117PD

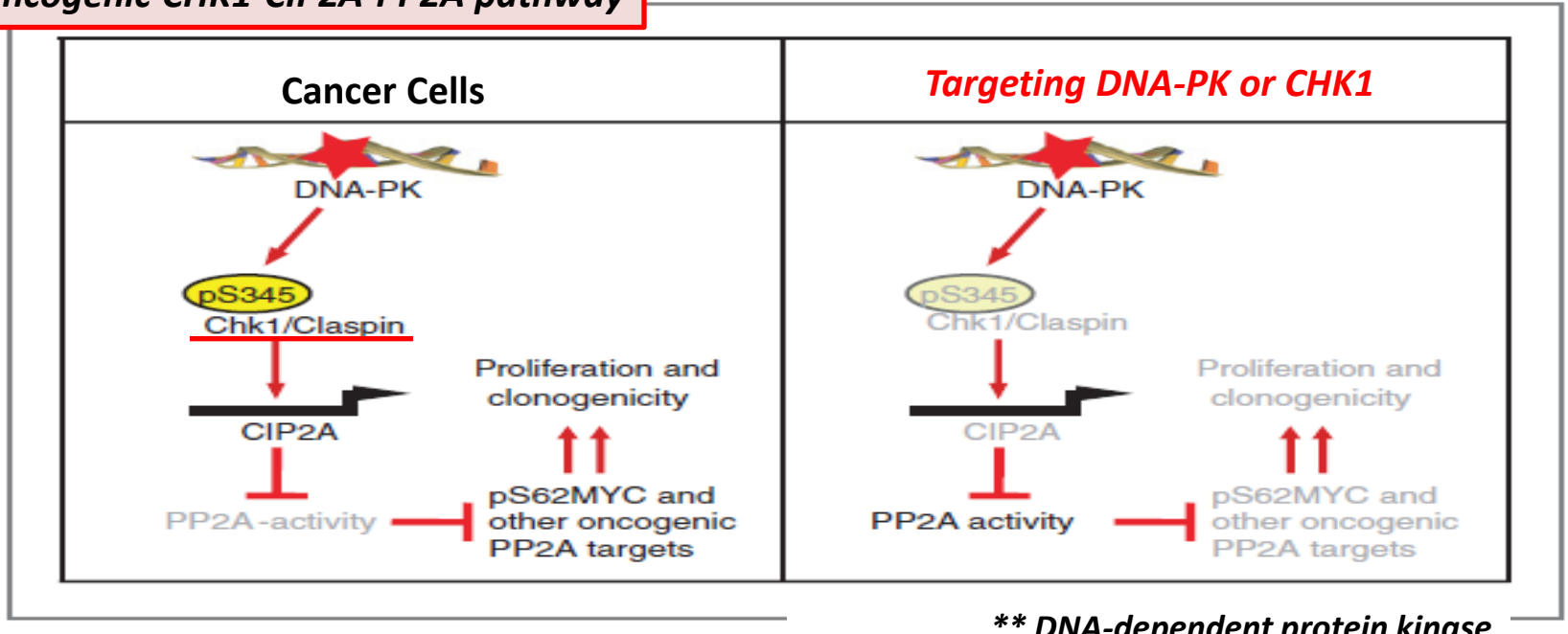
Clinical relevance of DNA damage modulator checkpoint kinase 1 (CHK1) and cancerous inhibitor of protein phosphatase 2A (CIP2A) in human gliomas

