

Molecular Pathology of Lung Cancer

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Financial Disclosure

- I have no significant relationships to disclose

Molecular pathology of lung cancer

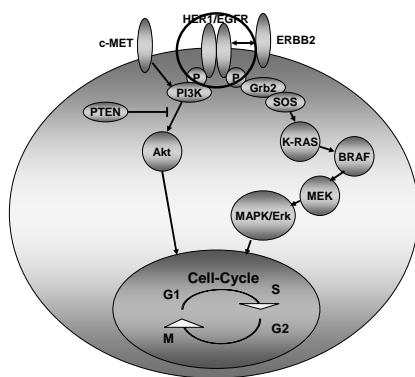
- Molecular analysis of non-small cell carcinoma, especially adenocarcinoma, has become an important clinical tool
- Chemotherapy can be individually tailored for specific molecular diagnoses

Cell Signaling Pathway

- Multiple pathways and branches with multiple targets (and multiple avenues for resistance...)
- Mutually exclusive mutations
 - *KRAS, EGFR, ALK, ERBB2, BRAF*
 - Together occur in 50% of lung adenocarcinoma
- The human epidermal growth factor receptor (HER) family of tyrosine receptors offers many targets for potential intervention
 - HER-1(ErbB-1) EGFR
 - HER-2(ErbB-2)
 - HER-3(ErbB-3)
 - HER-4(ErbB-4)

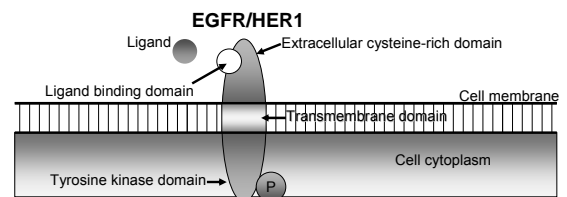
Rosell, et al. N Engl J Med 2009;361:958-967.

Lynch, et al. N Engl J Med 2004;350: 2129-2139.



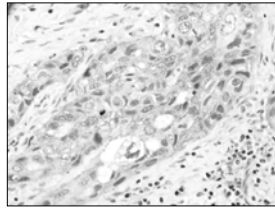
Epidermal Growth Factor Receptor

- EGFR is a transmembrane glycoprotein with extracellular ligand-binding domain, transmembrane domain and intracellular tyrosine kinase domain
- Activation of EGFR results in upregulation of survival signals downstream
- Some tumors can secrete their own ligands EGF or TGF- α
- EGFR status is an important guide for therapy of specific agents



EGFR analysis

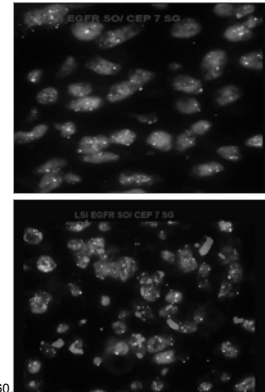
- Protein expression by immunohistochemistry
 - Membranous staining
 - Very nonspecific
 - Definition of overexpression varies from study to study
 - Some studies require only 1 cell to be positive
 - Easy test to administer
 - **NO CLINICAL APPLICABILITY**



Ray et al. Oncologist 2009;14:1116-1130.

EGFR analysis

- Gene copy number by FISH or CISH
 - Increase of gene copy number may correlate with abnormal protein
 - High polysomy defined as ≥ 4 copies in $\geq 40\%$ cells
 - Gene amplification ≥ 15 copies in $\geq 10\%$ cells
 - Studies have shown conflicting results regarding response to TKIs based on copy number gain



Chiosea, et al. Hum Pathol 2010;41: 1053-1060.

Sholl et al. Am J Clin Pathol 2010;133:922-934.

Image courtesy of Nagesh Rao, Ph.D.

EGFR analysis

- EGFR mutation by PCR and sequence analysis
 - At least 75% of NSCLC that respond to EGFR TKIs have identifiable EGFR mutations
 - EGFR mutation present in $\sim 15\%$ of lung adenocarcinomas
 - Different mutations in EGFR gene have different significance
 - **Favorable mutations show best correlation with prolonged progression free survival with tyrosine kinase inhibitors (TKIs)**
 - Phase III IPASS study – 261 EGFR mutated cases had significant PFS with gefitinib versus carboplatin and paclitaxel – conversely EGFR wild type did worse on gefitinib alone

Ray et al. Oncologist 2009;14:1116-1130.

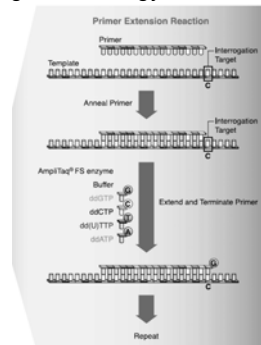
Rosell, et al. N Engl J Med 2009;361:958-967.

