Molecular Neuropathology of Gliomas

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Goals

1. Basic review of histopathologic glioma subtypes

2. Illustrate common genetic alterations in astrocytic and oligodendroglial neoplasms

3. Discuss the utility of molecular testing in the context of gliomas

Glioma tumor phenotype

Neoplastic proliferation capable of:

- Uncontrolled proliferation
- Avoiding cell death (apoptosis)
- Unlimited DNA replication
- Avoiding immune surveillance
- Inducing vascular supply
- Tissue invasion and/or metastasis

Histopathologic Subclassification of Brain Cancer

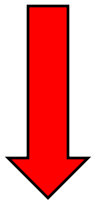
- Cellular morphology has been the foundation of brain cancer classification
- Histologic subtype is based on the concept that brain tumors arise from dedifferentiation of mature cells or from glial or neuronal precursors arrested in an earlier developmental stage (astrocytic, oligodendroglial or mixed)
- Tumor grade is based on the presence of histologic features giving an impression of the anticipated biologic behavior (WHO grade I through IV)

"INFILTRATION" and "INEVITABLE PROGRESSION IN GRADE"

WHO tumor grading of diffuse gliomas

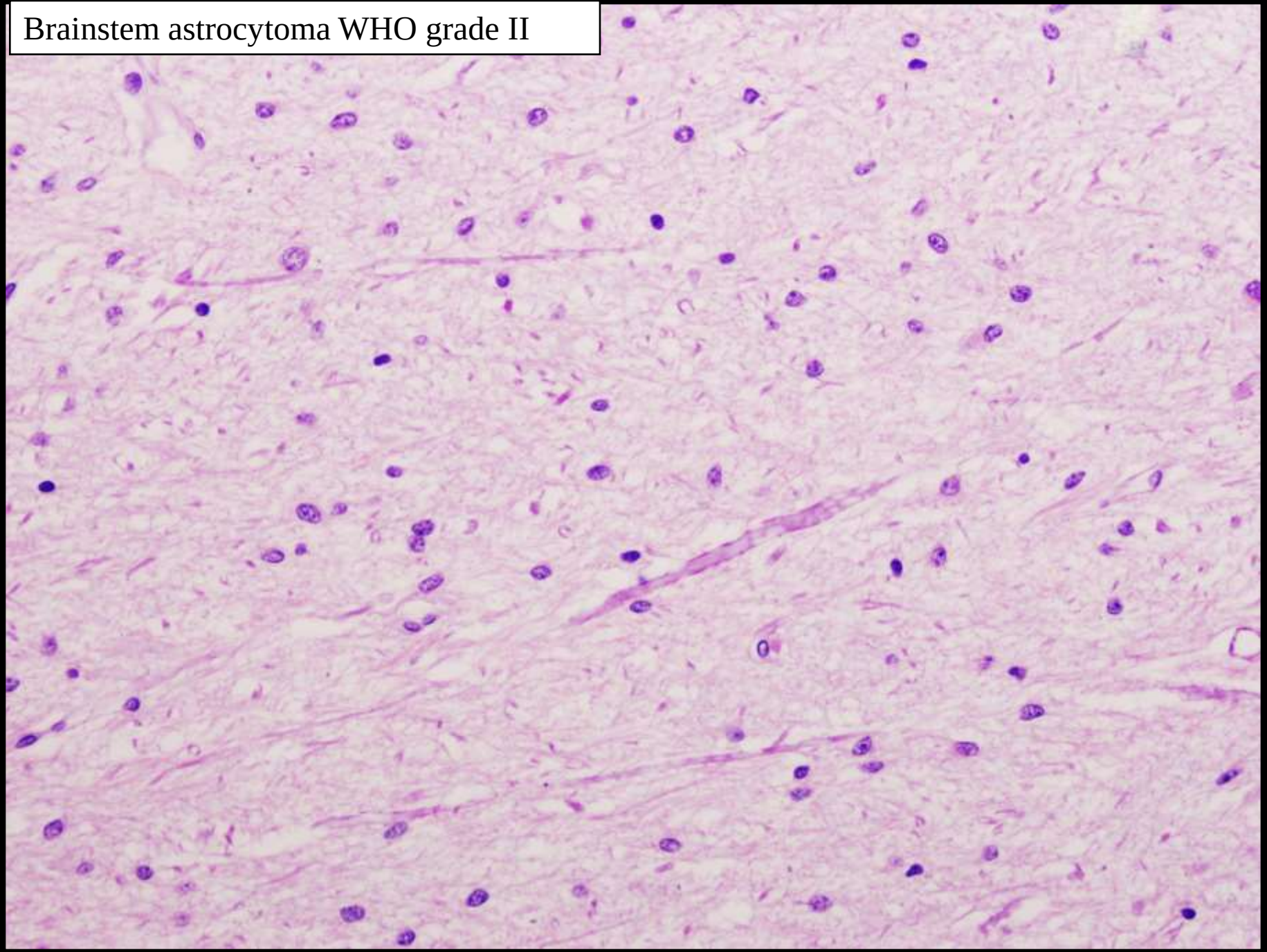
Grade	Histologic subtype		
	Astrocytic	**Mixed**	**Oligodendroglial**
I			
II	Astrocytoma	Oligoastrocytoma	Oligodendroglioma
III	Anaplastic astrocytoma	Anaplastic oligoastrocytoma	Anaplastic oligodendroglioma
IV	Glioblastoma	Glioblastoma with oligodendroglial component	

Cellularity
Nuclear atypia
Mitotic activity
Microvascular proliferation and/or necrosis

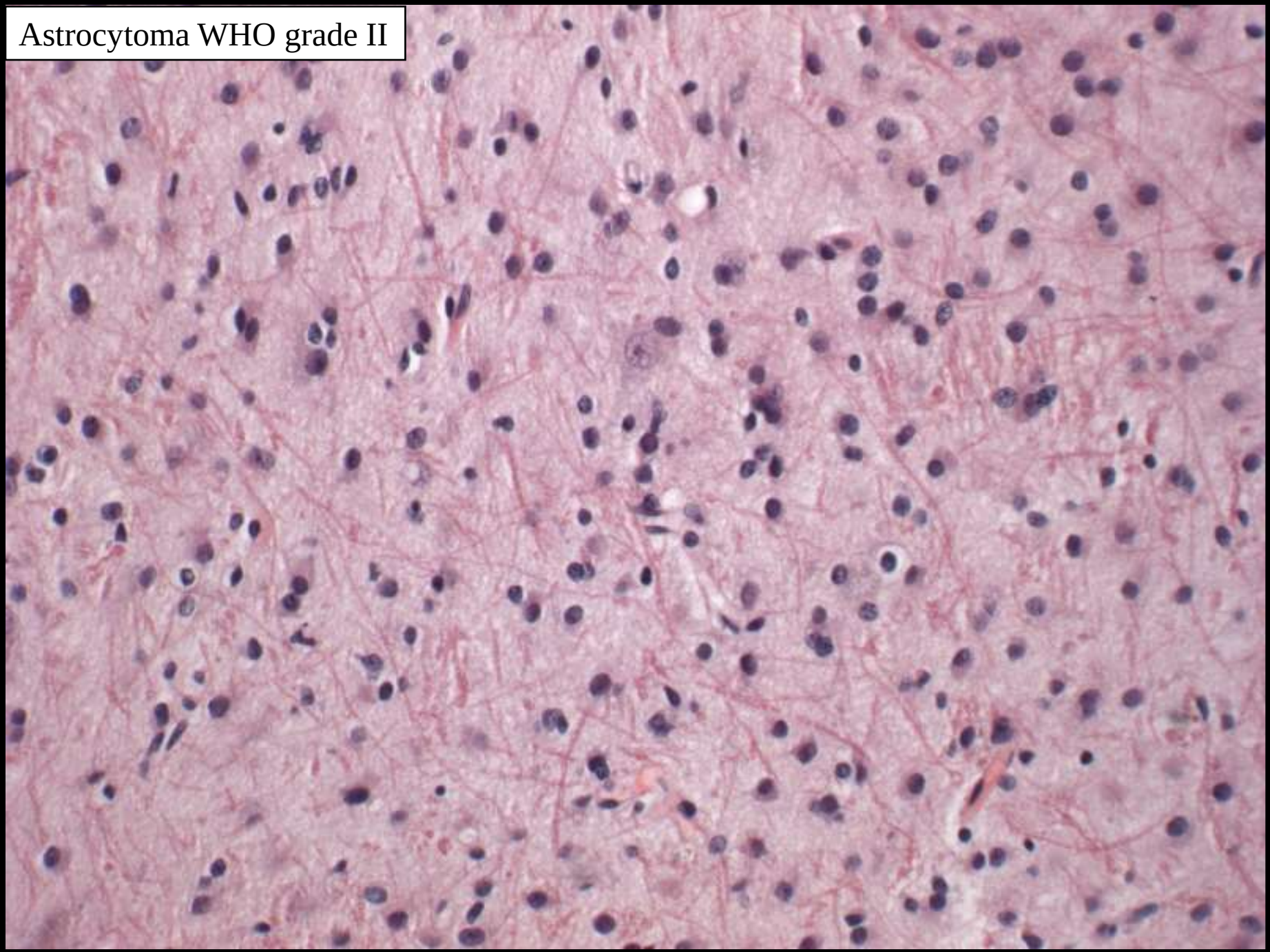


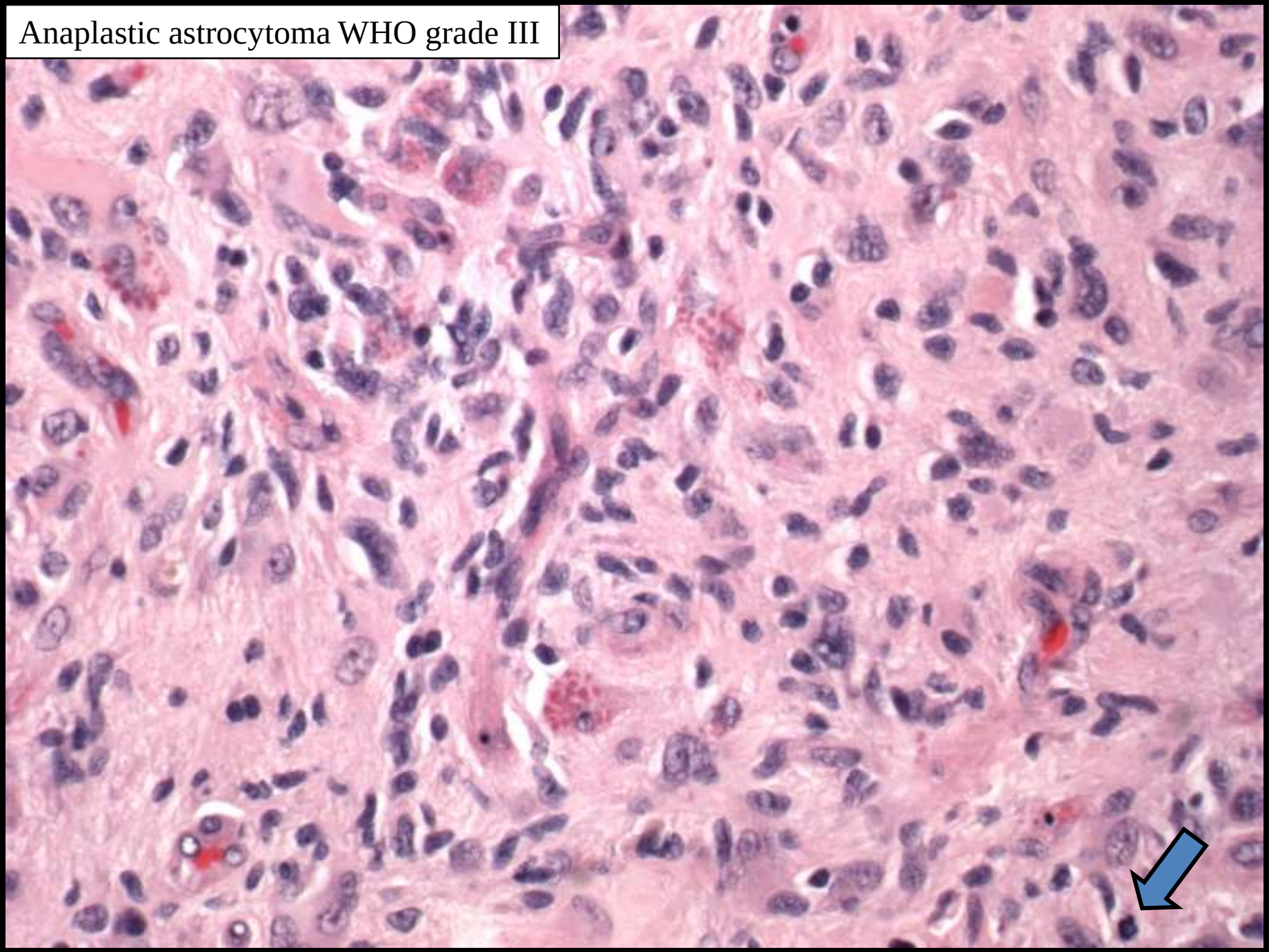
Grade I	Benign
Grade II	Low grade
Grade III	High grade
Grade IV	High grade

