Predictors of sensitivity and resistance mechanisms

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Vienna, Austria
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Session: Biologically based Treatment in head and neck squamous cell carcinoma

Disclosure slide

- Advisory Board (Merck KGaA)

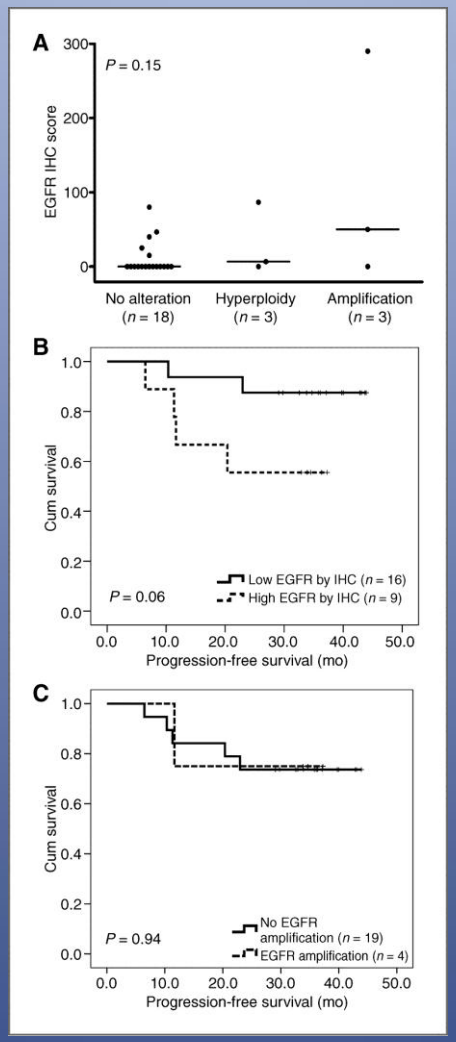
Potential mechanisms of resistance to EGFR-targeted therapies in HNSCC

Mechanisms of resistance	Examples
EGFR mutations	Extracellular domain (EGFRvIII)
Ras mutations	H-ras mutations
Epithelial-mesenchymal transition	Aberrant cortactin expression, delta-crystallin enhancer binding factor 1(E-cadherin repressor)
	Cyclin D1 upregulation
	PTEN mutations or decreased expression PI3KCA mutations,Akt amplification, EGFR phosphatase (PTPRS)
Activation of alternative and/or downstream pathways	Induction of alternative oncogenic pathways by EGFR blockade (i.e. STAT3)
	G-protein-coupled receptor mediated activation of EGFR (i.e. PDK1)
	Concomitant activation of Met, Her2, IGF-1R, Src kinases

Predictive biomarkers for response to EGFR-targeted therapies

EGFR by IHC

High tumor EGFR protein by IHC tended to be associated with reduced PFS in the cetuximab-treated cohort

