Molecular Biomarkers Beyond *MGMT* Promoter Hypermethylation: Hope or Hype?

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Standard of Care – Summary
(EANO Guidelines, Lancet Oncol 2014)

- There is no way around a histologic/molecular diagnosis
- Maximal safe resection
- Age + *MGMT* status may/should be taken into consideration
- Radiochemotherapy with temozolomide (six maintenance cycles) irrespective of the *MGMT* status
- 2-3 monthly clinical and MRI F/U
- Multiple options but no standard at recurrence!
  - Surgery/radiotherapy/nitrosoureas/bevacizumab
  - Immunotherapy/targeted therapies in trials

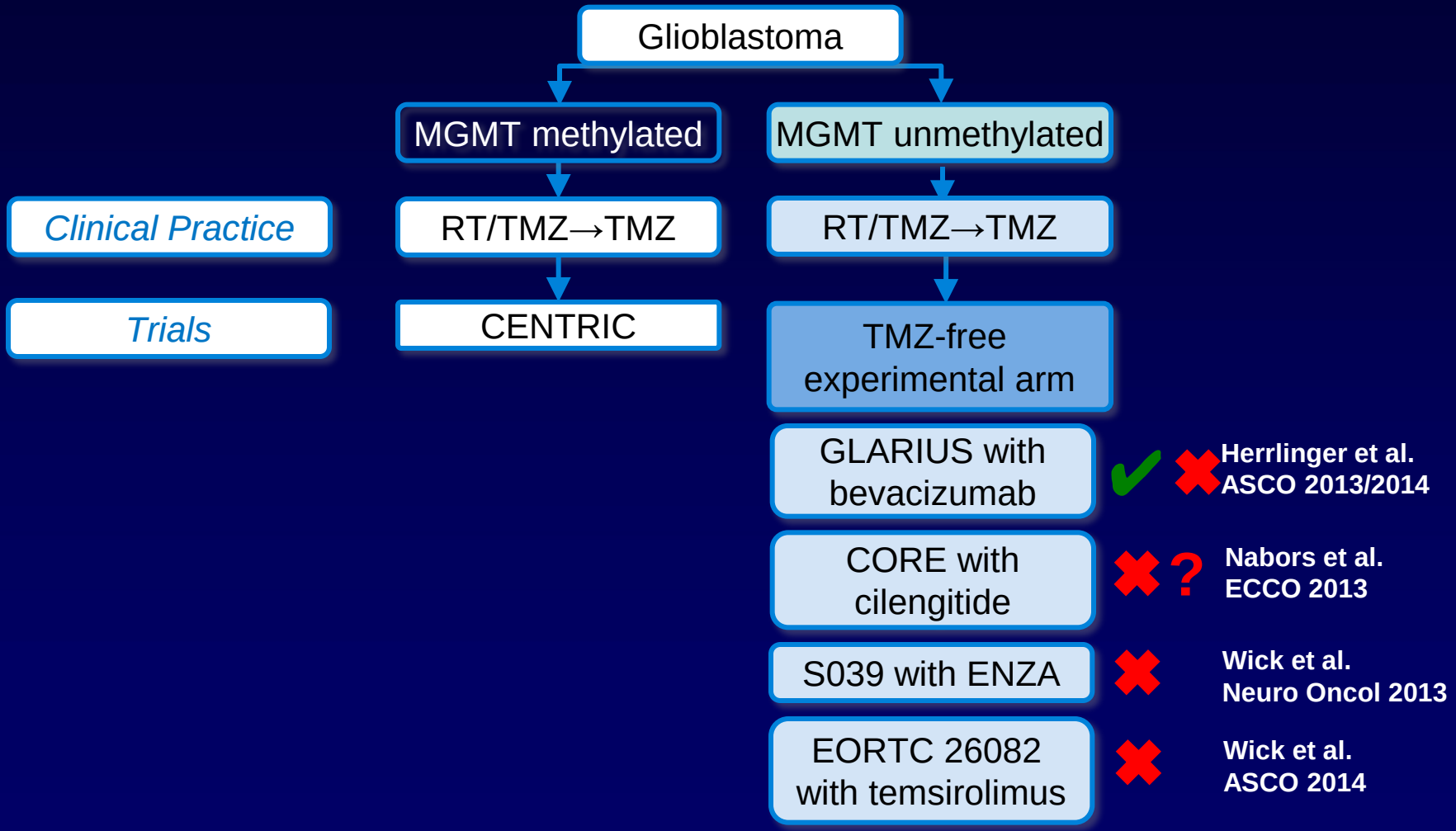
Weller M, et al. *Lancet Oncol.* 2014;15(9):e395-403.

Absence of Positive Prognostic Markers in Glioblastoma of the Elderly

- ***MGMT*** **promoter methylation occurs at similar frequency**
- ***IDH*** **mutations occur at a lower frequency[1]**
- **General methylation levels are low (eg, *PRDX*, *APNG*)[2]**
- **What is the impact of other biomarkers?**
- **Do gliomas in the elderly represent a separate disease entity?[3]**

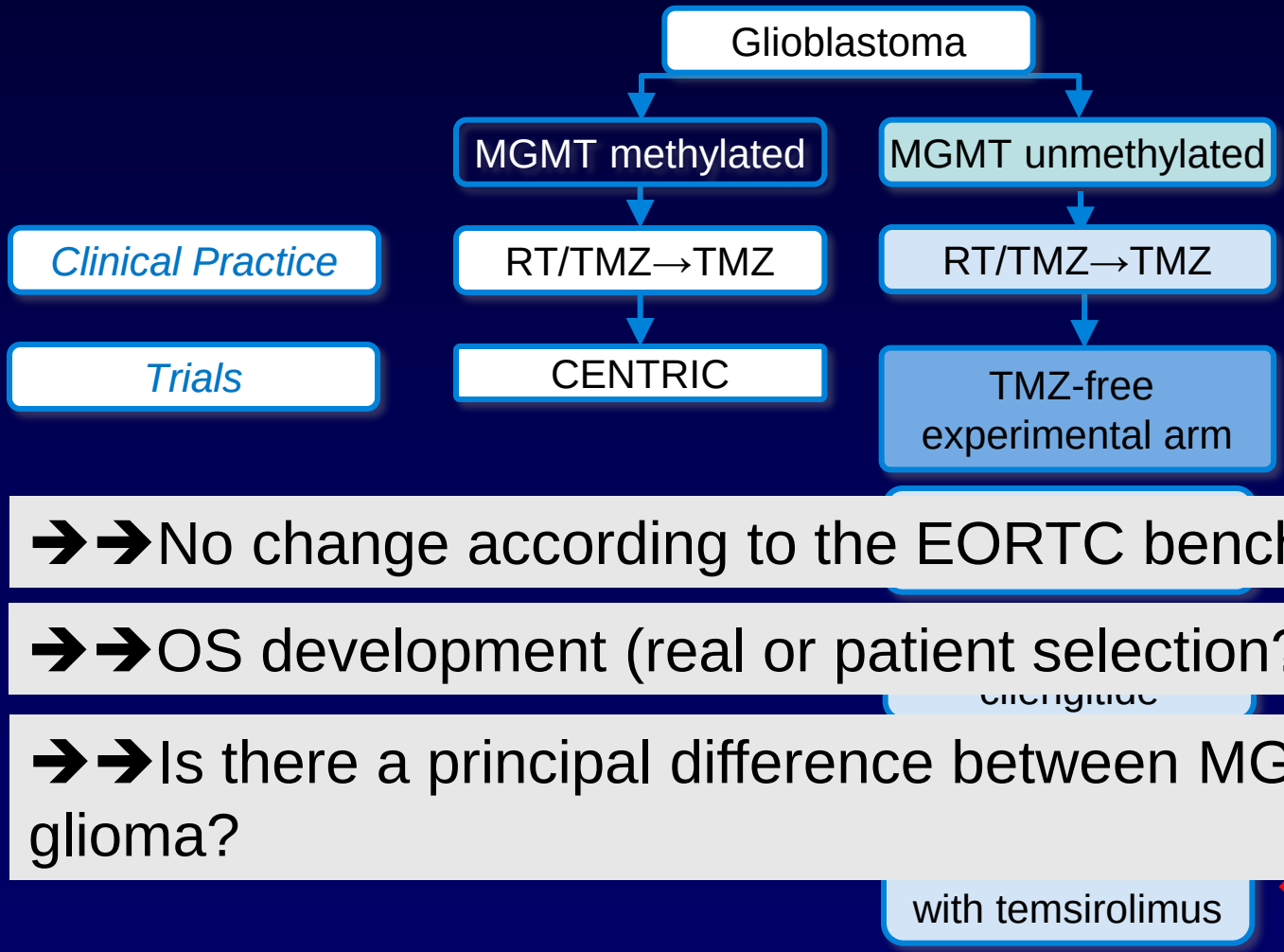
1. Reifenberger G, et al. *Int J Cancer.* 2011; 2. Wiestler B, et al. *Neuro Oncol.* 2013;15(8):1017-1026; 3. Sturm D, et al. *Cancer Cell.* 2012;22(4):425-437.

