elcc European Lung Cancer Conference

Systemic treatment in EGFR/ALK NSCLC patients: second line and beyond

10 years after the discovery of EGFR mutations screening and treatment are insufficient

SLCG performed the first large scale screening of EGFR mutations for erlotinib treatment *(Rosell, Moran Queralt NEJM 2009)*

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Presentation reviewed in N Karachaliou, R Rosell, D Morales-Espinosa, S Viteri. Expert Rev Anticancer Ther 2014

- Impaired immune response in EGFR mutant mice with PDL-1 expression and upregulation of IL-6 and TGFβ1. PD-1 blockage reduces tumor burden and increases survival *(Akbay et al. Cancer Discov. 2013).* *Anti-PD-1 and PDL-1 blocking antibodies in NSCLC* *(Brahmer et al. NEJM 2012; Topolian et al. NEJM 2012)*.

- Crizotinib an ATP-competitive aminopyridine inhibits tyrosine phosphorylation of ALK with an IC_{50} of 20-40 nM and response in 57% of patients with ALK-rearrangement positive lung cancer *(Kwak et al. NEJM 2010).* *55-60% of responses in crizotinib resistant ALK + NSCLC with second generation ALK inhibitors: ceritinib (LDK378), AP26113, alectinib* *(Doebele JTO 2014).*

- High frequency of EML4-ALK in thyroid cancer from atomic bomb survivors in Japan detected by a highly sensitive RT-PCR assay from archival paraffin blocks and *not confirmed by FISH or other methods* *(Kelly et al. PNAS 2014).*

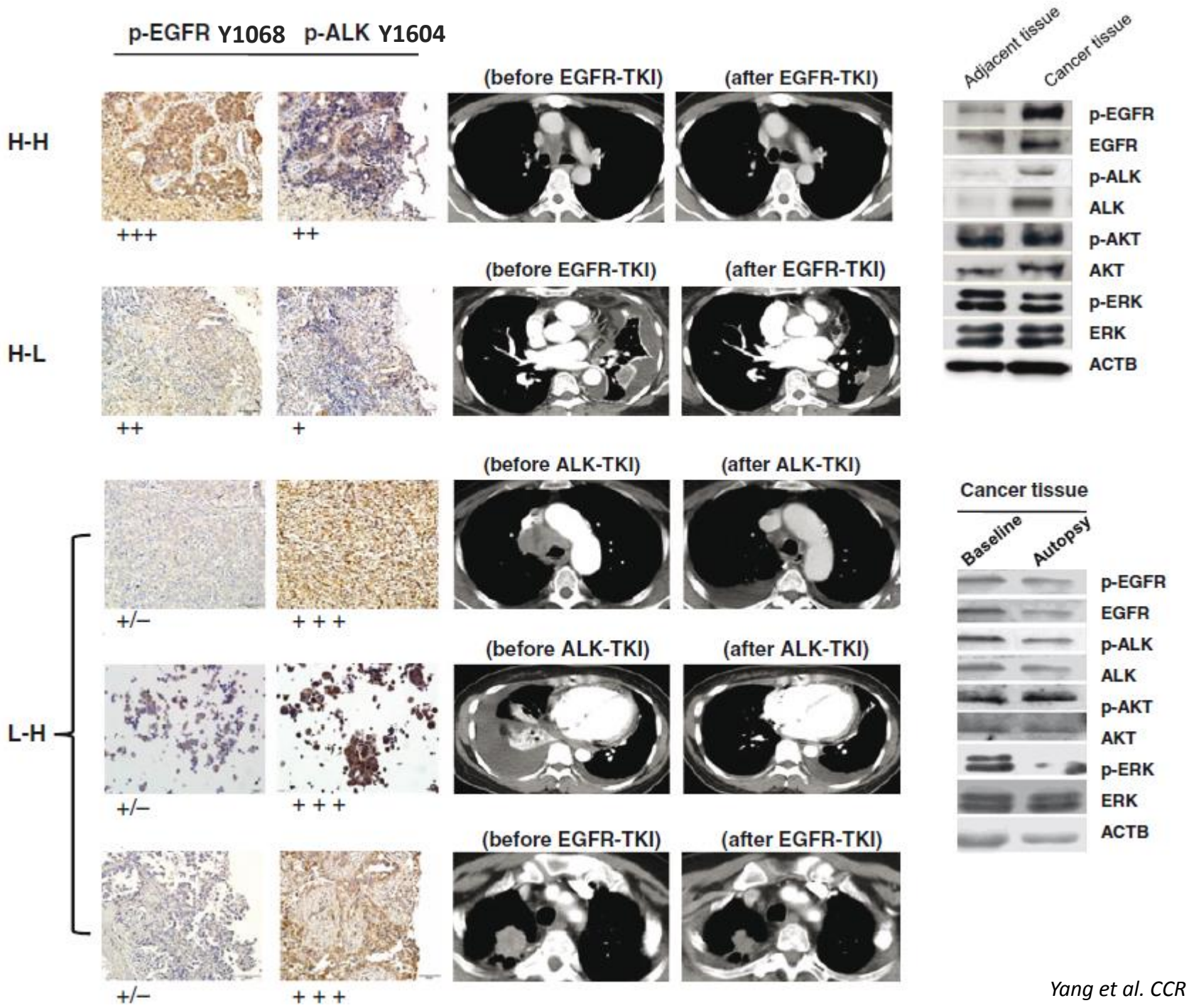
- Role of crizotinib either in combination with other agents in the relapse setting, or in combination with other agents in the upfront setting in ALK NSCLC, warrants investigation.

- Crizotinib resistance includes secondary resistance mutations and activation of bypass signaling pathways. *(Choi et al. NEJM 2010; Katayama et al. Science Trans Med 2012; Sasaki et al. Cancer Res 2011; Doebele et al. CCR 2012).* Also, EGFR/ALK co-alterations. *(Yang et al. CCR 2014; Karachaliou et al. JTO 2013)*

- Second line in ALK NSCLC: CBPD *(Ou et al. Annals of Oncol. 2014),* addition of local therapy *(Weickhardt et al. JTO 2012),* sequencing chemotherapy *(Browning et al. JTO 2013),* HSP90 inhibitors *(Sang et al. Cancer Discov. 2013)* and second generation ALK inhibitors ceritinib, AP26113, alectinib. *Alectinib inhibits ALK gatekeeper L1196M mutation in vitro (Sakamoto et al. Cancer Cell 2011).*

- *EGFR mutants can 'flare' after stopping EGFR TKI. EGFR mutants can respond to an EGFR TKI after wash-out. (Chmielecki et al. Science Trans Med 2011; Sequist et al. Science Trans Med 2011). FLARE (GFCP 03-2013) phase III trial second line chemotherapy w/wout erlotinib. T790M inhibitors (CO-1686, AZD9291 Arteaga & Engelman. Cancer Cell 2014).*

- *Biomarker oriented (BIM, AXL, STAT3) synthetic lethal combinations offer the potential for long term control.*