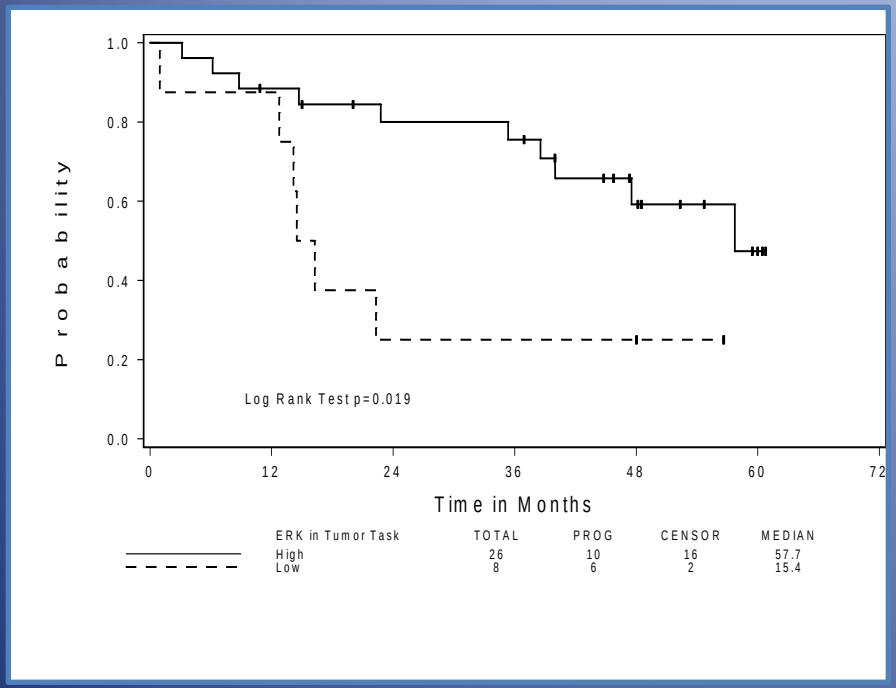


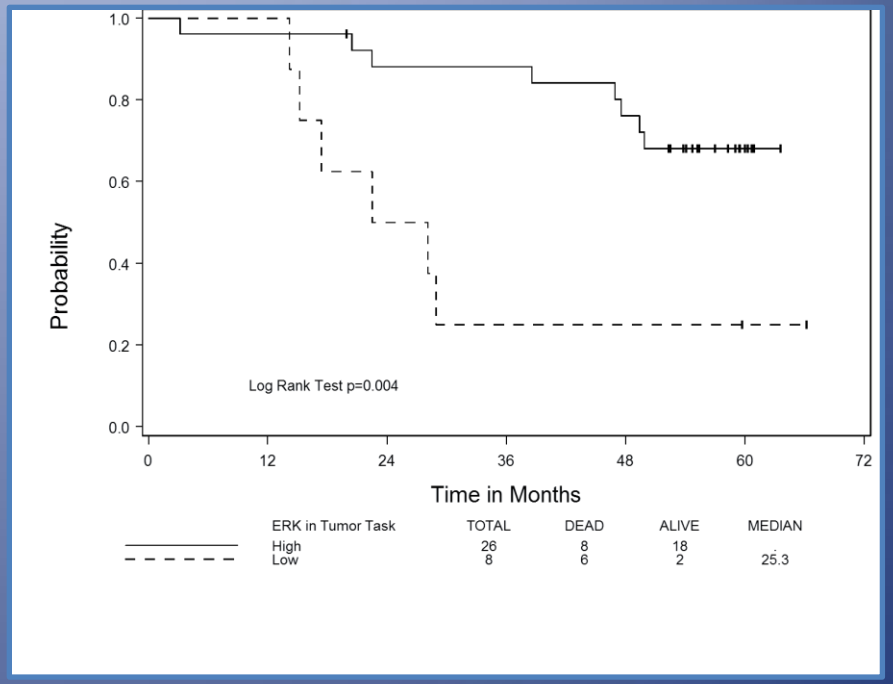
Wheeler S et al. Clin Cancer Res 2012;18:2278-2289

ERK

- Predictors for response to cetuximab in a prospective clinical trial (E2303)of patients (pts) with operable stage III/IV HNSCC phase II trial were analyzed on a tissue microarray
- EGFR, ERK1/2, Met, pAkt and STAT protein expression levels were assessed using automated quantitative protein analysis (AQUA)

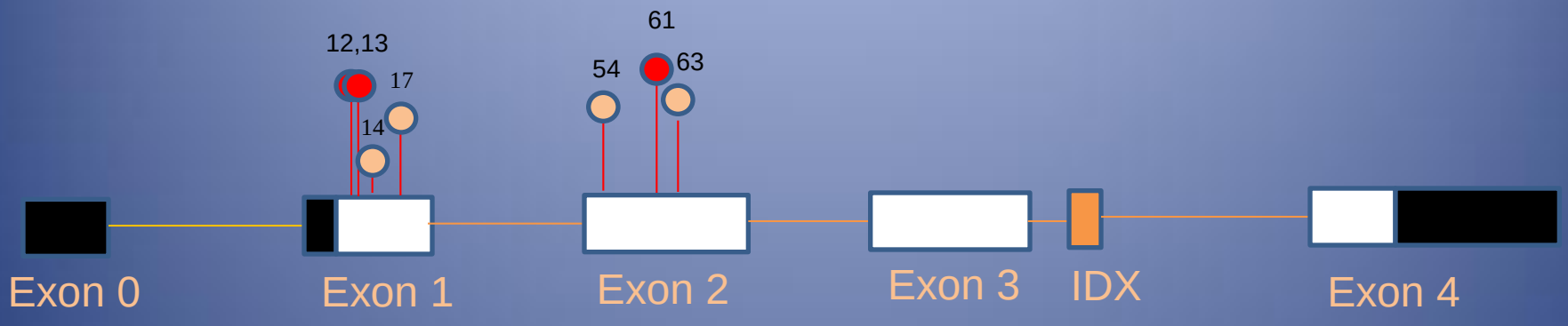
Progression-Free Survival and Overall Survival by ERK Status



