WCN Teaching Course

Glioblastomas :

from molecular markers to treatment decisions

Vienna, 24 September 2013

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Standards of care in newly diagnosed glioblastoma

- **Surgery**
  Histology
  Impact on survival in elderly patients
  Vuorinen et al. Acta Neurochir 2003;145:5-10
  Higher rate of complete resections improves progression-free survival rate at 6 months
  Stummer et al. Lancet Oncol 2006;7:392-401

- **Radiotherapy**
  Doubling of median survival
  Review, Laperriere et al. Radiother Oncol 2002;64:259-273

- **Pharmacotherapy**
  Temozolomide **Stupp et al. NEJM 2005;352:987-996**
  Nitrosoureas (Gliadel, Westphal et al. JNO 2003;5:79-88, Metaanalysis, systemic therapy, Lancet 2002;359:1011-1018)

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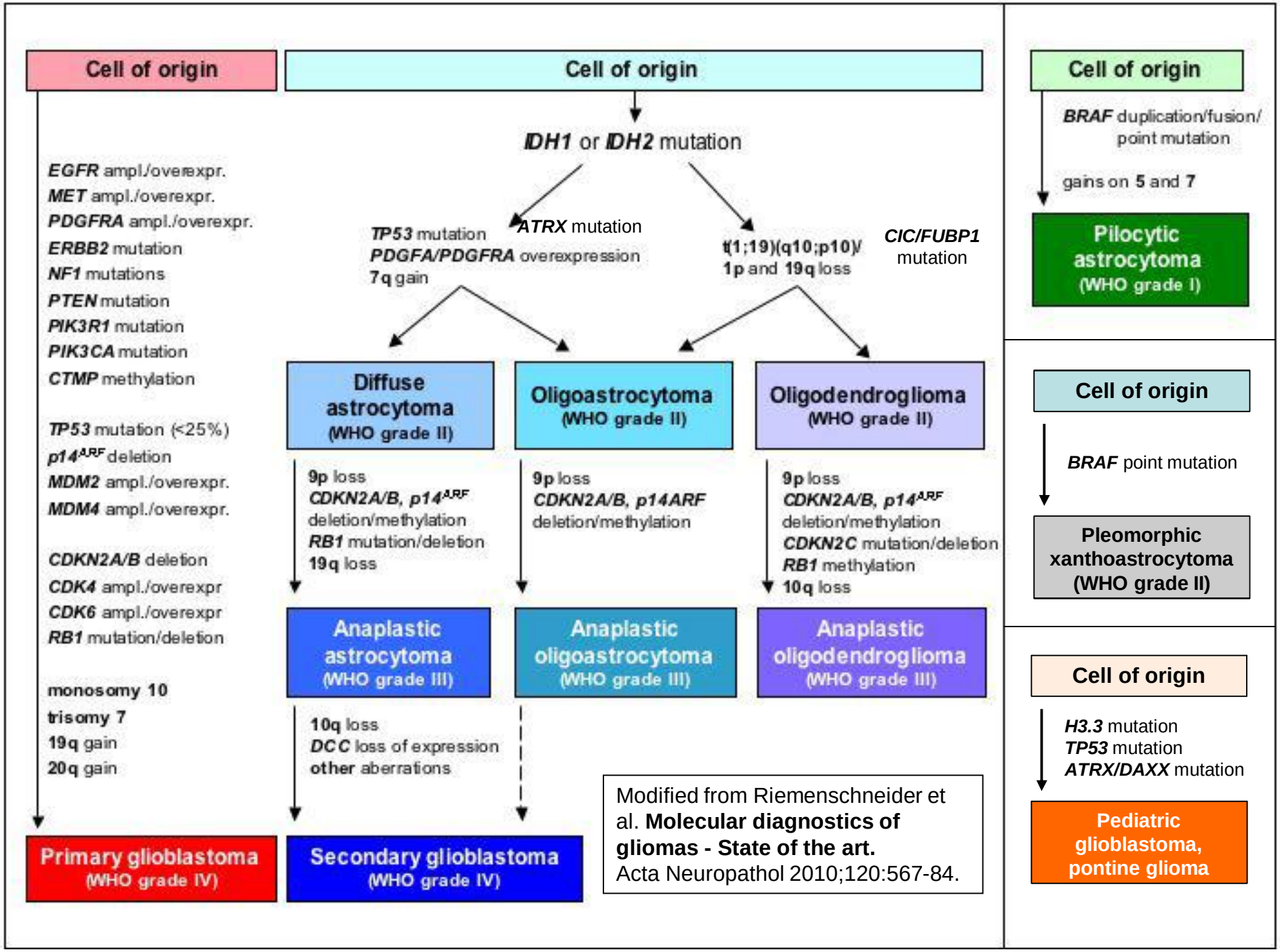
(No) standards of care in recurrent glioblastoma

- **Surgery**
  positive effect on survival in retrospective series
- **Radiotherapy**
  positive effect on survival in highly selected patient populations
- **Pharmacotherapy**
  Temozolomide superior to procarbazine
  Yung et al. Br J Cancer 2000;83:588–593
  Gliadel superior to placebo (?)
  Brem et al. Lancet 1995;345:1008-1012
  Bevacizumab (?)
  Friedman et al. J Clin Oncol 2009;27:4733-4740; Kreisl et al. J Clin Oncol 2009;27:740-745


Cell of origin
Cell of origin
IDH1 or IDH2 mutation
EGFR ampl./overexpr.
MET ampl./overexpr.
PDGFRA ampl./overexpr.
ERBB2 mutation
NF1 mutations
PTEN mutation
PIK3R1 mutation
PIK3CA mutation
CTMP methylation
TP53 mutation (<25%)
p14ARF deletion
MDM2 ampl./overexpr.
MDM4 ampl./overexpr.
CDKN2A/B deletion
CDK4 ampl./overexpr
CDK6 ampl./overexpr
RB1 mutation/deletion
monosomy 10
trisomy 7
19q gain
20q gain
TP53 mutation
PDGFA/PDGFRA overexpression
7q gain
ATRX mutation
t(1;19)(q10;p10)/
1p and 19q loss
CIC/FUBP1
mutation
Diffuse astrocytoma (WHO grade II)
Oligoastrocytoma (WHO grade II)
Oligodendroglioma (WHO grade II)
9p loss
CDKN2A/B, p14ARF
deletion/methylation
RB1 mutation/deletion
19q loss
9p loss
CDKN2A/B, p14ARF
deletion/methylation
9p loss
CDKN2A/B, p14ARF
deletion/methylation
CDKN2C mutation/deletion
RB1 methylation
10q loss
Anaplastic astrocytoma (WHO grade III)
Anaplastic oligoastrocytoma (WHO grade III)
Anaplastic oligodendroglioma (WHO grade III)
10q loss
DCC loss of expression
other aberrations
Primary glioblastoma (WHO grade IV)
Secondary glioblastoma (WHO grade IV)
Modified from Riemenschneider et al. Molecular diagnostics of gliomas - State of the art. Acta Neuropathol 2010;120:567-84.
Cell of origin
BRAF duplication/fusion/ point mutation
gains on 5 and 7
Pilocytic astrocytoma (WHO grade I)
Cell of origin
BRAF point mutation
Pleomorphic xanthoastrocytoma (WHO grade II)
Cell of origin
H3.3 mutation
TP53 mutation
ATRX/DAXX mutation
Pediatric glioblastoma, pontine glioma

How to assess the top biomarkers?

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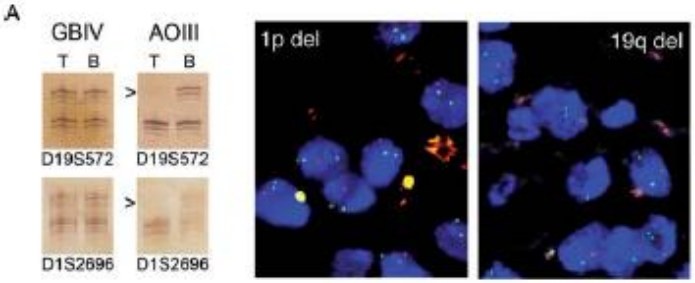
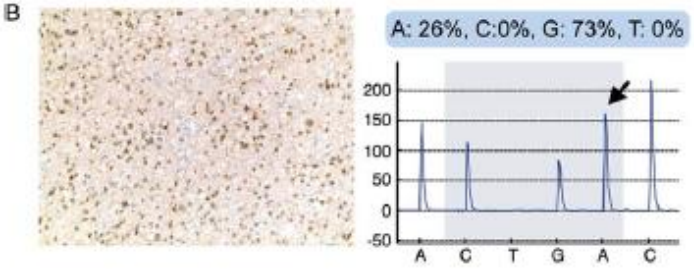
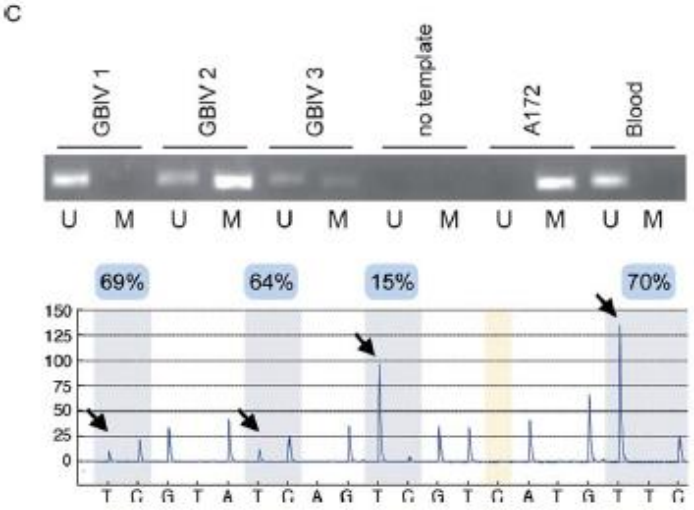
1p/19q



IDH



MGMT



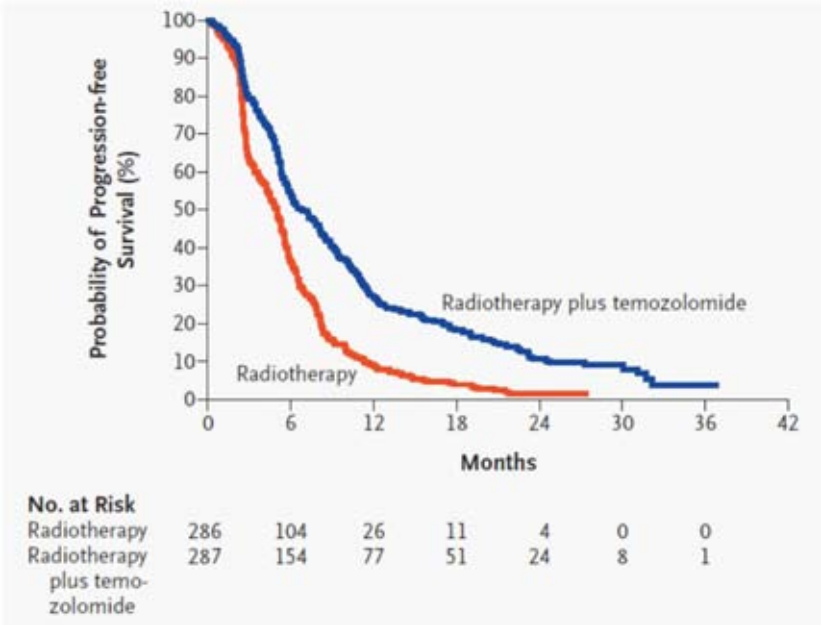
The NEW ENGLAND JOURNAL of MEDICINE

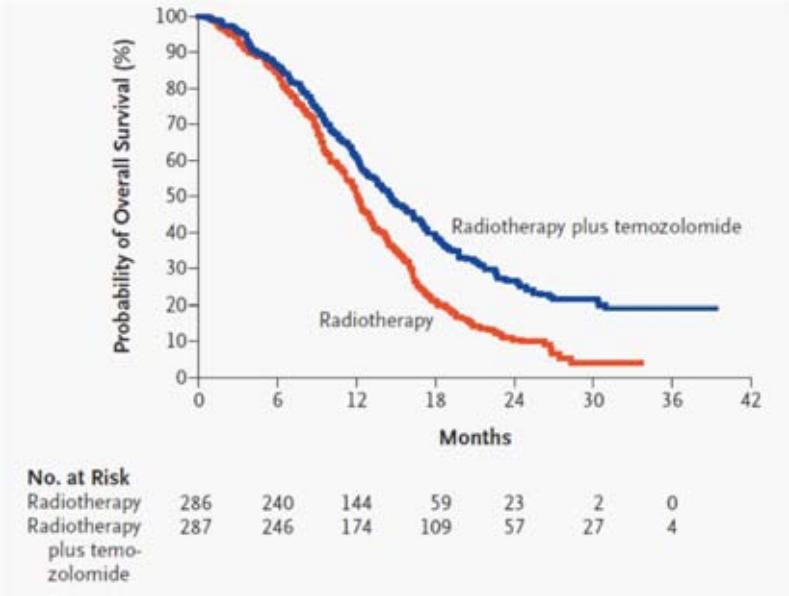
ORIGINAL ARTICLE

Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma

Roger Stupp, M.D., Warren P. Mason, M.D., Martin J. van den Bent, M.D., Michael Weller, M.D., Barbara Fisher, M.D., Martin J.B. Taphoorn, M.D., Karl Belanger, M.D., Alba A. Brandes, M.D., Christine Marosi, M.D., Ulrich Bogdahn, M.D., Jürgen Curschmann, M.D., Robert C. Janzer, M.D., Samuel K. Ludwin, M.D., Thierry Gorlia, M.Sc., Anouk Allgeier, Ph.D., Denis Lacombe, M.D., J. Gregory Cairncross, M.D., Elizabeth Eisenhauer, M.D., and René O. Mirimanoff, M.D., for the European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada Clinical Trials Group*

N Engl J Med 2005;352:987-96.





MGMT promoter methylation in malignant gliomas: ready for personalized medicine?