Yang et al. CCR 2014

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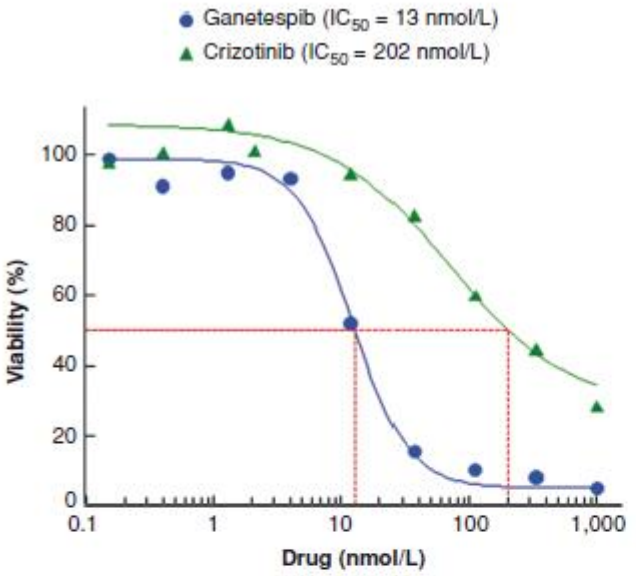

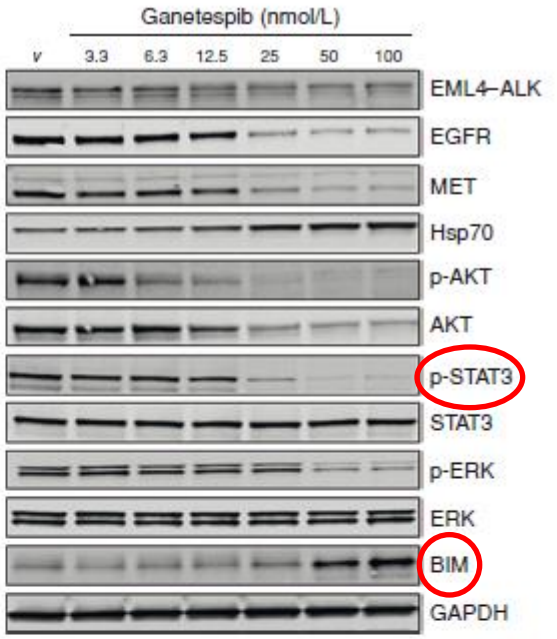

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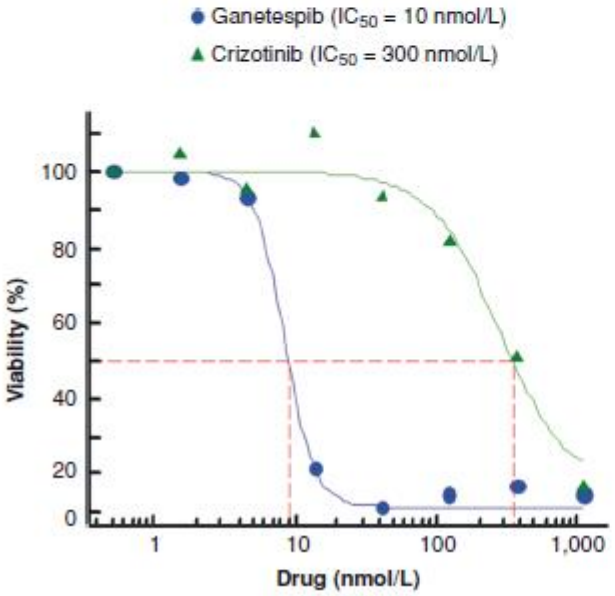
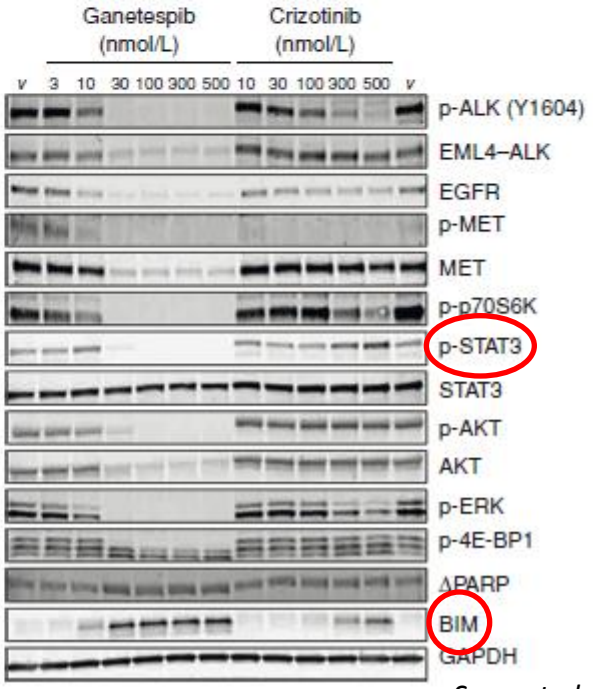


