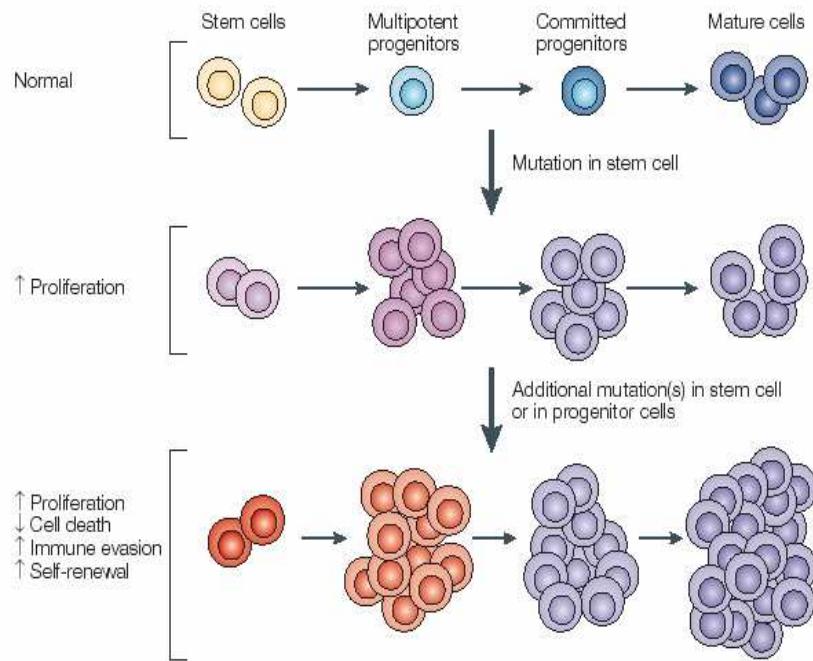
Glioma Cancer Stem Cells



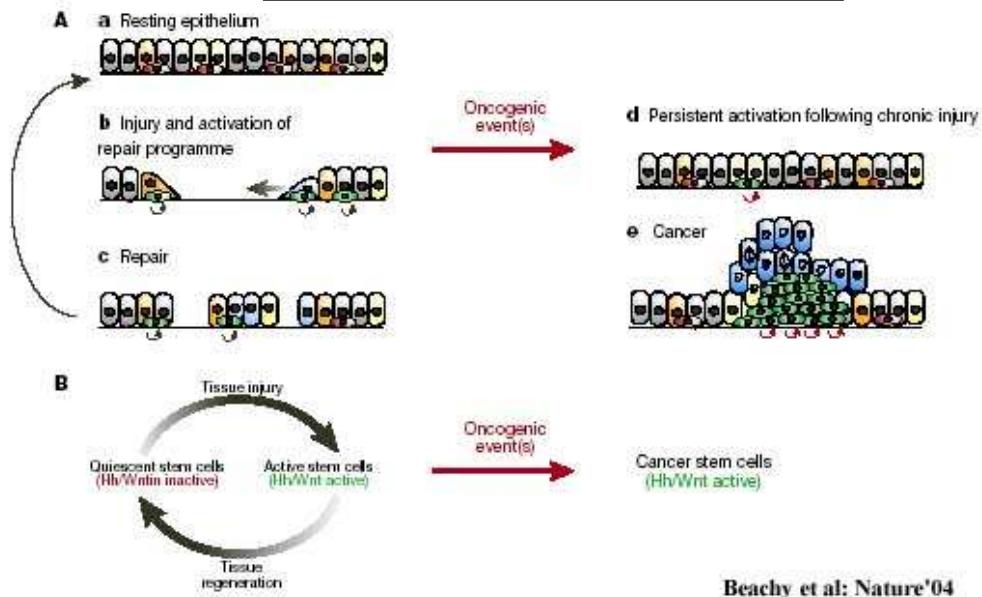
Singh, Dirks: Nature'04



Stem Cell Oncogenesis

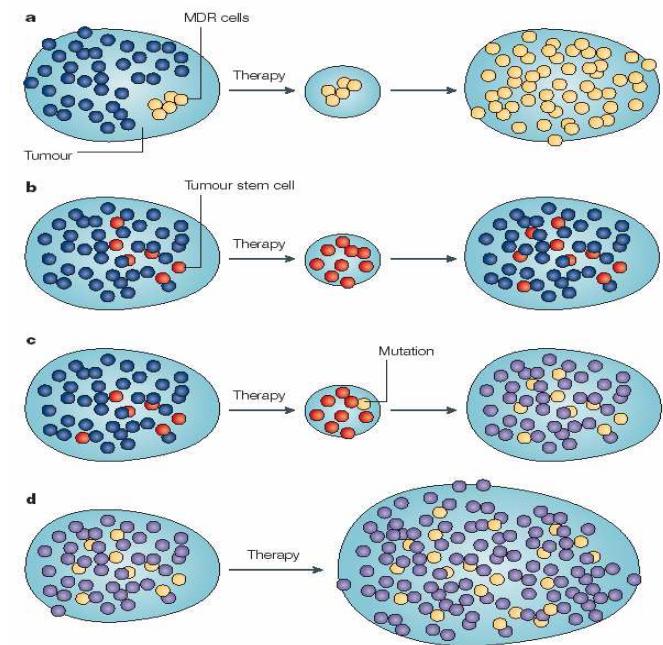


Chronic Injury & Oncogenesis



Cancer Stem Cells

Stem Cell Resistance



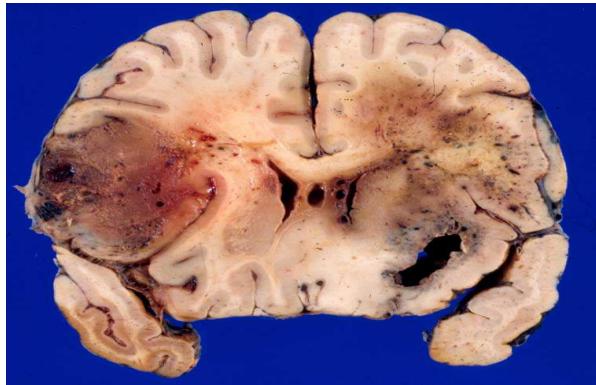
Dean: Nature'05

C.S.C.

- ?? Same genetic events
- ?? Same epigenetic events
- ?? Same susceptibility & resistance
- ?? Rx useful- “homing”

Beachy et al: Nature'04

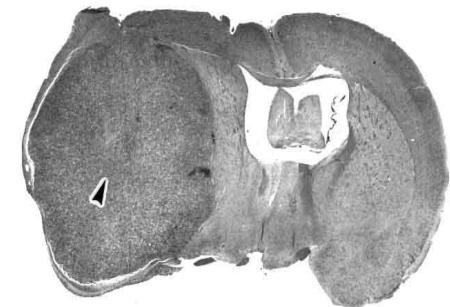
? Need for Spontaneous Glioma Models ?



Invasive; heterogenous; vascularized;
immunological response



Non-invasive; homogenous; poorly vascularized,
immunocompromised host



*Study progression

*Study *in vivo* interactions of implicated genetic aberrations

*Study the effects of genetic aberrations \longleftrightarrow ontogeny

*Study the effects of tumor cell \longleftrightarrow stromal cells (i.e. ECs)

*Gene-Discovery

*Pre-clinical testing platform for Rxs and their inter/tox etc.