CHk1 Targeting Reactivates PP2A Tumor Suppressor Activity in Cancer Cells

***Anchit Khanna* et al, Cancer Res, 2013**

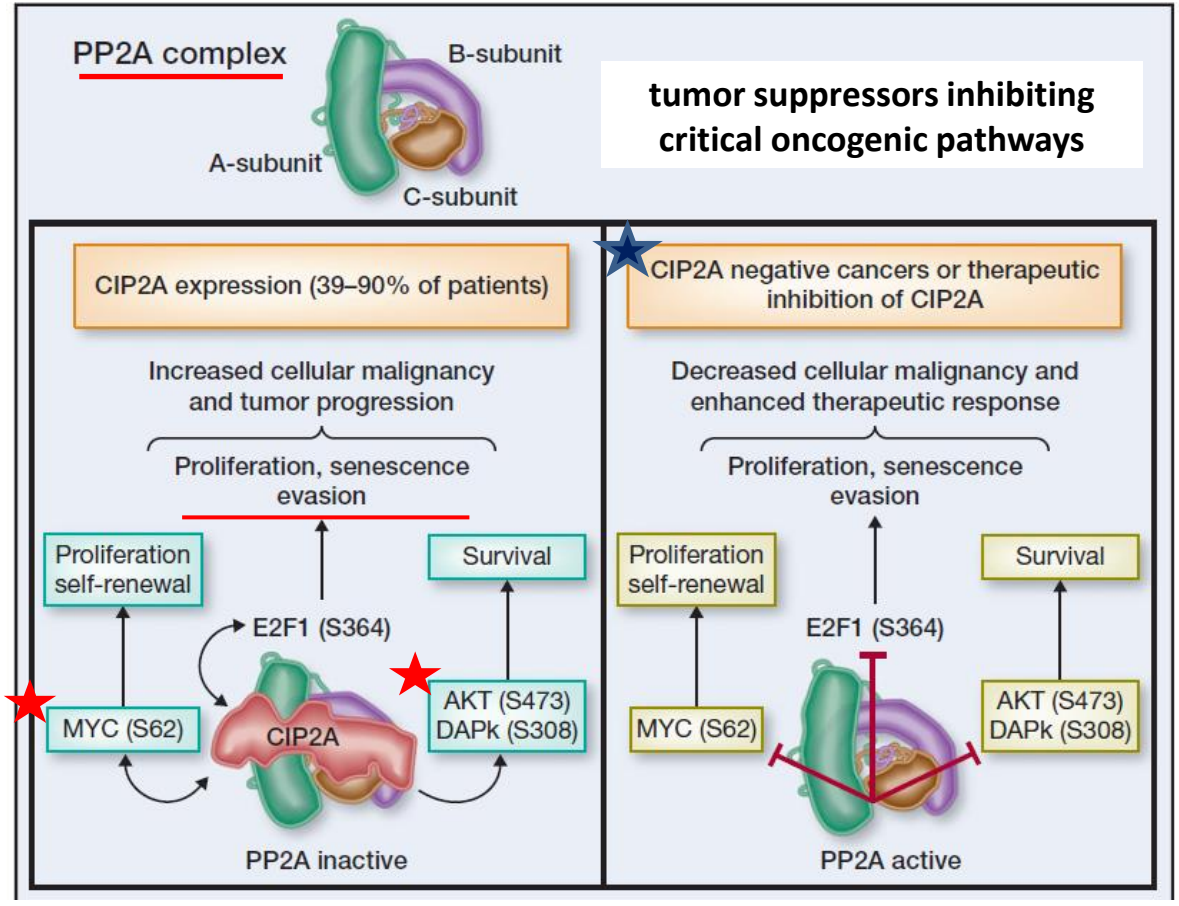
- ***Chronic activation of Chk1 in cancer cells promotes CIP2A transcription.***
- ***CIP2A-PP2A status in cancer cells as a PD marker for response to single agent CHK1-inhibitors.***

Oncogenic CHK1-CIP2A-PP2A pathway



*** DNA-dependent protein kinase***

CIP2A, a Potential Cancer Therapy Target



- ***Endogenous PP2A inhibitor in malignant cells***
- ***Amplified / overexpressed in 39%-90%*** **in almost all cancer types**
- ***Novel anticancer target based on PP2A reactivation***

Cancer Res, 2013

Clinical significance and biological function of CIP2A in human astrocytoma

Table I. Relationship between cancerous inhibitor of protein phosphatase 2A (CIP2A) and clinicopathological features

Clinical parameters		n	CIP2A Negative	CIP2A Positive	P-value
Age					
<45 years		67	33	34	0.301
≥45 years		68	27	41	
Gender					
Male		82	37	45	0.861
Female		53	23	30	
Grading					
I	**11.1%**	9	8	1	<0.001
II	**47.0%**	66	36	31	
III	***76.2%***	42	10	32	
IV	***61.1%***	18	7	11	

- ***CIP2A depletion*** **in astrocytoma cell lines** ***inhibit cell growth and anchorage-independent cell growth and increase apoptosis.***
- ***Molecular target*** **in human astrocytoma associated with biological aggressiveness**