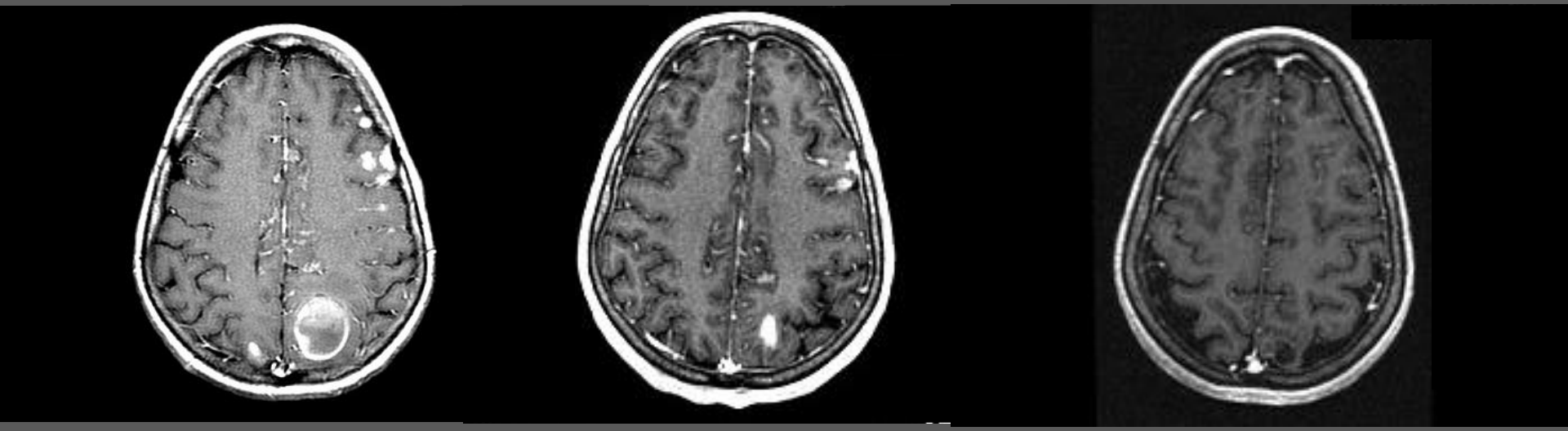


Sang et al. Cancer Discov. 2013

Dec 2011

Aug 2012

Feb 2014



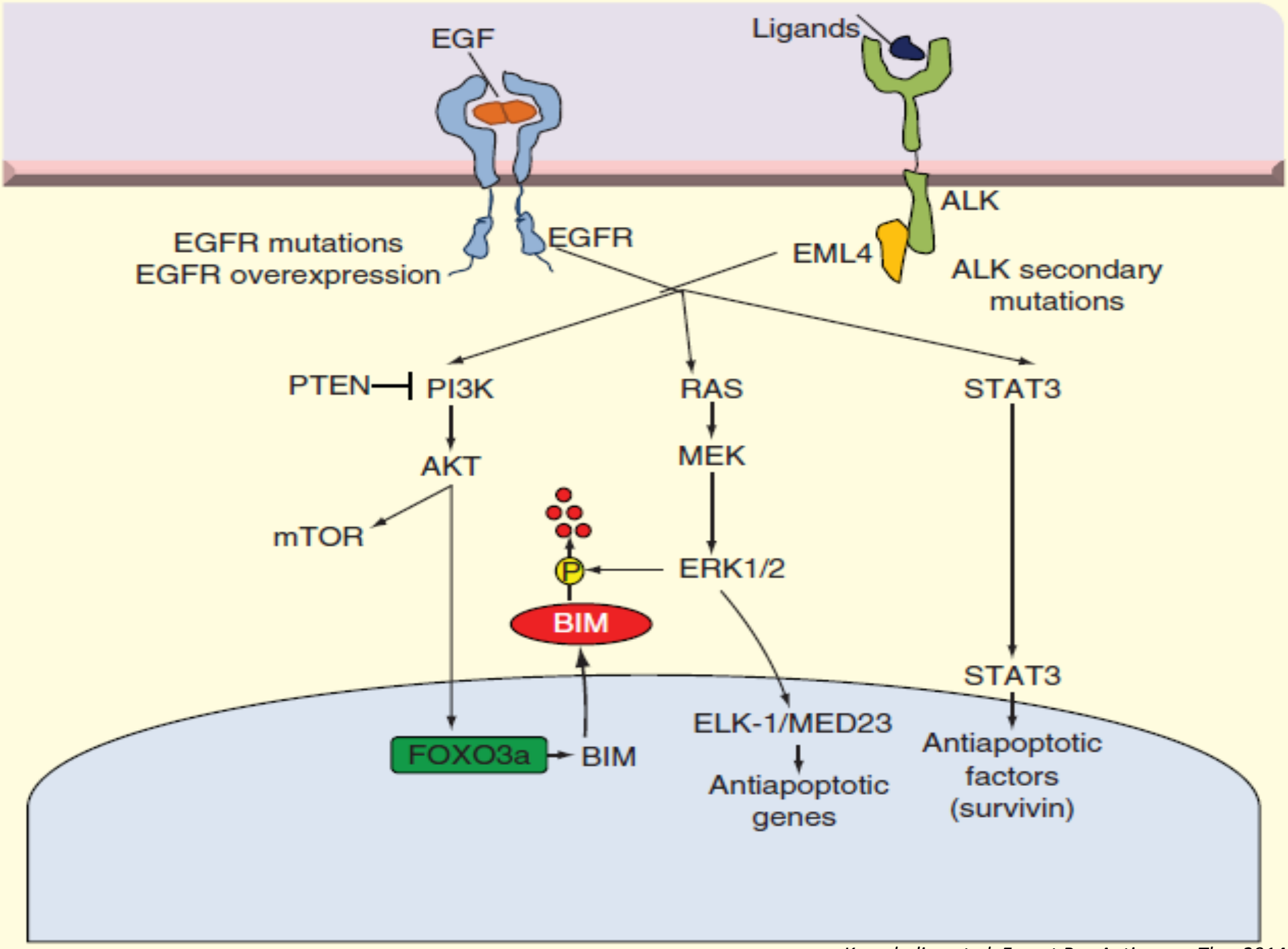
Courtesy S Viteri

Progression
After 1yr crizotinib

Partial response
After 8m ganetespib

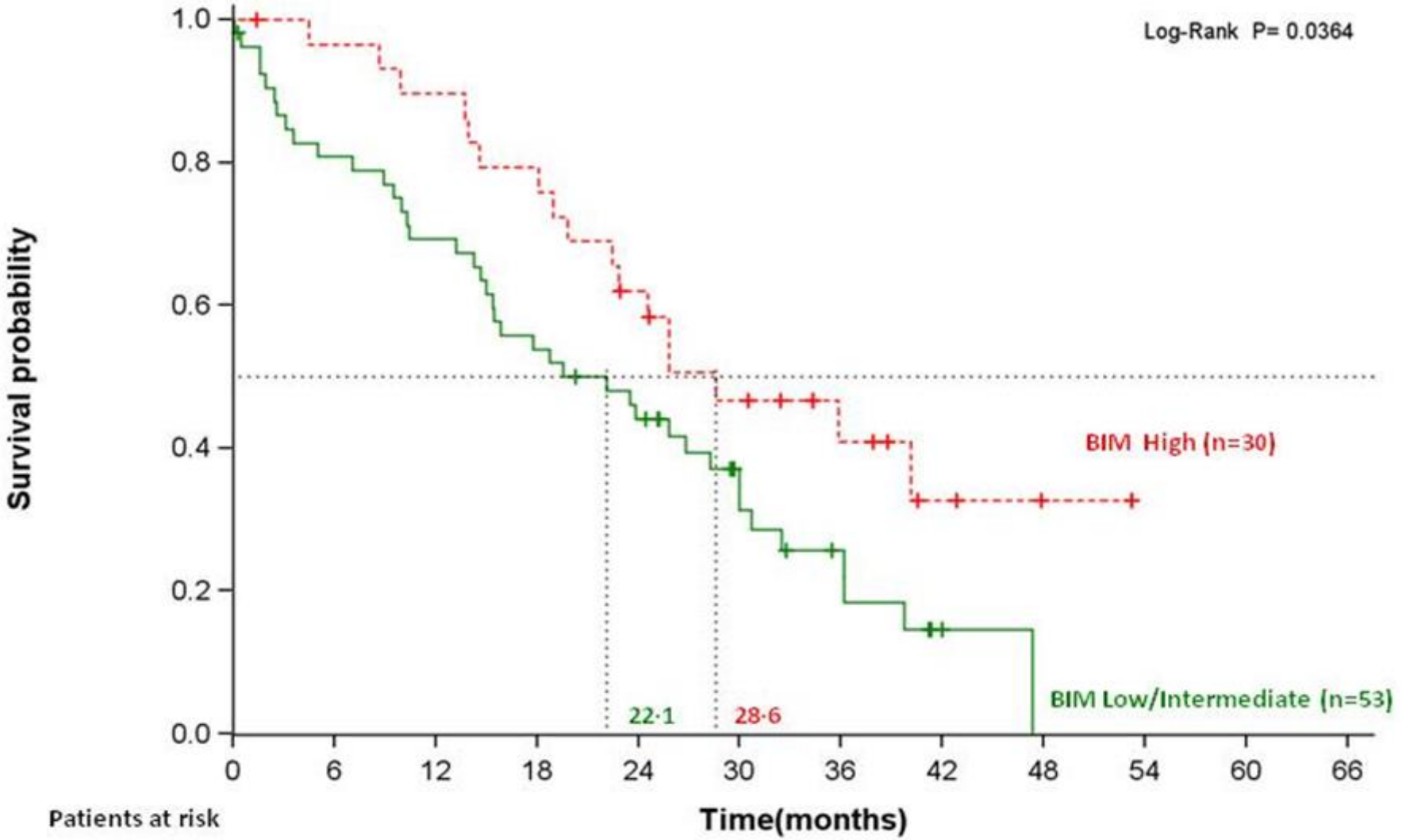
Almost CR
After 12m ceritinib (LDK378)