Sholl et al. Am J Clin Pathol 2010;133:922-934.

Molecular testing methodology

SNaPshot

- Based on multiplex PCR, primer extension, and capillary electrophoresis that can assess for numerous somatic mutations in multiple genes
- *AKT1, BRAF, EGFR, KRAS, MEK1, NRAS, PIK3CA, PTEN*
- PCR-based sizing assay can assess for insertions and deletions
- Robust and relatively inexpensive technique

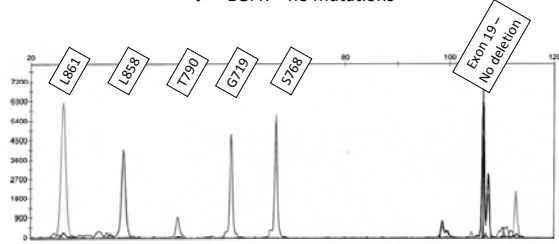


Zengliu Su, et al. J Mol Diagn 2011, 13:74-84.

Sequist LV, et al. Sci Transl Med. 2011 Mar 23;3(75):75ra26.

Slide courtesy of Joshua Deignan, Ph.D.

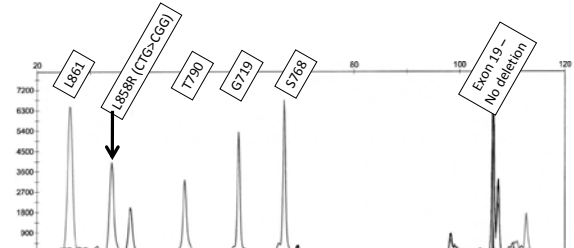
SNaPshot assay EGFR – no mutations



A – green T – red
C – black G – blue

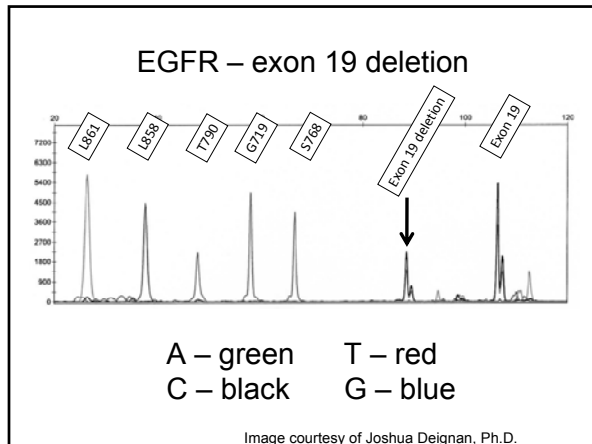
Image courtesy of Joshua Deignan, Ph.D.

EGFR – L858R mutation

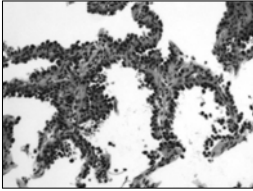


A – green T – red
C – black G – blue

Image courtesy of Joshua Deignan, Ph.D.



- ### EGFR mutations
- Exon 18
 - G719A, G719C, G719S; Mutation at codon 719
 - Associated with response to TKIs
 - Exon 19
 - Deletion of exon 19 Most common EGFR mutations
 - Associated with response to TKIs
 - Exon 20
 - T790M point mutation, S768I, exon 20 insertion
 - All show lack of response to TKIs
 - Exon 21
 - L858R, L861Q; missense mutations
 - Associated with response to TKIs

- ### EGFR mutations
- EGFR mutation (responsiveness to TKIs) most common in:
 - Never-smokers/light smokers
 - People of Asian ethnicity
 - Women
 - Well-differentiated adenocarcinoma
- 
- Do not assume genotype based on clinical phenotype – Testing is still necessary...
- Ding L, et al. Nature. 2008; 455:1069-75.

- ### EGFR specific treatments
- Cetuximab
 - Monoclonal antibody directed against extracellular domain of EGFR molecule – inhibits tumor growth
 - National Comprehensive Cancer Network recommends cetuximab plus vinorelbine and cisplatin in the setting of recurrent and widespread NSCLC if the following present:
 - Age ≥18
 - PS 0-2
 - EGFR by IHC (one or more positive cell)
 - No brain metastases
 - No prior chemotherapy/EGFR-targeted therapy
- Butts CA, et al. J Clin Oncol 2007;25:5777-578.
O'Byrne KY, et al. J Clin Oncol 2009;27(15 suppl):[abstract 8007].

The NEW ENGLAND
JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 20, 2004 VOL. 350 NO. 21

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib

Thomas J. Lynch, M.D., Daphne W. Bell, Ph.D., Raffaella Sordella, Ph.D., Sarada Gurubhagavatula, M.D., Ross A. Okimoto, B.S., Brian W. Brannigan, B.A., Patricia L. Harris, M.S., Sara M. Hasserlat, B.A., Jeffrey G. Supko, Ph.D., Frank G. Haluska, M.D., Ph.D., David N. Louis, M.D., David C. Christiani, M.D., Jeff Settleman, Ph.D., and Daniel A. Haber, M.D., Ph.D.

