

Challenges in Developing Clinical Biomarker Tests

Lessons Learned from Single Analyte Tests

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Lessons Learned from Single Analyte Tests

- 1. EGFR Mutation Testing**
2. BRAF and HER2 Testing
3. Chromosomal Rearrangements

Epidermal Growth Factor Receptor Mutations

PNAS

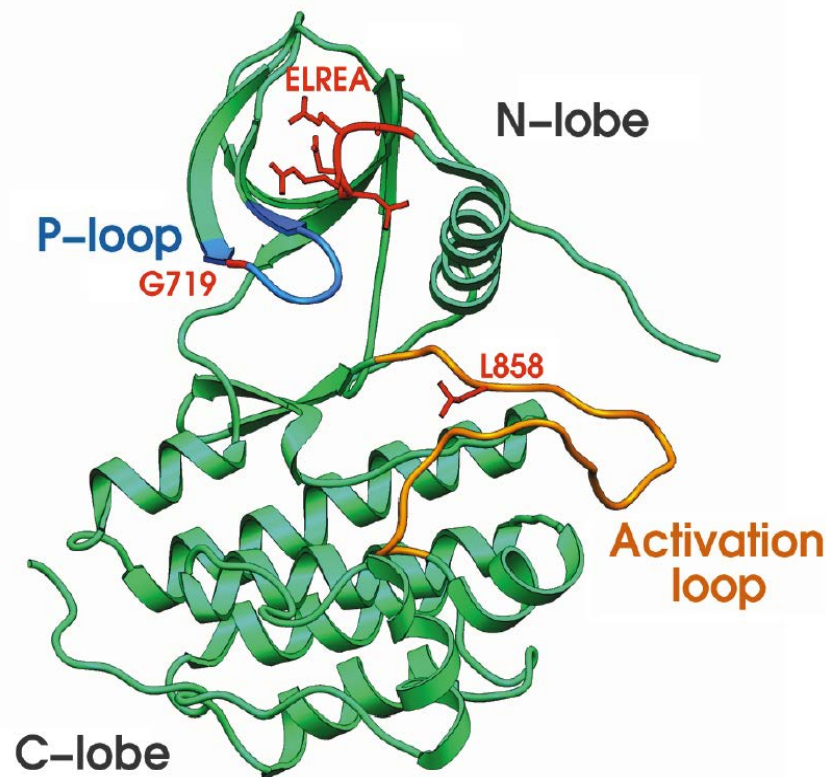
Pao et al 2004

**25 of 31 Patients with
Response to Gefitinib
had EGFR Mutation**



The NEW ENGLAND
JOURNAL of MEDICINE

Lynch et al 2004



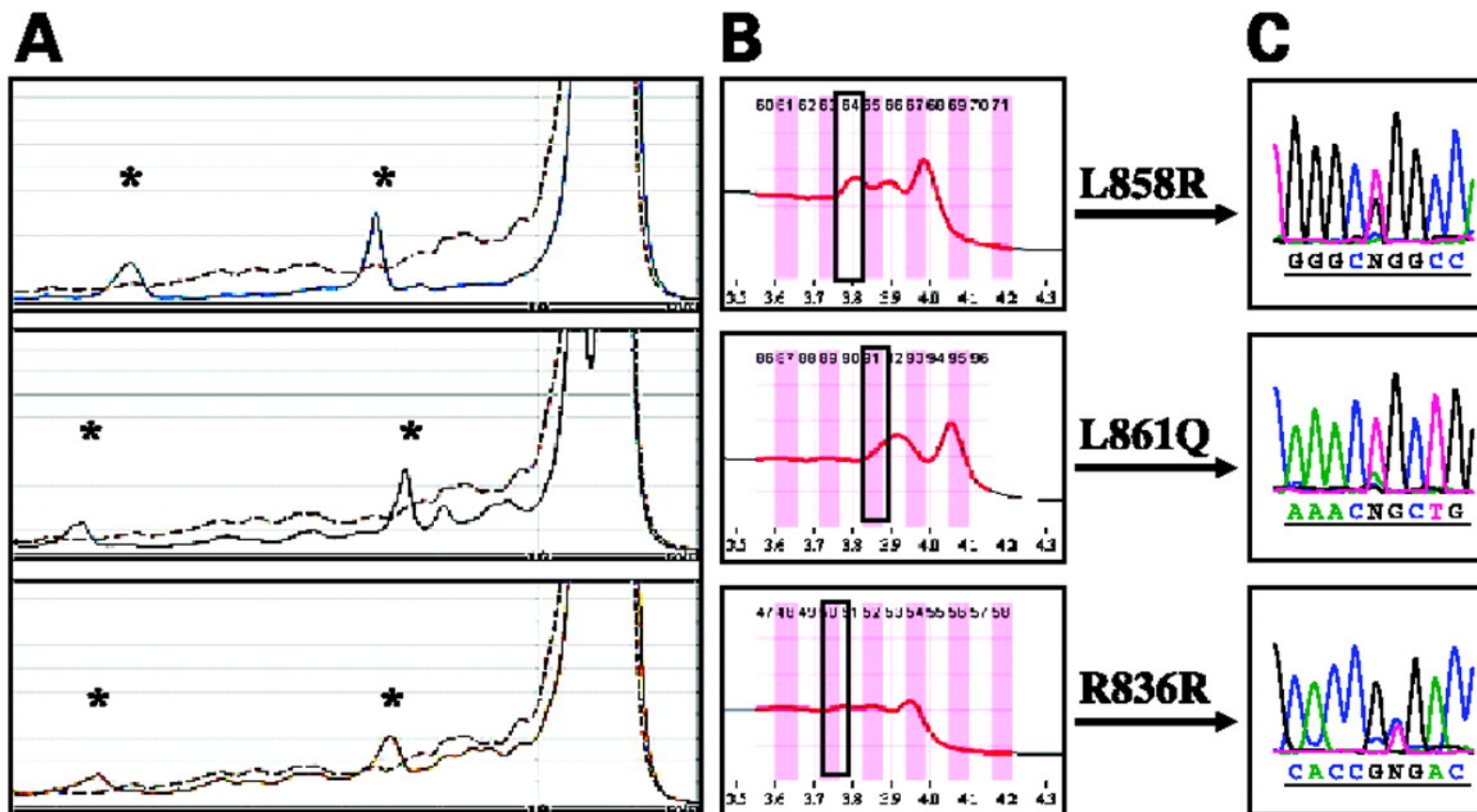
Science

Paez et al. 2004

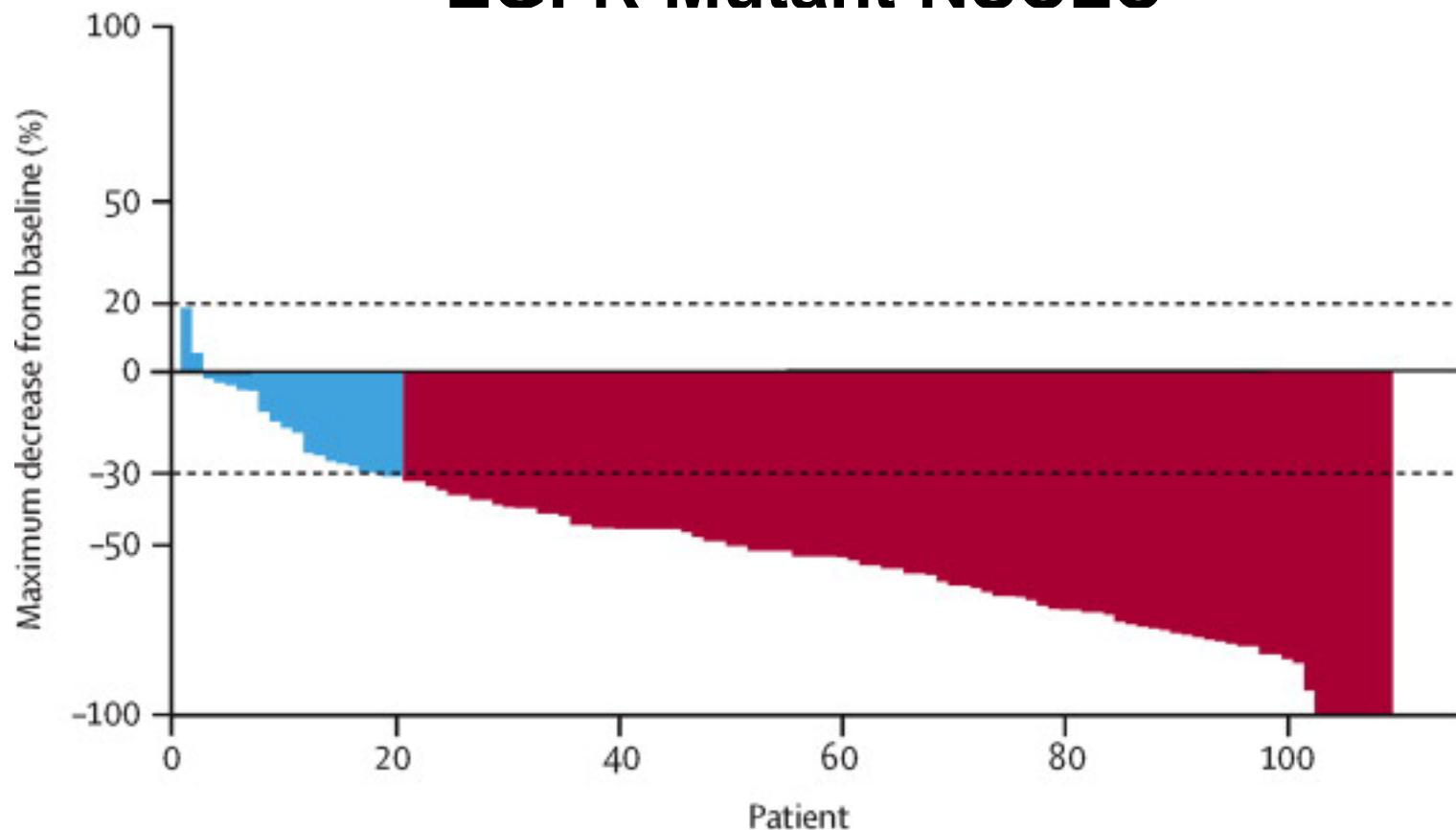
Epidermal Growth Factor Receptor Mutation Testing

- By August 2004 BWH, DFCI, and MGH referred patient material through the Laboratory of Molecular Medicine (LMM) at the Harvard Medical School/Partners HealthCare Center for Genetics for EGFR Mutation Testing
 - BWH and MGH Pathology, tumor slides DNA sent to LMM, tracing sent back to Path, interpreted, issued report
- September 2005 Genzyme Corporation announced the commercial availability of an EGFR mutation test to help identify patients likely to respond to therapies targeted for the treatment of non-small cell lung cancer (NSCLC).
 - Cells from specific tumor-rich areas are microdissected, followed by DNA extraction, PCR amplification, and bi-directional sequencing of exons 18 through 21 in tyrosine kinase domain of the EGFR gene. A team of 17 surgical pathologists and Ph.D. geneticists review the EGFR mutation analyses

SURVEYOR Analysis of EGFR and Sequencing Of 3 Patient Specimens with 3 Distinct Variants