Acquired ALK secondary mutations to crizotinib include ALK G1292R which also confers resistance to alectinib. ALK G1202R located at the solvent front of the ALK kinase domain exhibits high level of resistance to all other ALK inhibitors *(Ou et al. JTO 2014)*



Karachaliou et al. Expert Rev Anticancer Ther 2014

BIM – Bcl-2 interacting mediator of cell death



EURTAC – cutoff January 2013

Costa et al. CCR 2014

Predictive biomarkers for drug therapy combination in EGFR mutant and EML-ALK rearranged NSCLCs

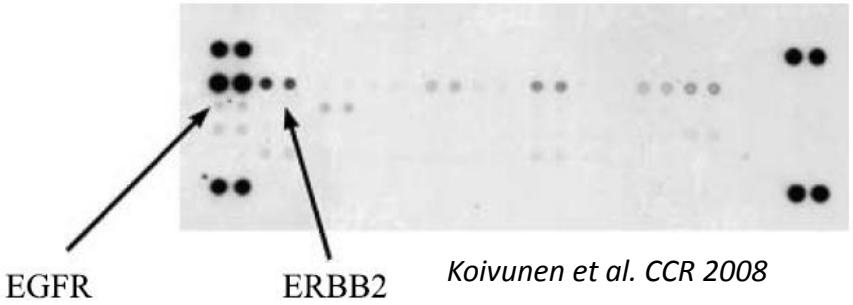
- BIM mRNA (*reported* *(D Costa et al. PLoS Med 2007; Cragg et al. PLoS Med. 2007; Gong et al. PLoS Med. 2007), well-established in EGFR mutant NSCLCs* *Faber et al. Cancer Discov. 2011; C Costa et al. CCR 2014*). Reported in EML4-ALK *(Tanizaki et al. BJC 2012; Katayama et al. Science Trans Med. 2012; Sang et al. Cancer Discov. 2013).*

- Shp2 reported in EGFRvIII GBM *(Zhan & O'Rourke Cancer Res 2004),* NPM-ALK *(Voena et al. Cancer Res 2007)* and mutant NSCLC *(Lazzara et al. Cancer Res 2010).* *Shp2 enhances MAPK signaling.*

- *TGFβR2, early adaptive escape resistance in EGFR mutant (reported* *Yao et al. PNAS 2010; Fan et al. Cancer Res 2011),* and MED12 mRNA in EGFR mutant and EML4-ALK, NSCLC (*reported* *Huang et al. Cell 2012; Rosell et al. NEJM 2013*)

- EGFR phosphorylation reported in ALK NSCLC *(Koivunen et al. CCR 2008; Katayama et al. Science Trans Med 2012)*

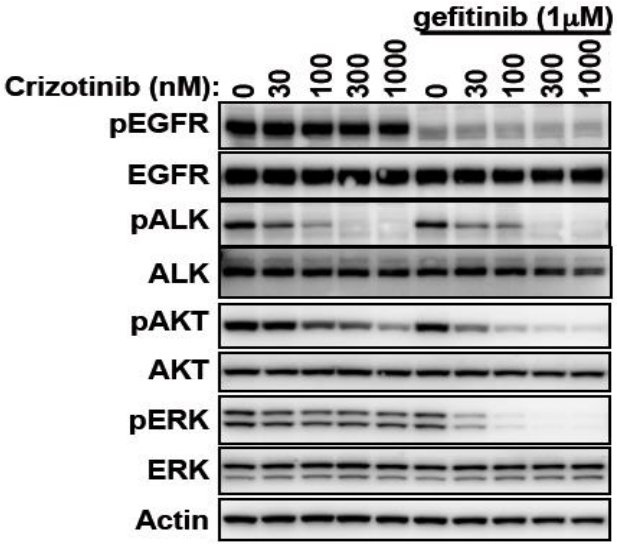
- Acquired ALK, EGFR and KRAS mutations *(Choi et al. NEJM 2010; Katayama et al. Science Trans Med 2012; Doebele et al. CCR 2012)*

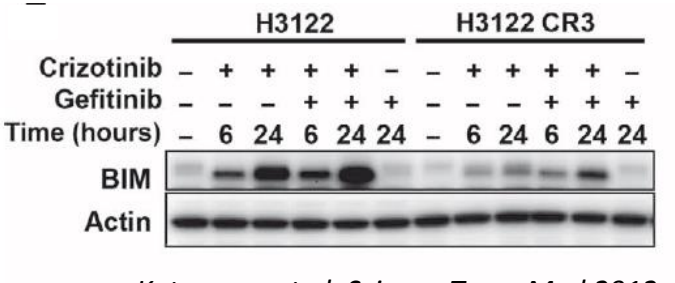


Koivunen et al. CCR 2008

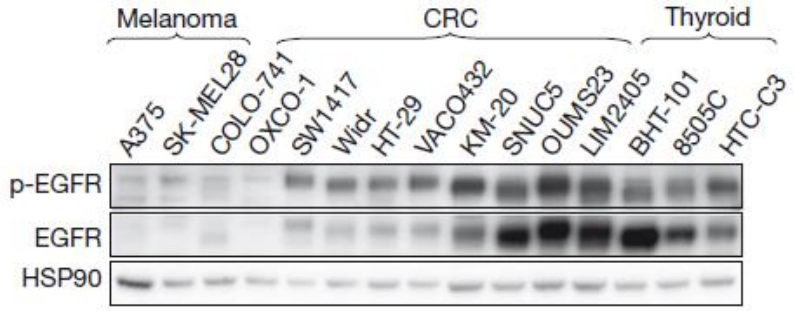
Katayama et al. Science Trans Med 2012

DFCI032 (EML4-ALK v1) resistant to TAE684 and sensitive to the combination with a dual EGFR TKI