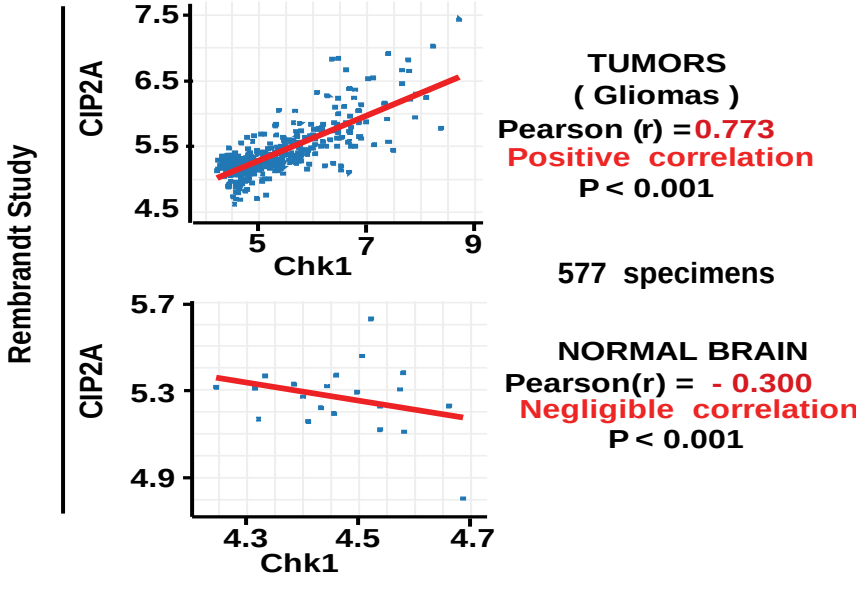
MOLECULAR MEDICINE REPORTS, 2013

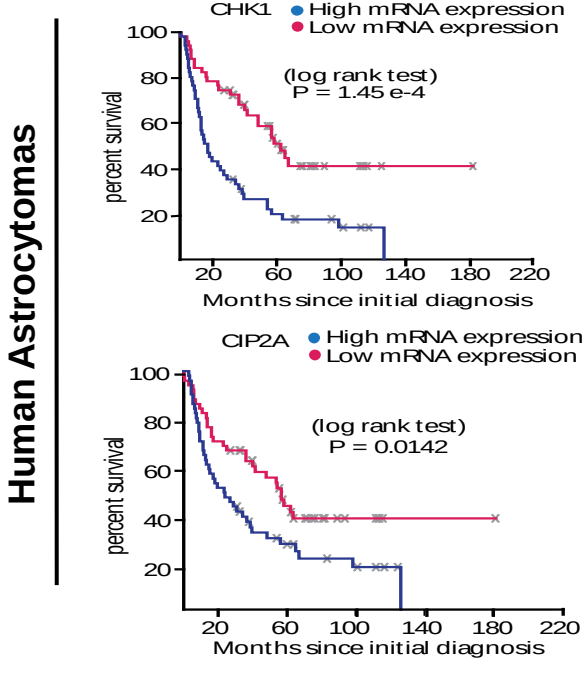
Clinical Relevance and Therapeutic role of Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) in Human Gliomas

Anchit Khanna 1 , Brett Stringer 2 , Bryan Day 2 , Kathleen Ensbey 2 , Andrew Boyd 2 , Kerrie McDonald 1 , John Pimanda1

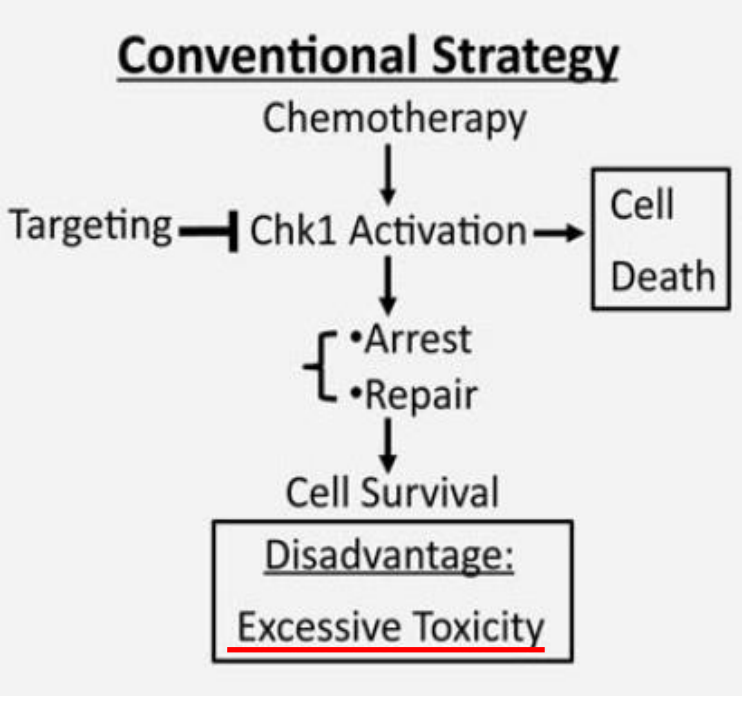
1. Lowy Cancer Research Centre, UNSW Medicine, University of New South Wales (UNSW), Sydney, Australia
2. QIMR-Berghofer Medical Research Institute, University of Queensland, Brisbane, Queensland, Australia

- ***Tumor-specific strong positive correlation for CHK1 and CIP2A expression in glioma***
- ***CHK1 and CIP2A as poor prognostic factors in glioma patients***





