Cancer pathways, Targeted Therapy and Resistance

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Disclosure

Nothing to disclose

Targeting Negative Feedback Regulators to Hyperactivate Oncogenic Signaling

BC CANCER

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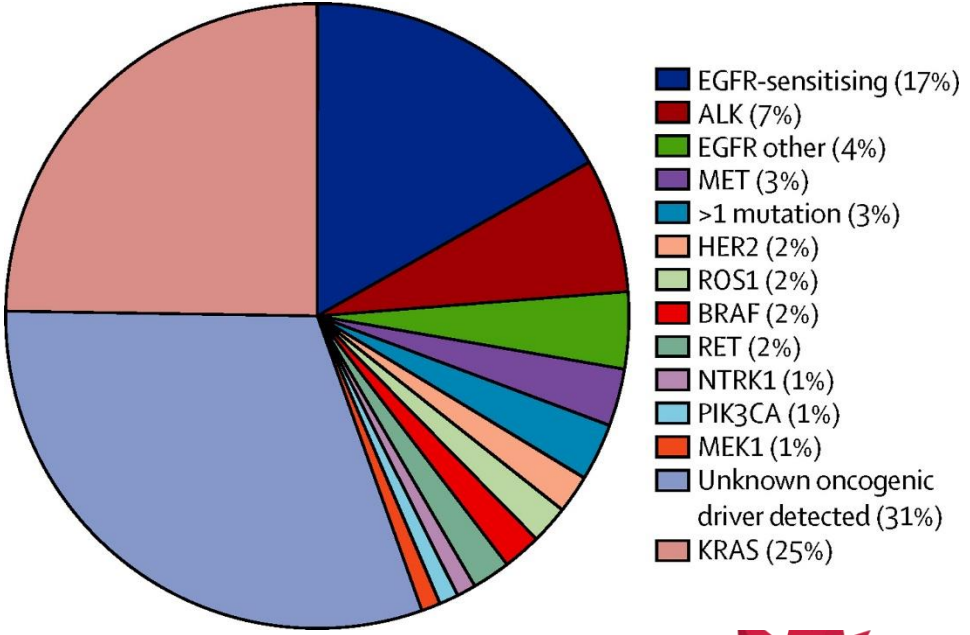
UBC

Issues with Targeted Therapy in Lung Adenocarcinoma

1. Approximately 30% of lung adenocarcinomas have unknown oncogenic driver mutation(s)

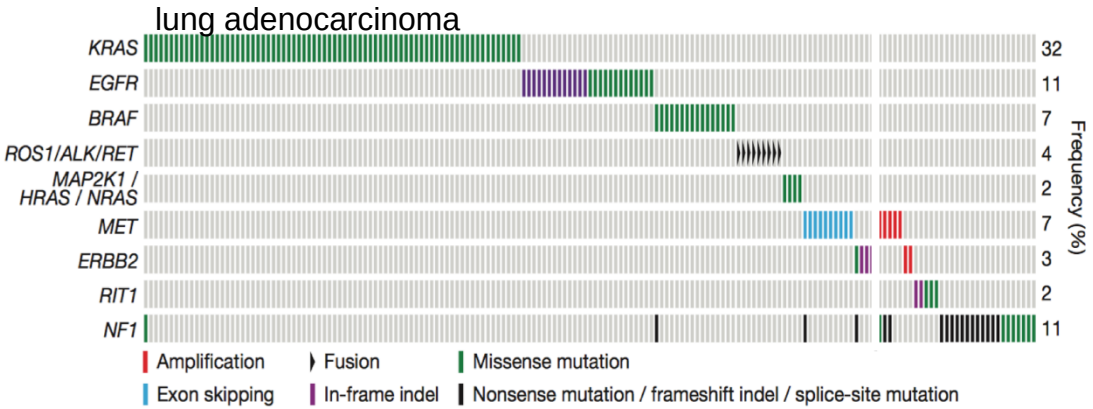
2. Not all identified driver genes in lung cancer are “druggable” – i.e. KRAS

3. Targeted therapies directed at established oncogenic drivers such as EGFR or ALK suffer from primary and required resistance

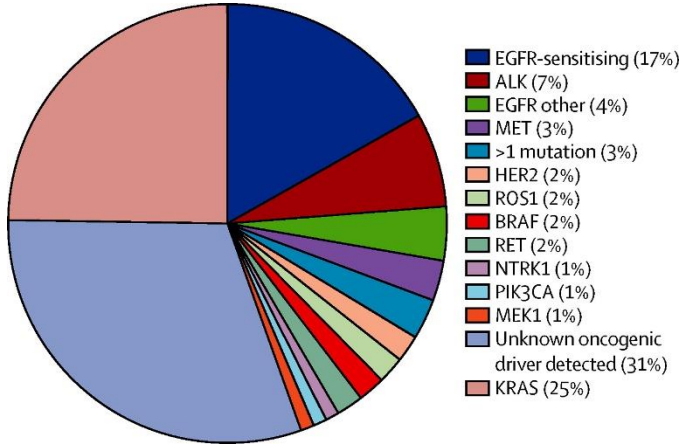


Hirsch et al 2017; Lancet

Genetic Mutations Driving Lung Adenocarcinoma Occur in Mutually Exclusive Manner

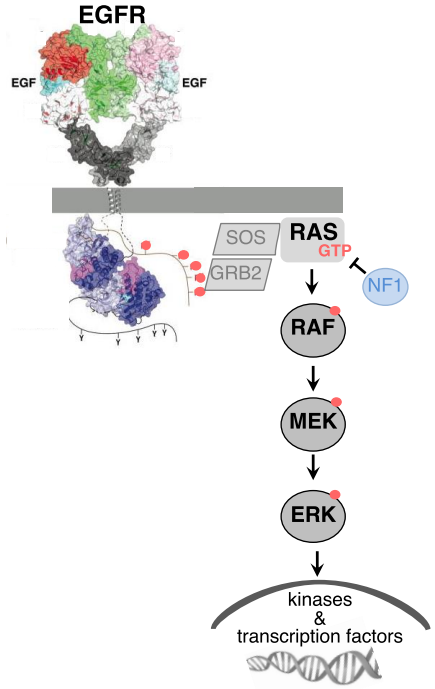


Comprehensive molecular profiling of lung adenocarcinoma
Meyerson M, TCGA. Nature 2014



Hirsch et al 2017; Lancet

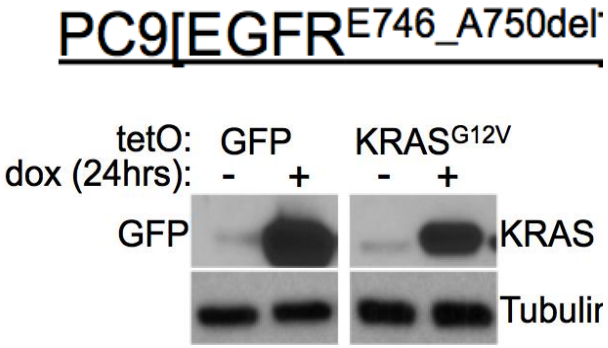
Why are mutations in KRAS and EGFR mutually exclusive in lung adenocarcinoma?

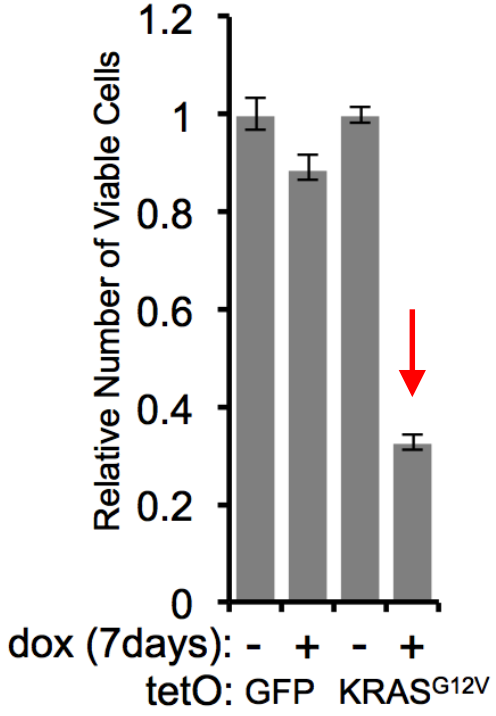


Two genes are in the same/overlapping signaling pathways

Functionally redundant?

Co-expression of mutant KRAS and EGFR results in reduced viability

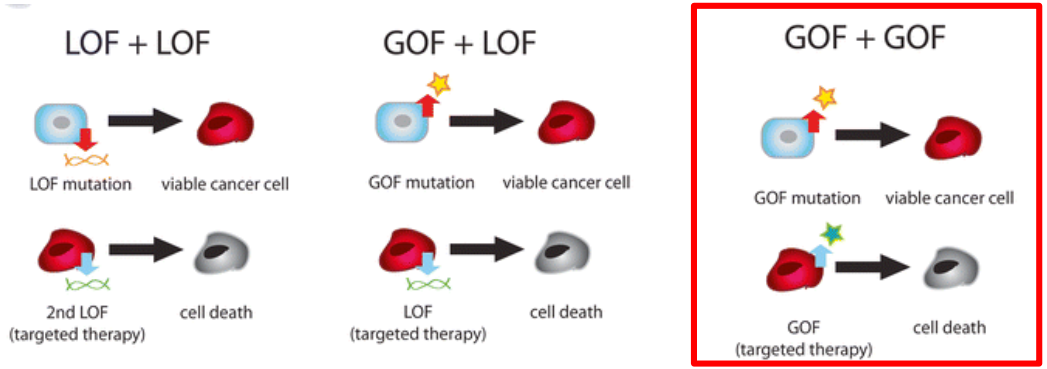




Similar results with H1975[EGFRmutant] and H358 [KRASmutant] cells and transgenic mouse models

Research Questions

- Why are cells intolerant to the combination of mutant EGFR and mutant KRAS?
- Does this reveal a vulnerability in the signaling pathway?
- Can this information be used to develop therapies?



