EGFR EXPRESSION AND AUTOPHAGY MODULATION IN GLIOBLASTOMA: IN VITRO RESULTS AND RELEVANCE FOR A CLINICAL SERIES

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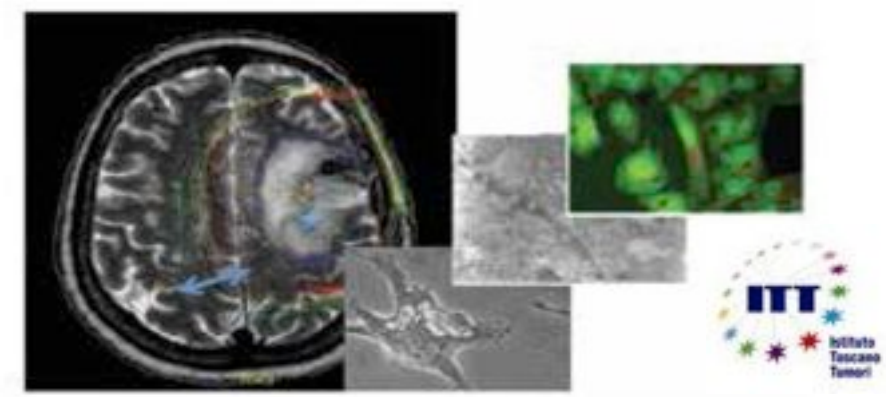

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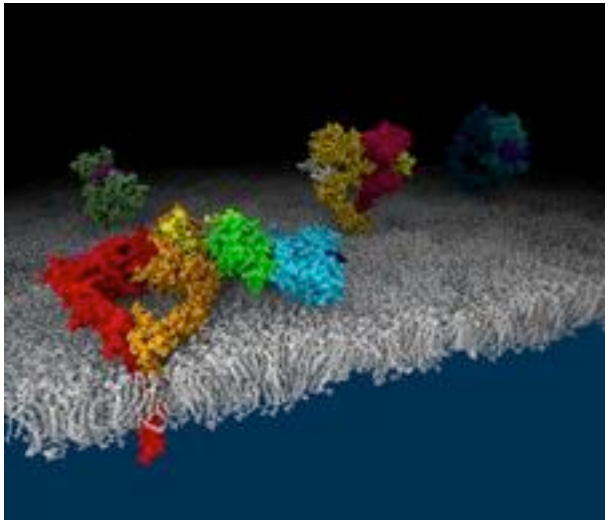
Background

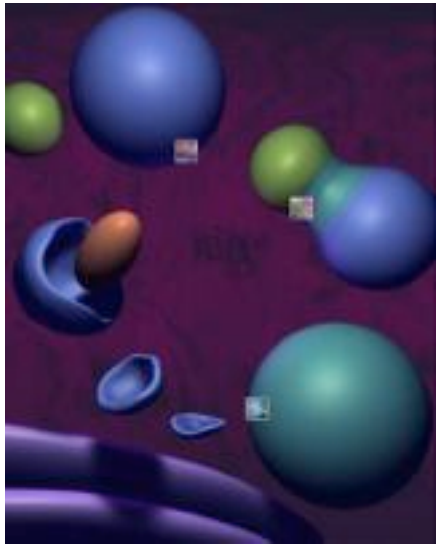
✓ *Locus amplification, gene overexpression, and genetic mutations of epidermal growth factor receptor (EGFR) exist in the majority of Glioblastomas (GB).*

✓ *Consequent modifications of its downstream signal pathway, involve also the down-regulation of the autophagy process, thus promoting the aggressive phenotype and radiation resistance of this tumor.*

Aim

*We **analize the interactions between EGFR expression and autophagy** in GB throughout IN VITRO experiments and its relevance in the CLINICAL setting.*





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Method and Materials

➢*IN VITRO MODULATION of EGFR and AUTOPHAGY :*

✓***EGFR expression profile and autophagy were modulated in human T98G and U373MG-GB cell coltures.***

✓***Cell migration capability was evaluated by a migration assay.***

✓***Radiosensitivity was tested by clonogenic assay after IR treatment.***

✓***Inhibition of EGFR expression and autophagy was achieved by specific siRNAs (siEGFR and siATG7).***

✓ ***Autophagy induction was obtained with a m-TOR inhibitor (Rapamycin.)***

Combined EGFR and Autophagy Modulation Impairs Cell Migration and Enhances Radiosensitivity in Human Glioblastoma Cells. ***Palumbo S****, Tini P, Toscano M, et Al.J Cell Physiol. 2014 Apr 1*