Conventional strategy with Checkpoint Kinase 1 (CHK1)

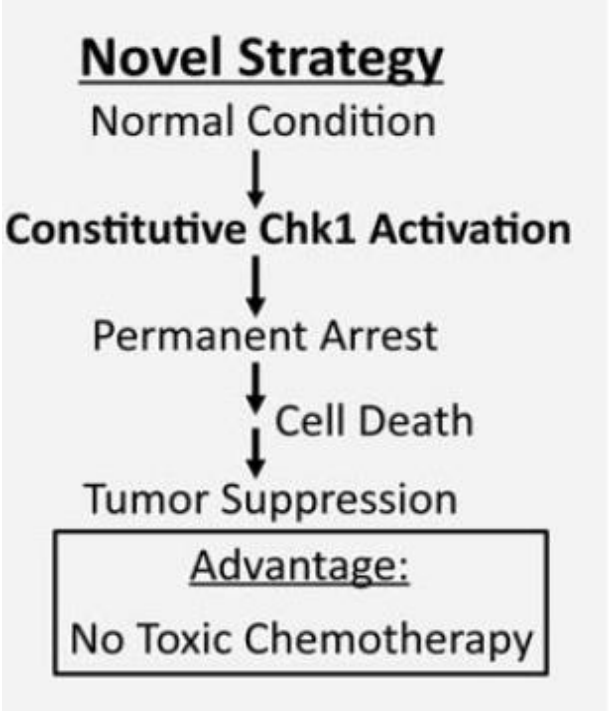


- **Serine/threonine kinase that coordinates various aspects of DNA repair**

- **Due to its central role in the DNA damage response (DDR), *CHK1* has engendered considerable interest as *a target for potentiating the efficacy of DNA-damaging chemotherapeutics and RT*.**

Pharmacology & Therapeutics, 2014

Mechanism of action for CHK1 inhibitor monotherapy



- **CHk1 is *constitutively active* in a variety of human tumors.**
- **CHK1 is *essential for cell cycle progression and survival* even *in the absence of any DNA-damaging treatment* in tumor cells.**
- **The novel strategy should significantly *reduce the toxicity of DNA-damaging agents*.**

Int. J. Cancer, 2014

CHK1 inhibitor monotherapy

LY260636 (Dual CHK1/CHK2 inhibitor)	**the only agent to date to be clinically evaluated as monotherapy**	**Phase 1 trial recruiting for single agent in advanced cancers, squamous cell and head and neck cancers (NCT01115790)**

- **A number of new generation CHK1 inhibitors have shown deleterious effects on normal cells.**

- **Identification and targeting of *cancer specific effectors of CHK1 signaling* has emerged as an attractive therapeutic alternative.**

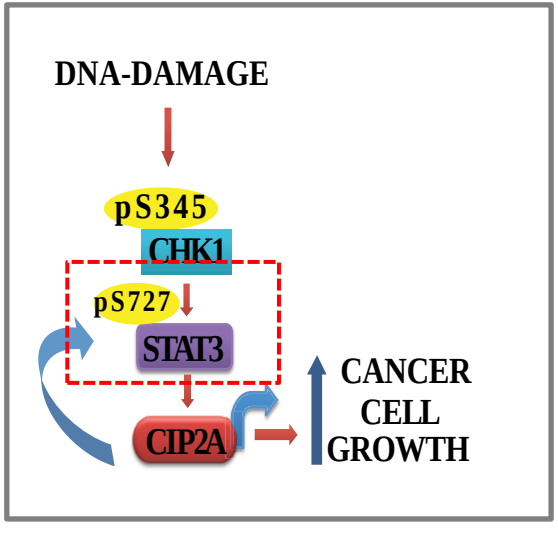
Pharmacology & Therapeutics, 2014

Clinical Relevance and Therapeutic role of Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) in Human Gliomas

Anchit Khanna 1 , Brett Stringer 2 , Bryan Day 2 , Kathleen Ensbey 2 , Andrew Boyd 2 , Kerrie McDonald 1 , John Pimanda1

1. Lowy Cancer Research Centre, UNSW Medicine, University of New South Wales (UNSW), Sydney, Australia
2. QIMR-Berghofer Medical Research Institute, University of Queensland, Brisbane, Queensland, Australia

