Anaplastic gliomas – update 2013

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Conflicts of Interest

- Advisory boards
  - Apogenix
  - Eli Lilly
  - Magforce
  - MSD
  - Roche
- Speaker's honoraria
  - MSD
  - Roche
- Industry Funding
  - Apogenix
  - Boehringer Ingelheim
  - Eli Lilly
  - MSD
  - Roche
- No immediate payments for any parts of this presentation

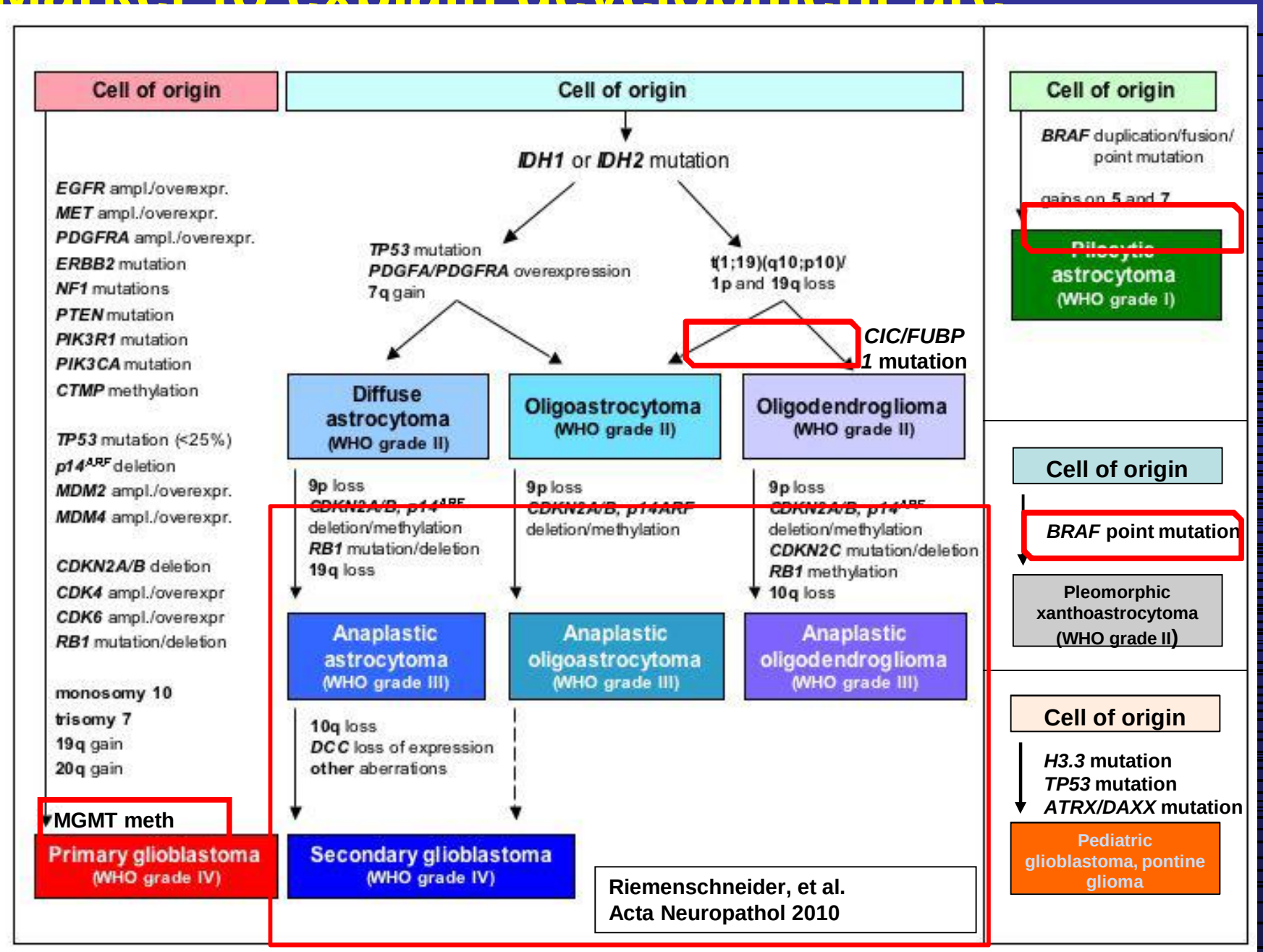
Learning objectives

- Relevance of the long-term analysis of the EORTC/RTOG trials in anaplastic oligodendroglial tumors
- Answer to the question of on state-of-the-art diagnosis and therapy of anaplastic gliomas
- Which biomarkers are necessary for the management of our patients?

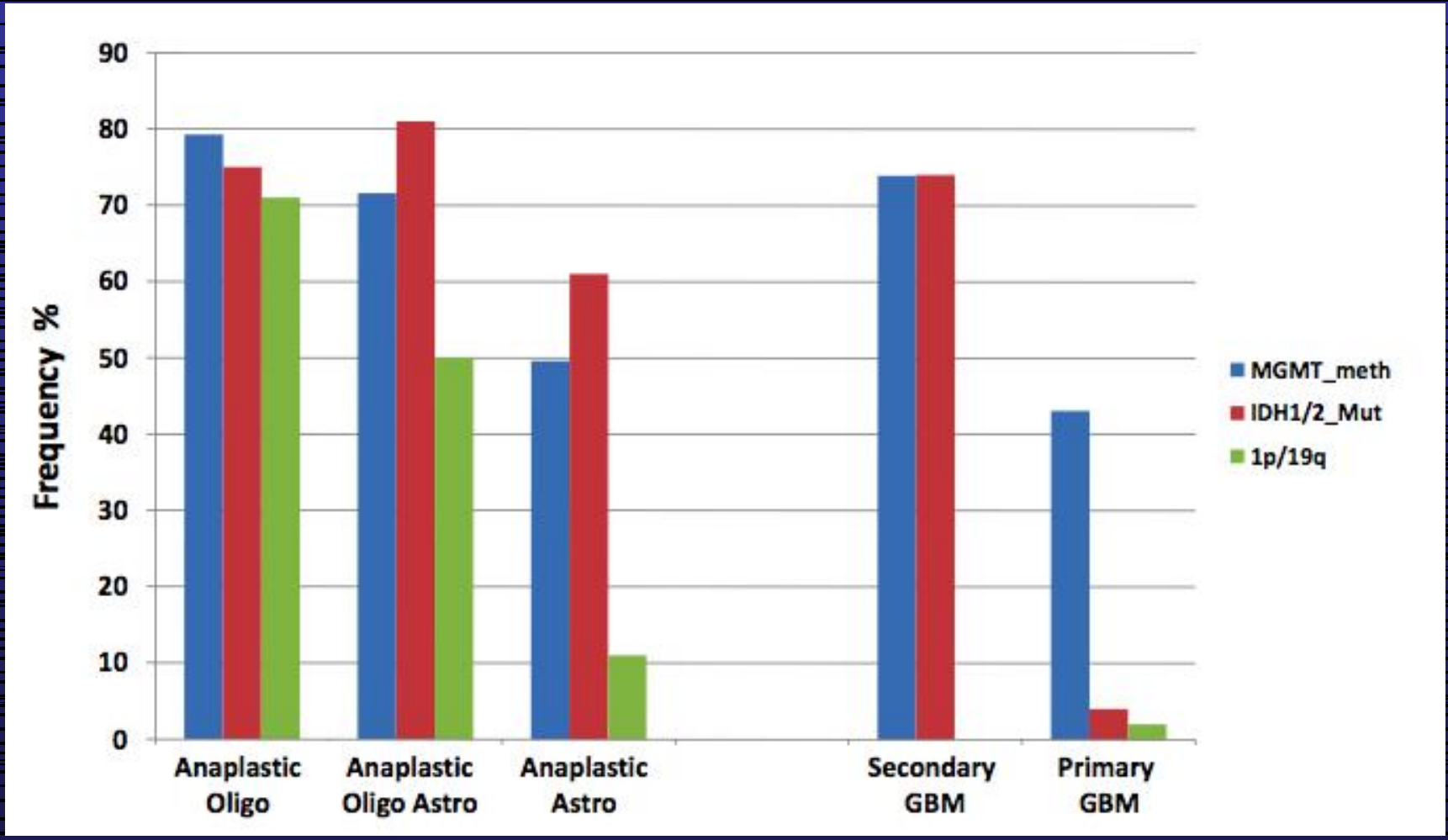
Structure

- Introduction into biomarkers in anaplastic gliomas
- „Good wine needs aging“
- Some considerations on the NOA-04 trial
- Pragmatic algorithm for molecularly based diagnoses

Marker to explain development are



Distinct frequencies of molecular markers in high-grade gliomas

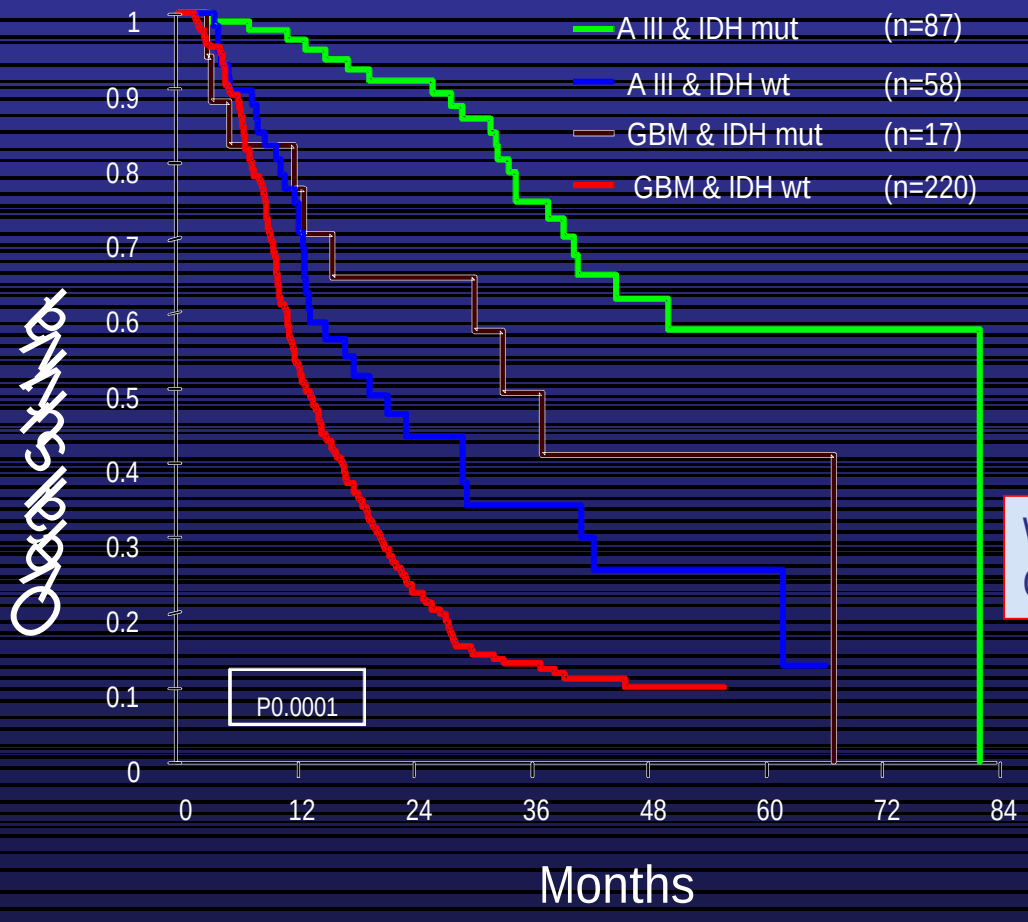


Riemenschneider et al., Target Oncol 2010

Important questions: isocitrate dehydrogenase

- Does a mutated gene have clinical consequences?
- Are IDH-mutated gliomas a separate entity?
- How can we make use of this specific glioma feature?

IDH: better to discriminate high-grade glioma than WHO grade?



WHO-Grade

- Anapl. astrocytoma IDH-mut
- GBM IDH-mut
- Anapl. astrocytoma IDH-wt
- GBM IDH-wt

each

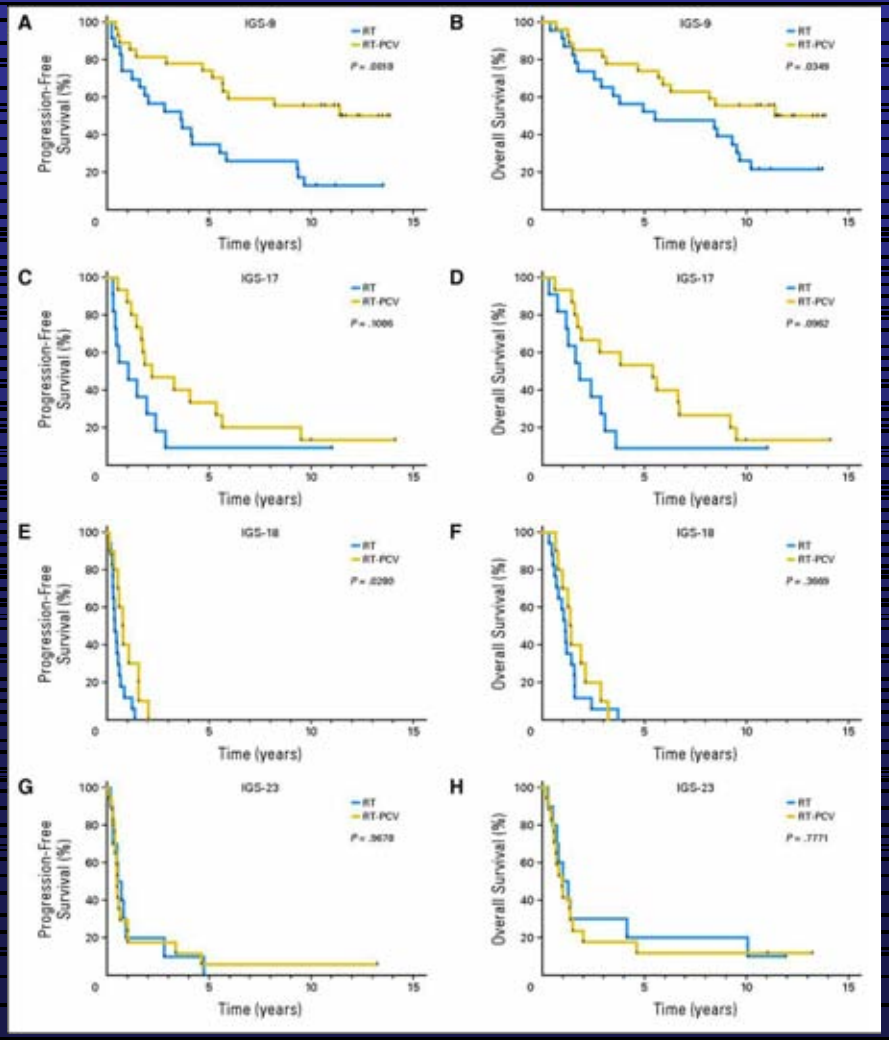
- MGMT meth.
- MGMT unmeth.