Glioblastoma: Trials According to *MGMT* Status



Wick W, et al. *Nat Rev Neurol.* 2014;10(2):372-385.

Glioblastoma: Trials According to *MGMT* Status



➔➔No change according to the EORTC benchmark

➔➔OS development (real or patient selection?)

➔➔Is there a principal difference between MGMT prof/def glioma?

inger et al. O 2013/2014

Nabors et al. ECCO 2013

ASCO 2014

Wick W, et al. *Nat Rev Neurol.* 2014;10(2):372-385.

Beyond Standard of Care – Biomarkers: Which Are Ready For Prime Time?

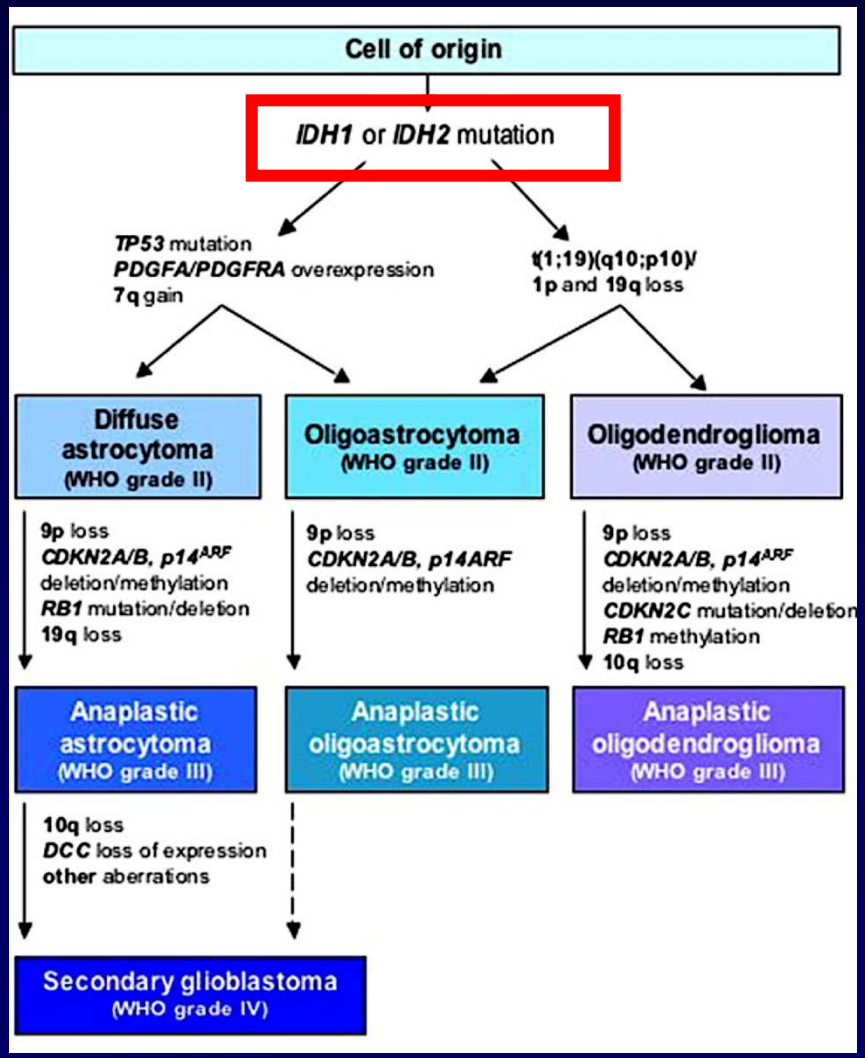
- There is no way around a histologic/molecular diagnosis
- Maximal safe resection
- Age + *MGMT* status may/should be taken into consideration
- What about the other biomarkers?
  - 1p/19q co-deletion – predicitve + irrelevant for glioblastoma
  - IDH1/G-CIMP
  - Philipps/TCGA classification
  - Imaging (as already discussed)
  - NDGR1, PTEN, CD95L...

Basic Principles

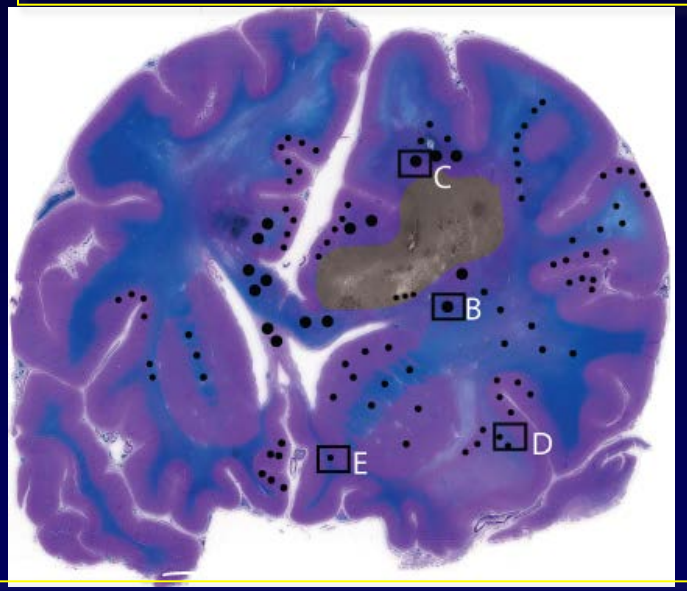
- There is a distinction between diagnostic, prognostic, and predictive biomarkers
- Biomarker development follows stringent rules, which include the hypothesis, confirmatory experiments, a technical validation, a clinical validation, and parallel development of a suitable test instrument

Biomarker – Gliomagenesis *Isocitrate Dehydrogenase (IDH)*

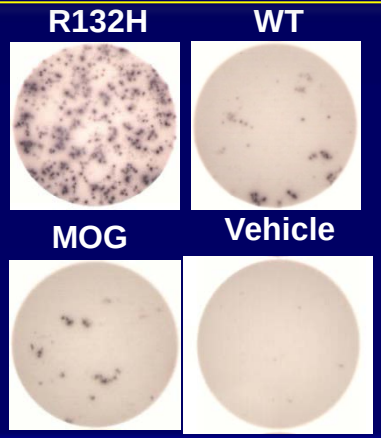
***IDH* mutations are early events in gliomagenesis**



