Continuous Evolution: Current and Emerging Indications for Molecular Pathology in Lung Cancer

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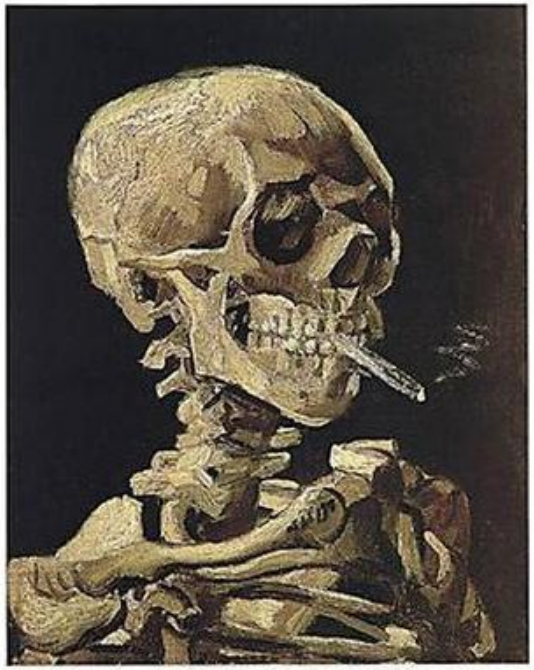


Annual Park City Pathology Update

Park City, Utah

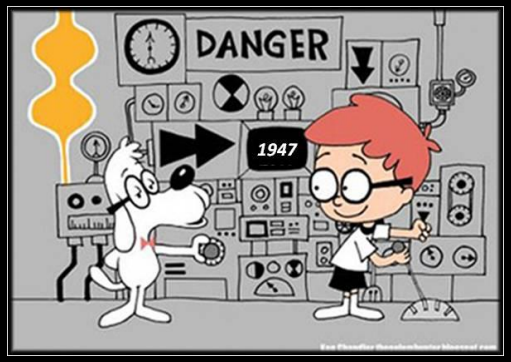
February 6, 2022

Introductions

I have no conflicts of interest

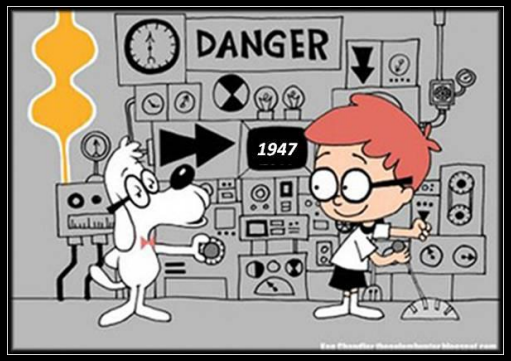
But there's always hope...

Case Presentation:



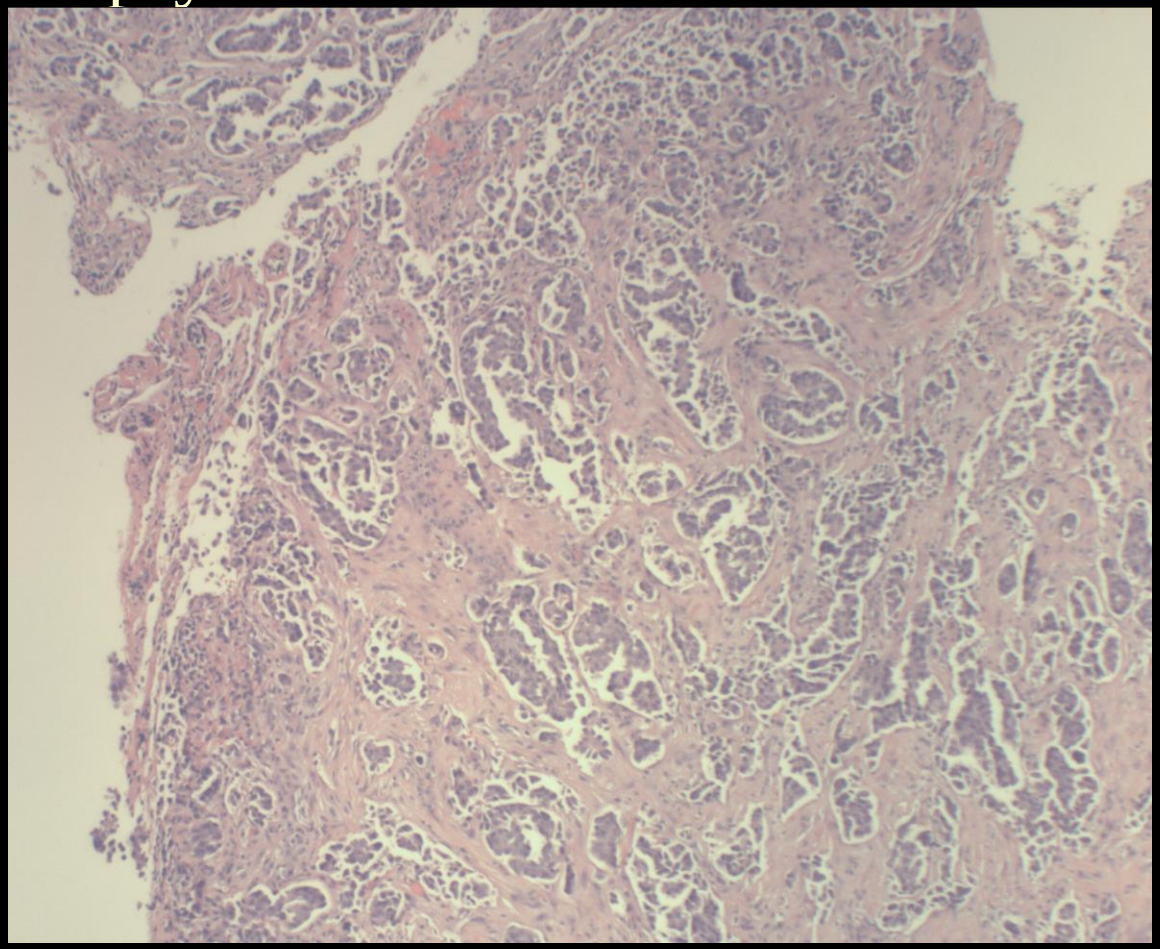
- 52 yr old female
- Symptoms (months):
  - Headaches
  - Light/dark sensitivity
  - Chronic dry cough
- MRI:
  - Enhancing dural mass

- Differential Dx:
  - Tumor
    - Meningioma
    - Lymphoma
    - Metastasis
    - Primary CNS tumor
  - Granuloma
    - Sarcoidosis
    - Tuberculosis
  - Chronic meningitis
    - Wegener's

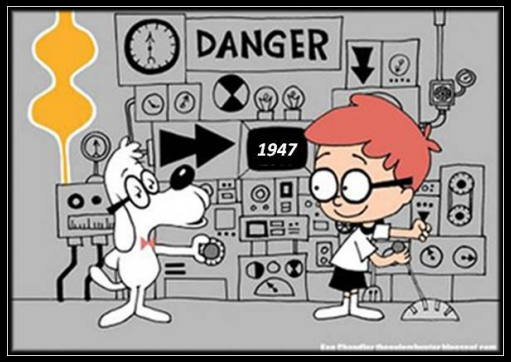


Case presentation:

- Brain biopsy:

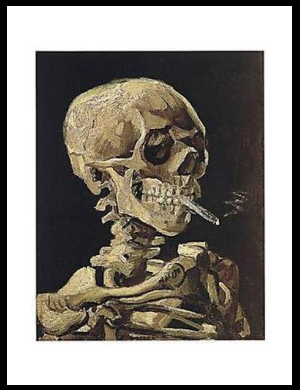


Case Presentation



- Follow-up imaging studies
  - 5 cm lung mass
  - Additional masses:
    - Lung (x2), liver (x2), bone
- Non-small Cell Lung Cancer, Stage IV