GFAP:V¹²Ha-Ras

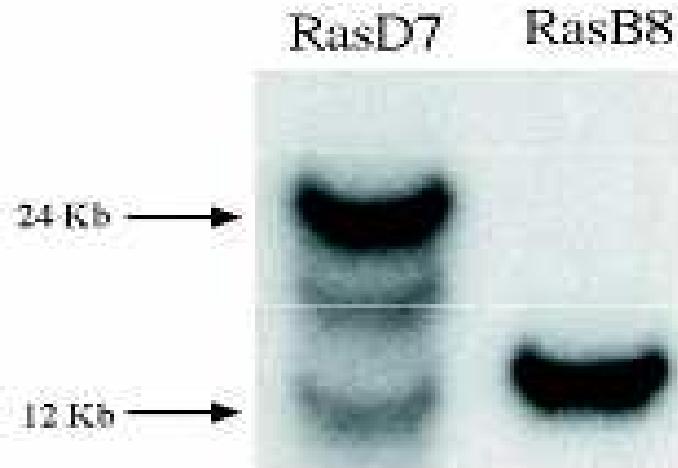
Southern Blot

ES cell-mediated transgenesis

Specific promoter  gene IRES lacZ
Not expressed in ES cells

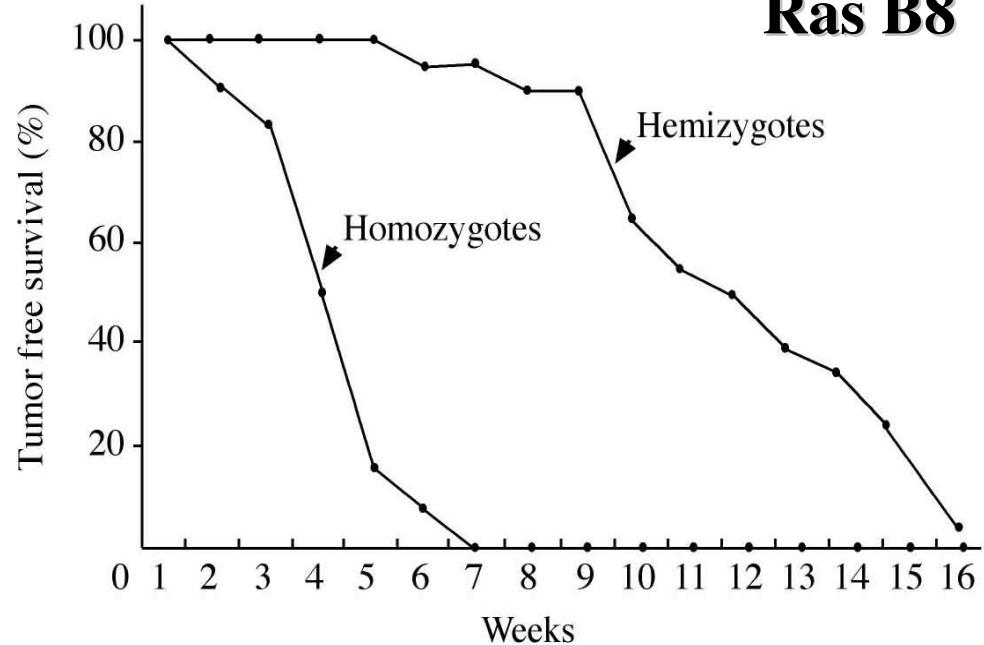
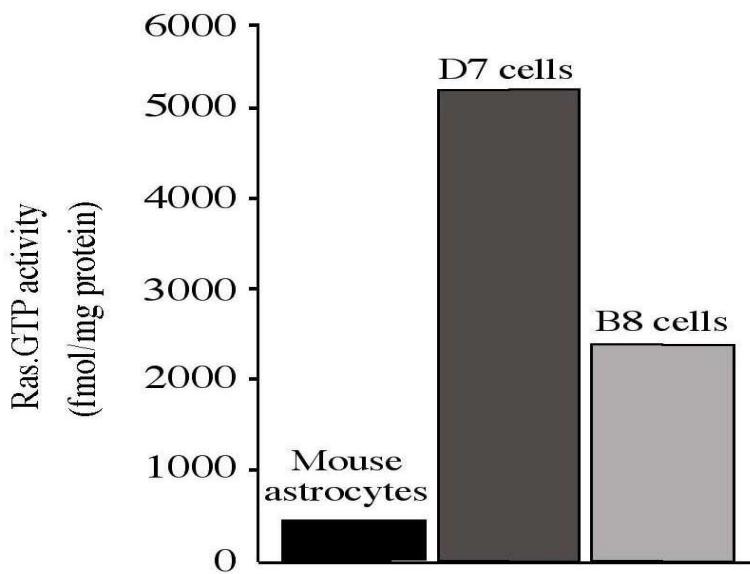