Hartmann et al., Acta Neuropathol 2010

Deutsches Gliomnetzwerk

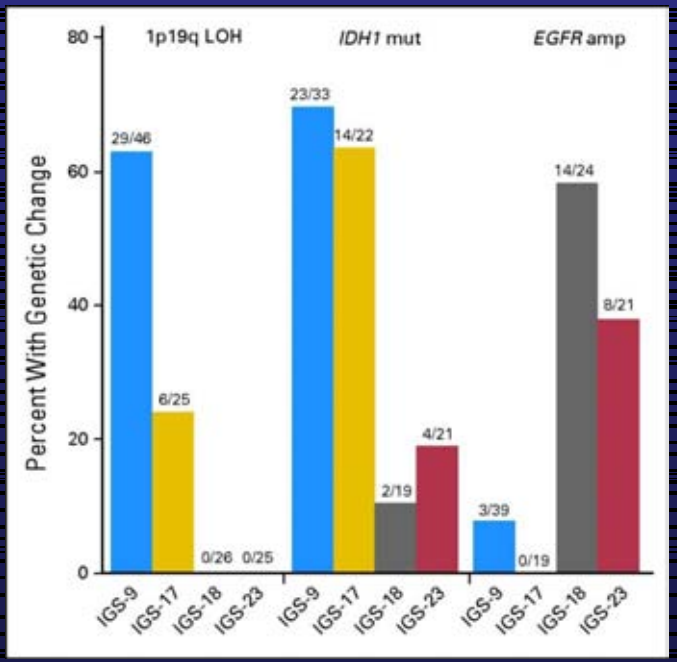
Gene expression profiling identifies 4 different clusters, associated with benefit to PCV

Kaplan-Meier survival curves of the four intrinsic glioma subtypes (IGSs) per treatment arm.



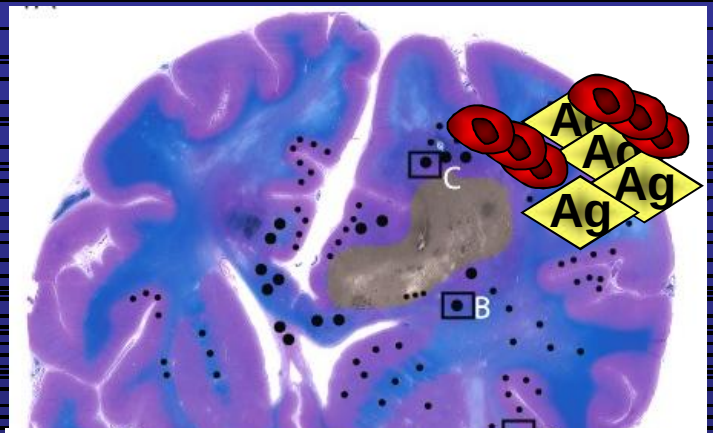
Erdem-Eraslan L et al. JCO 2013;31:328-336

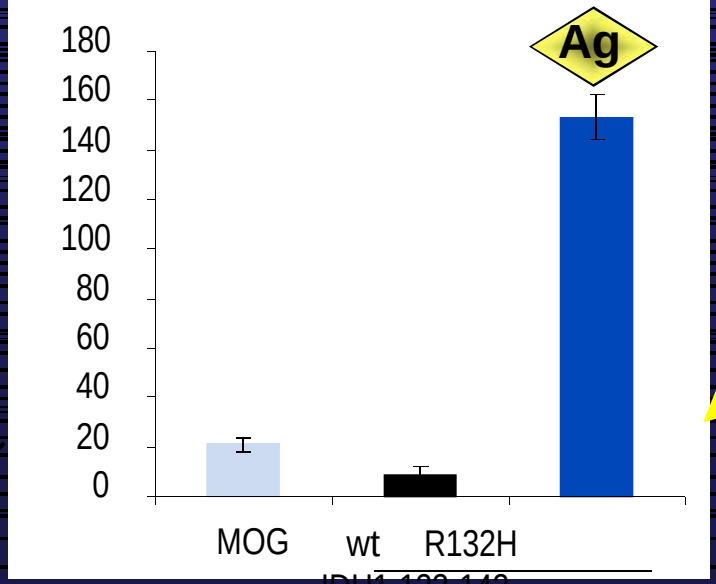
- Incomplete Association with 1p/19q, IDH status
- Two major responsive and two major unresponsive clusters
- Predictive value of clustering appears higher than 1p/19q or IDH alone

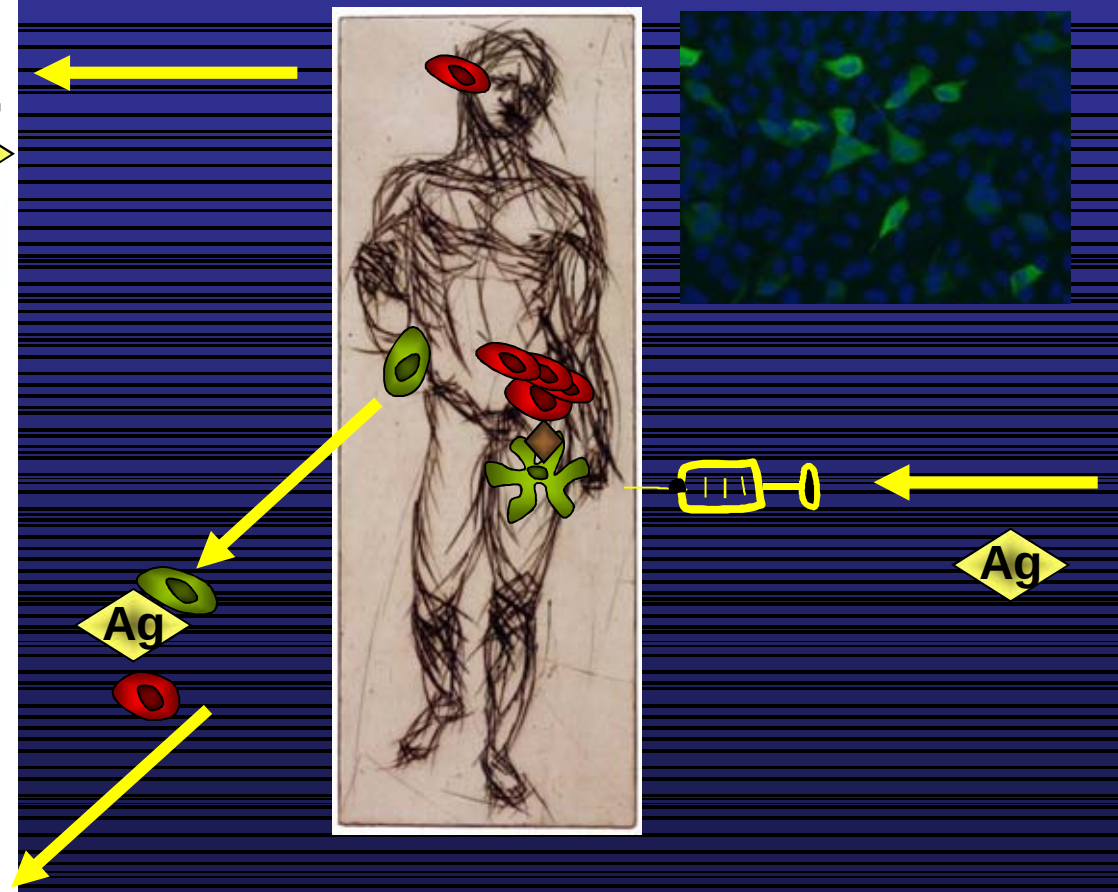


Genetic differences between intrinsic glioma subtypes (IGSs).

IDH1: diagnostic tool and potent(ial) target for immunotherapies







Sahm, et al. Arch Neurol 2011, Schumacher, Bunse et al.

AGI-5198, a selective R132H-IDH1 inhibitor, blocks *R*-2-hydroxyglutarate (*R*-2HG)

TS603 glioma cells with an endogenous heterozygous *R132H IDH1* mutation

- **AOIII, 1p/19q co-deletion**

AGI-5198 has been discovered in a high-throughput screen to block *R*-2HG production

Blockage of *mIHD1* impaired growth of *IDH1*-mutant, but not wt glioma cells *in vitro* and *in vivo* without rel. changes in genome-wide methylation

AGI-5198 induced demethylation of histone H3K9me3 and expression of astrocytic differentiation genes (*GFAP, AQP4* and *ATP1A2*)

Rohle et al. Science 2013, April 4

IDH1: value for clinical decision making?

Can we use the IDH status for diagnostic purposes?	Yes. IDH mutations are common in grade II and III gliomas and can aid in the differential diagnosis, e.g., from pilocytic astrocytomas and ependymomas which lack IDH mutations and show that gliomas are whole-brain disaeses
Can we use the IDH status for prognostic purposes?	Yes. IDH mutations are prognostically favourable across all glioma entities.
Can we use the IDH status as a predictive marker for clinical decision making?	Not alone, but as one factor to define the molecular background.
Can we do therapy based on IDH?	Probably, yes.

adapted from Weller et al., Neuro Oncol 2012

What do we know about *MGMT* and *IDH1 as biomarkers*?

MGMT is predictive for alkylating chemotherapy (temozolomide) in glioblastoma patients[1,2,3]

MGMT is just prognostic in anaplastic gliomas[4,5]

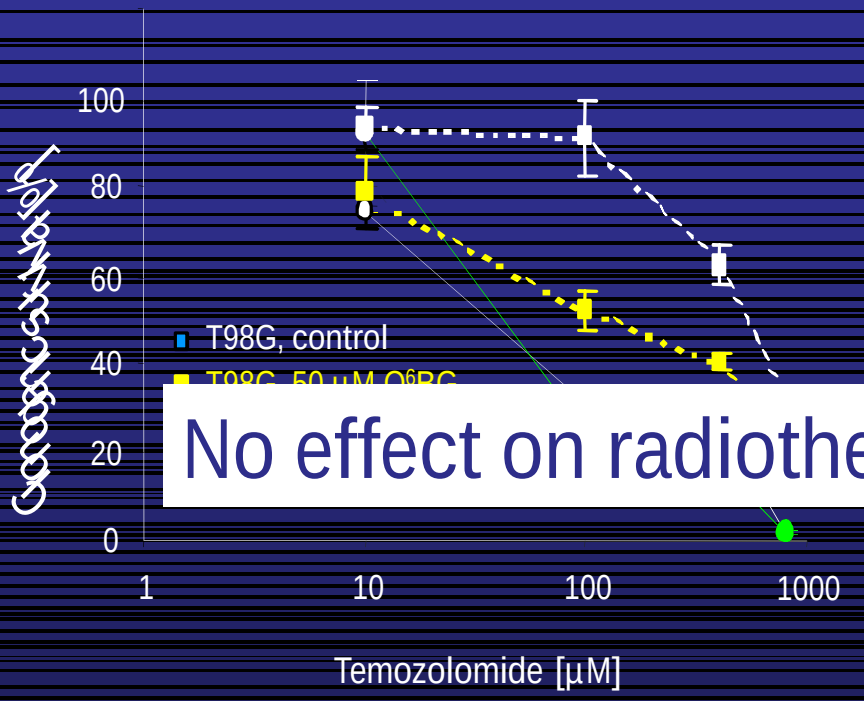
IDH1 is a diagnostic and merely prognostic biomarker

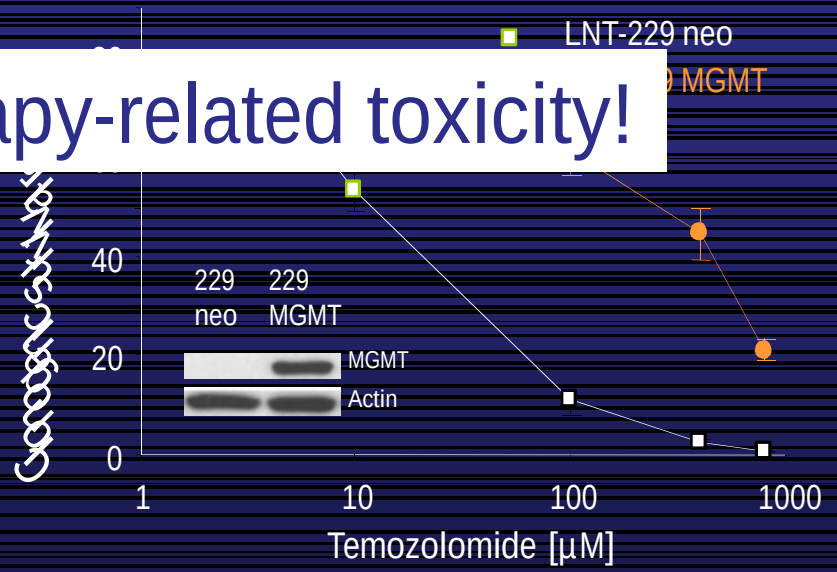
Background for the contextual role is not understood

1. Hegi et al. NEJM 2005
2. Wick et al. Lancet Oncol 2012
3. Malmström et al. Lancet Oncol 2012
4. Wick et al. J Clin Oncol 2009
5. Van den Bent et al. J Clin Oncol 2009

Biology of O6-Methyl-Guanyl-Methyltransferase

MGMT gene transfer protects MGMT-negative glioma cells from temozolomide-induced cell death





No effect on radiotherapy-related toxicity!