- ### Small molecule TKIs
- Gefitinib and Erlotinib
 - **Reversible** tyrosine kinase inhibitors – competitively inhibit ATP-binding site blocking downstream signaling
 - Orally active, well tolerated
 - Studies have looked at NSCLC, adenocarcinomas, EGFR mutated cancers
 - Studies are often geographically or clinically restricted
 - Presence of EGFR mutation is strong predictive marker of response
 - Phase III IPASS
 - Several studies have shown benefit of TKIs as second-line monotherapy versus placebo – some series have shown dramatic results
- Shepherd et al. N Engl J Med 2005;353:123-132.
Thatcher et al. Lancet 2005;366:1527-1537.

Gefitinib and Erlotinib

- Several studies do not show benefit when combined with standard first line therapy
 - Phase III Iressa NSCLC Trial Assessing Combination Treatment (INTACT)-1
 - INTACT-2
 - The Tarceva Lung Cancer Investigation Trial
- Maintenance therapy
 - The West Japan Thoracic Oncology Group performed a large study evaluating maintenance gefitinib. In patients with advanced NSCLC platinum based therapy for 3 cycles with maintenance gefitinib had longer PFS than patients with 6 cycles of platinum based therapy alone



N Engl J Med 2010;362:2380-8.

- PFS = 10.8 months (TKI) vs. 5.4 months (chemo)
- Recommend gefitinib as first line agent in pts with advanced lung cancer with EGFR mutation

Table 1. Baseline Characteristics of the Intention-to-Treat Population, According to Treatment Group.*

Characteristic	Gefitinib (N=114)	Carboplatin-Paclitaxel (N=114)
Sex — no. (%)		
Male	42 (36.8)	41 (36.0)
Female	72 (63.2)	73 (64.0)
Age — yr		
Mean	63.5±7.7	62.6±8.9
Range	43–75	35–75
Smoking status — no. (%)		
Never smoked	75 (65.8)	66 (57.9)
Previous or current smoker	39 (34.2)	48 (42.1)
ECOG performance status score — no. (%)		
0	54 (47.4)	57 (50.0)
1	59 (51.8)	55 (48.2)
2	1 (0.9)	2 (1.8)
Histologic diagnosis — no. (%)		
Adenocarcinoma	103 (90.4)	110 (96.5)
Large-cell carcinoma	1 (0.9)	0
Adenosquamous carcinoma	2 (1.8)	1 (0.9)
Squamous-cell carcinoma	3 (2.6)	2 (1.8)
Other	5 (4.4)	1 (0.9)
Clinical stage — no. (%)		
IIIB	15 (13.2)	21 (18.4)
IV	88 (77.2)	84 (73.7)
Postoperative relapse	11 (9.6)	9 (7.9)
Type of EGFR mutation — no. (%)		
Exon 19 deletion	58 (50.9)	59 (51.8)
L858R	49 (43.0)	48 (42.1)
Other	7 (6.1)	7 (6.1)

* Plus-minus values are means ±SD. ECOG denotes Eastern Cooperative Oncology Group.

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Maemondo M, et al. N Engl J Med. 2010;362:2380-8.

Maemondo M, et al. N Engl J Med. 2010;362:2380-8.

- No significant difference in overall survival (median and 2 year survival longer in gefitinib group, 30.5 vs 23.6 months and 61.4% vs. 46.7 %, respectively)
- Best time for treatment (first-line vs. second-line) undetermined.
 - Results suggest first-line may result in longer survival (response rate to gefitinib 73.7% vs. 58.5% second-line)

Resistance to EGFR TKIs

- Primary
 - Lack of any response to EGFR TKIs
 - most are EGFR wild-type
- Secondary
 - Develop after initial response (acquired)
 - **T790M point mutation in exon 20 accounts for 50% of secondary resistance**
 - May account for inherited susceptibility in rare germline mutations

Bell DW, et al. Nat Genet. 2005;37:1315-6.

Vikis H, et al. Cancer Res. 2007;67:4665-70.

Sequist LV, et al. Sci Transl Med. 2011 Mar 23;3(75):75ra26.

Resistance to *EGFR* TKIs

- MET amplification utilizes HER3 tyrosine receptor that bypasses HER1 (EGFR)
 - Occurs in ~15-20% of cases of secondary resistance
- *K-RAS* mutation in codon 12 or 13
- PTEN pathway downregulation

Sequist LV, et al. *Sci Transl Med.* 2011 Mar 23;3(75):75ra26.

Ray et al. *Oncologist* 2009;14:1116-1130.

Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors

Lecia V. Sequist,^{1,2,*} Belinda A. Waltman,^{2*} Dora Dias-Santagata,^{2,3*} Subba Digumarthy,^{2,4} Alexa B. Turke,^{1,2} Panos Fidas,^{1,2} Kristin Bergethon,³ Alice T. Shaw,^{1,2} Scott Gettinger,² Arjola K. Cosper,² Sara Akhavanfard,^{2,3} Rebecca S. Heist,^{1,2} Jennifer Temel,^{1,2} James G. Christensen,⁶ John C. Wain,^{1,2,7} Thomas J. Lynch,² Kathy Vernovsky,¹ Eugene J. Mark,^{2,3} Michael Lanuti,^{1,2,7} A. John Iafrate,^{2,3} Mari Mino-Kenudson,^{2,3} Jeffrey A. Engelman^{1,2†}

Sequist LV, et al. *Sci Transl Med.* 2011 Mar 23;3(75):75ra26.

Methods

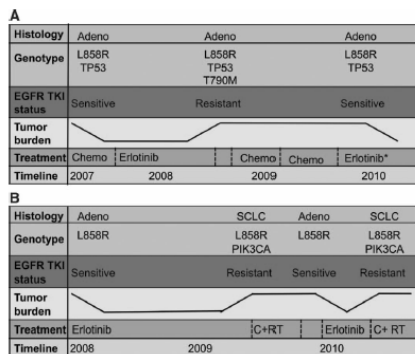
- 37 pts with known *EGFR* mutations and TKI treatment with response
- All re-biopsied at time drug resistance acquired
 - 18 had new T790M mutations (3 with EGFR amplification)
 - 2 had *MET* amplification
 - 2 had *PIK3CA* mutation
 - 5 underwent transformation to Small cell CA phenotype (1 with *PIK3CA* mutation)
 - 3 demonstrated EMT
 - 8 no-changes
- Original *EGFR* mutation still present in all cases

Sequist LV, et al. *Sci Transl Med.* 2011 Mar 23;3(75):75ra26.

Further follow up biopsies

- Three patients had further biopsies following 7-10 month periods without exposure to TKIs
 - L858R → L858R+T790M → L858R
 - Exon 19 del → Exon 19 del+T790M → Exon 19 del
 - L858R → L858R+*PIK3CA* → L858R
- At least 2 patients responded to a second trial of TKIs
- Resistance mechanisms appear to be lost without the continual selective pressure of a TKI

Sequist LV, et al. *Sci Transl Med.* 2011 Mar 23;3(75):75ra26.



Sequist LV, et al. *Sci Transl Med.* 2011 Mar 23;3(75):75ra26.

Bad *EGFR* mutations

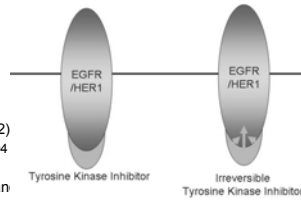
- Exon 20
 - Mutation of methionine for threonine at 790 (T790M) causes most cases of acquired resistance
 - Increases binding affinity for ATP
 - T790M indicates lack of responsiveness or shorter response in tumors prior to TKI treatment – even when found in minority of tumor cells
 - Insertion mutation in exon 20 are rare, also indicate inferior response to TKIs
- Can evaluate circulating tumor cells for *EGFR* mutations
 - Allows for detection of new mutations and predicts response and progression free survival

Maheswaran S, et al. *N Engl J Med* 2008;359:366-377.

Strategies to overcome EGFR TKI resistance

- Irreversible tyrosine kinase inhibitors

- Preclinical trials indicate activity versus T790M mutations
- Some irreversible TKIs inhibit multiple HER molecules (HER-1, HER-2)
 - BIBW 2992, PF00299804
- Clinical trials underway to determine effectiveness and toxicities as first line and secondary treatment
- Effectiveness increased with rapamycin



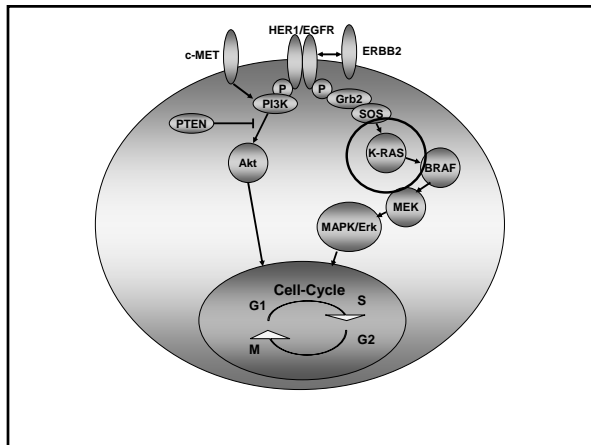
Li D, et al. Cancer Cell. 2007;12:81-93.
Ray et al. Oncologist 2009;14:1116-1130.