Method and Materials

➢*RELEVANCE IN THE CLINICAL SETTING EGFR and AUTOPHAGY MARKERS :*

✓ ***The clinical relevance of these markers was investigated out of a series of 156 consecutive GB patients undergoing a standard RT-TMZ protocol.***

✓***We analyzed the expression of EGFR (156 pts) and of the autophagy protein Beclin-1 (Beclin-1 Protein Cytoplasmatic Expression: BPCE) (81pts) on surgical samples.***

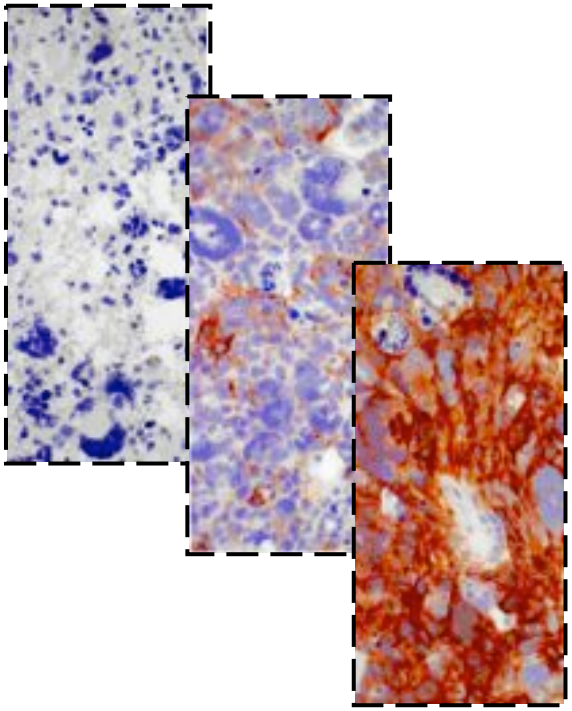
✓***We clustered patients according an expression score for EGFR and BPCE.***

✓***EGFR expression and BPCE were analyzed to find out potential correlation with incidence of multifocal disease and Overall survival (OS).***

Method and Materials

EGFR espression score

✓ *A Neuropathologist evaluated EGFR expression on surgical samples by IHC :*



IHC Intensity **+** ***% positive cells***

EGFR espression score

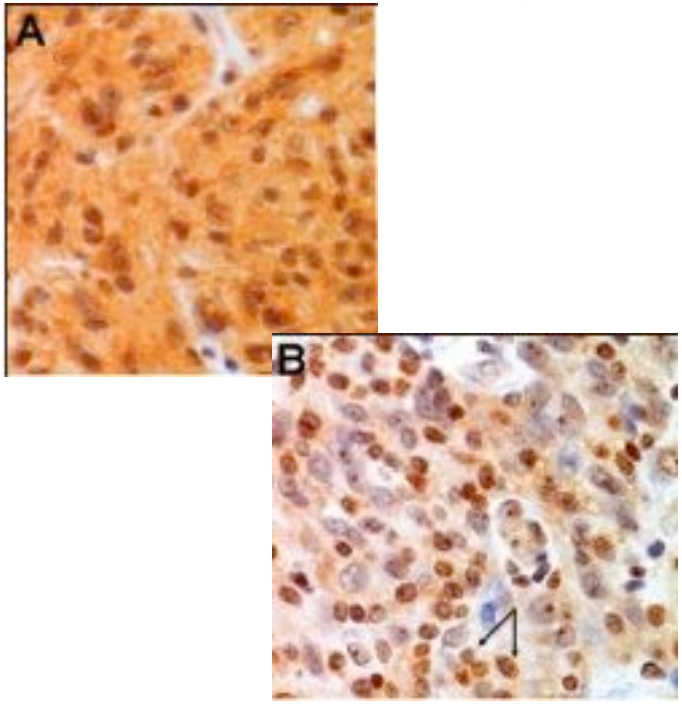
Low-negative EGFR expression

High EGFR expression

Method and Materials

Beclin-1 protein cytoplasmic expression score (BPCE)

✓ *A Neuropathologist evaluated the autophagy protein Beclin-1 expression score on surgical samples by IHC :*