Uniform expression in all mutant glioma



Spontaneous specific T cell response

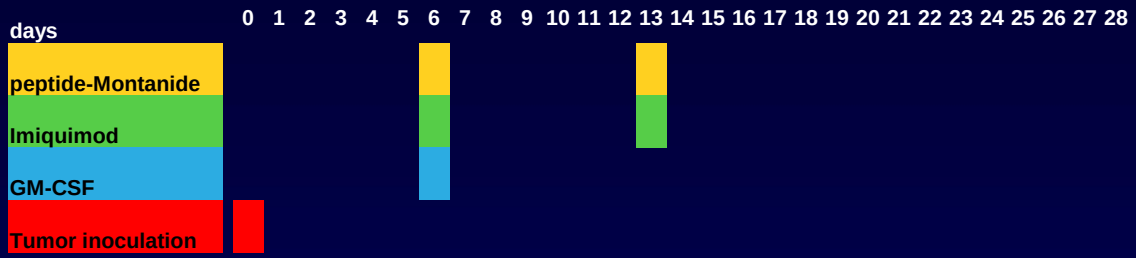


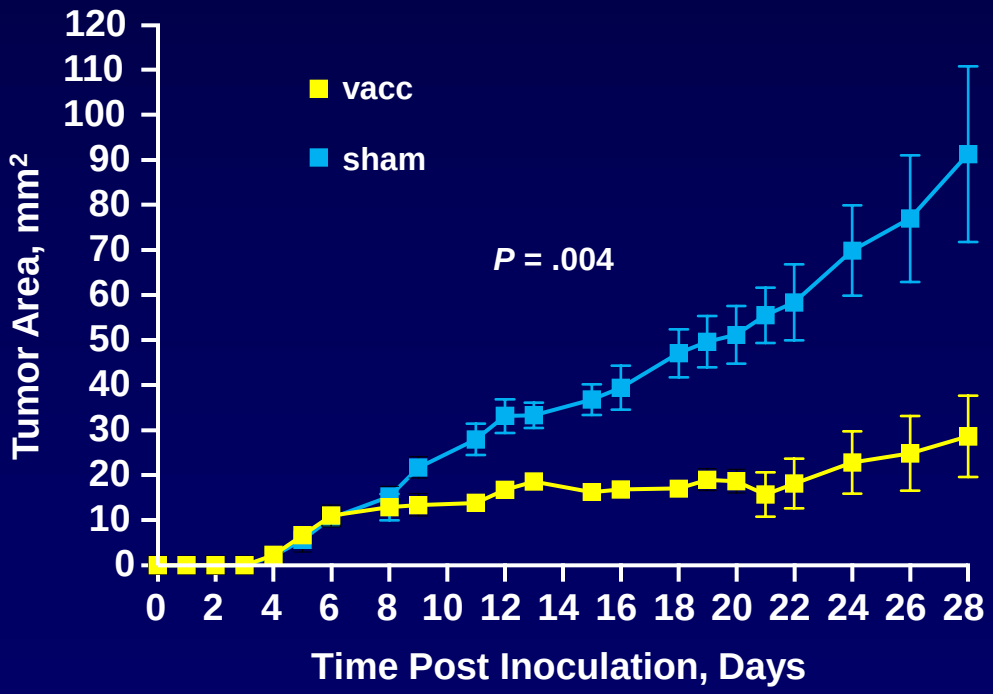
Basic Principles - *IDH*

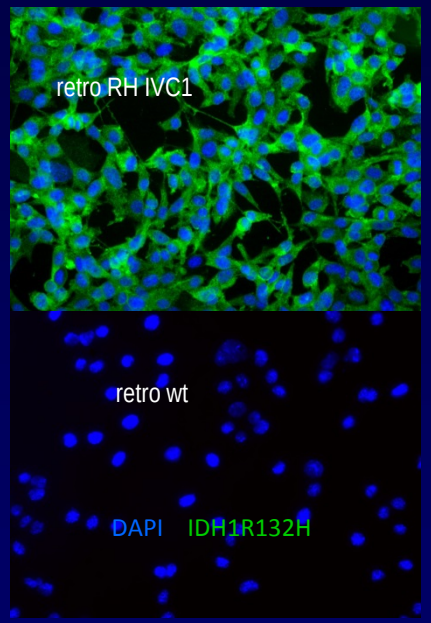
- ***IDH*** **is a diagnostic and prognostic biomarker[1]**
- **It separates two principle groups of glioma[2]**
- ***IDH1*** **may be a promising drug and immunotherapeutic target[3,4]**
- **In the RTOG 9402 trial, it showed predictive properties[5]**

1. Weller M, et al. *Neuro Oncol* 2013; 2. Weller M and Wick W. *Nat Rev Neurol.* 2014;10(2):68-70; 3.Schumacher T, et al. *Nature.* 2014;512(7514):324-327; 4. Röhle D, et al. *Science.* 2013;340(6132):626-630; 5. Cairncross JF, et al. *J Clin Oncol.* 2014;32(8):783-790.

Therapeutic IDH1R132H Peptide Vaccination Reduces IDH1R132H Tumor Growth





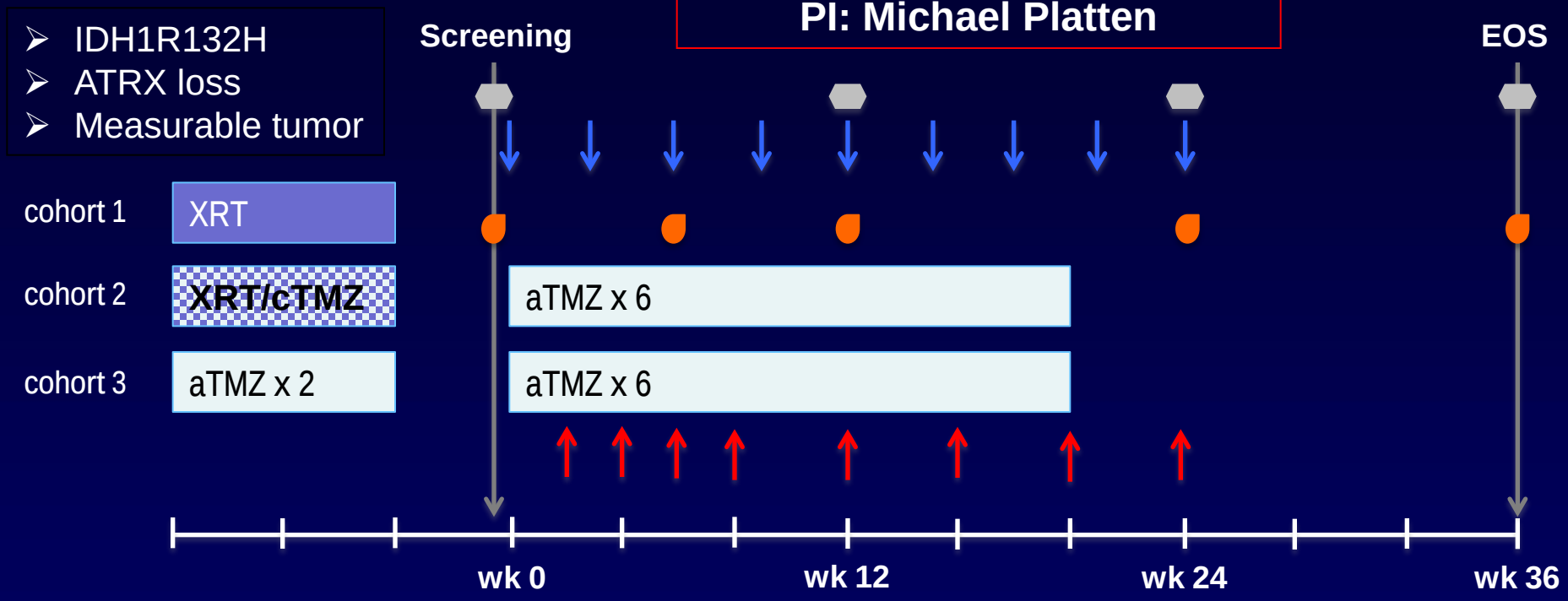


Schumacher T, et al. *Nature*. 2014;512(7514):324-327.

IDH1RpepvaccH (NCT-2013-0216, EudraCT 2014-000503-27)

PI: Michael Platten

- IDH1R132H
- ATRX loss
- Measurable tumor



XRT: radiotherapy (30 x 2 Gy; Mo-Fr)

aTMZ: adjuvant temozolomide (200 mg/m^2; d1-5 of 28-day cycles)

cTMZ: concomitant temozolomide (75 mg/m^2 daily for 6 weeks)

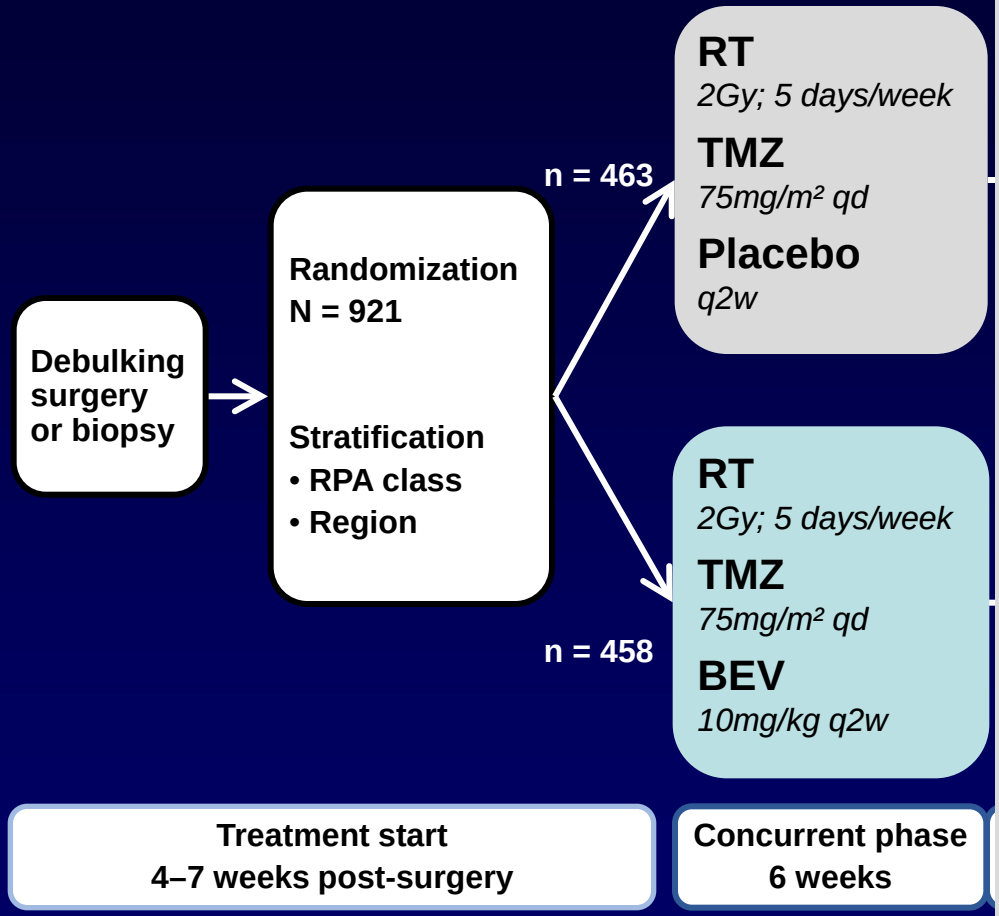
↓ anti PD-1 / PD-L1 (d1 of 21-day cycles)