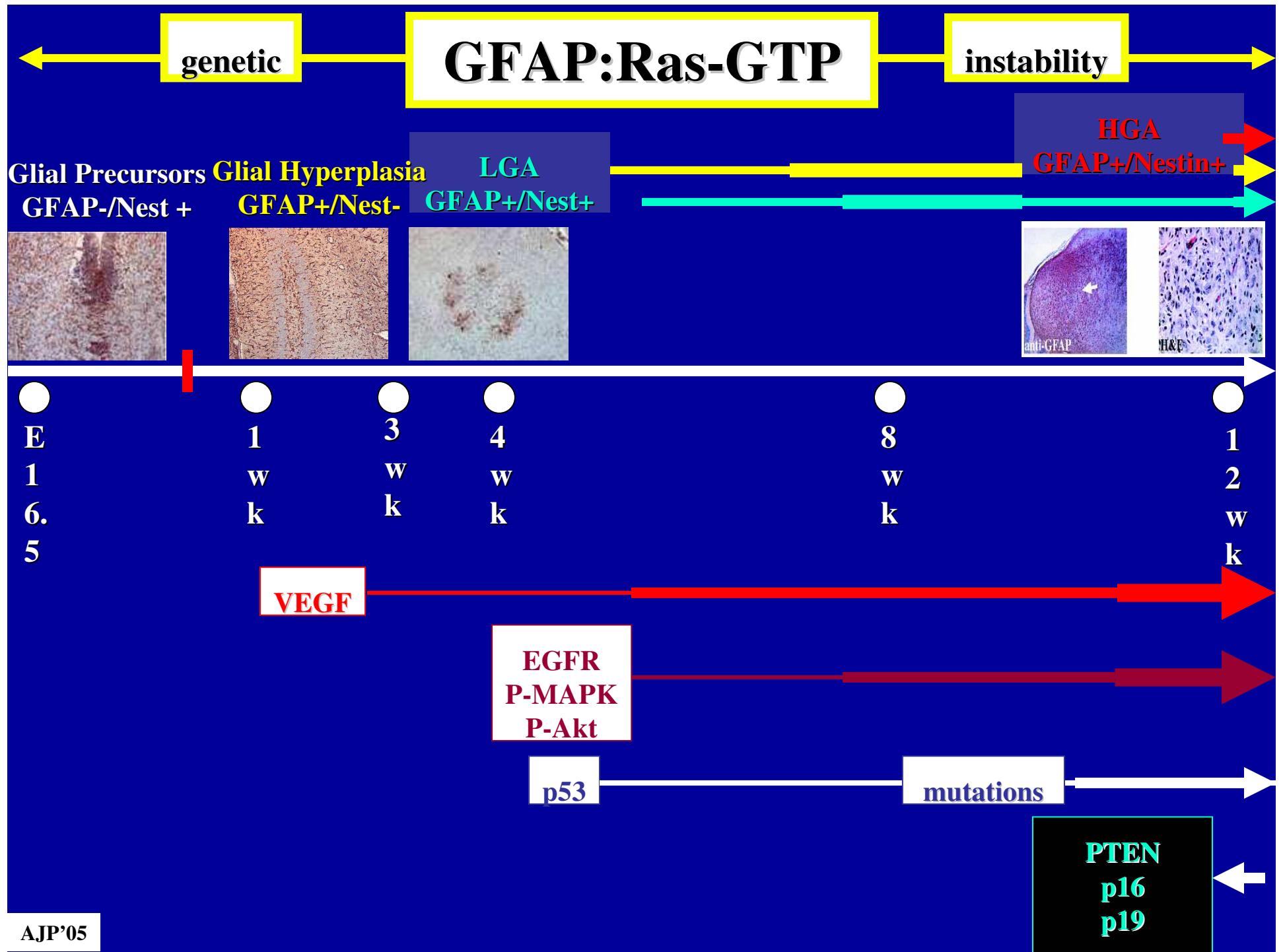
5% of clones



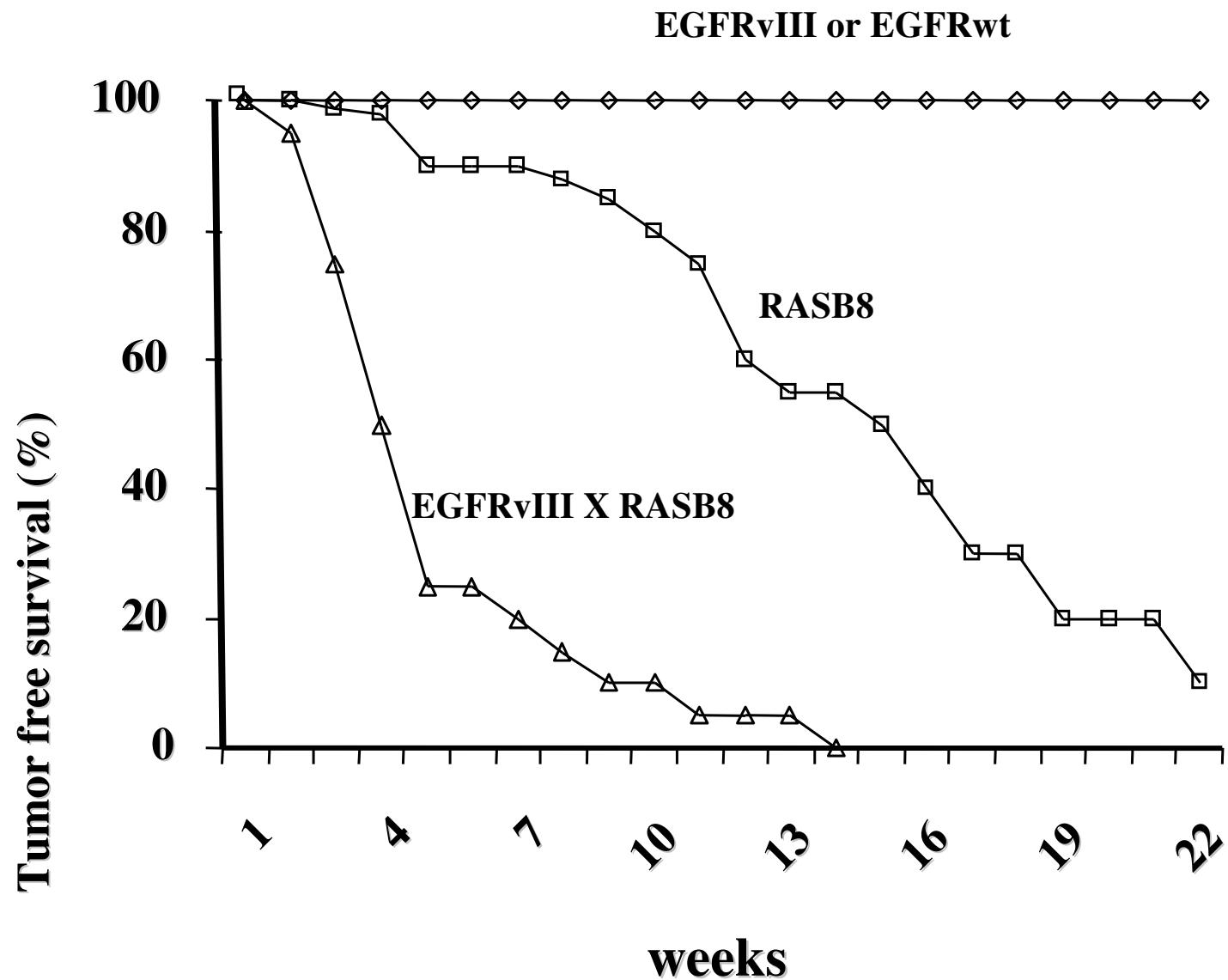
Ras B8

A



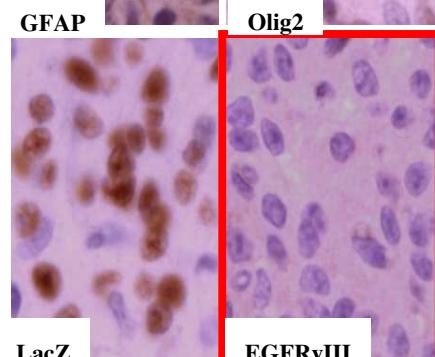
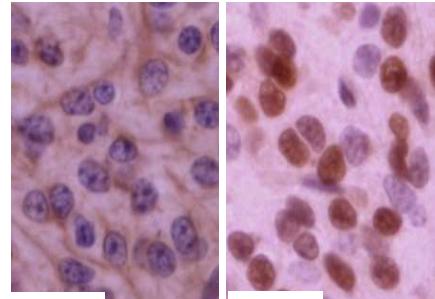
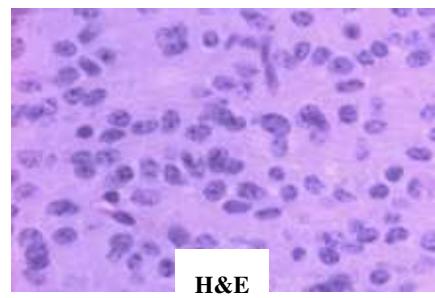