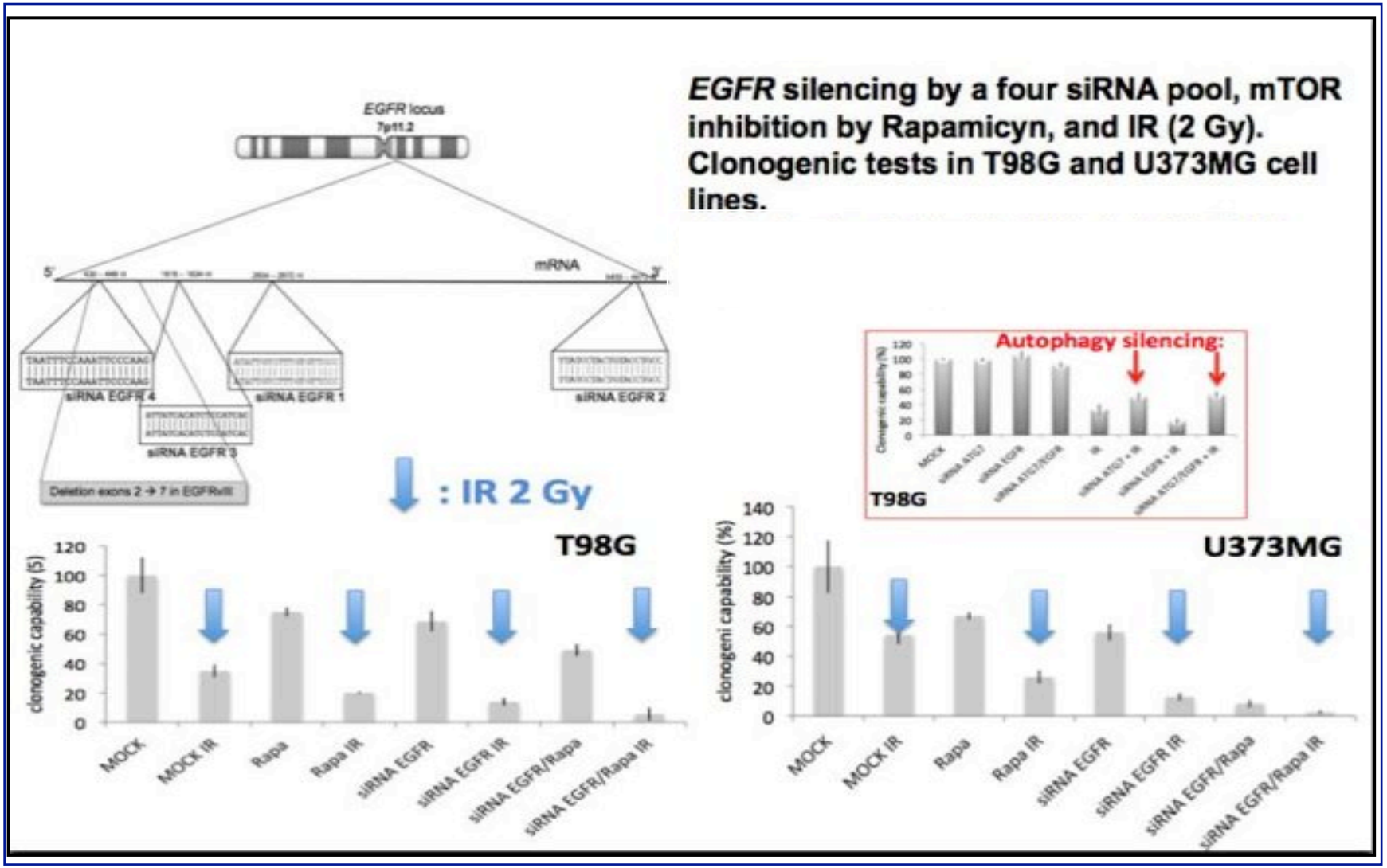
IHC Cytoplasmatic Intensity

BPCE score

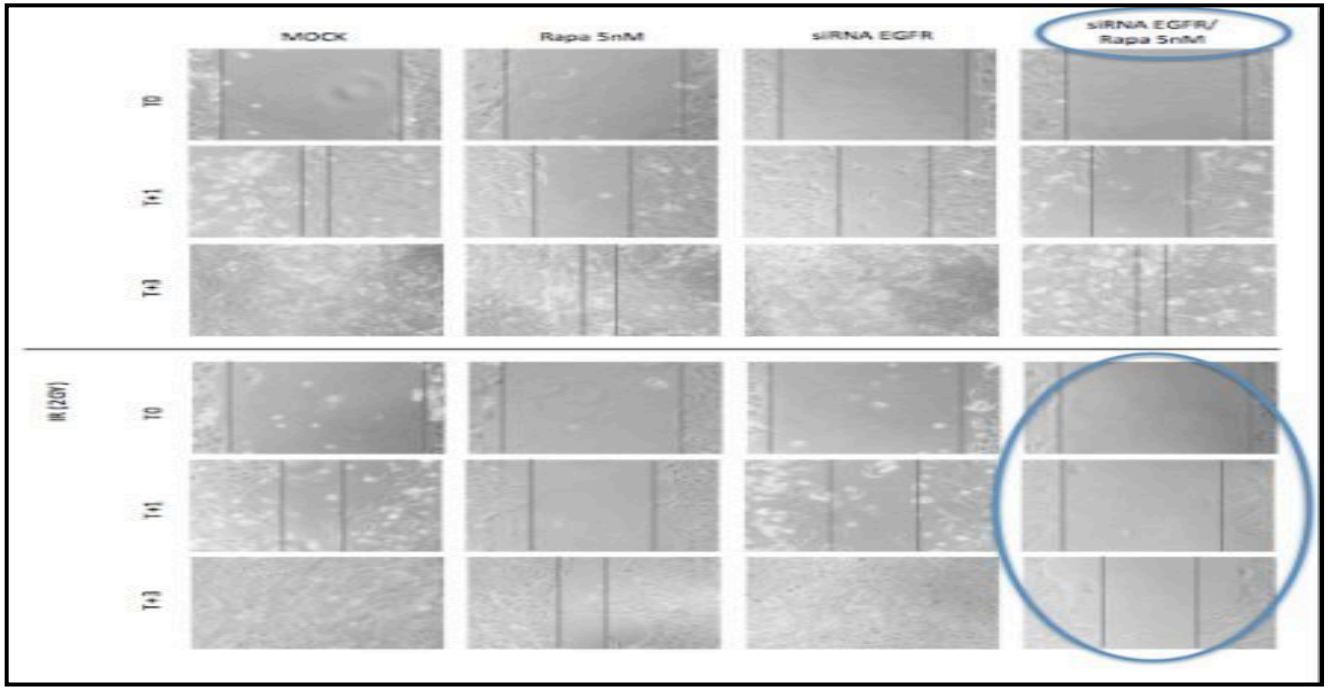
Low BPCE

High BPCE

Results


EGFR silencing by a four siRNA pool, mTOR inhibition by Rapamicyn, and IR (2 Gy). Clonogenic tests in T98G and U373MG cell lines.
EGFR locus
7p11.2
mRNA
siRNA EGFR 4
siRNA EGFR 1
siRNA EGFR 2
siRNA EGFR 3
Deletion exons 2 → 7 in EGFRvIII