Strategies to overcome EGFR TKI resistance

- Targeting multiple pathways

- Trials are underway evaluating the effectiveness of agents that inhibit VEGF receptor and EGFR – BMS-690514, ZD6474 (vandetanib)
- May depend on what the resistance is due to...
- Consider second trial of TKIs after TKI-free interval

Sequist LV, et al. Sci Transl Med. 2011 Mar 23;3(75):75ra26.



K-RAS mutation

- K-RAS is a GTPase signaling molecule downstream from EGFR
 - Mutation results in upregulation of signaling pathway beyond EGFR point
- K-RAS mutation may be seen in cases of EGFR protein overexpression (IHC) and increased gene copy number (FISH). However, K-RAS and EGFR mutations are generally mutually exclusive.
- More common in:
 - Smokers
 - Moderate-poorly differentiated non-small cell carcinoma
- Present in 20-30% of lung adenocarcinoma (twice as common as EGFR)

Massarelli E, et al. Clin Cancer Res 2007;13: 2890-2896.
Schmid K, et al. Clin Cancer Res 2009;15: 4554-4560.

KRAS Mutations and Primary Resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib

William Pao^{1,2}, Theresa Y. Wang¹, Gregory J. Riely², Vincent A. Miller², Ohlu Pan², Marc Ladanyi³, Maureen F. Zakowski³, Robert T. Heelan⁴, Mark G. Kris², Harold E. Varmus¹

¹ Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, ² Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, ³ Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, ⁴ Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America

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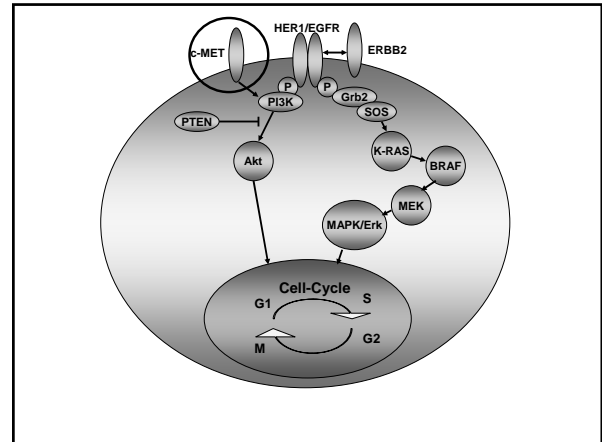
K-RAS mutation

- Predictive of poor prognosis in resected disease as well as resistance to treatment with erlotinib or gefitinib
 - Mao C, et al. Lung Cancer 2010;69:272-278.
- Used as a prognostic marker or screen for TKI non-responders
- No response to cetuximab treatment
 - O'Byrne KJ, et al. J Clin Oncol 2009;27(15 suppl):8007.
- Has been difficult to target therapeutically
- Downstream molecules (RAF and MEK) considered potentially better candidates for targeted therapy
 - Effective in transgenic mice with KRAS G12D mutations, phase II trials have been disappointing

Haura EB, et al. Clin Cancer Res. 2010;16:2450-7.

EGFR and K-RAS

- Well-differentiated tumor → EGFR test
- Poorly-differentiated tumor → KRAS test
- Does a positive status of one test obviate the need of testing for the other?
- Always testing KRAS first may be more cost effective
 - Dacic et al. Mod Pathol 2010;23:159-168.



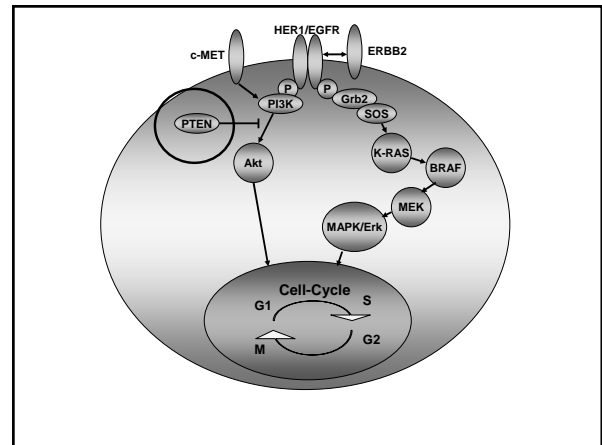
c-MET amplification

- May be a source of acquired resistance through activation of phosphatidylinositol 3-kinase (PI3K)
 - This bypasses the HER-1 (EGFR) pathway
- Detection of c-MET amplification by FISH may identify poor responders to TKIs
- May occur in combination with T790M mutation
- Accounts for 15-20% of secondary resistance
- May be another possible target for treatment
 - Multikinase inhibitor XL880

Qian F, et al. Cancer Res 2009; 69:8009-8016.