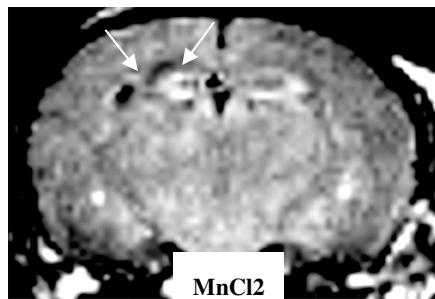


EGFR: Mouse Glioma Models

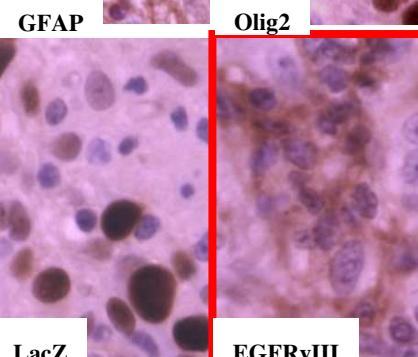
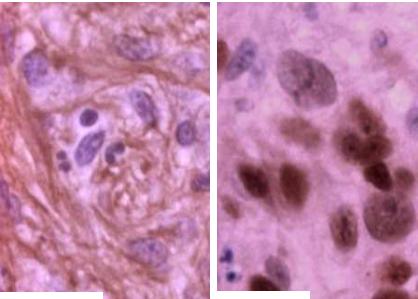
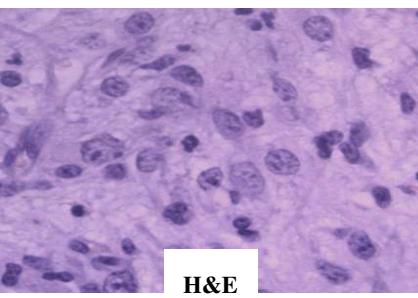
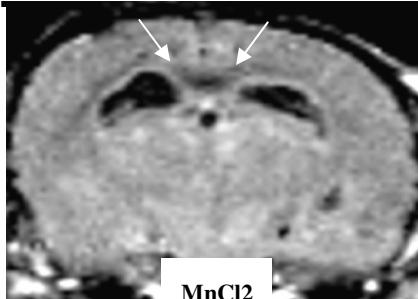