: IR 2 Gy
T98G
clonogenic capability (%)
MOCK
MOCK IR
Rapa
Rapa IR
siRNA EGFR
siRNA EGFR IR
siRNA EGFR/Rapa
siRNA EGFR/Rapa IR
Autophagy silencing:
T98G
U373MG
clonogeni capability (%)

Results

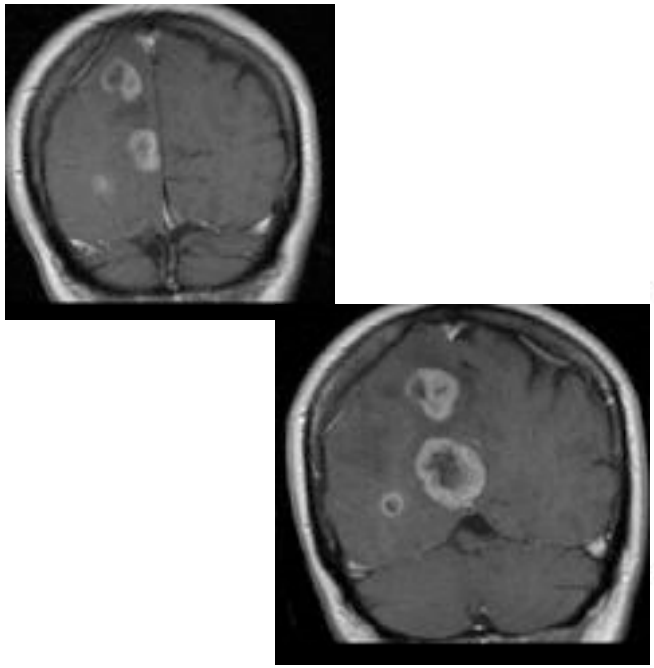


- *In vitro, combined si-EGFR and Rapamicin induced a decrease in cellular migration rates .*
- *In vitro, combined si-EGFR and Rapamicin increased IR response.*