O^6-Benzylguanine sensitizes MGMT-positive glioma cells for temozolomide-induced cell death

Hermisson et al. J Neurochem 2006

Methods to assess the *MGMT* status in gliomas

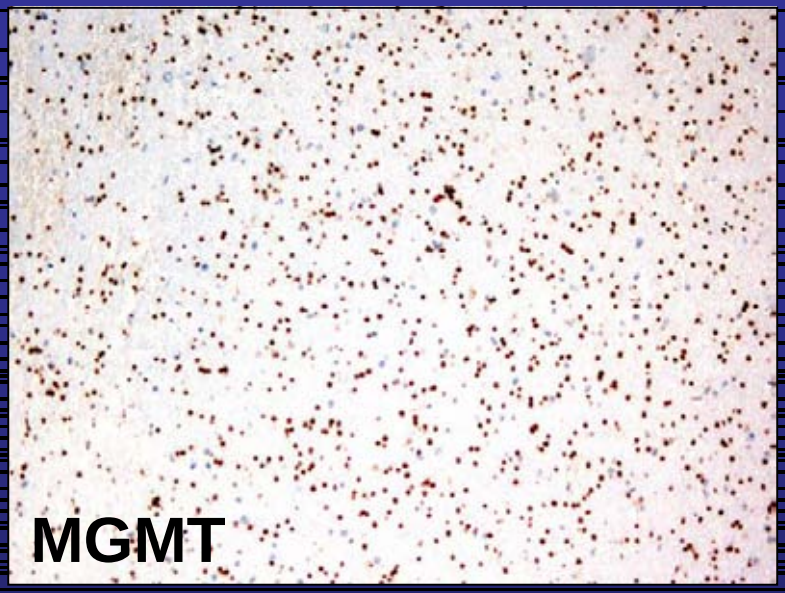
- **Promoter methylation analyses**
  - **methylation-specific PCR (MSP)**
  - **quantitative MSP**
  - **pyrosequencing**
  - **MS-MLPA**

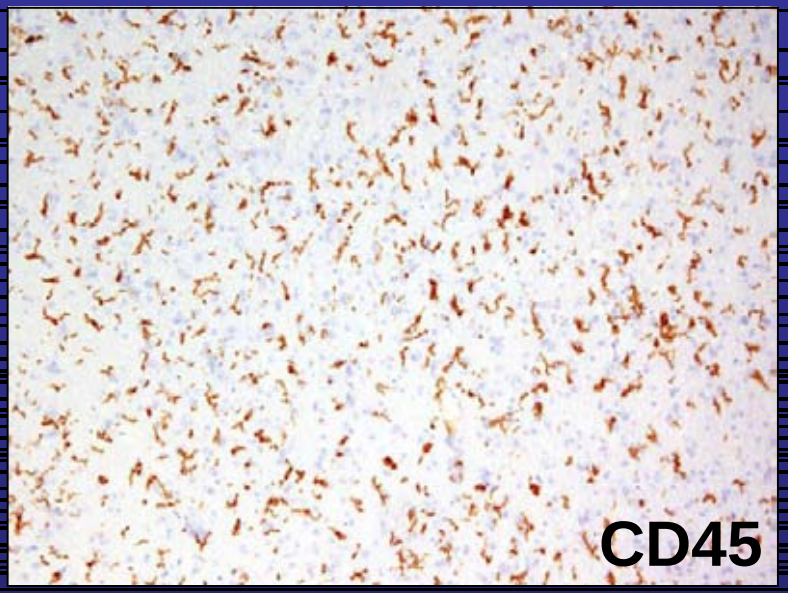
- **Expression analyses**
  - **protein: immunohistochemistry, Western blotting**

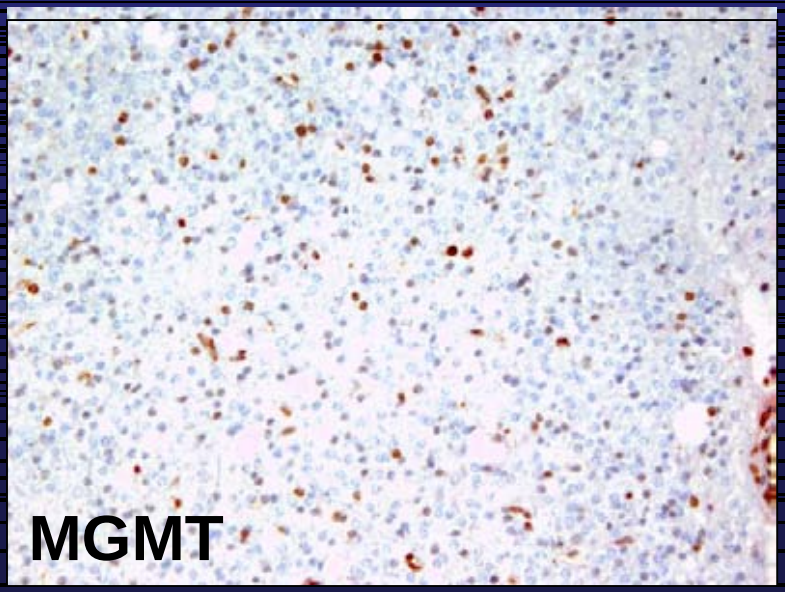
Ring trials and formal quality assurance is needed for MGMT testing!

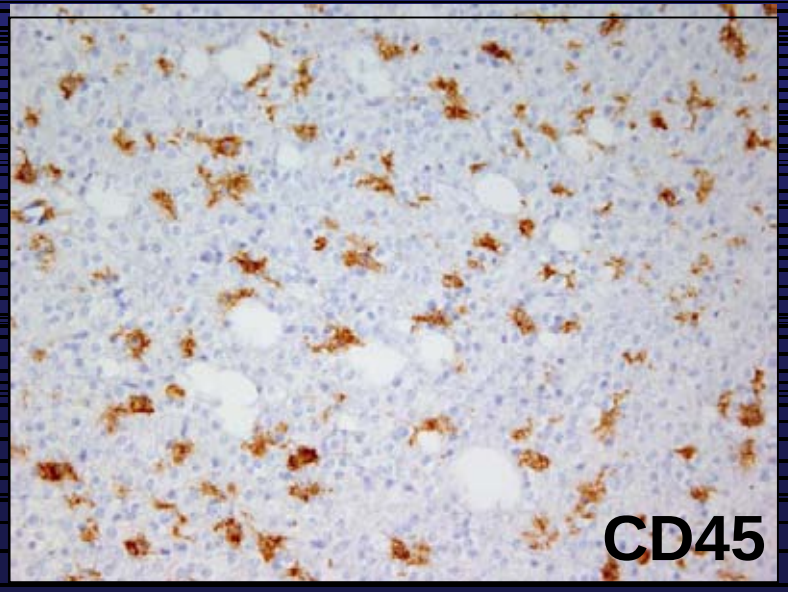
- **Biochemical assessment of enzymatic activity**

IHC is not reliable for the diagnostic assessment of the *MGMT* status

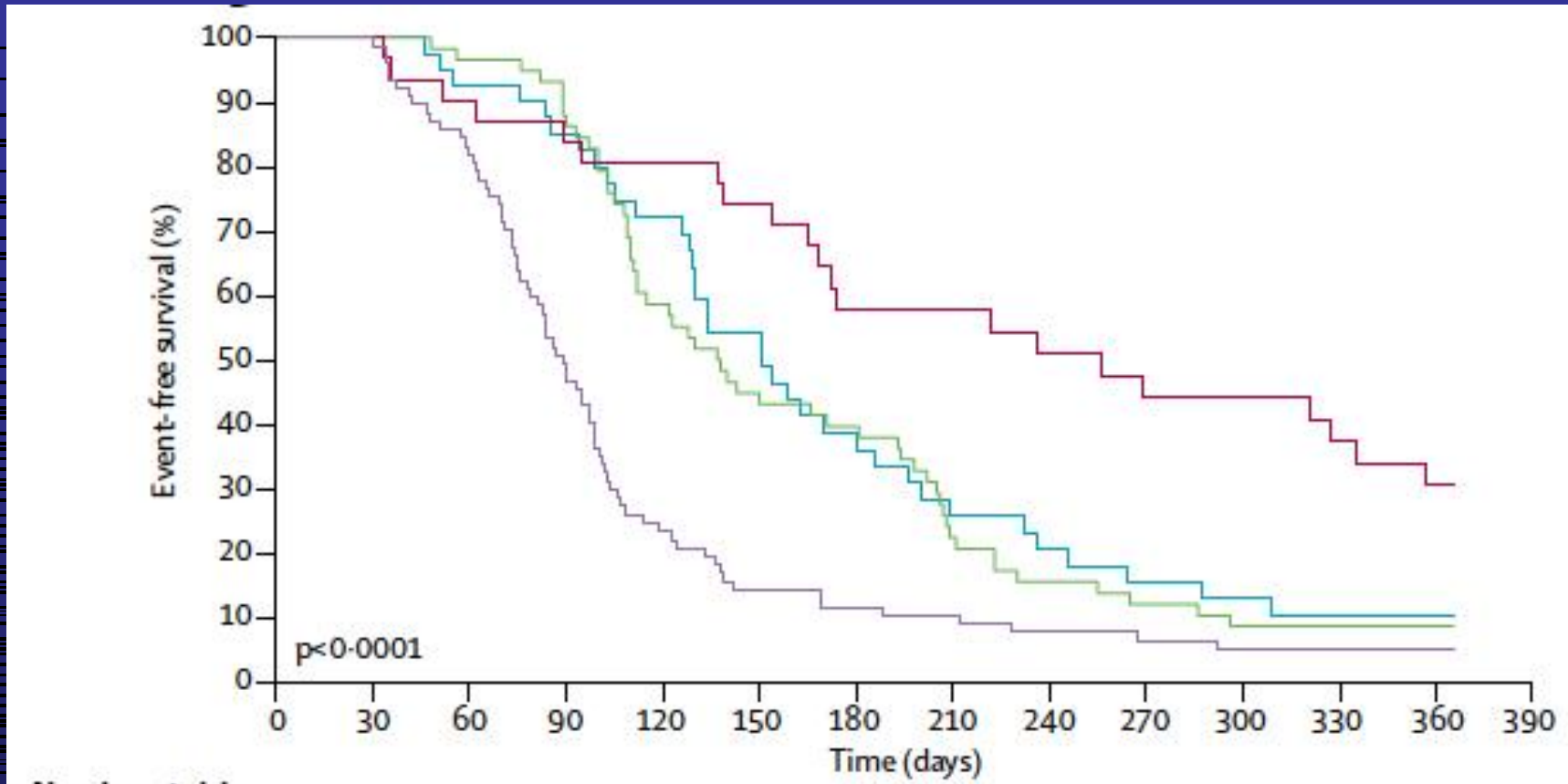








MGMT is a predictive biomarker in elderly



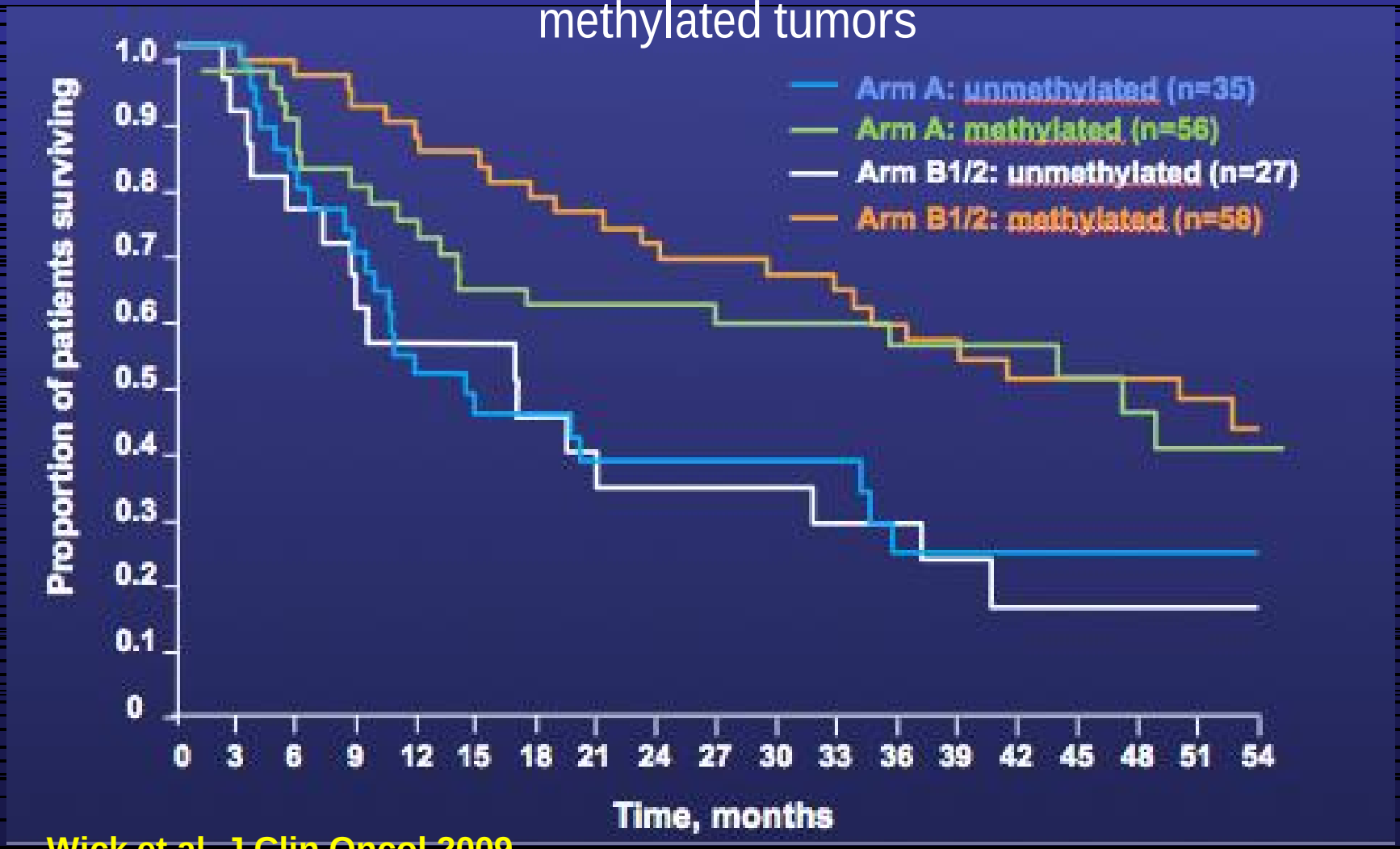
Number at risk

RT													
MGMT+	42	41	38	33	28	21	14	10	8	6	5	4	1	0
MGMT–	59	59	56	50	34	25	23	13	9	7	5	4	1	0
TMZ														
MGMT+	31	30	28	26	25	23	17	17	15	13	13	11	9	8
MGMT–	77	76	63	37	18	11	9	8	6	5	4	4	1	0