↑ IDH1R132H vaccine with imiquimod wk 2,4,6,8,12,16,20,24)

⬣ MRI + 2-hydroxyglutarate (2HG) magnetic resonance spectroscopy (MRS)

● Immune monitoring (IDH1R132H antibody ELISA, EliSpot)

Study Design - AVAglio



Study Objectives

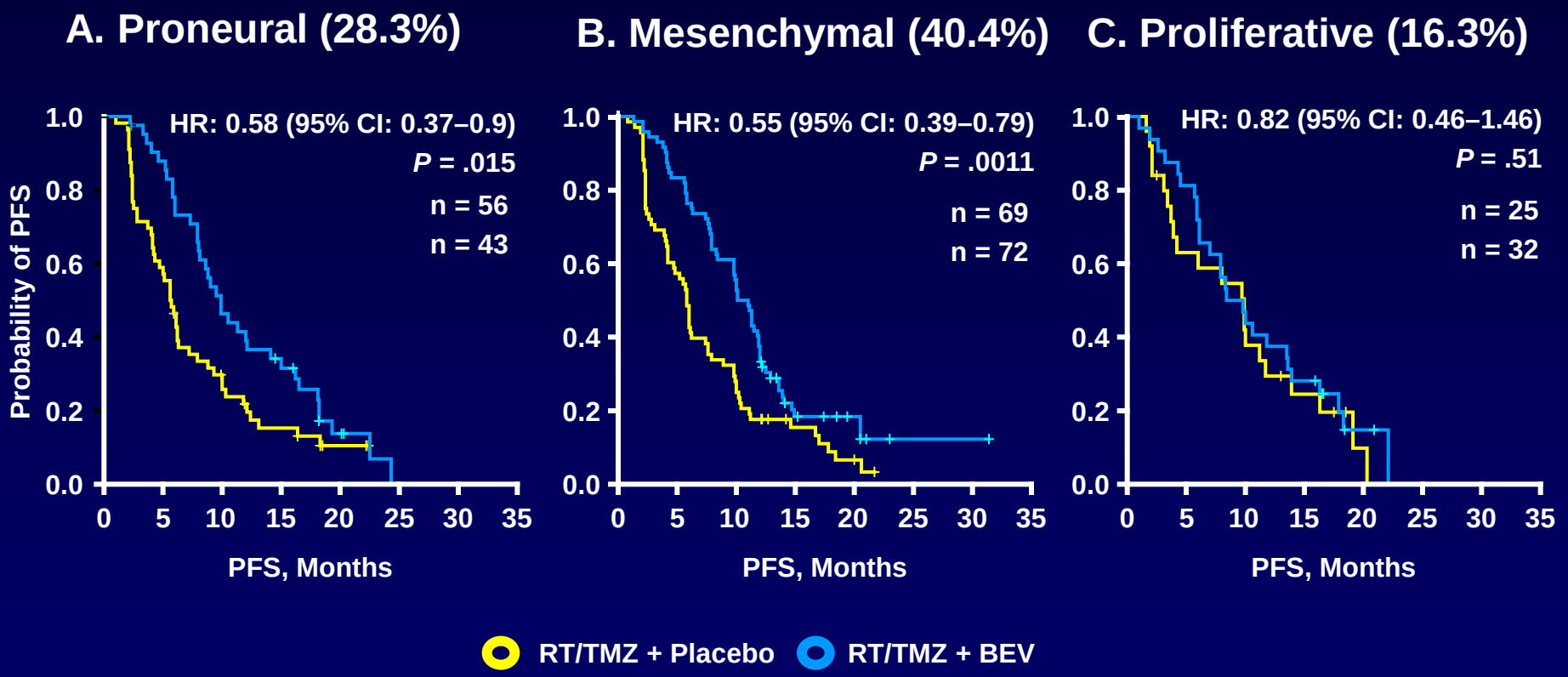
- **Co-primary objectives**
  - PFS (investigator assessed)
  - OS
- **Secondary objectives**
  - PFS (Independent Review Facility)
  - 1-year and 2-year survival rates
  - HRQoL
  - Safety

PD, progressive disease; qd, daily; q28d, every 28 days; q2w, every 2 weeks; q3w, every 3 weeks; RPA, recursive partitioning analysis; Tx, treatment

Chinot OL, et al. *N Engl J Med.* 2014;370(8):709-722.

Last patient in: March 2011

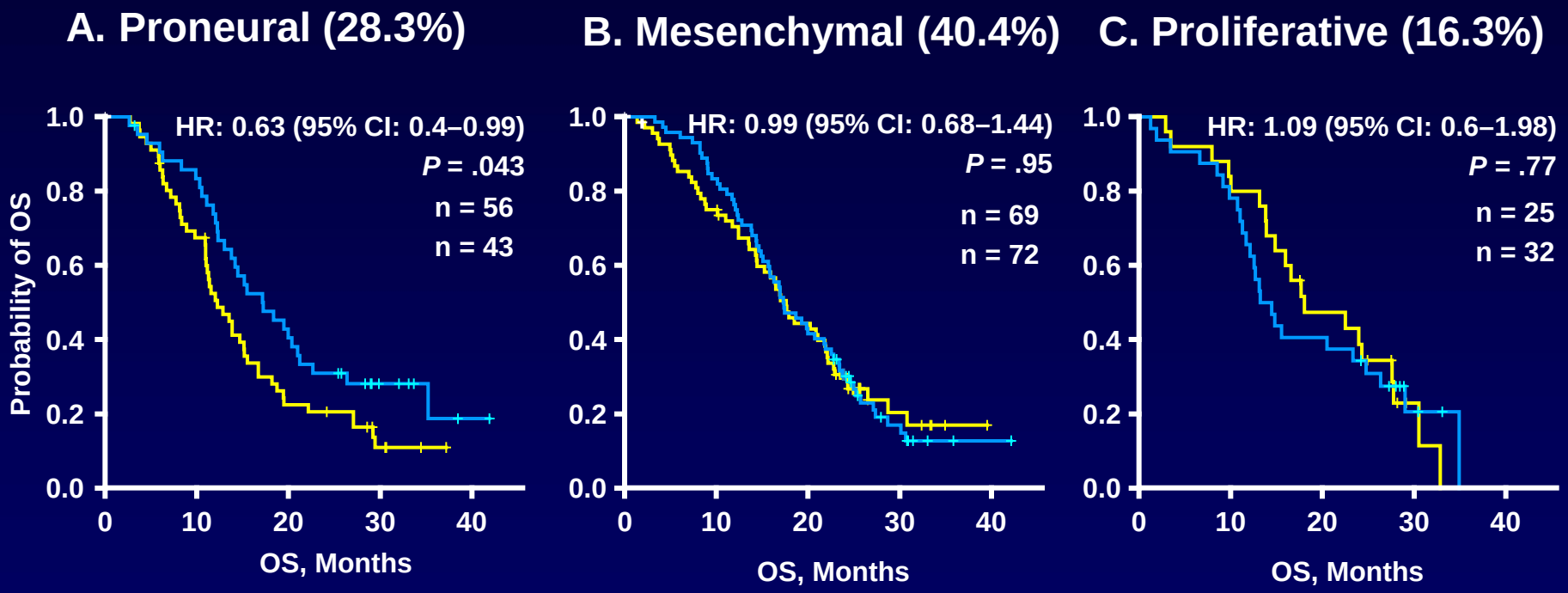
Bevacizumab Treatment Impacted PFS in Proneural and Mesenchymal Subtypes



Unclassified (n = 42, 12.3%) and *IDH1*-mutant samples (n = 10, 2.6%) not shown

Phillips H, et al. *J Clin Oncol.* 2014;32(5S): Abstract 2001.