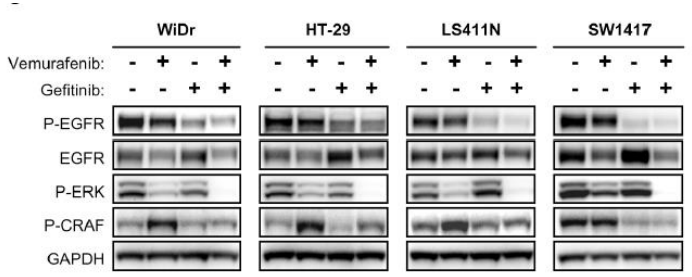




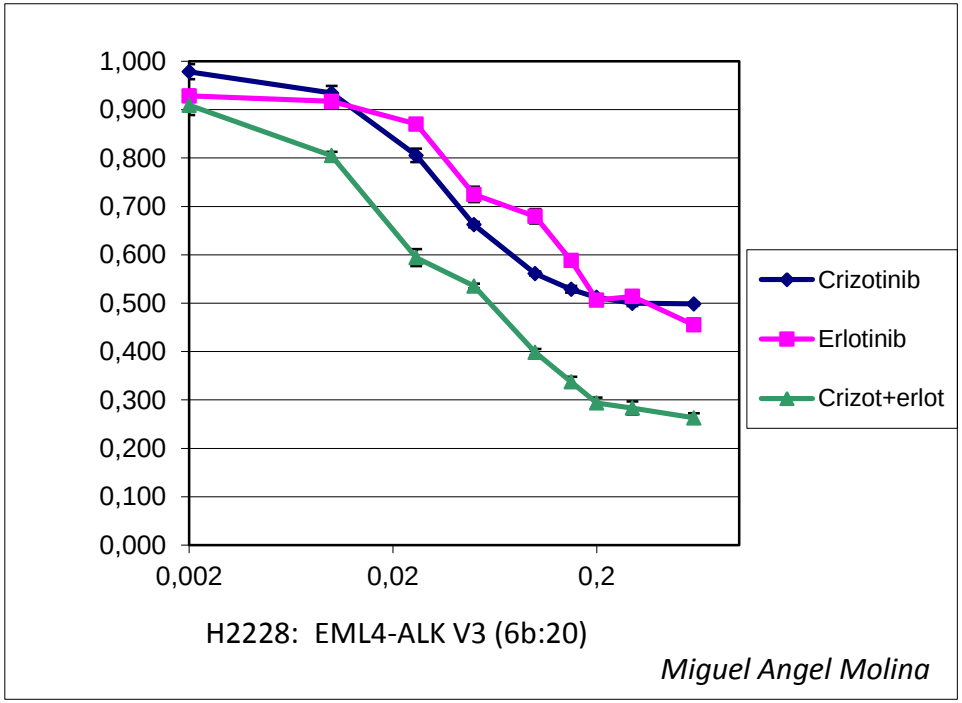
Katayama et al. Science Trans Med 2012

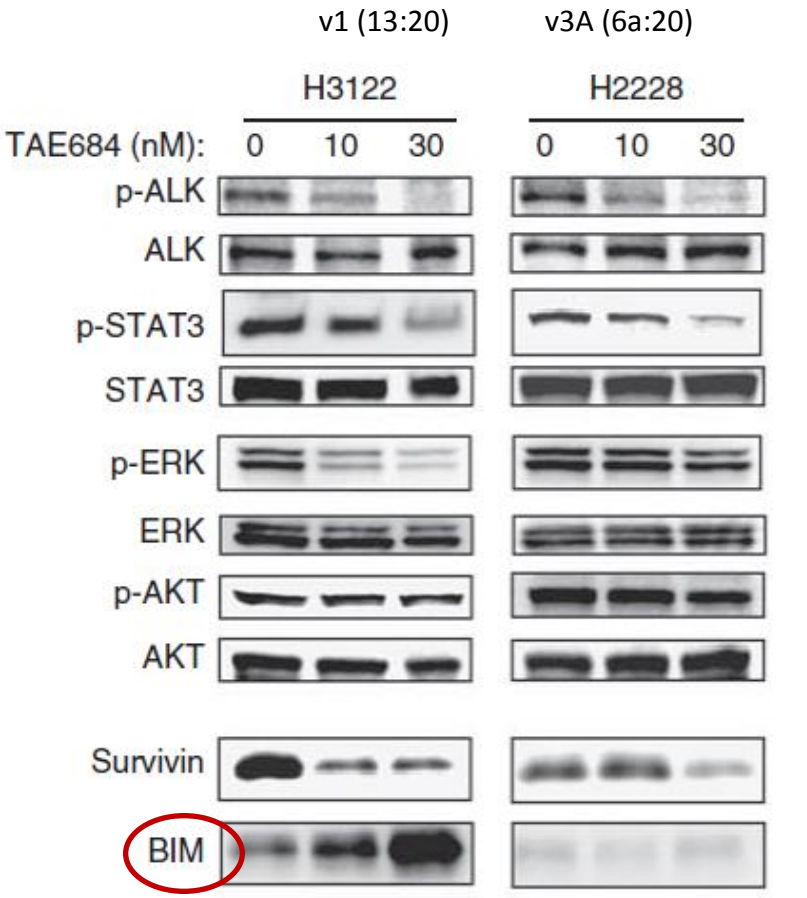


Prahallad et al. Nature 2012



Corcoran et al. Cancer Discovery 2012





Tanizaki et al. BJC 2012

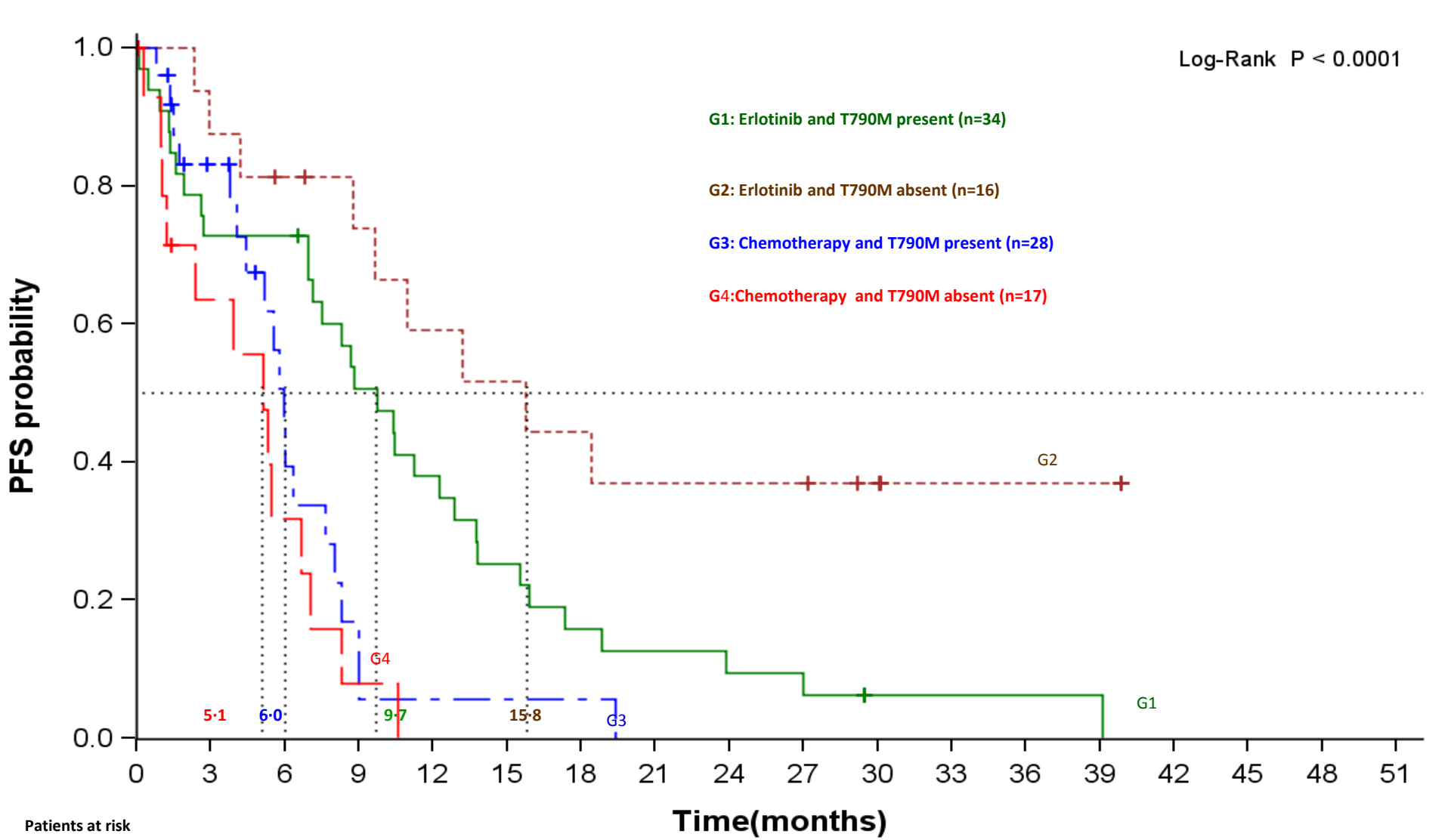
- BIM a companion diagnostic marker for *synthetic lethal combinations* in lung cancer with EGFR mutations, and possibly in ALK positive NSCLCs *(C Costa et al. CCR 2014; Faber et al. Cancer Discov 2011; Tanizaki et al. BJC 2012; Katayama Science Trans Med 2012; Sang et al. Cancer Discov. 2013).*

- T790M and BIM two-gene model for developing a predictive classifier in EGFR mutant NSCLC *(C Costa et al. CCR 2014).*
  - 100% response with high BIM and absence of T790M.