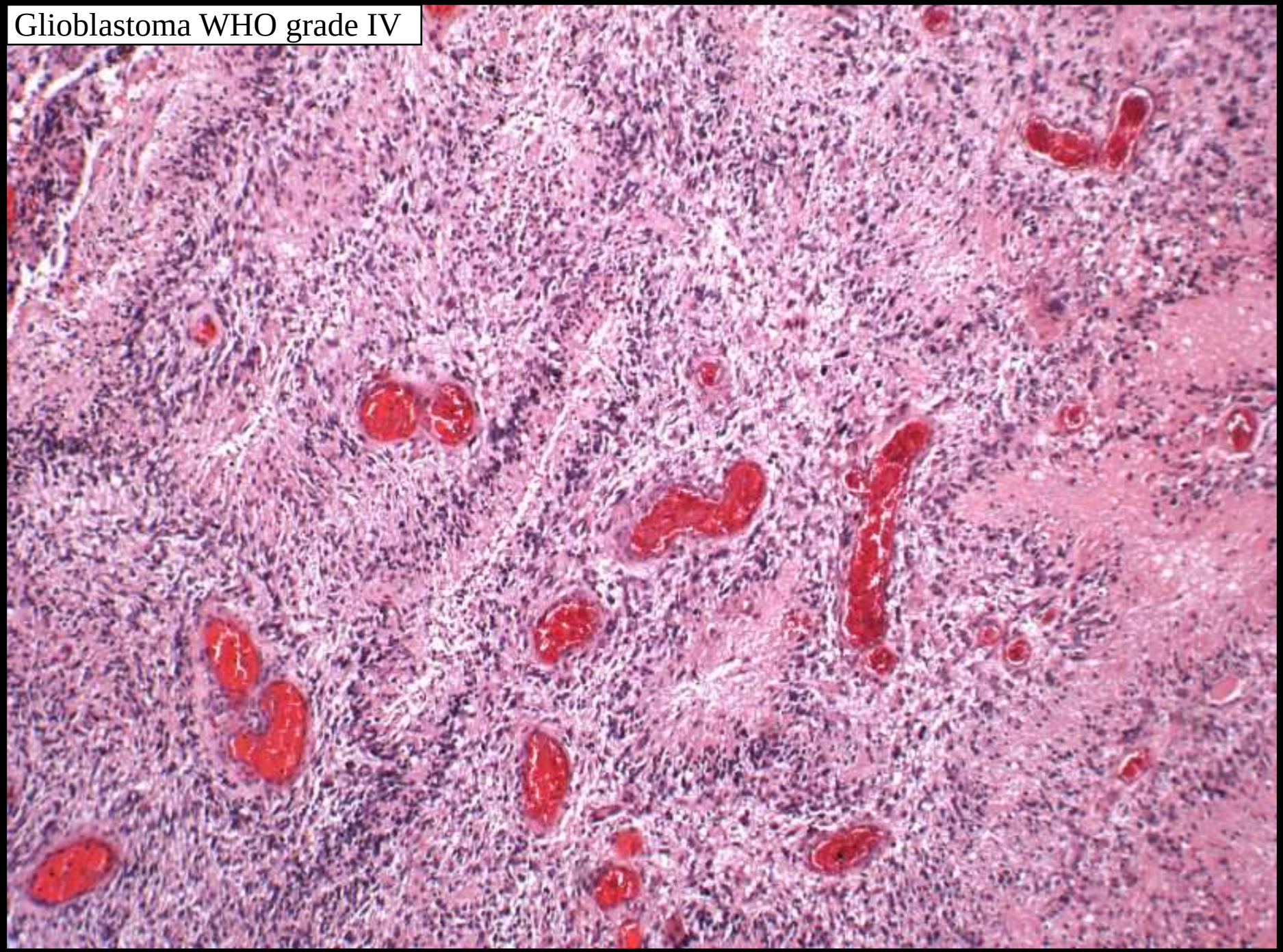
Brainstem astrocytoma WHO grade II



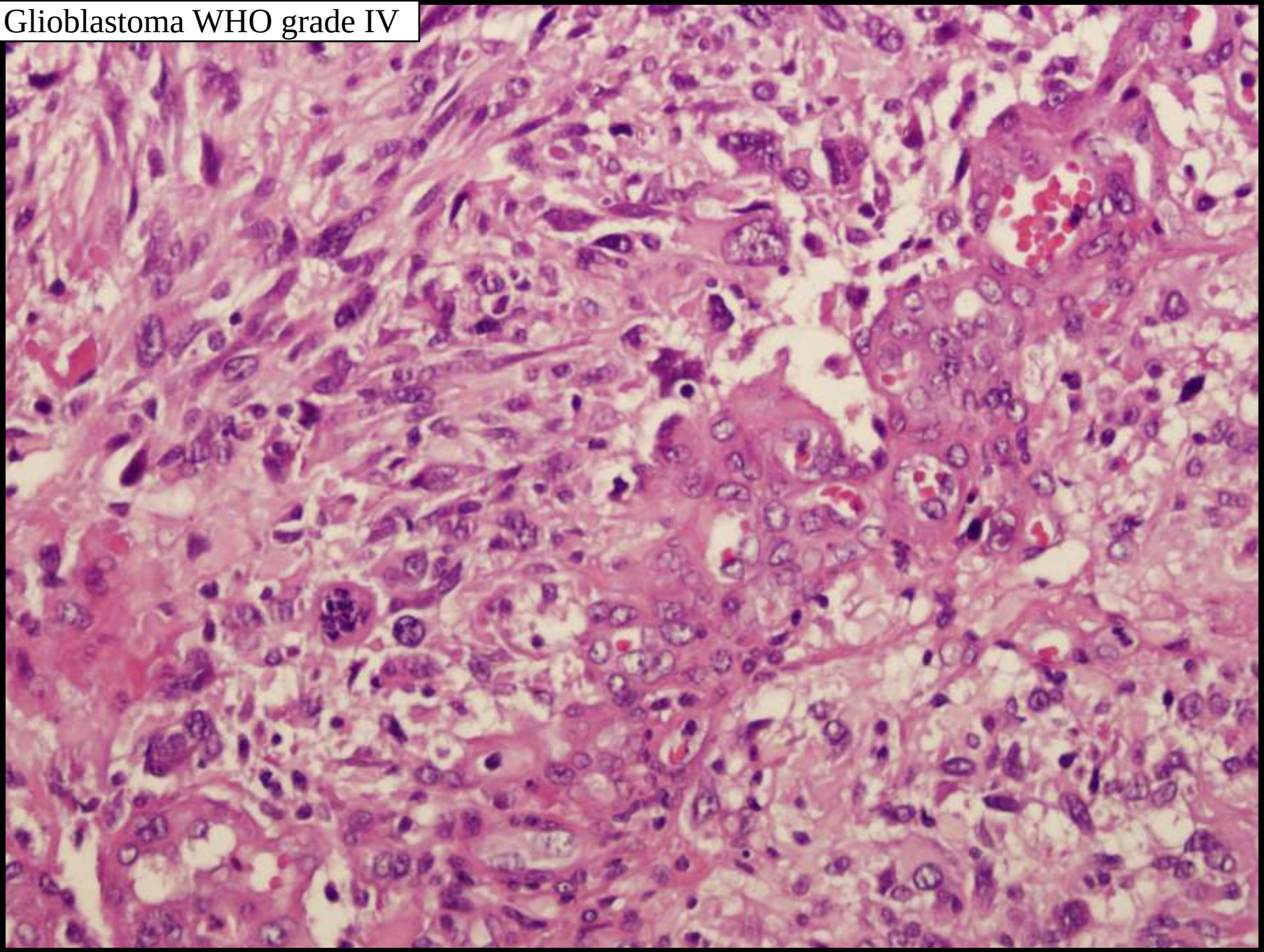
Astrocytoma WHO grade II

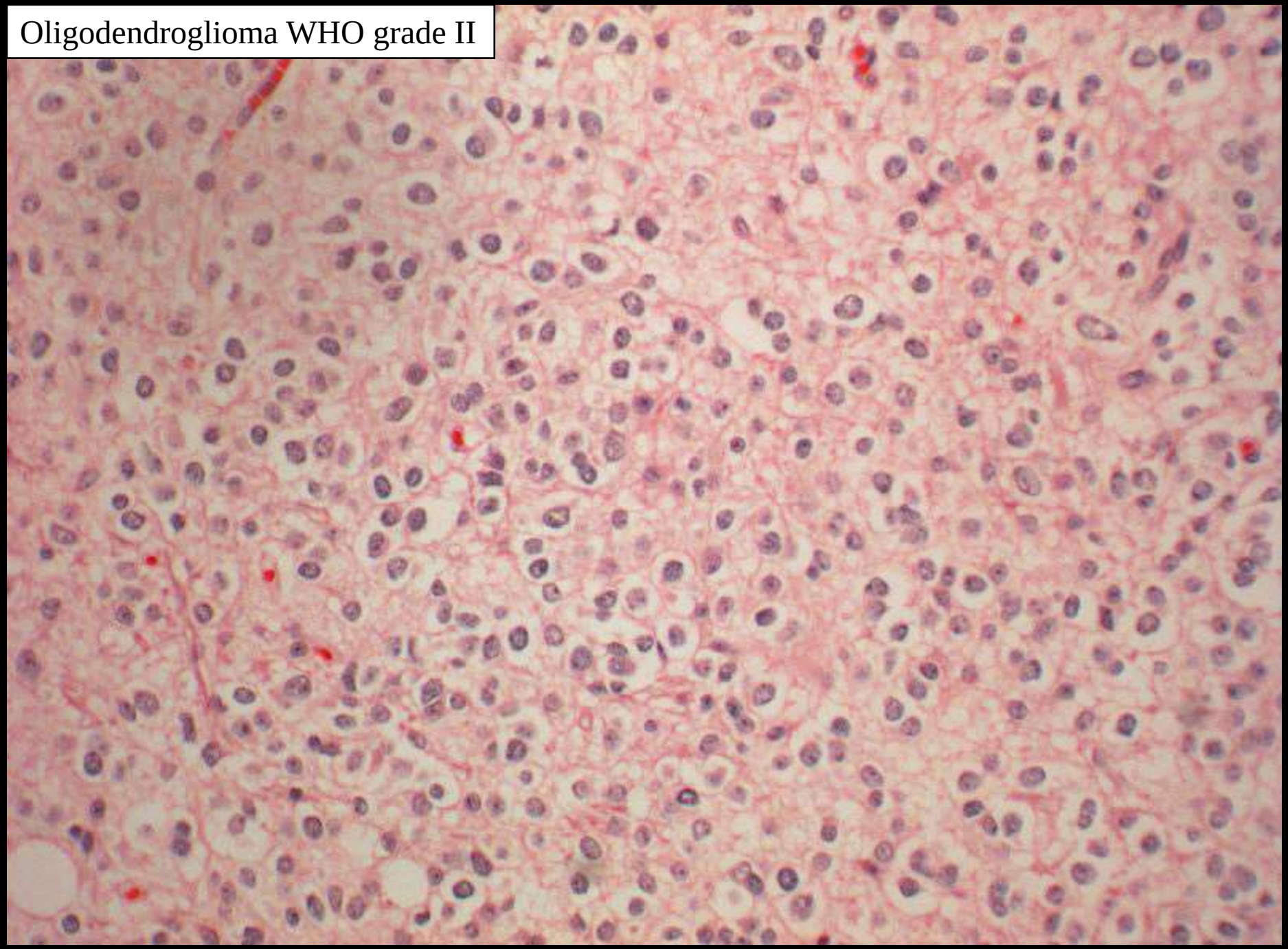



Anaplastic astrocytoma WHO grade III

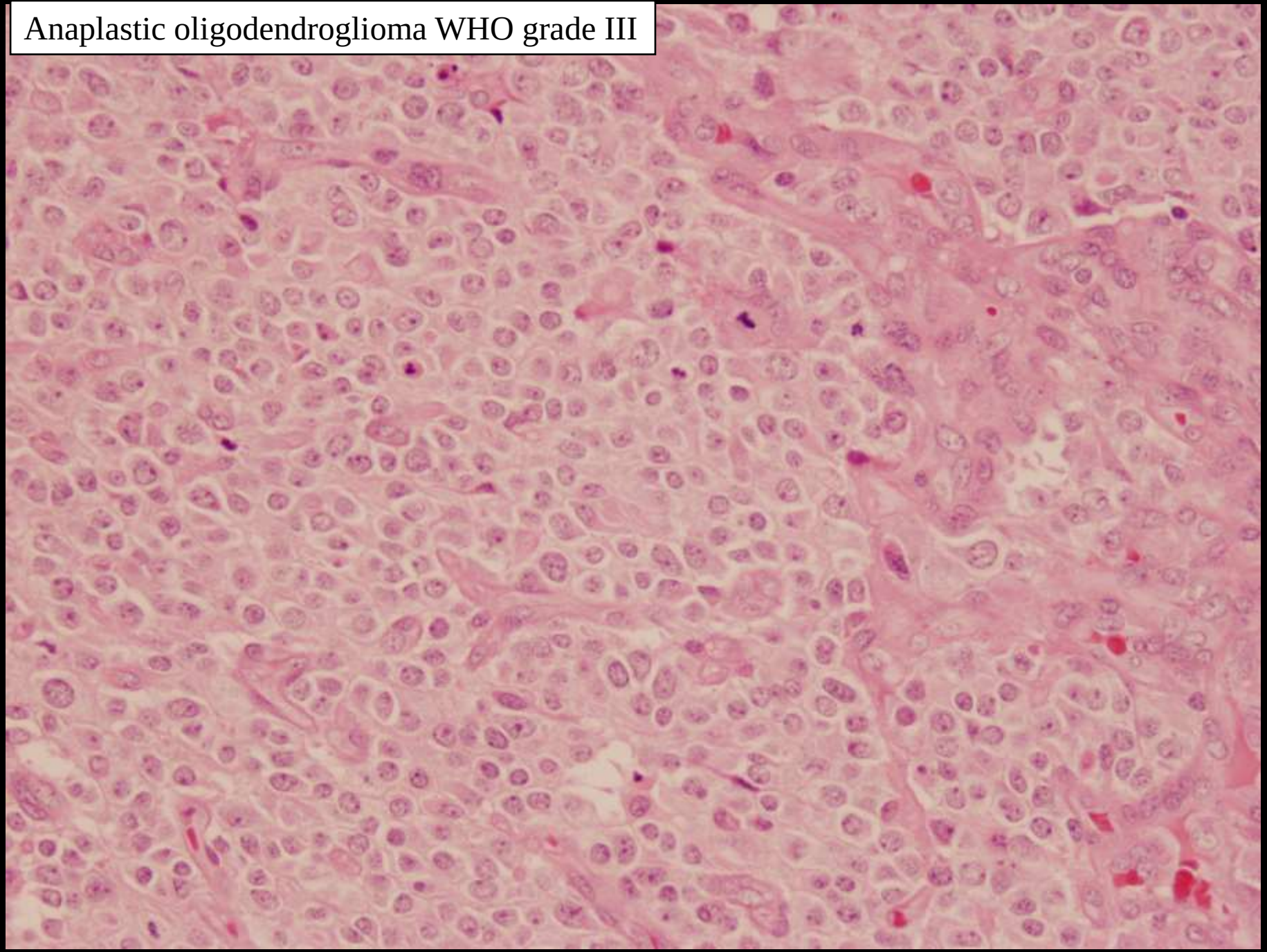

Glioblastoma WHO grade IV

Glioblastoma WHO grade IV




Oligodendroglioma WHO grade II

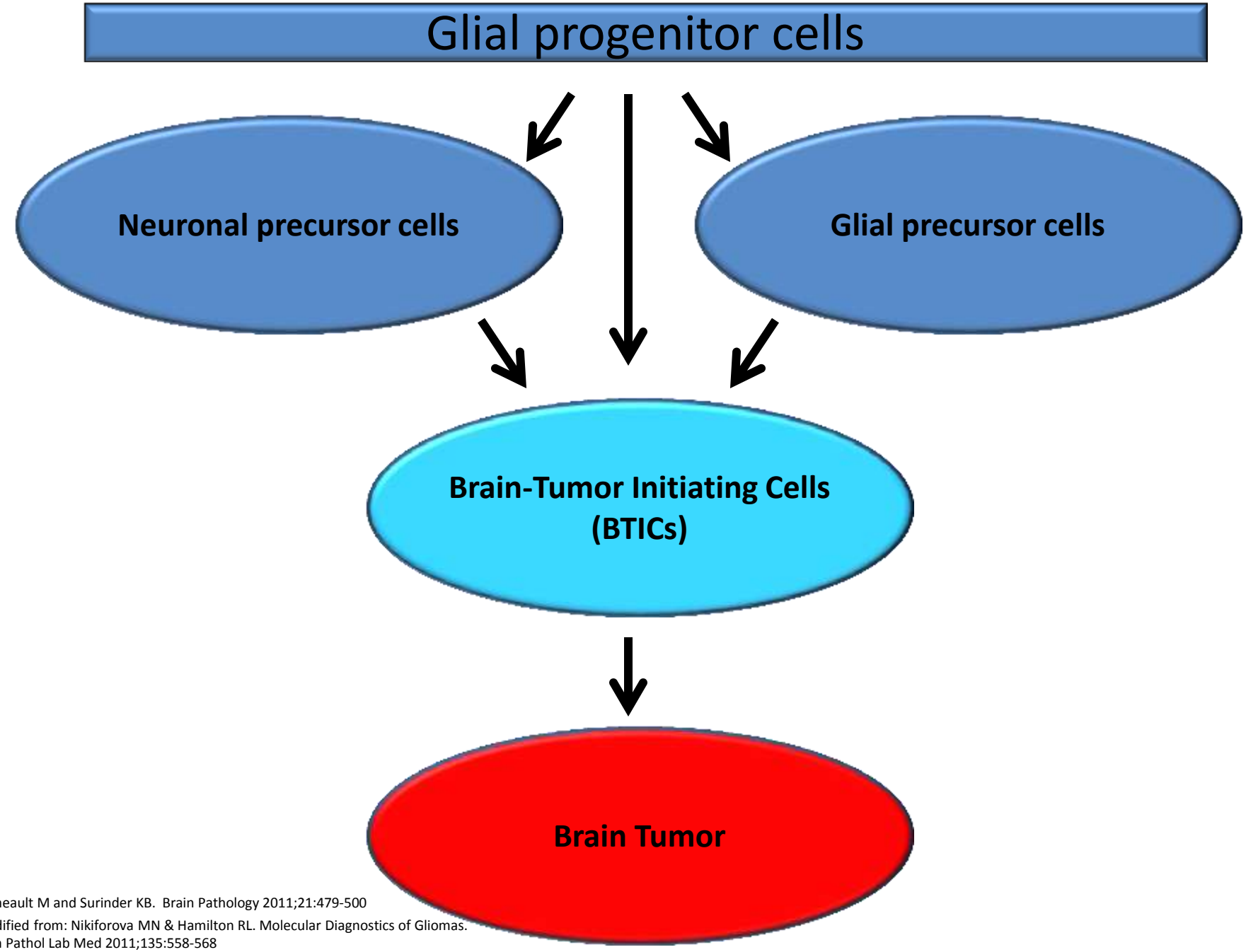
Anaplastic oligodendroglioma WHO grade III



Gliomas account for:

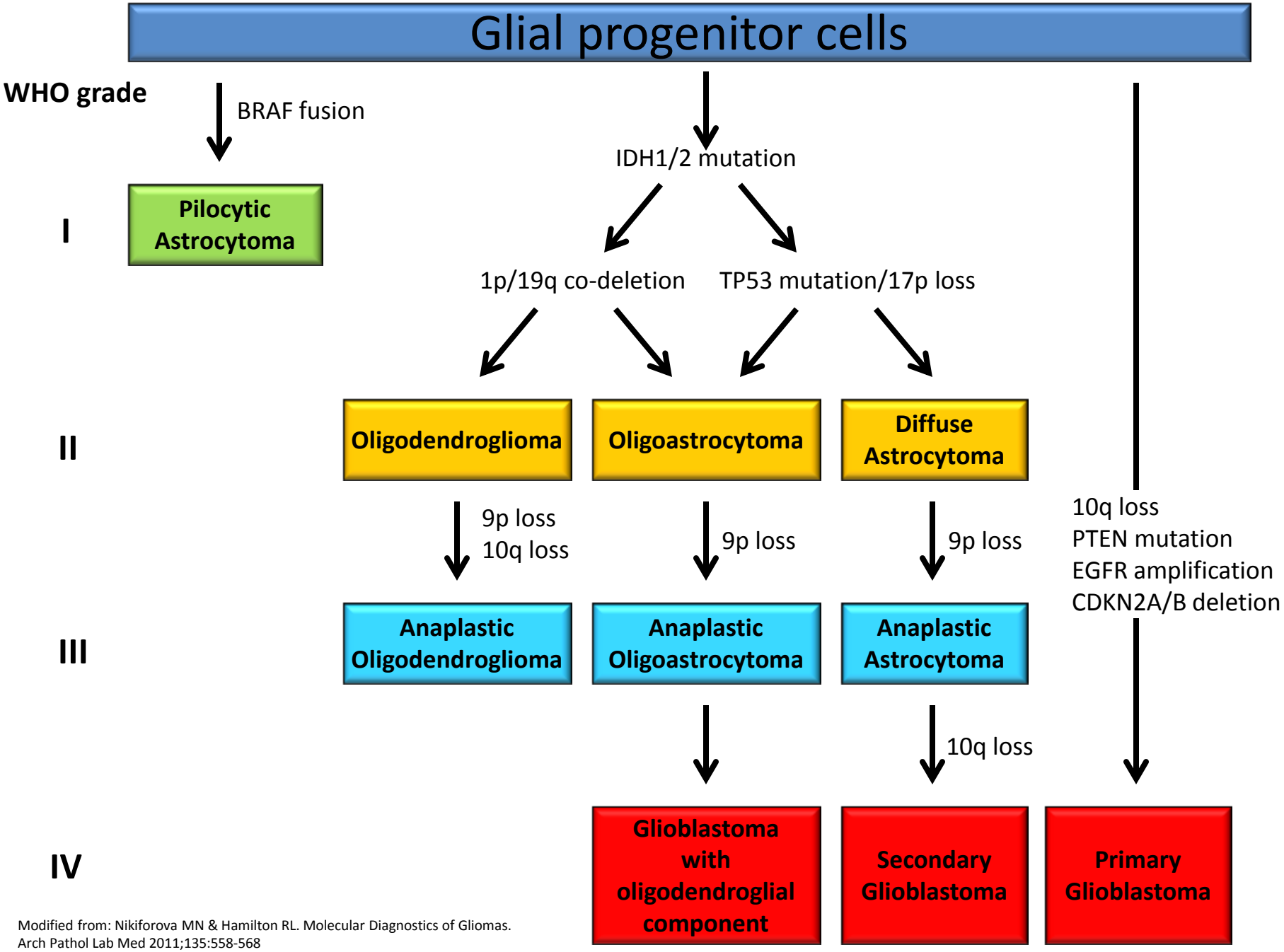
- 30% of all CNS tumors
- 80% of primary malignant brain tumors

	WHO grade	Percentage of gliomas	5 year survival
Astrocytoma	II	1.6%	50%
Anaplastic astrocytoma	III	6.7%	30%
Glioblastoma (15% of all CNS tumors) **Primary** (80-85% of GBM) **Secondary**	IV	55%	5%
Oligodendroglioma 5-18% of all gliomas <1% of pediatric gliomas	II	2-5%	75% (39-75%)
Anaplastic oligodendroglioma 1.8% of all gliomas	III	3.5%	23-41%
Oligoastrocytoma	II	~	58%
Anaplastic oligoastrocytoma	III	~	36%



Mimeault M and Surinder KB. Brain Pathology 2011;21:479-500

Modified from: Nikiforova MN & Hamilton RL. Molecular Diagnostics of Gliomas. Arch Pathol Lab Med 2011;135:558-568



Modified from: Nikiforova MN & Hamilton RL. Molecular Diagnostics of Gliomas. Arch Pathol Lab Med 2011;135:558-568

Why test?

- Potential type(s) of information obtained:
  - **Diagnostic**: Aid in rendering a morphologic diagnosis
  - **Prognostic**: Educated guess at a tumor's behavior without the influence of treatment
  - **Predictive**: Response of tumor to therapy

Average Prevalence of Genetic Abnormalities in Gliomas and Their Clinical Utility

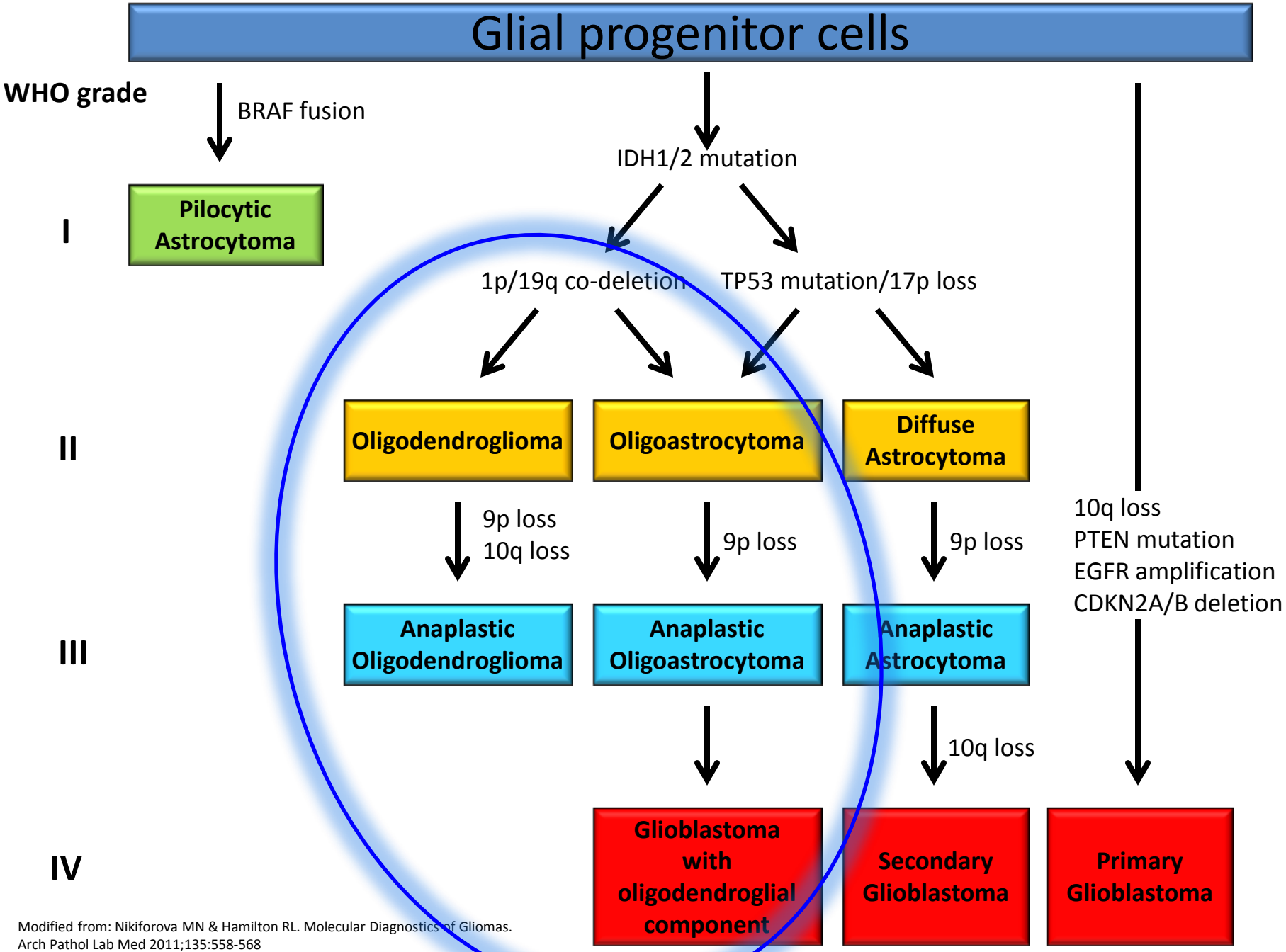
Tumor type WHO grade	BRAF fusion	1p/19q deletion	IDH mutation	MGMT methylation	10q LOH/ PTEN mutation	EGFR EGFRvIII
Pilocytic astrocytoma WHO I	60-80 %			<20%		
Oligodendroglioma WHO II		80-90%	80-94%	60-93%		
Anaplastic oligodendroglioma WHO III		50-70%	90%	50-75%	<10%	
Diffuse astrocytoma WHO II			70-90%	40-45%		
Anaplastic astrocytoma WHO III			70%	50%	35-60%	
Secondary GBM WHO IV			80-85%	40-60%	60-70%/<5%	
Primary GBM WHO IV			<5%	40%	80%/15-40%	~40%
Clinical utility	Diagnostic	Diagnostic Prognostic Predictive	Diagnostic Prognostic Predictive?	Predictive	Prognostic	Diagnostic Predictive?
Targeted therapy?	Yes?	No	No	Yes	No	Yes

Modified from: Nikoforova MN and Hamilton RL. Molecular Diagnostic of Gliomas.
Arch Pathol Lab Med 2011;135:558-568

Goals

1. Illustrate common genetic alterations in astrocytic and oligodendroglial neoplasms
   - **Co-deletion of 1p and 19q**
   - MGMT methylation
   - IDH1 and IDH2 mutations
   - Basic introduction to signaling pathways commonly involved in gliomas