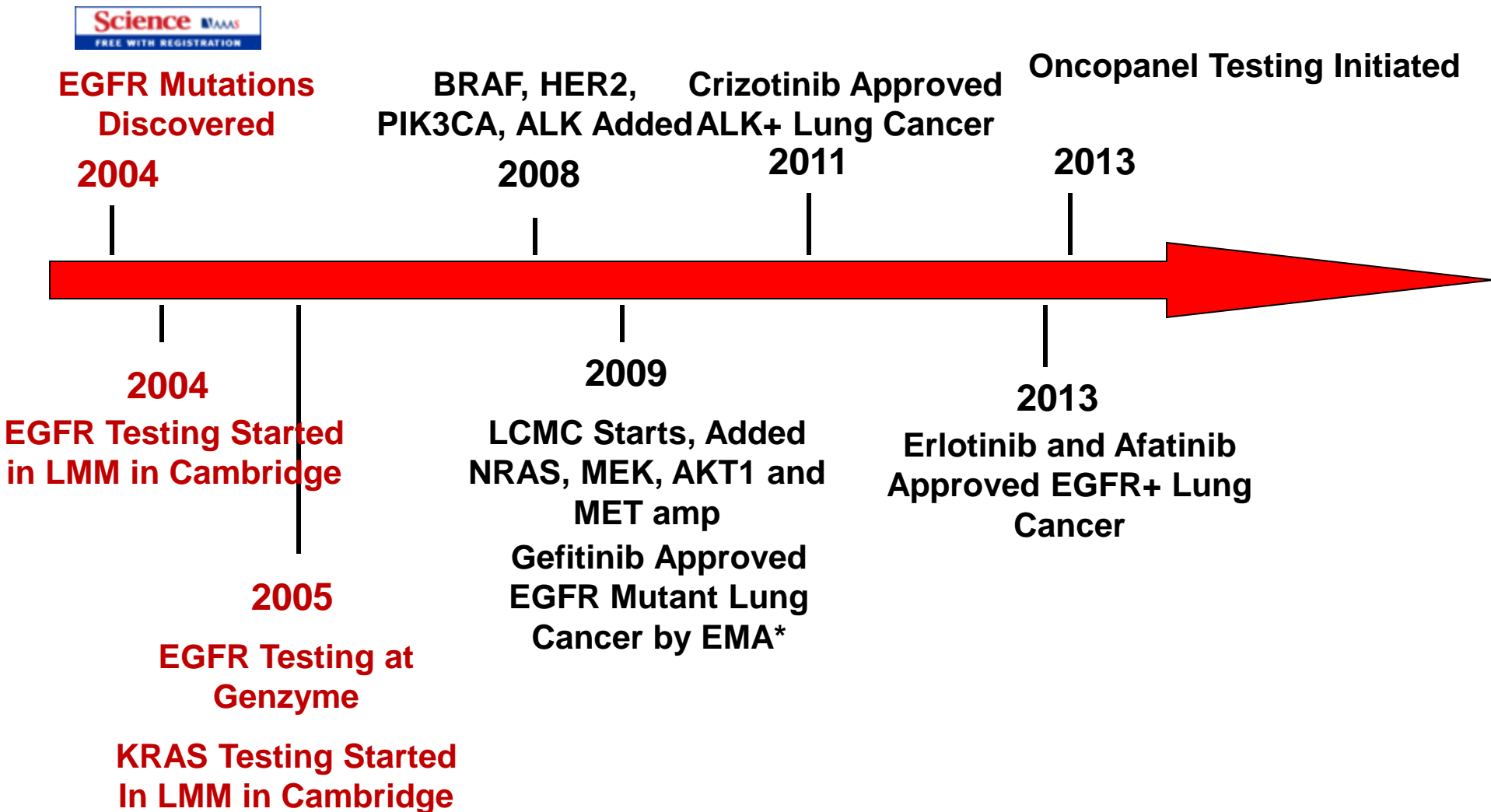


Tumor Responses to Afatinib in EGFR Mutant NSCLC



Yang et al. Lancet Oncology 2012; 13:539

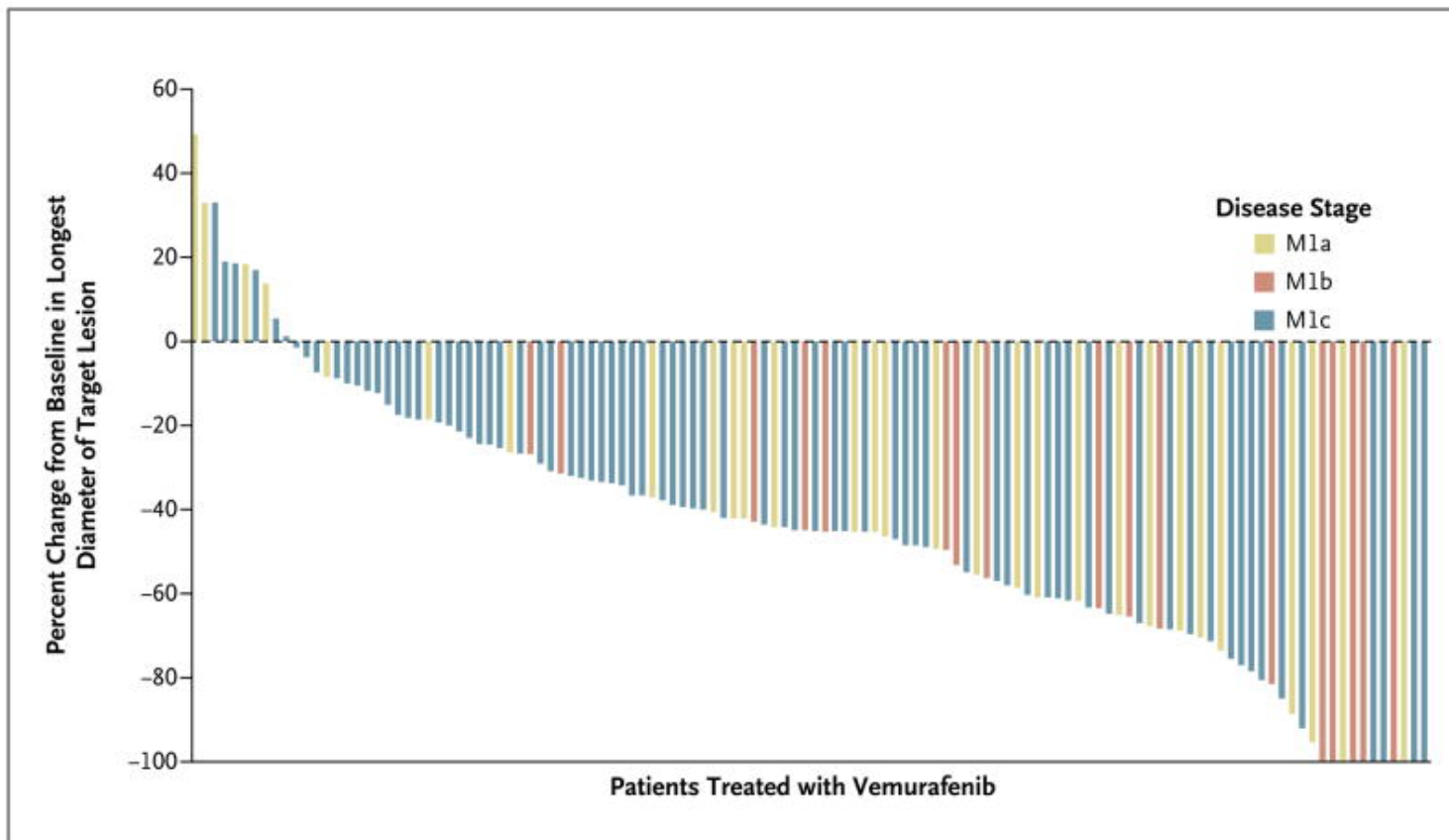
Genotyping Timeline