  - 31.6% response with low BIM and presence of T790M – *intrinsic resistance.*

- Clinical validation of the T790M, BIM and other biomarkers in the BELIEF ETOP trial (erlotinib/bevacizumab) and GOAL-SLCG trial (gefitinib/olaparib).

- Pretreatment T790M for management with selective T790M inhibitors *(Lee et al. Cancer Discov 2013).* AXL a diagnostic marker for *intrinsic* (H820 cells) or *acquired* resistance (PC9 resistant cell lines).

- AXL an upstream regulator of EMT induction *(Asiedu et al. Oncogene 2014).*



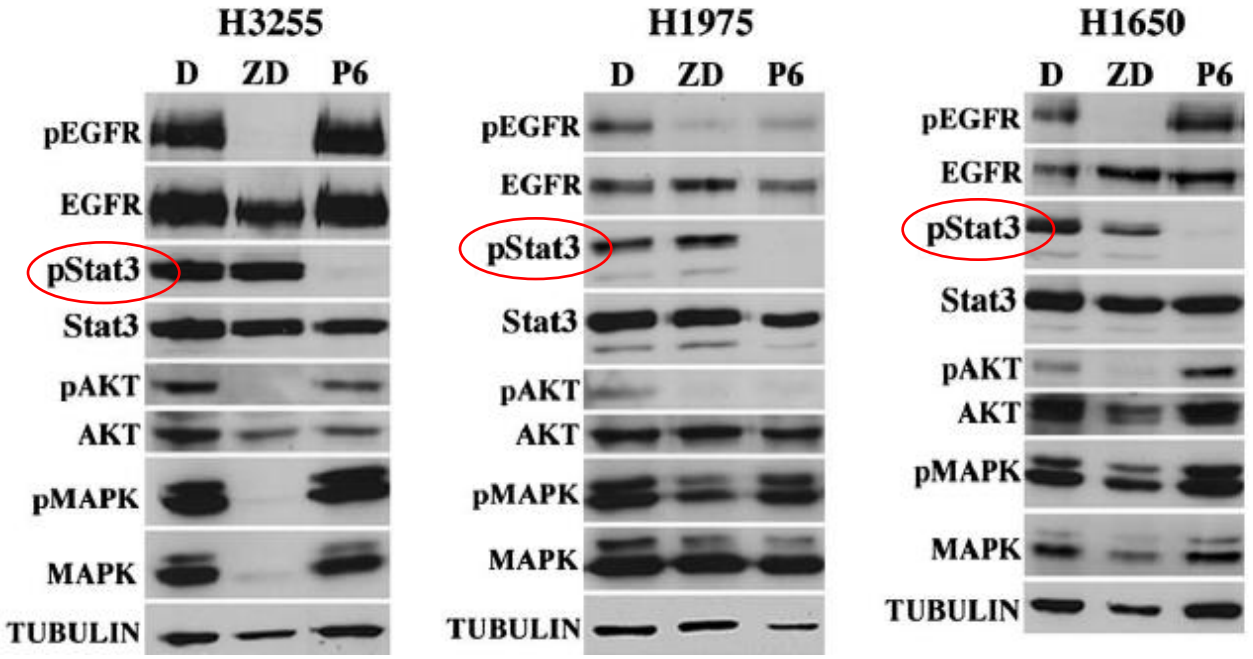
Patients at risk

G1	34	24	24	16	12	8	5	4	3	2	1	1	1	1	0	0	0	0
G2	16	14	12	10	8	7	6	5	5	5	3	1	1	1	0	0	0	0
G3	28	17	8	3	1	1	1	0	0	0	0	0	0	0	0	0	0	0
G4	17	8	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