Bevacizumab Treatment Impacted OS in the Proneural Subtype*



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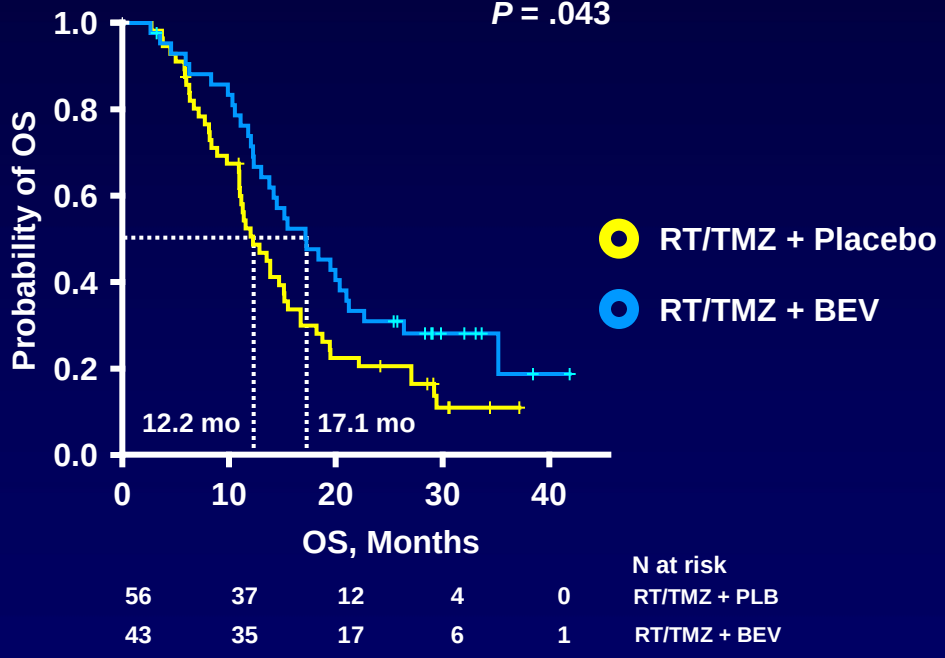
Bevacizumab Treatment Impacted OS in the Proneural Subtype

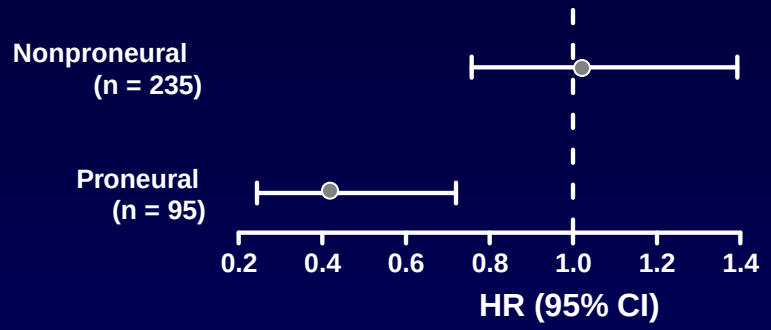
Univariate analysis of Proneural subtype

HR: 0.63 (95% CI: 0.4–0.99)

P = .043



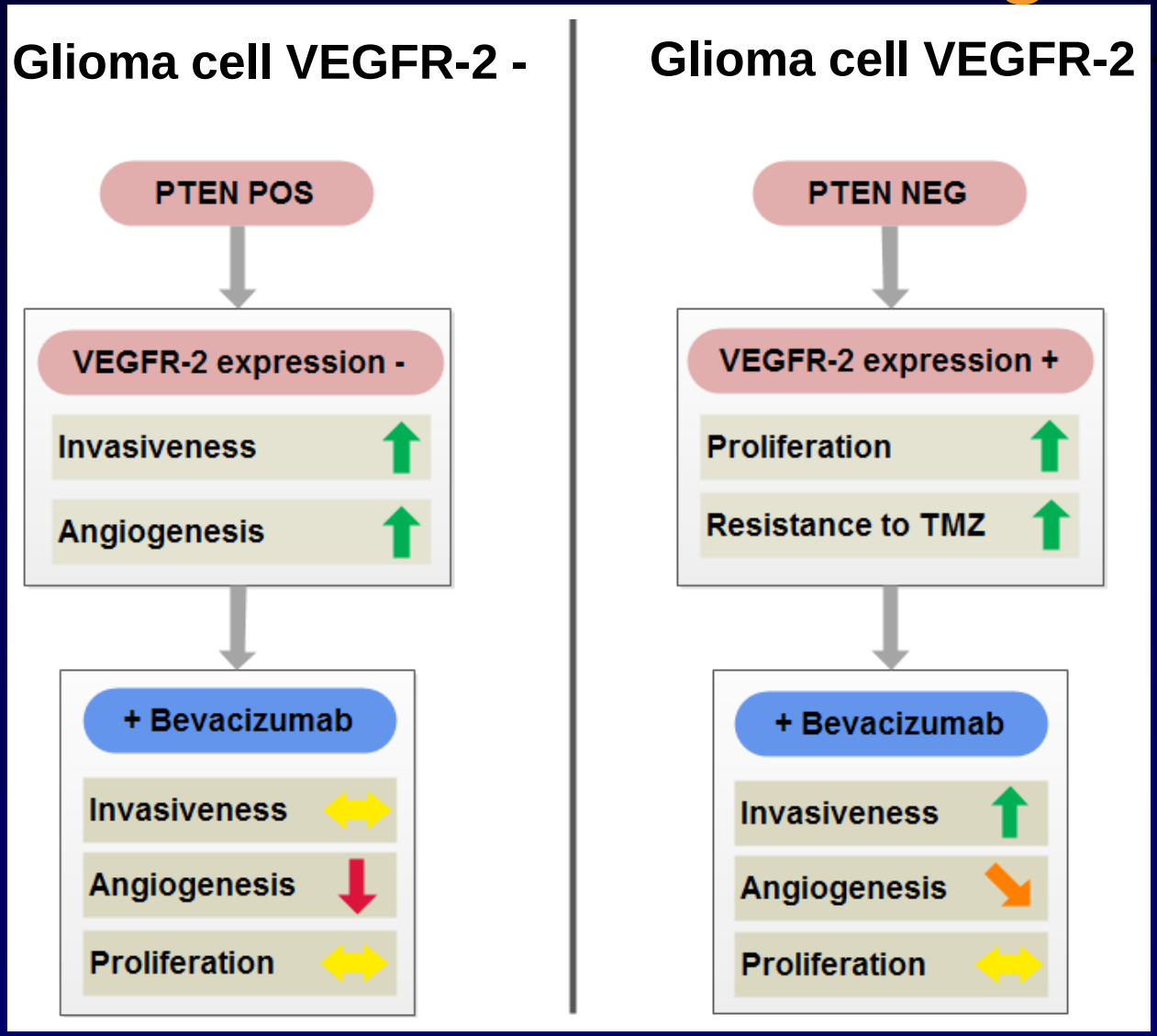
Multivariate* analysis of all BM-evaluable cases



- **Proneural tumors**
  - **Statistically significant BEV benefit**
  - **HR: 0.42 (95% CI: 0.24–0.72), *P* = .002**
- **Nonproneural tumors**
  - **No evidence for BEV effect**
  - **HR: 1.03 (95% CI: 0.76–1.39), *P* = .863**
- **Statistically significant interaction between proneural subtype and BEV (*P* = .012)**

- *Multivariate Cox-proportional hazards, covariates: age, corticosteroids, extent of resection, Karnofsky performance score, mini-mental state examination score, RPA class, *MGMT* status, World Health Organization performance score, gender
- n = 10 patients with *IDH1*-mutant tumors were excluded and n = 9 patients were excluded due to missing covariate information

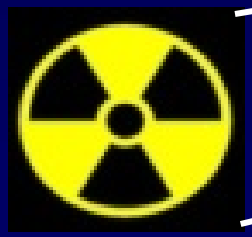
PTEN/VEGFR-2 Defines Therapeutically Relevant Glioblastoma Subgroups