Lessons Learned from Single Analyte Tests

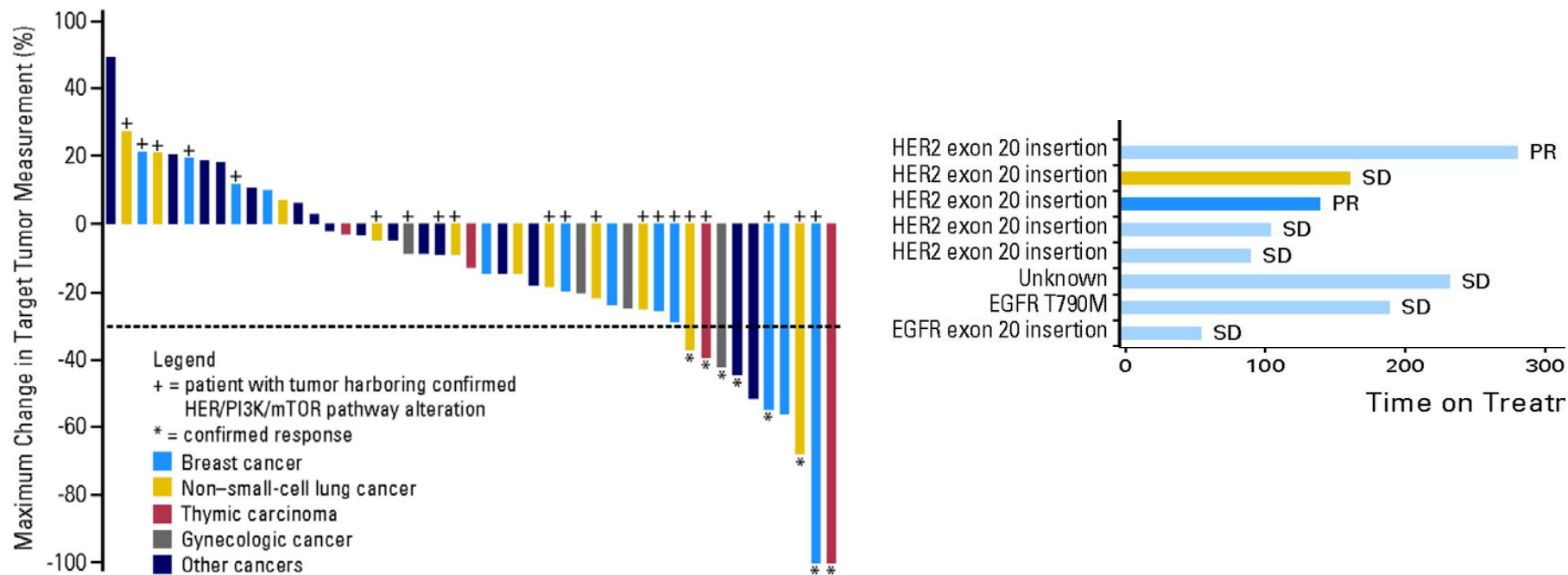
1. EGFR Mutation Testing
2. **BRAF and HER2 Testing**
3. ALK and ROS1 Chromosomal Rearrangements