Wick et al. Lancet Oncol 2012

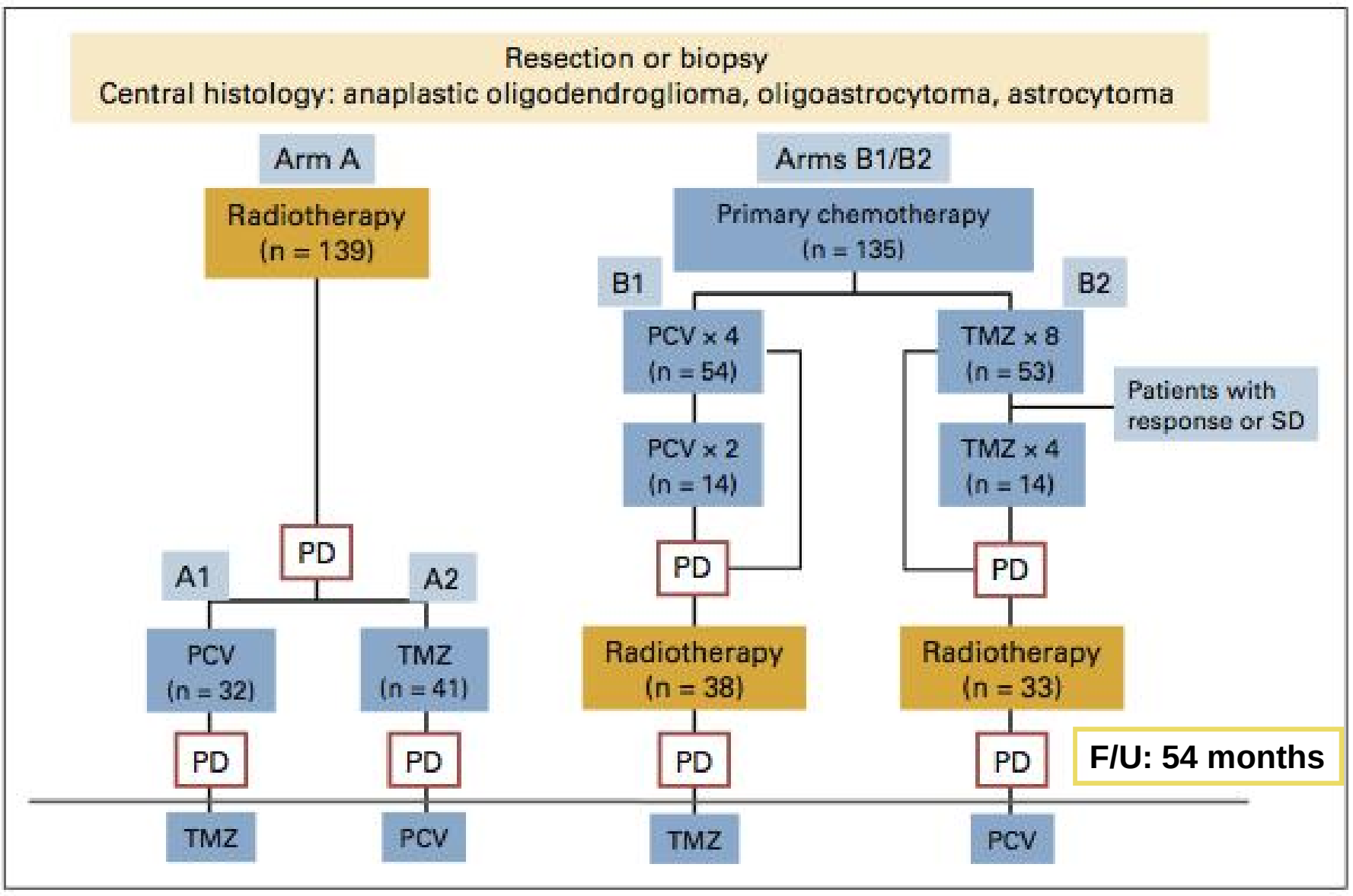
MGMT is a prognostic biomarker in anaplastic glioma

Newly diagnosed anaplastic glioma: efficacy of RT improved in methylated tumors

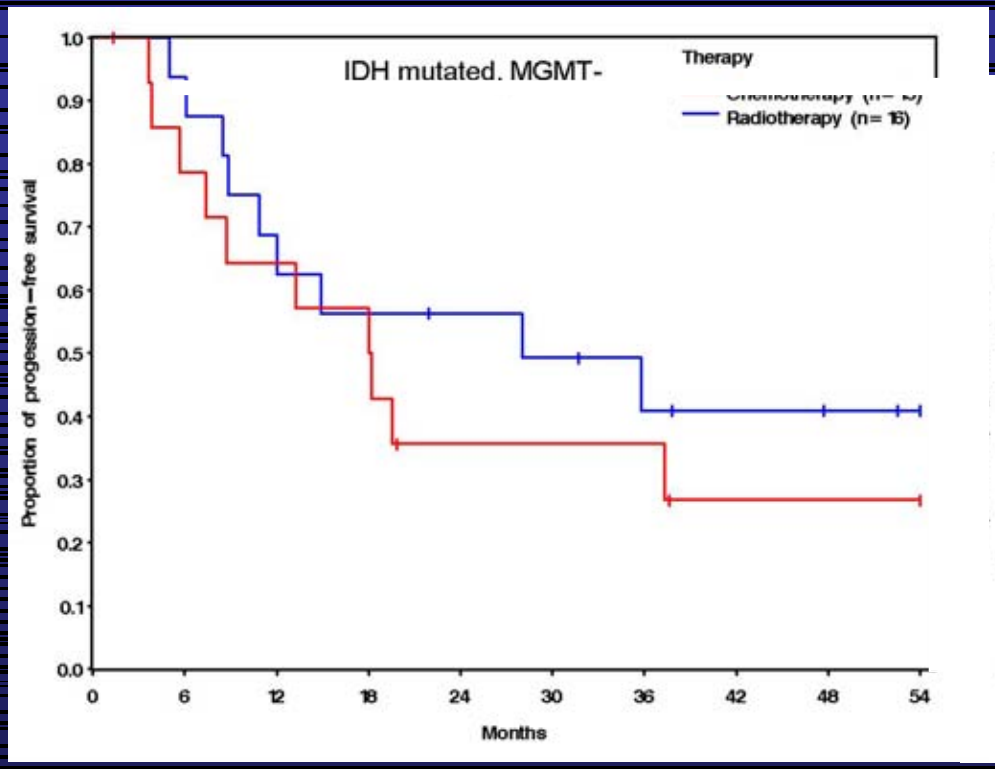


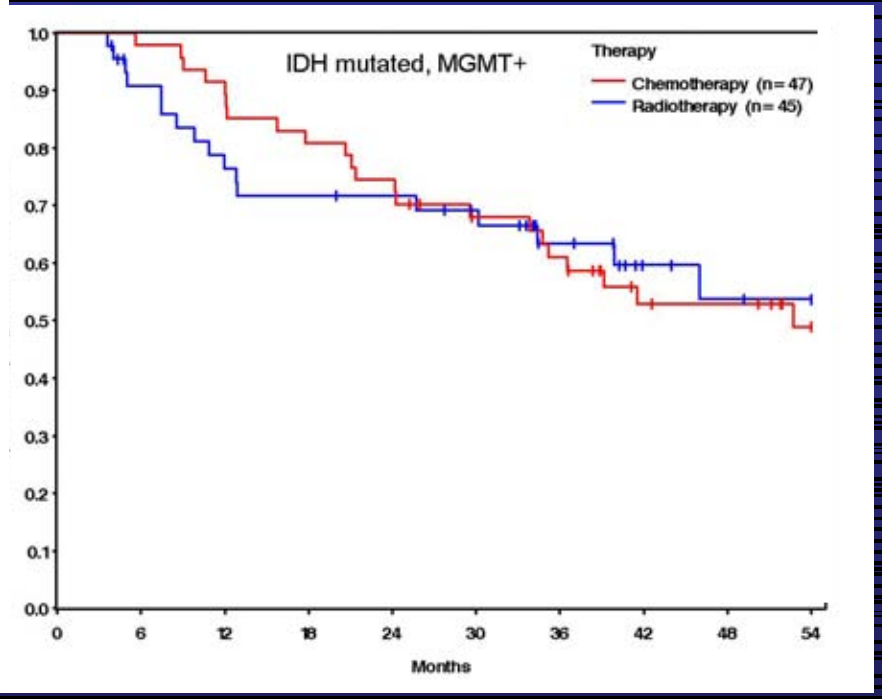
Wick et al. J Clin Oncol 2009

NOA-04 trial design



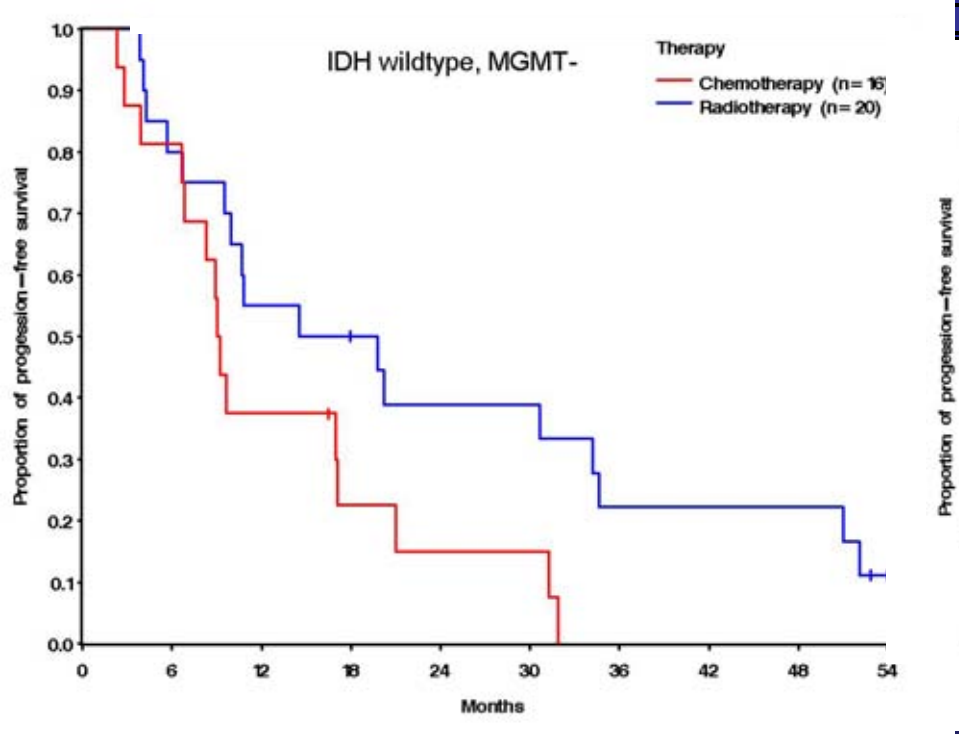
Interaction between *MGMT* and *IDH1*?

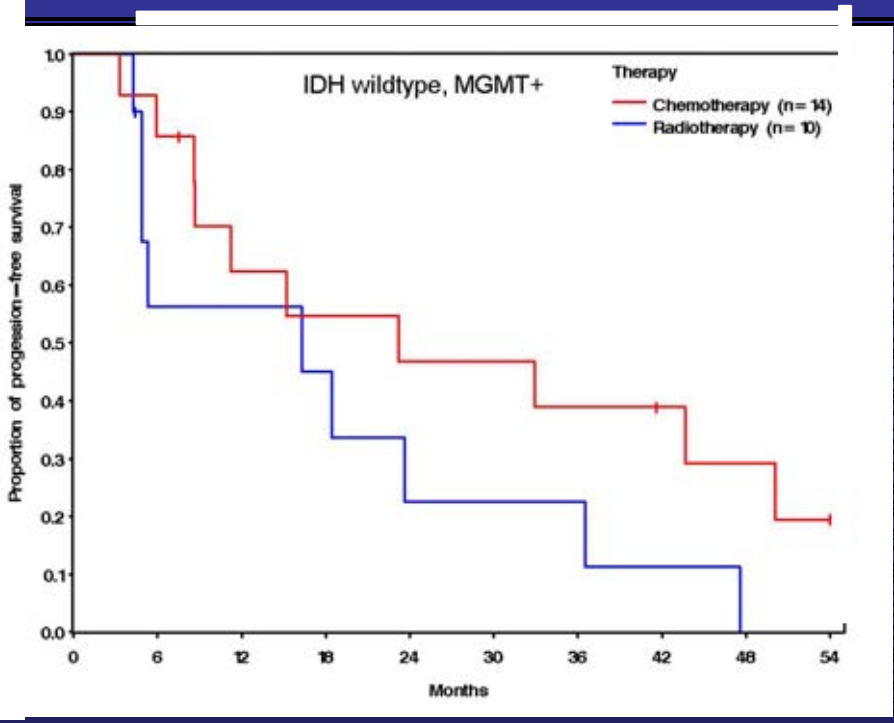




- Strong prognostic impact of IDH mutations for RT and chemotherapy
- Prognostic impact of MGMT for RT or chemotherapy in patients with IDH-mutated tumours

Interaction between *MGMT* and *IDH1*!