Michael Weller, Roger Stupp, Guido Reifenberger, Alba A. Brandes, Martin J. van den Bent, Wolfgang Wick and Monika E. Hegi

Nat. Rev. Neurol. **6**, 39–51 (2010)

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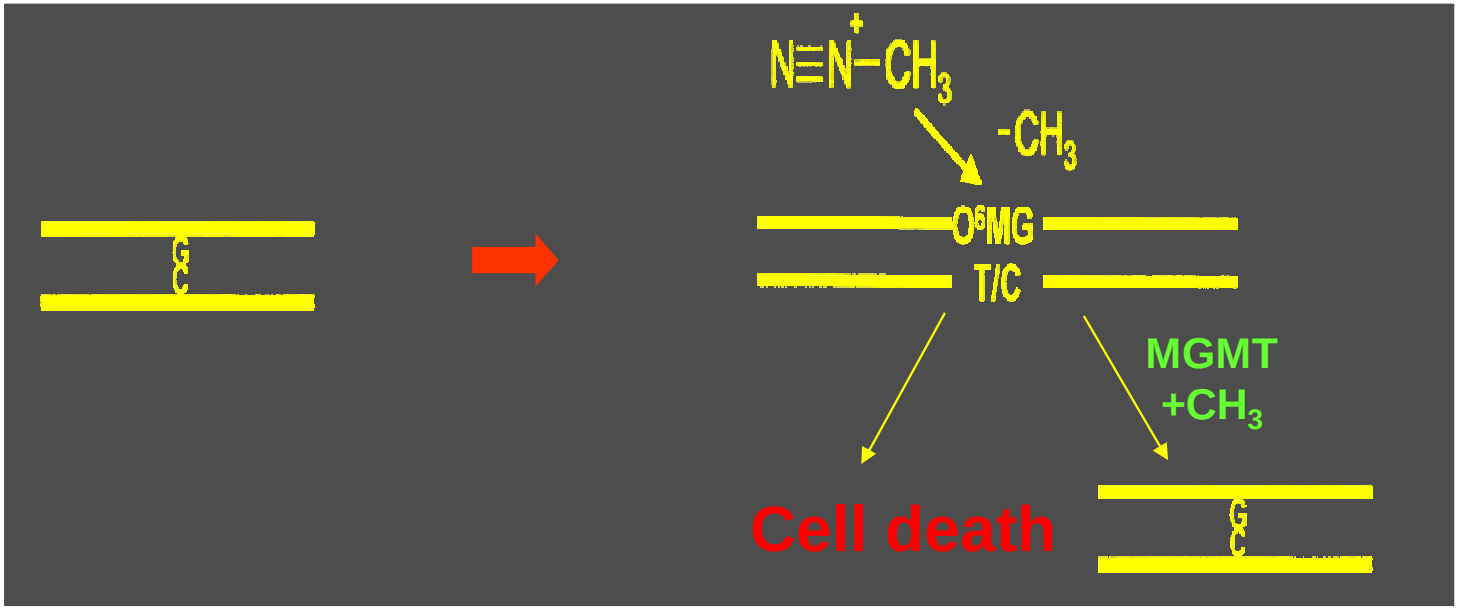
O^6-Methylguanin-methyltransferase (MGMT, AGAT), a DNA repair protein, counteracts the effect of alkylating agents:



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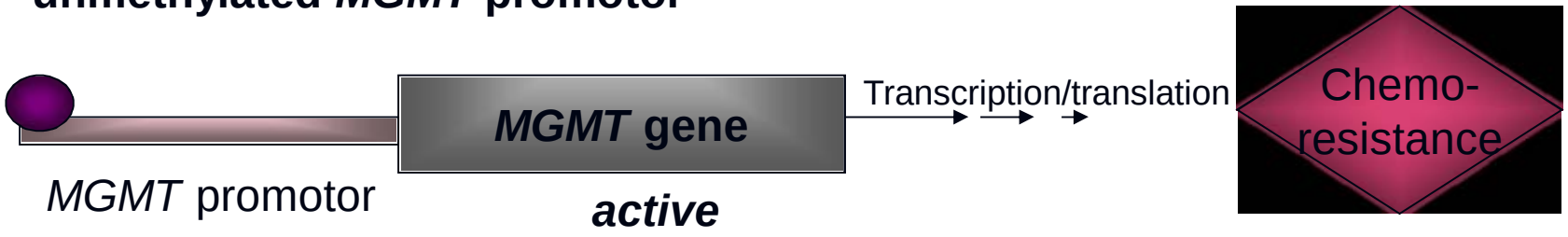
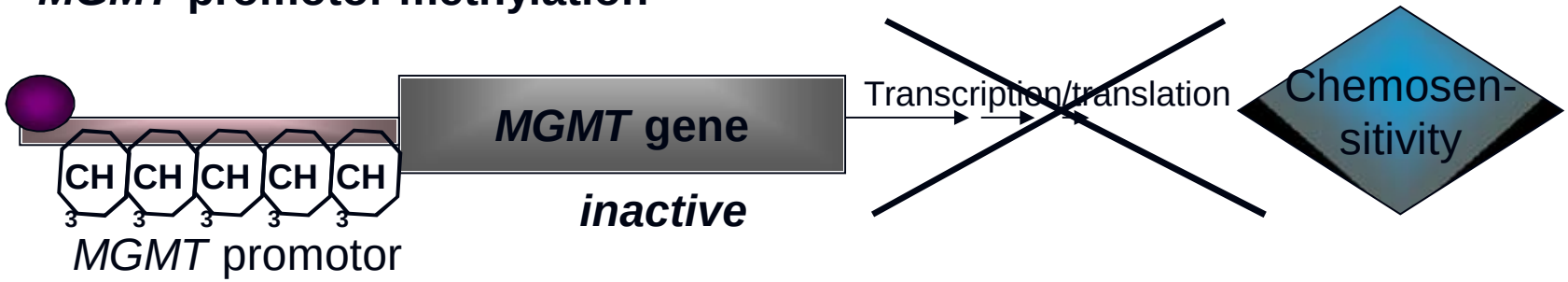
unmethylated *MGMT* promotor



***MGMT* promotor methylation**



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.

N Engl J Med 2005;352:997-1003.

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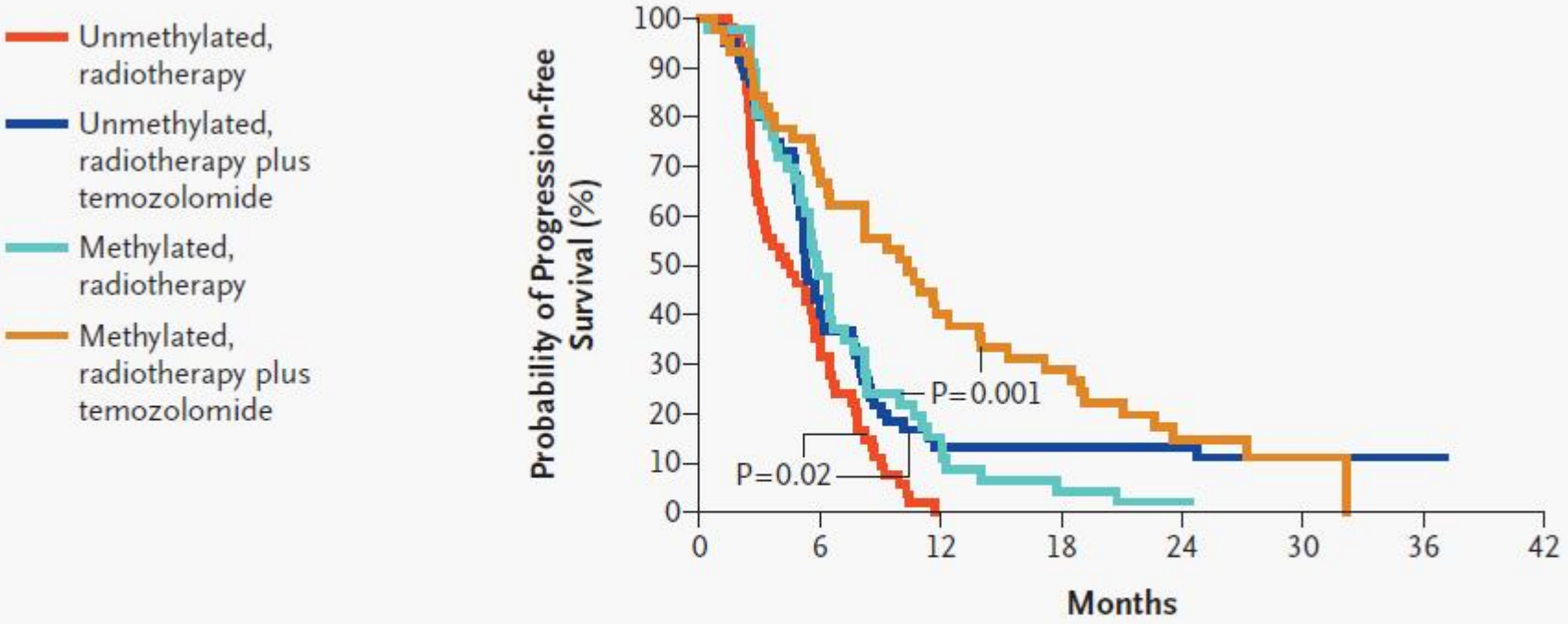
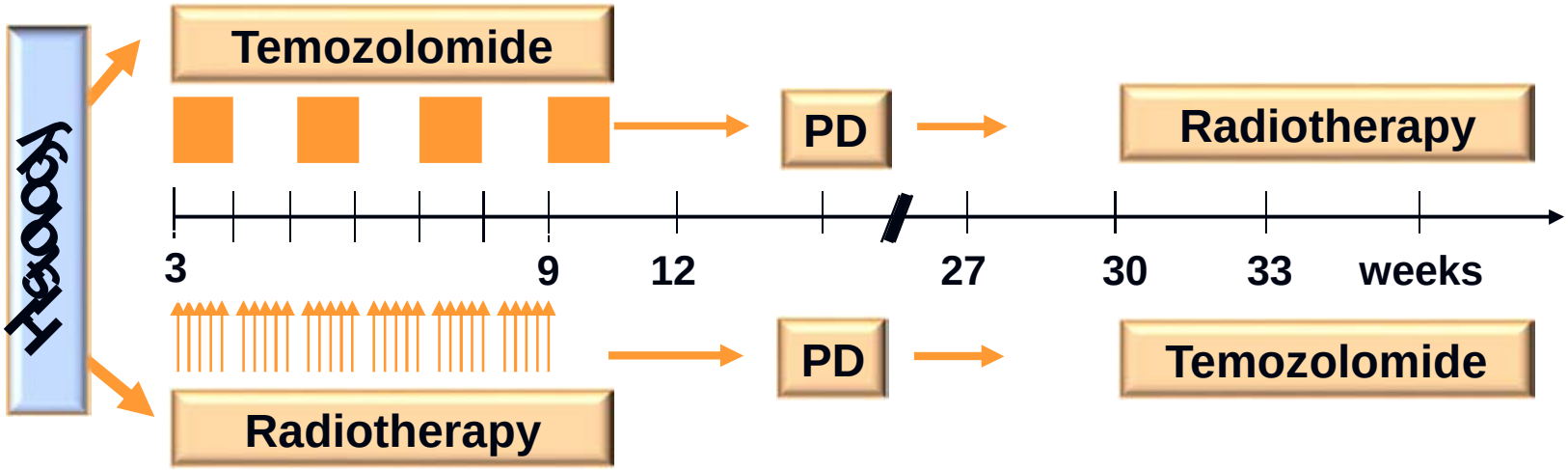


No. at Risk

Unmethylated, radiotherapy	54	19	0	0	0	0	0
Unmethylated, radiotherapy plus temozolomide	60	24	8	8	7	4	1
Methylated, radiotherapy	46	22	7	2	1	0	0
Methylated, radiotherapy plus temozolomide	46	31	18	13	6	2	0

NOA-08/Methvsalem: Design

Temozolomide *(one week on/one week off)* vs radiotherapy in the primary treatment of anaplastic astrocytoma and glioblastoma in older patients: a randomized phase III study



TMZ 100 mg/m^2 po/day for 7 days every 14 days until failure of therapy, to be adjusted in 25-mg steps

Focal radiotherapy daily — 30 x 1.8-2 Gy to a total 54–60 Gy

Deutsche Krebsgesellschaft e.V.
Gütesiegel A

TMZ is not inferior to RT

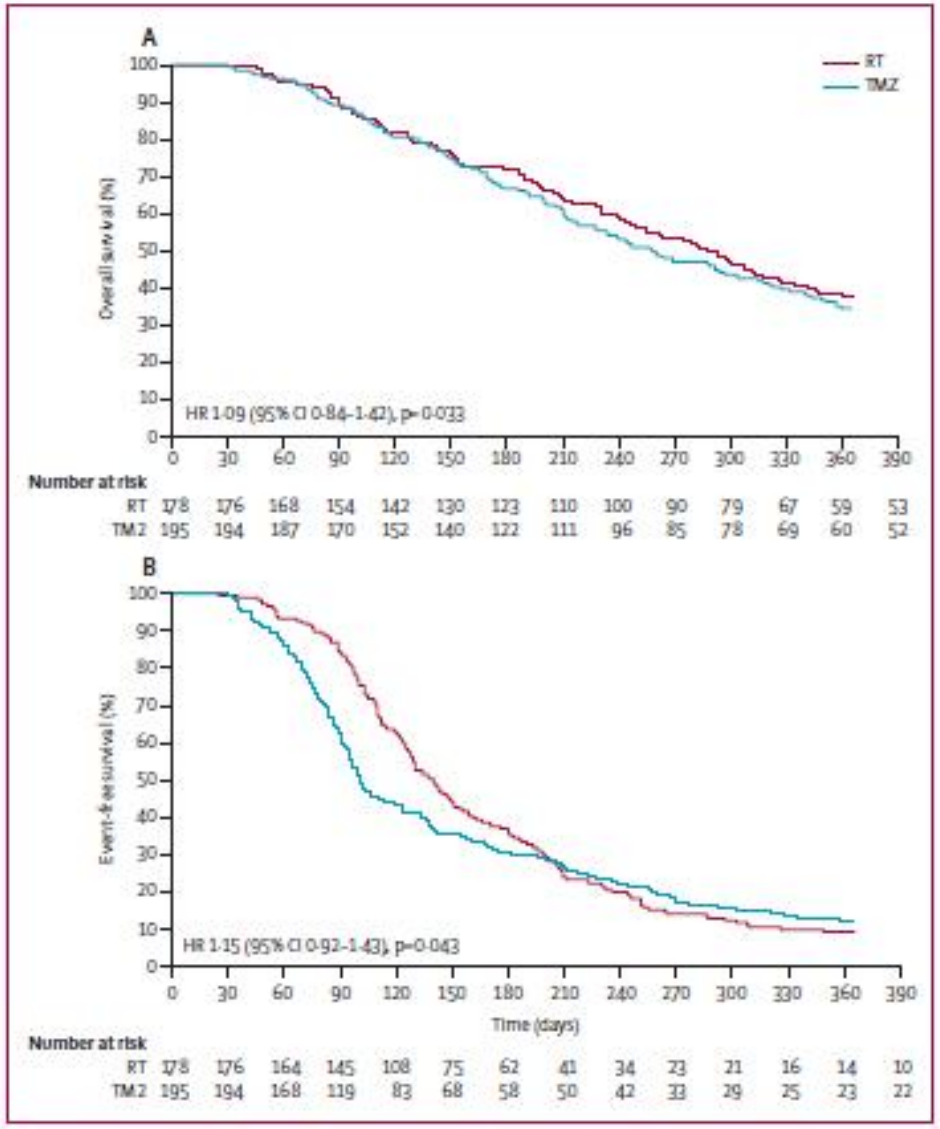


Figure 2: **Kaplan-Meier analysis of overall and event-free survival**
(A) Overall survival. (B) Event-free survival presented as non-proportional curves, which are deemed non-problematic in the context of non-inferiority. RT=radiotherapy. TMZ=temozolomide. HR=hazard ratio.