Results

Clinical and pathological characteristics

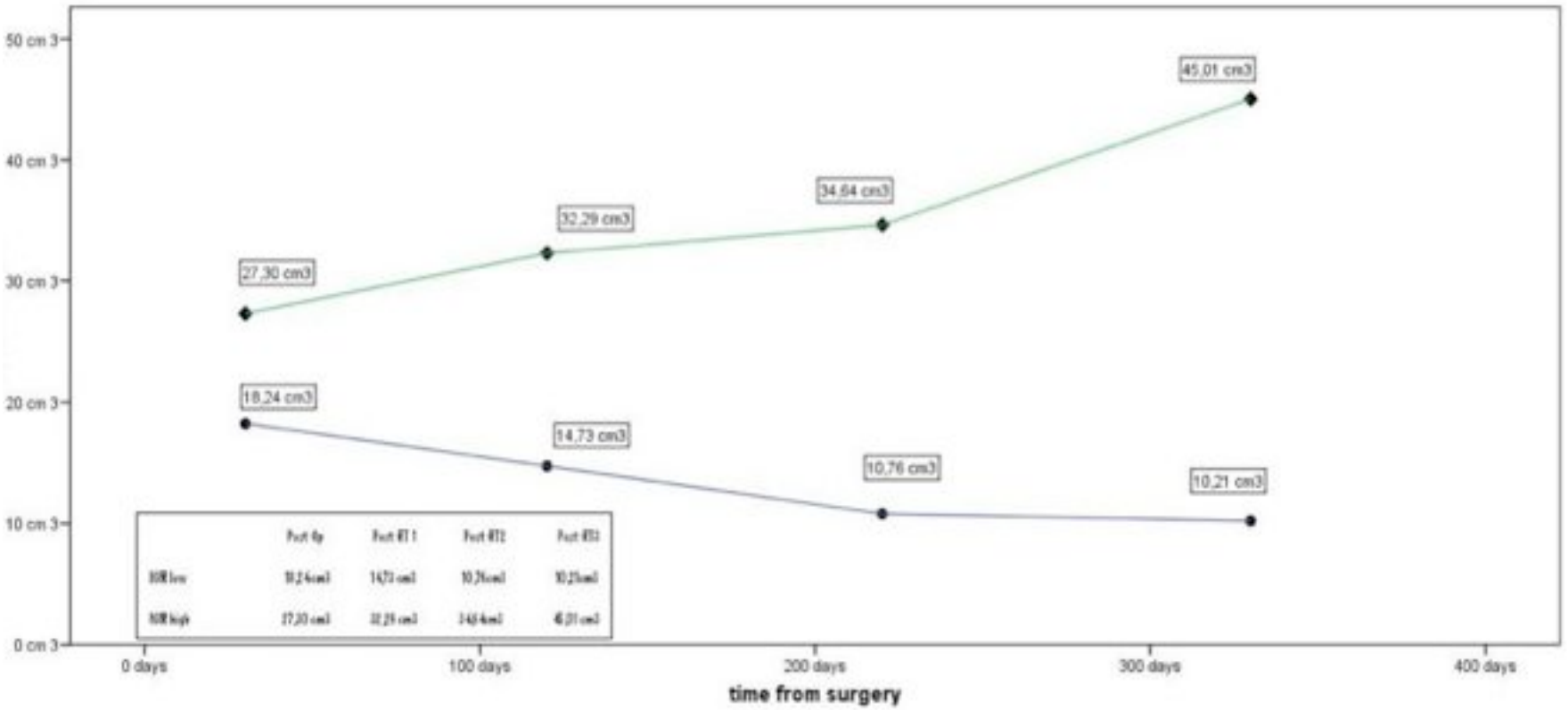
Patients: n°156 (EGFR and multicentric/unicentric disease)



		multicentricità sincrona		
		sì	no	Total
espressioneEGFR	Low	3	59	62
	High	23	71	94
Total		26	130	156

✓*In high EGFR expression group: we found an increased incidence of multicentric disease (p=0,03)*