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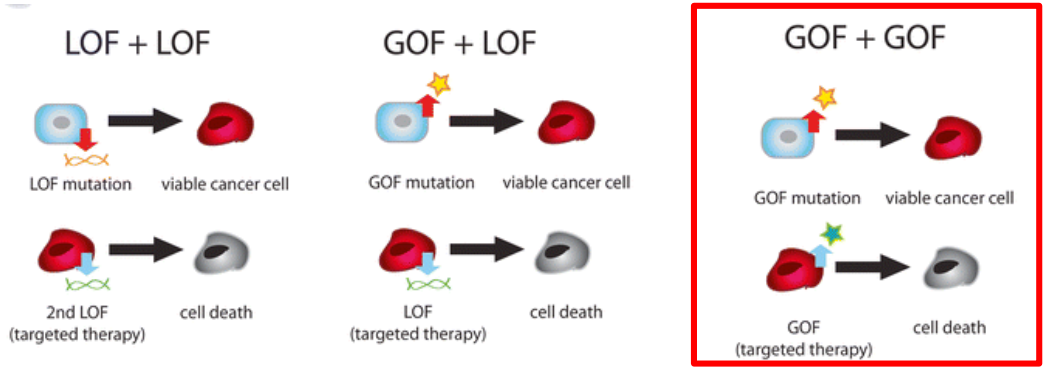
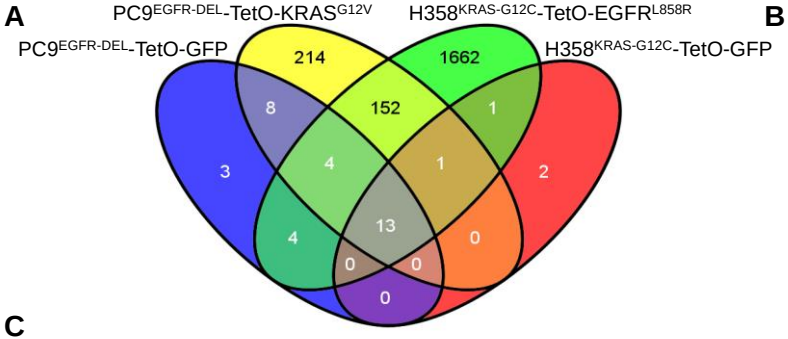


Expression of mutant KRAS/EGFR increases MAPK signaling

A

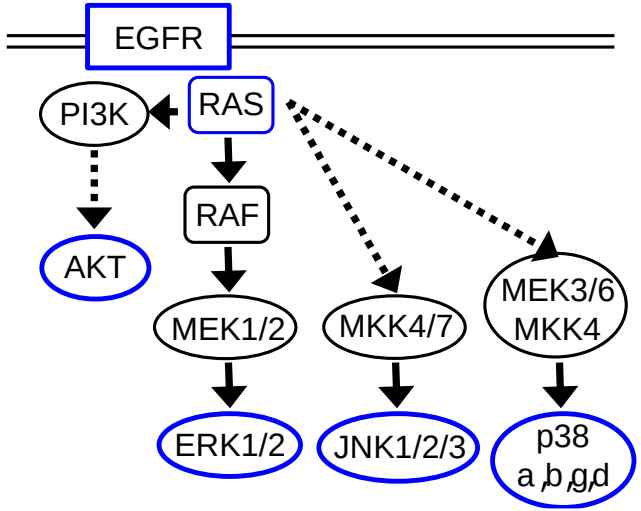


B

C

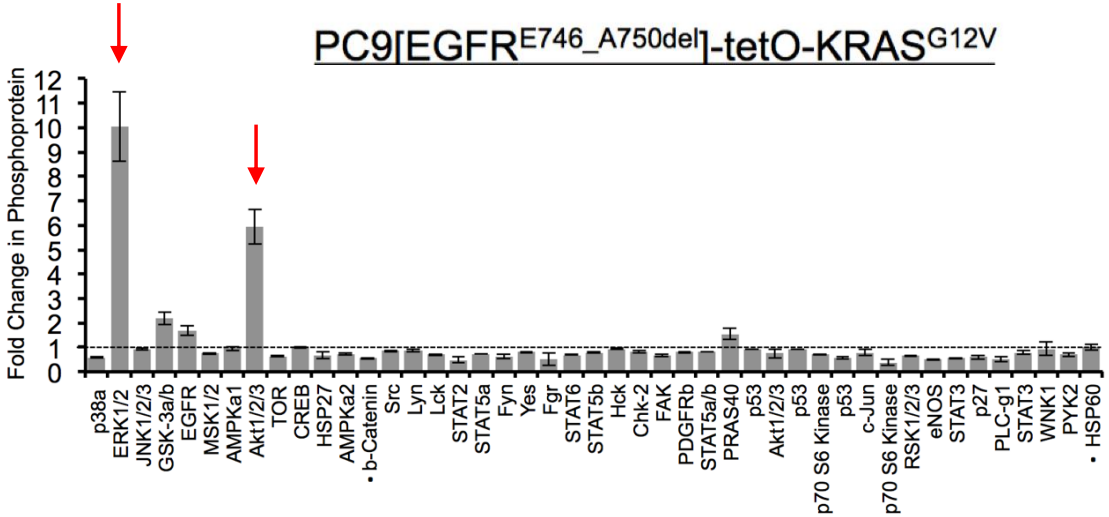
Ingenuity Canonical Pathways	P-Value	Activation Status
p38 MAPK Signaling	2.24E-04	Upregulated
ERK/MAPK Signaling	4.90E-04	Upregulated
IL-6 Signaling	1.91E-03	Upregulated
NRF2-mediated Oxidative Stress Response	2.29E-03	Downregulated
PAK Signaling	4.17E-03	Upregulated
TGF-β Signaling	4.37E-03	Downregulated
PPAR Signaling	4.79E-03	Downregulated
Paxillin Signaling	6.17E-03	Upregulated
Cholecystokinin/Gastrin-mediated Signaling	7.08E-03	Upregulated
Rac Signaling	7.41E-03	Upregulated

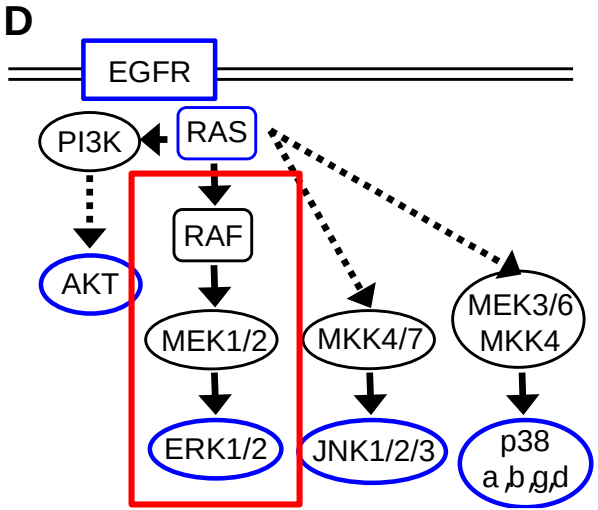
D



Unni*, Lockwood*, Zejnullahu, Lee-Lin, Varmus 2015 eLIFE

Temporal Phosphoarray Analysis Reveals ERK as Potential Mediator of Lethality



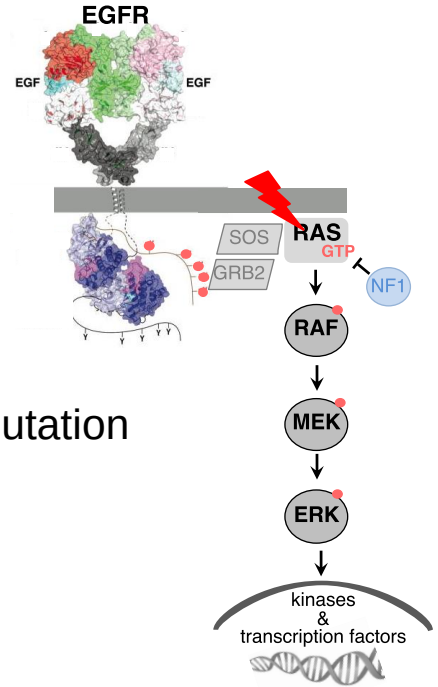


Does overactive ERK kill cancer cells?

Unni, Oh, Harbourne, Wild, Varmus*, **Lockwood***, 2018 BioRvix – in revision eLIFE

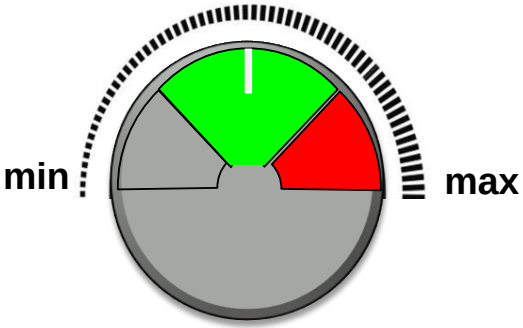
WCLC 2018

A new way to kill cancer?