2. Discuss the utility of molecular testing in the context of gliomas



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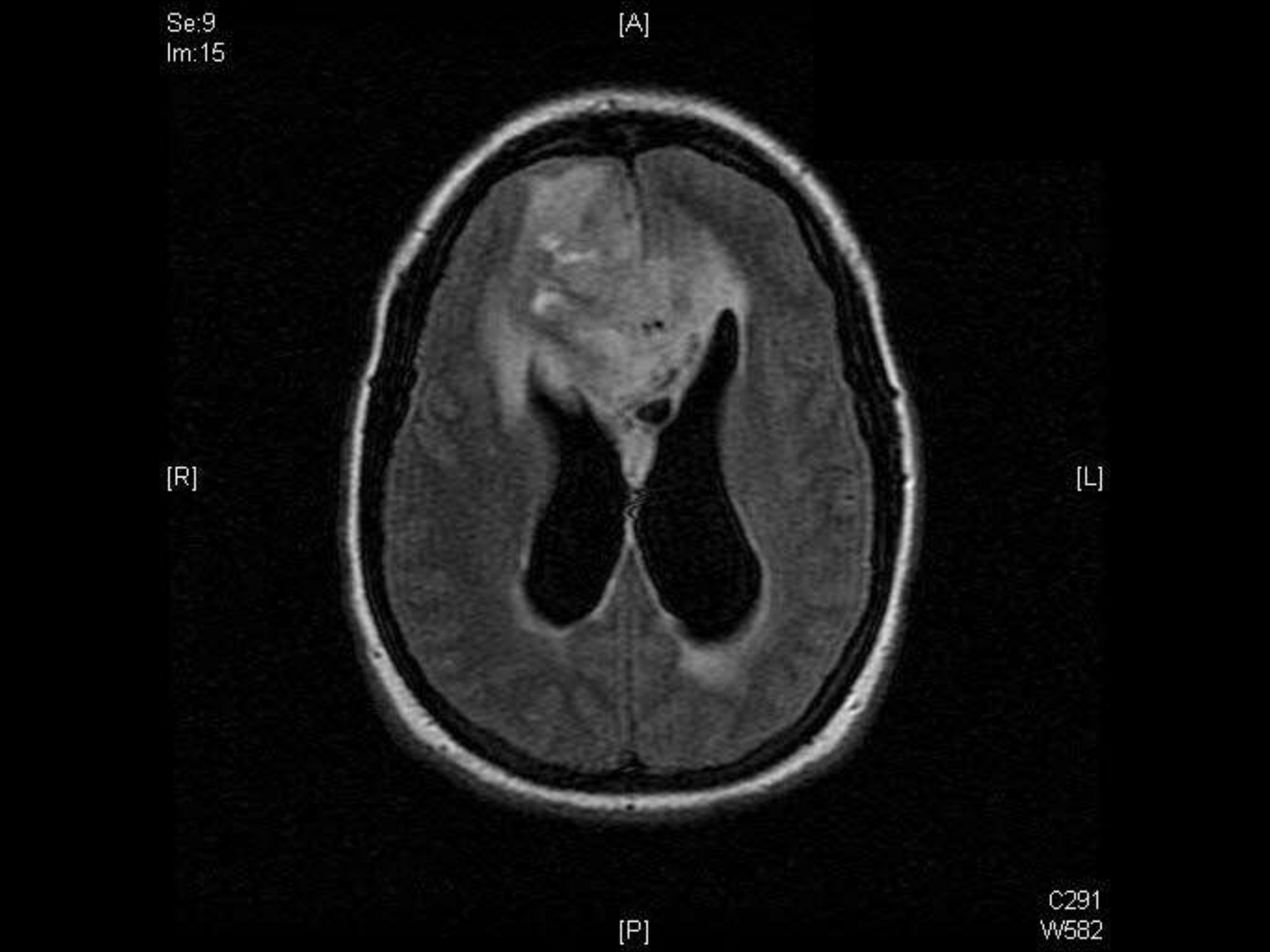
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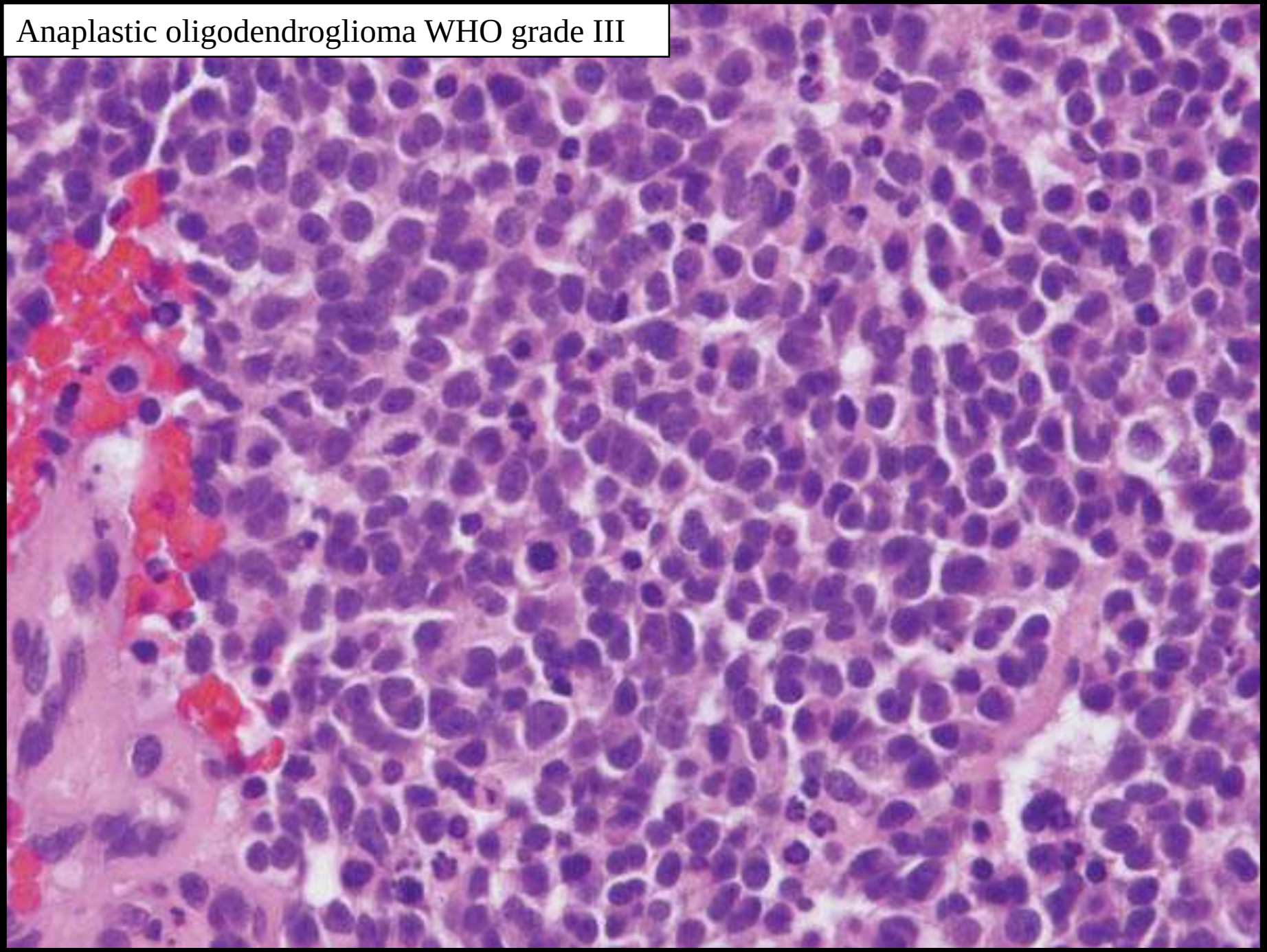
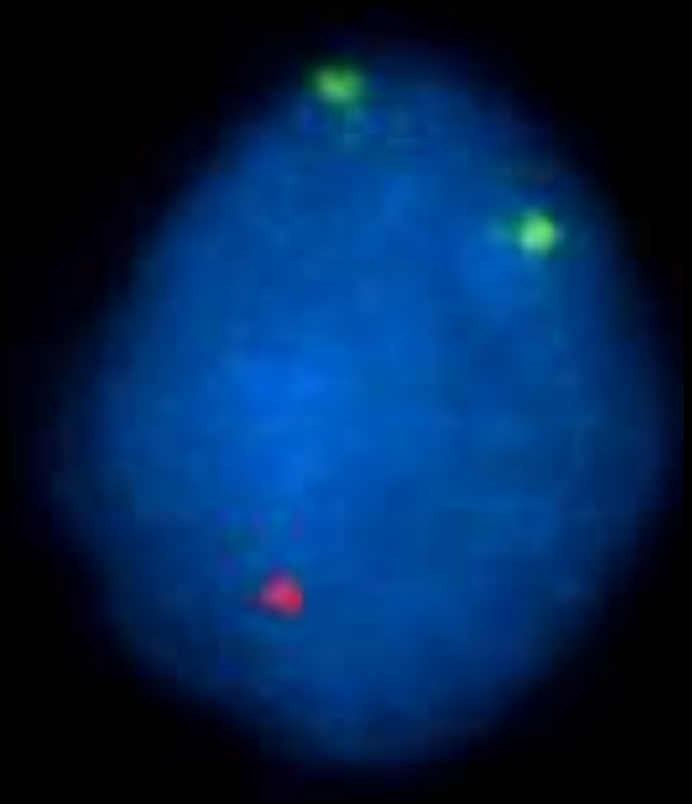
C291
W582

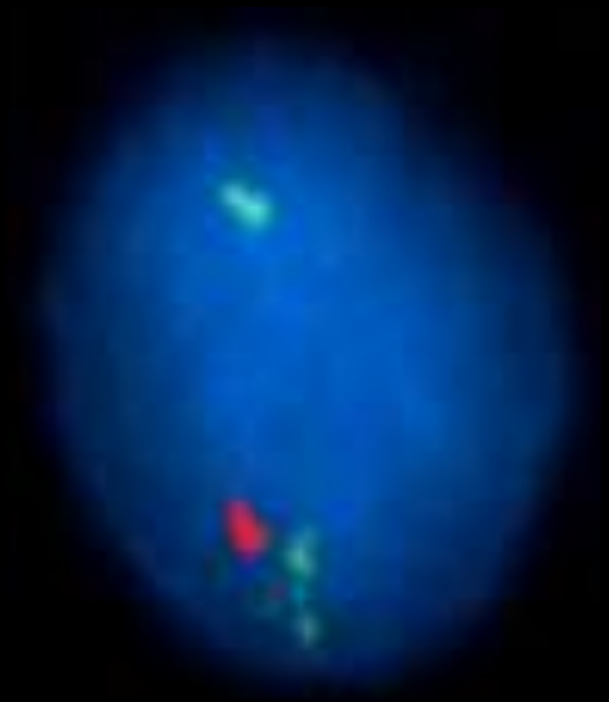
Anaplastic oligodendroglioma WHO grade III



1p36 SO/1q25 SGn



19q13 SO/19p13 SGn



Diagnosis:

- Anaplastic oligodendroglioma, WHO grade III
- 1p and 19q co-deletion identified

Successful Chemotherapy for Newly Diagnosed Aggressive Oligodendroglioma

David R. Macdonald, MD,*† Laurie E. Gaspar, MD,† and J. Gregory Cairncross, MD*†

We treated 3 patients with newly diagnosed aggressive oligodendroglioma with chemotherapy prior to radiotherapy and observed responses. All had residual or progressive tumor following initial surgery. We used a combination called PCV-3 because recurrent anaplastic oligodendroglioma responds predictably to this regimen. Chemotherapy for response induction followed by radiotherapy for consolidation is feasible and effective initial treatment for this tumor.

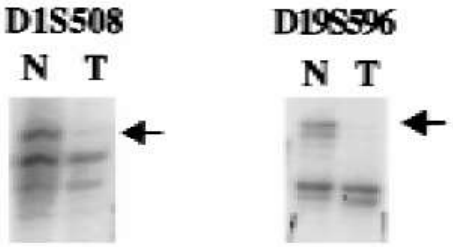
Macdonald DR, Gaspar LE, Cairncross JG.
Successful chemotherapy for newly diagnosed aggressive oligodendroglioma.
Ann Neurol 1990;27:573–574

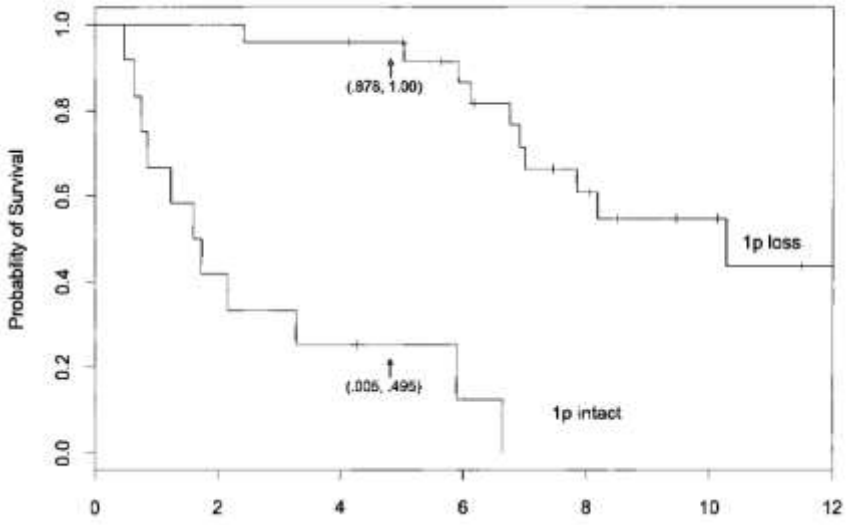
PCV = procarbazine, CCNU (lomustine), & vincristine
Currently use the oral alkylating agent temozolomide

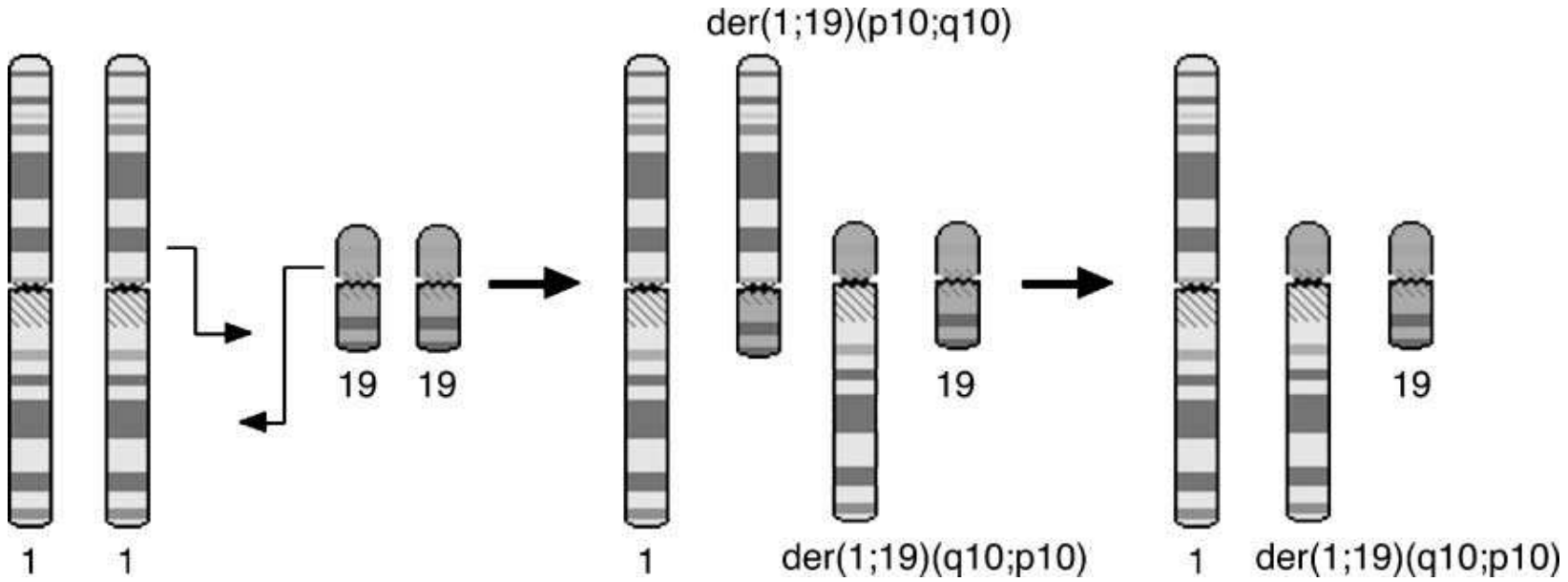
J Natl Cancer Inst 1998;90:1461-1467

Specific Genetic Predictors of Chemotherapeutic Response and Survival in Patients With Anaplastic Oligodendrogliomas

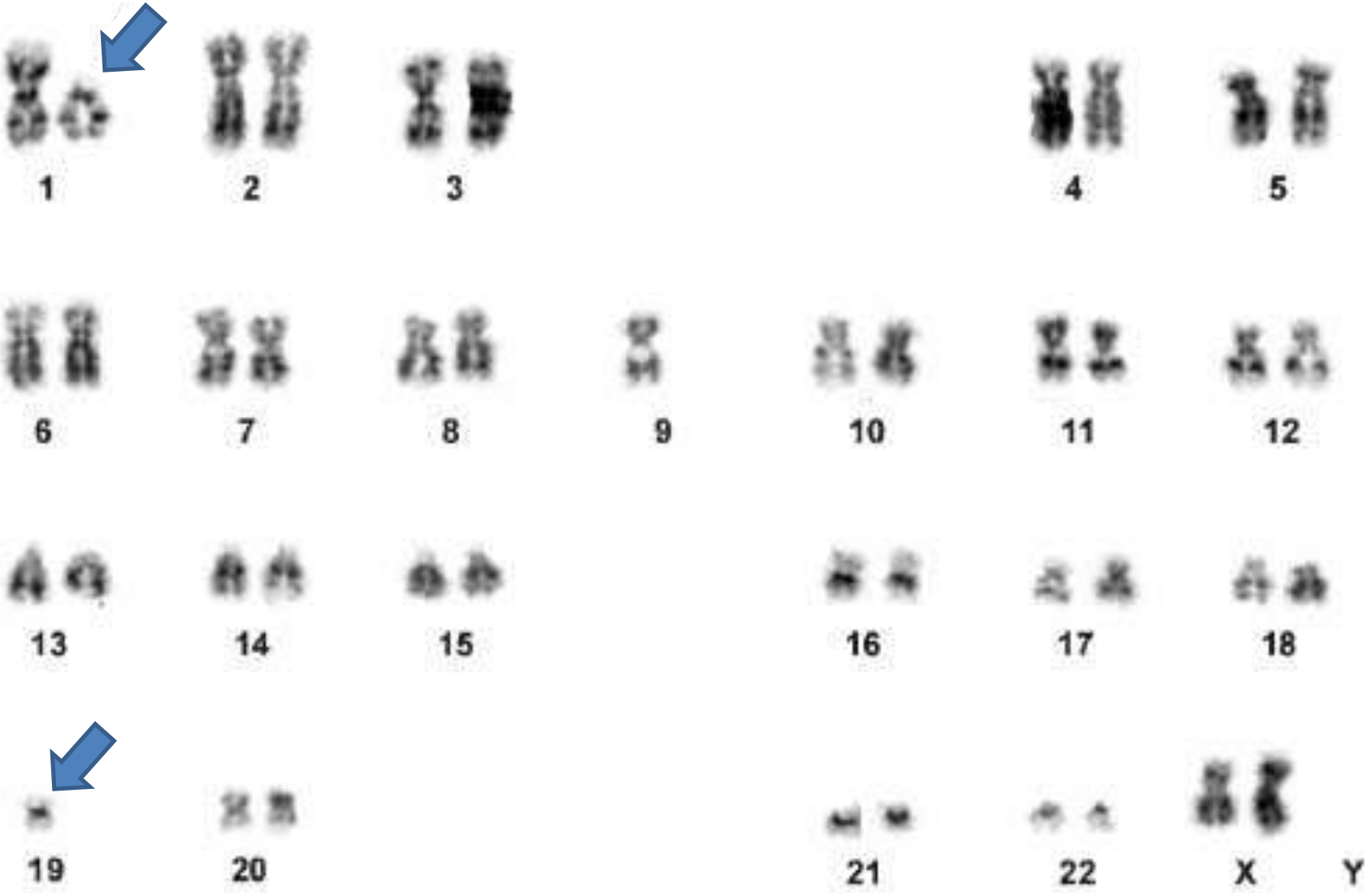
J. Gregory Cairncross, Keisuke Ueki, Magdelena C. Zlatescu, David K. Lisle, Dianne M. Finkelstein, Robert R. Hammond, Jonathan S. Silver, Paul C. Stark, David R. Macdonald, Yasushi Ino, David A. Ramsay, David N. Louis







From: Griffen CA, et al.
Identification of der(1;19)(q10;p10) in Five Oligodendrogliomas Suggests Mechanism of Concurrent 1p and 19q Loss.
J Neuropathol Exp Neurol 2006;65:988-994



Griffen CA, et al. Identification of der(1;19)(q10;p10) in Five Oligodendrogliomas Suggests Mechanism of Concurrent 1p and 19q Loss. J Neuropathol Exp Neurol 2006;65:988-994

1p/19q co-deletion

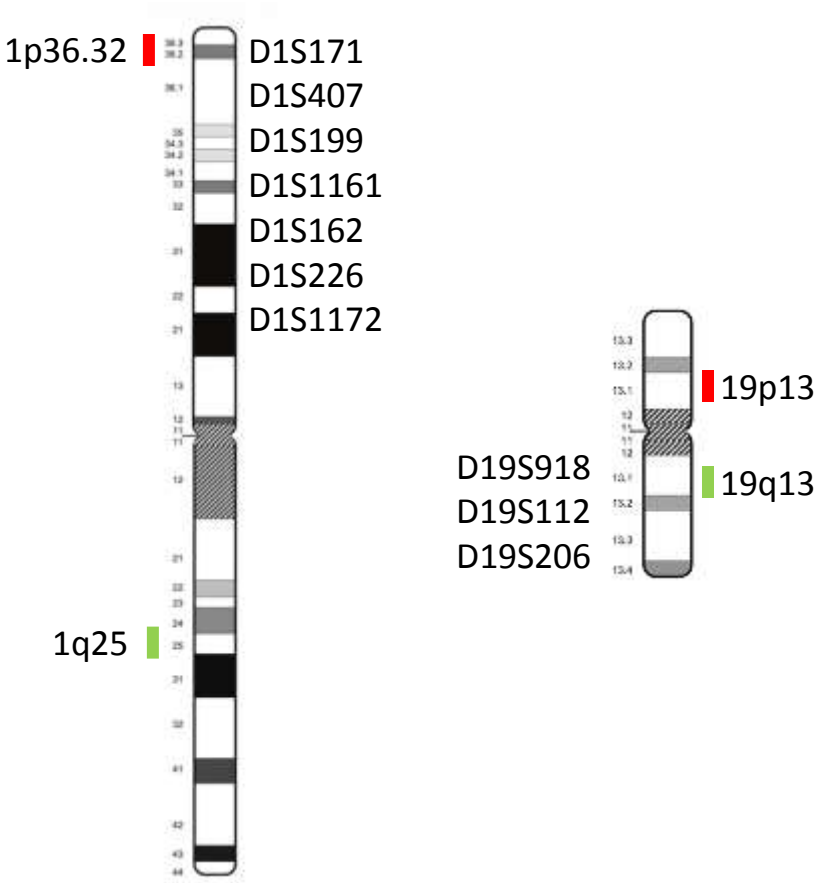
Clinical utility

- Diagnostic:
  - Positive result may be used to confirm morphologic impression of oligodendroglioma (seen in 50% - 80% of oligodendroglial neoplasms)
- Prognostic:
  - Longer survival
- Predictive:
  - Genetic indicator of “responder phenotype”
  - Complete neuroradiologic response:
    - ~50% in cases with co-deletion of 1p & 19q
    - <25% in cases without co-deletion 1p & 19q
  - Survival (mean)
    - 10 years in cases with co-deletion of 1p & 19q
    - ~2 years in cases without co-deletion
  - Gene(s) and/or mechanism(s) not yet identified