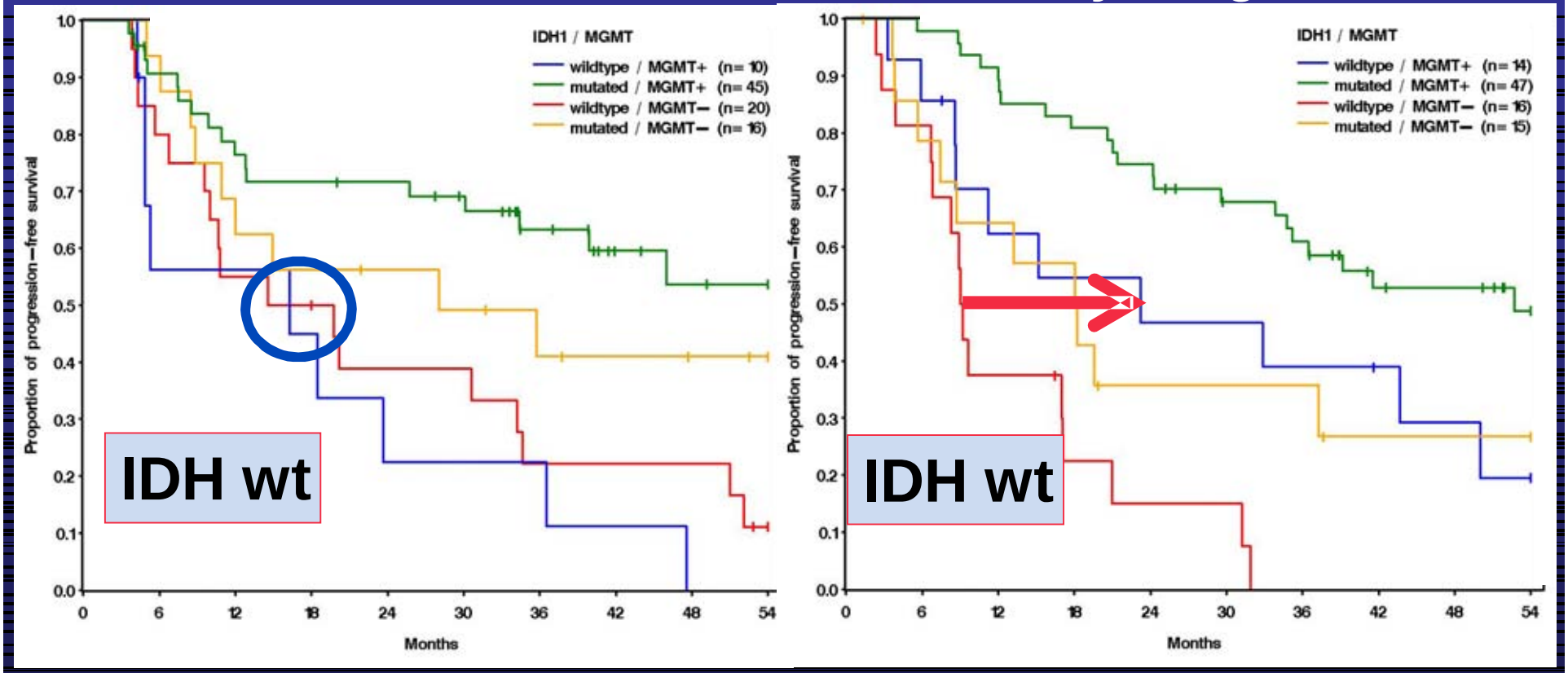




- Predictive role for MGMT for chemotherapy in patients with IDH-wt tumours

Interaction between *MGMT* and *IDH1*!

Radiotherapy **Alkylating CT**



- Data from the GGN/NOA-08 cohort (n=109)
- Higher median age compared to the NOA-04 cohort

Wick et al. Neurology 2013

MGMT is a predictive biomarker in glioblastoma

Can I use the MGMT status for diagnostic purposes?	No.
Can I use the MGMT status for prognostic purposes?	Yes. MGMT promoter methylation is positively prognostic in anaplastic glioma patients treated with RT or chemotherapy or both (NOA-04, EORTC 26951).
Can I use the MGMT status as a predictive marker for clinical decision making?	Yes. MGMT promoter methylation predicts benefit from alkylating agent chemotherapy in glioblastoma (EORTC 26981), is particularly useful in the elderly (NOA-08 and Nordic Elderly Trial) and may be used in IDH-wt anaplastic gliomas

adapted from Weller et al., Neuro Oncol 2012

Important questions: 1p/19q

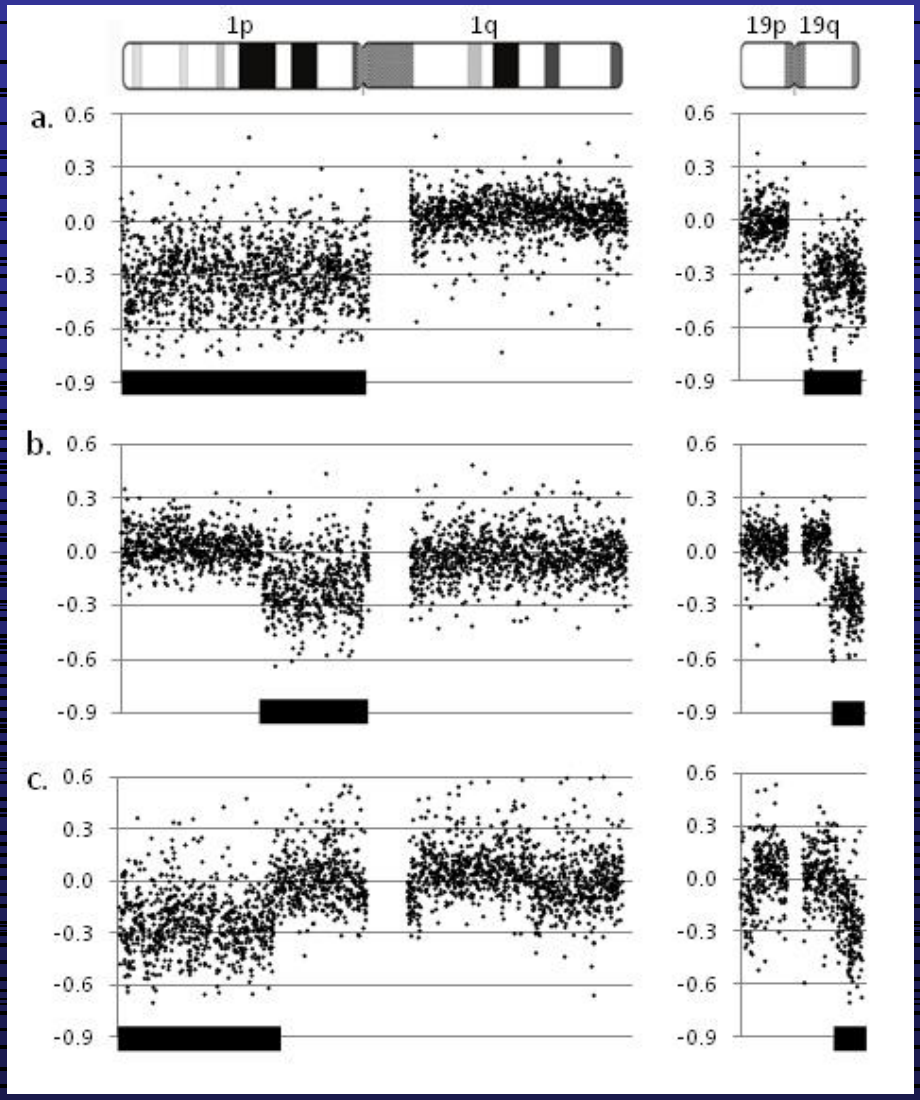
- Why are 1p and 19q frequently co-deleted in oligodendroglial tumors?
- Which genes are the relevant tumor suppressors on 1p and 19q?
  CIC, FUBP1 (Bettegowda et al. Science 2011)
- Why are 1p/19q losses associated with favorable response to therapy and better prognosis?
- How to assess 1p/19q deletion in glioma diagnostics?

Methods to assess the 1p/19q deletion status

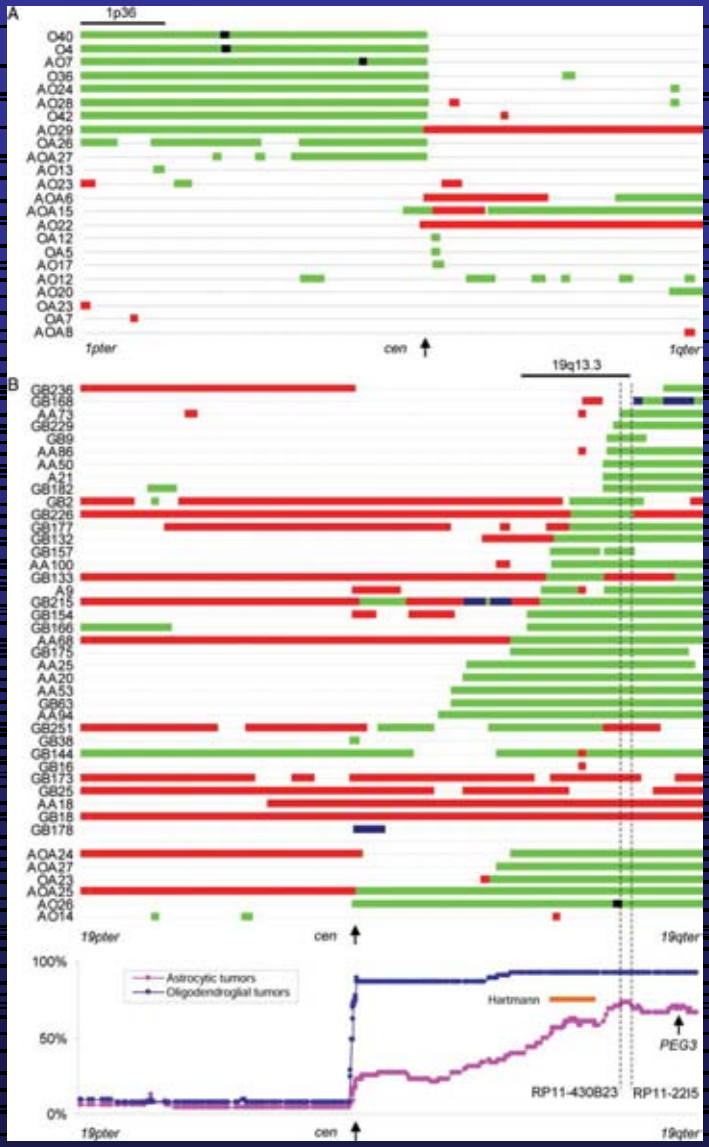
Most commonly used methods for 1p/19q deletion testing			
(Fluorescence) in situ hybridization ((F)ISH)	fresh frozen or FFPE tumor tissue	signal ratio target versus control clone in individual cells	best method on archival specimens / difficult to quantify, labor-intensive
Loss of heterozygosity (LOH) analysis	fresh frozen or FFPE tumor tissue plus additional patient blood sample	gel-based detection of allelic imbalance, comparative evaluation of the same set of loci in tumor and blood DNA	better to test for multiple loci along a chromosomal arm to differentiate partial from complete losses / requires blood sample / allelic imbalance may not only be caused by allelic loss but also by allelic gain
Multiplex ligation dependent probe amplification (MLPA)	fresh frozen or FFPE tumor tissue	ratio target versus reference probe	multiple loci (up to 45) can be assessed in a single experiment

Molecular diagnostics of gliomas - state of the art. M. J. Riemenschneider, J. W. Jeuken, P. Wesseling, G. Reifenberger. Acta Neuropathol, 2010

Pitfalls in the 1p/19q deletion testing

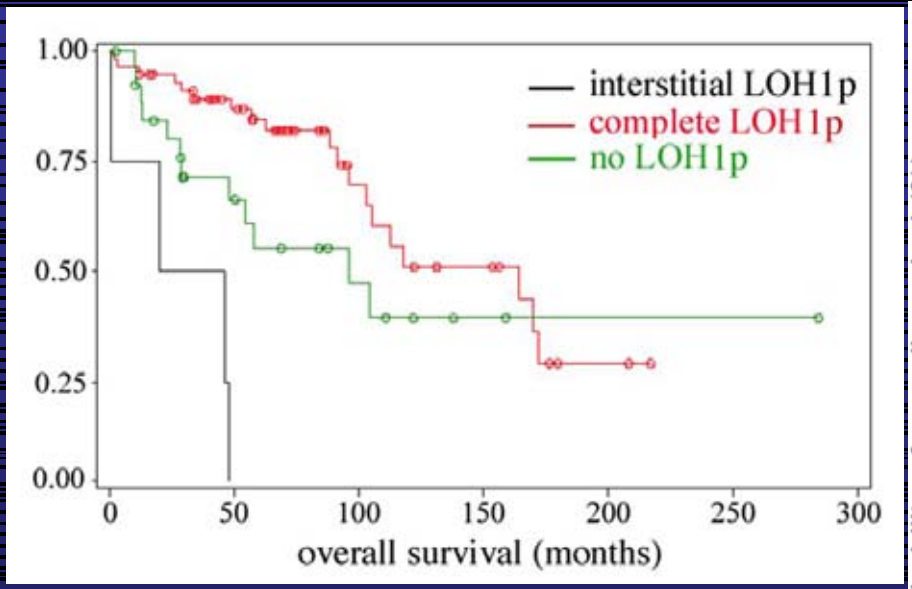


Riemenschneider et al. Acta Neuropathol, 2010

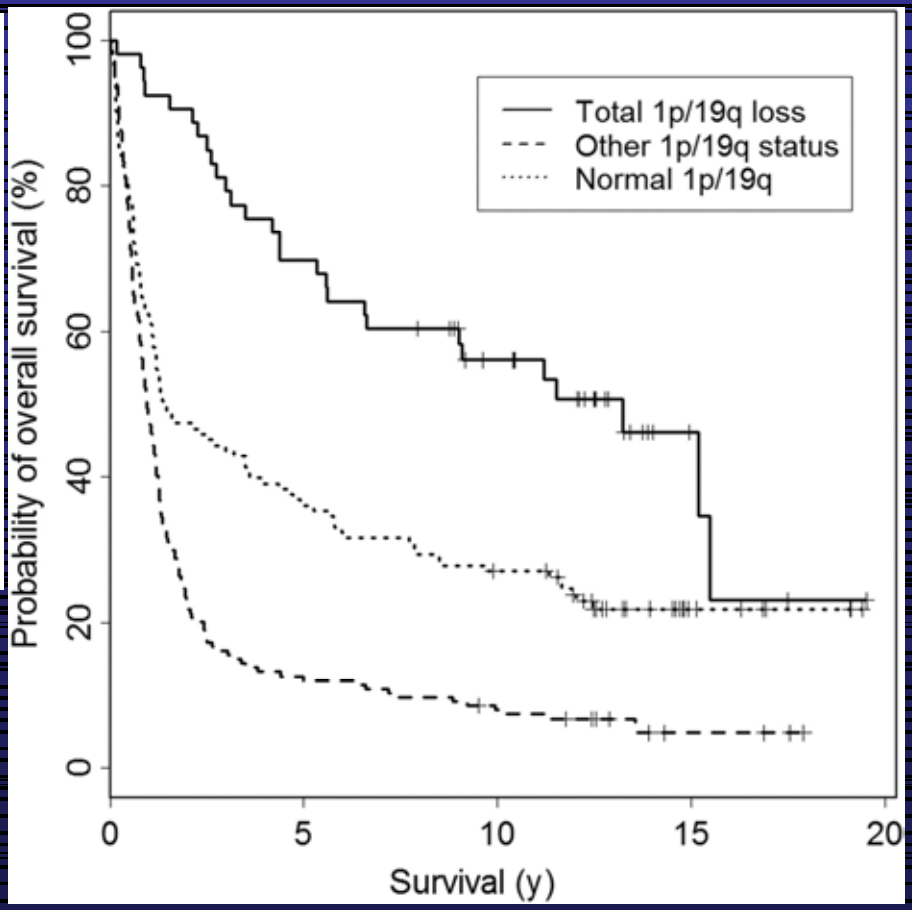


Vogazianou et al. Neuro Oncol 2010

Partial 1p/19q losses are associated with unfavorable prognosis



Felsberg et al. Brain Pathology 2004



Vogazianou et al. Neuro Oncol 2010

Clinical relevance of 1p/19q codeletion in patients with anaplastic gliomas

1p and 19q loss

→ 100% response to PCV chemotherapy

→ Median survival: 10 years

Homozygous *CDKN2A* deletion

→ No response to PCV chemotherapy

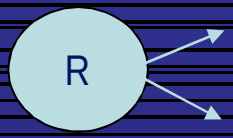
→ Median survival: 2-3 years

Cairncross *et al*., J Natl Cancer Inst 1998; 90: 1473-9

- RTOG 9402: Anapl. oligodendrogliomas and oligoastrocytomas
  RT vs. RT+PCV (289 patients) (Cairncross et al., JCO 24: 2707-14, 2006)
- EORTC 26951: Anapl. oligodendrogliomas and oligoastrocytomas
  RT vs RT+PCV (368 patients) (van den Bent et al., JCO 24:2715-22, 2006)
- NOA-04: Anapl. oligodendrogliomas, oligoastrocytomas and astrocytomas
  RT vs. PCV or TMZ (318 patients) (Wick et al., JCO 27:5874-80, 2009)