EGFRvIII in high not low grade transformed astrocytes and oligodendrocytes

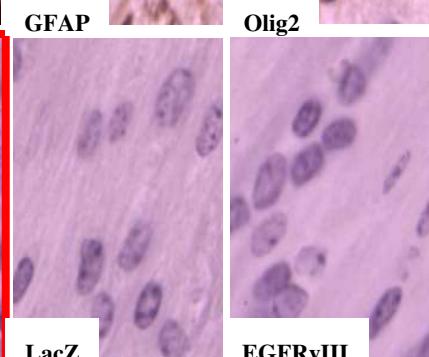
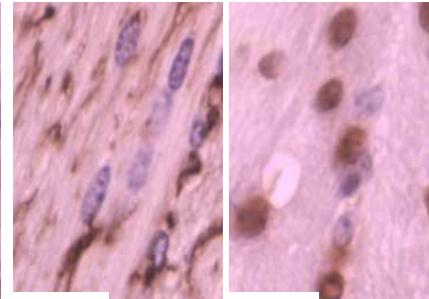
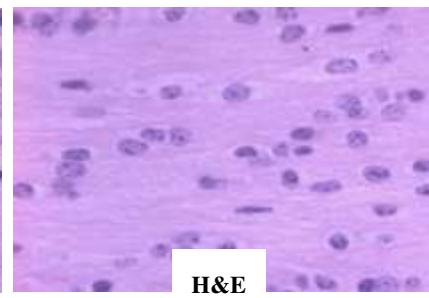
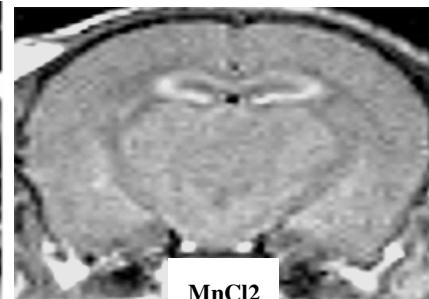
A: Ad: EGFRvIII X RasB8
LG O-A