MGMT is a prognostic biomarker

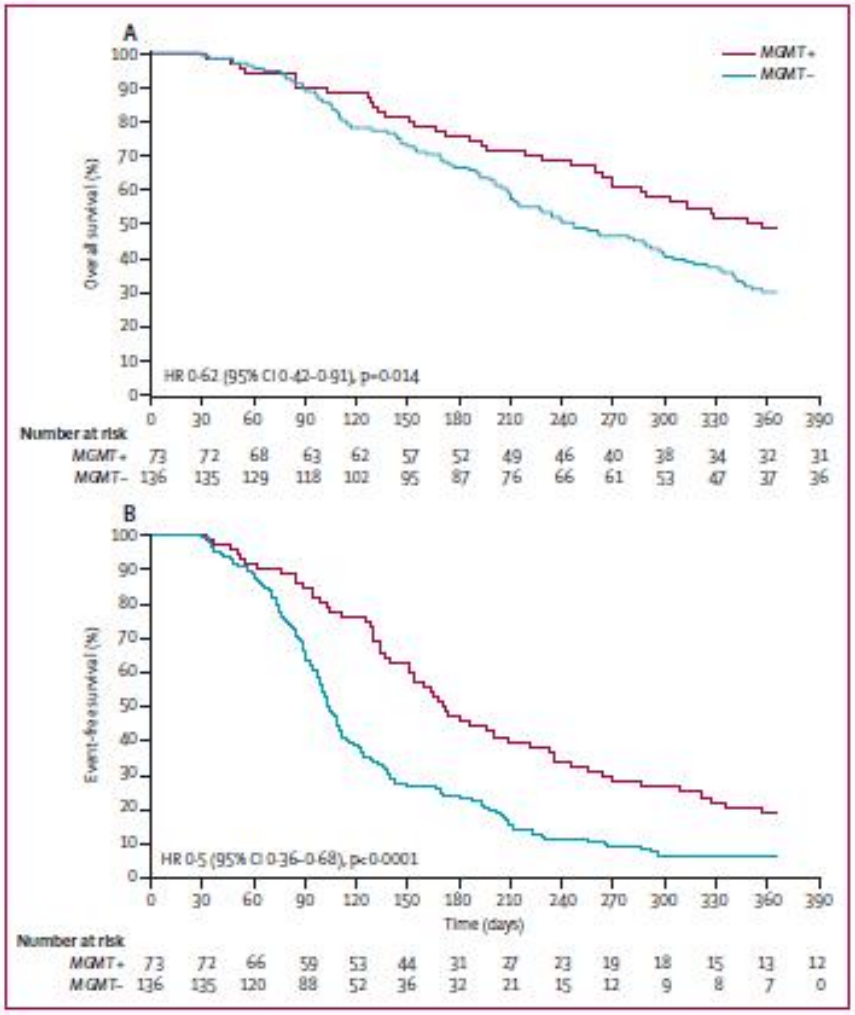
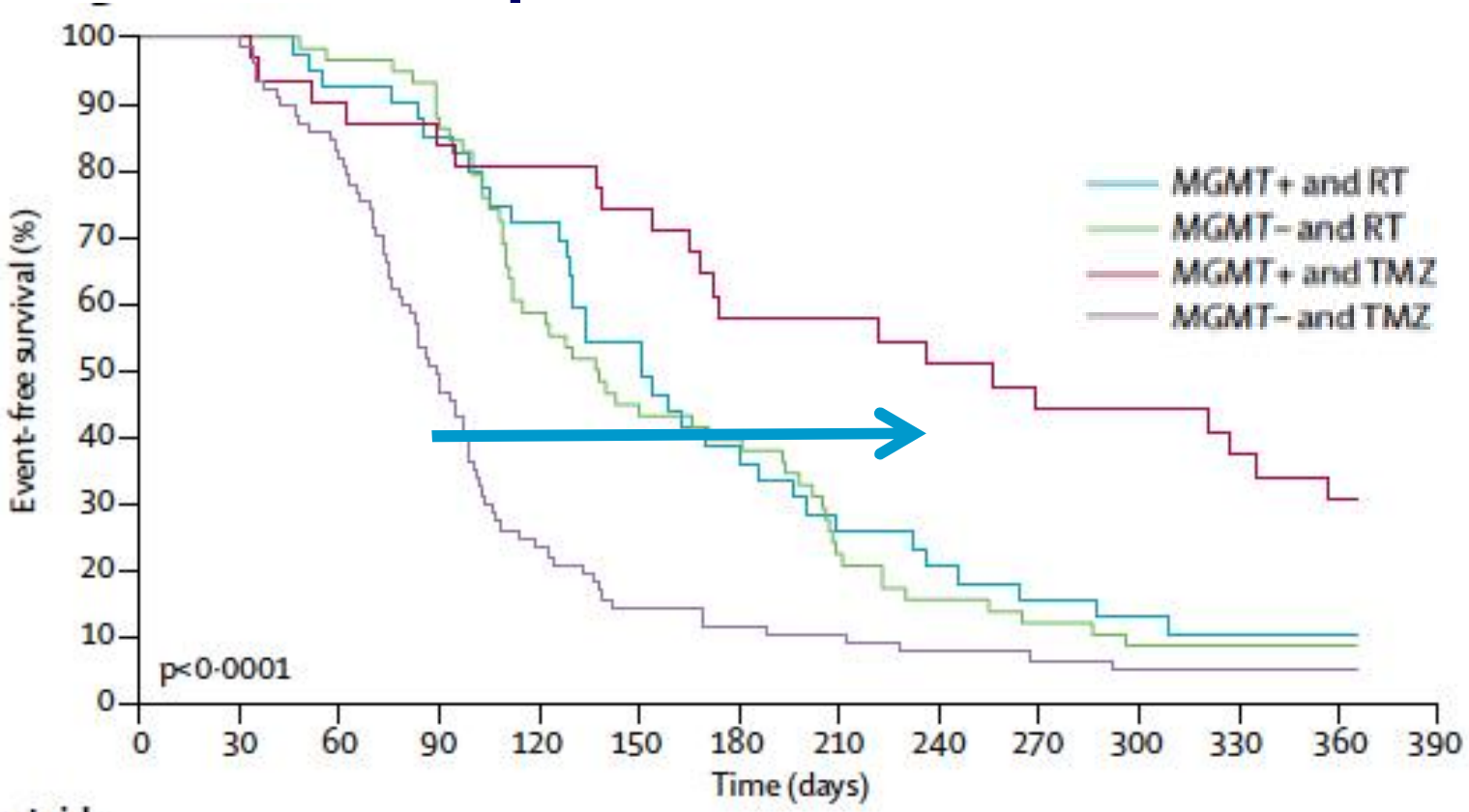


Figure 3: **Kaplan-Meier analysis of overall and event-free survival in relation to MGMT promoter methylation status**
(A) Overall survival. (B) Event-free survival. HR=hazard ratio.

MGMT is a predictive biomarker



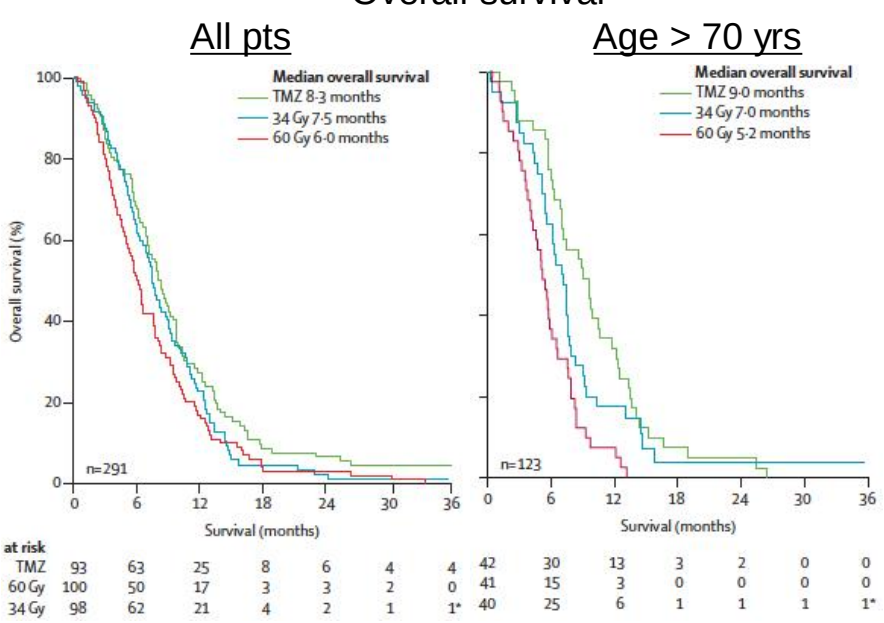
Number at risk

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

Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial

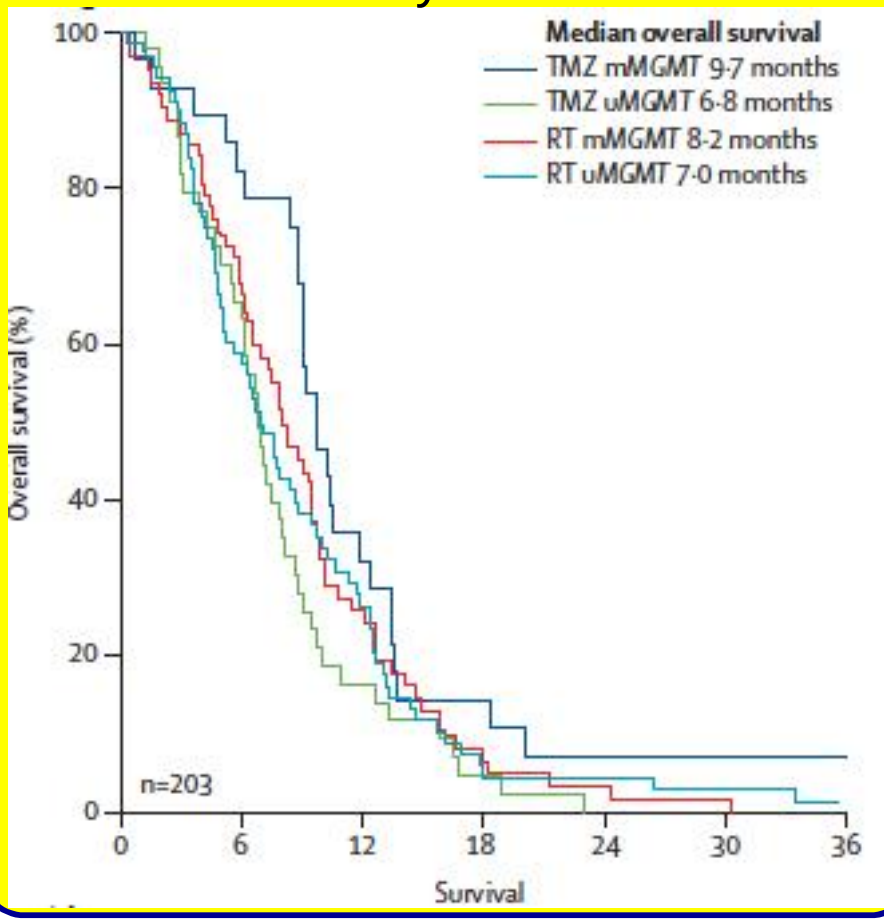
Annika Malmström, Bjørn Henning Grønberg, Christine Marosi, Roger Stupp, Didier Frappaz, Henrik Schultz, Ufuk Abacioglu, Björn Tavelin, Benoit Lhermitte, Monika E Hegi, Johan Rosell, Roger Henriksson, for the Nordic Clinical Brain Tumour Study Group (NCBTSG)

Overall survival



MGMT methylated: TMZ > RT

unmethylated: RT > TMZ



Lancet Oncol 2012

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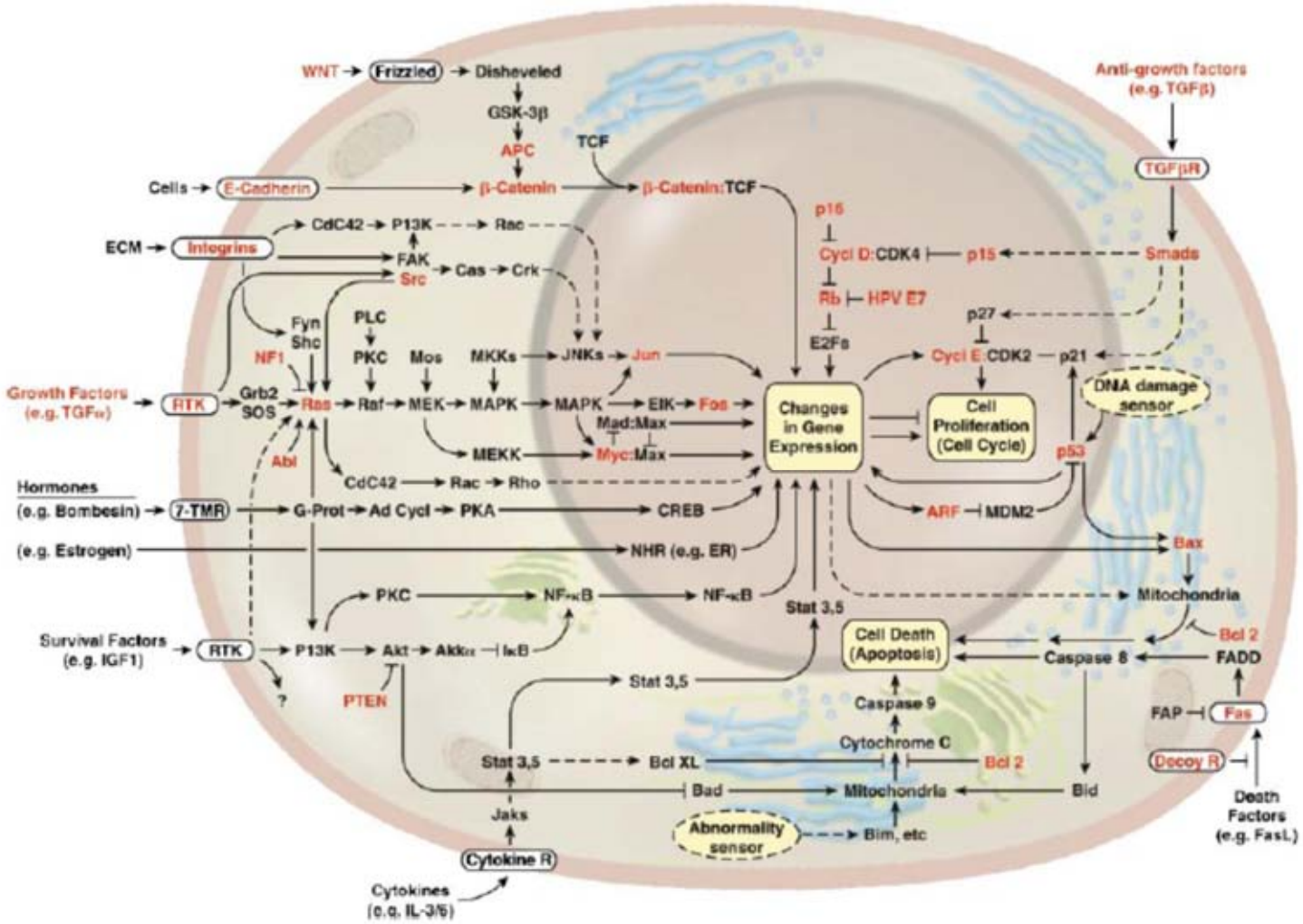
How do we select targets for intervention?