International trials for anaplastic oligodendroglial tumors

RTOG 94-02

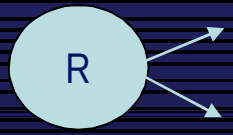


PCV* + RT — Prim. endpoint = PFS

RT — Sec. endpoint = OS

1994 - 2003: 289 pat. included
KPS ≥ 60
88% resections
70% grade III
Genetic of 206 tumors: 92 (46%) LOH 1p/19q

EORTC 26951

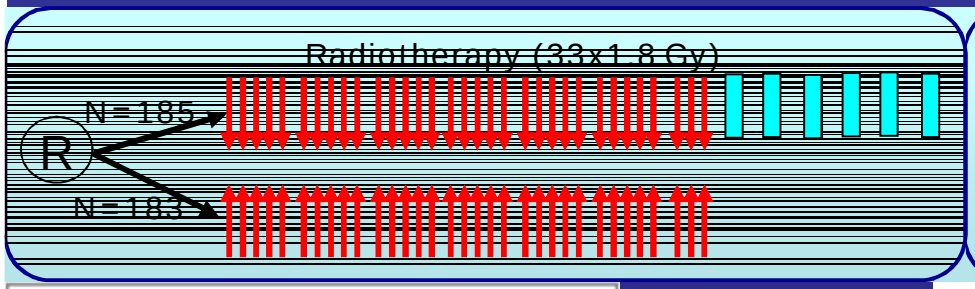


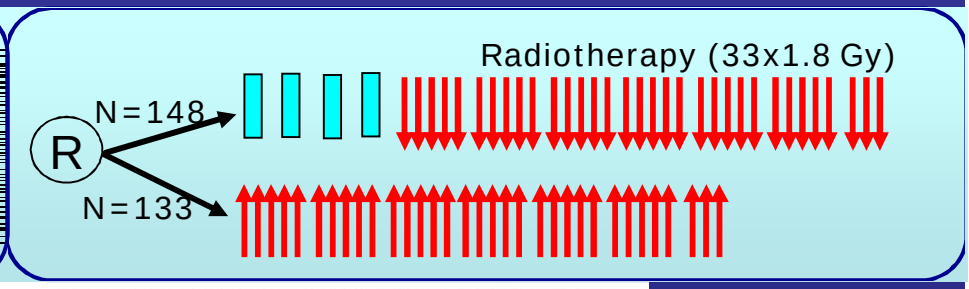
RT + PCV — Prim. Endpoint = OS

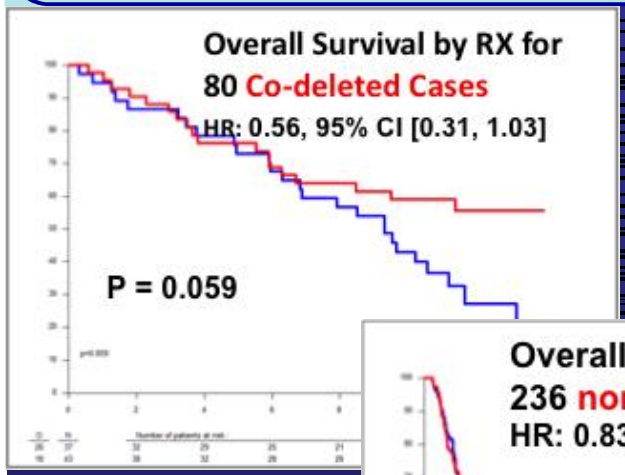
RT — Sec Endpoint = PFS

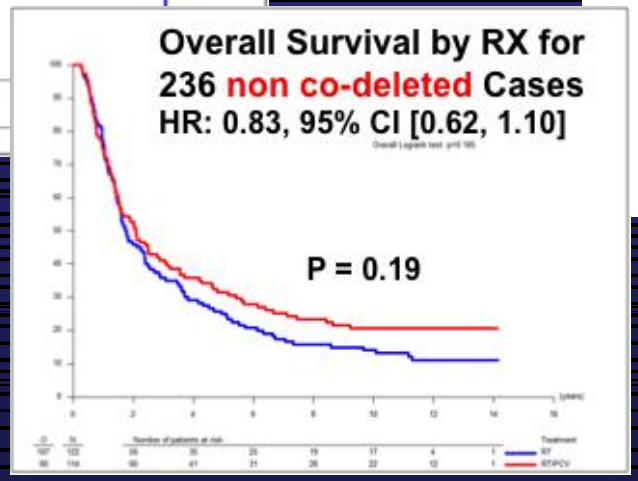
1995-2003: 368 pat. Included
KPS ≥ 60
64% resections
50-85% grade III

Prolonged survival with (neo-) adjuvant PCV chemotherapy

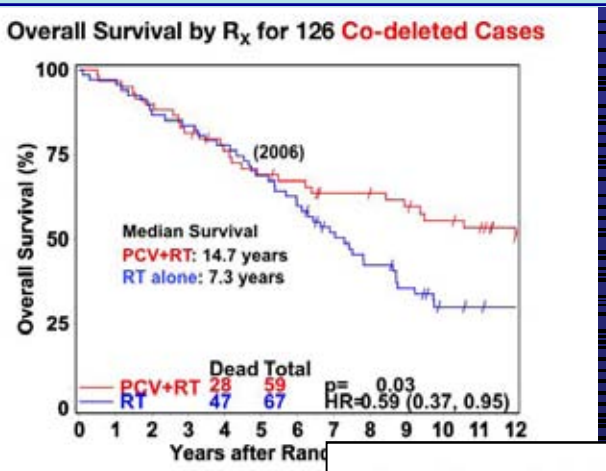


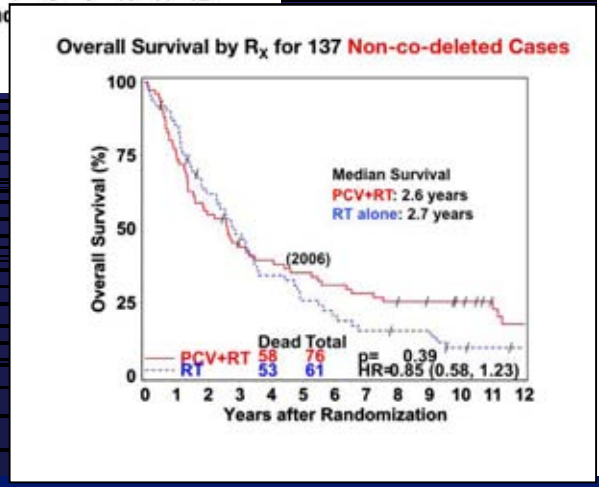






van den Bent et al. J Clin Oncol 2012 Oct 15





Cairncross et al. J Clin Oncol 2012 Oct 15

1p/19q codeletion is a predictive Biomarker in oligodendroglial tumors

	RTOG 9402		EORTC 26951	
	RT	PCV+RT	RT	RT+PCV
PFS, 1p/19q intact				
OS, 1p/19q intact	2.7	2.6	1.8	2.1
PFS, 1p/19q deleted				
OS, 1p/19q deleted	7.3	14.7	9.3	Not reached

van den Bent et al. J Clin Oncol 2012; Cairncross et al. J Clin Oncol 2012

Diagnostic value of the 1p/19q codeletion

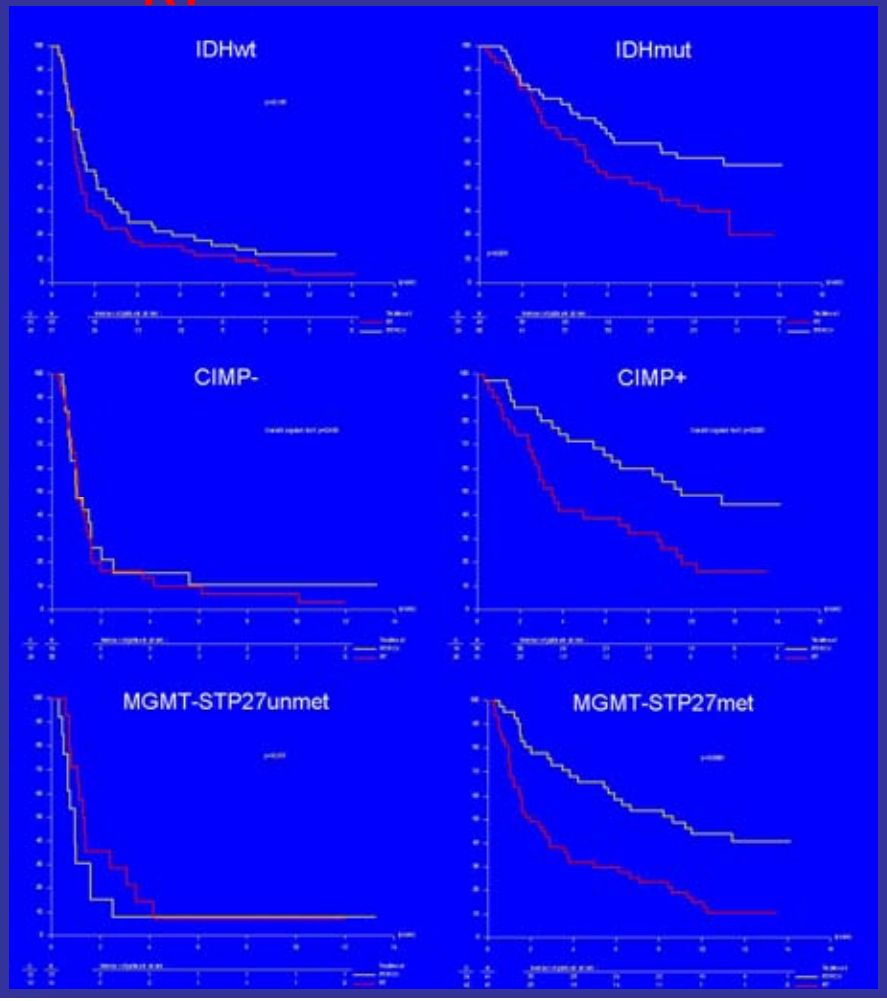
Can we use the 1p/19q status for diagnostic purposes?	Sometimes. The presence of the 1p/19q codeletion supports the diagnosis of an oligodendroglial tumor.
Can we use the 1p/19q status for prognostic purposes?	Yes. The 1p/19q codeletion is a strong prognosticator in anaplastic glioma patients treated with RT or alkylating agent chemotherapy or both. Its role in low-grade gliomas is less clear, but likely to be similar.
Can we use the 1p/19q status as a predictive marker for clinical decision making?	Yes. RTOG 9402 and EORTC 26951 suggest that the 1p/19q codeletion is a predictive marker for improved survival for patients treated with PCV in addition to RT versus RT alone. Whether this holds true for TMZ, too, is not known.