Significance

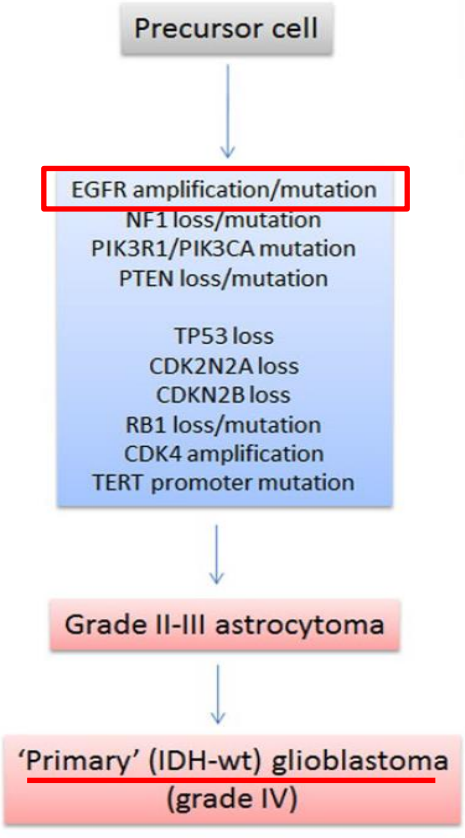


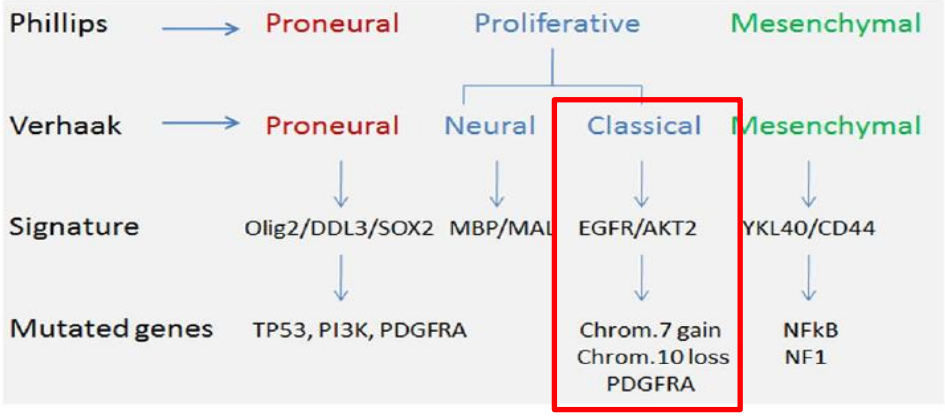
- ***First identification of STAT3 as a transcriptional mediator for CHK-CIP2A pathway in human GBMs***
- ***Identification of CIP2A as a cancer specific target downstream of constitutively active CHK1 and STAT3 signaling in human GBMs***
- ***Suggestion of CIP2A as a biomarker to stratify patients for CHK1 and/or STAT3 inhibitor in human GBMs***

119PD

Role of EGFR as a prognostic factor in primary glioblastoma

EGFR in Primary Glioblastoma





- **Amplified / Over-expressed in Primary GBM (30%–70%)**
- **EGFRvIII in 50% of GBMs with EGFR amplification**
- **Each alteration in EGFR has important clinical implications for diagnosis, prognosis, or therapeutic investigation.**

Acta Neuropathol, 2015
Cancers, 2013

Role of EGFR as a Prognostic Factor in Primary Glioblastoma

Karthikeyan Perumal[1], Sushama V Patil[2], Mahadev Potharaju[1]
1Radiation Oncology, Apollo Specialty Hospital, Chennai, IN,
2Surgical & Neuropathology, Apollo Specialty Hospital, Chennai, IN

- **44 Primary GBM patients who underwent gross total excision and undergoing adjuvant CCRT with TMZ**
- **Quantitative & Qualitative evaluation of EGFR expression status using *IHC***
- ***No statistically significant impact based on EGFR status for the RFS or OS***
- ***Complex relationship of EGFR positivity, treatment response, RFS and OS***

Well-designed studies **are necessary to determine the value of EGFR markers in patients with GBM.**

ARTICLE

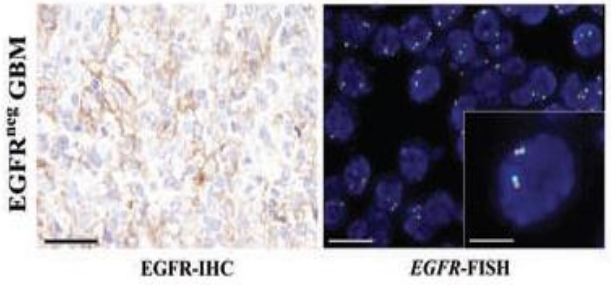
EGFR Amplified and Overexpressing Glioblastomas and Association With Better Response to Adjuvant Metronomic Temozolomide