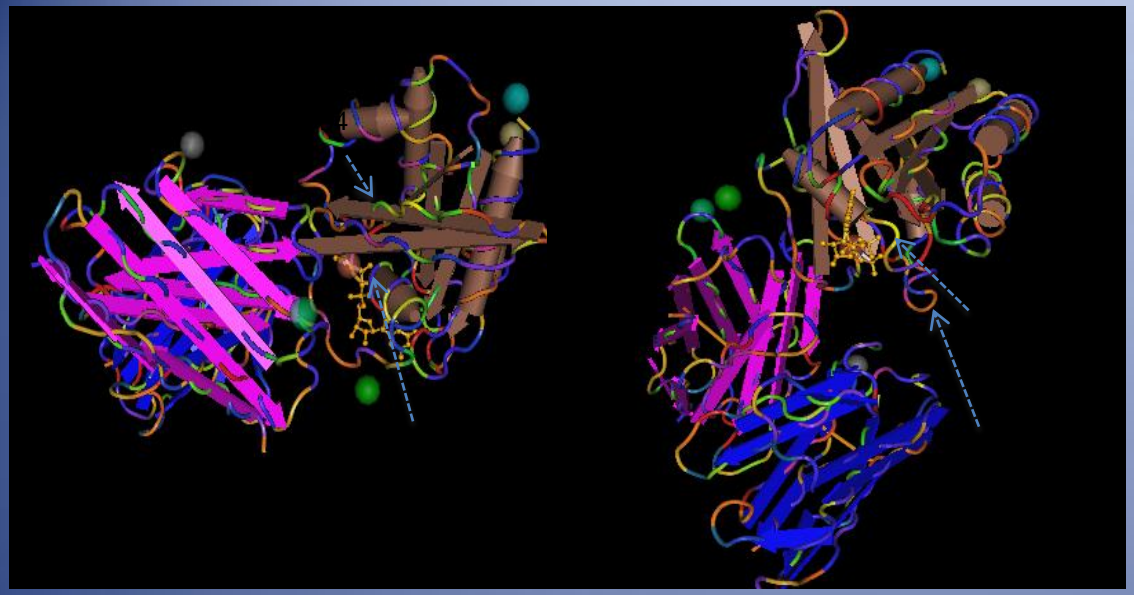


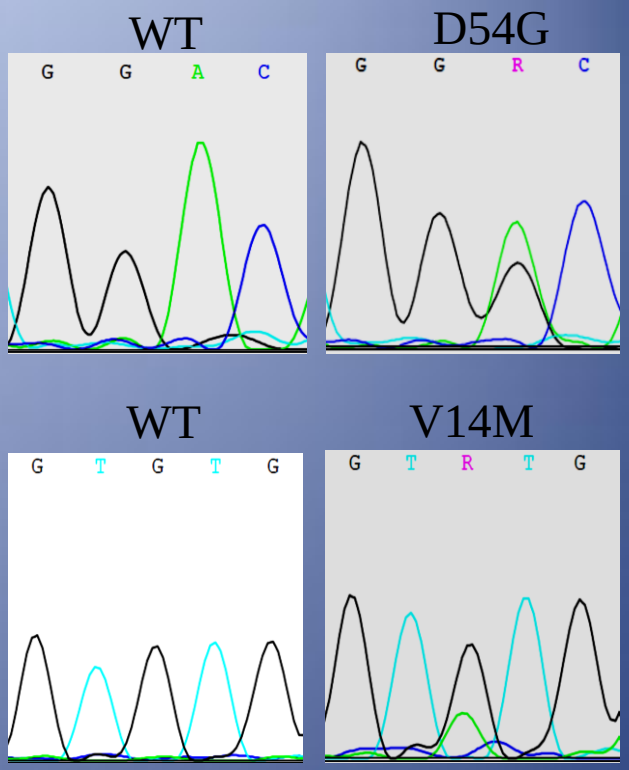
Psyrri et al: ASCO 2012

Mutational analysis of *HRAS*



Mutation detection analysis showed that 11 of 158 (**6.96%**)HNSCC specimens contained mutations at hotspot codons 12,13 and 61





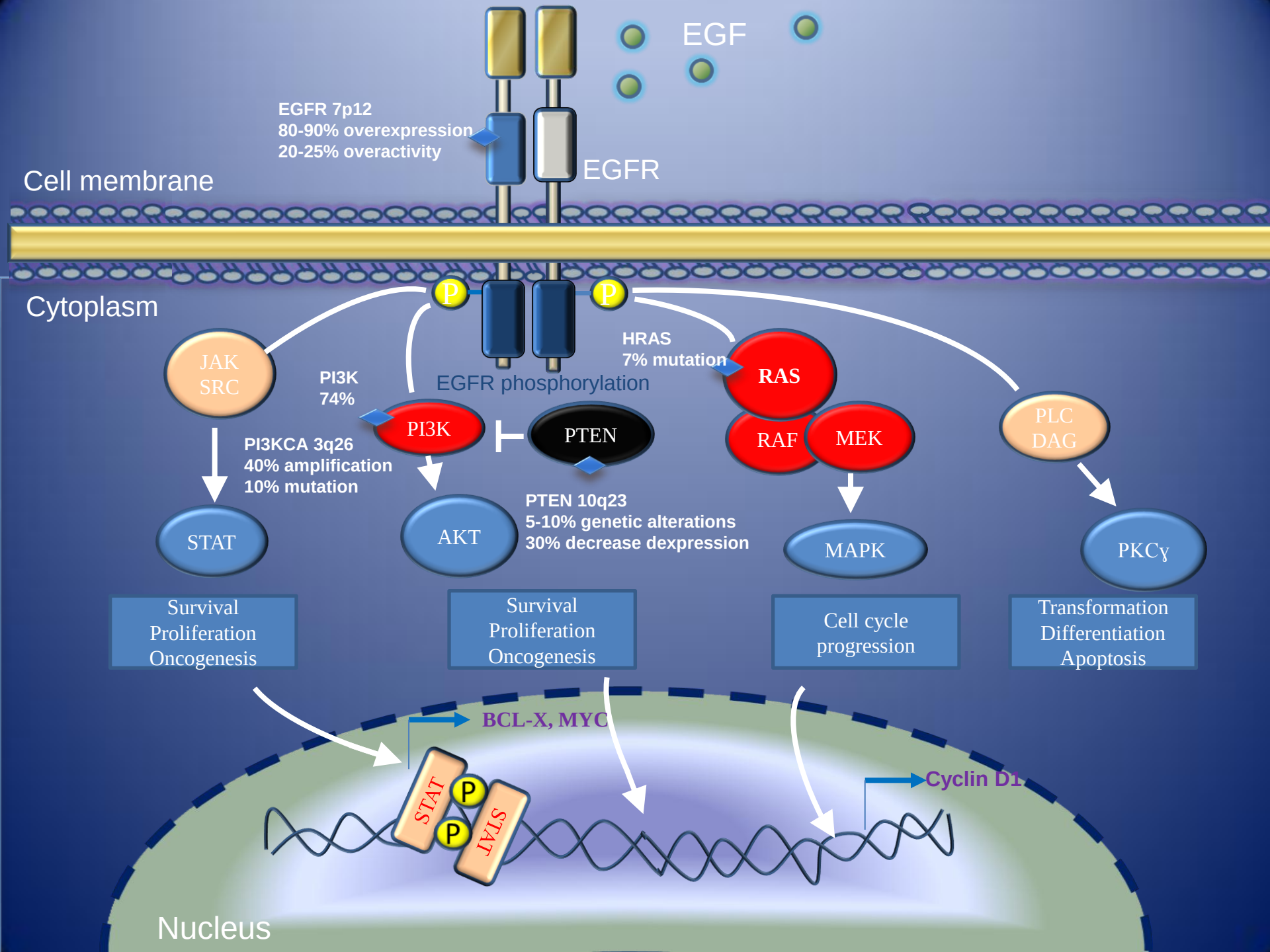
We also detected two mutations in codon 14 (V14M), one in codon 17 (S17N), one in codon 54 (D54G) and one in codon 63 (E63K). Our analysis showed that **3.16**% HNSCC samples contained rare HRAS mutations.