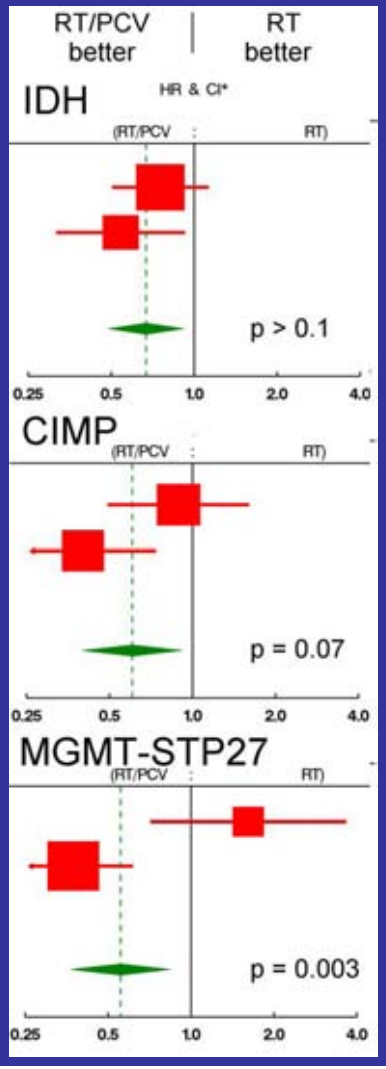
Weller et al., Neuro Oncology 2012

Overall survival: molecular parameters and treatment effects

RT/PCV

RT





Interaction tests:

- **No significance for IDH**

(n = 194)

- **Borderline statistical significance for CIMP**

(n = 115)

- **High statistical significance for MGMT-STP27**

(p = 0.003, n = 115)

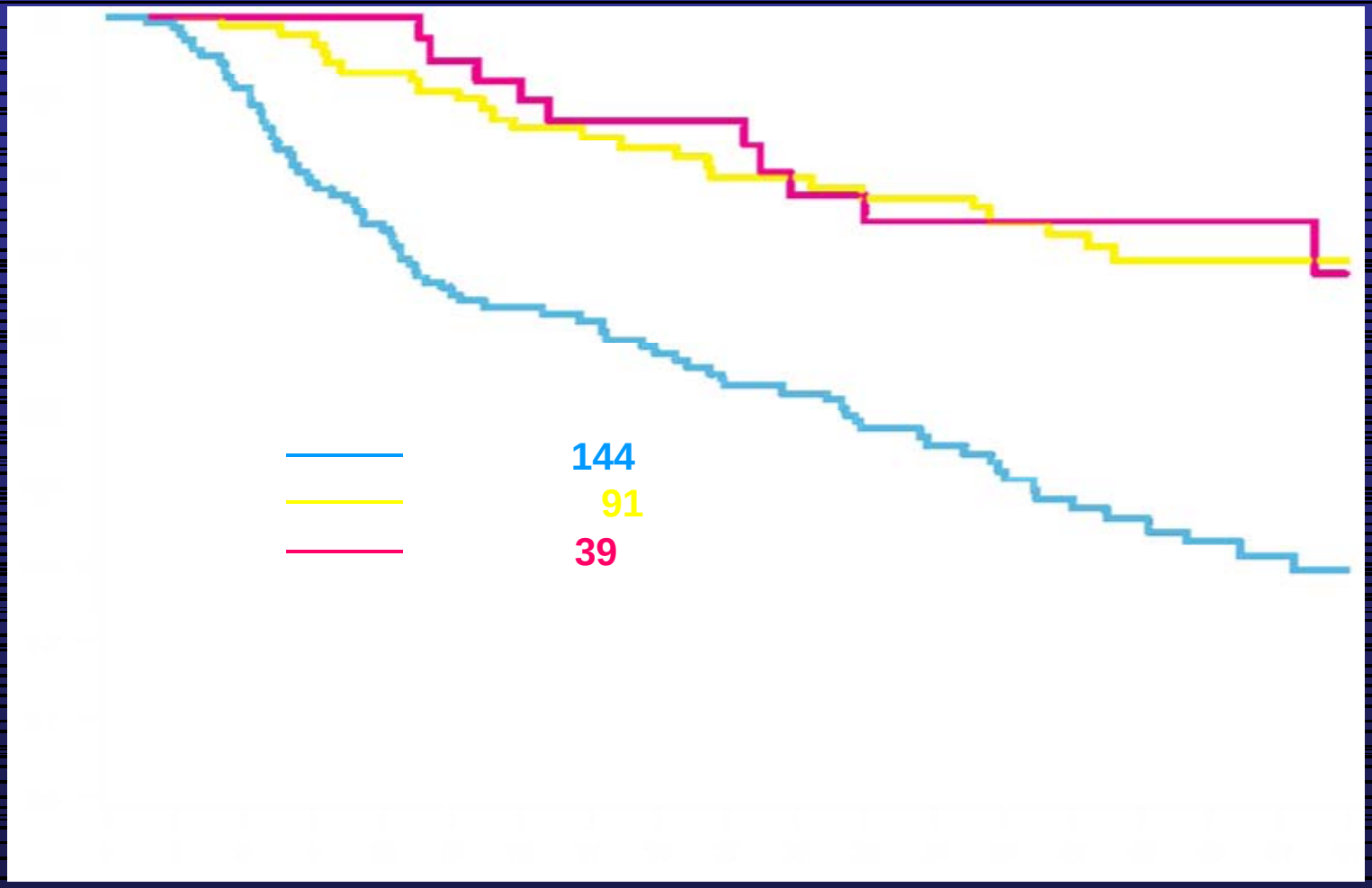
EORTC *The future of cancer therapy*

van den Bent Clin Canc Res 2013

Important questions: What else defines anaplastic astrocytoma?

- Why do anaplastic oligodendrogliomas and oligoastrocytomas have a similar course in the NOA-04 trial?
- What are the factors involved in radio-/chemoresistance in addition to MGMT?
- What are the next clinical steps?
- How to make practical use of the new data?

NOA-04: Primary endpoint according to histologies



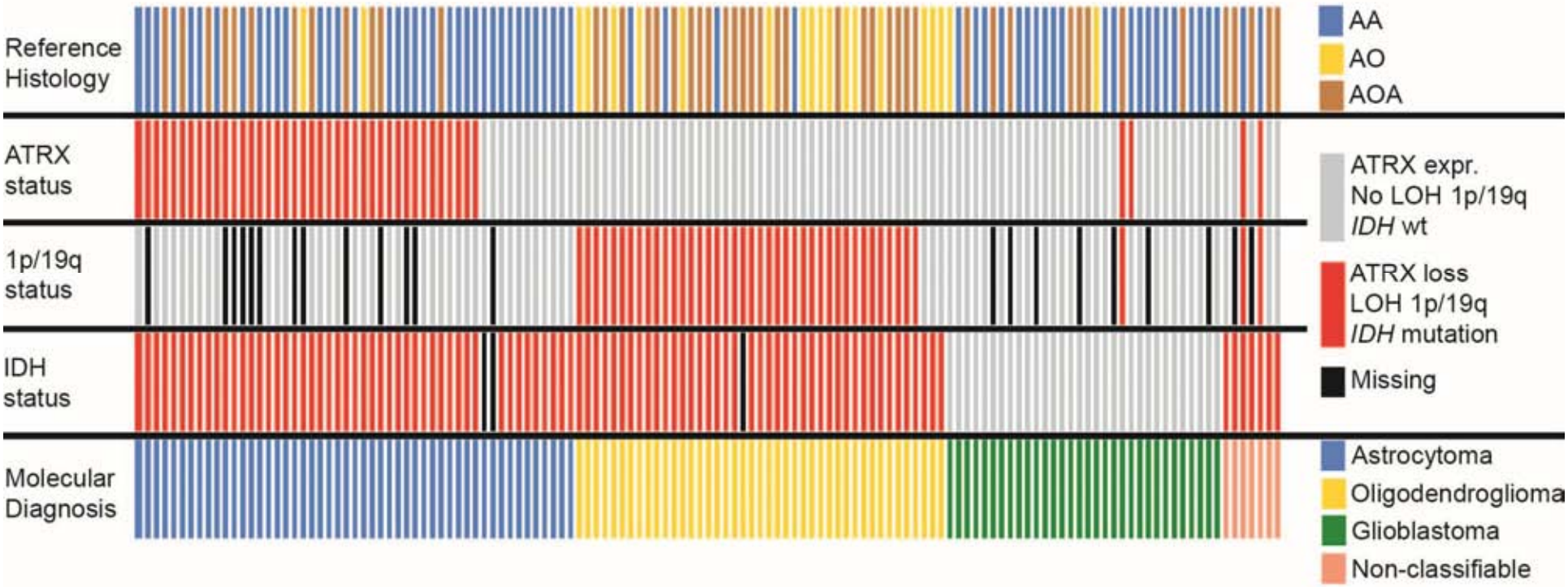
a-thalassemia/mental-retardation-syndrome-X-linked (ATRX)

- **Mutations and loss of expression of ATRX in 30% of pediatric glioblastomas and 7% of adult glioblastomas[1]**
- **Mutations are inactivating and lead to a loss of protein expression[1,2]**
- **ATRX loss assessed by immunohistochemistry 27% in grade II and 41% in grade III gliomas in adults[2]**
- **Association with astrocytic >> oligodendroglial tumors**
- **Association with *IDH* mutation and inverse correlation with 1p/19q co-deletion[3]**

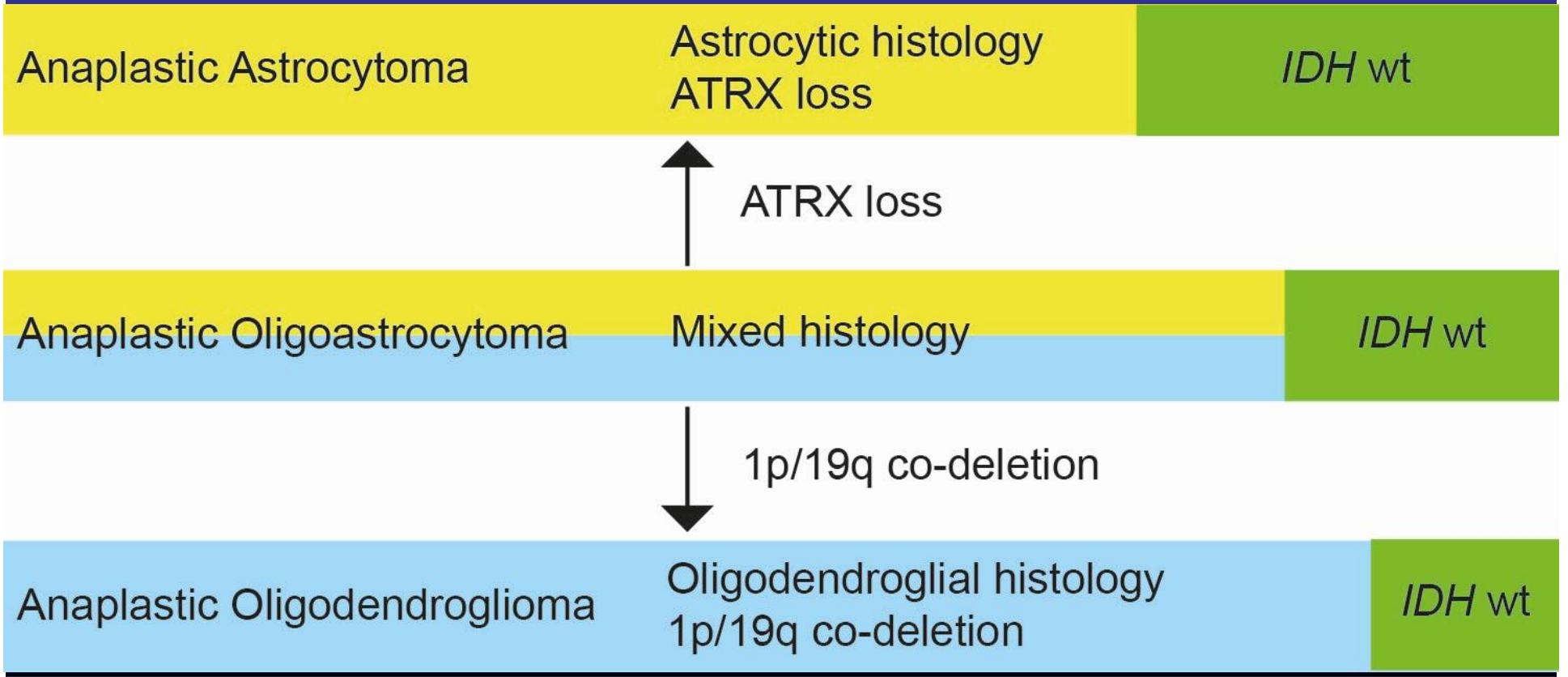
[1]Heaphy et al. Science 2011; [2]Liu et al. et al. Acta Neuropathol 2012

[3]Jiao et al. Oncotarget 2012

Classification of anaplastic glioma: molecular markers aid to understand the subgroups

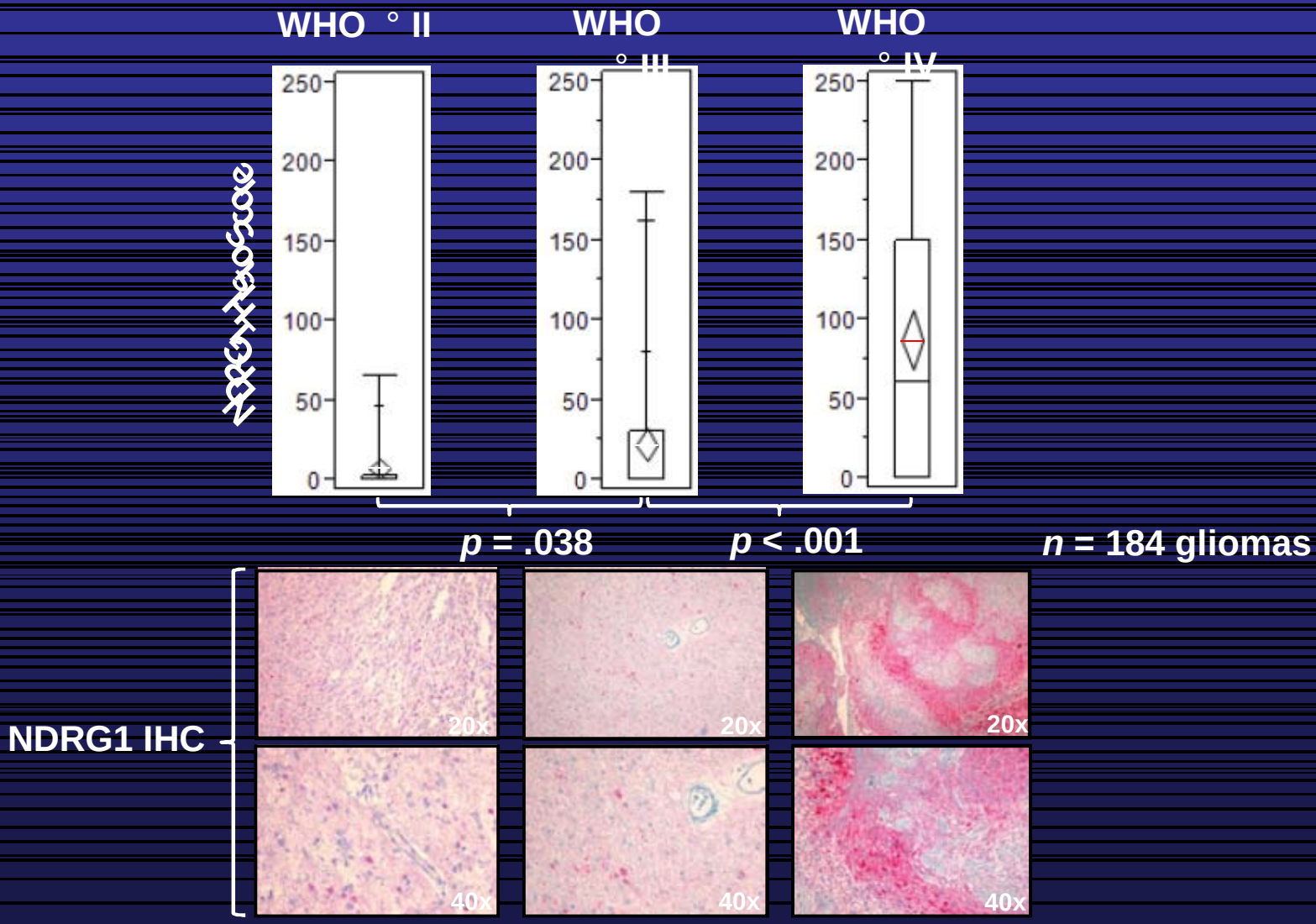


ATRX loss refines the classification of anaplastic glioma and is a favorable prognostic marker