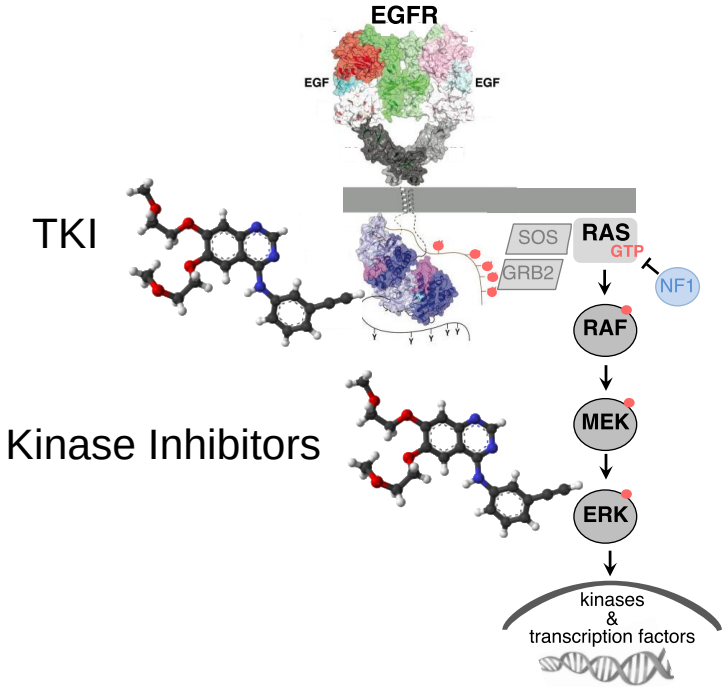
Single Mutation

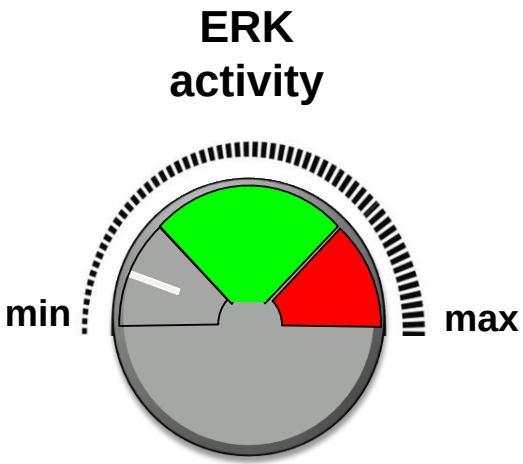
ERK activity



Pathway On - Cancer Grows

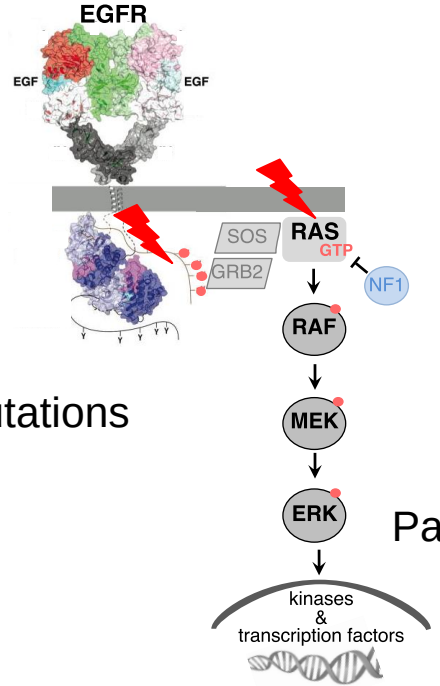
A new way to kill cancer?





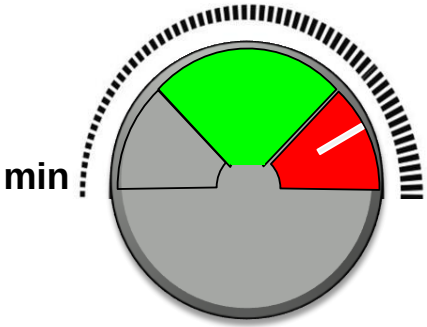
Pathway Off - Cancer Dies

A new way to kill cancer?



Multiple Mutations

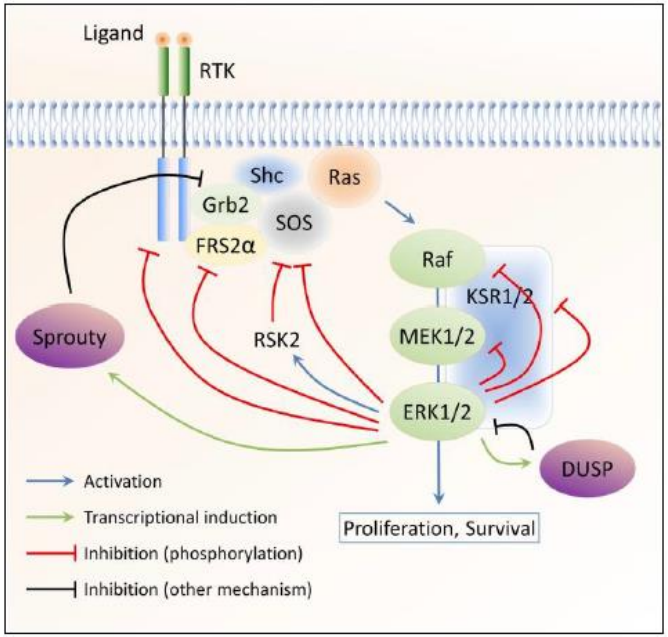
ERK activity



Pathway Hyperactive – Cancer Dies

How can we exploit this hyperactivation vulnerability in the MAPK pathway?

Negative Feedback Phosphatases Regulate EGFR-RAS-MEK-ERK Signaling



- In normal cells, activated in order to down regulate pathway activity
- Typically considered tumor suppressors
- Our data suggests too much ERK activation is lethal
- Do lung adenocarcinomas with mutations in EGFR or KRAS depend on these phosphatases to achieve optimal ERK signaling and cell growth?

Summary

- Hyperactivation of ERK, through co-expression of mutant oncogenes, induces lethality in lung adenocarcinoma cells

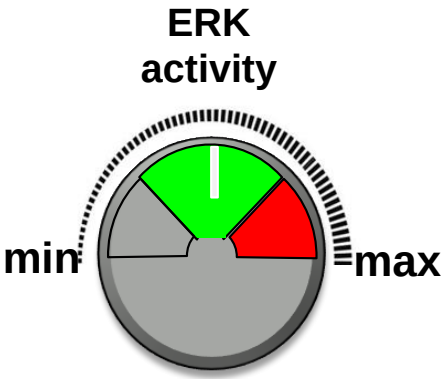
-*DUSP6,* an ERK phosphatase, is differentially expressed in mutant lung adenocarcinoma tumors

-Inhibition of DUSP6 is lethal to lung adenocarcinoma cell lines carrying mutations in *KRAS* or *EGFR*

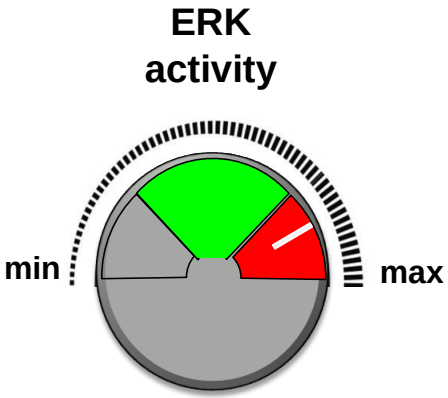
-DUSP6 buffers ERK activity, and mutations in KRAS or EGFR confer dependency on this phosphatase to ensure viable ERK levels

Take Home Messages

-maintaining a specific ERK activation level/zone may underlie mutual exclusivity mutation patterns



Intact Feedback – Cancer Grows



Feedback Inhibition – Cancer Dies

-strategies to acutely activate ERK, through the inhibition of *negative* regulators like DUSP6 may be a therapeutic strategy in tumors with EGFR or KRAS mutations

PEBC

Cancer Epigenetics and Biology Program
Programa d'Epigenètica i Biologia del Càncer
Programa de Epigenética y Biología del Cáncer

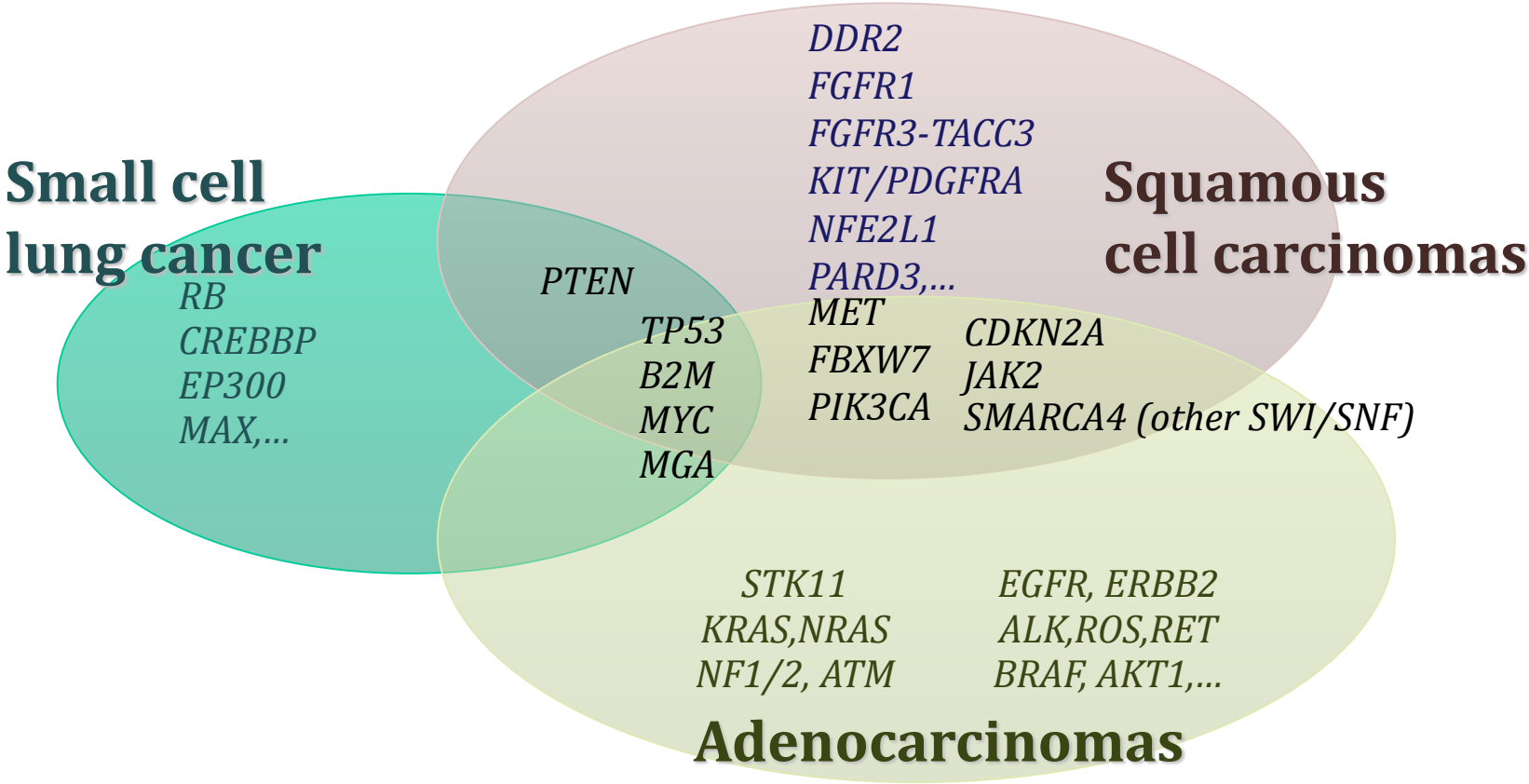
IDIBELL

DEFECTS OF THE SWI/SNF OR MYC/MAX PATHWAYS IN LUNG CANCER:

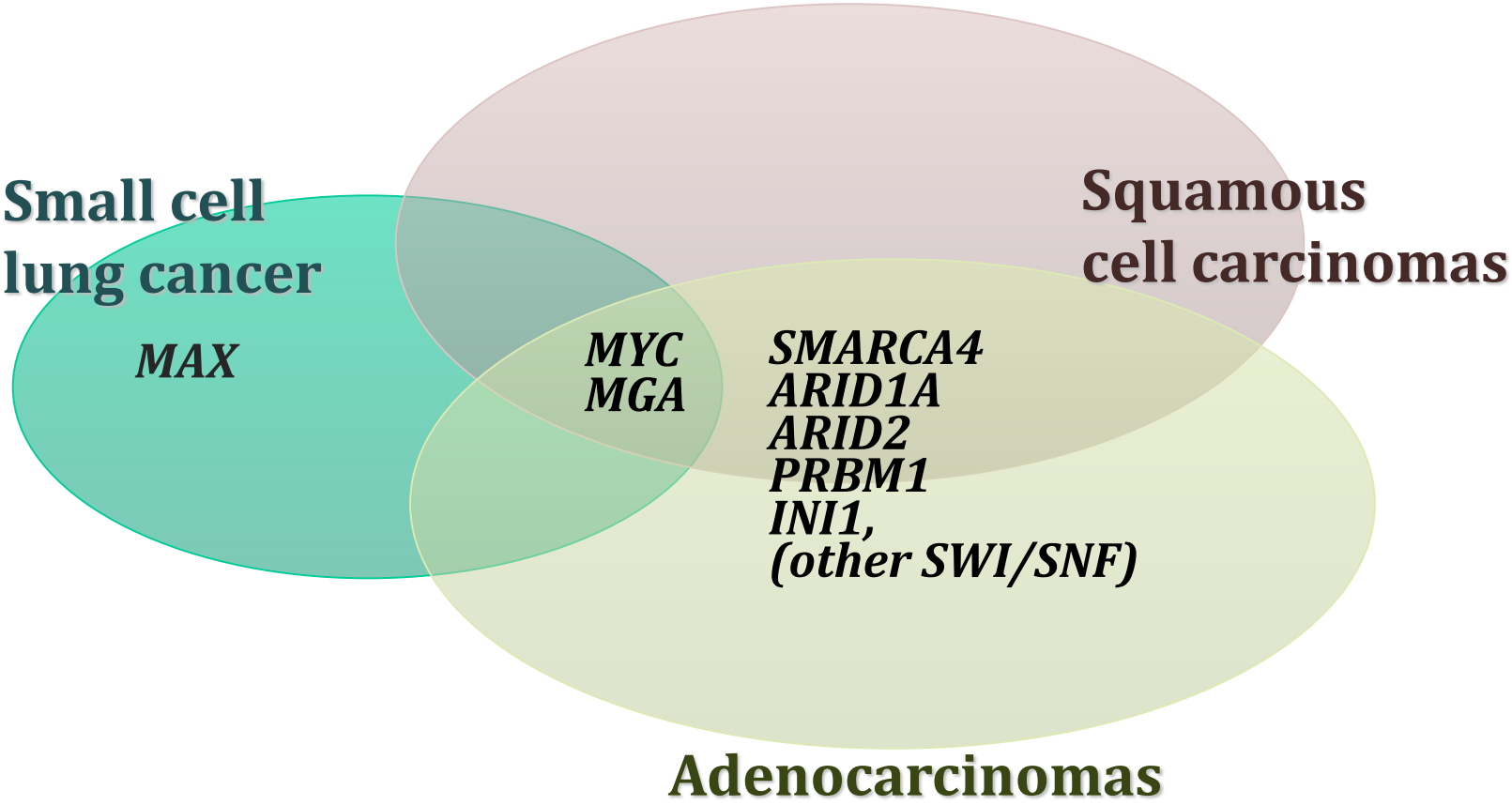
EFFECTS IN CELL DIFFERENTIATION AND THERAPEUTIC OPPORTUNITIES

Montse Sanchez-Cespedes
Head of the Genes & Cancer Group
Cancer Epigenetics & Biology Program-PEBC
Bellvitge Biomedical Research Institute-IDIBELL
Barcelona, Spain

The genetic alteration profile of lung cancer is specific of the different histopathologies



The involvement of the SWI/SNF and MYC/MAX networks in lung cancer



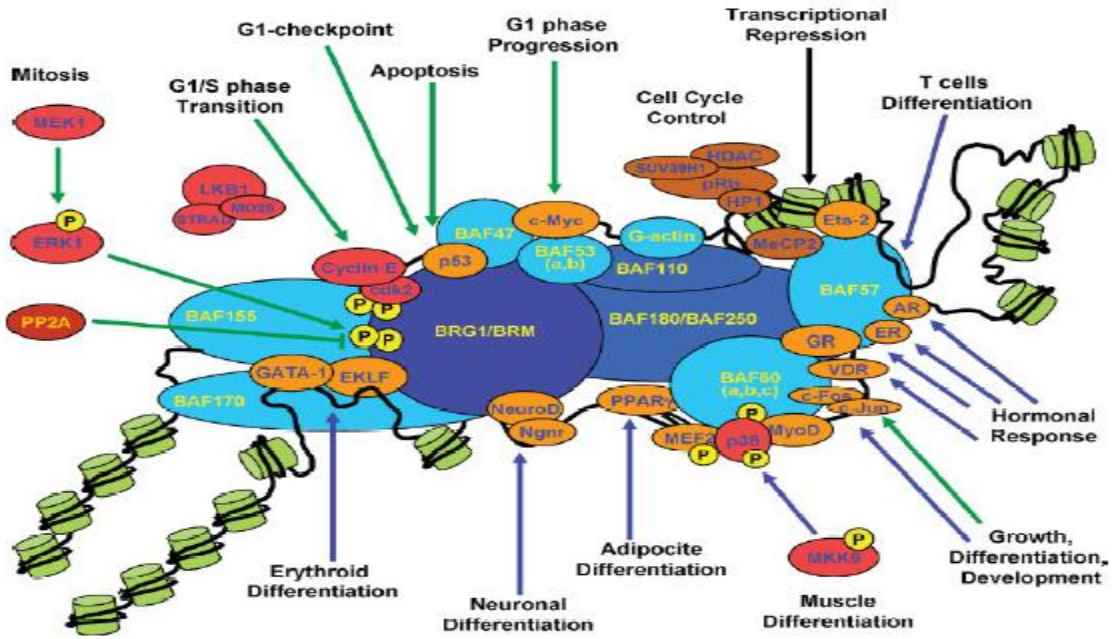
The BRG1 (also SMARCA4) tumor suppressor

BRG1 belongs to the SWI/SNF chromatin remodeling complex

BRG1 (and other components of the complex) bind important oncogenes and tumor suppressors

The complex is composed of one ATPase (BRM or BRG1), and a variable composition of BRG1-associated factors (BAFs)

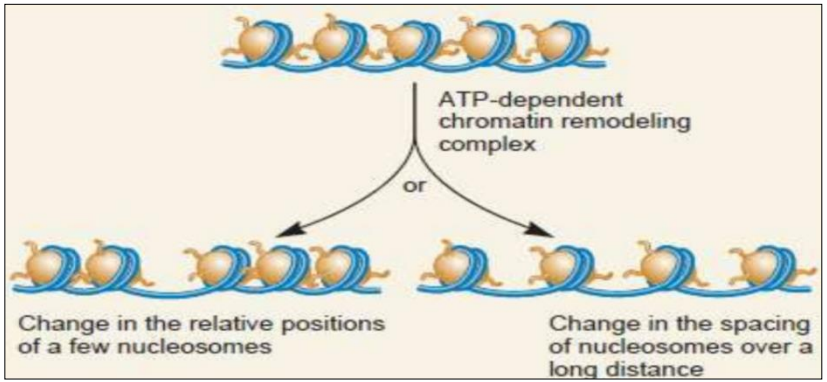
SWI/SNF complex controls the transcriptional activity of NR and other TF



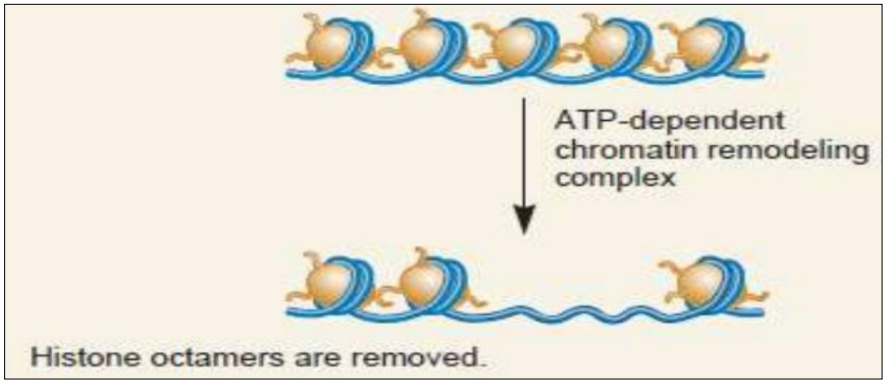
Medina *et al*. **Hum Mut** 2008
Romero et al. **Oncogene** 2017 (review)

The SWI/SNF chromatin remodelling complex alters the position and composition of nucleosomes to change the structure of the chromatin from the closed to the open conformation