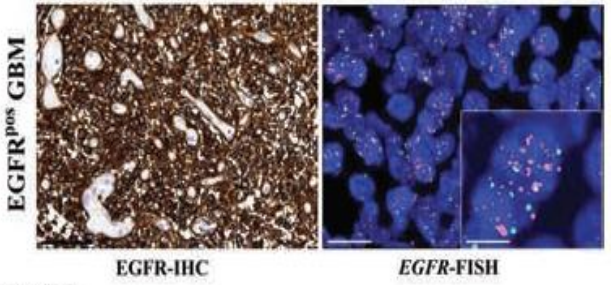
J Natl Cancer Inst, 2015

Manuela Cominelli*, Salvatore Grisanti*, Stefania Mazzoleni, Caterina Branca, Luciano Buttolo, Daniela Furlan, Barbara Liserre, Maria Fausta Bonetti, Daniela Medicina, Vilma Pellegrini, Michela Buglione, Roberto Liserre, Serena Pellegatta, Gaetano Finocchiaro, Piero Dalerba, Fabio Facchetti, Marina Pizzi, Rossella Galli, Pietro Luigi Poliani

Affiliations of authors: Pathology (MC, BL, MFB, DM, VP, FF, PLP) and Pharmacology Units (CB, MP), Department of Molecular and Translational Medicine, University of Brescia and National Institute of Neuroscience, Italy; Medical Oncology (SG), Neurosurgery (LB), Radiation Oncology (MB), and Neuroradiology Departments (RL), Spedali Civili of Brescia, University of Brescia, Italy; Neural Stem Cell Biology Unit, Division of Regenerative Medicine, Stem Cells & Gene Therapy, San Raffaele Scientific Institute, Milan (SM, RG); Pathology Unit, Department of Surgical and Morphological Sciences, University of Insubria, Italy (DF); Neurological Institute Besta, Milan, Italy (SP, GF); Herbert Irving Comprehensive Cancer Center, Department of Pathology & Cell Biology and Department of Medicine, Division of Digestive and Liver Diseases, Columbia University, New York, NY (PD); IRCCS San Camillo Hospital, Venice, Italy (MP).
* Authors contributed equally to this work.

- ***EGFR-amplified/overexpressing GBMs strongly benefit from metronomic TMZ–based therapies*** **due to activated EGFR/PI3K/Akt axis.**
- ***EGFR amplification*** **: FISH & IHC**



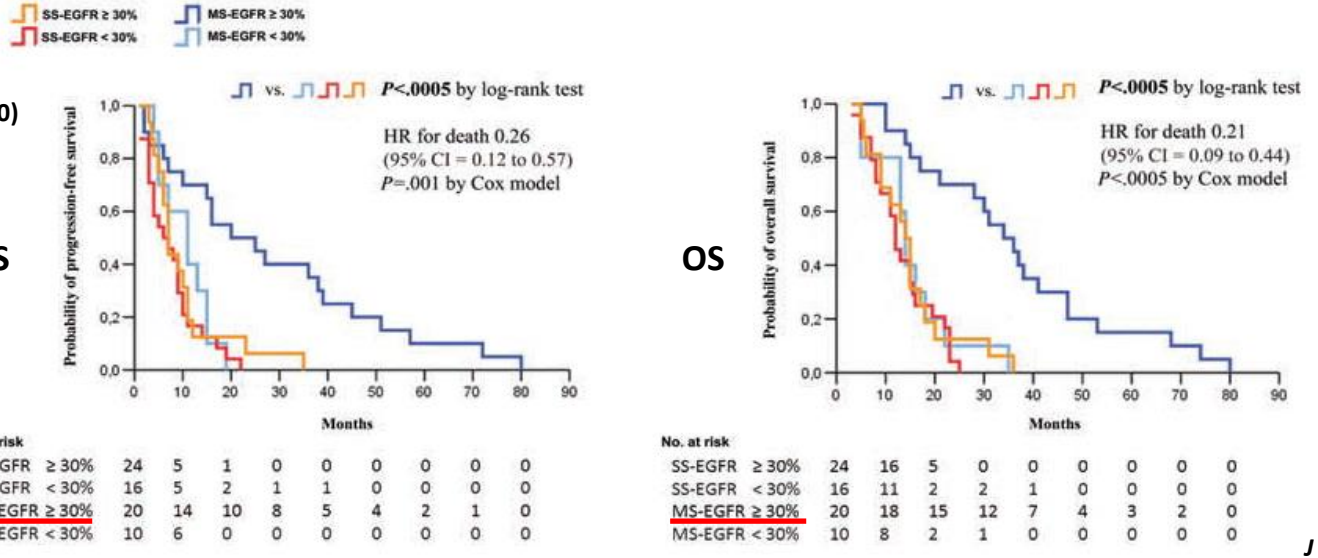


**** EGFR expression in more than 30% of tumor cells is highly predictive of EGFR amplification.**

	Immunohistochemistry: EGFR ≥ 30%	Immunohistochemistry: EGFR < 30%
FISH: Not amplified	14 (31.8%)	23 (88.5%)
FISH: Amplified	30 (68.2%)	3 (11.5%)