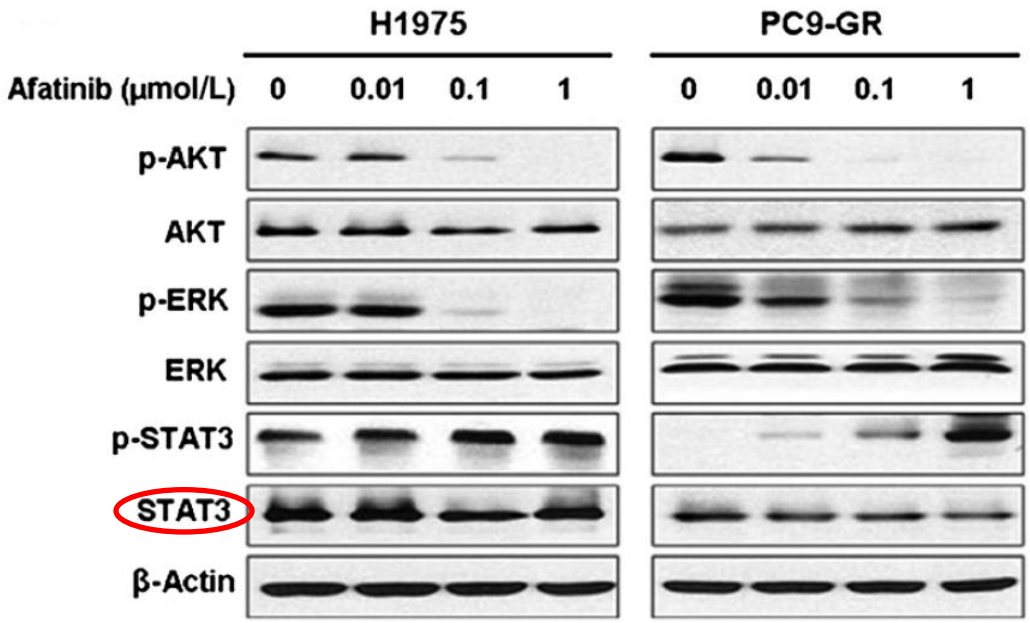
EURTAC – cutoff January 2013

C Costa, Molina, Drozdowskyj et al. CCR 2014

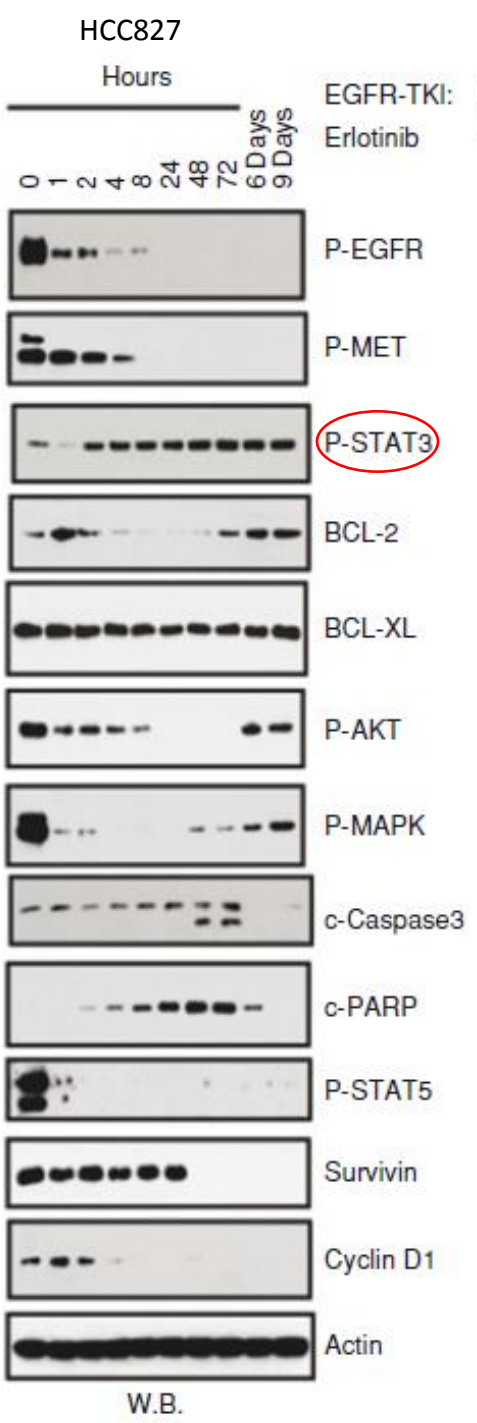
Author	No. of Patients	Histology	Tissue	Percentage of Patients with Pretreatment T790M Mutations Detected by Each Technique	PFS/TTP *(with vs without T790M mutation)*	Allelic Dilution	Dose-Dependent Effect of T790M
Inukai et al [1]	280	ADC, SCC, EGFR mt and wt	FF & FFPE	Direct sequencing: 0.36% Mutant-enriched PCR: 3.6%	no response to gefitinib	1:1000	--
Maheswaran et al [2]	26	ADC, EGFR mt and wt	FFPE	Allele-specific SARMS assay: 38%	7.7 vs. 16.5 m *P* < 0.001	1:500	--
Fukuoka et al [3]	261	ADC, EGFR mt	FFPE	DNA sequencing: 0% Multithreaded electronic PCR sequencing: 0% ARMS: 4.2%	no association with EGFR-TKI resistance	--	--
Oh et al [4]	147	ADC, SCC, LCC, EGFR mt and wt	FFPE	PNA-clamping PCR: 8.2% Direct sequencing: 0%	--	1:10000	--
Querings et al [5]	24	ADC, SCC, EGFR mt and wt	FF & FFPE	Sanger sequencing/pyrosequencing: 0% Massively parallel sequencing: 27%	--	--	--
Rosell et al [6]	129	ADC, EGFR mt	FFPE	TaqMan assay-PNA: 35%	12 vs 18 m *P* = 0.05	1:5000	--
Fujita et al [7]	38	ADC, EGFR mt	FF	SARMS: 0% Colony Hybridization Assay: 79%	--	1:1000	*T790M levels & mTTF:* nil, 0%: 7m low, 0–0.5%: 7m high, ≥ 0.5%: 41m
Su et al [8]	73	ADC, SCC, EGFR mt and wt	FFPE	MALDI-TOF MS: 31.5% Direct sequencing: 2.7%	6.7 vs 10.2 m *P* < 0.05	1:1000	--
Lee et al [9]	11	ADC, EGFR mt	FFPE	Targeted deep sequencing: 9%	immediate disease progression		
Ye et al [10]	36	ADC, EGFR mt	FF & FFPE	Mutant-enriched PCR FF: 2.8% FFPE: 41.7%	--	1:1000	--
Lee et al (Cancer 2014)	124	ADC, EGFR mt	FFPE	MALDI-TOF MS: 25%	6.3 vs 11.5 m *P* < 0.001	--	>2%: 2.4m <2%: 6.7m *P* = 0.009
Costa et al (CCR 2014)	95	ADC, EGFR mt	FFPE	TaqMan assay-PNA: 65.26% TMDA: 24.2%	9.7 vs 15.8 m 10.4 vs 10.8 m	1:5000 1:100	>0.7%: 8.7m <0.7%: 10.4m