Tumor Responses to Vemurafenib in V600E BRAF Mutant Melanoma



Sossman et al. NEJM 2012; 366:707

Tumor Responses to Neratinib plus Temsirolimus in HER2 Mutated NSCLC



Genotyping Timeline



**EGFR Mutations
Discovered**

2004

**BRAF, HER2,
PIK3CA, ALK Added**

2008

**Crizotinib Approved
ALK+ Lung Cancer**

2011

Oncopanel Testing Initiated

2013

2004
**EGFR Testing Started
in LMM in Cambridge**

2005

**EGFR Testing at
Genzyme**

**KRAS Testing Started
in LMM in Cambridge**

2009
**LCMC Starts, Added
NRAS, MEK, AKT1 and
MET amp**

**Gefitinib Approved
EGFR Mutant Lung
Cancer by EMA***

2013
**Erlotinib and Afatinib
Approved EGFR+ Lung
Cancer**

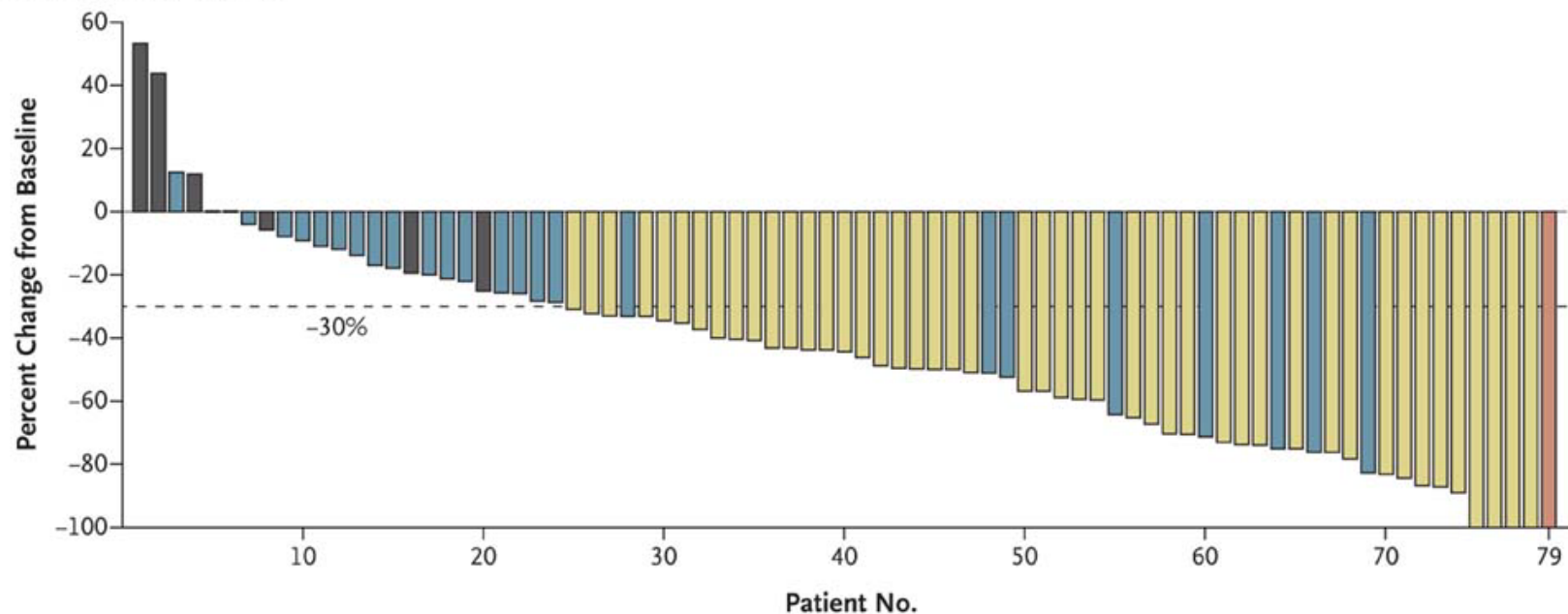
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Tumor Responses to Crizotinib in ALK Rearranged Non-Small-Cell Lung Cancer

■ Disease progression ■ Stable disease ■ Partial response ■ Complete response

A Percent Change in Tumor Burden

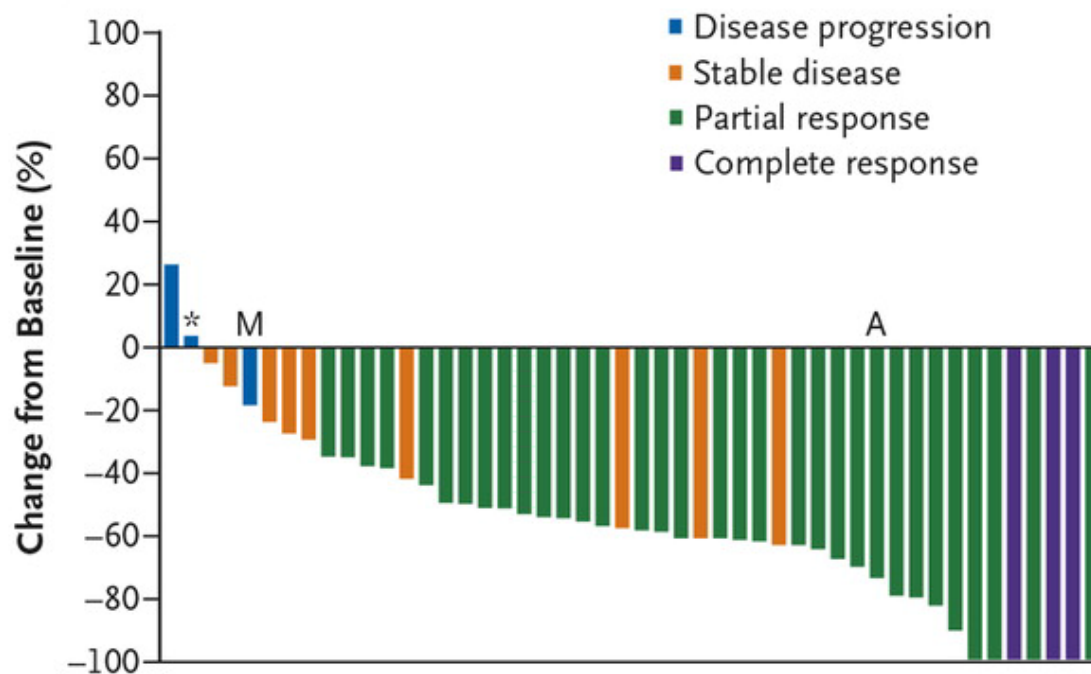


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Kwak et al. 2010 363(18) 1693-1703

Tumor Responses to Crizotinib in ROS1-Rearranged Non-Small-Cell

A Best Response



ALK Chromosomal Rearrangements

- FISH Testing

- 2011 The FDA granted accelerated approval to crizotinib for the treatment of patients with locally advanced or metastatic NSCLC that is ALK-positive as detected by an FDA-approved test. The FDA approved the Vysis ALK Break-Apart FISH Probe Kit¹
- The Vysis ALK Break Apart FISH Probe Kit is intended to detect rearrangements involving the ALK gene via fluorescence in situ hybridization (FISH) in formalin-fixed paraffin-embedded (FFPE) NSCLC tissue specimens. If a chromosome rearrangement at the 2p23 ALK breakpoint region has occurred, one orange and one green signal separated by at least two signal diameters will be seen. If the average percent positive cells is $\geq 15\%$ ($\geq 15/100$), the sample is considered positive²