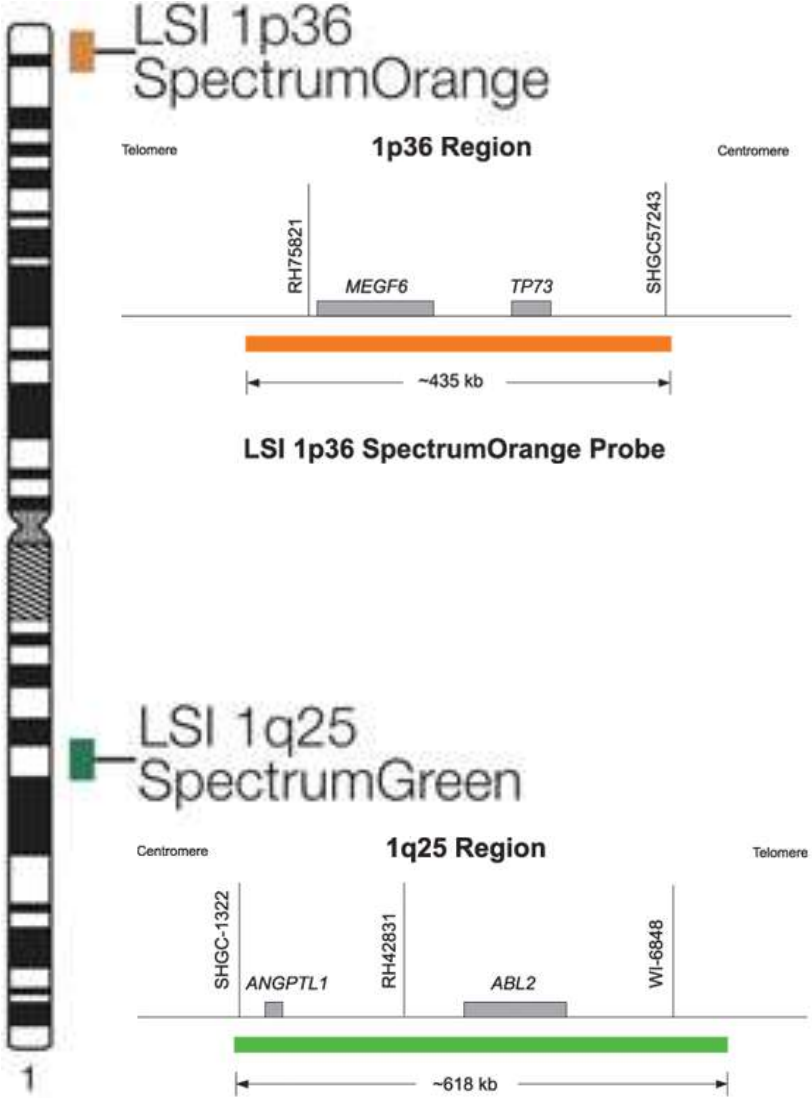
1p/19q co-deletion

Test methodologies

- FISH
- Microsatellite
- CGH
- MLPA

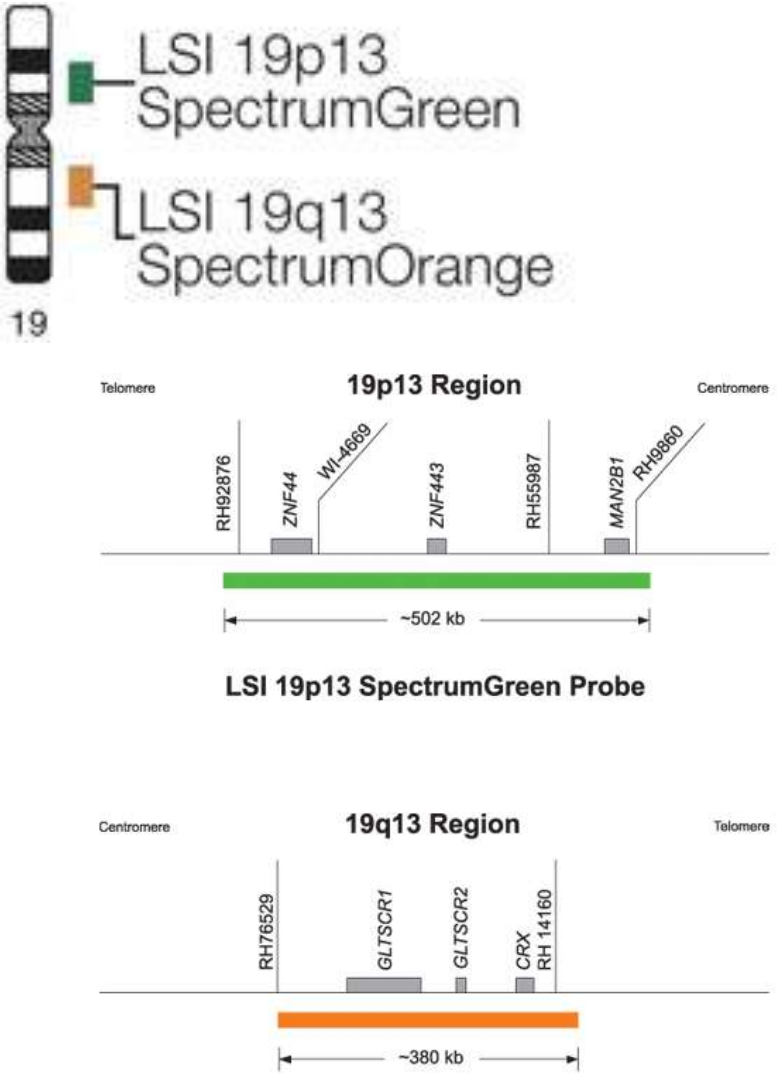


Modified from: http://atlasgeneticsoncology.org



LSI 1p36 SpectrumOrange Probe

LSI 1q25 SpectrumGreen Probe



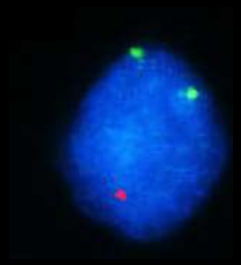
LSI 19p13 SpectrumGreen Probe

LSI 19q13 SpectrumOrange Probe

FISH 1p deletion

1p36 SO/1q25 SGn

Normal (two alleles)



Abnormal (loss of one copy of 1p)

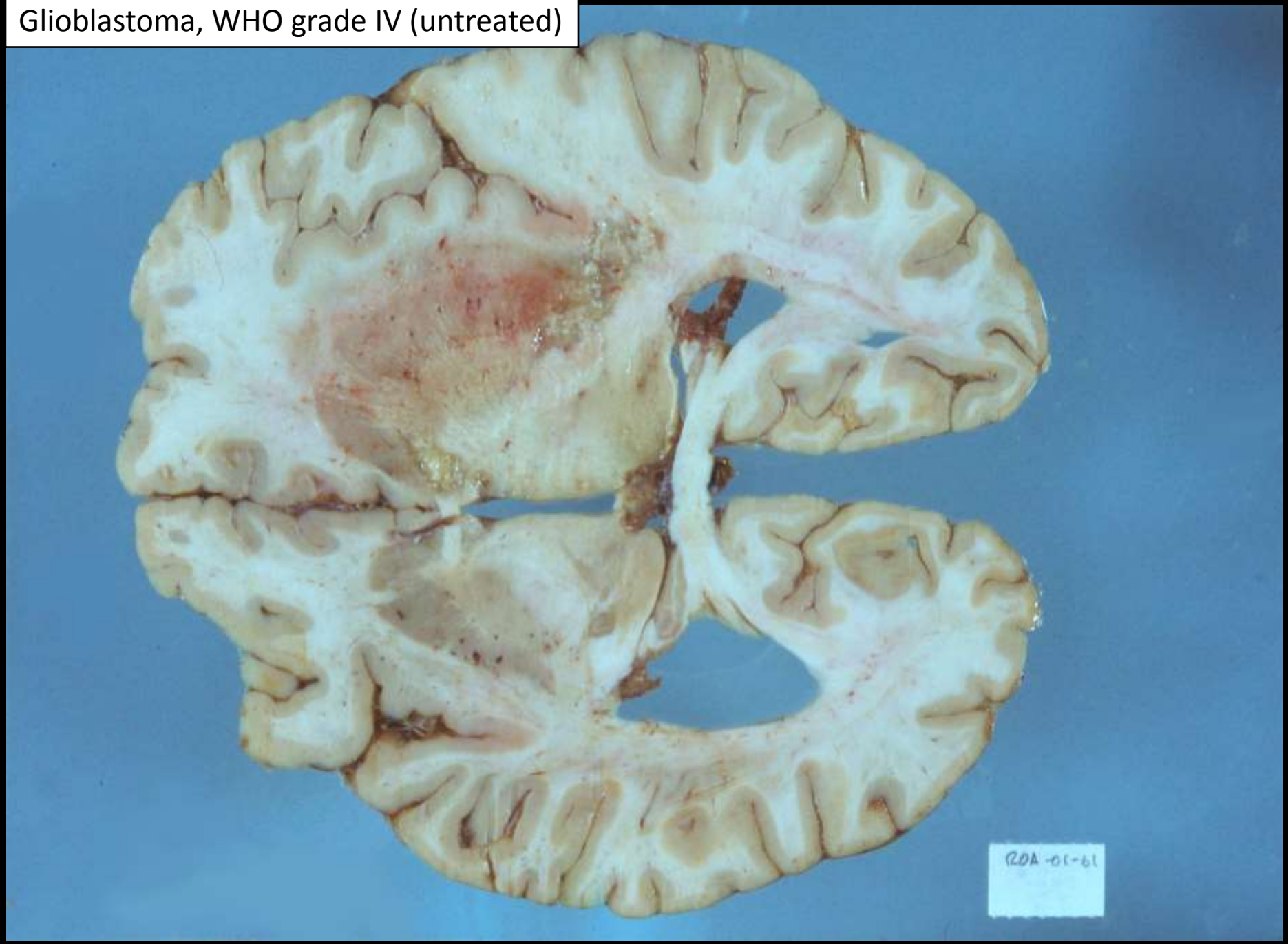
Abnormal (polysomy with relative loss of 1p)

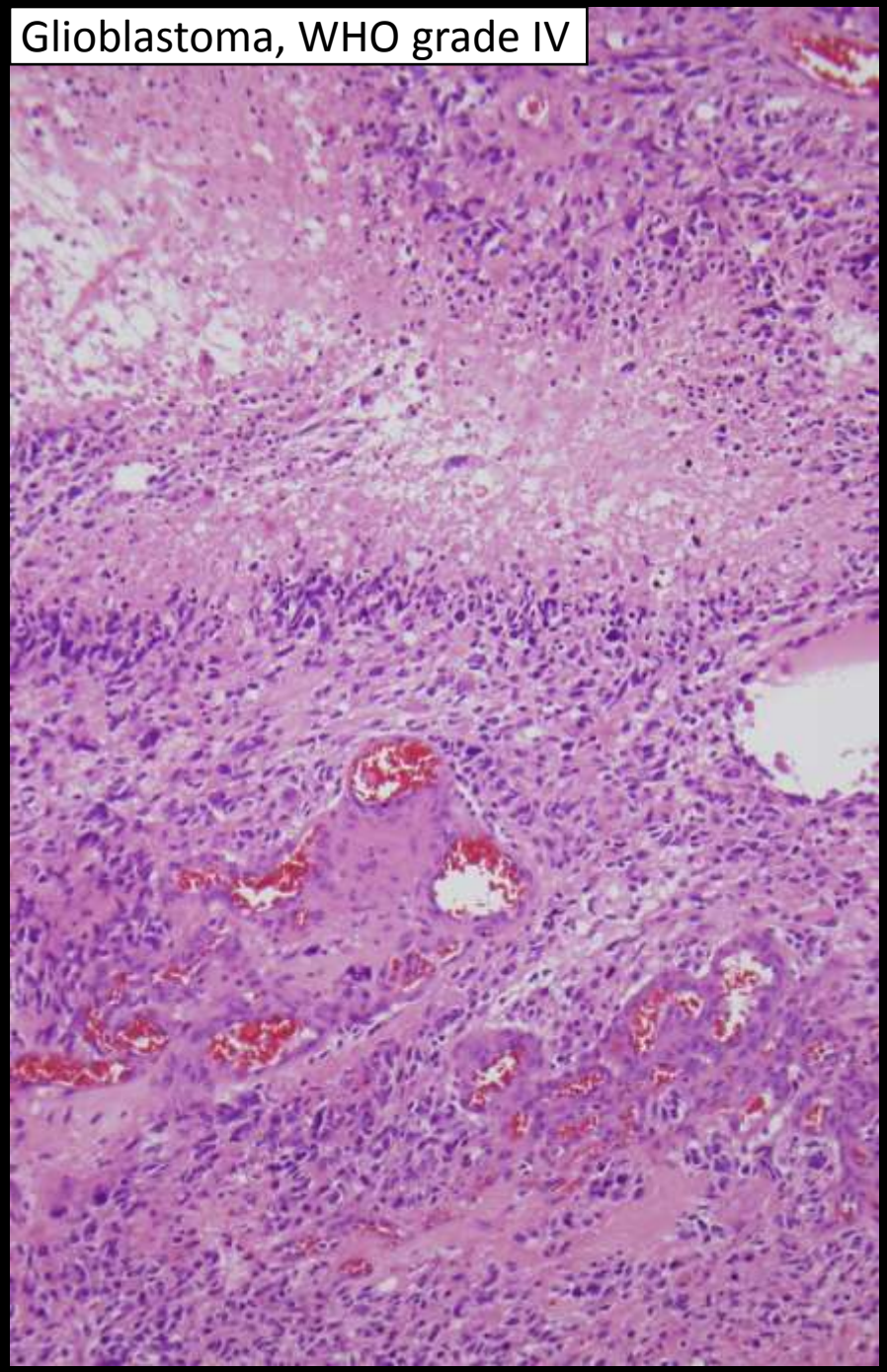
Goals

1. Illustrate common genetic alterations in astrocytic and oligodendroglial neoplasms
   - Co-deletion of 1p and 19q
   - **MGMT methylation**
   - IDH1 and IDH2 mutations
   - Basic introduction to signaling pathways commonly involved in gliomas

2. Discuss the utility of molecular testing in the context of gliomas

Glioblastoma, WHO grade IV (untreated)




Glioblastoma, WHO grade IV

Clinical significance of MGMT promoter hypermethylation in GBM (Hegi, et al 2005)

- Survival rates when treated with concomitant and adjuvent (maintenance) temozolomide and radiotherapy:

	Survival rates	
Treatment	**2 year**	**5 year**
Temozolomide + radiotherapy	49%	14%
Radiotherapy alone	24%	5%

Hegi ME, et al. MGMT gene silencing and Benefit from Temozolomide in Glioblastoma. NEJM 2005;352:997-1003

NEJM 2005;352:997-1003 The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma

Monika E. Hegi, Ph.D., Annie-Claire Diserens, M.Sc., Thierry Gorlia, M.Sc., Marie-France Hamou, Nicolas de Tribolet, M.D., Michael Weller, M.D., Johan M. Kros, M.D., Johannes A. Hainfellner, M.D., Warren Mason, M.D., Luigi Mariani, M.D., Jacoline E.C. Bromberg, M.D., Peter Hau, M.D., René O. Mirimanoff, M.D., J. Gregory Cairncross, M.D., Robert C. Janzer, M.D., and Roger Stupp, M.D.

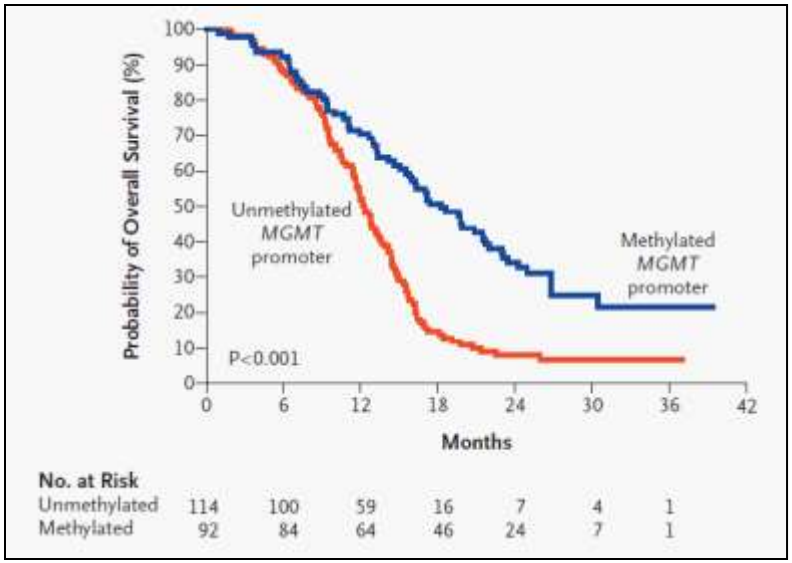


Figure 2. Kaplan–Meier Estimates of Overall Survival, According to *MGMT* Promoter Methylation Status.

The difference in survival between patients with a methylated *MGMT* promoter (92 patients, 65 of whom died) and those with an unmethylated *MGMT* promoter (114 patients, 105 of whom died) was highly significant ($P<0.001$ by the log-rank test), indicating that the *MGMT* methylation status has prognostic value. In the group of patients with a methylated *MGMT* promoter, there was a risk reduction of 55 percent (hazard ratio for death, 0.45; 95 percent confidence interval, 0.32 to 0.61), as compared with the group with an unmethylated *MGMT* promoter.

Hegi ME, et al. NEJM 2005;352:997-1003

Overall Survival

Progression Free Survival

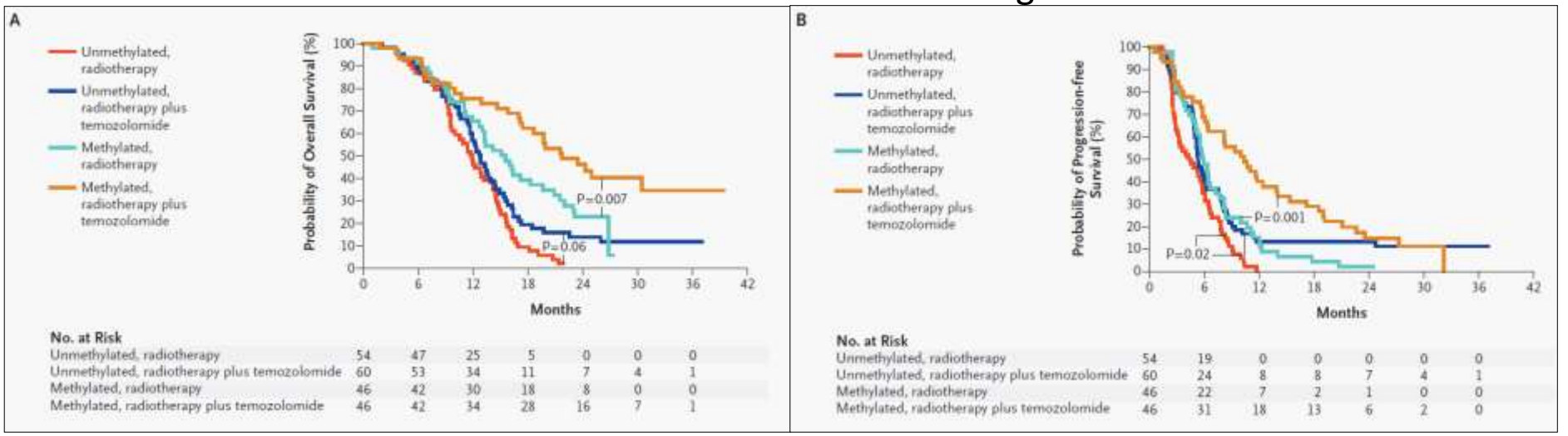
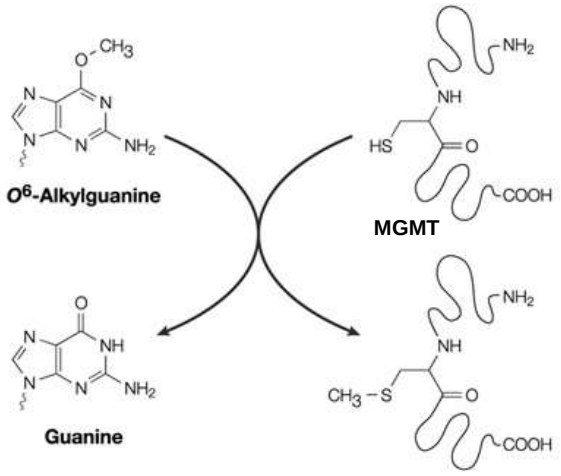


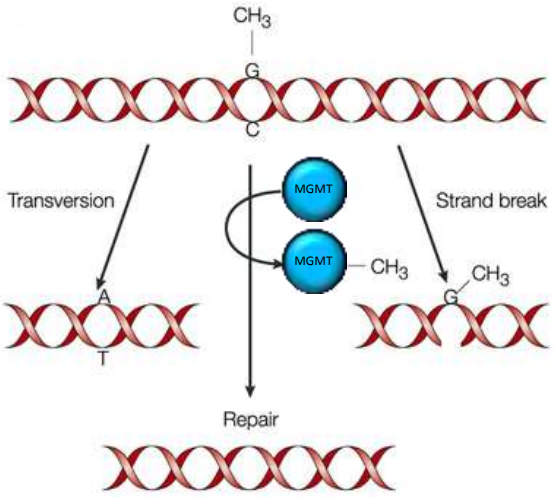
Figure 3. Kaplan–Meier Estimates of Overall and Progression-free Survival, According to *MGMT* Promoter Methylation Status and Random Assignment to Temozolomide plus Radiotherapy or Radiotherapy Alone.

The Kaplan–Meier estimates for overall survival indicate that the group of patients with a methylated *MGMT* promoter who were randomly assigned to temozolomide and radiotherapy (46 patients, 40 of whom had progression and 27 of whom died) had a 49 percent risk reduction (hazard ratio for death, 0.51; 95 percent confidence interval, 0.31 to 0.84), as compared with the group with a methylated *MGMT* promoter who were randomly assigned to radiotherapy only (46 patients, 45 of whom had progression and 38 of whom died) (Panel A). An unmethylated *MGMT* promoter and random assignment to temozolomide and radiotherapy (60 patients, 53 of whom had progression and 52 of whom died) yielded a risk reduction of 31 percent (hazard ratio for death, 0.69; 95 percent confidence interval, 0.47 to 1.02), as compared with an unmethylated *MGMT* promoter and random assignment to radiotherapy only (54 patients, all of whom had progression and 53 of whom died). In order to display a possible effect of salvage treatment on overall survival, in particular in the group of patients with a methylated *MGMT* promoter who were randomly assigned to radiotherapy alone. Kaplan–Meier curves are also shown for progression-free survival (Panel B) in a similar manner.