***P*<.0005**, Pearson's chi square test

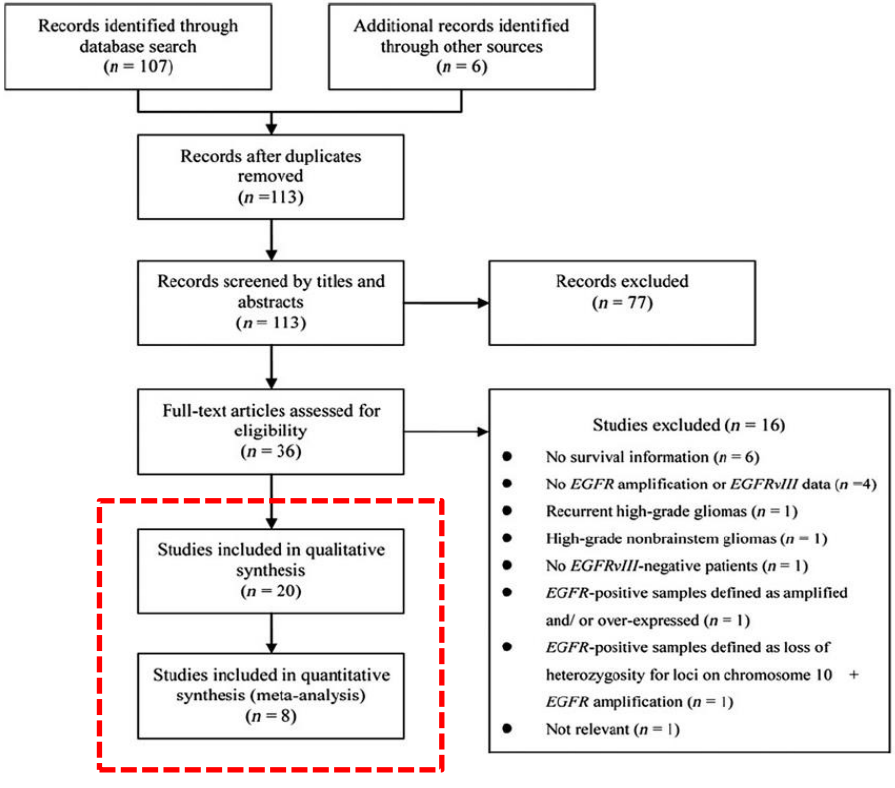
MS = metronomic (n = 30)
SS = standard (n=40)



J Natl Cancer Inst, 2015

Prognostic value of EGFR amplification and EGFRvIII in glioblastoma: *Meta-analysis*

Acta Neurol Scand, 2015



*** GBM : 15 studies**

*** Anaplastic astrocytoma : 5 studies**

Table 1 Summary of the studies included in the meta-analysis

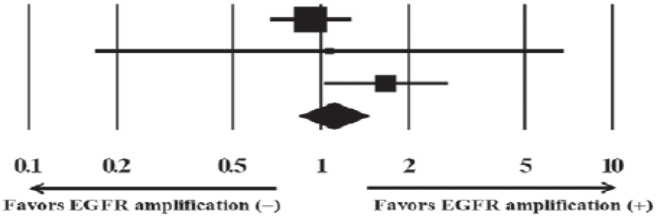
First author (year of publication)	Study type	Number of cases
Glioblastoma		
Weller (2014)	Cohort	184
Bieńkowski (2013)	Cohort	83
Lv (2012)	Prospective phase II trial	35
Srividya (2010)	Cohort	140
Viana-Pereira (2008)	Cohort	55
Pelloski (2007)	NA	268
Houillier (2006)	Cohort	220
Layfield (2006)	Retrospective	32
Kleinschmidt-DeMasters (2005)	Cohort	20
Quan (2005)	Retrospective	107
Shih (2005)	NA	75
Batchelor (2004)	Cohort	140
Aldape (2004)	NA	105
Shinojima (2003)	Cohort	87
Hurtt (1992)	NA	14
Torp (1992)	NA	20
Anaplastic astrocytoma		
Gulati (2010)	Retrospective	31
Kouwenhoven (2009)	RCT	202
Idbaih (2008)	NA	52
Aldape (2004)	NA	63
Smith (2001)	NA	63

Prognostic value of EGFR amplification and EGFRvIII in glioblastoma: *Meta-analysis*

Acta Neurol Scand, 2015

- **No significant difference in OS between EGFR amplification (+) and EGFR amplification (-) GBMs**