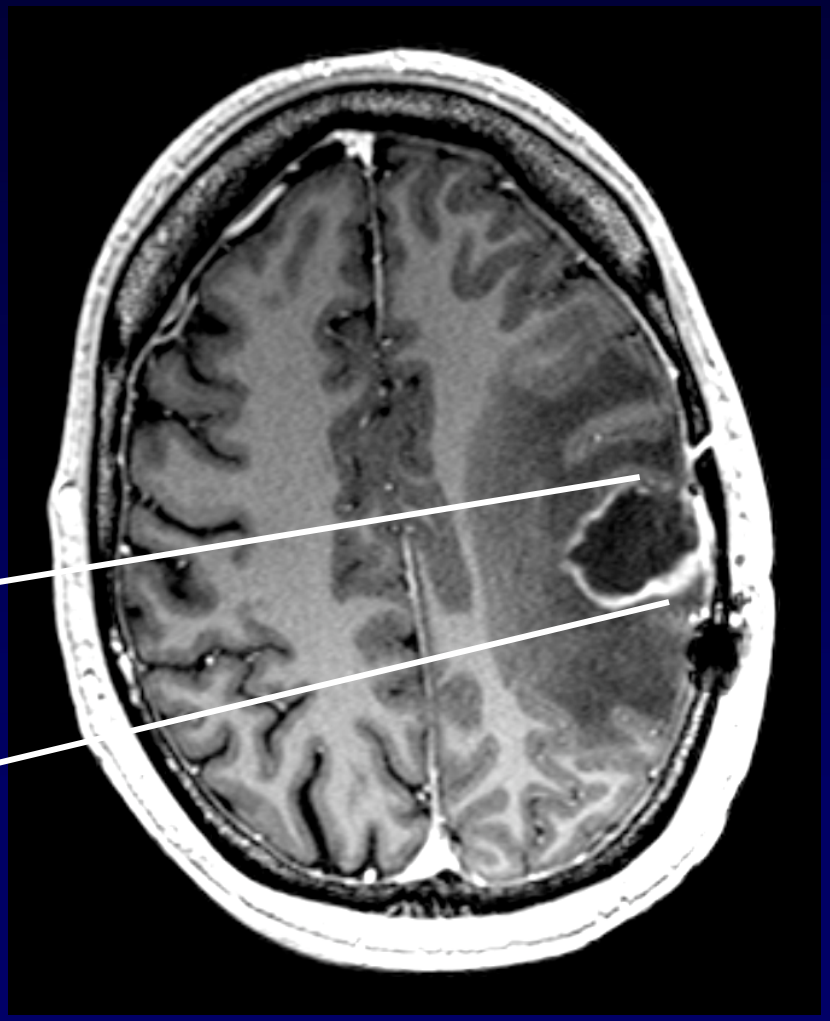


Weiler M, et al. EANO 2014. Abstract O06.

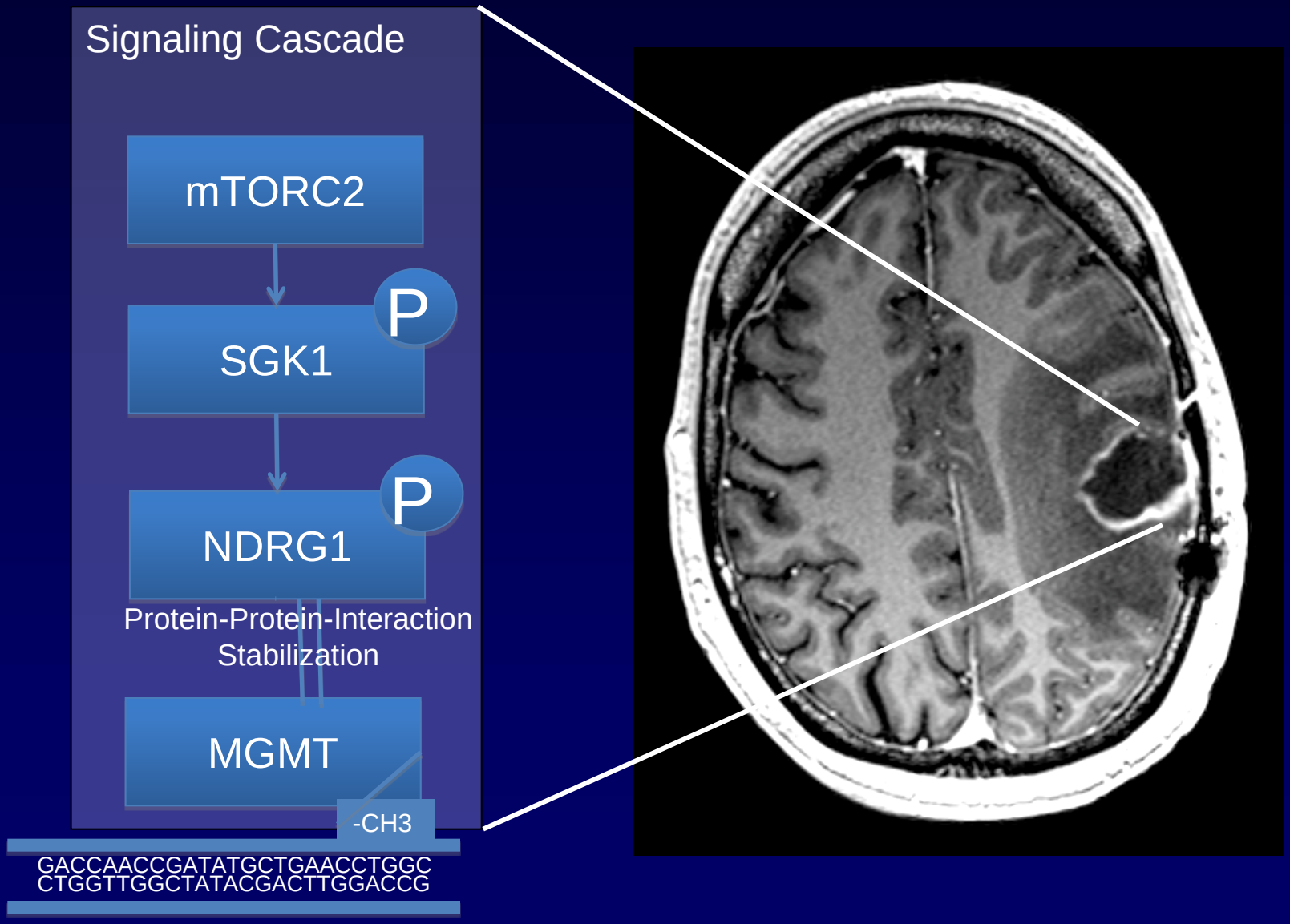
Adaptive Biomarkers: Tumor Hypoxia & O_2-Dependent Niches

Hypothesis:
Hypoxic microenvironments in glioblastoma serve as germ centers for more aggressive and treatment-resistant tumor cell phenotypes