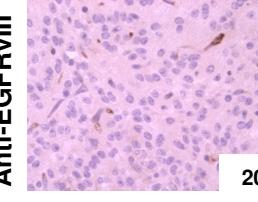
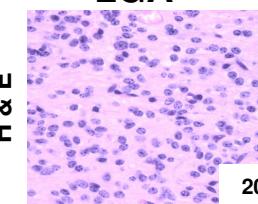
B: Ad:EGFRvIII X RasB8
HG O-A



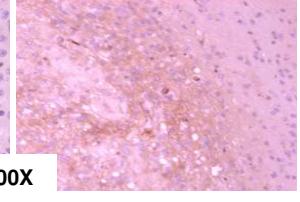
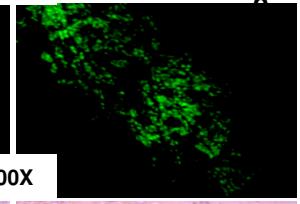
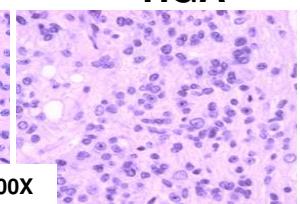
C: Ad:GFP X WT
No tumor



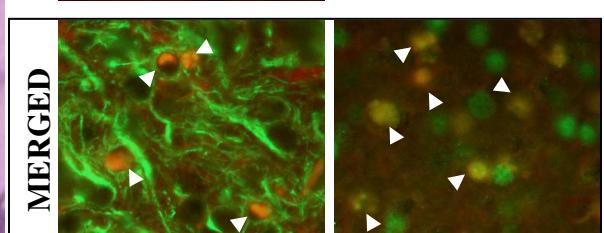
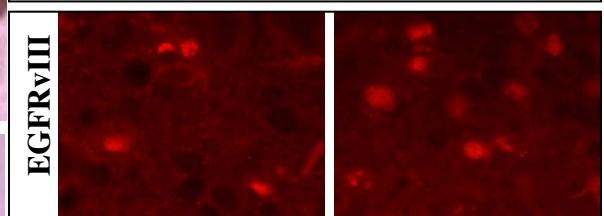
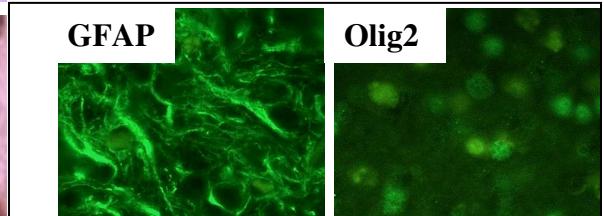
LGA