- Proliferation? Migration? Invasion?
- Understand tumor biology and interfere with tumor-related processes
- Understand glioma biology and interfere with glioma-related processes
- Glioma targets or host targets?

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Douglas Hanahan* and Robert A. Weinberg†



Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

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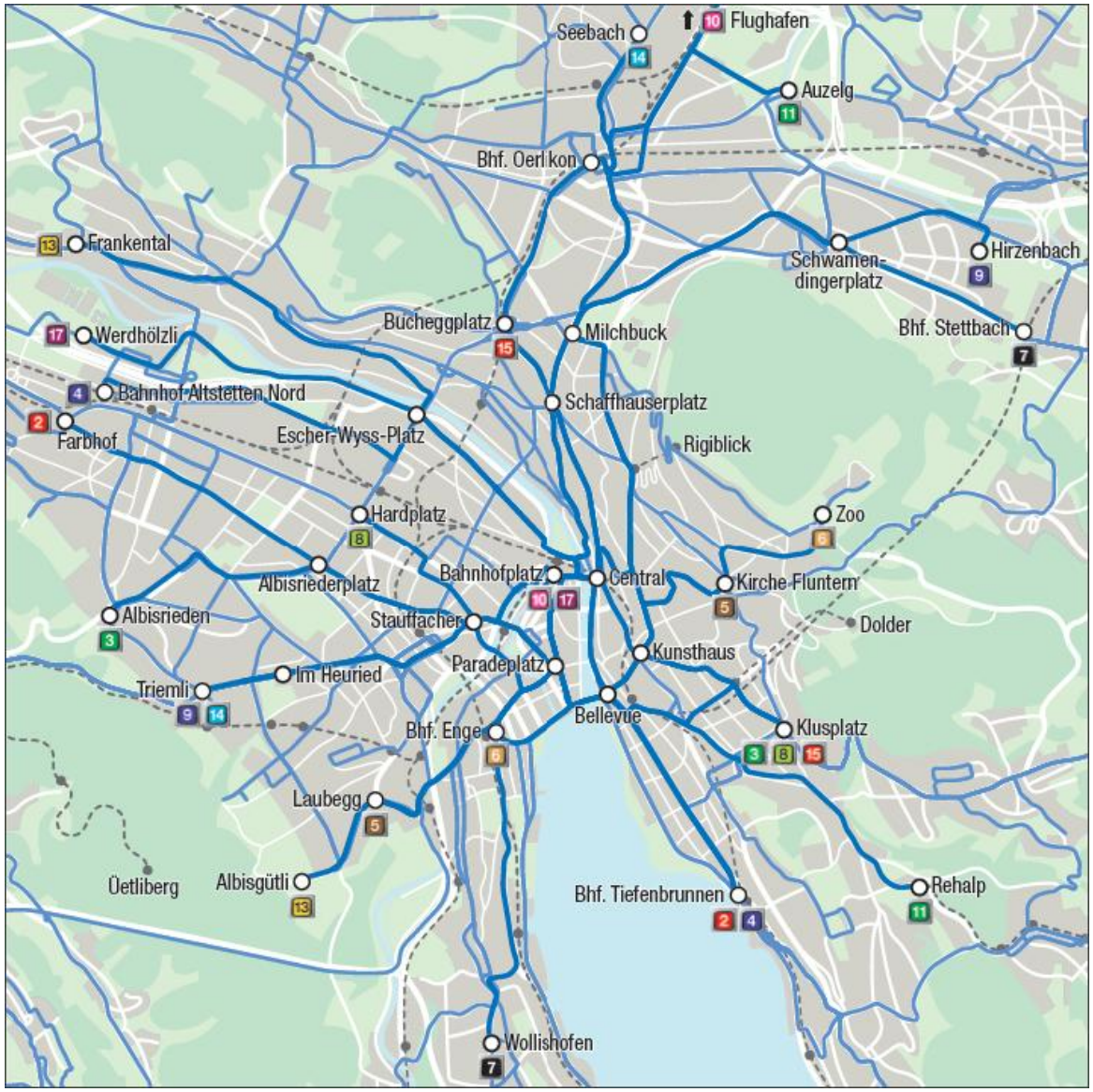

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Rationale for anti-angiogenic treatment

- **Cancers are angiogenic**
- **Starvation kills**
- **Inhibition of angiogenesis should be well tolerated**
- **The target might be non-neoplastic (*not moving*) and therefore stable: resistant to the development of resistance**
- **Potential for synergy with radiotherapy and chemotherapy**

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Anti-angiogenic therapies for glioblastoma may not have to target proper endothelial cells

A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry

Soufiane El Hallani,[1] Blandine Boisselier,[1] Florent Peglion,[1] Audrey Rousseau,[2] Carole Colin,[3] Ahmed Idbaih,[1,4] Yannick Marie,[1] Karima Mokhtari,[2] Jean-Léon Thomas,[1] Anne Eichmann,[5] Jean-Yves Delattre,[1,4] Andrew J. Maniotis[6] and Marc Sanson[1,4]

doi:10.1093/brain/awq044 Brain 2010: 133; 973–982 | 973

LETTER

doi:10.1038/nature09624

Glioblastoma stem-like cells give rise to tumour endothelium

Rong Wang[1,2,3], Kalyani Chadalavada[4], Jennifer Wilshire[5], Urszula Kowalik[1], Koos E. Hovinga[1,6], Adam G
Margaret Leversha[4], Cameron Brennan[1,3,7] & Viviane Tabar[1,2,3]

LETTER

doi:10.1038/nature09557

Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells

Lucia Ricci-Vitiani[1]*, Roberto Pallini[2]*, Mauro Biffoni[1], Matilde Todaro[3], Gloria Invernici[4], Tonia Cenci[5], Giulio Maira[2], Eugenio Agostino Parati[4], Giorgio Stassi[3,6], Luigi Maria Larocca[5] & Ruggero De Maria[1,7]

Transdifferentiation of glioblastoma cells into vascular endothelial cells

Yasushi Soda[a], Tomotoshi Marumoto[a,b], Dinorah Friedmann-Morvinski[a], Mie Soda[a], Fei Liu[a], Hiroyuki Michiue[c], Sandra Pastorino[d], Meng Yang[e], Robert M. Hoffman[e,f], Santosh Kesari[d], and Inder M. Verma[a,1]

4274–4280 | PNAS | March 15, 2011 | vol. 108 | no. 11

Molecular targets of anti-angiogenic therapies investigated in glioblastoma

Wick et al. Neuro-Oncology 2011;13:566-79

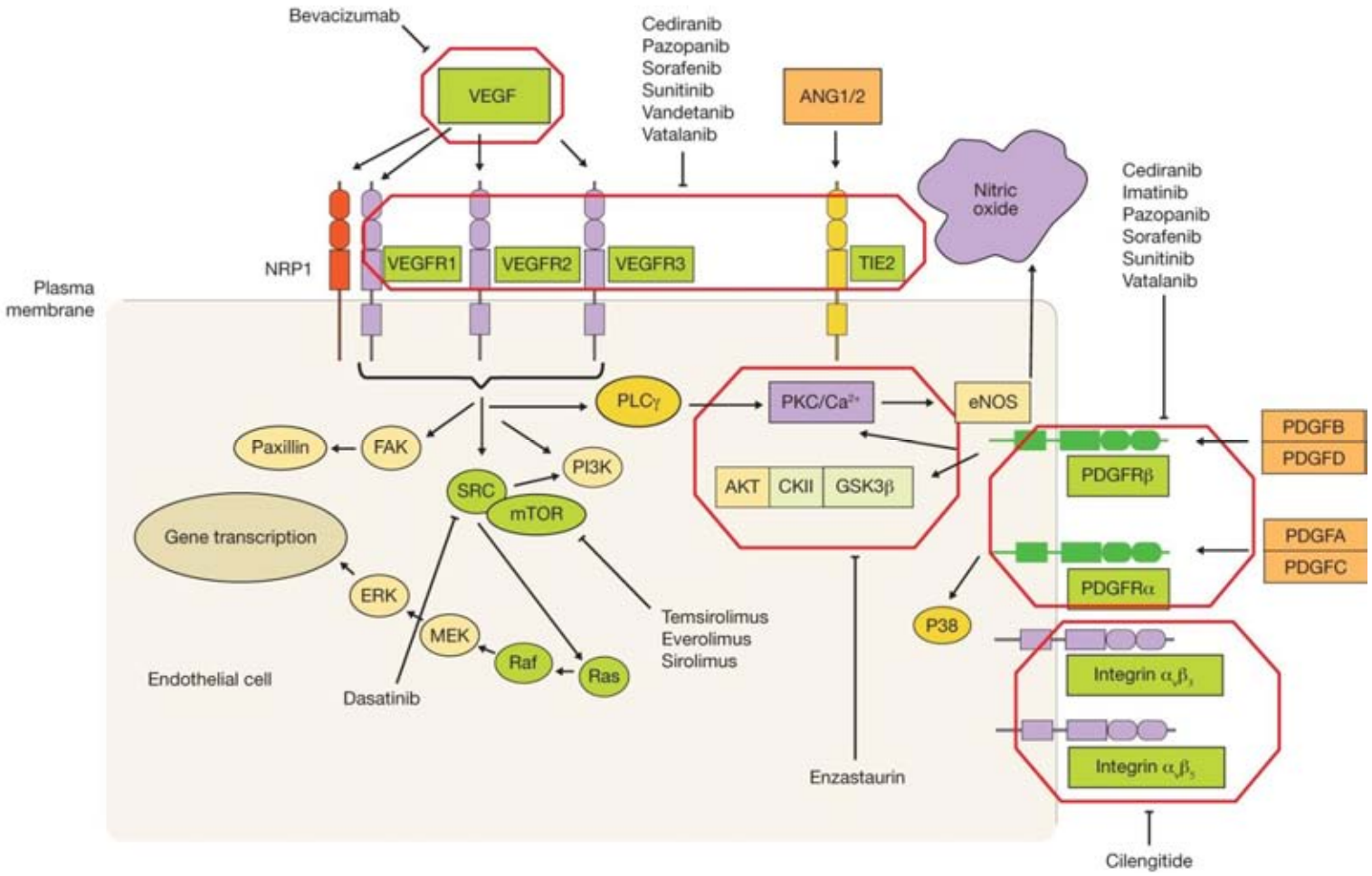

Fig. 2. Molecular targets of antiangiogenic therapies investigated in glioblastoma. ANG indicates angiopoietin; CKII indicates casein kinase II; eNOS indicates endothelial nitric oxide synthase; ERK indicates extracellular signal-regulated kinases; FAK indicates focal adhesion kinase; GSK3β indicates glycogen synthase kinase 3β; MEK indicates mitogen-activated protein kinase kinase; mTOR indicates mammalian target of rapamycin; PDGF(R) indicates platelet-derived growth factor (receptor); PI3K indicates phosphatidylinositol 3-kinase; PKC indicates protein kinase C; PLCγ indicates phopholipase Cγ; and VEGF(R) indicates vascular endothelial growth factor (receptor).

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Resistance to antiangiogenic treatment

- **Primary resistance: the target is not expressed**
- **Primary resistance: the target is expressed, but not essential**
- **Acquired resistance: the tumor may escape by switching to another mode of angiogenesis**
- **Acquired resistance: the tumor may escape by switching from angiogenic to infiltrative phenotype**
- **Escape pathways from anti-angiogenic treatments may be diverse and agent- and target-specific**

Candidate antiangiogenic agents in glioblastoma

- Enzastaurin: PKC- antagonist
- Bevacizumab: VEGF antibody
- Cediranib: VEGF receptor antagonist (TKI)
- Aflibercept (VEGF trap): VEGFR/IgG fusion protein
- Cilengitide: Integrin antagonist
- RO5323441: PlGF antibody

Candidate antiangiogenic agents in glioblastoma

- Enzastaurin: PKC- antagonist
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- Cediranib: VEGF receptor antagonist (TKI)
- Aflibercept (VEGF trap): VEGFR/IgG fusion protein
- Cilengitide: Integrin antagonist
- RO5323441: PlGF antibody

AVAGlio and RTOG-0825 Rationale

- **Conditional approval by FDA in 2009 for recurrent glioblastoma**
- **Safety and tolerability confirmed in phase II**
- **Promising PFS and OS data in phase II**