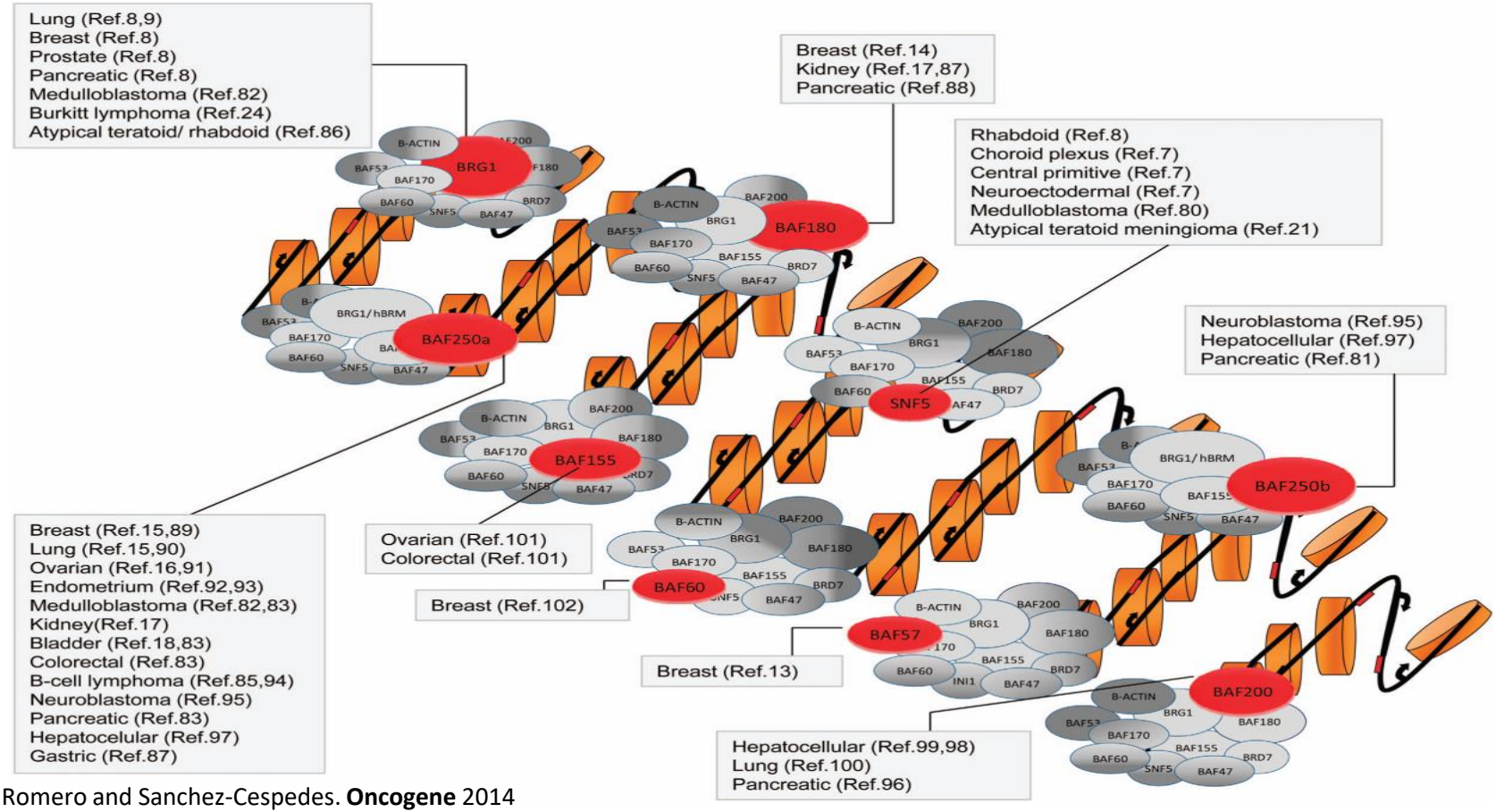
Change the location of nucleosomes



Remove histones from the DNA

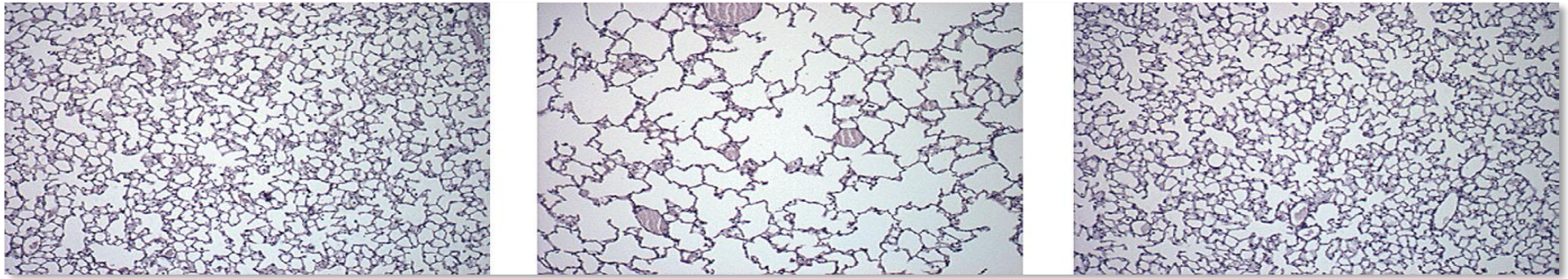


The SWI/SNF complex is widely mutated in human cancer



Romero and Sanchez-Cespedes. **Oncogene** 2014

LUNG HOMEOSTASIS AND NUCLEAR RECEPTOR BIOLOGY



Retinoic acid (RA):

-Induces the regeneration of lung alveoli in experimentally damaged adult rat lungs (*Massaro & Massaro 1997*).

-Its deficiency generates lung tumors in mice (*Saffiotti et al, 1967*).

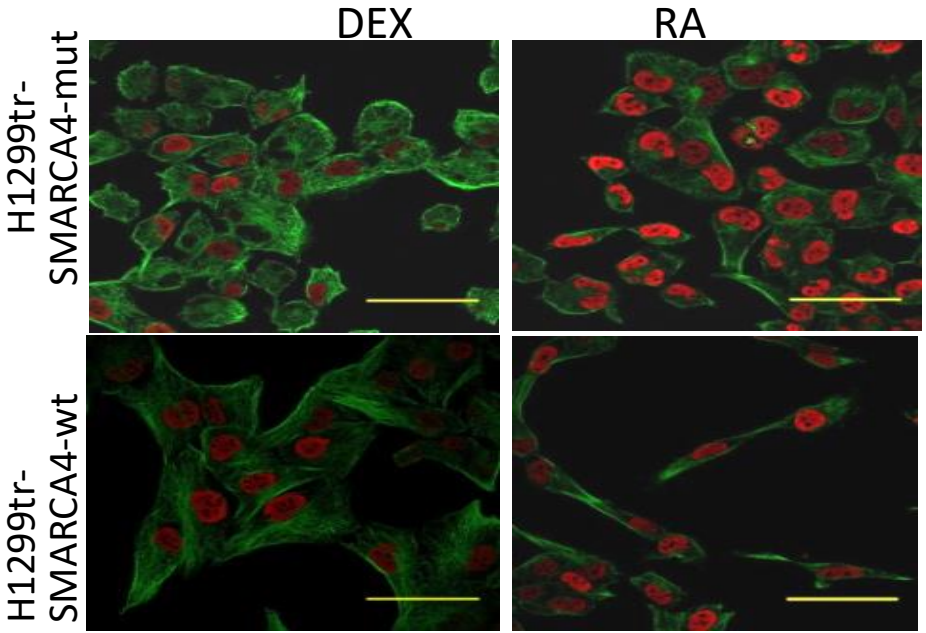
Corticoids:

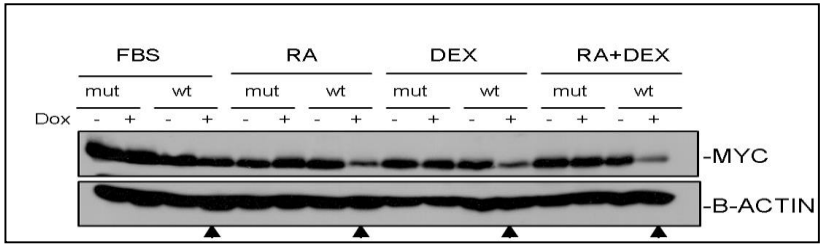
- A failure to respond to GCs, which are involved in resolving inflammation of the lung epithelia, constitute a risk factor for lung cancer, especially in smokers (*Shi et al, 2009*).

-Prevent the normal formation of alveoli in the rat (*Massaro et al. 1985; Tschanz et al. 1995*).

-Accelerate alveolar wall thinning and decreases cell number by inhibiting replication (*M. Maden and M. Hind, 2004*).

SMARCA4/BRG1 is required to mediate the response to corticoids/ Retinoids and MYC down-regulation