HGA



HGA



LacZ

EGFRvIII

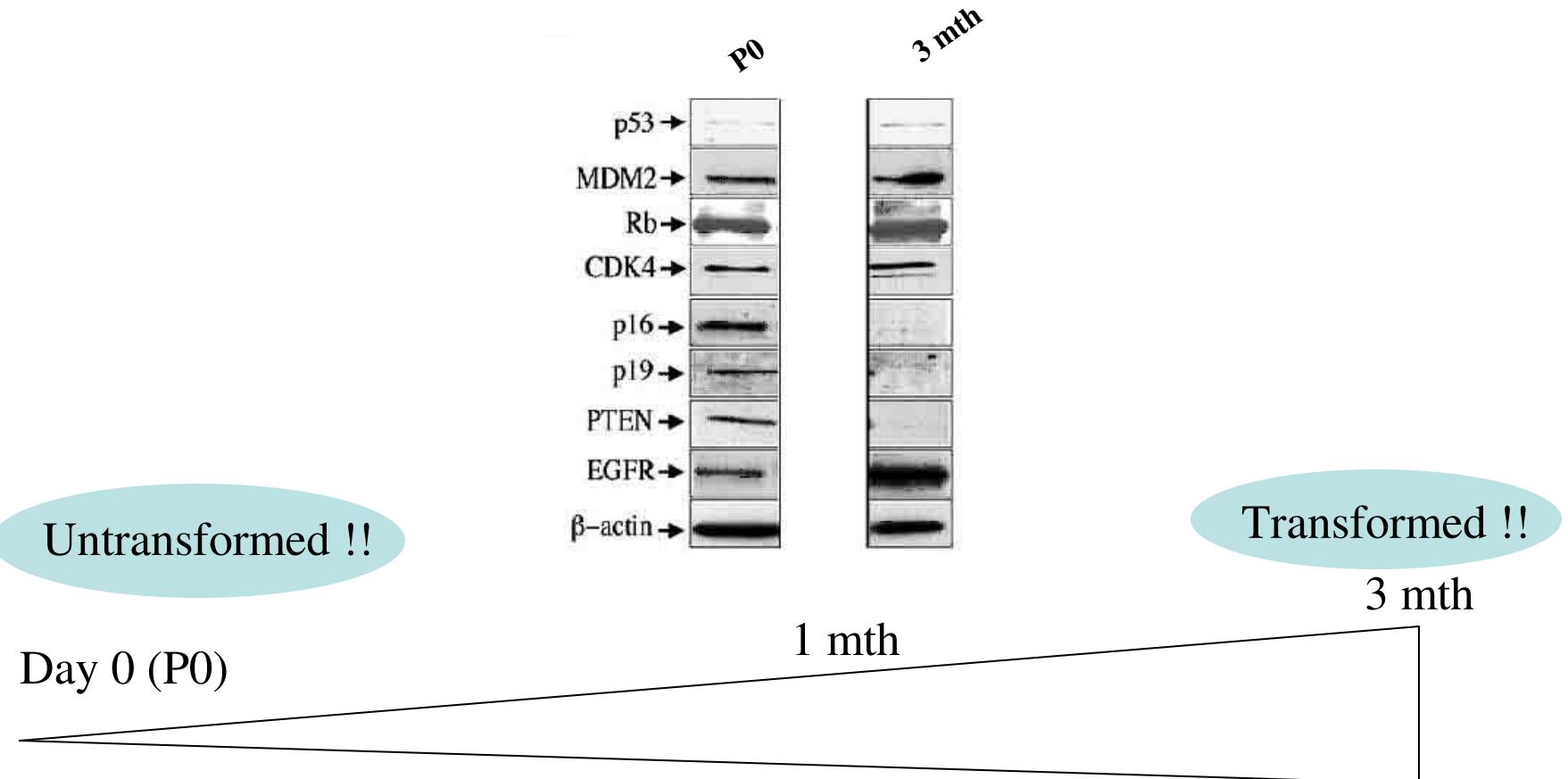
LacZ

EGFRvIII

LacZ

EGFRvIII

Gene Discovery: Gene Trapping



**P0 astrocytes from
GFAP:12VHaRas
-non-transformed
(soft agar assay)**

**??Growth
in Soft
Agar??**

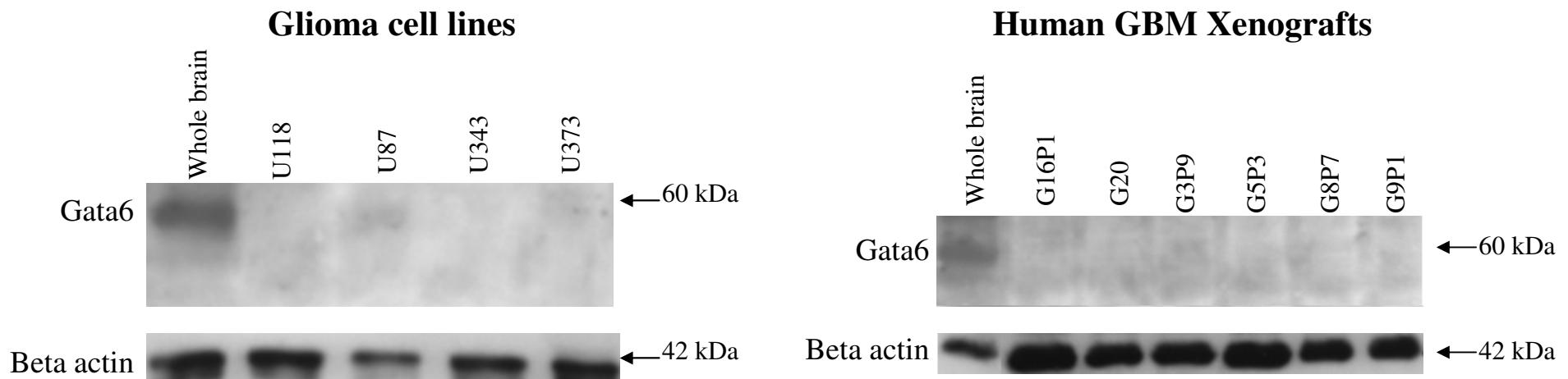
**??Growth
in Nod-
Scids??**

**Clone &
Check in
Human/
transgenic
astrocytomas**

**??Fn
??Make
Transgenics**

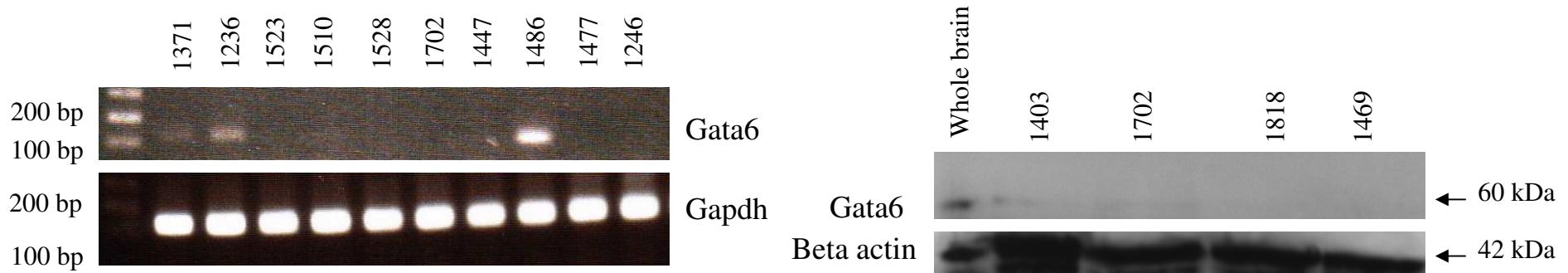
Etc.