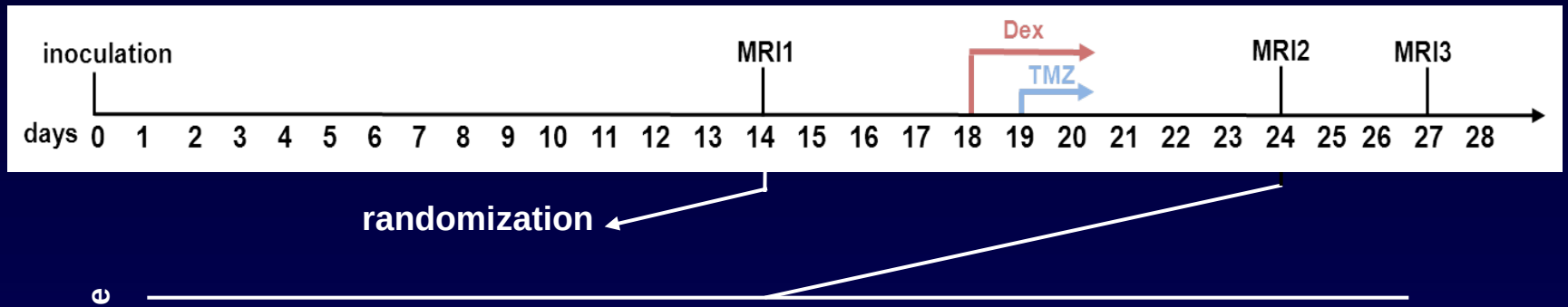


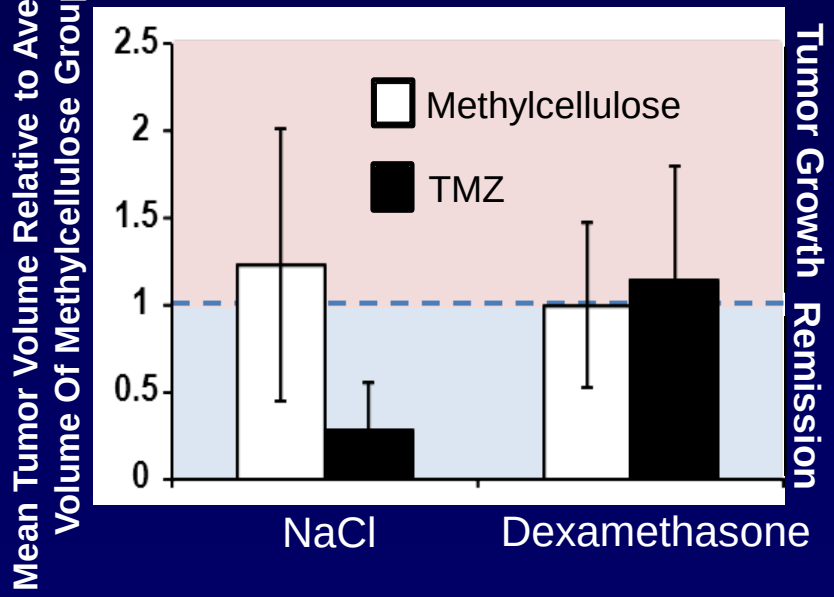


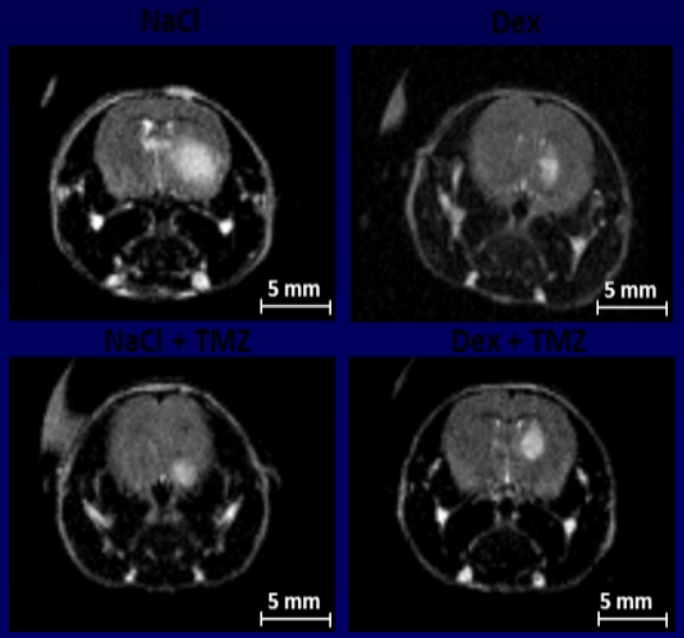
EGFR-Downstream Signaling Cascade – N-myc Downstream Regulated Gene (NDRG)



Resistance to TMZ is Enhanced by Corticosteroids



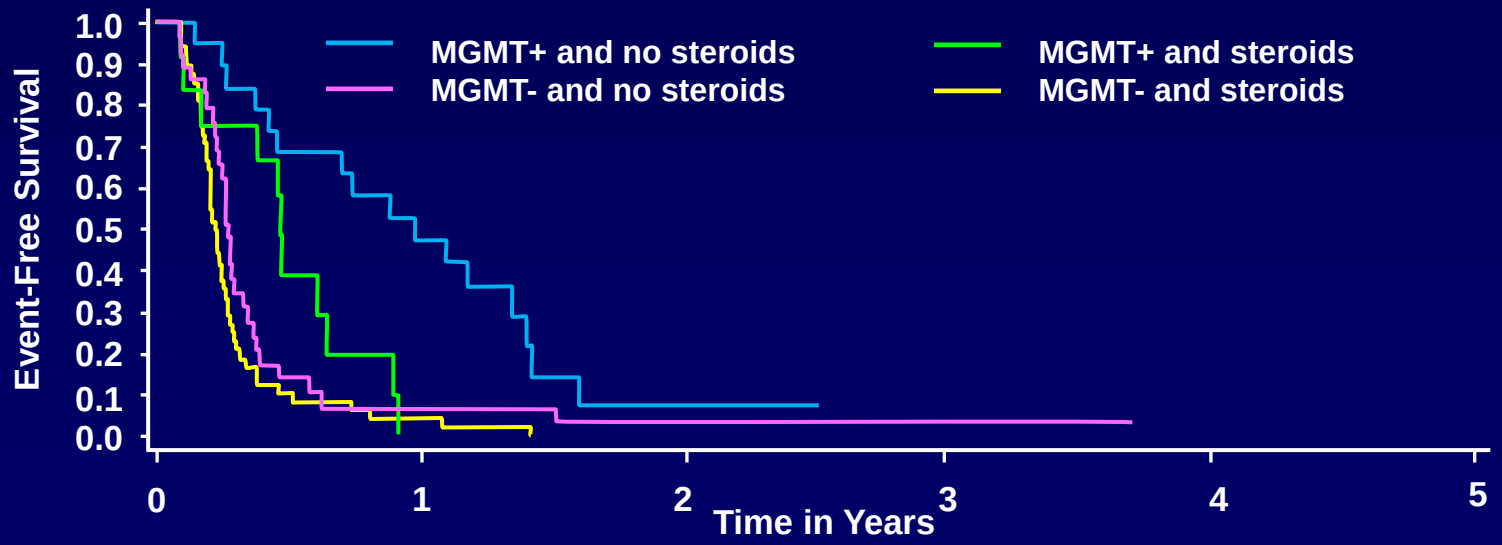




Steroids Eliminate the Advantage From *MGMT* Promoter Methylation

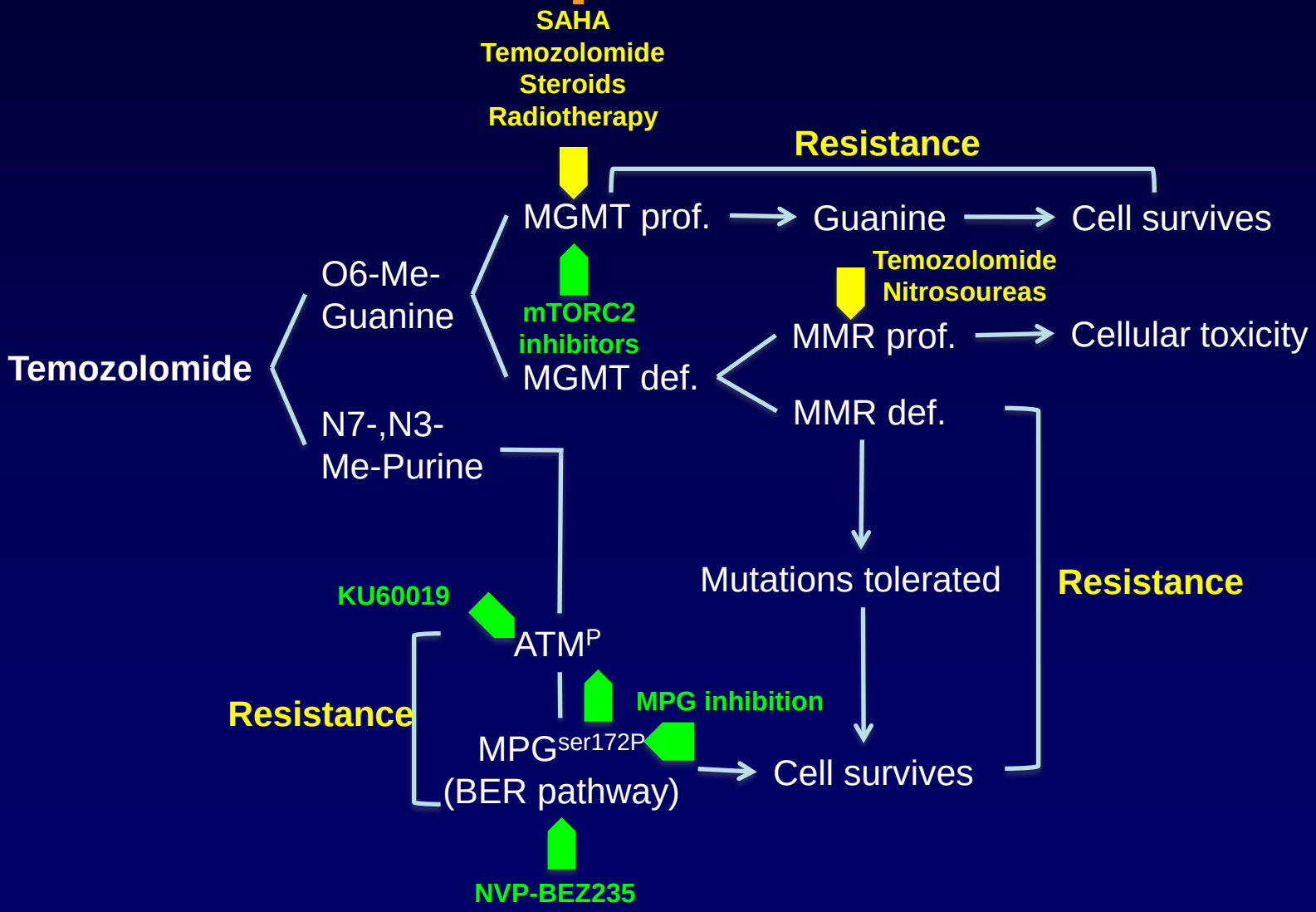
Event-free survival in elderly patients of the NOA-08 trial according to treatment and steroid use