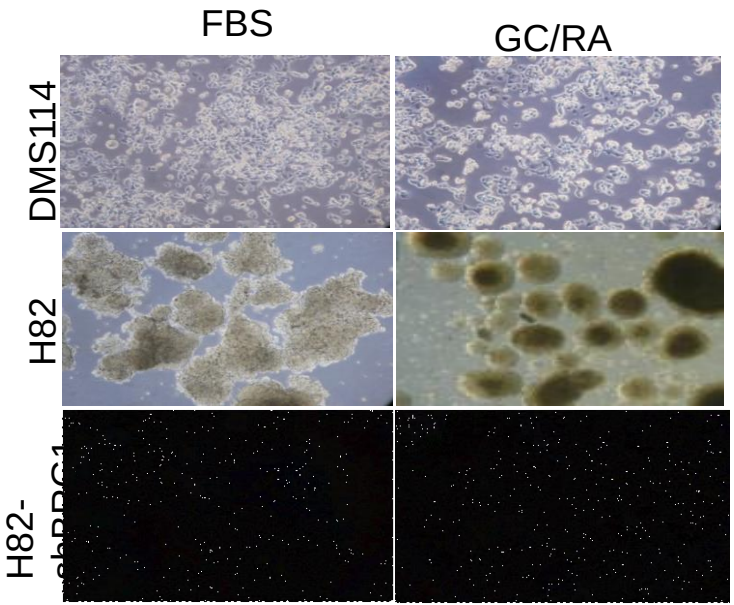


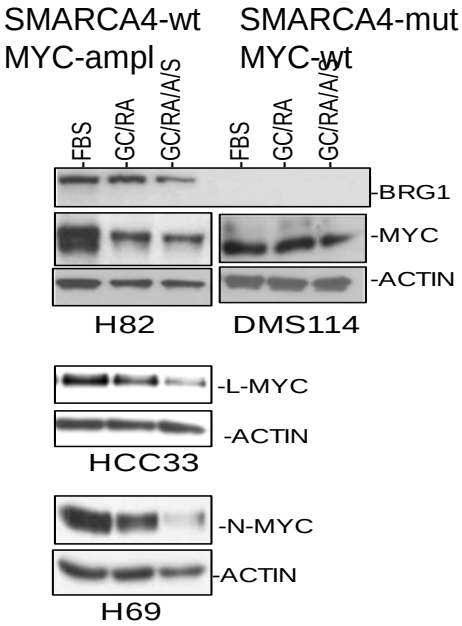


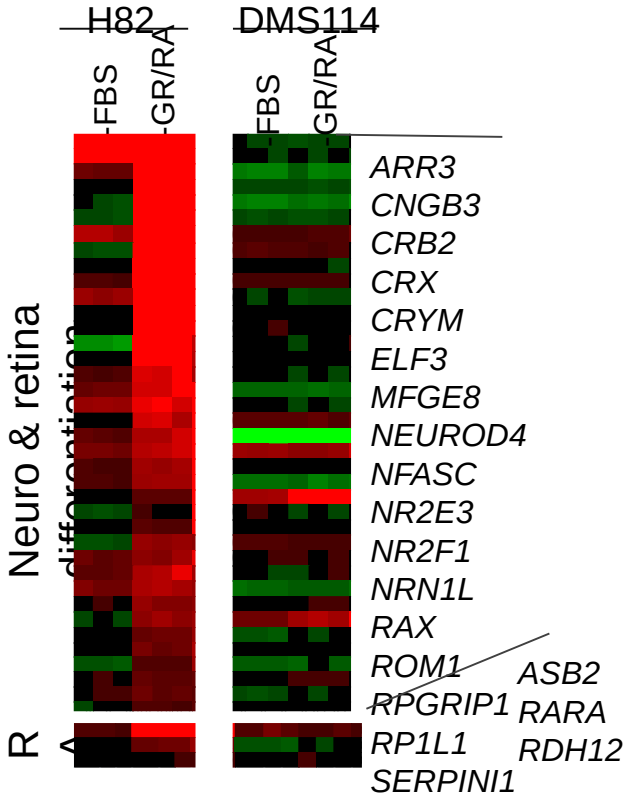
Romero OA et al. **EMBO Mol Med** 2012

GC/RA treatment promotes cell differentiation in SCLC-derived cells that are wt for SMARCA4 (BRG1) and carry MYC-activation.

H82: *SMARCA4* wt & MYC amplified
DMS114: *SMARCA4* mutant & MYC wt



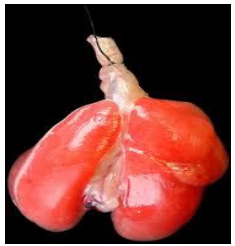


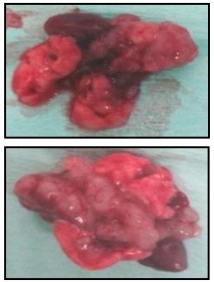


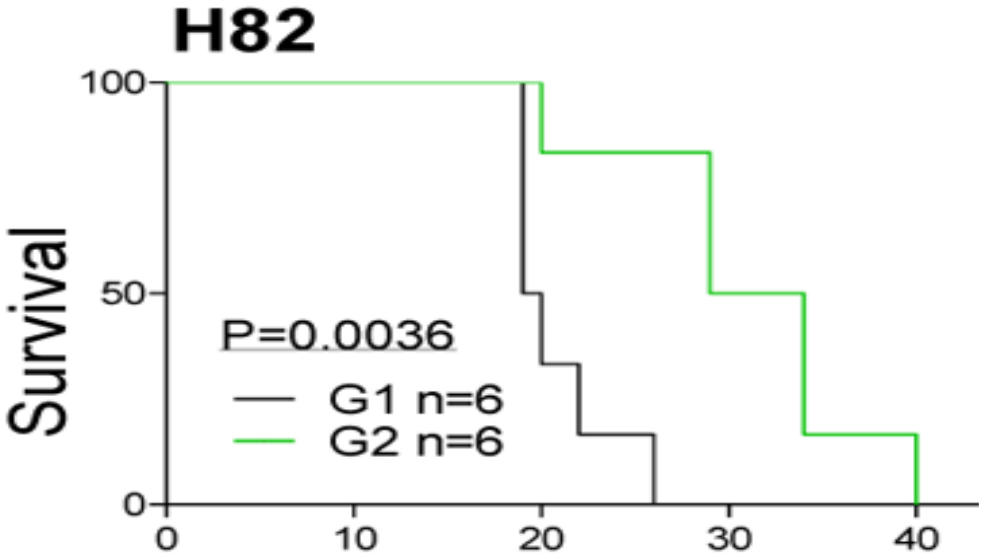
Romero OA et al. **Oncogene 2017**

EFFECTS OF GC/RA IN TUMOR GROWTH, *IN VIVO*

Ortothopic mice model







Increased OS
[HR] =12.3, 95%
[CI] = 2.27 to 66.8

Group treatment	OS (Days)
G1-vehicle	19.5 ± 3
G2-GC/RA	31.5 ± 7

Conclusions

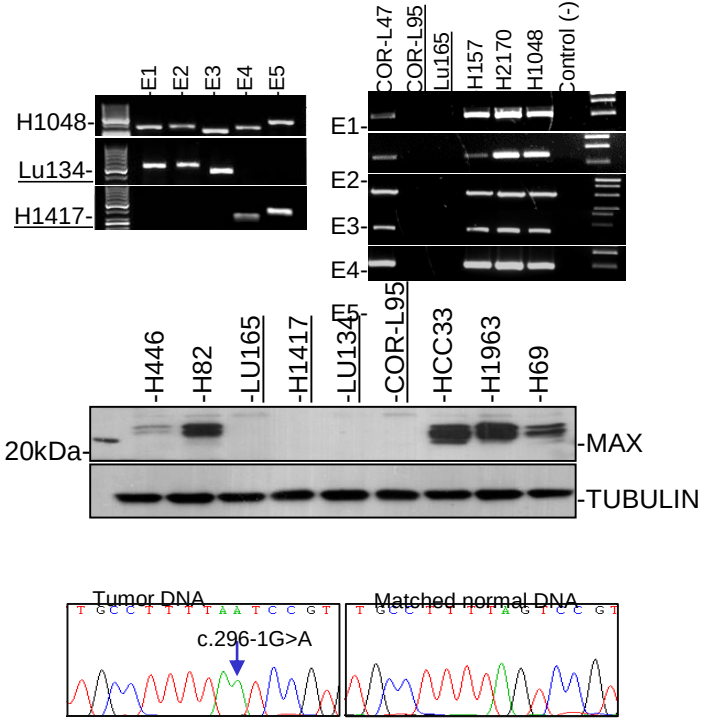
-Through *SMARCA4* inactivation, the cancer cell abolishes the regulation of MYC and prevents the appropriate control of gene expression, promoting cancer development.

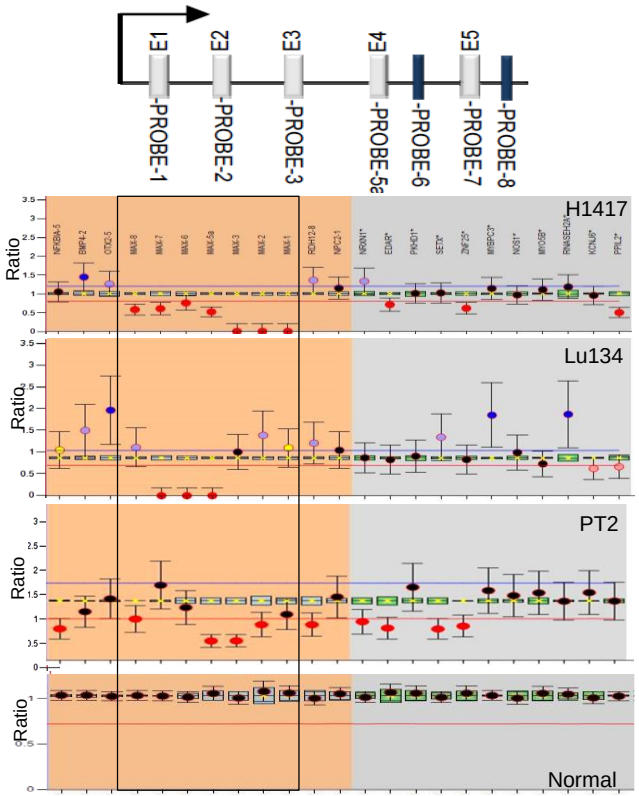
-In contrast to SMARCA4-mutant, MYC-amplified cells are amenable to cell growth inhibition using a combination of GC/RA and, possible other epigenetic drugs. This could be exploited, clinically.

OPEN QUESTION: Does the inactivation of other members of the SWI/SNF or MYC/MAX pathways have the same effect?

MAX is recurrently inactivated in SCLCs

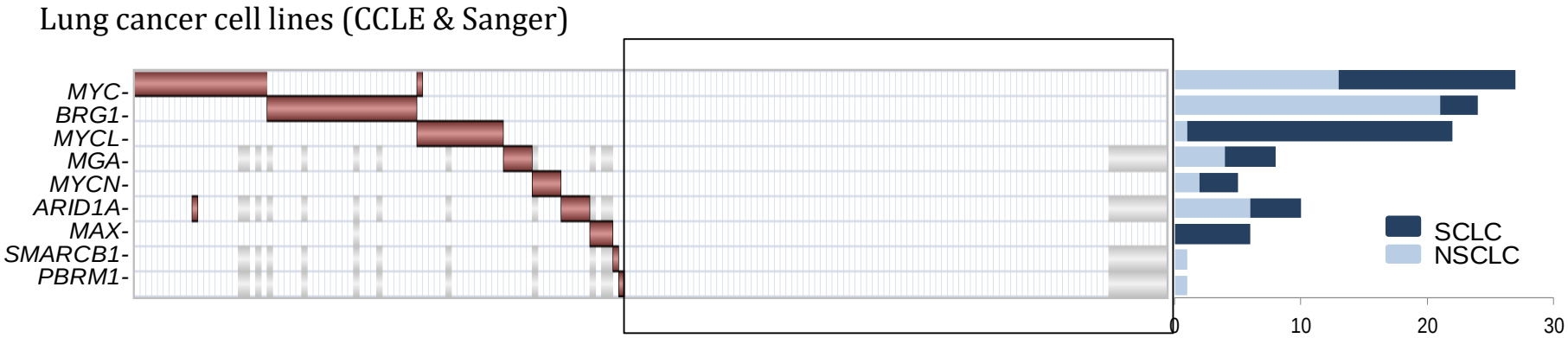
List of the *MAX* alterations found (10%) among the 98 SCLCs





Romero et al. **Cancer Discovery,** 2014

Alterations at the MAX /MYC and at the SWI/SNF pathways are mutually exclusive



ARE THERE OTHER GENES ALTERED WITHIN THIS NETWORK???