O^6-methylguanine-DNA methyltransferase (MGMT gene)

- AKA "O^6-alkylguanine-DNA alkyltransferase (AGT)"
- DNA repair protein
  - Chromosome 10q26
  - Removes methyl groups from the O^6 position of guanine and to a lesser extent the O^4 position of thymine (suicide protein; degraded after acts)
  - Unique: acts alone to remove DNA adducts (all others part of a complex)





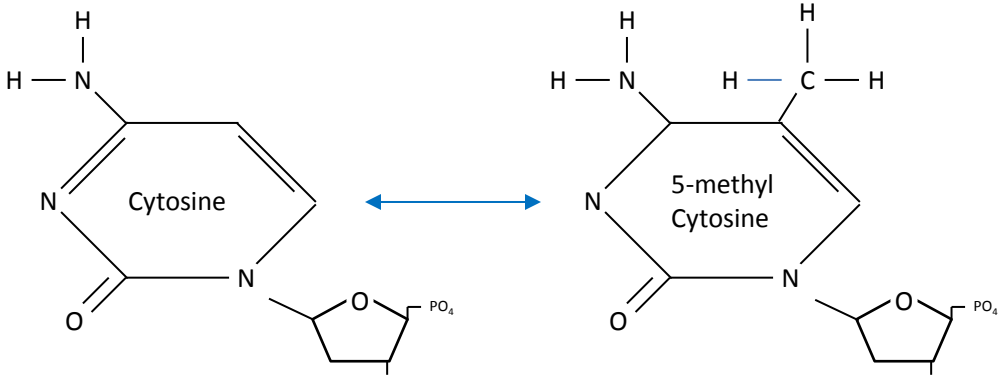
O^6-methylguanine is mutagenic
Repair is essential:
- DNA polymerase stalls at these sites
- Wrong base inserted >90% of time
- Leads to GC > AT and TA > GC mutations, or strand breaks

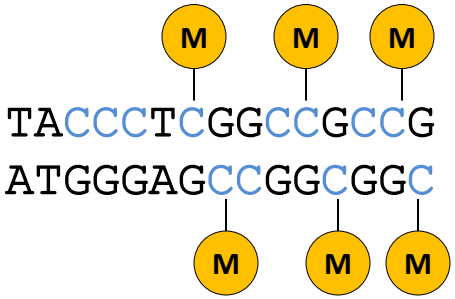
Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Canc 2004;4:296-307

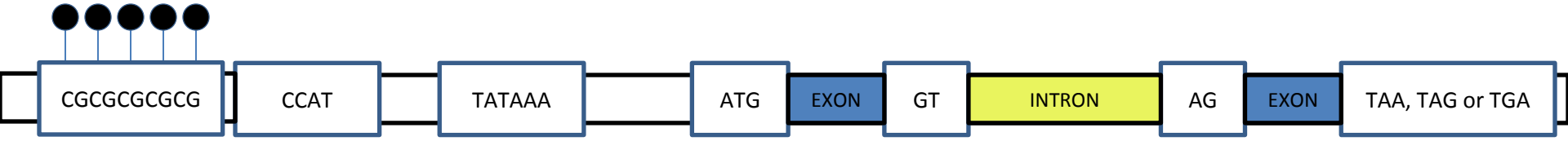
O^6-methylguanine-DNA methyltransferase (MGMT gene)

- Normal cells
  - Protection from O^6-methyl guanine induced DNA alterations
- Tumor cells
  - Intact or increased expression is protective against alkylating chemo-therapeutic agents (temozolomide and others)
  - Decreased expression of MGMT via promoter hypermethylation (epigenetic silencing) sensitizes tumor cells to alkylating agents
    - Present in ~40% of primary glioblastomas
    - Associated with favorable response to alkylating chemotherapy (predictive)
    - Potentially a double edged sword
      - Decreased expression increases sensitivity to Temozolomide
      - May promote mutation/tumor evolution via increased rate of mutation

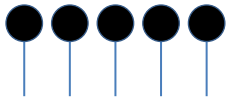
Promoter methylation (epigenetic)







CpG islands



Methylated

* MGMT gene has 97 CpGs in its promoter region

Modified from Leonard: Molecular Pathology in Clinical Practice

MGMT promoter methylation

Clinical utility

- Predictive:
  - Response to alkylating agent-type chemotherapy
- Test methodologies
  - Sodium bisulfite treatment followed by:
    - Sequencing
      - Sanger
      - Methylation-specific pyrosequencing
    - Methylation specific PCR (MSP)
    - Methylation sensitive High Resolution Melting Point Analyis (MS-HRM)
  - Other
    - Methylation specific MLPA (no bisulfite step)
    - MGMT IHC (poor interobserver reproducibility)

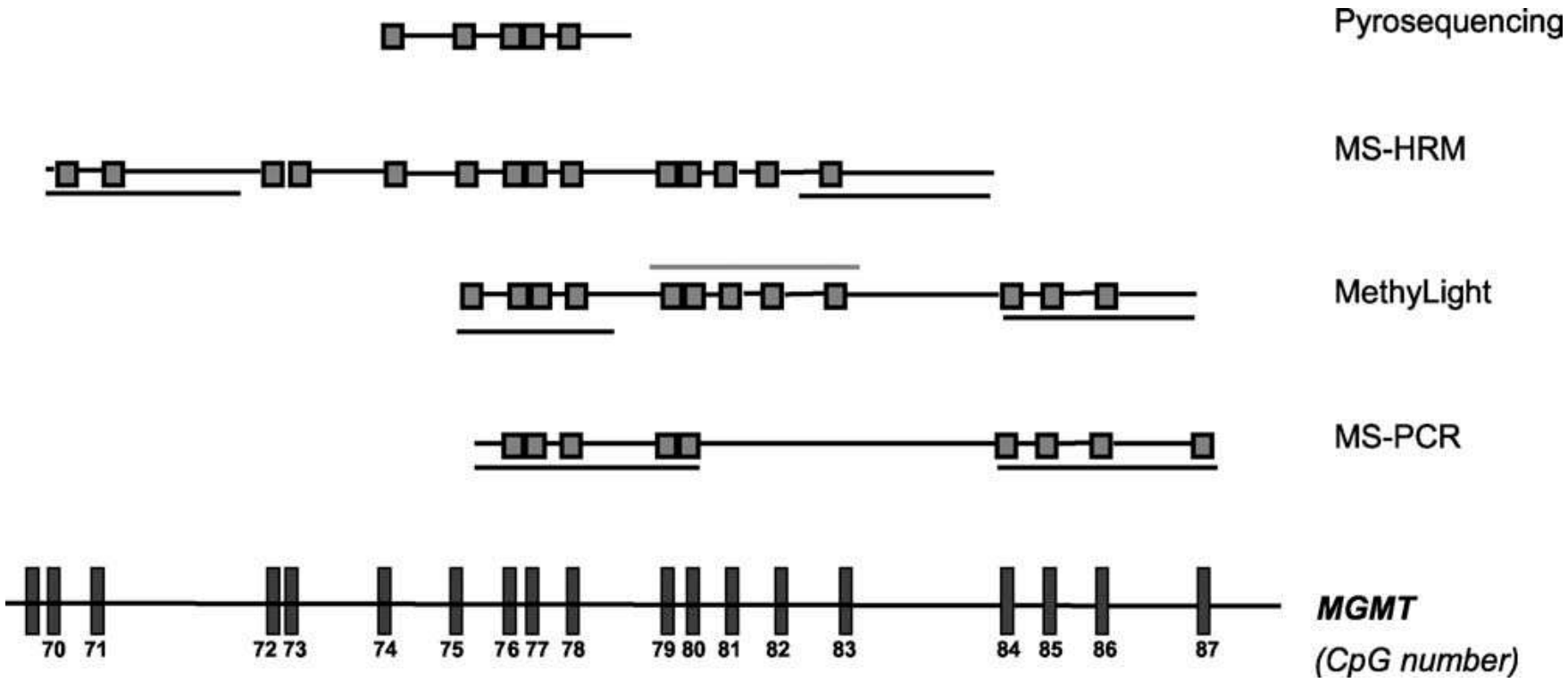


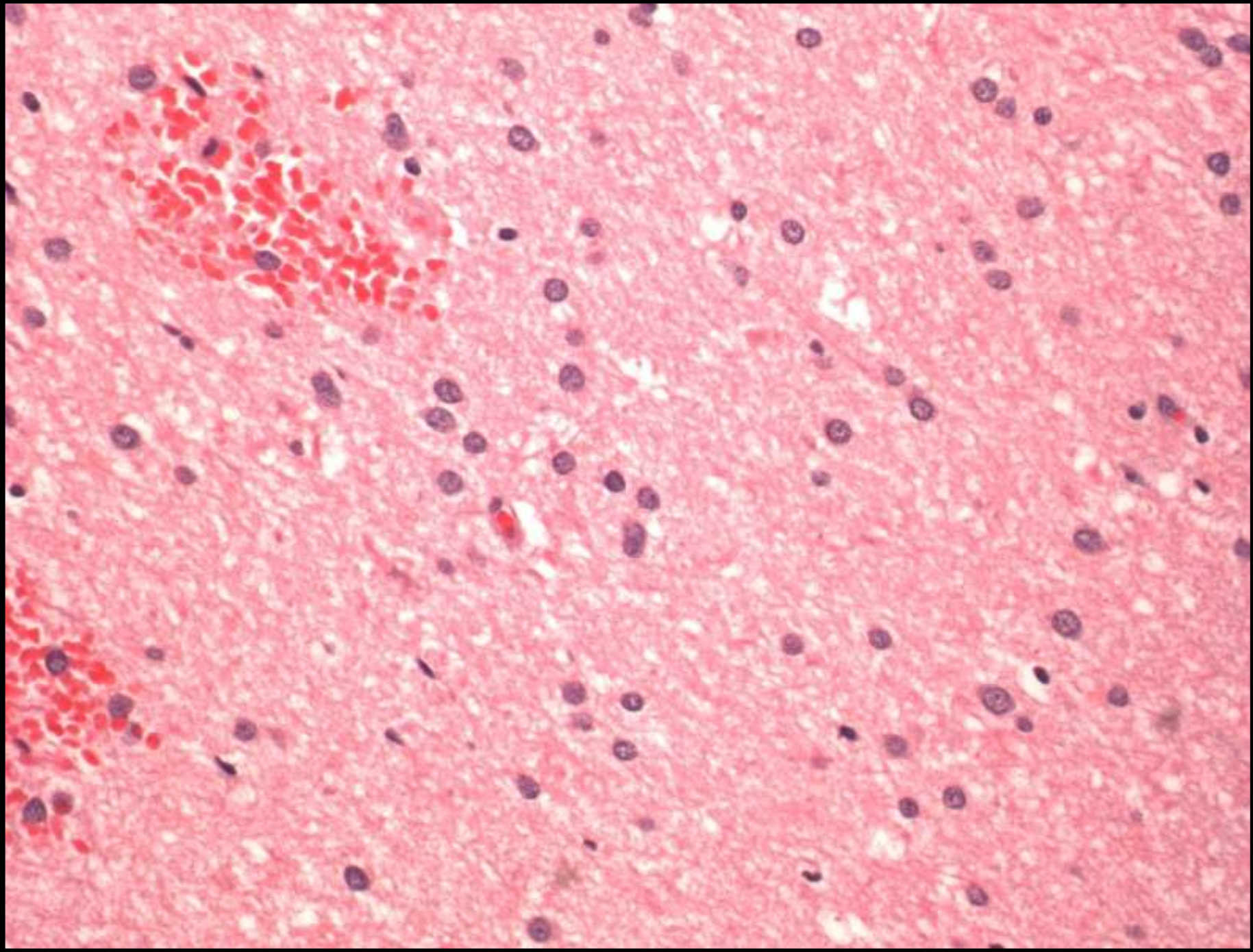
Figure 1. CpGs are interrogated by the different techniques. The figure shows at the bottom a part of the MGMT gene; each gray rectangle represents a CpG site. Gray squares indicate the CpGs interrogated by the different assays. For pyrosequencing, CpGs numbers 74, 75, 76, 77, and 78 are tested (referred to as PYRCpG1, PYRCpG2, PYRCpG3, PYRCpG4, and PYRCpG5 in the text). Black lines represent the position of the primers for assays with a specific amplification step, and the gray line shows the position of the specific probe used for MethyLight. MS-HRM, methylation-sensitive high-resolution melting; MS-PCR, methylation-specific polymerase chain reaction.

Quillien V, et. Al. Comparative Assessment of 5 Methods (Methylation-Specific Polymerase Chain Reaction, MethyLight, Pyrosequencing, Methylation-Sensitive High-Resolution Melting , and Immunohistochemistry) to analyze o^6-Methylguanine-DNA-Methyltransferase in a Series of 100 Glioblastoma Patients. Cancer 2012; Epub ahead of print

Goals

1. Illustrate common genetic alterations in astrocytic and oligodendroglial neoplasms
   - Co-deletion of 1p and 19q
   - MGMT methylation
   - **IDH1 and IDH2 mutations**
   - Basic introduction to signaling pathways commonly involved in gliomas

2. Discuss the utility of molecular testing in the context of gliomas



Isocitrate dehydrogenase (IDH1 and IDH2) gene

- In 2008 genomic analysis of GBMs identified frequent IDH1 mutations
  - Associated with:
    - Younger age
    - Secondary-type GBM
    - Increased overall survival

Parsons DW, et. al. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. Science 2008;321:1807-18-12

An Integrated Genomic Analysis of Human Glioblastoma Multiforme

D. Williams Parsons,[1,2*] Siân Jones,[1*] Xiaosong Zhang,[1*] Jimmy Cheng-Ho Lin,[1*] Rebecca J. Leary,[1*] Philipp Angenendt,[1*] Parminder Mankoo,[3] Hannah Carter,[3] I-Mei Siu,[4] Gary L. Gallia,[4] Alessandro Olivi,[4] Roger McLendon,[5] B. Ahmed Rasheed,[5] Stephen Keir,[5] Tatiana Nikolskaya,[6] Yuri Nikolsky,[7] Dana A. Busam,[8] Hanna Tekleab,[8] Luis A. Diaz Jr.,[1] James Hartigan,[9] Doug R. Smith,[9] Robert L. Strausberg,[8] Suely Kazue Nagahashi Marie,[10] Sueli Mieko Oba Shinjo,[10] Hai Yan,[5] Gregory J. Riggins,[4] Darell D. Bigner,[5] Rachel Karchin,[3] Nick Papadopoulos,[1] Giovanni Parmigiani,[1] Bert Vogelstein,[1†] Victor E. Velculescu,[1†] Kenneth W. Kinzler[1†]

Glioblastoma multiforme (GBM) is the most common and lethal type of brain cancer. To identify the genetic alterations in GBMs, we sequenced 20,661 protein coding genes, determined the presence of amplifications and deletions using high-density oligonucleotide arrays, and performed gene expression analyses using next-generation sequencing technologies in 22 human tumor samples. This comprehensive analysis led to the discovery of a variety of genes that were not known to be altered in GBMs. Most notably, we found recurrent mutations in the active site of isocitrate dehydrogenase 1 (*IDH1*) in 12% of GBM patients. Mutations in *IDH1* occurred in a large fraction of young patients and in most patients with secondary GBMs and were associated with an increase in overall survival. These studies demonstrate the value of unbiased genomic analyses in the characterization of human brain cancer and identify a potentially useful genetic alteration for the classification and targeted therapy of GBMs.

Parsons DW, et. al. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. Science 2008;321:1807-18-12

Isocitrate dehydrogenase (IDH1 and IDH2) gene

Follow-up studies

- Mutation present in 60-90% of WHO grade II & III diffuse gliomas (both astrocytomas and oligodendrogliomas)
  - Frequently co-exists with p53 mutation in astrocytoma
  - Frequently co-exists with 1p/19q codeletion in oligodendrogliomas
  - Rare in primary GBM
  - Absent in pilocytic astrocytoma
  - IDH2 mutations more frequent in oligodendrogliomas
    - But still much less common than IDH1
- Not identified in non-neoplastic conditions
- Associated with better outcome

Isocitrate dehydrogenase (IDH1 and IDH2) genes

- Function
  - Oxidative decarboxylation of isocitrate to α-ketoglutarate
  - Two subclasses of enzyme
    - 3 utilize NAD + as electron acceptor
    - 2 utilize NADP+ as electron acceptor
  - Form homodimer
- IDH1 2q33.3
  - Cytosolic and peroxisomal NADP+ dependent IDH
- IDH2 15q26.1
  - Mitochondrial NADP+ dependent IDH

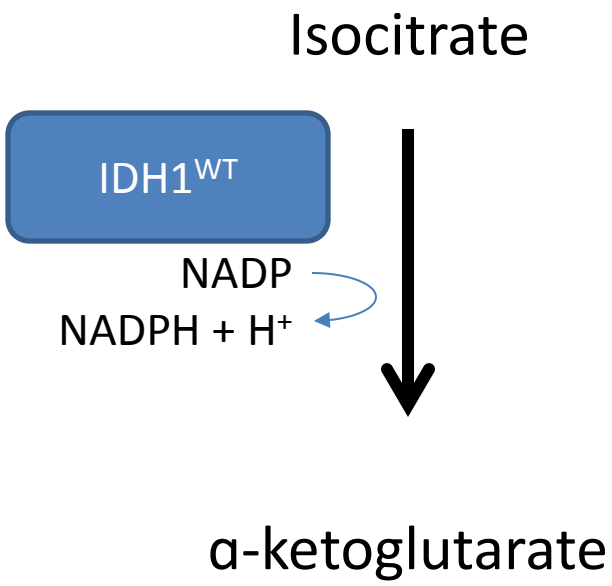
IDH1 and IDH2 gene mutation pathogenesis

- Mutational hotspot for heterozygous mutations at arginine residue
  - IDH1 R132 in 71% of gliomas
    - Most commonly R132H (93.2%); R132C (3.7%), R132S (1.3), R132G (1.2%), and R132L (0.5%), R132V (0.1%)
  - IDH2 R172 in 5% of gliomas
    - Most commonly R172K, R172M, R172G

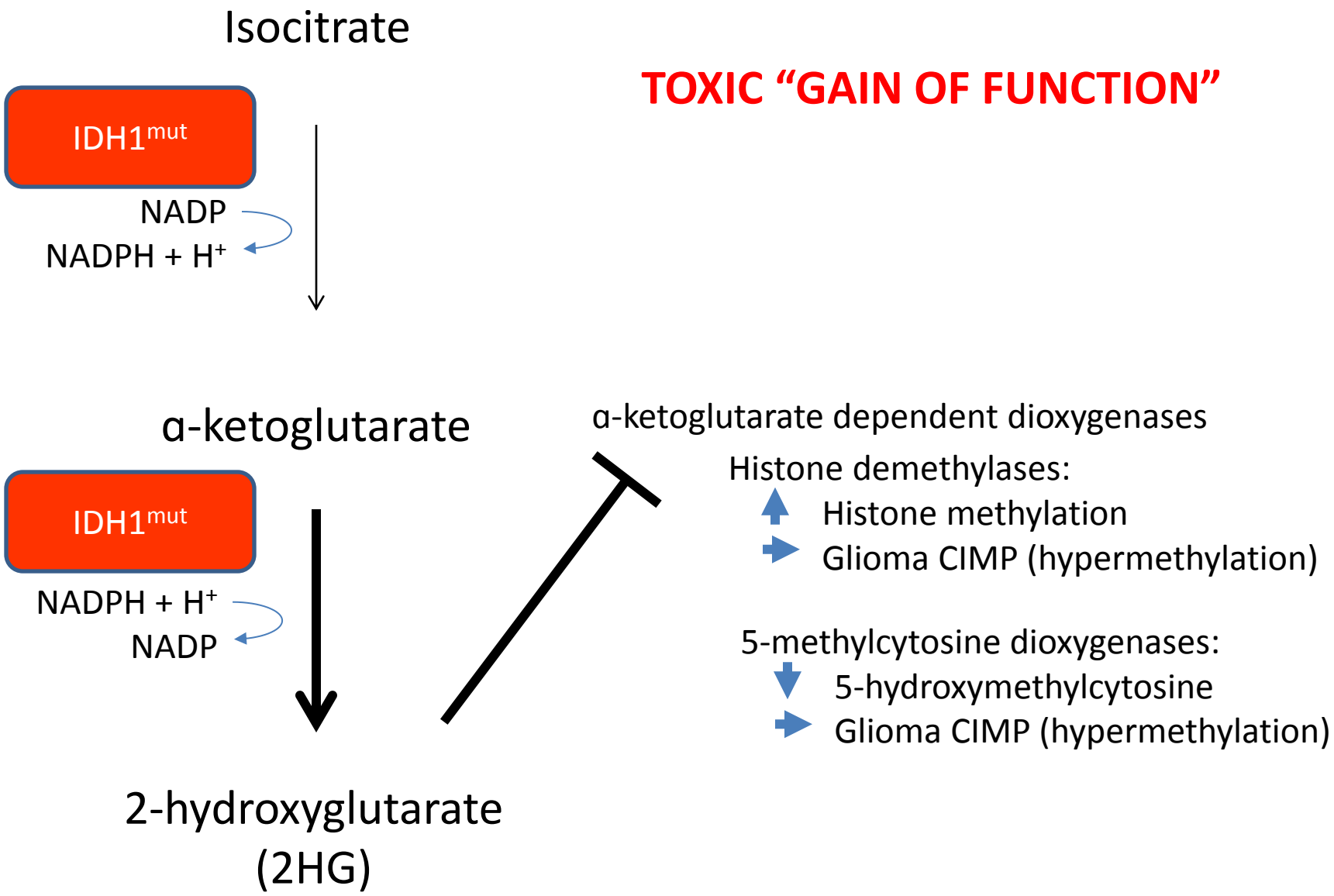
von Deimling A, et. al. The Next Generation of Glioma Biomarkers: MGMT Methylation, BRAF Fusions and IDH1 mutations. Brain Pathology 2011;21:74-87

IDH1 and IDH2 gene mutation pathogenesis

- Proposed mechanism(s) of disease
  - Gain of function
    - Reduces α-ketoglutarate to R-2-hydroxyglutarate (2HG)
      - Elevated risk of malignant tumors in patients with inborn errors of 2HG metabolism
        - 2HG competitively inhibits a variety of α-ketoglutarate dependent enzymes, including:
          - Histone demethylases
          - TET family of 5mC hydoxylases
      - Reaction consumes alpha-ketoglutarate and NADPH
        - Compounds susceptibility to oxidative stress



Malzkorn B, et al. Unraveling the Glioma Epigenome – From Molecular Mechanisms to Novel Biomarkers and Therapeutic Targets. Brain Pathology 2011;21:619-632



TOXIC "GAIN OF FUNCTION"

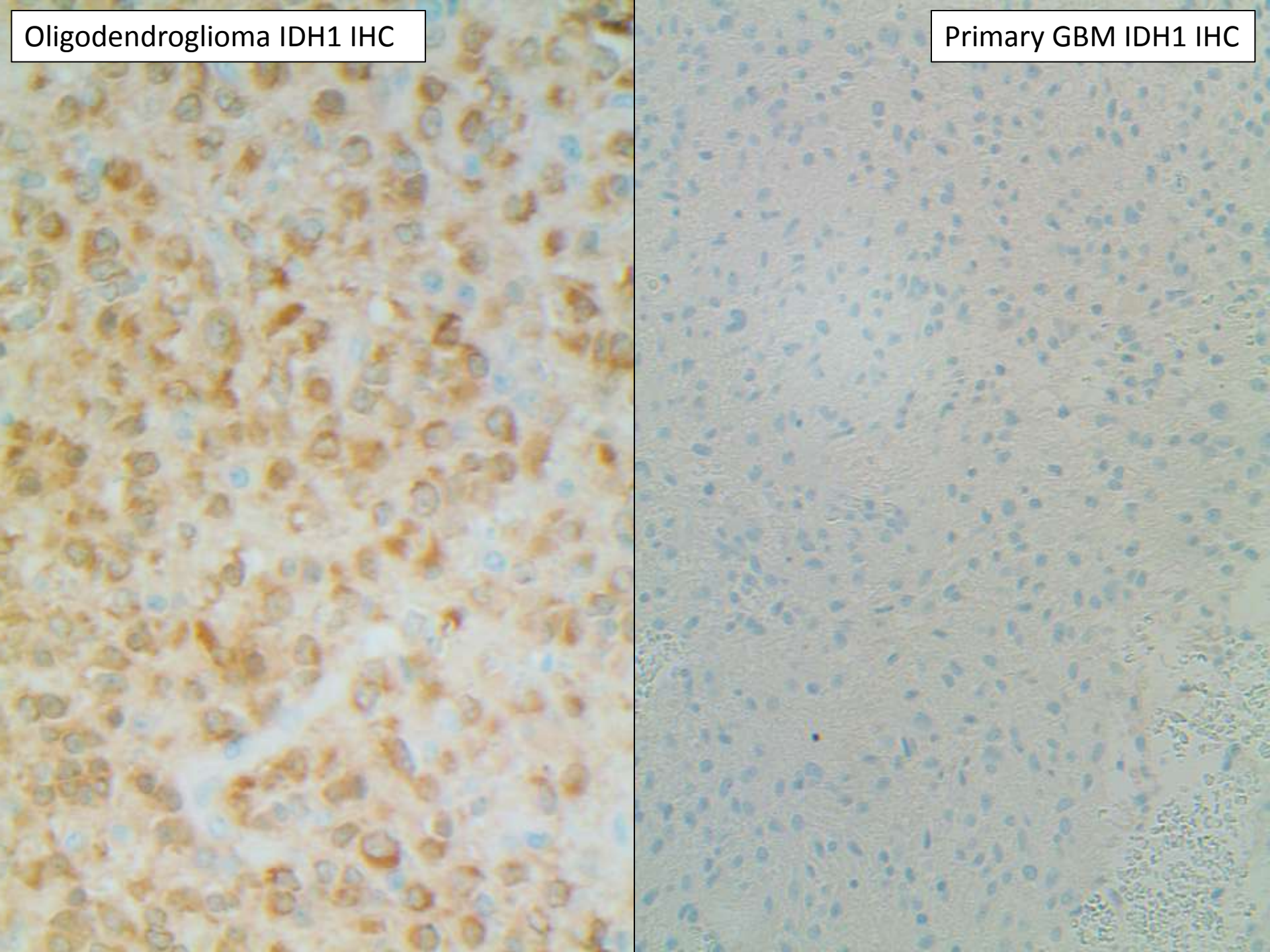
Malzkorn B, et al. Unraveling the Glioma Epigenome – From Molecular Mechanisms to Novel Biomarkers and Therapeutic Targets. Brain Pathology 2011;21:619-632

IDH1 and IDH2 gene mutation pathogenesis

- Proposed mechanism(s) of disease
  - Loss of function
    - Decreased ability to catalyze decarboxylation of isocitrate to α-ketoglutarate
      - Decrease in α-ketoglutarate and NADPH renders cell more susceptible to oxidative stress
    - Associated with increased level of hypoxia-inducible factor-1α (HIF1) transcription factor

IDH1 and IDH2 gene mutation

- Clinical utility
  - Diagnostic
    - Neoplastic versus reactive glial proliferation (IDH1 R132H IHC)
      - Misses 10% of gliomas with other IDH R132 mutations
      - Misses all IDH2 mutations
  - Prognostic: longer survival
  - Predictive: no specific therapy but associated with better response
- Test methodologies
  - Sequencing
  - Polymerase chain reaction (PCR) based assays
  - IDH1 R132H immunohistochemical stain (IHC)


Oligodendroglioma IDH1 IHC
Primary GBM IDH1 IHC

Published in final edited form as:
Cancer Cell. 2010 May 18; 17(5): 510–522. doi:10.1016/j.ccr.2010.03.017.

Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma

Houtan Noushmehr[1,*], Daniel J. Weisenberger[1,*], Kristin Diefes[2,*], Heidi S. Phillips[3], Kanan Pujara[3], Benjamin P. Berman[1], Fei Pan[1], Christopher E. Pelloski[4], Erik P. Sulman[4], Krishna P. Bhat[2], Roel G.W. Verhaak[5,6], Katherine A. Hoadley[7,8], D. Neil Hayes[7,8], Charles M. Perou[7,8], Heather K. Schmidt[9], Li Ding[9], Richard K. Wilson[9], David Van Den Berg[1], Hui Shen[1], Henrik Bengtsson[10], Pierre Neuvial[10], Leslie M. Cope[11], Jonathan Buckley[1,12], James G. Herman[11], Stephen B. Baylin[11], Peter W. Laird[1,**,†], Kenneth Aldape[2,**], and The Cancer Genome Atlas Research Network

Significance

Glioblastoma (GBM) is a highly aggressive form of brain tumor, with a patient median survival of just over one year. The Cancer Genome Atlas (TCGA) project aims to characterize cancer genomes to identify means of improving cancer prevention, detection, and therapy. Using TCGA data, we identified a subset of GBM tumors with characteristic promoter DNA methylation alterations, referred to as a glioma CpG Island Methylator Phenotype (G-CIMP). G-CIMP tumors have distinct molecular features, including a high frequency of IDH1 mutation and characteristic copy-number alterations. Patients with G-CIMP tumors are younger at diagnosis and display improved survival times. The molecular alterations in G-CIMP tumors define a distinct subset of human gliomas with specific clinical features.

Highlights

- Identification of a CpG island methylator phenotype (G-CIMP) in gliomas
- G-CIMP is tightly associated with *IDH1* mutation
- G-CIMP patients are younger at diagnosis and display improved survival
- G-CIMP is more prevalent among low- and intermediate-grade gliomas

Mechanistic link between IDH1 mutation and altered epigenome of gliomas

- Noushmehr et al.
  - Detected hypermethylation of many gene loci in 8.8% of GBMs in The Cancer Genome Atlas project
  - G-CIMP (Glioma-CpG Island Methylator Phenotype)
    - Common in secondary GBMs
    - Common in other diffuse gliomas
      - Particularly oligodendrogliomas
    - Associated with a proneural gene expression profile and prolonged survival
    - Strongly associated with IDH1 mutations
      - IDH1 mutation identified in 18/23 (78%) of G-CIMP^{+} GBMs

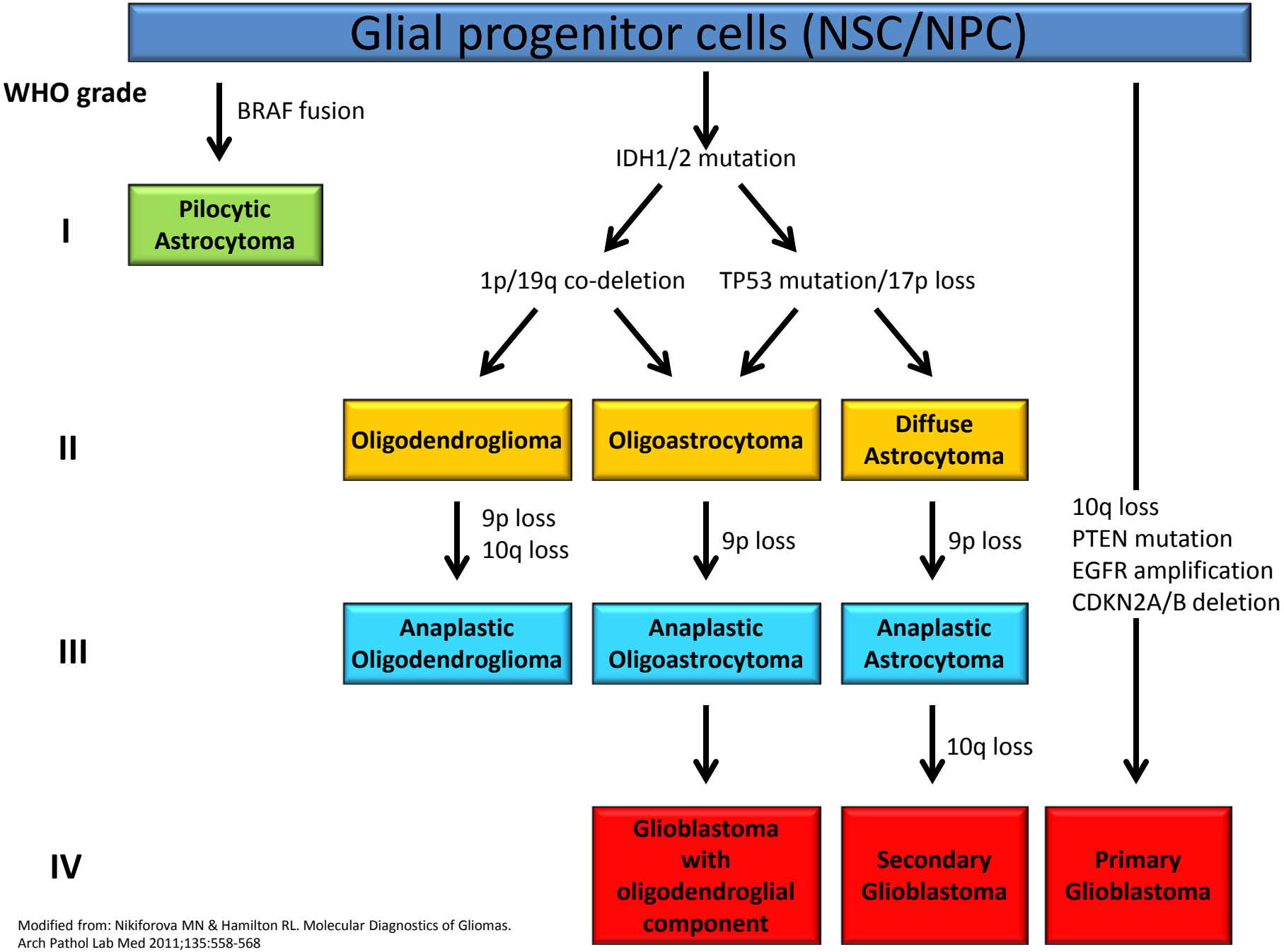
Noushmehr H, et al. Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Gliomas. Cancer Cell 2010;17:510-522

Significance of IDH1 and/or IDH2 mutation status

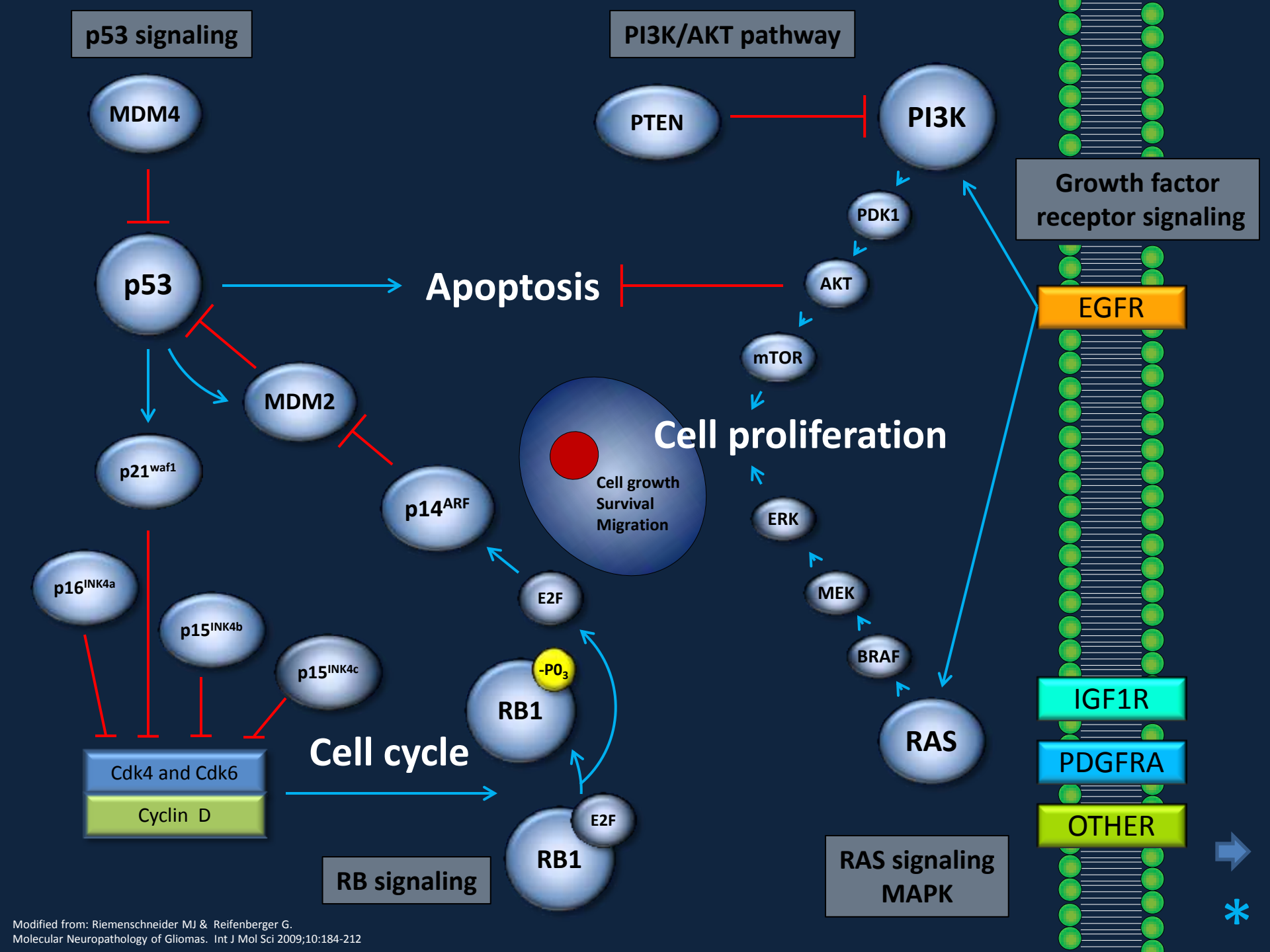
- Appears to be one of the earliest mutations in diffuse gliomas in the oligodendroglioma and astrocytic (secondary GBM) pathways
  - Shared mutation trait suggests origin from a common precursor cell type for oligodendroglioma and astrocytoma
- Favorable prognostic factor
- Association with G-CIMP phenotype

Goals

1. Illustrate common genetic alterations in astrocytic and oligodendroglial neoplasms
   - Co-deletion of 1p and 19q
   - MGMT methylation
   - IDH1 and IDH2 mutations
   - **Basic introduction to signaling pathways commonly involved in gliomas**
     A. EGFR
     B. BRAF
     C. PTEN
     D. TP53
2. Discuss the utility of molecular testing in the context of gliomas

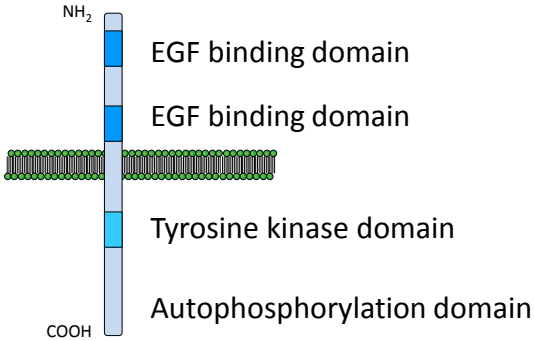


Modified from: Nikiforova MN & Hamilton RL. Molecular Diagnostics of Gliomas. Arch Pathol Lab Med 2011;135:558-568



Modified from: Riemenschneider MJ & Reifenberger G.
Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

Epithelial derived growth factor (EGFR1)

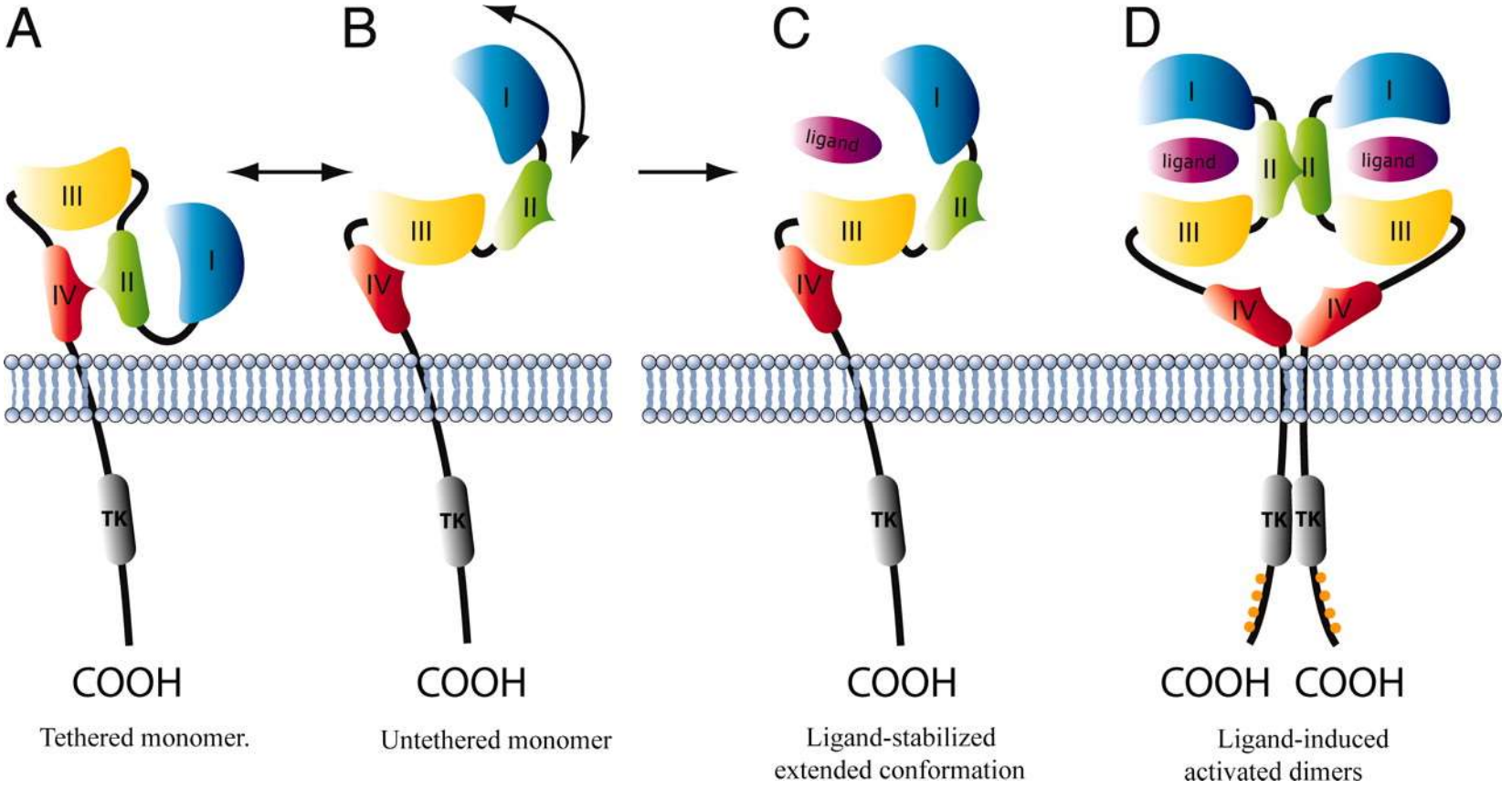


- Oncogene
- Receptor tyrosine kinase
  - Transmembrane glycoprotein
    - N-terminal extracellular ligand binding domain
    - Transmembrane lipophilic segment
    - C-terminal intracellular region including tyrosine kinase (TK) domain
- Chromosome 7p12
- Multiple ligands
  - Epithelial derived growth factor (EGF)
  - Transforming growth factor (TGF)-alpha
- Alterations most common in primary glioblastoma (40-50%)
  - Overexpression of normal protein and/or amplification of gene
  - Mutation
    - EGFRvIII (most common; 40%)
    - EGFRc958 (less common; 20%)

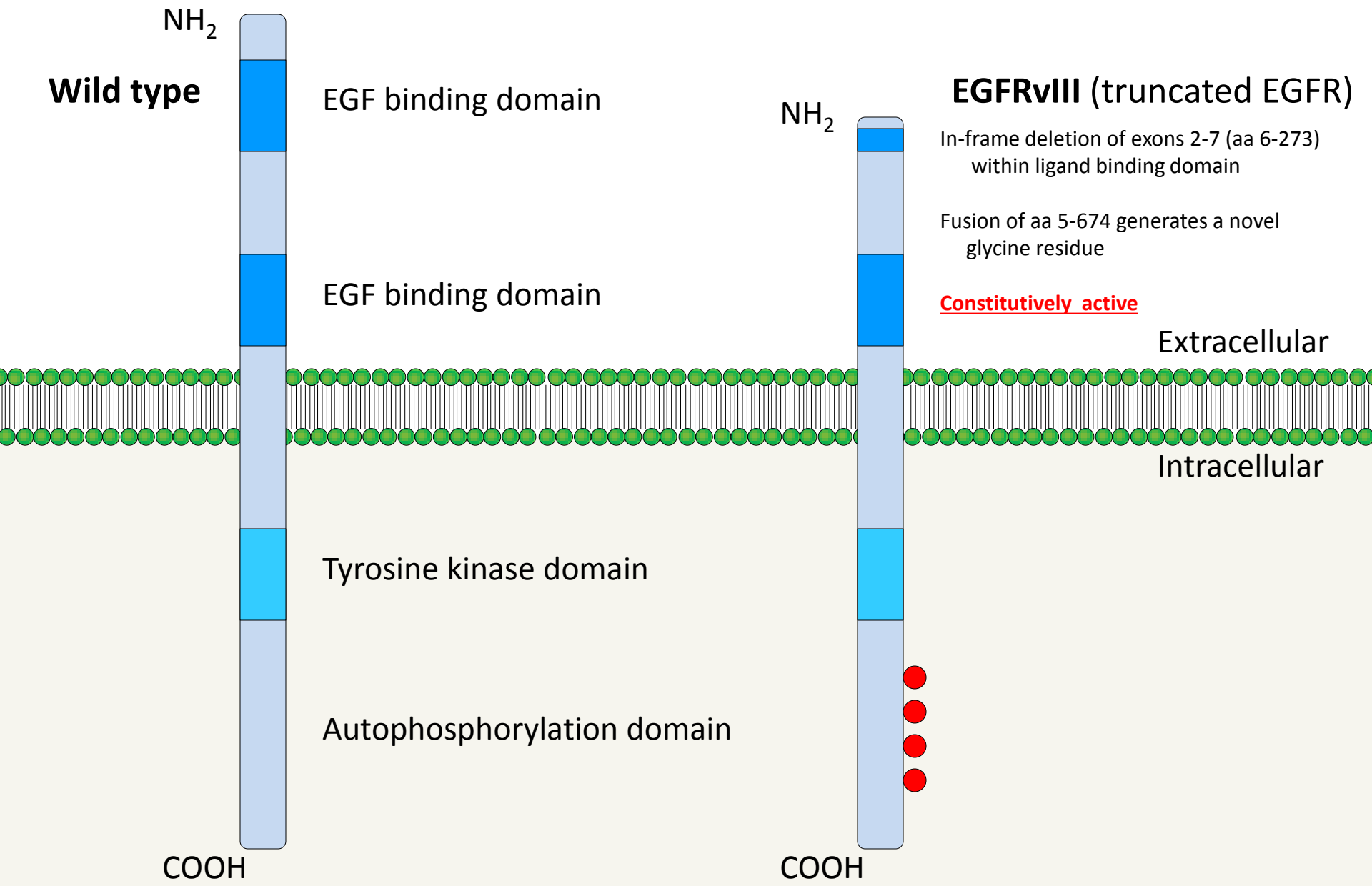
HER family of membrane receptors		
Receptor	**Tyrosine kinase activity**	**Major ligands**
HER1 (EGFR)	Yes	EGF, amphiregulin, TGF-alpha
HER2 (ERBB2)	Yes	None
HER3	No	Heregulin
HER4	Yes	Heregulin