Bean J, et al. Proc Natl Acad Sci USA 2007;104:20932-20937.

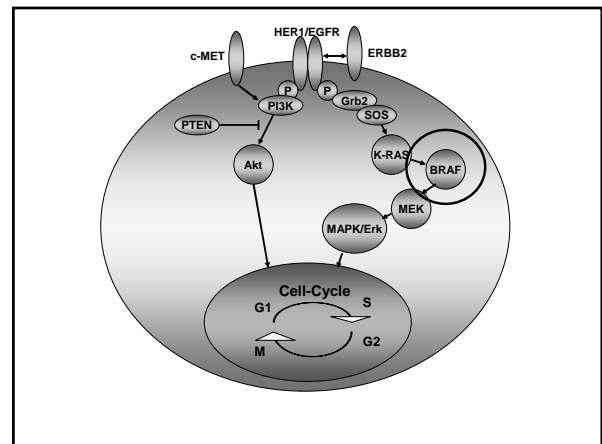
Beau-Faller, et al. J Thorac Oncol 2008;3: 331-339.



PTEN pathway

- PTEN negatively regulates PI3K/Akt pathway – promotes apoptosis
- If PTEN function is lost there is resistance to apoptosis and reduced response to TKIs
- Loss of PTEN expression associated with poor response

Tang JM, et al. Lung Cancer 2006;51:181-191.



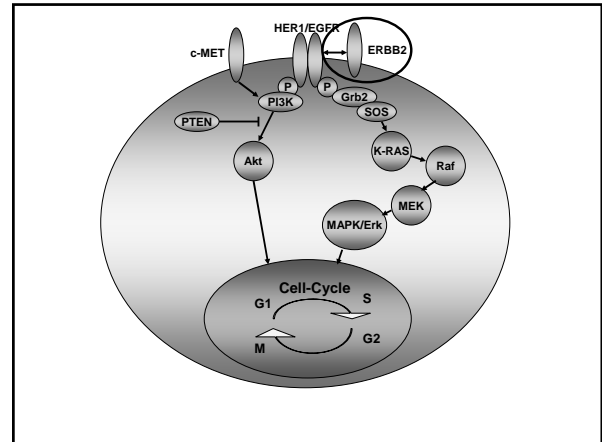
B-RAF

- *B-RAF* is a downstream serine/threonine kinase in the *EGFR* signaling pathway
- Mutations in exon 15 have been found in 1-4% of lung adenocarcinomas
- Mutation may indicate a worse response to EGFR TKIs but can offer a different target for treatment
- PLX4032, a *B-RAF* inhibitor, has demonstrated effectiveness in melanoma cases – effectiveness in lung CA remains to be seen
- *B-RAF* inhibitors may activate MAPK pathway through *C-RAF*.
 - May be contraindicated in *KRAS* mutant tumors

Hatzivassiliou G, et al. Nature. 2010;464:431-5.

Shigematsu H, et al. Cancer Res 2005; 65:1642-1646.

Schmid K, et al. Clin Cancer Res 2009;15: 4554-4560.

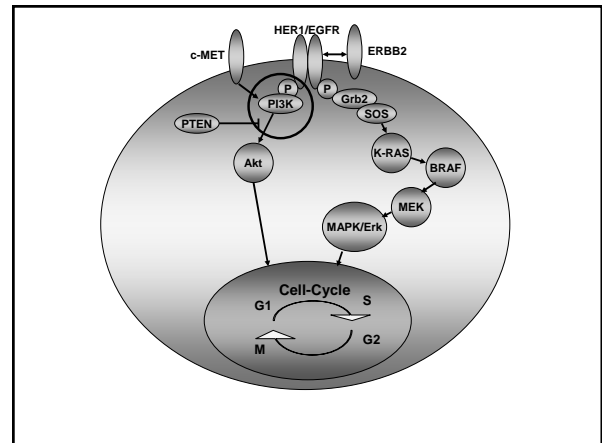


ERBB2 (HER2)

- Mutations in the ERBB2 kinase domain seen in 2-4% of lung adenocarcinomas
- Small in-frame insertions in exon 20 of the kinase domain
- Dimerizes with self or other ERBB molecules
- In vitro studies have shown response to irreversible TKIs with the ERBB2 mutation

Shigematsu H, et al. Cancer Res 2005; 65:1642-1646.

Lee J, et al. Cancer Letters 2006;237:89-94.