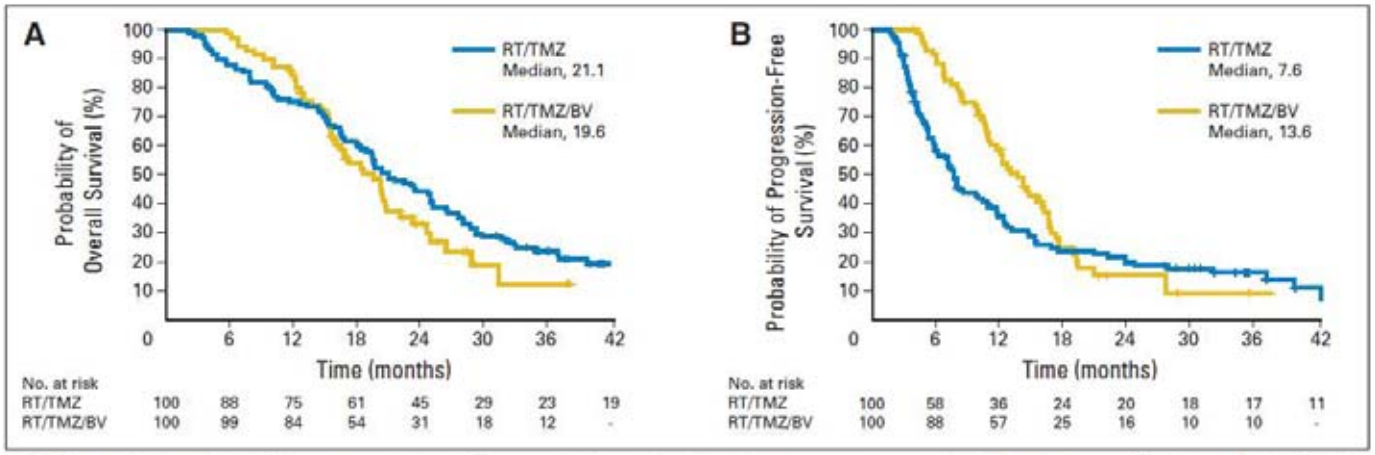
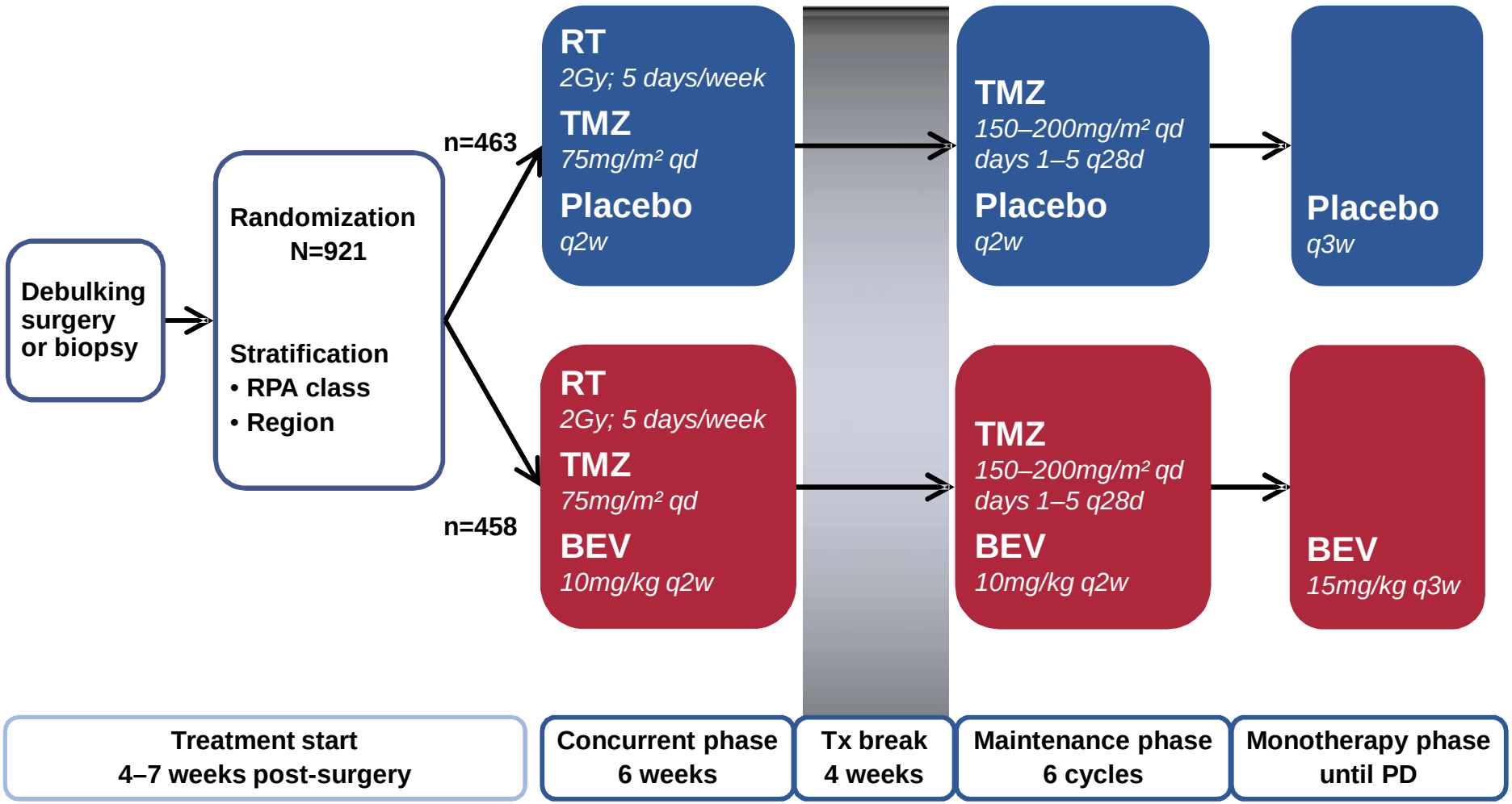


Fig 2. Kaplan-Meier analysis of (A) overall survival and (B) progression-free survival comparing current study group (gold; radiation therapy [RT] + temozolomide [TMZ] + bevacizumab [BV]) with University of California, Los Angeles/Kaiser Permanente Los Angeles control group (blue; RT + TMZ). Use of first-line BV shows early benefit in progression-free survival [Fleming(1,0) weighted log-rank test $P < .005$] and trended toward worse overall survival with later follow-up [Fleming(0,1) weighted log-rank test $P < .06$].

AVAGlio Design



Last patient in: March 2011

BEV = bevacizumab; PD = progressive disease; RPA = recursive partitioning analysis; RT = radiotherapy; TMZ = temozolomide; Tx = treatment; qd = daily; q28d = every 28 days; q2w = every 2 weeks; q3w = every 3 weeks

RTOG 0825 Design

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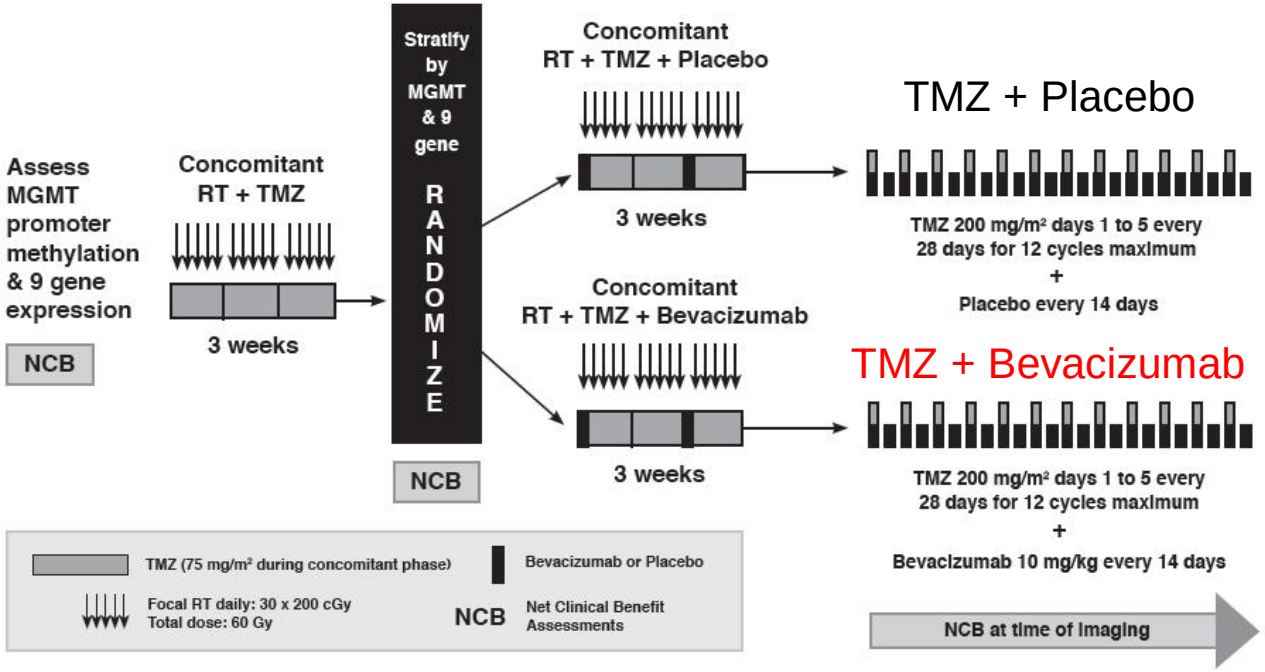
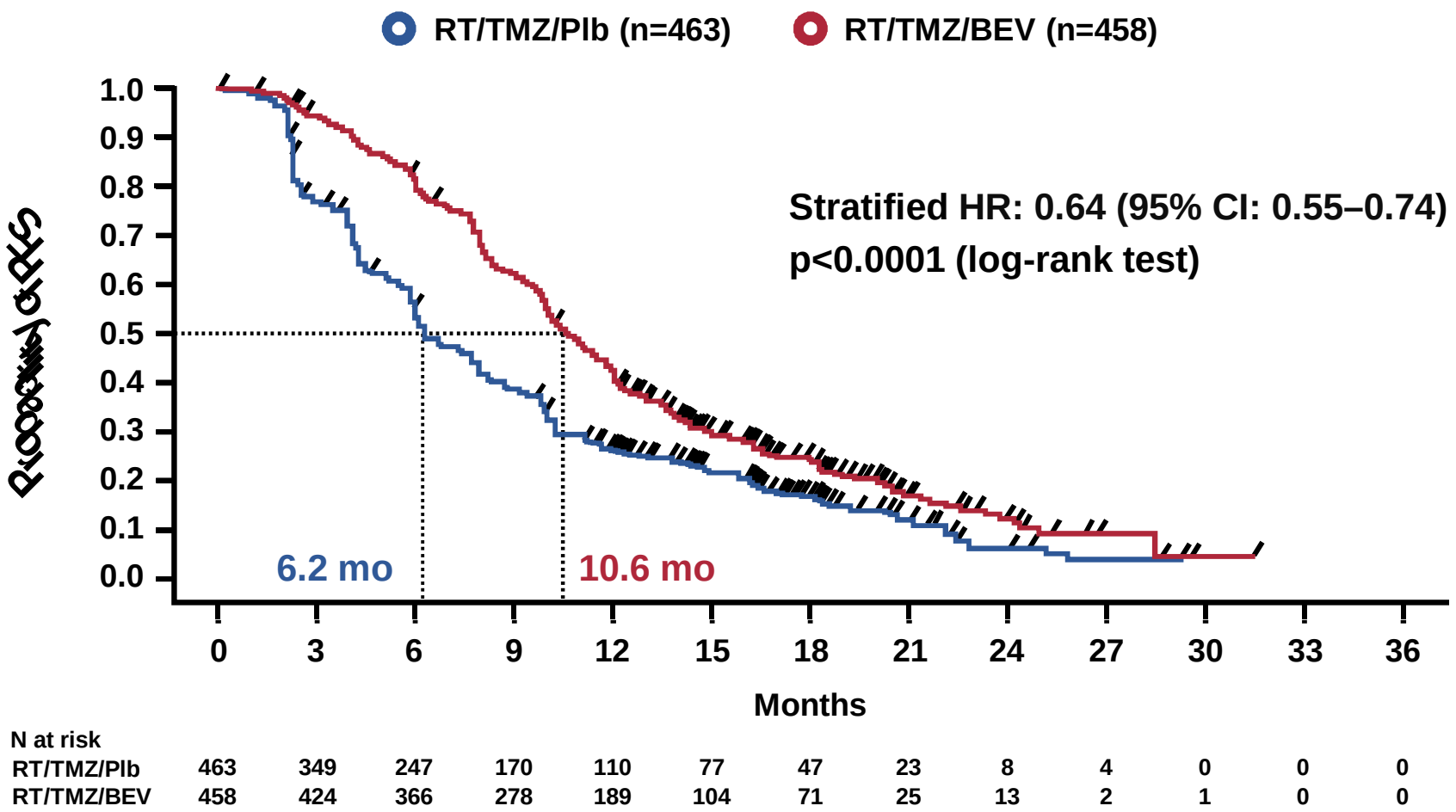
- **Primary objectives: determine if the addition of bevacizumab to standard chemoradiation improves OS or PFS in newly diagnosed GBM**
- **Secondary objectives:**
  - **Compare toxicity profiles**
  - **Compare symptom burden, HRQOL and neurocognitive function (Net Clinical Benefits-NCB)**
  - **Identify a selected patient subset with bevacizumab benefit based on MGMT, 9-gene molecular profile, and RPA**



Treatment arm blind broken at progression.

Built in crossover or continuation of bevacizumab.

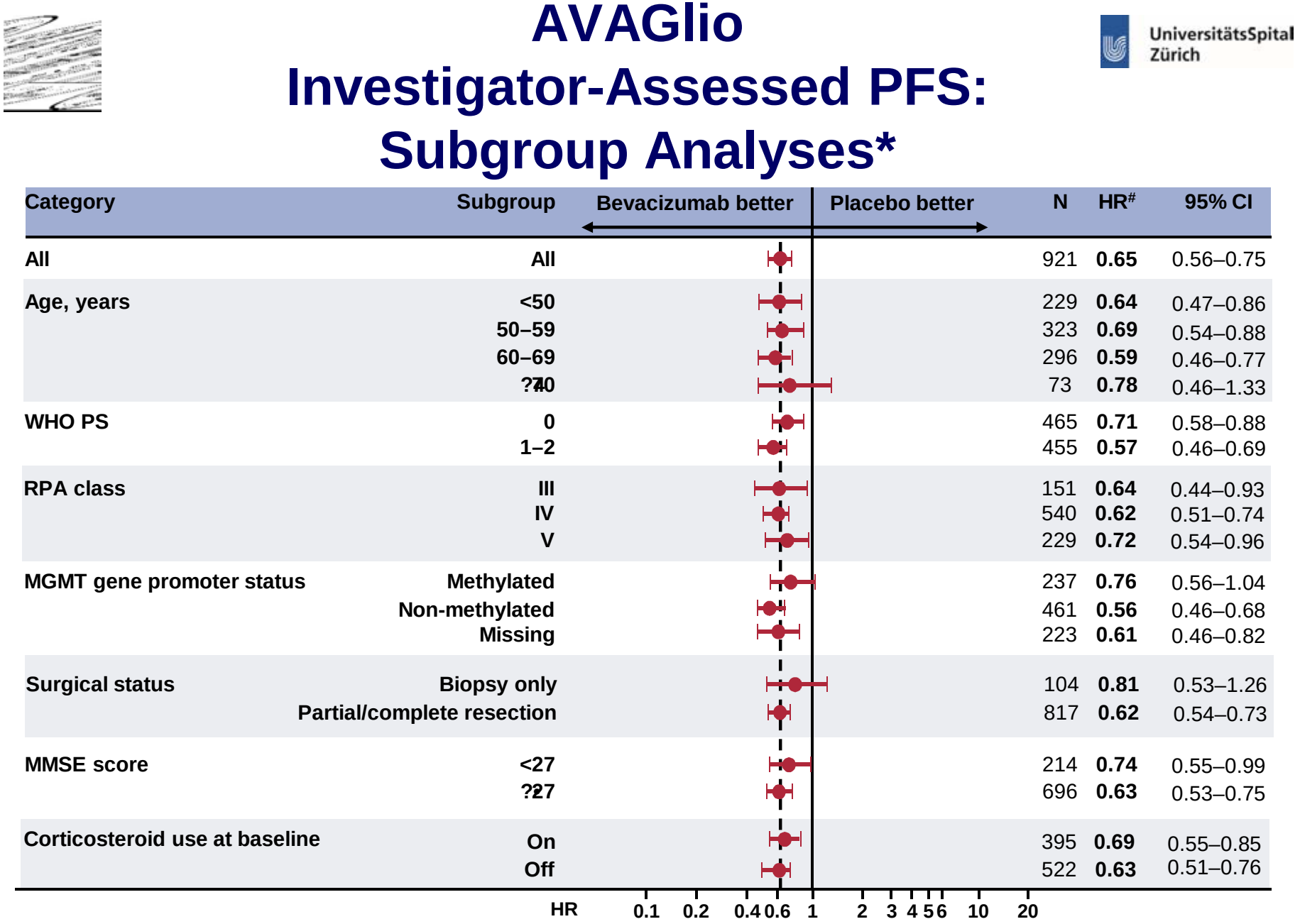
AVAGlio
Investigator-Assessed PFS (Co-Primary Endpoint)