From: Gutierrez CG and Schiff R. HER2: Biology, Detection, and Clinical Implications. Arch Pathol Lab Med 2011; 135:55-62

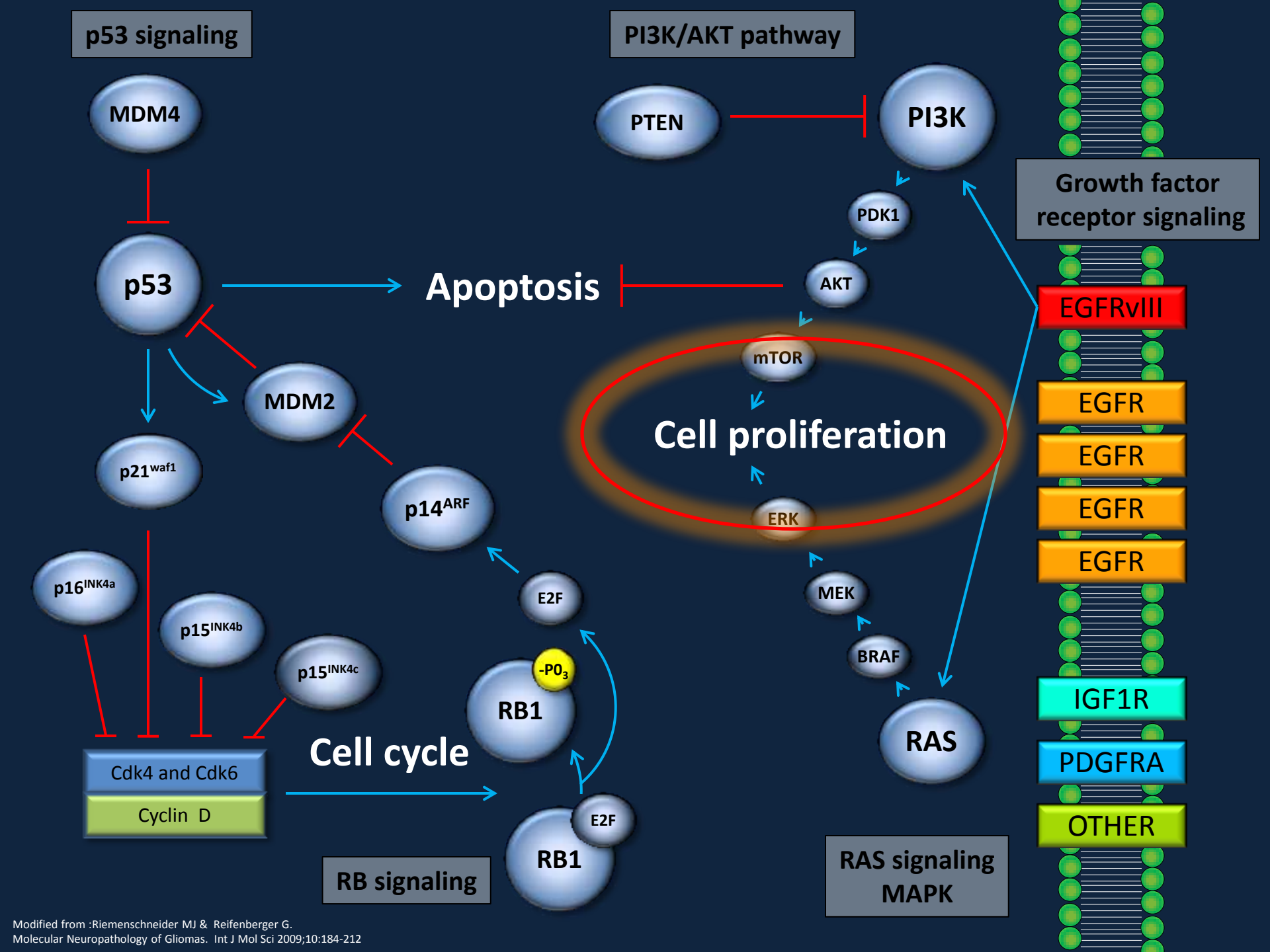
Model for molecular mechanism of ligand-induced EGFR activation.



Lammerts van Bueren J J et al. PNAS 2008;105:6109-6114



Modified from: Siwak DR, et. al. Targeting Epidermal Growth Factor Receptor in Epithelial Ovarian Cancer: Current Knowledge and Future Challenges. J Oncol 2010;Epub 2009 Nov 19



Modified from :Riemenschneider MJ & Reifenberger G.
Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

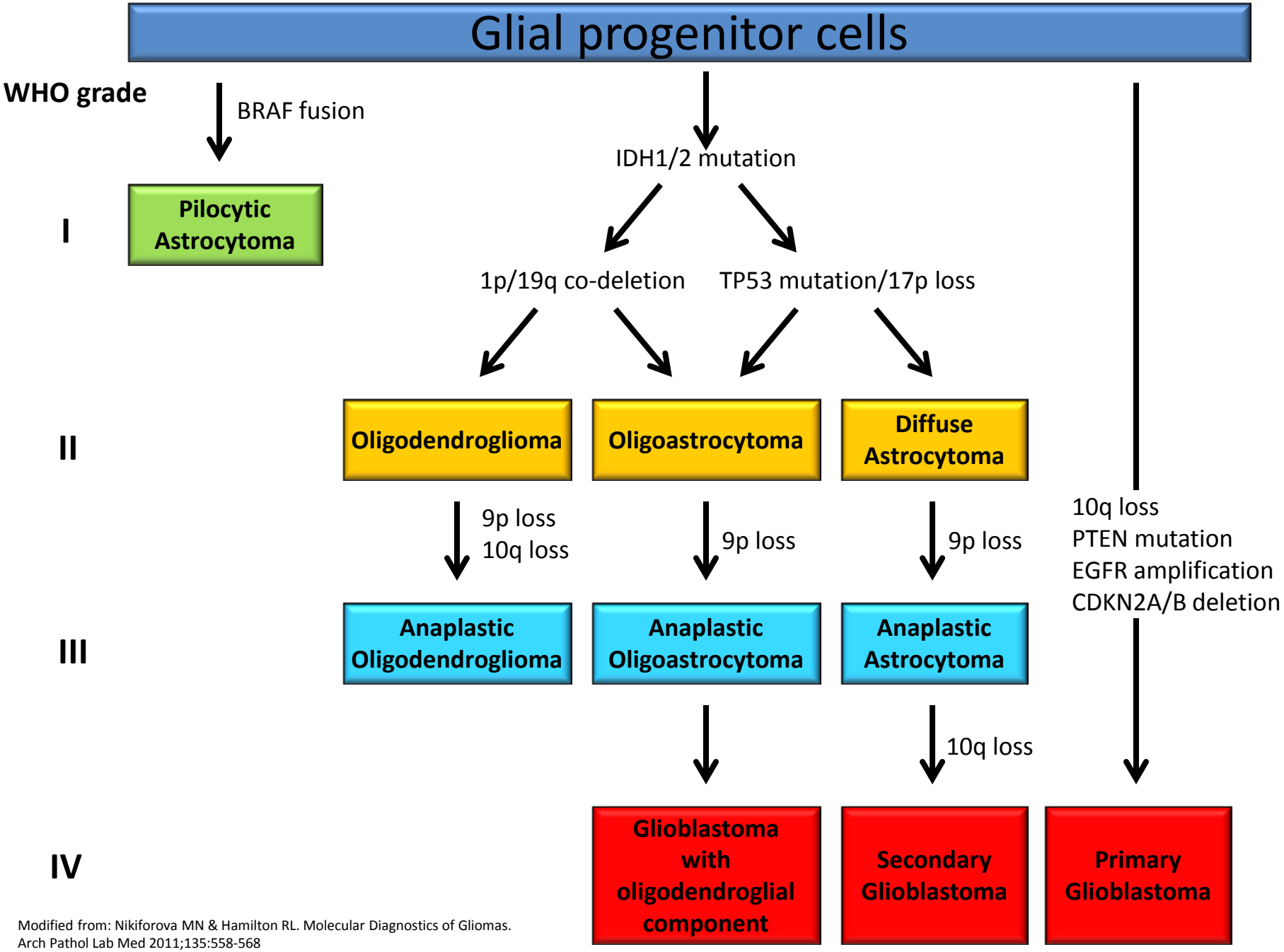
EGFR testing

Clinical utility

- Diagnostic:
  - Primary GBM versus Secondary GBM
  - Undersampled GBM (may highlight scattered cells)
- Prognostic:
  - questionable

Test methodologies

- EGFRvIII
  - Immunohistochemistry (*patent issues)
  - Polymerase chain reaction (PCR) based assays
- EGFR gene copy number (amplification)
  - Fluorescence in-situ hybridization (FISH)
- EGFR protein expression
  - Immunohistochemistry



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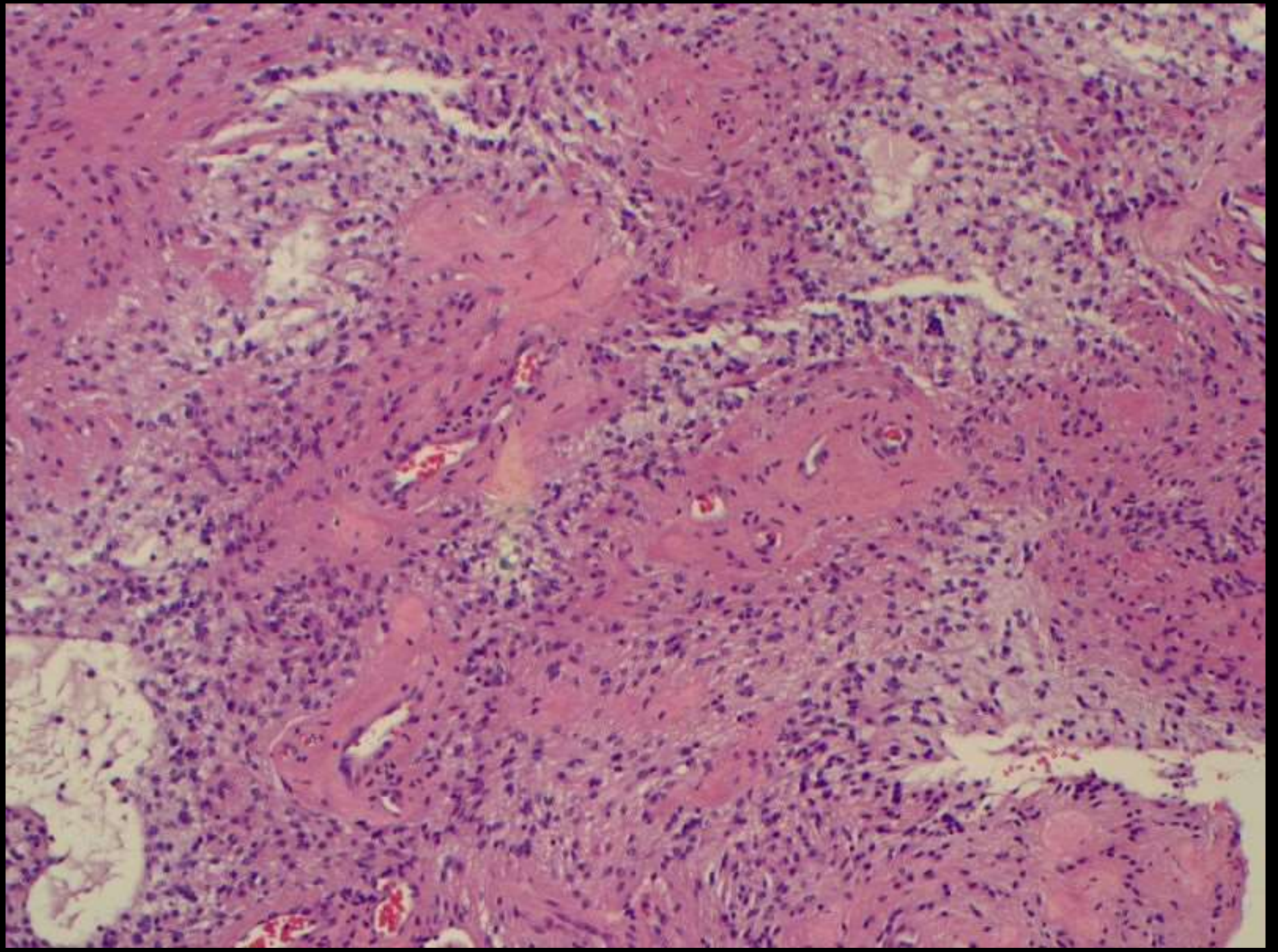
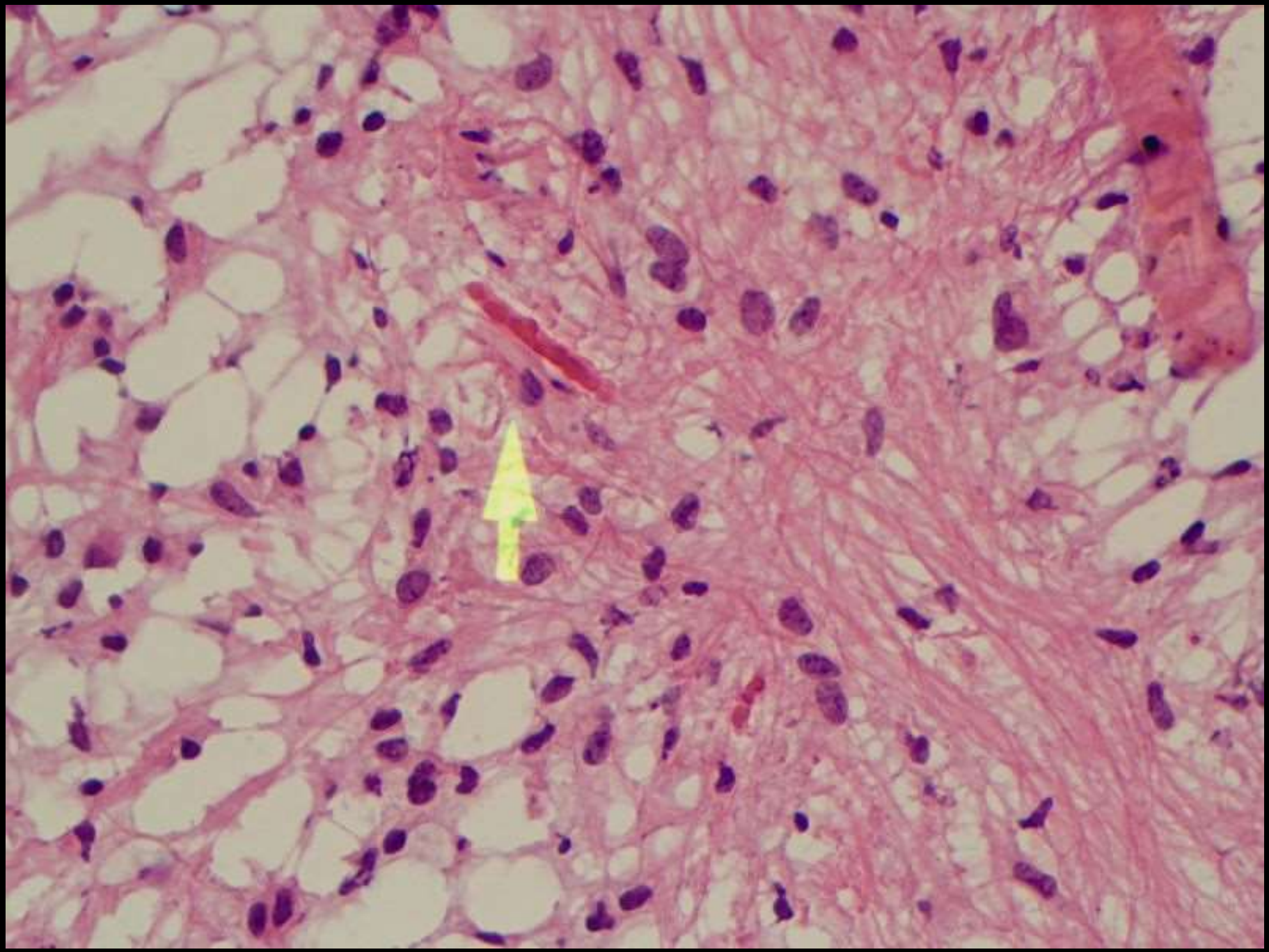




v-raf murine sarcoma viral oncogene homolog B1 (BRAF)

- Member of *raf* family of serine/threonine protein kinases
  - A-raf
  - B-raf
  - C-raf
- Component of mitogen-activated protein kinase (MAPK) pathway
- Chromosome 7q34
  - Pseudogene located on X chromosome

BRAF

Activating alterations identified in a variety of tumors

- Melanoma, ovarian carcinoma, thyroid carcinoma, colorectal carcinoma, and glioma
  - Mutations (many)
    - Most common = p.V600E (valine > glutamate)/c.1799T>A)
  - Translocation/fusion
    - AKAP9-BRAF
    - KIAA1549-BRAF
      - Non-random ~2MB tandem duplication with breakpoint clustering in KIAA1549 (uncharacterized gene) and BRAF
      - Results in fusion of 5’ portion of KIAA1549 with 3’ portion BRAF
  - Amplification

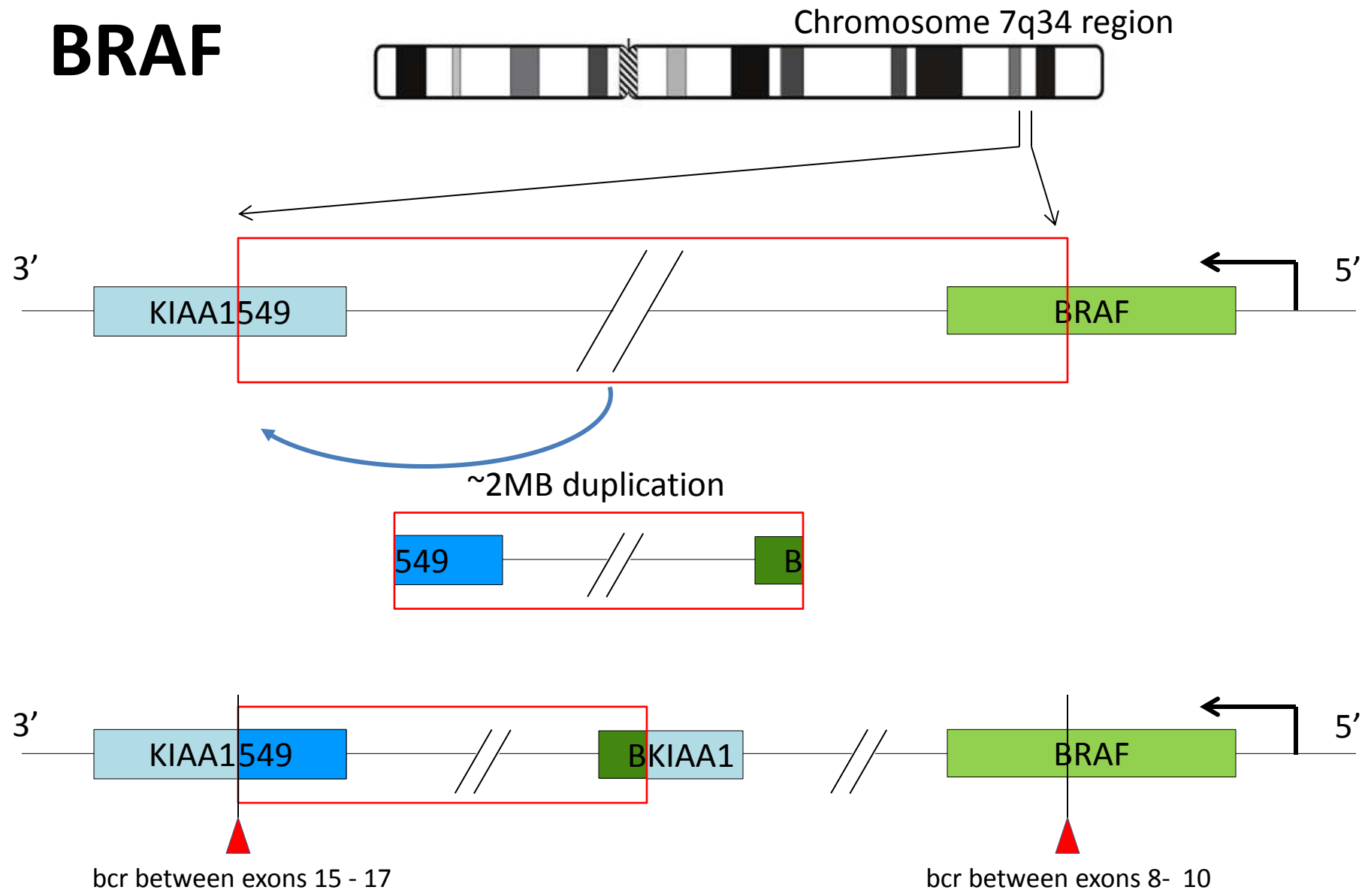
KIAA1549:BRAF fusion (tandem duplication)

- Pilocytic astrocytoma (60-73% of tumors)
  - Infratentorial 57%
  - Optic 59%
  - Supratentorial 19%

- No statistically significant prognostic impact
- Not identified in NF1 associated cases

Lin A, et. al. BRAF Alterations in Primary Glial and Glioneuronal Neoplasms of the Central Nervous System With Identification of 2 Novel KIAA1549:BRAF Fusion Variants. J Neuropathol Exp Neurol 2012: 71; 66-72