Results



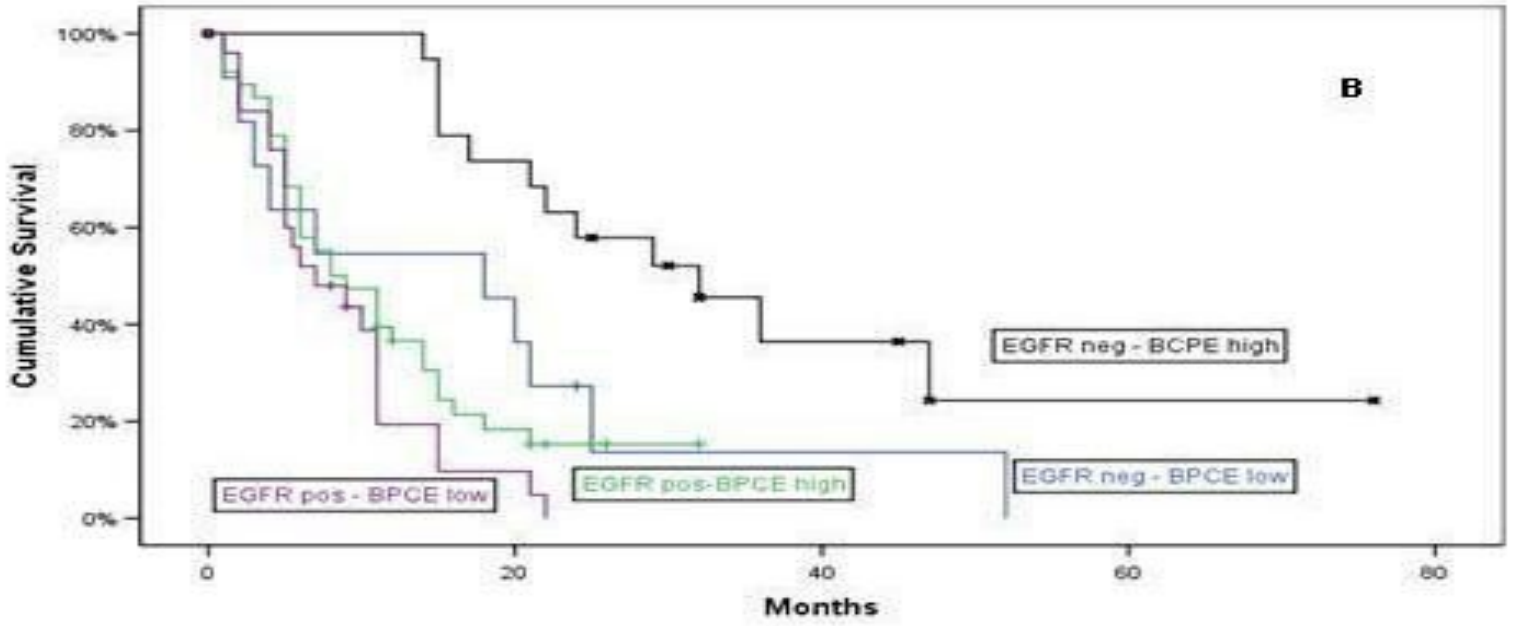
✓ ***MRI-based tumor growth evaluation according to EGFR expression subgroups (Low EGFR vs. High EGFR expression)***

Results

Clinical and pathological characteristics

Patients: n°81 (CLUSTERED FOR EGFR EXPRESSION and BPCE)

Follow-up (mean) : **12 months**



✓*In high EGFR group, BPCE didn't correlated with OS (p=0,163).*

✓*In low-negative EGFR group, BPCE strongly correlated with OS: median 18 months in low- BPCE versus 35 months of in high-BPCE pts (p=0,02)*

Conclusion

✓*Our results suggest that the relationship between EGFR expression and autophagy regulation could play a key role in invasion and growth patterns, and intrinsic radioresistance of GB.*

✓*A novel combined EGFR-autophagy modulation strategy can be hypothesised, to overcome the intrinsic GB radioresistance, thus improving the effectiveness of current standard of treatments of GB.*

✓*Other predictive biomolecular markers of local aggressiveness deserve further investigation in GB, for more through mechanistic knowledge of the EGFR-PIK3-AKT-mTOR signal pathway, to this purpose.*

Grazie per l'attenzione!!!

"L'ironia e l'intelligenza sono sorelle di sangue. "