Wiestler B et al. Acta Neuropathol 2013

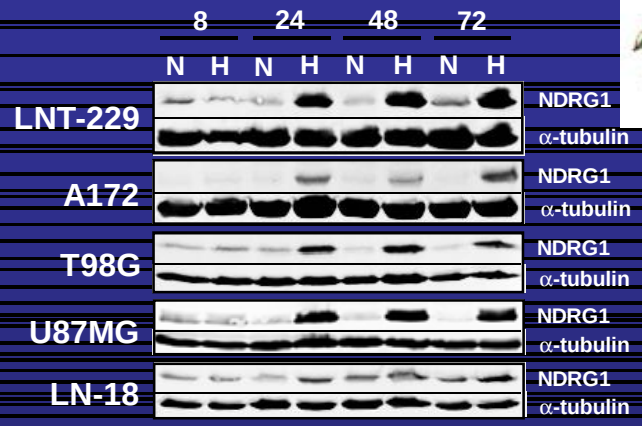
N-myc downstream regulated gene is induced in glioma with increased malignancy

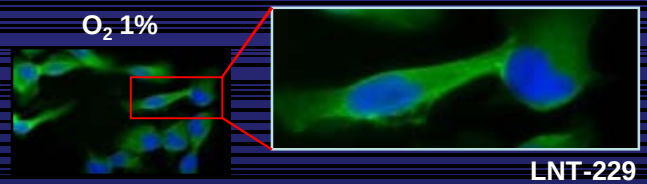


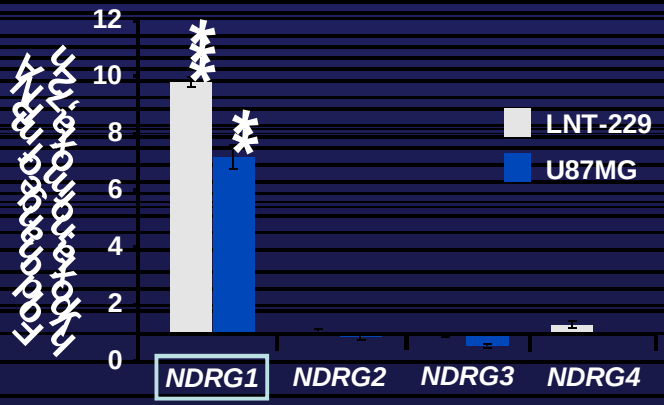
NDRG1 – basic background

NDRG1…

- § is a member of the N-myc downstream regulated gene family that belongs to the α/ -hydrolase superfamily;
- § has a genomic location on 8q24;
- § encodes a 43-kDa protein with intracellular localization;
- § is involved in stress and hormone responses, cell growth, and differentiation;
- § deficiency leads to Schwann cell dysfunction, and the *NDRG1* knock-out mouse develops demyelinating polyneuropathy;
- § is causative for hereditary motor and sensory neuropathy-Lom (CMT4D);



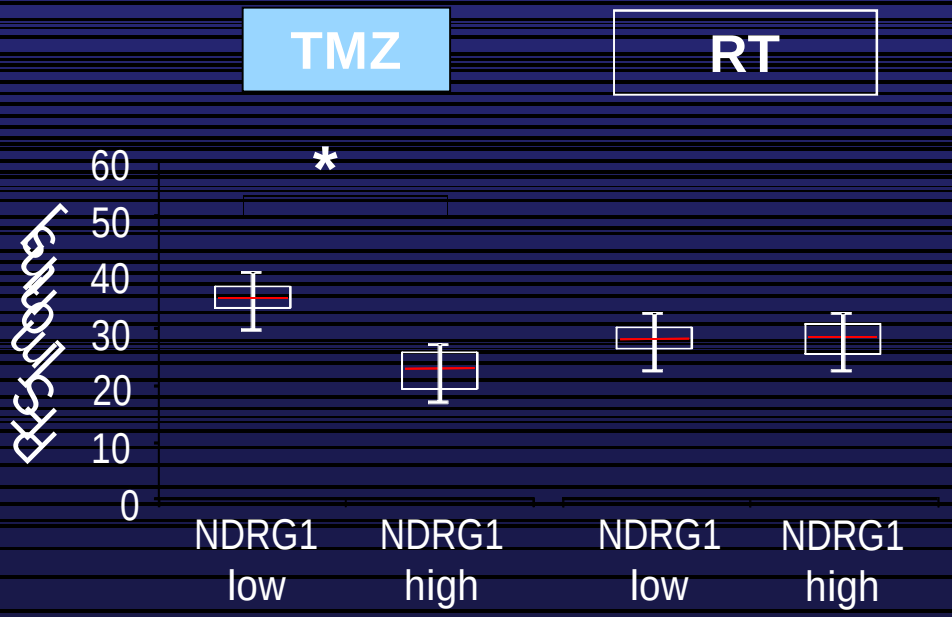




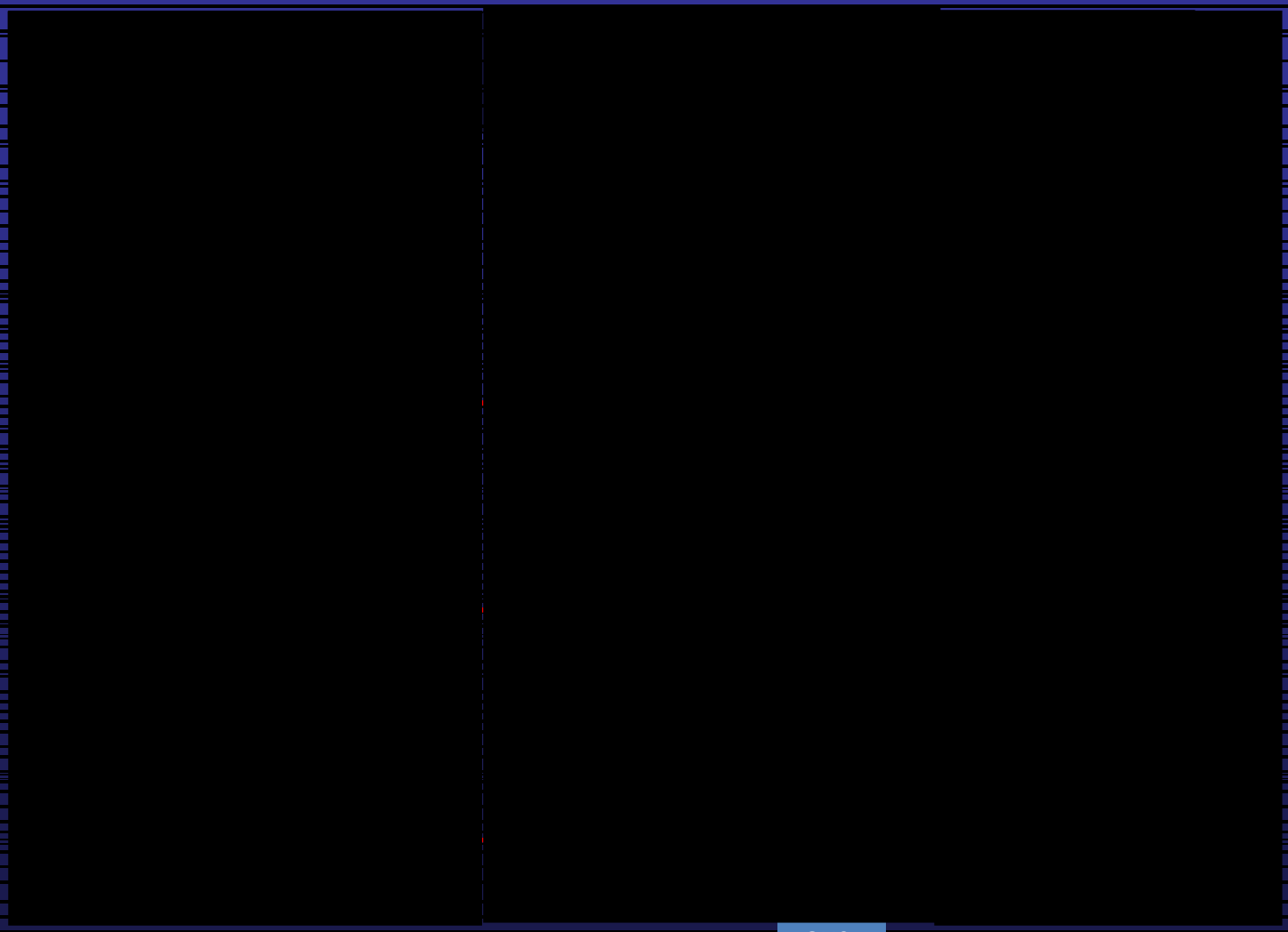
NDRG1 mediates resistance to chemotherapy in anaplastic gliomas

NOA-04 data set

	TMZ (n=31)	Radiotherapy (n=23)
NDRG1 negative/weak (PFS, months, n=31)	35.9 (32.1 – 39.8)	29.5 (27.0 – 32.1)
NDRG1 intermediate/strong (PFS, months, n=23)	24.3 (21.1 – 27.5) $p < .05$	30.9 (28.7 – 33.0) $p = .87$



mTOR/ NDRG1 processes are influenced at multiple levels



Weiler, Blaes et al.

NOA-04: Potential additional (hypothesis-generating) lessons from the trial?

- Anaplastic astrocytoma
  - lower frequency but same prognostic impact of 1p/19q?
- „Similar“ monotherapy efficacy of PCV and TMZ (to be demonstrated also for the long-term F/U)?
- Efficacy of chemotherapy alone for the long-term benefits might be as good as RT/PCV
  - seperation of the RT/chemotherapy curves in the codeleted patients?

Update anaplastic gliomas!

1p/19q codeletion and IDH1 (+ MGMT) are molecular markers that trigger treatment decisions

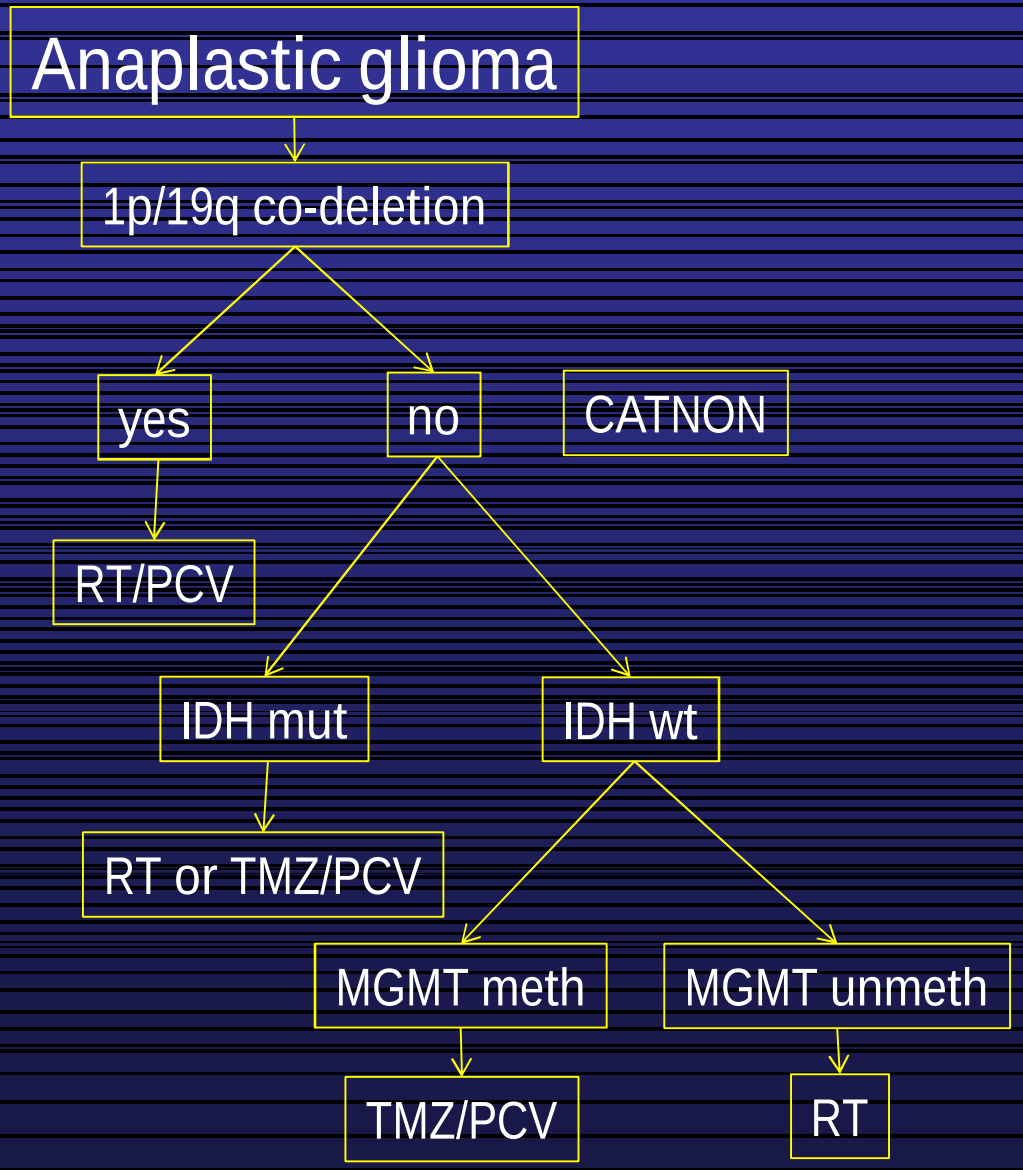
Sole radiotherapy for 1p/19q codeleted tumours no longer warranted

Interaction of IDH1 and MGMT

ATRX as a marker for astrocytoma

NDRG1 as a potential marker for intervention

A practical approach to biomarker testing in anaplastic gliomas



Histology

1p/19q analysis (FISH)

IDH mutation analysis (ICH, sequencing)

MGMT analysis (MSP, pyrosequecing)

Thank you!

Deutsche Krebshilfe
HELFEN. FORSCHEN. INFORMIEREN.

NCT

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