¹<http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm376058.htm>

²http://www.abbottmolecular.com/static/cms_workspace/pdfs/US/Vysis_ALK_FISH_Probe_Kit_PI.pdf

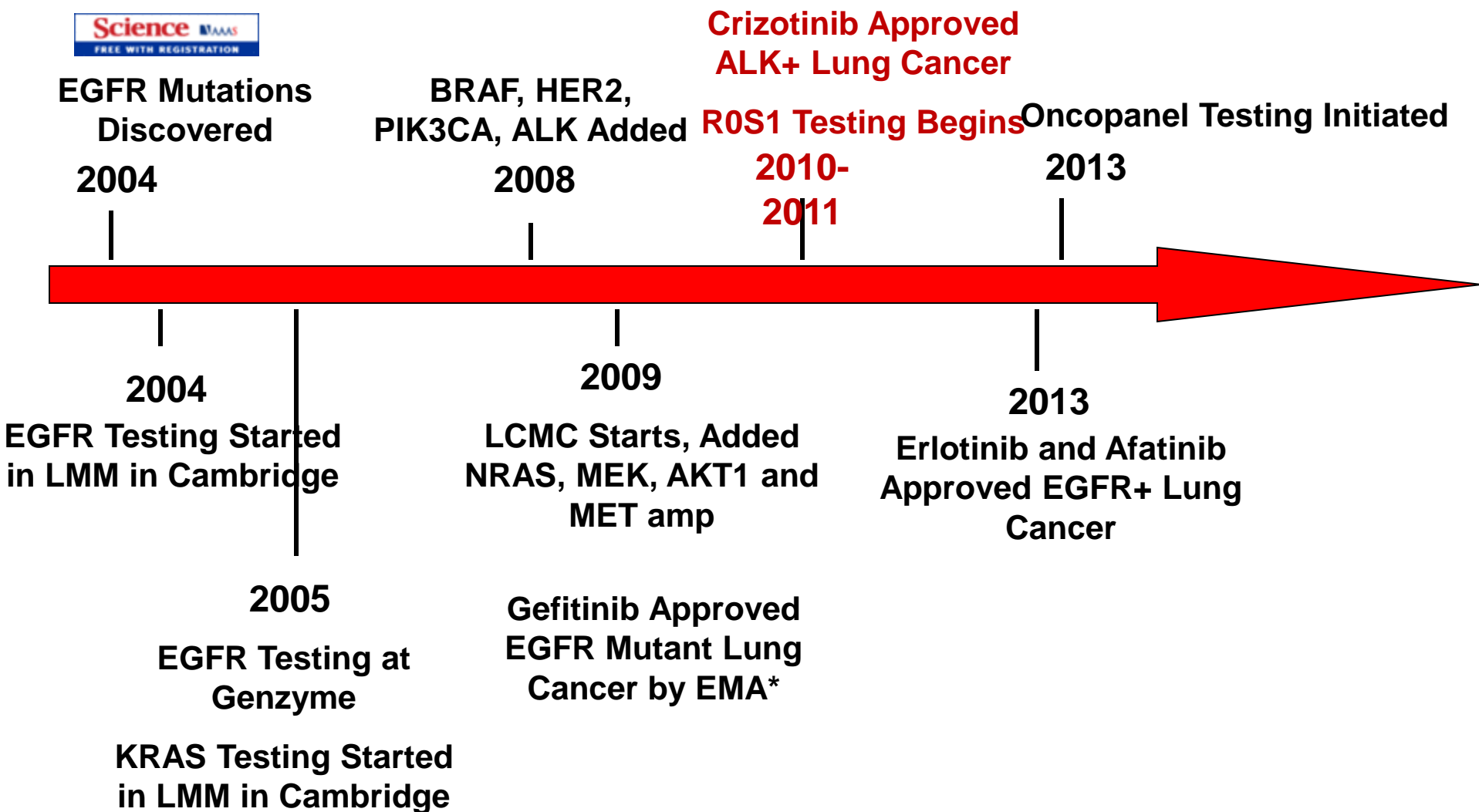
ALK Chromosomal Rearrangements

- FISH Testing

- Break-apart FISH using green and red labeled sequences flanking the *ROS1* gene. Images were captured and positive cases were defined as tumors harboring more than 15% of cells with split signals.