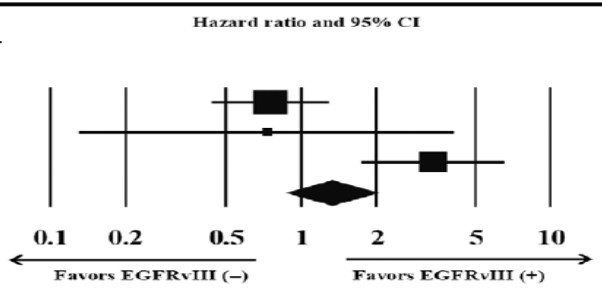
Study name	Hazard ratio	Lower limit	Upper limit	Z	P	Relative weight (Fixed)
Srividya (2010)	0.920	0.668	1.267	−0.511	0.609	68.33
Viana-Pereira (2008)	1.070	0.168	6.812	0.072	0.943	2.04
Shinojima (2003)	1.670	1.028	2.714	2.070	0.038	29.63
Pooled	**1.101**	**0.845**	**1.434**	**0.715**	**0.475**	



Heterogeneity: $Q = 4.04$, df(Q) = 2, $P = 0.133$, $I^2 = 50.51\%$

- **No significant difference in OS between EGFRvIII (+) and EGFRvIII (-) GBMs**

Study name	Hazard ratio	Lower limit	Upper limit	Z	P	Relative weight (Fixed)
Weller (2014)	0.750	0.438	1.284	−1.048	0.294	68.33
Viana-Pereira (2008)	0.730	0.130	4.095	−0.358	0.721	2.04
Pelloski (2007)	3.370	1.744	6.513	3.614	0.000	29.63
Pooled	**1.321**	**0.881**	**1.981**	**1.347**	**0.178**	



Heterogeneity: $Q = 12.47$, df(Q) = 2, $P = 0.002$, $I^2 = 83.96\%$

EGFR as a prognostic factor in primary GBM

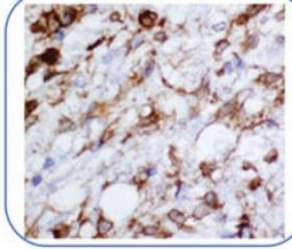
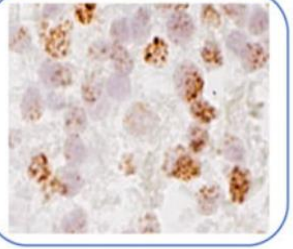
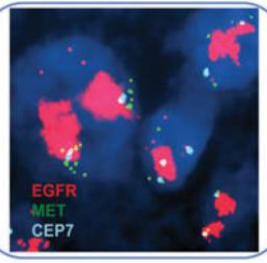
- **Multiple reports over EGFR alterations (overexpression, amplification, mutated form) and prognosis in patients with GBM have yielded *conflicting results*.**

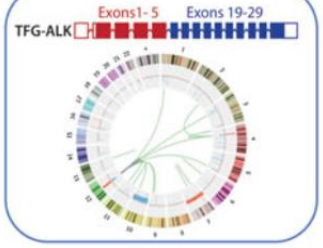
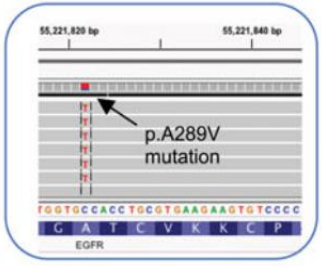
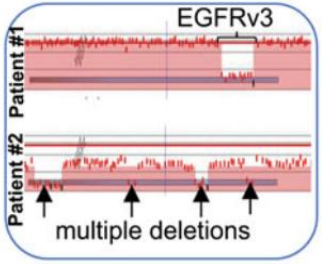
- **Some studies have shown an *adverse correlation*, while others indicated a *neutral or even favorable association* with longer survival.**

Limitations of previous studies exploring EGFR status

- **Significant heterogeneity among tumors among the included studies**
- ***Different methods for determining EGFR amplification and EGFRvIII expression***

Assays for EGFR assessment in GBM

	Diagnostic Assay	Characteristics
IHC		*Expression (Protein)* WT and EGFRvIII Single Cell Rapid Low Investment
CISH		*Copy number* Single Cell Rapid Low Investment
FISH		*Copy number/Fusion* Single Cell Low multiplex possible Moderate Investment

Anchored Multiplex PCR		*Fusion/Mutation/Exp* Multiplex Targeted Rapid Low cost
Targeted Exome Seq		*Mutation/Copy/Fusion* Multiplex Targeted Limited Copy/Fusion
Whole genome aCGH / SNP		*Copy number* Multiplex Whole genome Intragenic deletions Subclonal limitations

Neuro-Oncology, 2014