N at risk													
RT/TMZ/Plb	463	349	247	170	110	77	47	23	8	4	0	0	0
RT/TMZ/BEV	458	424	366	278	189	104	71	25	13	2	1	0	0

BEV = bevacizumab; CI = confidence interval; HR = hazard ratio; mo = months; PFS = progression-free survival; Plb = placebo; RT = radiotherapy; TMZ = temozolomide

AVAGlio
Investigator-Assessed PFS: Subgroup Analyses*



Category	Subgroup	N	HR#	95% CI
All	All	921	**0.65**	0.56–0.75
Age, years	<50	229	**0.64**	0.47–0.86
	50–59	323	**0.69**	0.54–0.88
	60–69	296	**0.59**	0.46–0.77
	?70	73	**0.78**	0.46–1.33
WHO PS	0	465	**0.71**	0.58–0.88
	1–2	455	**0.57**	0.46–0.69
RPA class	III	151	**0.64**	0.44–0.93
	IV	540	**0.62**	0.51–0.74
	V	229	**0.72**	0.54–0.96
MGMT gene promoter status	Methylated	237	**0.76**	0.56–1.04
	Non-methylated	461	**0.56**	0.46–0.68
	Missing	223	**0.61**	0.46–0.82
Surgical status	Biopsy only	104	**0.81**	0.53–1.26
	Partial/complete resection	817	**0.62**	0.54–0.73
MMSE score	<27	214	**0.74**	0.55–0.99
	?27	696	**0.63**	0.53–0.75
Corticosteroid use at baseline	On	395	**0.69**	0.55–0.85
	Off	522	**0.63**	0.51–0.76

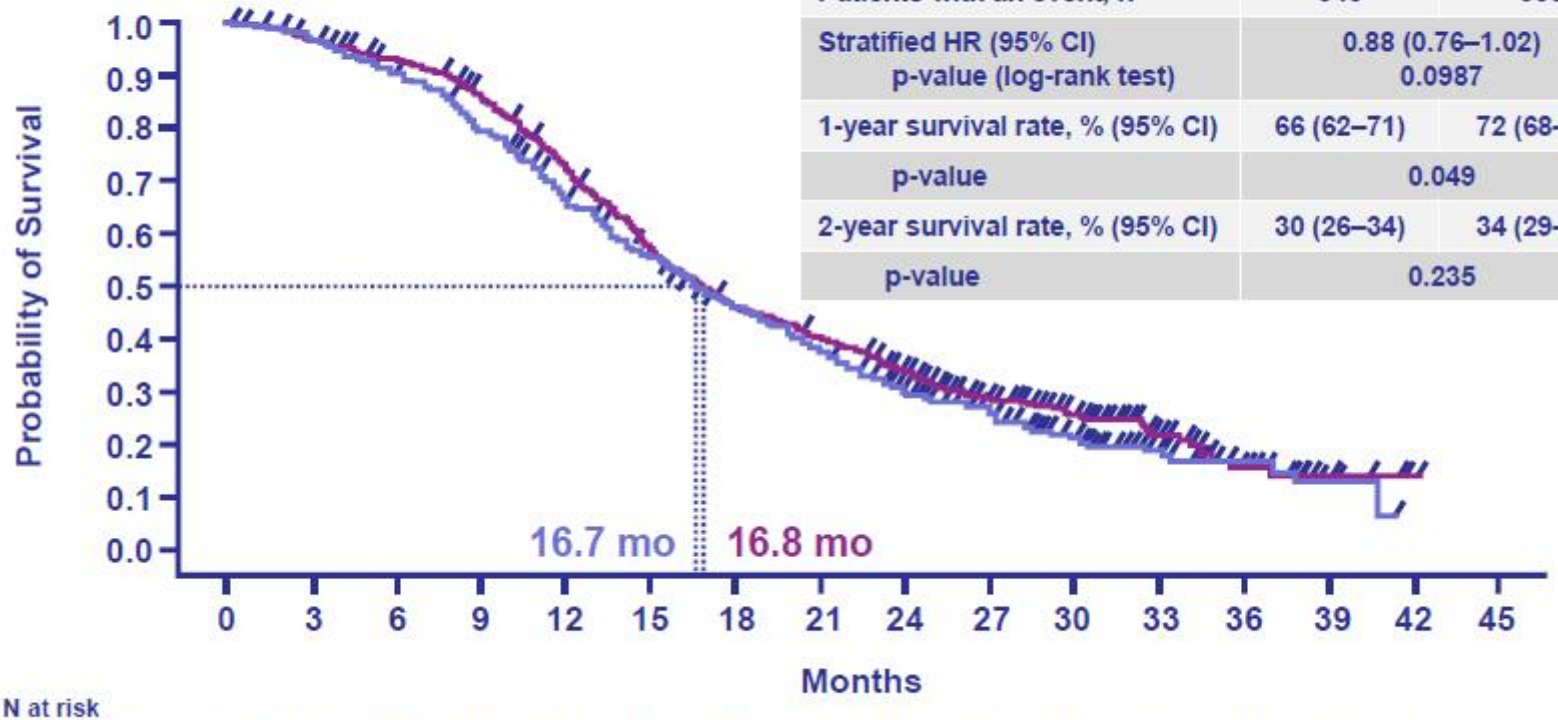
*Selected subgroups only; #Unstratified analysis

CI = confidence interval; HR = hazard ratio; MGMT = methylguanine-DNA methyltransferase; MMSE = mini-mental state examination; PFS = progression-free survival; RPA = recursive partitioning analysis; WHO PS = World Health Organization performance status

AVAGlio

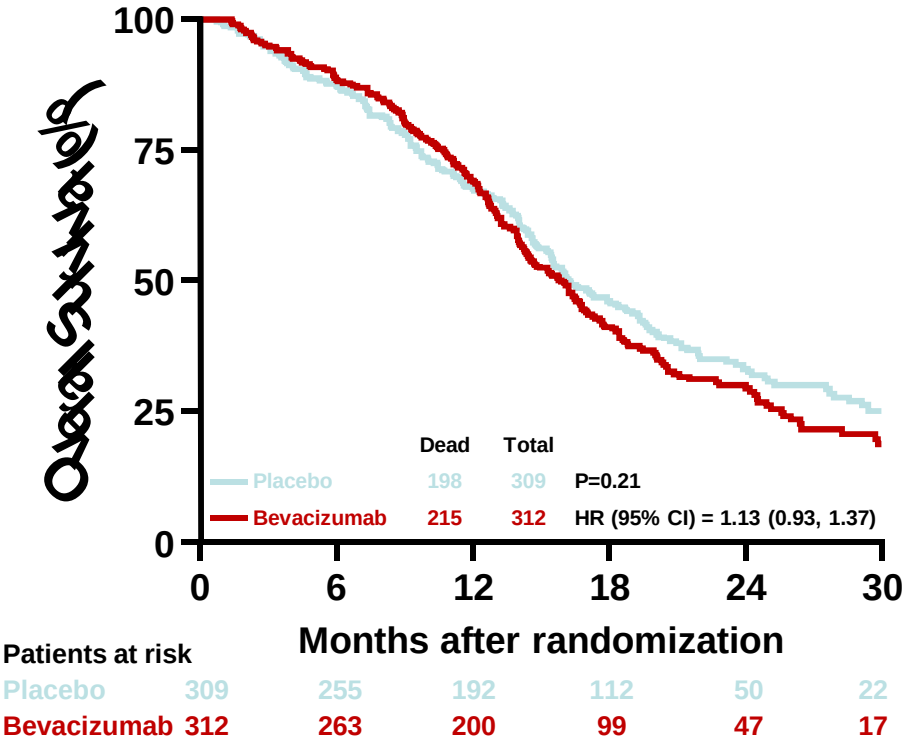
Overall Survival (Co-Primary Endpoint)

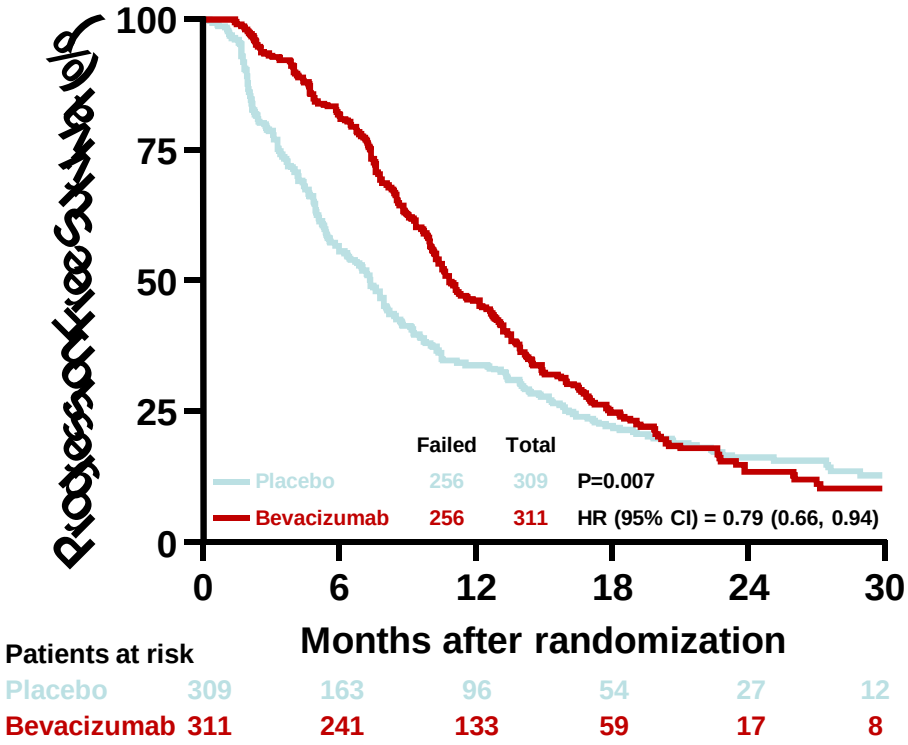
	RT/TMZ/Plb (n=463)	RT/TMZ/BEV (n=458)
Patients with an event, n	346	333
Stratified HR (95% CI)	0.88 (0.76–1.02)	
p-value (log-rank test)	0.0987	
1-year survival rate, % (95% CI)	66 (62–71)	72 (68–77)
p-value	0.049	
2-year survival rate, % (95% CI)	30 (26–34)	34 (29–38)
p-value	0.235	



N at risk																
RT/TMZ/Plb	463	444	405	355	293	245	201	163	118	84	53	28	15	6	0	0
RT/TMZ/BEV	458	440	421	387	322	253	203	176	139	91	61	27	11	4	1	0

RTOG-0825 - Survival



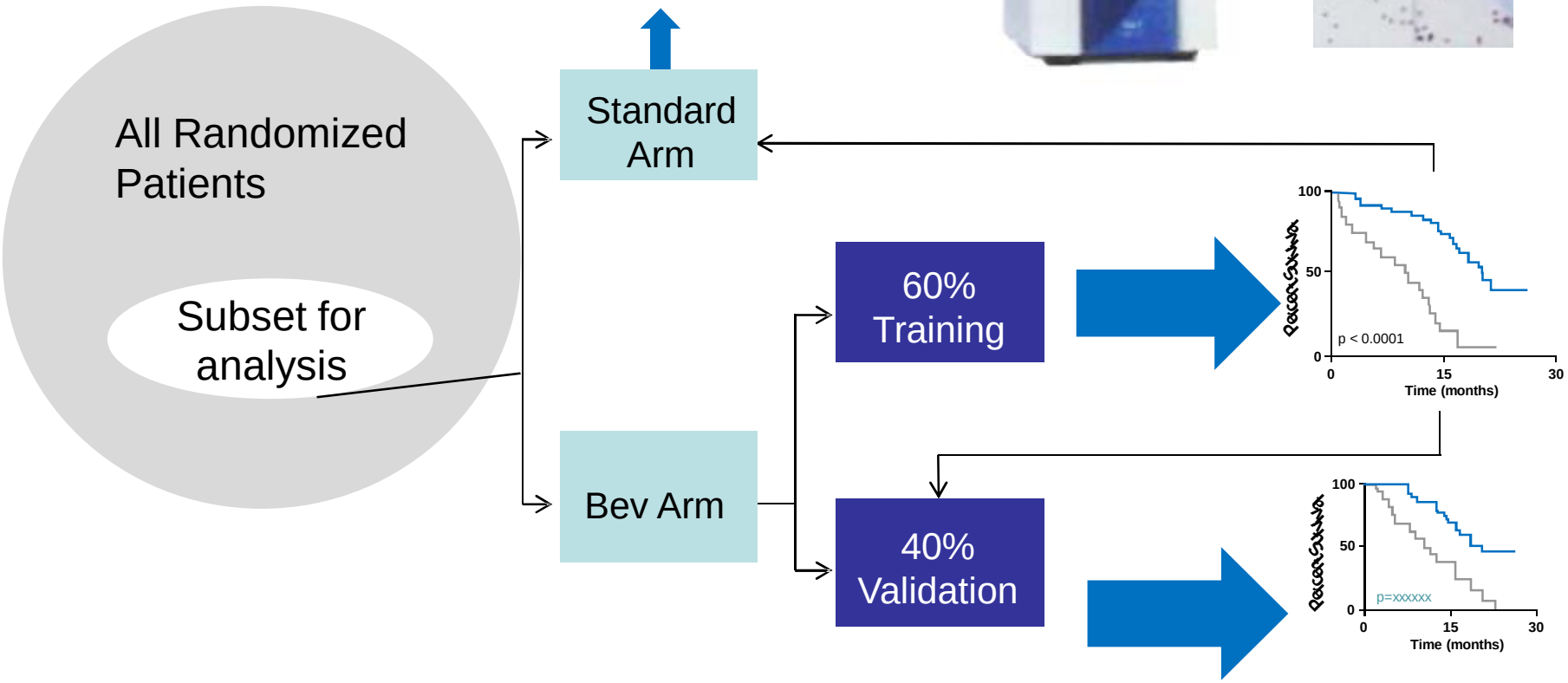


Median overall survival
- Placebo: 16.1 months
- Bevacizumab: 15.7 months
- HR (bev/placebo:1.13 (95%CI:0.93,1.37))
- p = 0.21

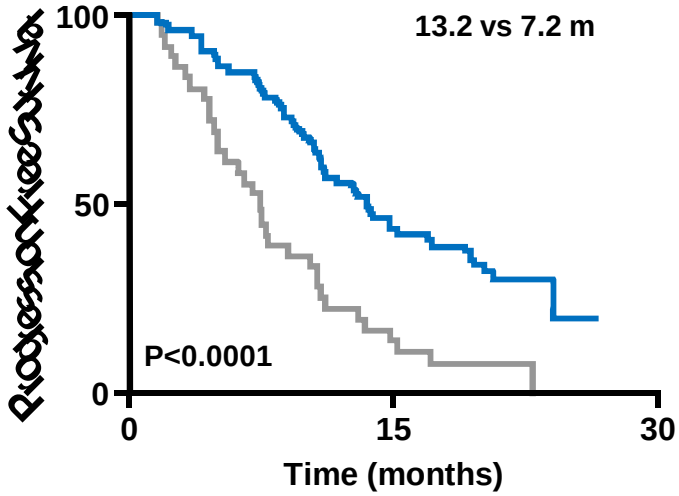
Median progression free survival
- Placebo: 7.3 months
- Bevacizumab: 10.7 months
- HR (bev/placebo:0.79 (95%CI:0.66,0.94))
- p = 0.007