Bergethon J Clin Oncol 2012; 30:863

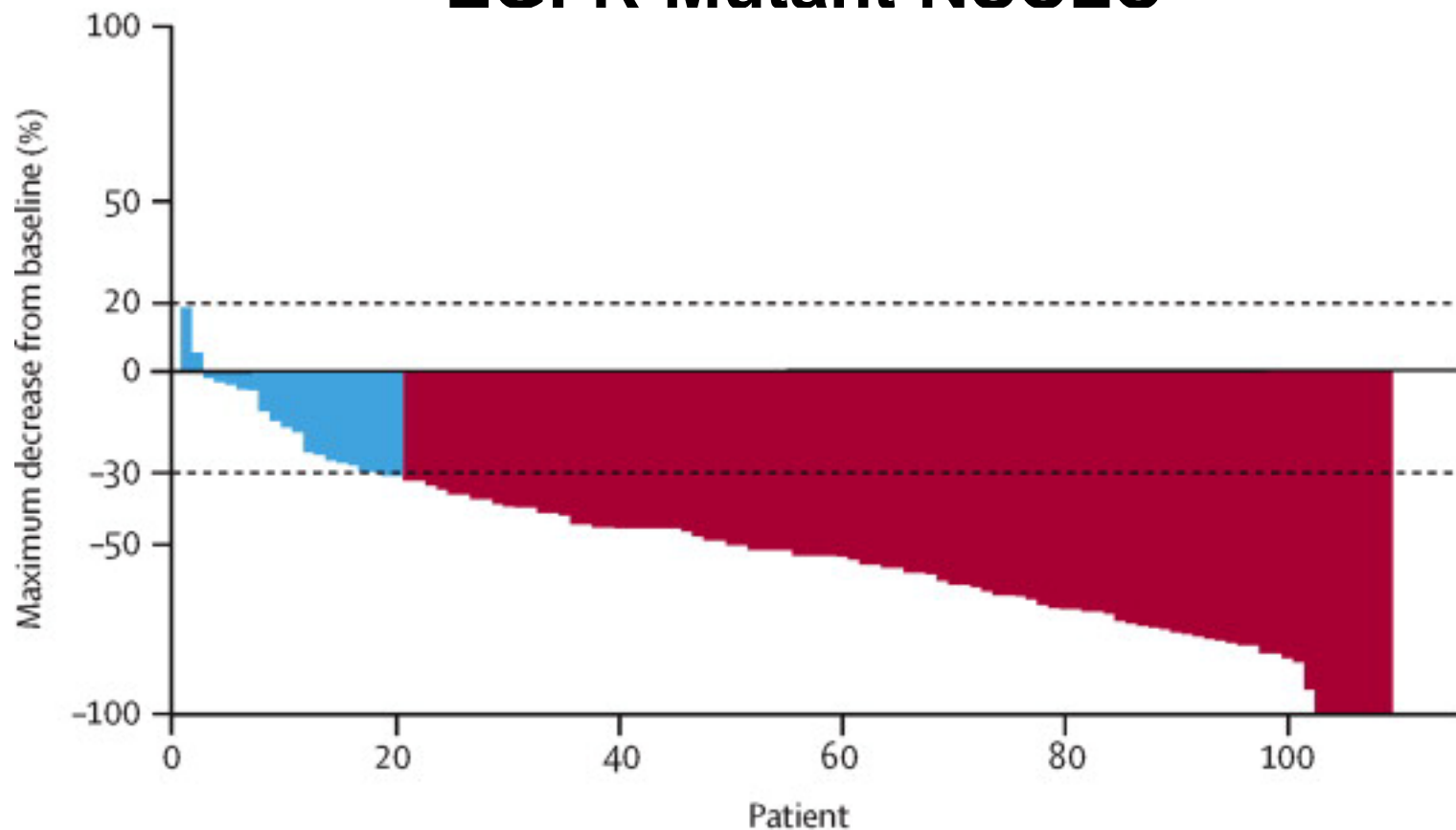
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EGFR-TKI Approvals for EGFR mutant NSCLC

- The FDA approved erlotinib for the first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations. This indication for erlotinib is being approved concurrently with the cobas® EGFR Mutation Test¹
- The FDA approved afatinib for the first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R substitution mutations as detected by an FDA-approved test. The FDA approved the *therascreen* EGFR RGQ PCR Kit (QIAGEN) for detection of these EGFR 19 mutations²

¹www.cancer.gov/cancertopics/druginfo/fda-erlotinib-hydrochloride

²www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm360574.htm

Mutation Detection Accuracy

- Cobas EGFR Mutation Test Kit
 - The **cobas**® EGFR Mutation Test is a real-time PCR test for the qualitative detection and identification of prespecified mutations in exons 18, 19, 20 and 21 of the EGFR gene in DNA derived from formalin-fixed paraffin-embedded (FFPET) NSCLC tumor tissue¹
- Therascreen EGFR RGQ PCR Kit
 - The **therascreen** EGFR RGQ PCR Kit is a real-time PCR test for the qualitative detection of 29 different exon 19 deletions and exon 21 (L858R) substitution mutations of EGFR gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) NSCLC tumor tissue.²

¹<http://molecular.roche.com/assays/Pages/cobasEGFRMutationTest.aspx>

²<http://www.qiagen.com/us/products/molecular-diagnostics-na/personalized-healthcare-na/egfr-rgq-pcr/detection-and-analysis/therascreen-egfr-rgq-pcr-kit-na>

Foundation Medicine Test (\$5,800)

- Applies next-generation sequencing in a unique manner to identify all 4 types of genomic alterations across drivers of solid tumors
- Sequences the coding region of 315 cancer-related genes plus introns from 28 genes often rearranged or altered in cancer to a typical median depth of coverage of greater than 500X.
- FoundationOne detects all classes of genomic alterations, including base substitutions, insertions and deletions (indels), copy number alterations (CNAs) and rearrangements using routine FFPE sample