In total, HRAS mutation analysis showed that **10.13%** HNSCC specimens harbor mutations in HRAS gene that affect the protein function and specificity

HRAS status and clinical outcome

- Patients bearing tumors with mutated *HRAS* had inferior mean OS (22.13vs 35.20, **p=0.02)** and a non-significant trend for inferior mean DFS

- Patients had received various treatments such as surgery plus/minus RT and various chemotherapy regimens. A subgroup analysis of 38 patients treated with cetuximab-based regimens showed that wt Hras was associated with higher likelihood of attaining CR or PR to treatment of borderline significance (**p=0.06**) due to small sample size

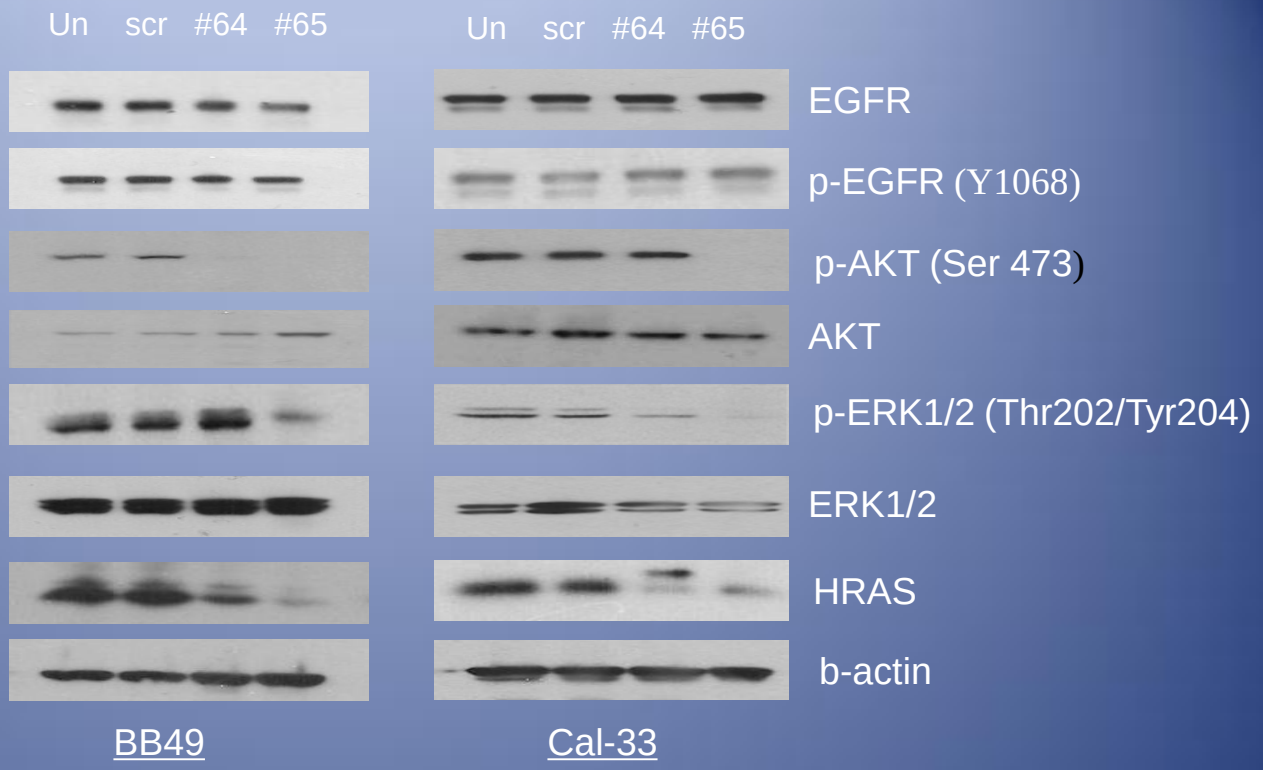

EGF
EGFR 7p12
80-90% overexpression
20-25% overactivity
EGFR
Cell membrane
Cytoplasm
P
P
JAK
SRC
PI3K
74%
EGFR phosphorylation
HRAS
7% mutation
RAS
PI3K
PTEN
RAF
MEK
PLC
DAG
PI3KCA 3q26
40% amplification
10% mutation
PTEN 10q23
5-10% genetic alterations
30% decrease dexpression
STAT
AKT
MAPK
PKCγ
Survival
Proliferation
Oncogenesis
Survival
Proliferation
Oncogenesis
Cell cycle
progression
Transformation
Differentiation
Apoptosis
BCL-X, MYC
Cyclin D1
STAT
STAT
P
P
Nucleus

RAS and PI3K

- We have developed a model cell system to study the impact of HRAS and PIK3CA mutations in cetuximab resistance in HNSCC

- To investigate whether activating mutations in downstream targets of EGFR can lead to resistance to EGFR blockade in head and neck cancer, we infected a group of cetuximab resistant HNSCC cell lines bearing mutations in *HRAS* (BB49) or *PIK3CA* (Cal-33) and a group of wild- type *HRAS/PI3K* cetuximab resistant HNSCC cell lines (UM-SCC-11A, UM-SCC-6) with lentivirus expressing shRNA that targets the HRAS gene or control shRNA.

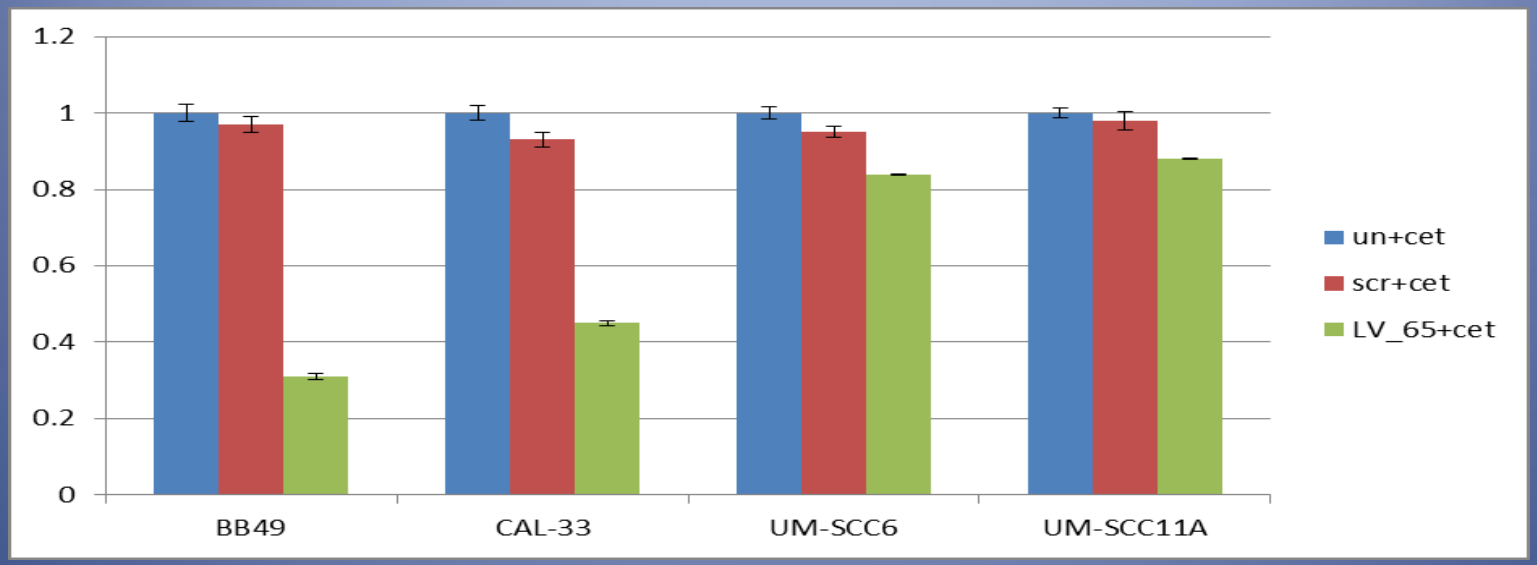
HRAS silencing



- HRAS silencing did not affect the expression levels of EGFR, p-EGFR , AKT and ERK1/2. However, HRAS downregulation was found to be associated with a significant reduction of phospho ERK1/2 and phospho AKT in HRAS/PI3K mutated cell lines