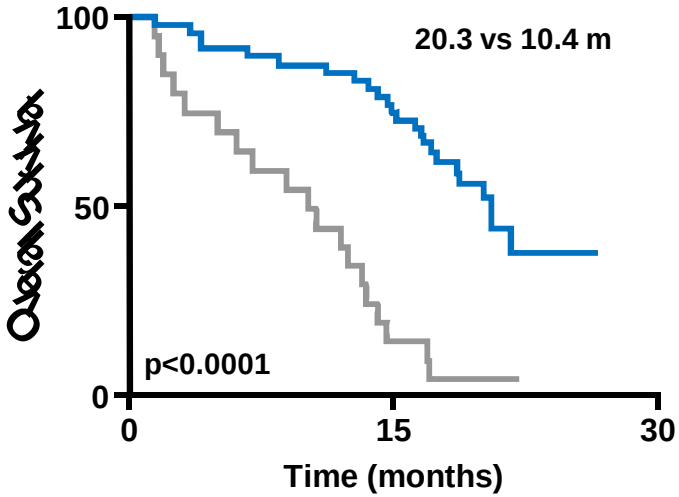
Neither OS or PFS achieved pre-specified endpoints

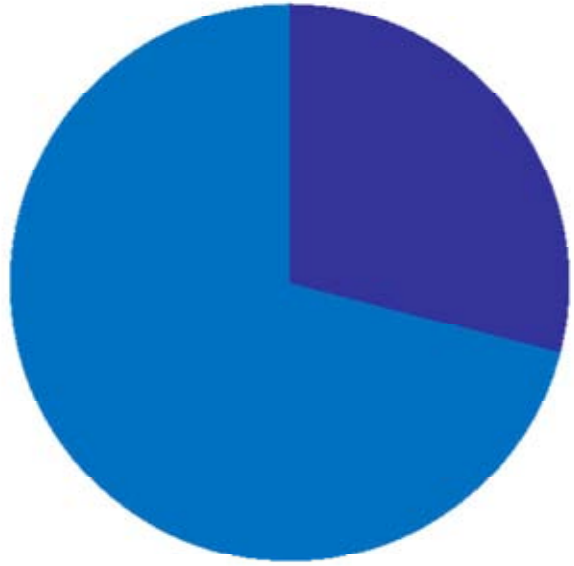
Methodology



PRoB-GBM Predicts Bevacizumab Responders

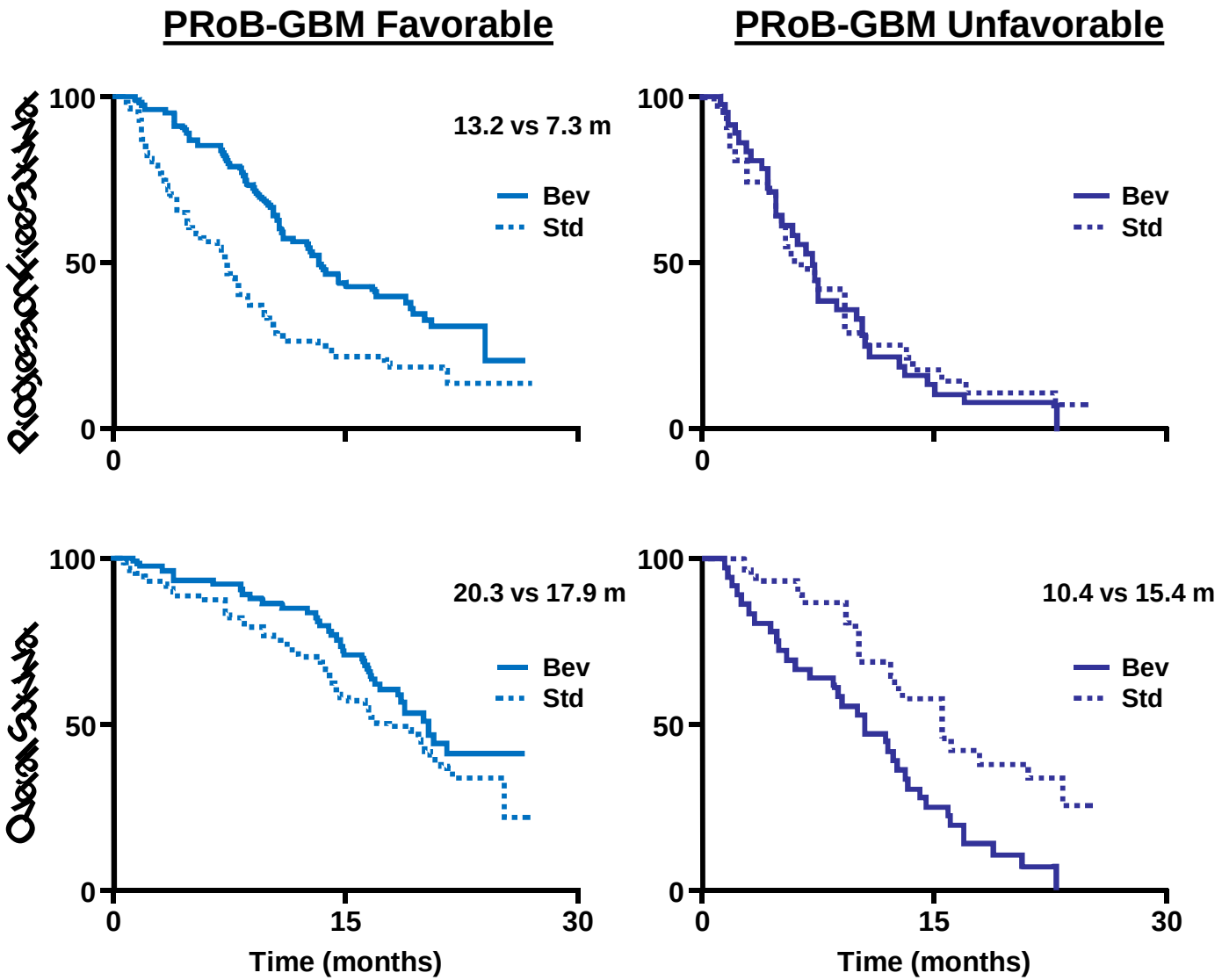






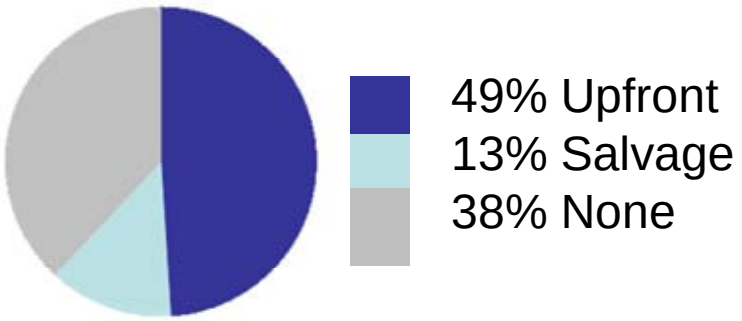
71% ProB-GBM Favorable
29% PRoB-GBM Unfavorable

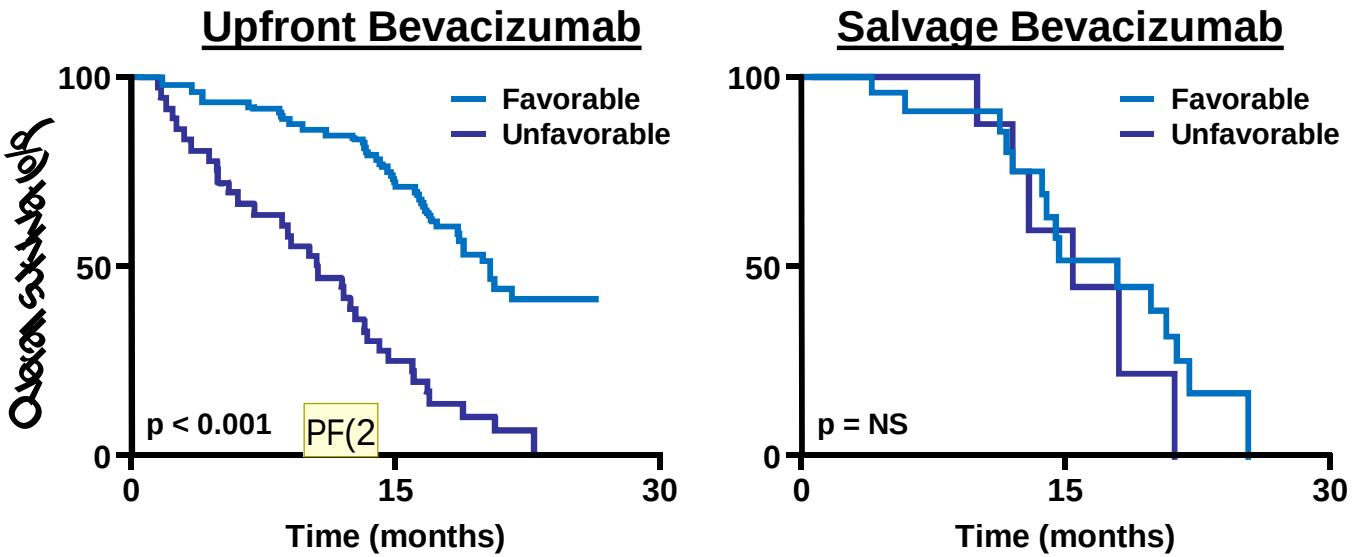
PRoB-GBM by Treatment Arm



PRoB-GBM Not Predictive for Salvage Bevacizumab

- **Patients could be unblinded at recurrence**
- **Those on standard arm could be given bevacizumab at recurrence**
- **Examined PRoB-GBM in salvage arm**





PF(2 The original slide says $p < 0.0001$

Pierre Fichelson (HI), 07/06/2013

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Review

The EGFRvIII variant in glioblastoma multiforme

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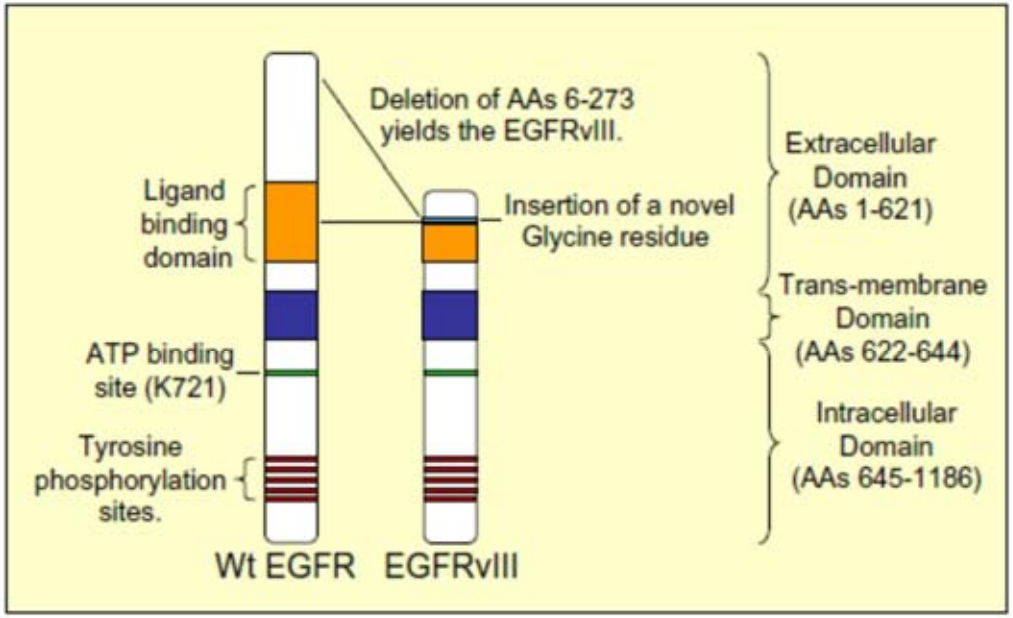
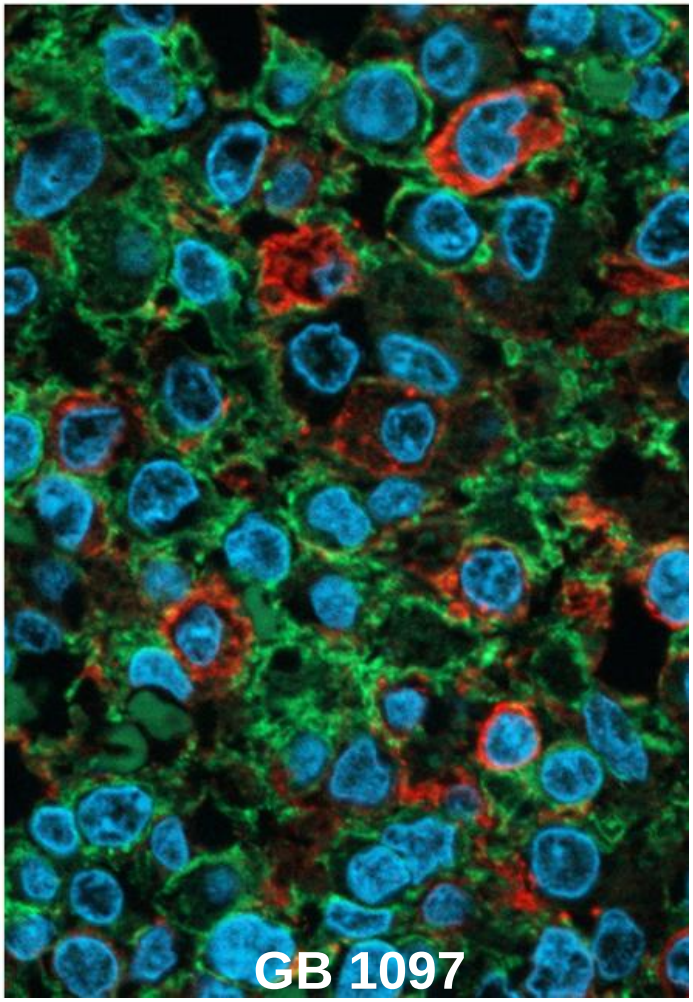
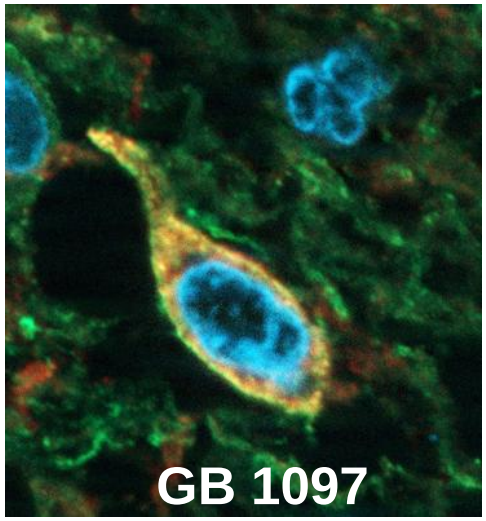


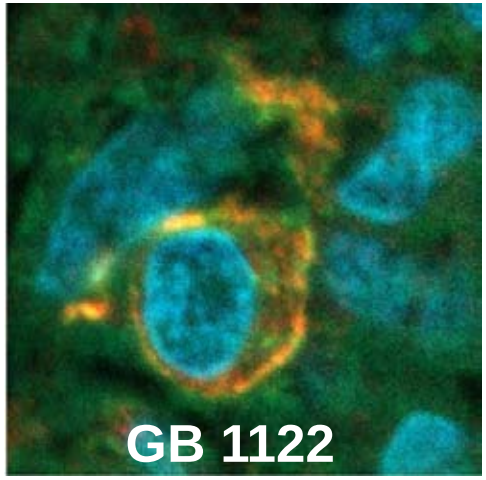
Fig. 1. Schematic of the epidermal growth factor receptor (EGFR)vIII truncation. The EGFRvIII variant receptor is characterized by a deletion of exons 2–7 of the wild type (Wt) *EGFR* gene. This results in an in-frame truncation of amino acids (AA) 6 to 273 in the extracellular domain of the full length protein, yielding a constitutively active variant receptor that can not bind ligand. The EGFRvIII also contains a novel glycine residue inserted at the fusion junction.