NOA-08 Cohort*	Radiotherapy (n = 176)		TMZ (n = 195)	
Steroids	Yes (n = 140)	no (n = 36)	Yes (n = 98)	Yes (n = 97)
EFS, days (95% CI)	140 (128-154)	143 (112-202) *P* = .912	91 (84-101)	130 (100-169) *P* = .0001



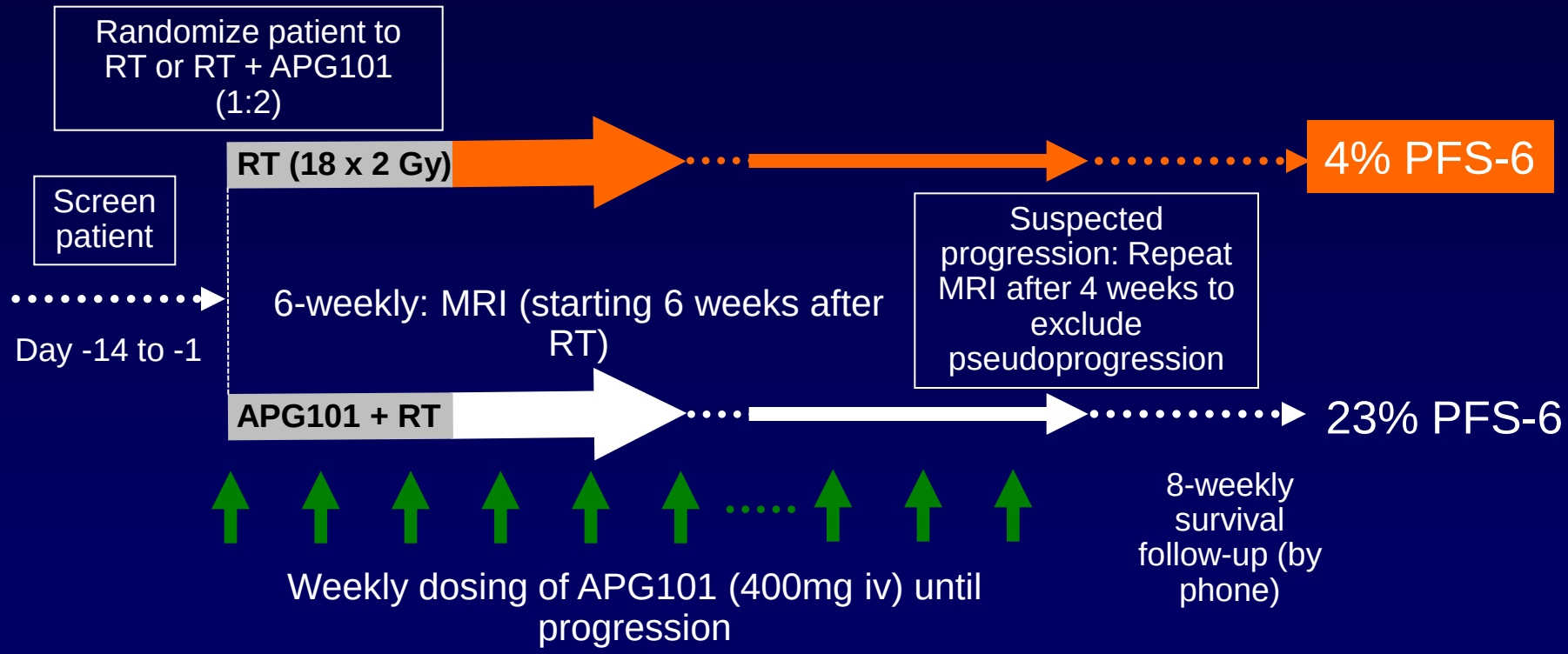
Weiler M, et al. *Proc Natl Acad Sci U S A*. 2014;111(1):409-414.

TMZ Resistance Is Modified at Multiple Levels



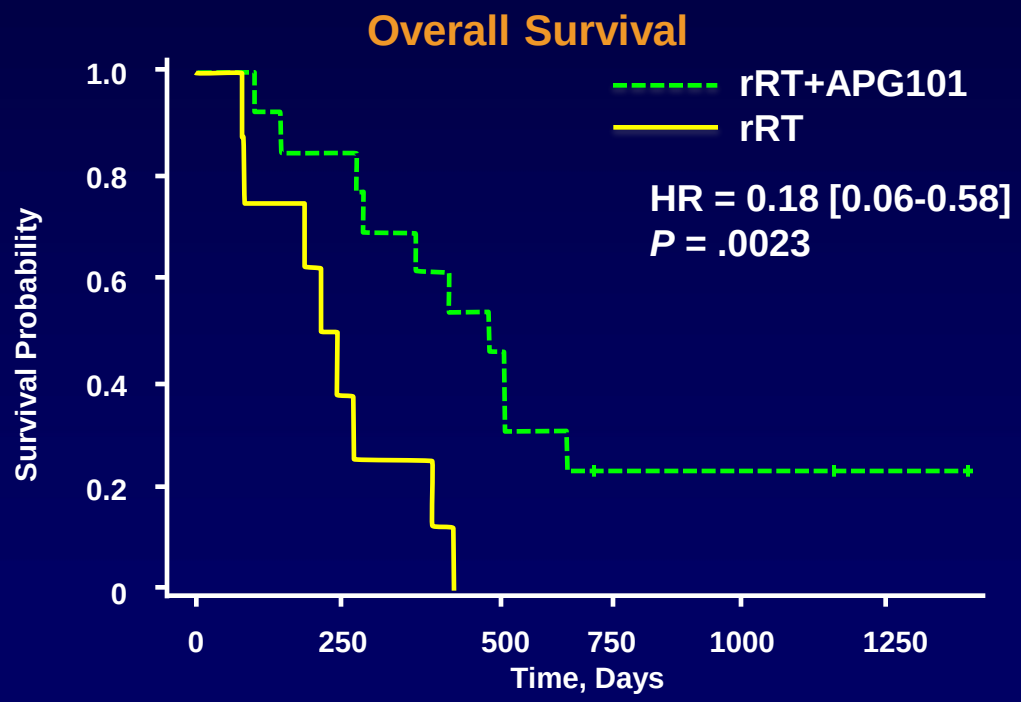
Wick W, et al. *Cancer Discov.* 2014;4(10):1120-1122.

APG-101: A Phase II, Randomized, Open-label, Multi-Center Study of Weekly APG-101 + Re-Irradiation Versus Re-Irradiation in the Treatment of Patients With First or Second Progression of Glioblastoma



APG101_CD_002: Biomarker for Overall Survival

- 450k arrays revealed differential methylation in CD95-pathway targets
- Development of a mass array for CpG2 in the CD95L promoter



CD95L Methylation Low

Wick W, et al. *Clin Cancer Res.* In Press.

Take-Home Messages

- **Alkylator resistance in glioblastoma is chiefly mediated by *MGMT*, but other factors (*NDRG1*) need consideration, eg, as therapeutic targets**
- ***IDH* is diagnostic, prognostic, and potentially predictive, as well as a novel therapeutic target**
- **Relevance of the proneural subtype in an independent cohort is needed to develop it as a biomarker; it has to be ascertained that patient cohorts with a potential detriment are readily discovered**
- **PTEN/VEGFR2 or CD95L have a revival, this time as potential predictive biomarkers in glioblastoma**