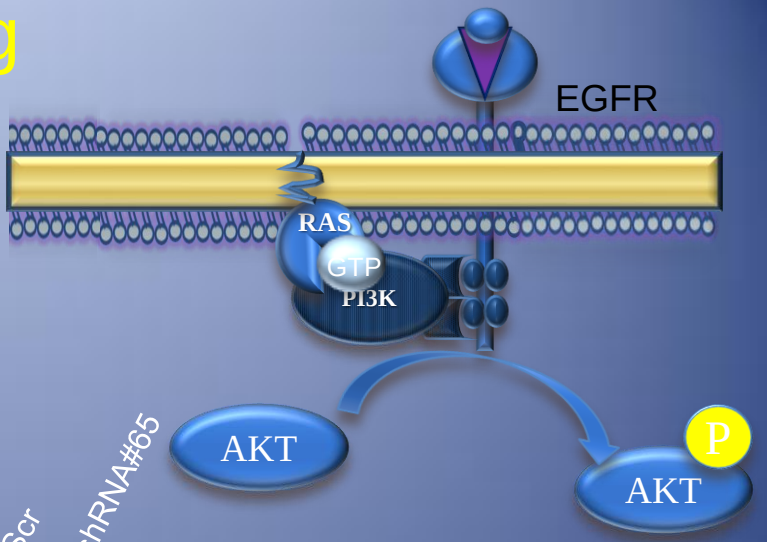
HRAS silencing in combination with Cetuximab treatment

MTT VIABILITY ASSAY

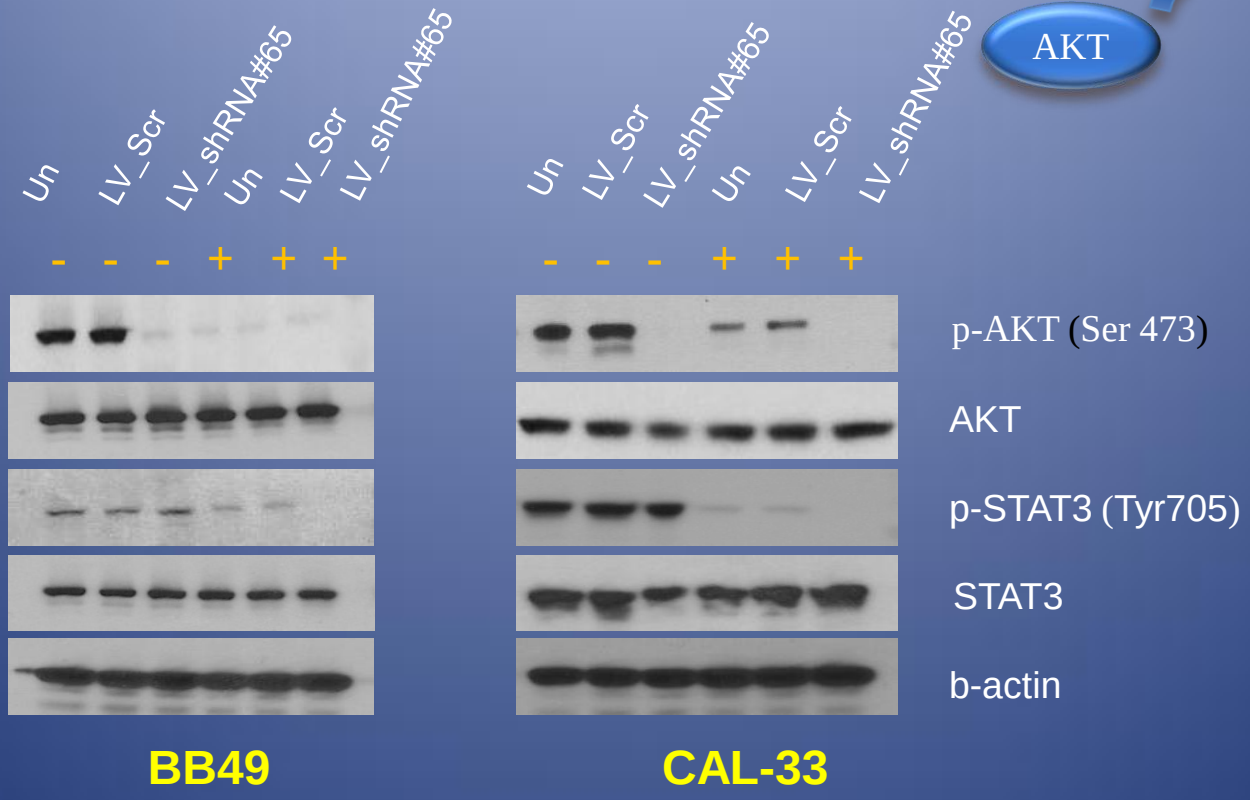


Treatment with 50 nM Cetuximab suppressed almost completely the proliferation of HRAS/PI3K mutated compared to HRAS/PI3K wild type HNSCC cell lines that were infected by LV_#65shRNA

Cetuximab plus HRAS silencing



Cetuximab : (50nM)