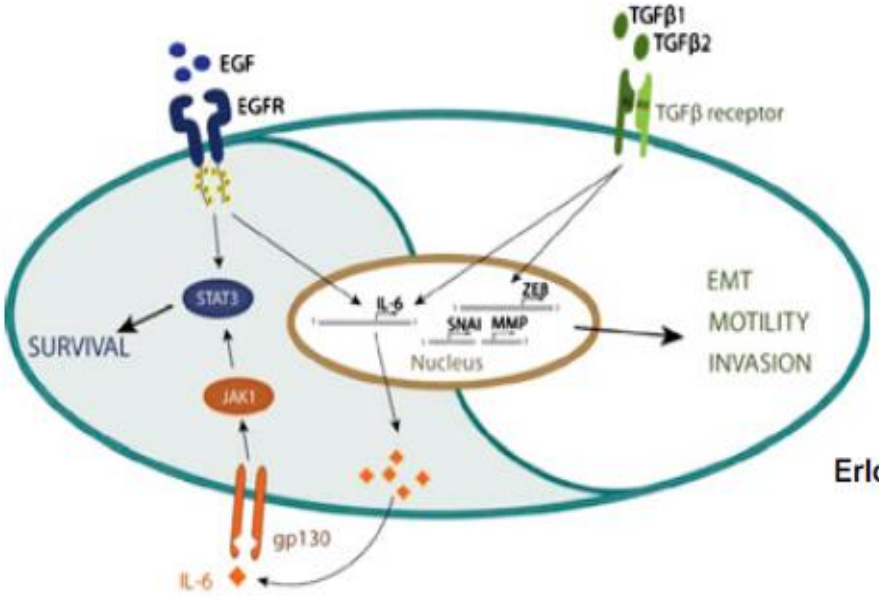


Gao et al. J Clin Investigation 2007



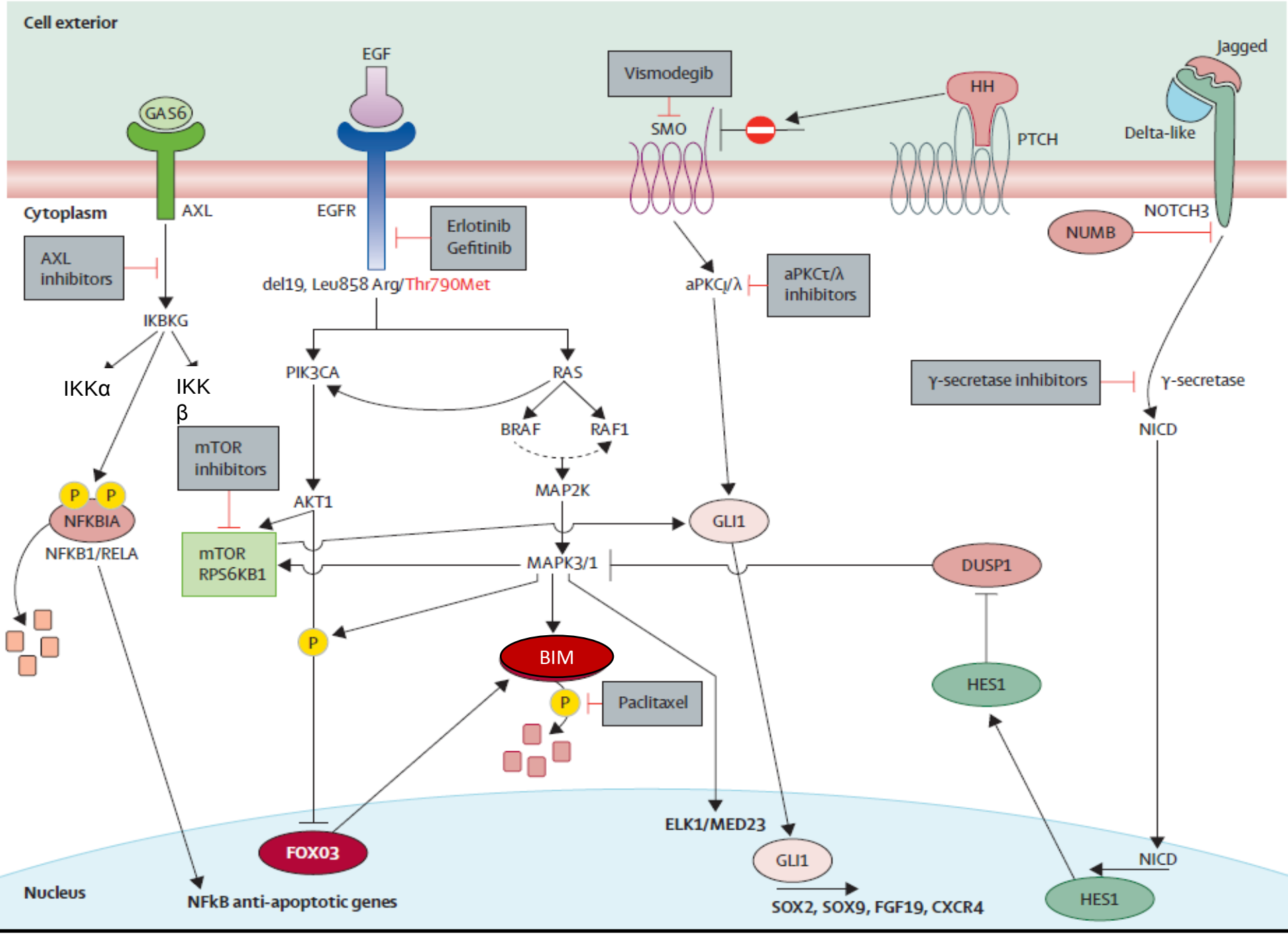
Kim et al. Mol Cancer Ther. 2012





TGFβ-IL6/STAT3 with upregulation of BCL-XL and Bcl2 in erlotinib resistant cells *(Yao et al. PNAS 2010)*

Early adaptive drug escape to erlotinib with activation of **BCL-2/BCL-2XL** associated with a proliferative quiescence state *(Fan et al. Cancer Res 2011)*



Rosell, Bivona, Karachaliou. Lancet. 2013

Conclusions

•BIM mRNA a predictive biomarker of response in EGFR mutant NSCLC *(C Costa et al. CCR 2014)* and potentially in ALK rearranged NSCLCs.

•*TGFβ2-IL6-STAT3-Bcl2 signaling a potential adaptive resistance mechanism in EGFR mutant tumors and in ALK with high BIM expression.*

• Frequent coexistence of T790M could be a target for selective T790M inhibitors or other synthetic lethal approaches (third generation EGFR TKIs).

•AXL indicator of EMT. Low BIM a potential indicator of AXL or GAS6 overexpression. Triple therapy with EGFR TKIs plus AXL and STAT3 blockers (i.e. taxanes) could prolong survival.

•Research warranted in ALK NSCLC patients. EGFR activation and EGFR mut/ALK co-alterations should be assessed.

•*Docetaxel or paclitaxel act as STAT3 inhibitors (Walker et al. Mol Pharmacol. 2010), shedding light on 'flare' after stopping EGFR TKIs, indicating that addition of taxanes can have a benefit through STAT3 inhibition.*