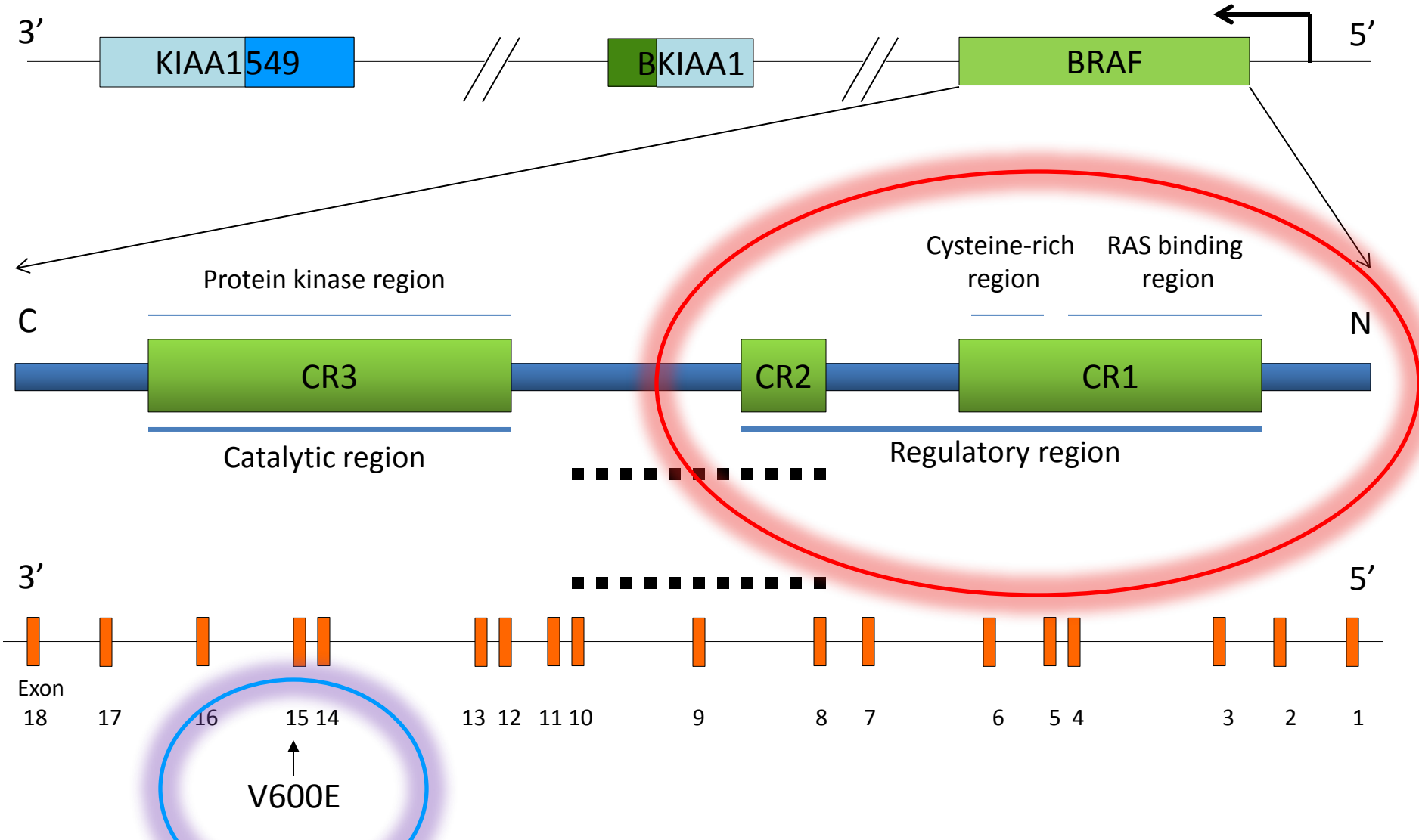
BRAF



Modified from: Cin H, et. al. Oncogenic FAM131B-BRAF fusion resulting from 7q34 deletion comprises an alternative mechanism of MAPK pathway activation in pilocyytic astrocytoma. Acta Neuropathol 2011;121:763-774

Modified from: Sievert AJ, et. Al. Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide Polymorphism-based genotype arrays reesults in a novel BRAF fusion gene. Brain Pathol 2009;19:449-458

BRAF



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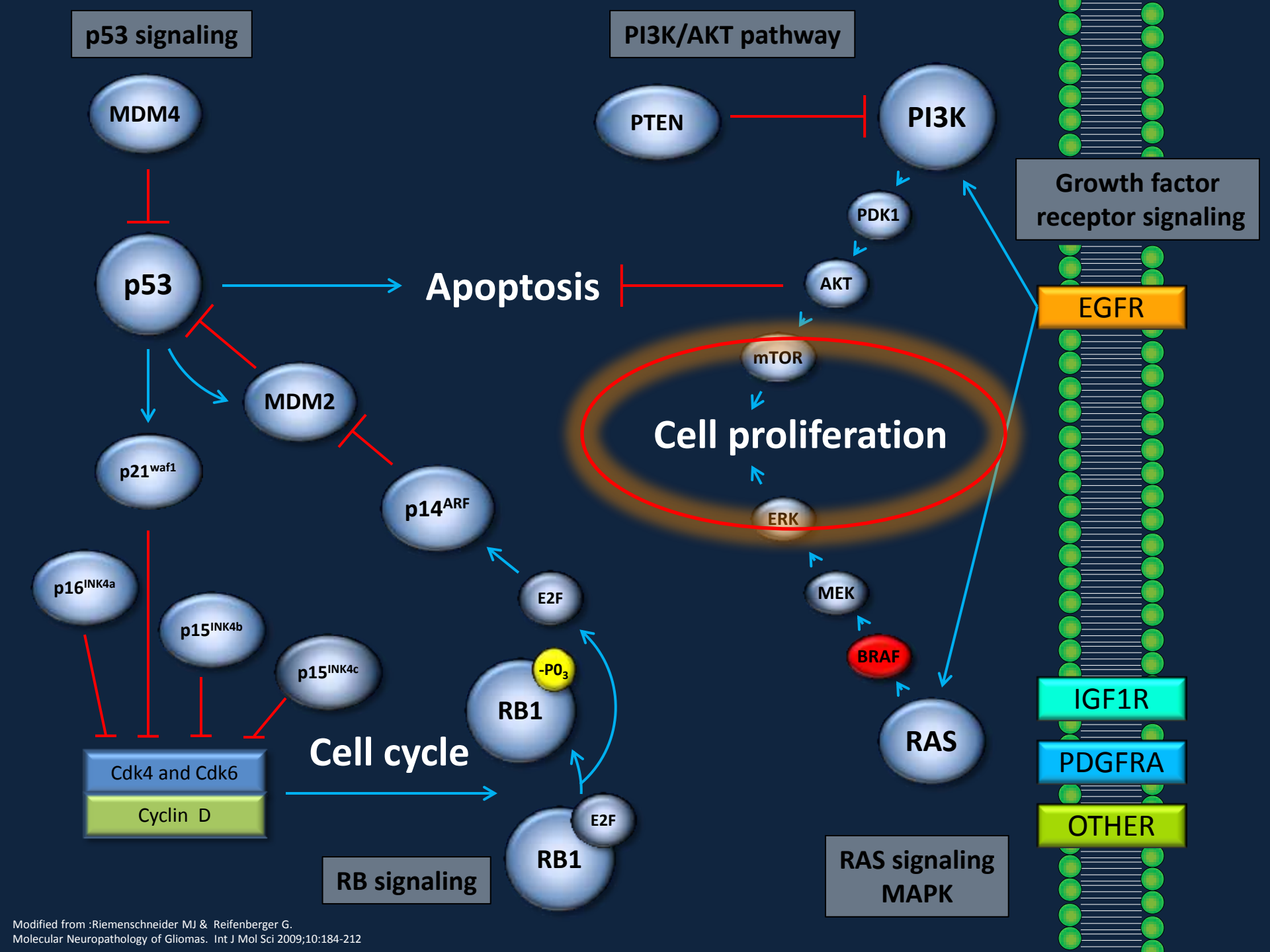


Modified from :Riemenschneider MJ & Reifenberger G.
Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

BRAF mutation

Clinical utility

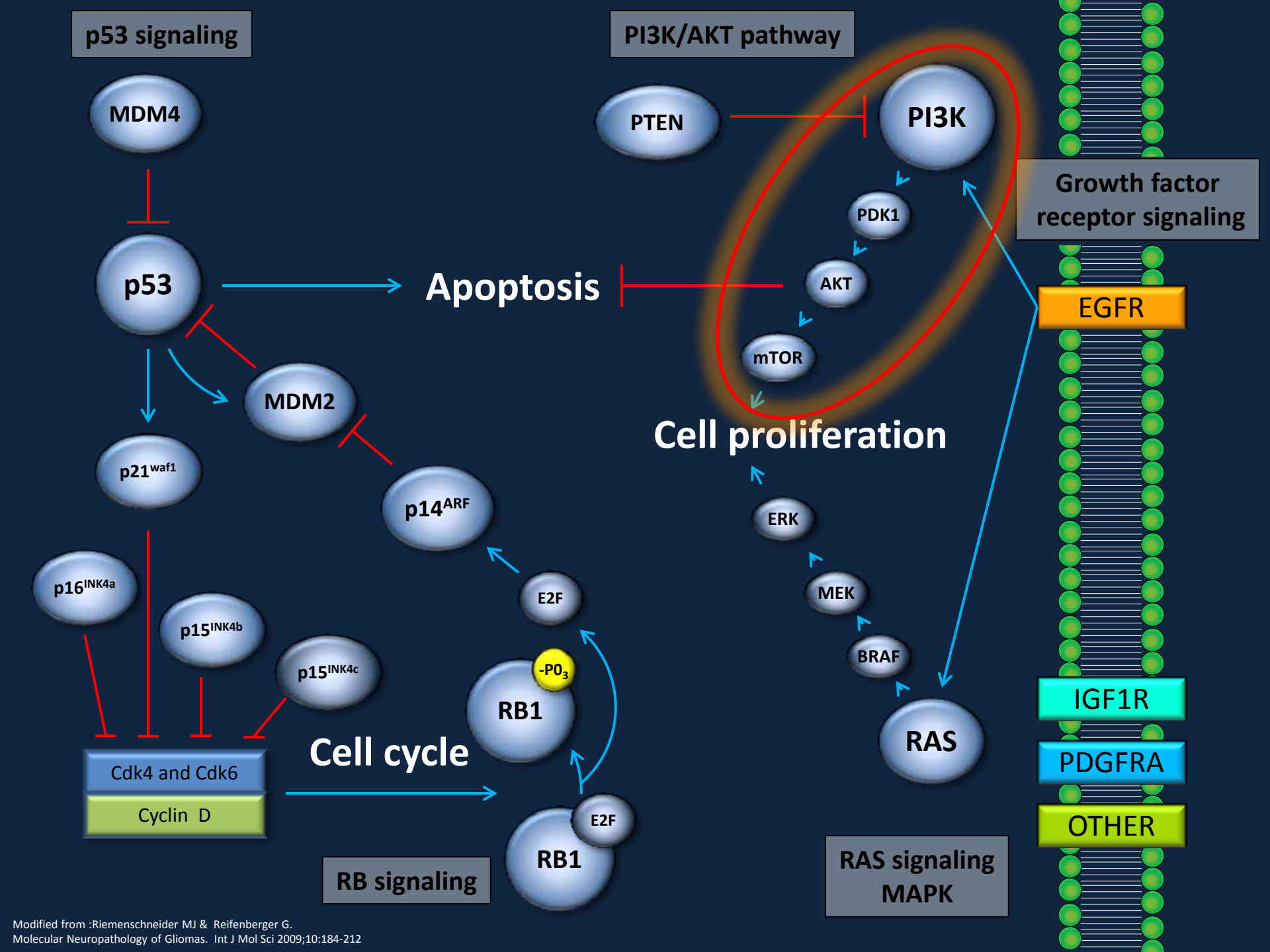
- Diagnostic:
  - May use to differentiate pilocytic astrocytoma versus diffuse glioma
- Prognostic:
  - Questionable
    - anti-MEK and anti-BRAF targeted therapies currently undergoing trials

Test methodologies:

- Polymerase chain reaction (PCR) based assays
- Sequencing
- Fluorescence in-situ hybrization (FISH)
- Immunohistochemistry (V600E)

Phosphatase and Tensin Homolog (PTEN)

- Tumor suppressor gene
- Chromosome 10q23.3
  - Encodes protein phosphatidylinositol-3, 4, 5-triphospate 3-phosphatase
  - De-phosphorylates phosphoinositide substrates
  - Negatively regulates intracellular levels of phosphatidylinositol-3, 4, 5-trisphosphate
  - Negatively regulates AKT/PI3K signaling pathway
    - Loss of PTEN leads to constitutive activation of AKT/PI3K signaling pathway
- Mutations identified in up to 30% of GBM (most commonly primary GBM)



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Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

PTEN loss/mutation

Clinical utility

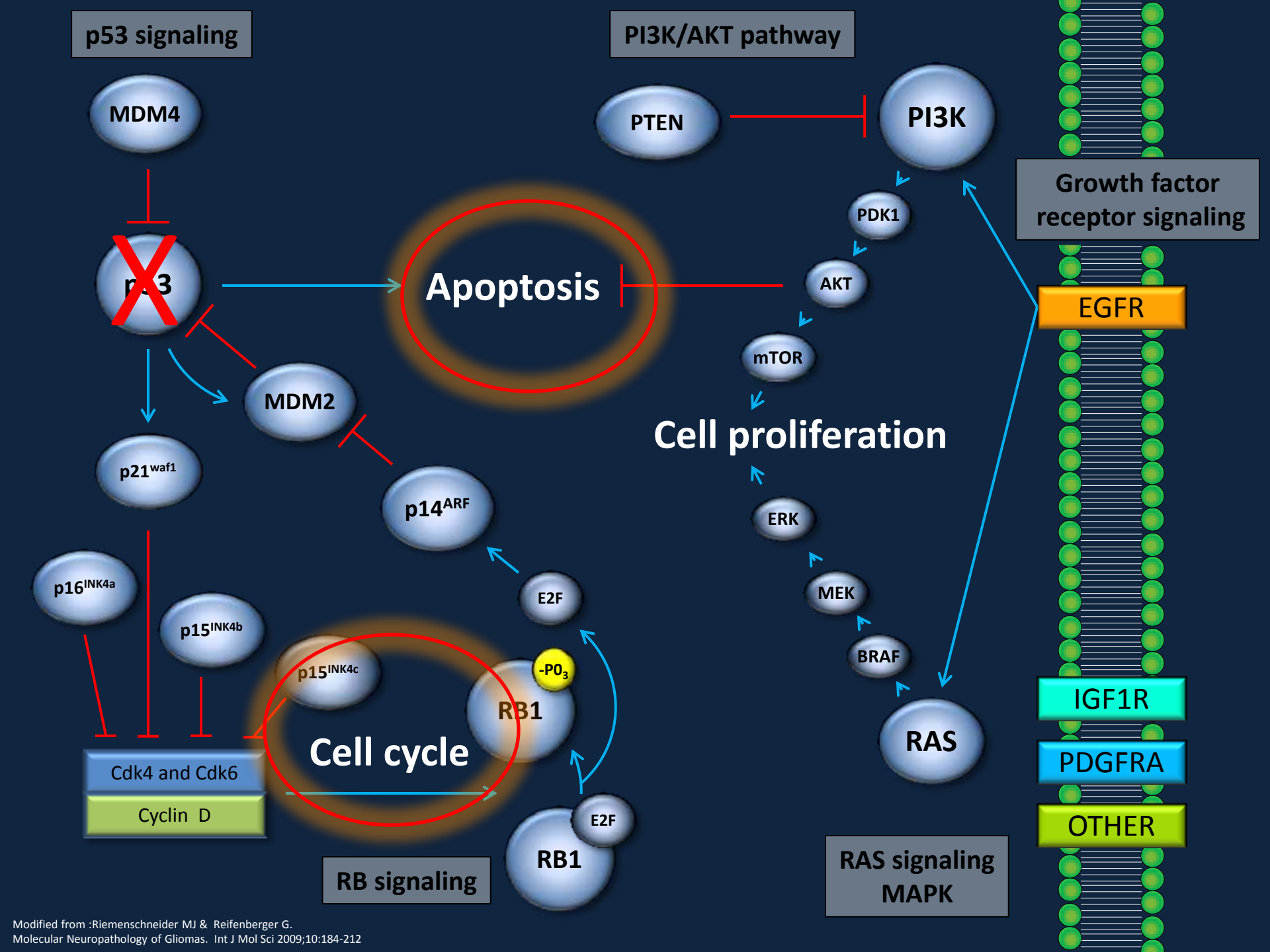
- Prognostic:
  - Typically seen in high grade gliomas

Test methodologies

- Fluorescence in-situ hybridization (FISH)
- Sequencing

Tumor protein 53 (TP53)

- Tumor suppressor gene
- Chromosome 17p13.1
- Encodes p53 protein
- DNA binding protein
  - Regulates target genes to induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism
- Mutations (cause failure to fail to bind to consensus DNA sequence)
  - Somatic
  - Germline (Li-Fraumeni syndrome)



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TP53 loss/mutation

- Clinical utility
  - Diagnostic
    - Neoplastic versus reactive astrocytic proliferation (IHC)

- Test methodologies
  - Immunohistochemistry (IHC)
  - Fluorescence in-situ hybridization (FISH)
  - Sequencing

Summary

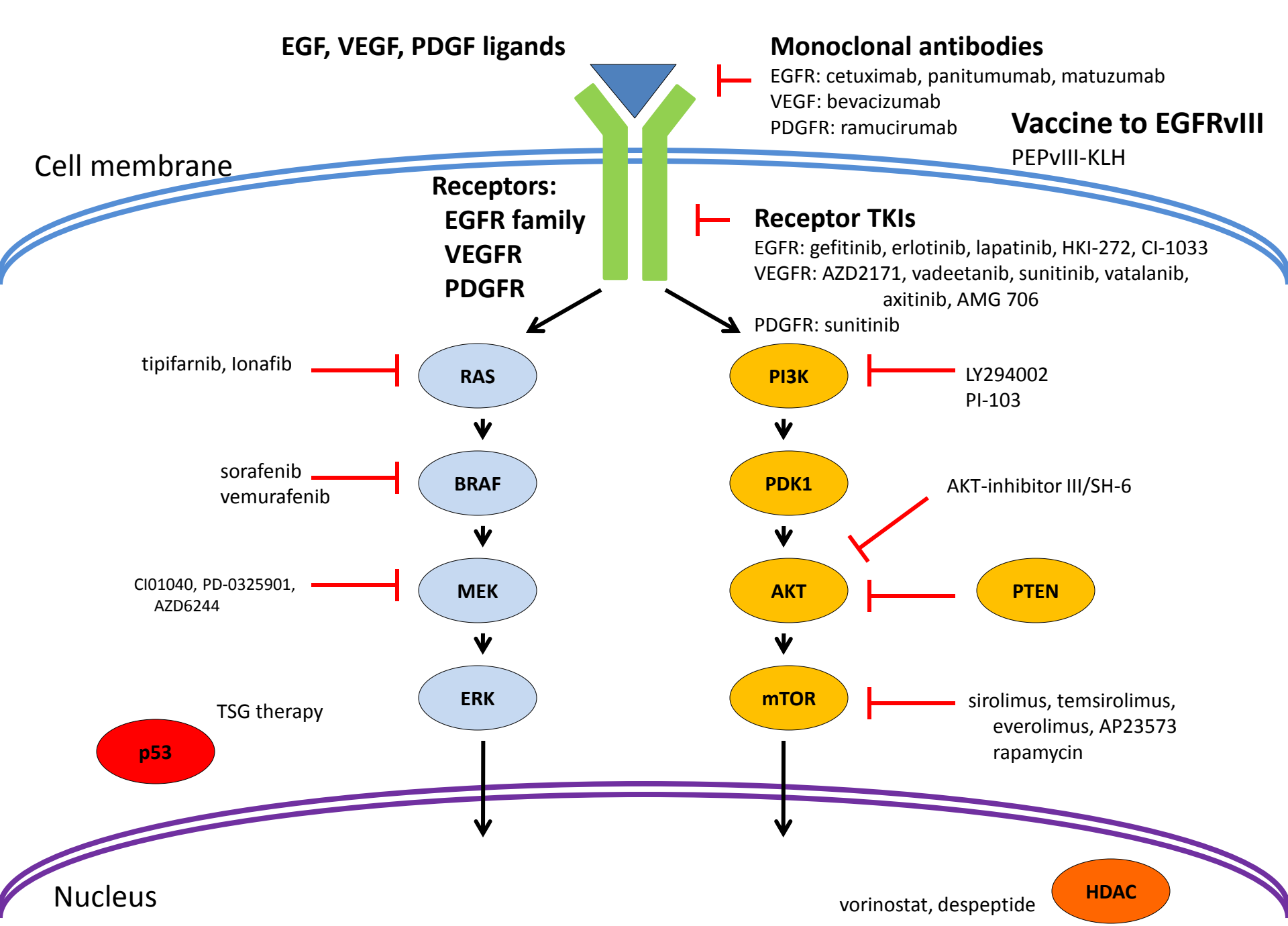
- The WHO histologic classification scheme has some limitations in predicting biologic behavior of gliomas

- Molecular abnormalities including 1p/19q codeletion, MGMT promoter methylation, IDH1/2 mutations, and BRAF mutations are now more commonly being utilized alongside histopathology for improved diagnostic, prognostic and predictive purposes

Summary

- Recent large scale integrative genomic analyses have identified glioma subclasses with characteristic molecular signatures

- Molecular subclassification of gliomas may allow for more effective patient treatment by enabling a more targeted approach to tumor therapy



Modified from: Dacic S. Molecular Diagnostics of Lung Carcinomas. Arch Pathol Lab Med 2011;135:622-629

References:

- Dacic S. Molecular Diagnostics of Lung Carcinomas. Arch Pathol Lab Med 2011;135:622-629
- Riemenschneider MJ & Reifenberger G. Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212
- Mimeault M and Surinder KB. Complex Oncogenic Signalling Networks Regulate Brain Tumor-Initiating Cells and Their Progenies: Pivotal Roles of Wild-Type EGFR, EGFRvIII Mutation and Hedgehog Cascades and Novel Multitargeted Therapies. Brain Pathology 2011;21:479-500
- Nikiforova MN & Hamilton RL. Molecular Diagnostics of Gliomas. Arch Pathol Lab Med 2011;135:558-568
- Malzkorn B, et al. Unraveling the Glioma Epigenome – From Molecular Mechanisms to Novel Biomarkers and Therapeutic Targets. Brain Pathology 2011:619-632.
- Noushmehr H, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. 2010;17:510-522.
- Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Canc 2004;4:296-307
- Szulwach KE, et.al. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nature Neuroscience 2011;14:1607-1616
- Jin SG, et. Al. 5-Hydromethylcytosine is strongly depleted in human cancers but its levels do not correlate with IDH1 mutations. Canc Res Nov 3, 2011 Published Online

References:

- Lin A, et. al. BRAF Alterations in Primary Glial and Glioneuronal Neoplasms of the Central Nervous System With Identification of 2 Novel KIAA1549:BRAF Fusion Variants. J Neuropathol Exp Neurol 2012: 71; 66-72
- Wang R, et. al. Glioblastoma stem-like cells give rise to tumour endothelium. Nature 2010;468:829-836
- Theeler BJ, et. al. Moving toward molecular classification of diffuse gliomas in adults. Neurology 2012;79:1917-1926
- Huse JT, et. al. Molecular Subclassification of Diffuse Gliomas: Seeing Order in Chaos. Glia 2011;59:1190-1199
- Goodenberger ML, et. al. Genetics of adult glioma. Cancer Genetics 2012;205:613-621
- TCGA. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-1068