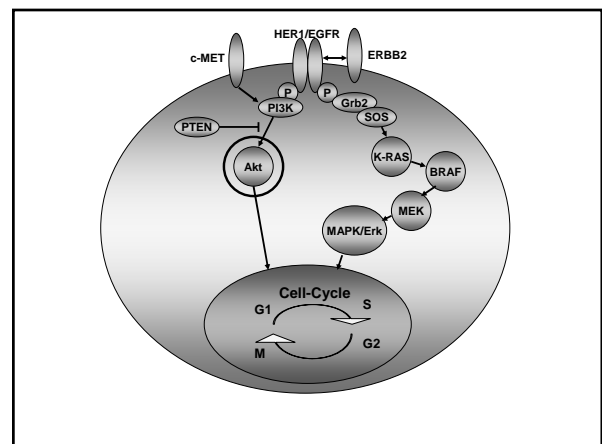


Phosphatidylinositol 3-kinase (PI3K)

- Mutations found in 1% to 2% of *PIK3CA* subunit of PI3K in lung cancers
- Mutation found to be oncogenic in animal models
- No response in cell lines at this time.
- One study demonstrated mutations in *PIK3CA* in 5 of 139 (3.6%) of Japanese lung cancer patients and 4-6% of lung carcinoma cell lines. Cell lines and tumors with these mutations are sensitive to a variety of PI3-kinase inhibitors that are in current clinical trials.

Engelman JA, et al. Nat Med. 2008;14:1351-6.

Sequist LV, et al. Sci Transl Med. 2011 Mar 23;3(75):75ra26.



AKT1

- AKT1 E17K mutations have been found in lung carcinomas at low frequency, 0.6-2.0% of lung carcinoma specimens.
- Downstream from PI3K
 - AKT1 and PI3K inhibitors may have important therapeutic benefit for patients with this mutation.
- Reported in both squamous cell and adenocarcinoma

Bleeker FE, et al. *Oncogene*, 2008.
Malanga D, et al. *Cell Cycle*, 2008. 7:665-9.

NRAS

- NRAS is a common oncogene in a variety of human cancers. The NRAS Q61 mutation has been identified in 3 of 188 (1.6%) lung adenocarcinomas in one study.

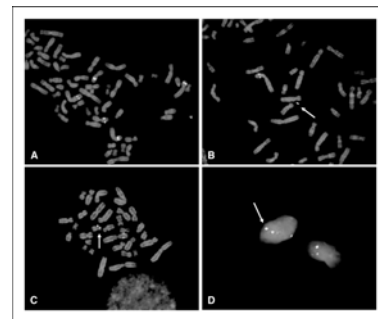
Ding, L., et al. *Nature* 455:(7216), 1069-1075. 2008.

EML4-ALK translocation

- Fusion of echinoderm microtubule-associated protein like-4 (EML4)–anaplastic lymphoma kinase (ALK)
- Found in up to 7% of Japanese adenocarcinoma, 3% in American studies
- More common in never/light smokers
- EML4-ALK tends occur in the absence of K-RAS and EGFR mutations

Sasaki T, et al. *Euro J Cancer* 2010;46 1773–1780.
Martelli MP, et al. *Am J Pathol* 2009;174:661–670.

Detection of EML4-ALK using FISH.



Koivunen J P et al. *Clin Cancer Res* 2008;14:4275-4283

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ALC Clinical Cancer Research

EML4-ALK translocation

- Detect by FISH or PCR
- PCR may be technically more difficult and does not distinguish between translocation in non-tumor tissue
- ALK offers target for treatment, PF-02341066 (crizotinib)
- ALK kinase inhibitors may be clinically effective for treatment of lung adenocarcinomas with EML4-ALK translocation –phase I trial demonstrate encouraging response rates

Koivunen JP, et al. *Clin Cancer Res* 2008;14:4275-4283.
Kwak EL, et al. *J Clin Oncol* 2009;27:15s [abstract 3509].

ALK resistance

- Reported acquired resistance alleles of *EML4-ALK*, ALK C1156Y, L1196M, and F1174L

Choi YL, et al. *N Engl J Med*. 2010;363:1734-9.

Tumor Suppressor Genes

- *TP53, STK11, CDKN2A, APC, RB1, NF1*
- Harder to target therapy
- May target activated genes downstream of inactive tumor suppressor gene

Future directions

- Large panel assay of multiple high and low frequency mutations
 - High throughput is possible
 - Further studies are necessary
- Gene profiling
 - Requires multicenter validation
 - Tissue handling issues
- Exon directed sequencing
 - High frequency of *TP53* and *KRAS* mutations
 - Whole-exome and whole-genome sequencing will reveal more detailed analysis of tumor DNA mutation status

References

- Lynch TJ, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350: 2129–2139.
- Rosell R, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009;361:958-967.
- Ray M, Salgia R, Vokes, E. The role of EGFR inhibition in the treatment of non-small cell lung cancer. *Oncologist* 2009;14:1116-1130.
- Chiosea S, Shuai Y, Cieleply K, Nikiforova MN, Dacic S. EGFR FISH-positive lung adenocarcinoma. *Hum Pathol* 2010;41: 1053–1060.
- Sholl, et al. *EGFR* Mutation best predicts TKI Response. *Am J Clin Pathol* 2010;133:922-934.