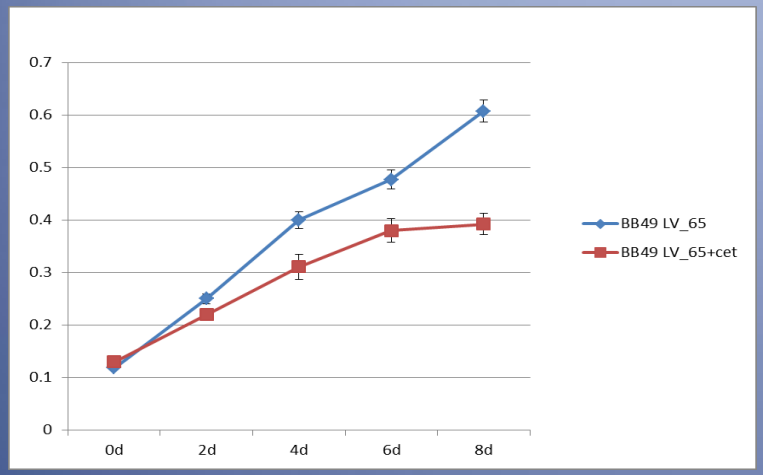
Un: Uninfected

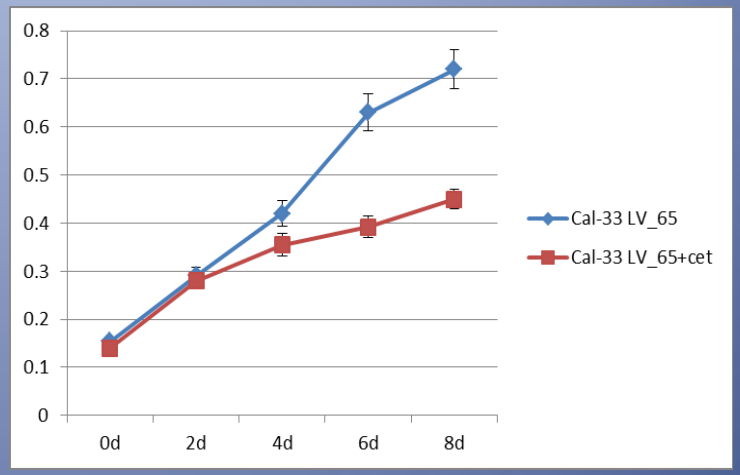
LV_Scr: Infected with lentivirus expressing control shRNA