Romero et al. **Cancer Discovery,** 2014

Conclusions

-*MAX* is mutated in SCLC and, thus, constitute a *bona-fide* tumor suppressor gene involved in its development.

OPEN QUESTIONS:

Has MYC any role, at all, in MAX-deficient cells?

If not, how do cells without MYC activity proliferate?

-Deletion of SMARCA4 in MAX-deficient cells is synthetic lethal, heralding potential clinical implications.

NYU Langone MEDICAL CENTER

Stimulating anti-Tumor immunity through enhancing T-cell activation

Kwok-Kin Wong, MD, PhD

Epigenetics regulation in cancer: HDAC inhibitors

- Histone deacetylases (HDAC) catalyze the removal of acetyl groups from lysine residues in histones and non-histone proteins thereby regulating many cellular processes

- Pan or isozyme-specific HDACi have gained attention in oncologic applications due to their reported cytostatic effects in cancer models

- Emerging data highlight their **immuno-regulatory** properties in various inflammatory settings

Epigenetics regulation in cancer: Bromodomain inhibitors

- Bromodomains are unique amino acid domains which act as readers of lysine acetylation thus are involved in epigenetic regulation

- The utility of inhibitors of bromodomain proteins (BrDi) are also being explored in many cancer indications
  - JQ1, an inhibitor of the BET family of bromodomain proteins (BRD2,3,4, and BRDT) has shown efficacy in hematologic malignancies such as AML and multiple myeloma

- A number of ongoing clinical trials are exploring therapeutic efficacy of BrDi in solid cancers

- There is paucity of data on their effects on tumor-associated immune cells

Collaborative project with Nathanael Gray laboratory

What does various kinase inhibition do to T cells?

Top hits of kinase inhibitors that activate T cells *in vitro*

Rank	Vendor ID	Chemical_Name	Zscore_1	Zscore_2	Zscore_AVG
1	361551	GSK-3 Inhibitor X	28.58	35.89	32.23
2	361550	GSK-3 Inhibitor IX	25.07	34.88	29.98
3	402081	Indirubin Derivative E804	20.78	24.43	22.60
4	559396	SB 220025	14.69	27.59	21.14
5	402086	Indirubin-3'-monoxime, 5-Iodo-	10.18	17.16	13.67
6	420320	KT5720	10.81	13.17	11.99
7	219476	Cdk4 Inhibitor	7.01	16.07	11.54
8	124029	Akt Inhibitor XII, Isozyme-Selective, Akti-2	5.54	15.29	10.41
9	572650	SU9516	8.23	12.12	10.18
10	420126	JAK3 Inhibitor VI	8.23	11.47	9.85
11	234501	Compound 401	2.83	15.49	9.16
12	189405	Aurora Kinase Inhibitor III	7.11	6.48	6.80
13	260962	DNA-PK Inhibitor III	5.18	6.23	5.71
14	440206	LY 294002, 4-NH2	3.33	7.54	5.43
15	361553	GSK-3b Inhibitor XI	5.72	4.87	5.30
16	181305	Arcyriaflavin A, Synthetic	3.89	5.67	4.78
17	375670	Herbimycin A, Streptomyces sp.	5.64	3.66	4.65

compound library:
protein kinase inhibitor

Total compounds screened:
244

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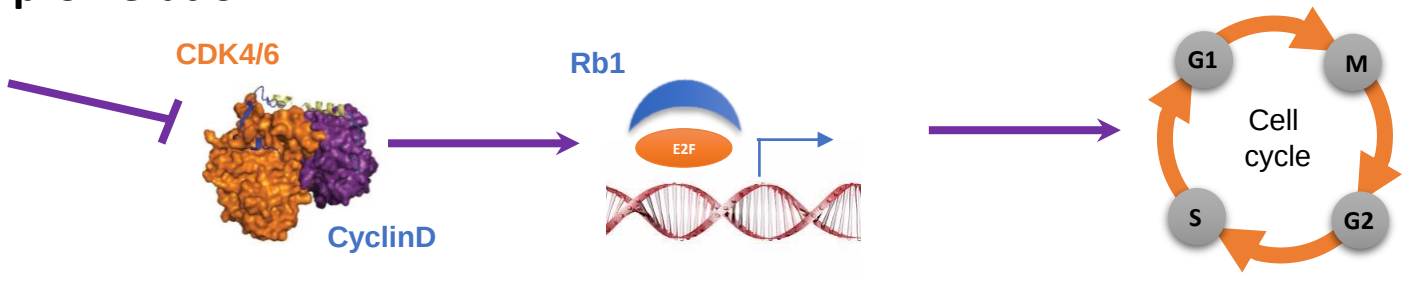
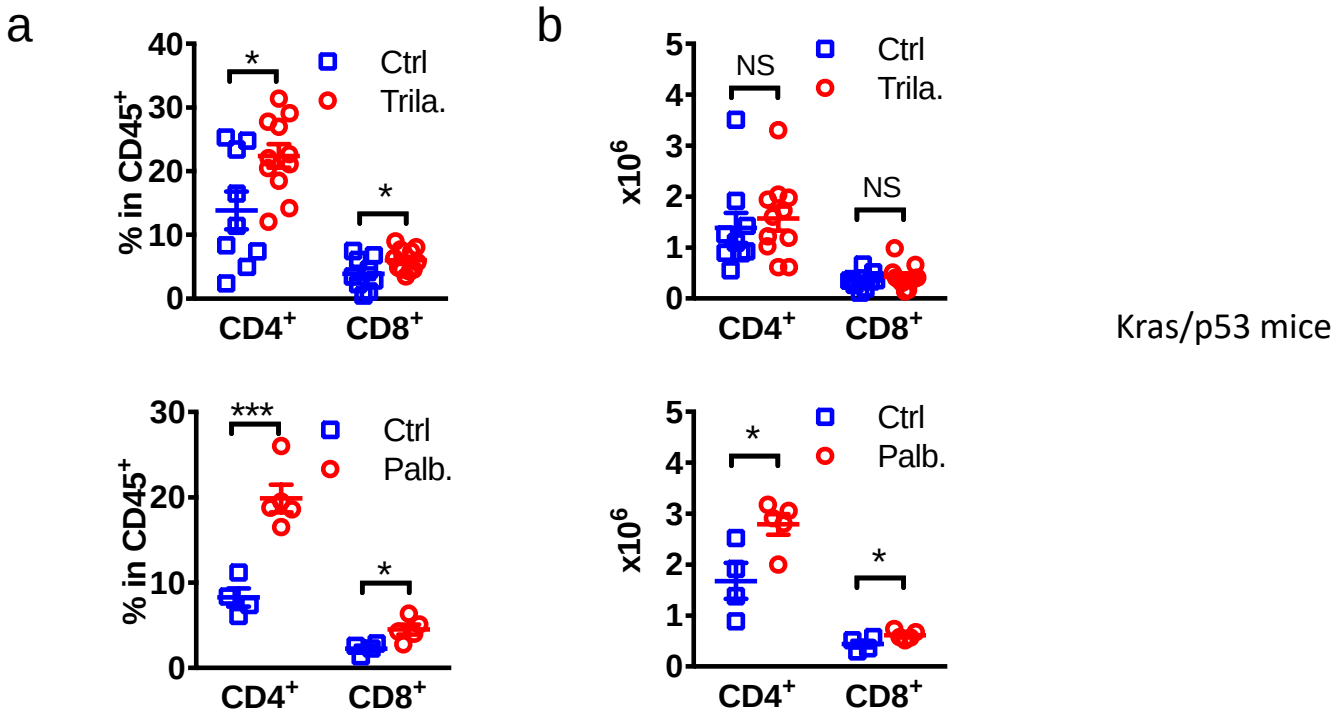
CDK4/6 inhibitor

- **Cyclin-dependent kinase (CDKs) mediates cell cycle progression. Among them, CDK4/6 regulates transition from G1 to S phase through Rb1**
- **Inactivation of G1/S phase checkpoint is often found in many types of cancer, including Rb1, CDKN2A inactivation or CCND1 amplification, which leads to CDK4/6 activation**
- **CDK4/6 is also required for hematopoietic stem and progenitor cells proliferation**

Palbociclib
Trilaciclib
Abemaciclib
Ribociclib
…



CDK4/6 inhibition increases T cell infiltration in tumor *in vivo*



Kras/p53 mice

Deng J, et al. *Cancer Discov*. 2018

Clinical advances of CDK4/6 inhibitors

Drug	Status	Approval Date	Cancer Type
PD0332991(Palbocilib)	Approved	03/31/2017	Breast Cancer
G1T28 (Trilaciclib)	Phase II		SCLC, TNBC
G1T38	Phase I/II		Breast Cancer, EGFRm NSCLC
LY2835219 (Abemaciclib)	Approved	02/26/2018	HR+, HER2- metastatic Breast Cancer
LEE011 (Ribociclib)	Approved	03/13/2017	HR+/HER2- advanced Breast Cancer

Key risks: Neutropenia, Hepatobiliary toxicity, lymphopenia

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Conclusions

HDAC, BET and CDK4/6 inhibitors all have differential effects on various immune cells within the lung cancer tumor immune microenvironment

Specific small molecule inhibitors can be combined with immunotherapies to increase rate, depth and duration of response.