www.foundationmedicine.com

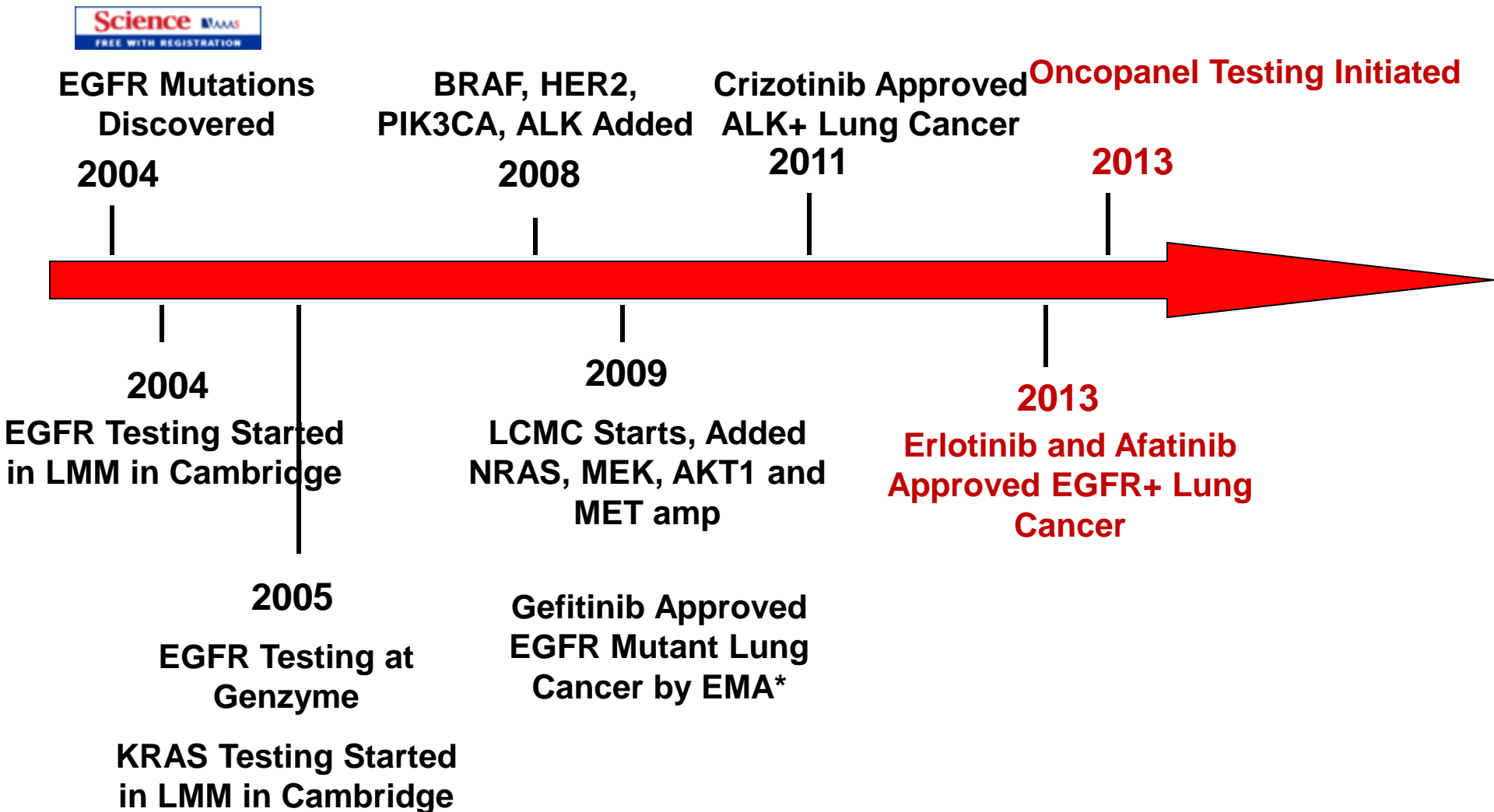
Oncopanel Gene List in 2014

Exons: ABL1, AKT1, AKT2, AKT3, ALK, ALOX12B, APC, AR, ARAF, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AURKA, AURKB, AXL, B2M, BAP1, BCL2, BCL2L1, BCL2L12, BCL6, BCOR, BCORL1, BLM, BMPR1A, **BRAF**, BRCA1, BRCA2, BRD4, BRIP1, BUB1B, CARD11, CBL, CBLB, CCND1, CCND2, CCND3, CCNE1, CD274, CD58, CD79B, CDC73, CDH1, CDK1, CDK2, CDK4, CDK5, CDK6, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK2, CIITA, CREBBP, CRKL, CRLF2, CRTCL1, CRTCL2, CTNNB1, CUX1, CYLD, DDB2, DDR2, DICER1, DIS3, DMD, DNMT3A, **EGFR**, EP300, EPHA3, EPHA5, EPHA7, **ERBB2**, ERBB3, ERBB4, ERCC2, ERCC3, ERCC4, ERCC5, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXT1, EXT2, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP9, FLCN, FLT1, FLT3, FLT4, GATA3, GATA4, GATA6, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GPC3, GSTM5, H3F3A, HNF1A, HRAS, ID3, IDH1, IDH2, IGF1R, IKZF1, IKZF3, JAK2, JAK3, KDM6A, KDM6B, KDR, KIT, KRAS, LMO1, LMO2, LMO3, MAP2K1, MAP2K4, MAP3K1, MAPK1, MCL1, MDM2, MDM4, MECOM, MEF2B, MEN1, MET, MITF, MLH1, MLL, MLL2, MPL, MSH2, MSH6, MTOR, MUTYH, MYB, MYBL1, MYC, MYCL1, MYCN, MYD88, NBN, NF1, NF2, NFE2L2, NFKBIA, NFKBIZ, NKX2-1, NOTCH1, NOTCH2, NPM1, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PARK2, PAX5, PDCD1LG2, PDGFRA, PDGFRB, PHF6, PHOX2B, PIK3C2B, PIK3CA, PIK3R1, PIM1, PMS1, PMS2, PNRC1, PRAME, PRDM1, PRF1, PRKAR1A, PRKCI, PRKCZ, PRKDC, PRPF40B, PRPF8, PSMD13, PTCH1, PTEN, PTK2, PTPN11, RAD21, RAF1, RARA, RB1, RBL2, REL, RET, RFWD2, RHPN2, ROS1, RPL26, RUNX1, SBDS, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMC3, SMO, SOCS1, SOX2, SOX9, SRC, SRSF2, STAG1, STAG2, STAT3, STAT6, STK11, SUFU, SUZ12, SYK, TCF3, TCF7L1, TCF7L2, TERT, TET2, TNFAIP3, TP53, TSC1, TSC2, U2AF1, VHL, WRN, WT1, XPA, XPC, XPO1, ZNF217, ZNF708, ZRSR2.

Introns: ABL1, AKT3, **ALK**, BCL2, BCL6, BRAF, CIITA, EGFR, ETV1, EWSR1, FGFR1, FGFR3, FUS, IGH@, IGK@, IGL@, JAK2, MLL, MYC, NPM1, PAX5, PDGFRA, PDGFRB, RAF1, RARA, RET, **ROS1**, TRA@, TRB@, TRG@.

Courtesy of Neal Lindeman

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