LV_shRNA#65: Infected with lentivirus expressing shRNA #65

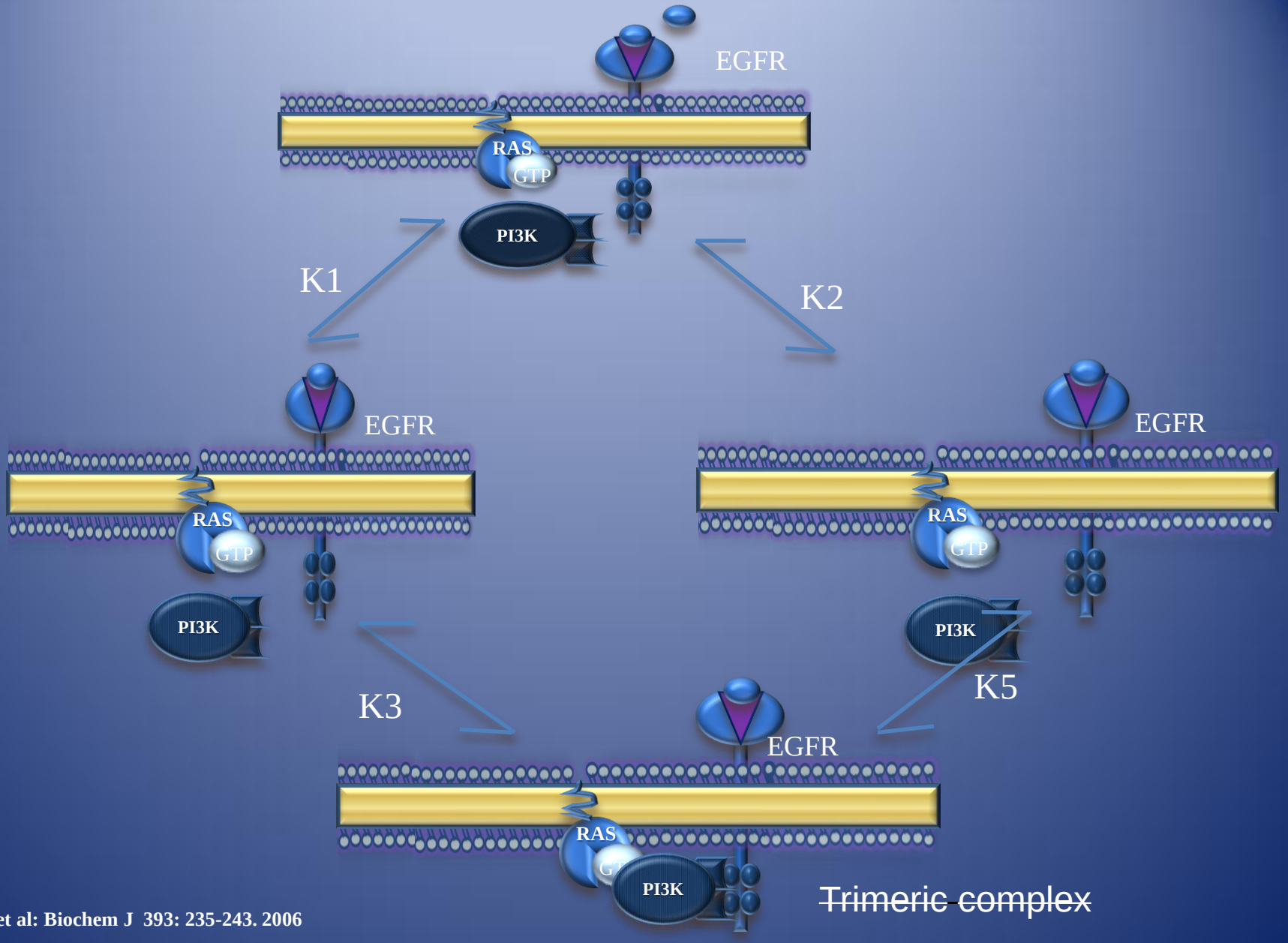
MTT VIABILITY ASSAY

Optical density





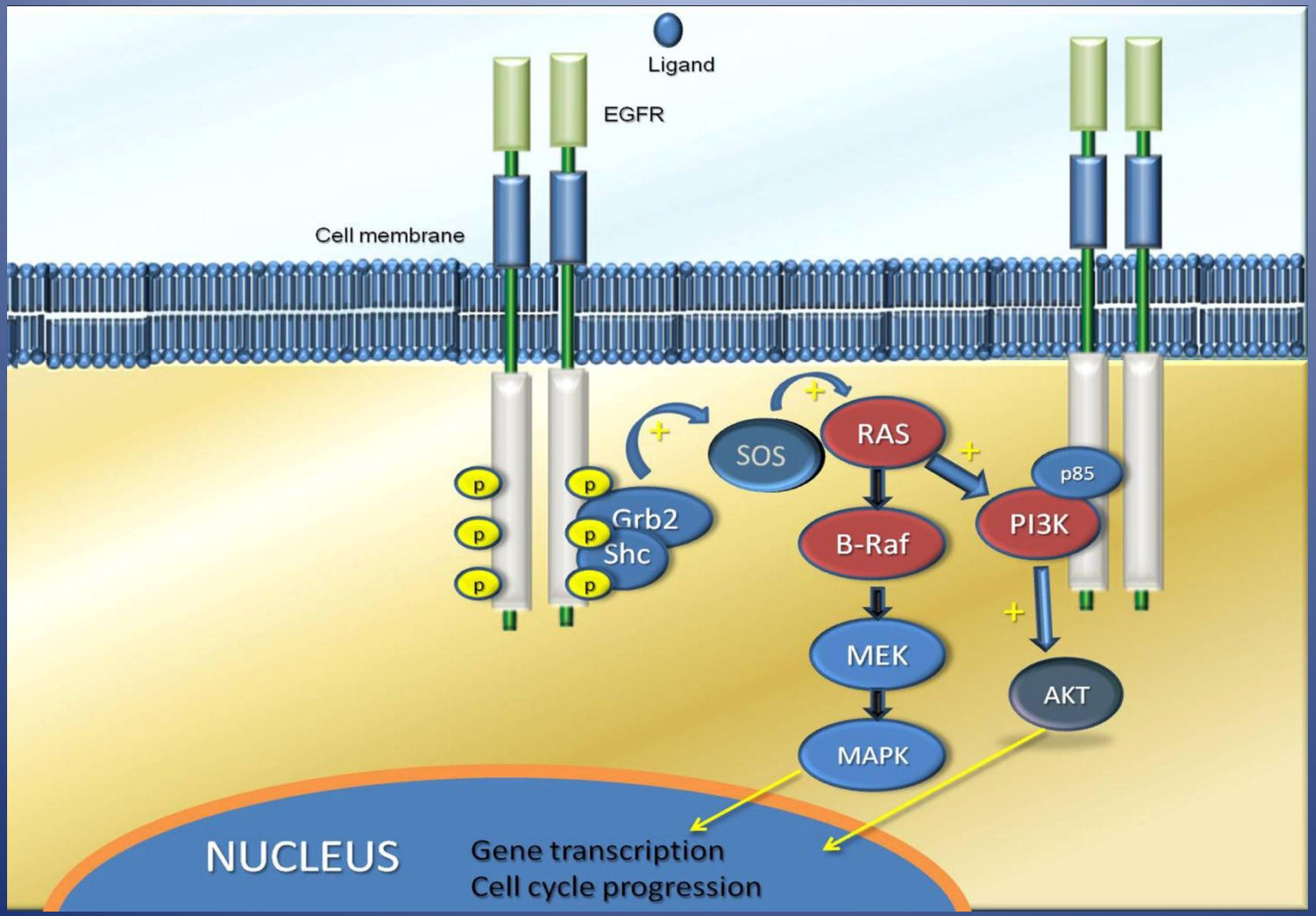
A direct comparison of the proliferative rate between cetuximab treated and untreated LV_#65shRNA infected BB49 and Cal-33 cells confirmed the restoration of sensitivity to cetuximab in these cells after HRAS silencing.



Kaur et al: Biochem J 393: 235-243. 2006

~~Trimeric complex~~

Molecular crosstalk between EGFR, RAS, PI3K pathways



Conclusions

- Currently, no biomarker is predictive for response to cetuximab in HNSCC
- *HRAS* genetic alteration is a frequent event in HNSCC
- Cell lines bearing *HRAS* mutation are resistant to cetuximab and *HRAS* silencing renders these cells sensitive
- HRAS silencing suppresses the ability of activated PI3K to promote the phosphorylation of AKT

Thank you