Future Directions

Identify additional novel synergistic combination

Addressing drug resistance beyond kinase domain mutations

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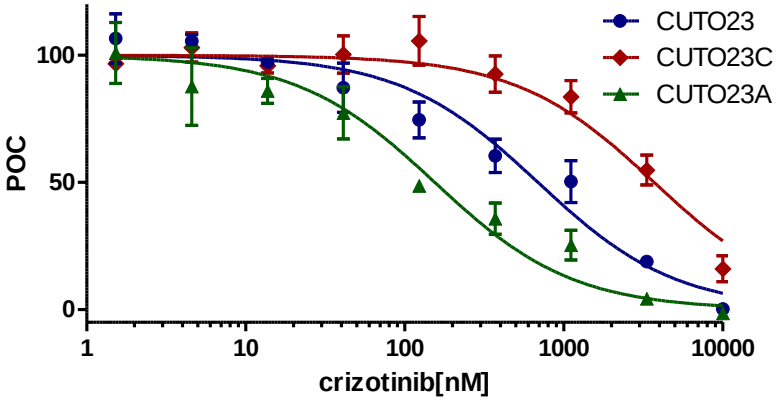
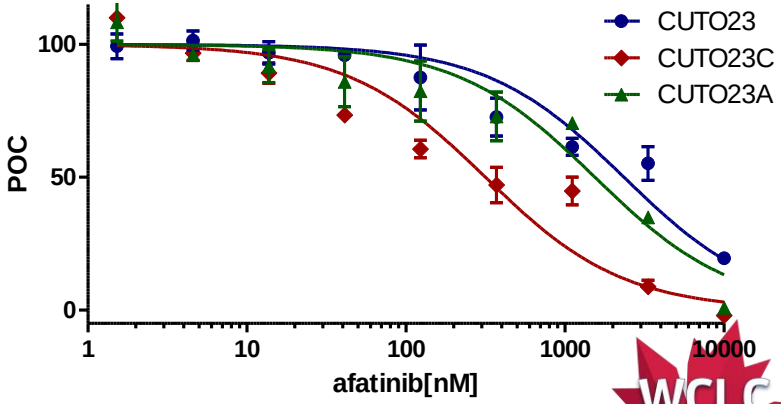
Outline

1. Kinase domain mutations
2. Bypass signaling
    a. New oncogenic mutations
    b. Activation of bypass pathways without mutation
3. Cancer cell state change
    a. EMT (Epithelial to mesenchymal transition)
    b. Histologic transformation

Plasticity of resistance suggests that cells can rapidly reprogram

CUTO23A *ROS1* ← 10nM afatinib — CUTO23 — 50nM crizotinib → CUTO23C *ErbB2*

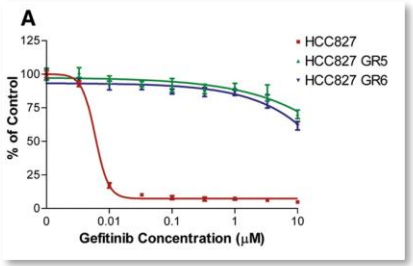




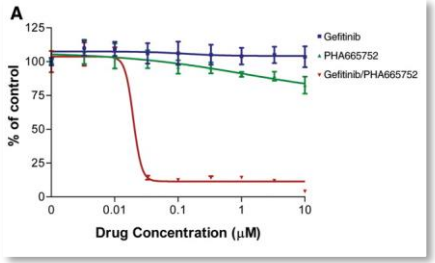
CUTO23 (*CD74-ROS1* with HER2-mediated resistance)

Bypass signaling by MET in EGFR TKI resistance

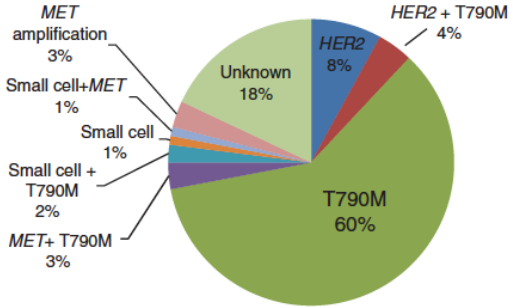
MET gene amp induces EGFR TKI resistance



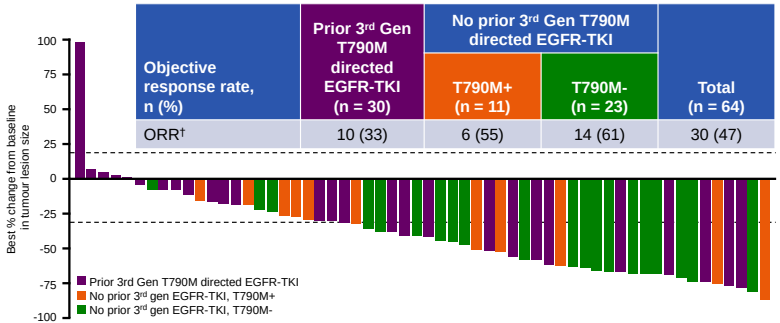
Combination EGFR + MET inhibition overcomes resistance



MET gene amplification~6-10% of EGFR TKI resistance



Tatton trial demonstrates effectiveness of METi savolitinib + osimertinib in MET+ resistance



Anti-tumour activity in all MET+ pts* n=64

Engelman et al., Science 2007

Yu et al., Clin Can Res 2013

Ahn et al., WCLC 2017

EMT as a mechanism of resistance to ALK inhibitors

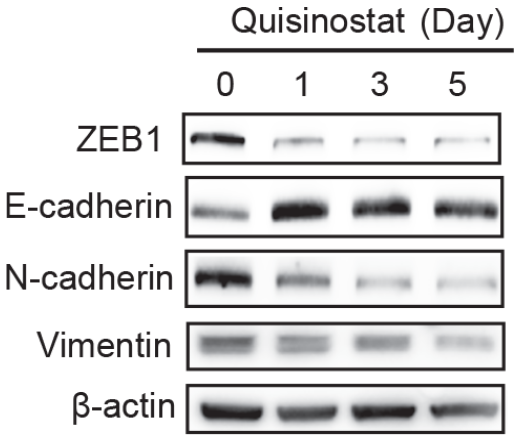
EMT in crizotinib-resistant tumors[1]

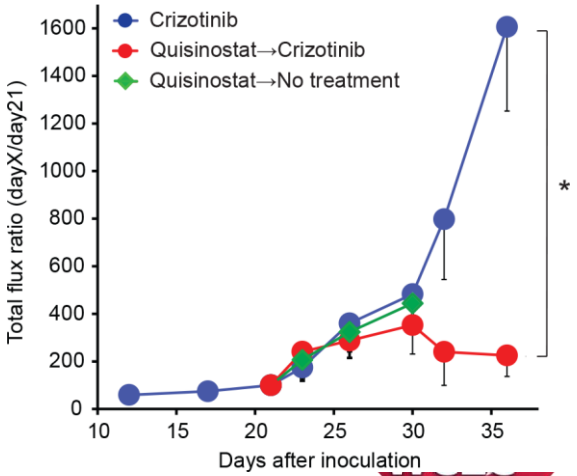
Patient ID	ALK resistance mutation	Vimentin	E-cadherin
MGH023-2	ALK F1174C	**Positive**	**Negative**
MGH034-2	WT	**Positive**	**Negative**
MGH049-1	WT	Positive	Positive
MGH051-2	ALK G1202R	Positive	Positive
MGH061-1	WT	Negative	Positive
MGH065-2	ALK L1196M	**Positive**	**Negative**
MGH067-1	ALK L1196M	**Positive**	**Negative**
MGH084-1	ALK I1171N, C1156Y	Negative	Positive
MGH089-1	WT	Negative	Positive
MGH092-1	ALK G1202del	Negative	Positive
MGH902-1	WT	**Positive**	**Negative***
MGH908-1	WT	Negative	Positive

*Partial loss

5/12 (42%) with **EMT**

Reversal of EMT and crizotinib resistance using an HDAC inhbiitor[2]

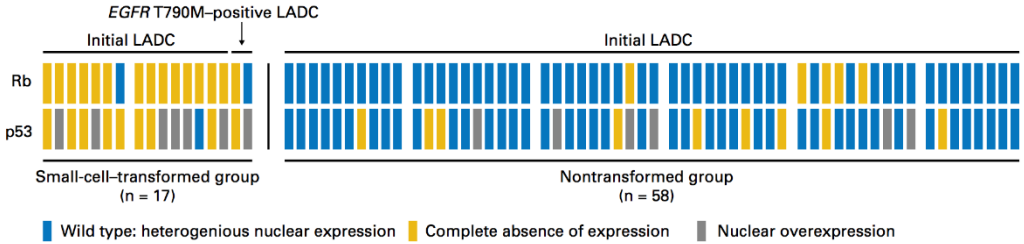




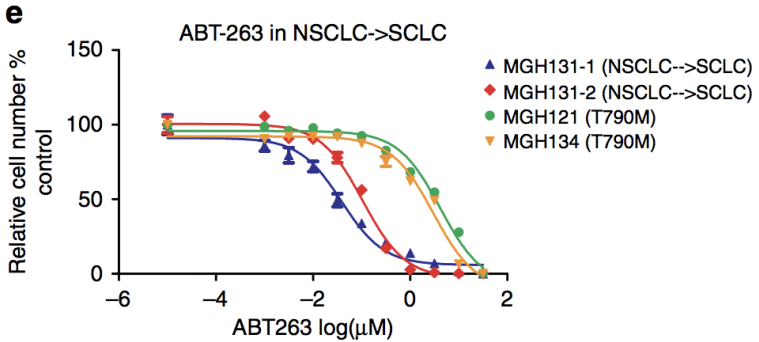
[1]Gainor et al., Can Discovery 2015, [2]Fukuda et al., WCLC 2016

Prediction and potential treatments for SCLC transformation

Rb and P53 loss predict SCLC transfomation[1]



SCLC transformed cell lines show sensitivity to BCL-2 family inhibitors[2]



	MGH131-1	MGH131-2	MGH121	MGH134
IC_{50} (μM)	0.039	0.11	4.2	3.0

Non-precision strategies to overcome off-target resistance mechanisms

- Chemotherapy
- Chemotherapy/IO combinations
  - IMpower150 (carbo/pac/bev/pembro) KN-042 (carbo/pem/pembro)
- ~~TKI/IO combinations~~
  - osimertinib/durvalumab and crizotinib/nivolumab
- Consolidation treatment with radiation therapy
  - LCT following 4 cycles of chemo or 3 months of TKI (Gomez et al., Lancet Onc 2016)
- Combination therapies upfront
  - TKI/anti-angiogenic (JO25567/NEJ026: erlotinib + bevacizumab)
  - chemo+ TKI (NEJ009: gefitinib + carbo/pem)

Conclusions

- Non-kinase domain mutations account for only 40-60% of resistance mechanisms
- Many resistance mechanisms are difficult to diagnose with standard clinical assays (even NGS panels)
- Resistance mechanisms beyond secondary kinase mutations do not have approved therapies yet in NSCLC
- Precision trials to match combination therapies with mechanisms of resistance have been rare
- Case reports of combination targeted therapies offer some guidance, but critical to report successes and failures including toxicities
- Non-precision therapies at current time remain the mainstay of treatment

ΕΥΧΑΡΙΣΤΩ