References

- Zengliu Su, Dora Dias-Santagata, MarKeesa Duke, et al. A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. *J Mol Diagn* 2011, 13:74–84.
- Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med*. 2011 Mar 23;3(75):75ra26.
- Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008; 455:1069-75.
- Butts CA, et al. Randomized phase II study of gemcitabine plus cisplatin, with or without cetuximab, as first-line therapy for patients with advanced or metastatic non small-cell lung cancer. *J Clin Oncol* 2007;25:5777-578.
- O'Byrne KY, et al. Molecular and clinical predictors of outcome for cetuximab in non-small cell lung cancer: Data from the FLEX study. *J Clin Oncol* 2009;27(15 suppl):[abstract 8007].

References

- Shepherd FA, et al. Erlotinib in previously treated non-small cell lung cancer. *N Engl J Med* 2005;353:123-132.
- Thatcher N, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non small-cell lung cancer: Results from a randomized, placebo-controlled, multicentre study (Iressa survival evaluation in lung cancer). *Lancet* 2005;366:1527-1537.
- Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010;362:2380-8.
- Bell DW, Gore I, Okimoto RA, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet*. 2005;37:1315-6.
- Vikis H, Sato M, James M, et al. EGFR T790M is a rare lung cancer susceptibility allele with enhanced kinase activity. *Cancer Res*. 2007;67:4665-70.
- Maheswaran S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med* 2008;359:366-377.

References

- Li D, Shimamura T, Ji H, et al. Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy. *Cancer Cell*. 2007;12:81-93.
- Massarelli E, et al. KRAS Mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non small cell lung cancer. *Clin Cancer Res* 2007;13: 2890-2896.
- Schmid K, et al. EGFR/KRAS/BRAF Mutations in primary lung adenocarcinomas and Corresponding locoregional lymph node metastases. *Clin Cancer Res* 2009;15: 4554-4560.
- Pao W, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005 Jan;2:e17. Epub 2005 Jan 25.
- Mao C, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. *Lung Cancer* 2010;69:272-278.

References

- Haura EB, Ricart AD, Larson TG, *et al.* A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. *Clin Cancer Res.* 2010;16:2450-7.
- Dacic S, *et al.* EGFR/KRAS predictors in lung adenocarcinoma. *Mod Pathol* 2010;23:159-168.
- Qian F, *et al.* Inhibition of Tumor Cell Growth, Invasion, and Metastasis by EXEL-2880 (XL880, GSK1363089), a Novel Inhibitor of HGF and VEGF Receptor Tyrosine Kinases. *Cancer Res* 2009; 69:8009-8016.
- Bean J, *et al.* *MET* amplification occurs with or without *T790M* mutations in *EGFR* mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007;104:20932-20937.
- Beau-Faller, *et al.* *MET* Gene Copy Number in Non-small Cell Lung Cancer. *J Thorac Oncol* 2008;3: 331-339.

References

- Tang JM, *et al.* Phosphorylated Akt overexpression and loss of PTEN expression in non-small cell lung cancer confers poor prognosis. *Lung Cancer* 2006;51:181-191.
- Hatzivassiliou G, Song K, Yen I, *et al.* RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature.* 2010;464:431-5.
- Shigematsu H, *et al.* Somatic Mutations of the HER2 Kinase Domain in Lung Adenocarcinomas. *Cancer Res* 2005; 65:1642-1646.
- Lee J, *et al.* ERBB2 kinase domain mutation in the lung squamous cell carcinoma. *Cancer Letters* 2006;237:89-94.
- Engelman JA, Chen L, Tan X, *et al.* Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med.* 2008;14:1351-6.

References

- Bleeker FE, Felicioni L, Buttitta F, *et al.* AKT1(E17K) in human solid tumours. *Oncogene*, 2008.
- Malanga D, Scrima M, De Marco C, *et al.* Activating E17K mutation in the gene encoding the protein kinase AKT1 in a subset of squamous cell carcinoma of the lung. *Cell Cycle*, 2008. 7:665-9.
- Sasaki T, *et al.* The biology and treatment of EML4-ALK non-small cell lung cancer. *Euro J Cancer* 2010;46 1773-1780.
- Martelli MP, *et al.* EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. *Am J Pathol* 2009;174:661-670.
- Kwak EL, *et al.* Clinical activity observed in a phase I dose escalation trial of an oral c-met and ALK inhibitor, PF-02341066. *J Clin Oncol* 2009;27:15s [abstract 3509].
- Koivunen JP, *et al.* EML4-ALK Fusion Gene and Efficacy of an ALK Kinase Inhibitor in Lung Cancer. *Clin Cancer Res* 2008;14:4275-4283.

References

- Choi YL, Soda M, Yamashita Y, *et al.* EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med.* 2010;363:1734-9.