GATA6 expression is lost in the human glioma specimens

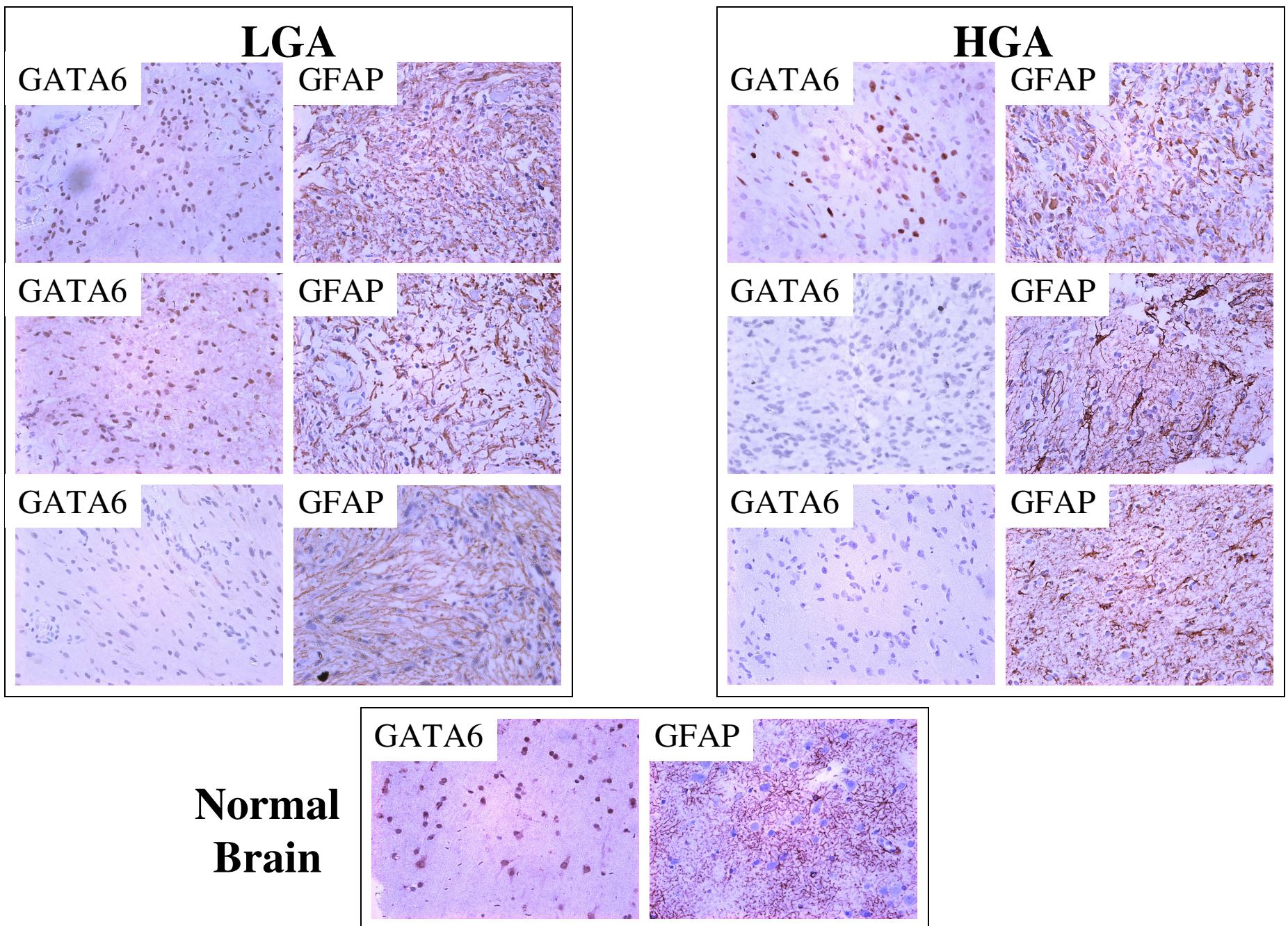


RT-PCR/Western blot analyses on human operative specimens

Expression present	Expression Absent
2 GBM	2 Mixed glioma
1 Meningioma	19 GBM



GATA6 expression is lost in LGA (33%) and HGA (>80%)



- ? Simultaneous XRT + Chemo ??? +/- Biologicals
- ? Cocktails: Anti-prolif +/- Anti-angiogenic
+/- Anti-invasion +/- Pro-apoptotic
- ? Future: PK and efficacy pre-clinical screening in GEMs
- ? Future: Rx dictated by molecular profile of tumor: tissue; imaging
- ? Future: Rx dictated by tumor micro-environmental influences on molecular targets
- ? Future: Targeting “CSC”
- ? Future: Able to shift targeted Rx as tumor biology shifts