Coexpression of wild-type EGFR and EGFRvIII expression in glioblastoma

UniversitätsSpital Zürich





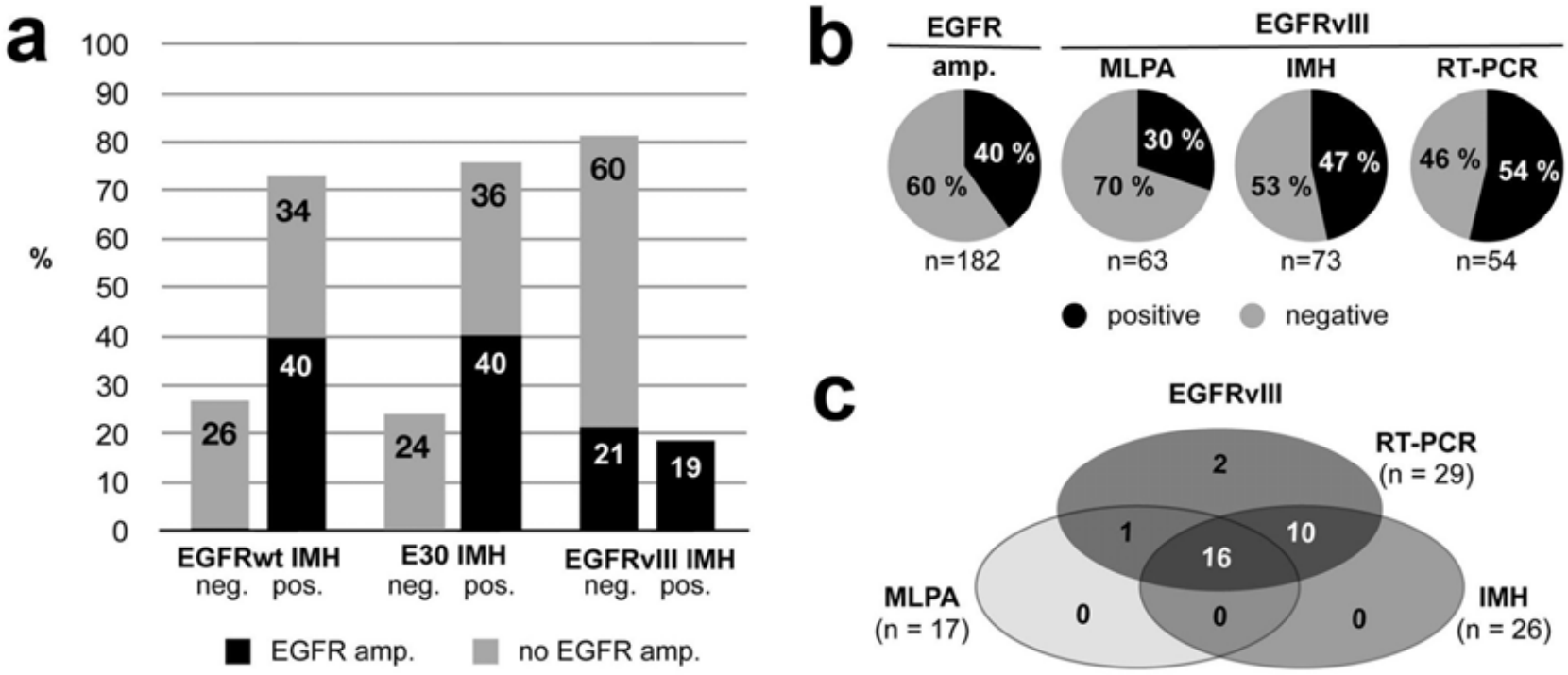


EGFRwt (3C6)

EGFRvIII (L8A4)

Overlap

How should we assess the EGFR status?



Deutsches Gliomnetzwerk

Helfen. Forschen. Informieren.

Immunologic Escape After Prolonged Progression-Free Survival With Epidermal Growth Factor Receptor Variant III Peptide Vaccination in Patients With Newly Diagnosed Glioblastoma

John H. Sampson, Amy B. Heimberger, Gary E. Archer, Kenneth D. Aldape, Allan H. Friedman, Henry S. Friedman, Mark R. Gilbert, James E. Herndon II, Roger E. McLendon, Duane A. Mitchell, David A. Reardon, Raymond Sawaya, Robert J. Schmittling, Weiming Shi, James J. Vredenburgh, and Darell D. Bigner

J Clin Oncol 28:4722-4729. © 2010 by American Society of Clinical Oncology

ABSTRACT

Purpose

Immunologic targeting of tumor-specific gene mutations may allow precise eradication of neoplastic cells without toxicity. Epidermal growth factor receptor variant III (*EGFRvIII*) is a constitutively activated and immunogenic mutation not expressed in normal tissues but widely expressed in glioblastoma multiforme (GBM) and other neoplasms.

Patients and Methods

A phase II, multicenter trial was undertaken to assess the immunogenicity of an *EGFRvIII*-targeted peptide vaccine and to estimate the progression-free survival (PFS) and overall survival (OS) of vaccinated patients with newly diagnosed *EGFRvIII*-expressing GBM with minimal residual disease. Intradermal vaccinations were given until toxicity or tumor progression was observed. Sample size was calculated to differentiate between PFS rates of 20% and 40% 6 months after vaccination.

Results

There were no symptomatic autoimmune reactions. The 6-month PFS rate after vaccination was 67% (95% CI, 40% to 83%) and after diagnosis was 94% (95% CI, 67% to 99%; n = 18). The median OS was 26.0 months (95% CI, 21.0 to 47.7 months). After adjustment for age and Karnofsky performance status, the OS of vaccinated patients was greater than that observed in a control group matched for eligibility criteria, prognostic factors, and temozolomide treatment (hazard ratio, 5.3; $P = .0013$; n = 17). The development of specific antibody ($P = .025$) or delayed-type hypersensitivity ($P = .03$) responses to *EGFRvIII* had a significant effect on OS. At recurrence, 82% (95% CI, 48% to 97%) of patients had lost *EGFRvIII* expression ($P < .001$).

Conclusion

EGFRvIII-targeted vaccination in patients with GBM warrants investigation in a phase III, randomized trial.

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Immunologic Escape After Prolonged Progression-Free Survival With Epidermal Growth Factor Receptor Variant III Peptide Vaccination in Patients With Newly Diagnosed Glioblastoma

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Table 3. *EGFRvIII* Immunohistochemistry Before and After Vaccination

Before Vaccination	At Recurrence
Positive	Negative
Positive	Negative
Positive	Negative
Positive	Positive (< 1%)
Positive	Negative
Positive	Negative
Positive	Negative
Positive	Negative
Positive	Negative
Positive	Positive
Positive	Negative

NOTE. Percent negative after vaccine is 82% (95% CI, 48% to 97%) or nine of 11; binomial test $P < .001$.

Abbreviation: *EGFRvIII*, epidermal growth factor receptor variant III.

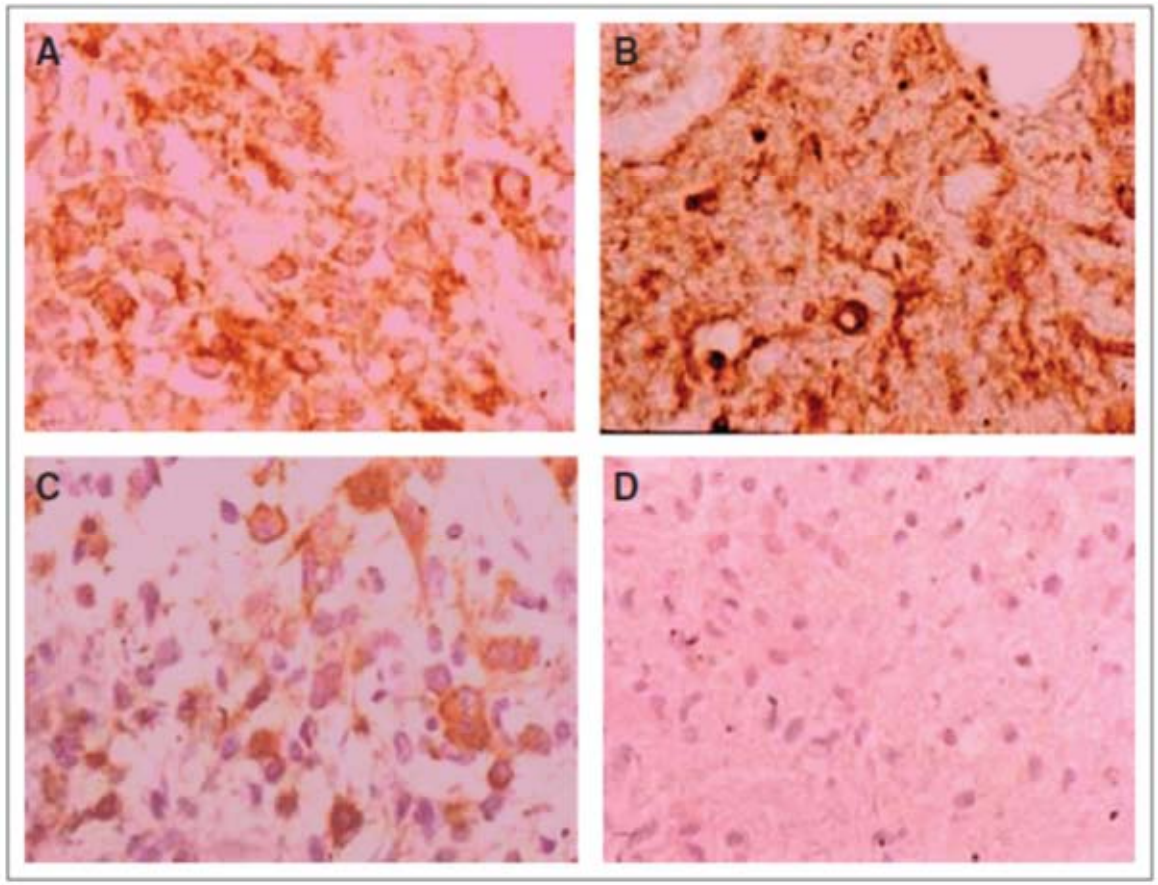
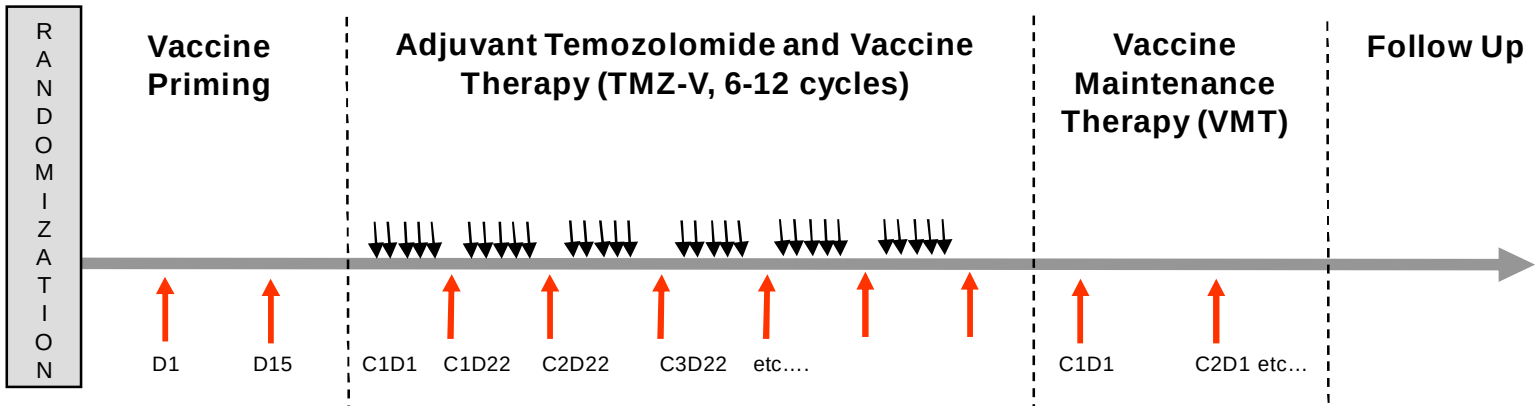


Fig 3. Epidermal growth factor receptor (EGFR) and EGFR variant III (*EGFRvIII*) immunohistochemistry of a patient with glioblastoma multiforme (GBM). Staining with (A) EGFR and (B) *EGFRvIII* before vaccine. (C) Preservation of EGFR staining but (D) specific loss of *EGFRvIII* staining at recurrence after vaccination.

ACT IV Study Design



- Dose vaccine days 1 and 15 of Vaccine Priming cycle
- Start cycle within 4 days after randomization and within 7-14 days after completion of CRT

- Vaccine or control (KLH) is administered Day 22 of each TMZ cycle
- Begin TMZ no sooner than 6 days after administration of the second vaccine priming dose
- Begin TMZ no sooner than 28 days after completion of CRT
- Begin TMZ when ANC ≥ 1000/µL and platelets ≥ 100,000/µL
- TMZ dosed days 1-5 of each 28 day cycle

If no disease progression after TMZ, continue dosing vaccine every 28 days (Day 1 ±3 days of each 28 day cycle) until intolerance or disease progression

Follow-up for overall survival every 12 weeks after disease progression

Temozolomide Dosing

Vaccine or Control (KLH) Dosing

CRT Chemoradiation Therapy

Treatment will be discontinued upon disease progression, unacceptable treatment-related toxicity, or patient refusal to continue study treatment