Lung Cancer is Bad

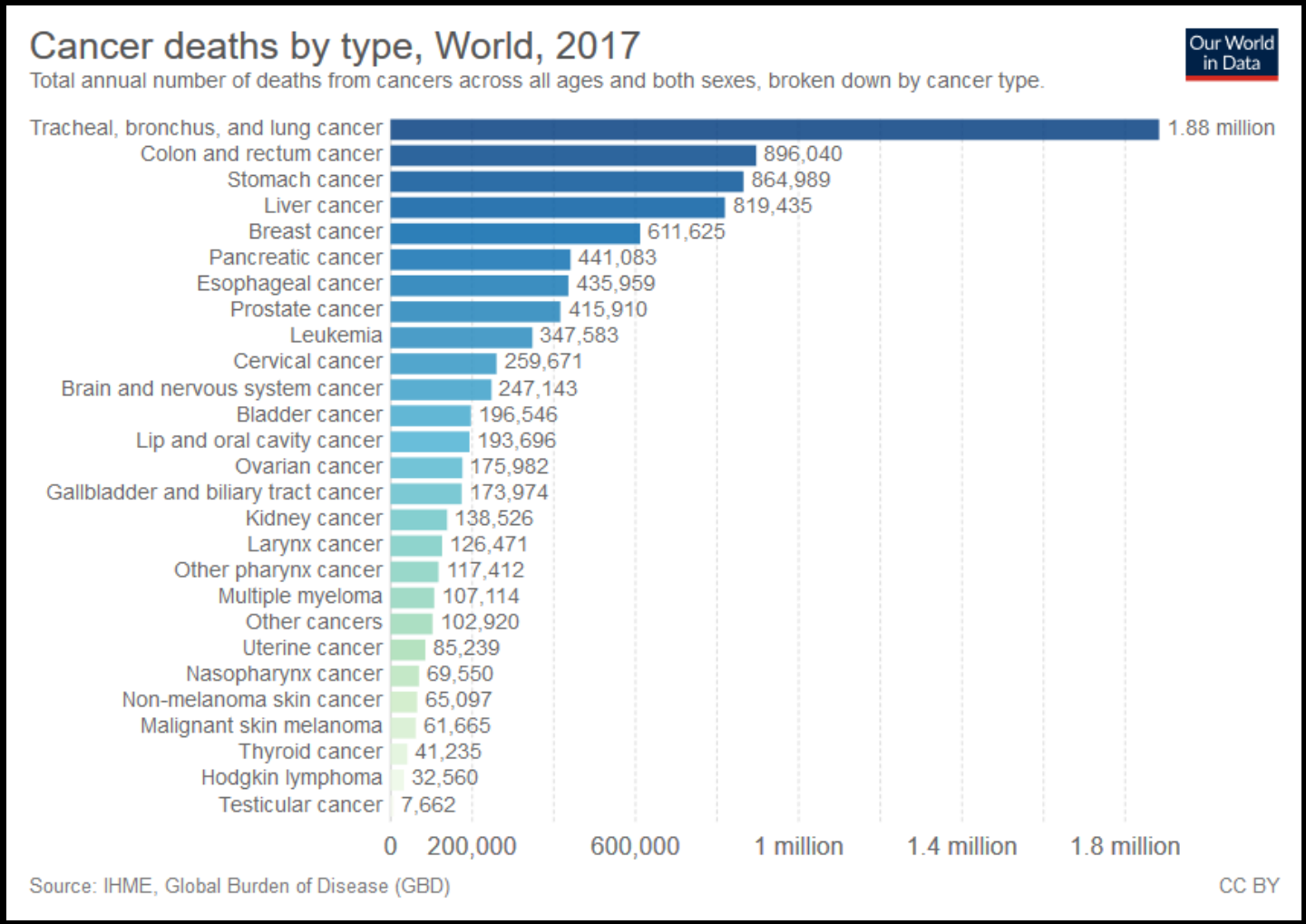


Van Gogh, 1885

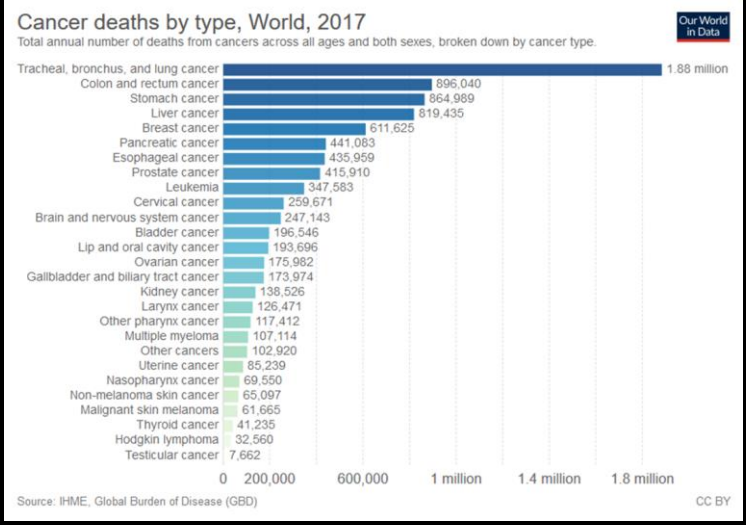
- Survival @ 18 months:
  - Stage I: 49 – 65%
  - Stage II: 39 - 55%
  - Stage III: 4 – 15%
  - Stage IV: 1%
- Median survival:
  - Stage I/II: 17 – 32 months
  - Stage III: 9 - 22 months
  - Stage IV: 16 – 36 weeks

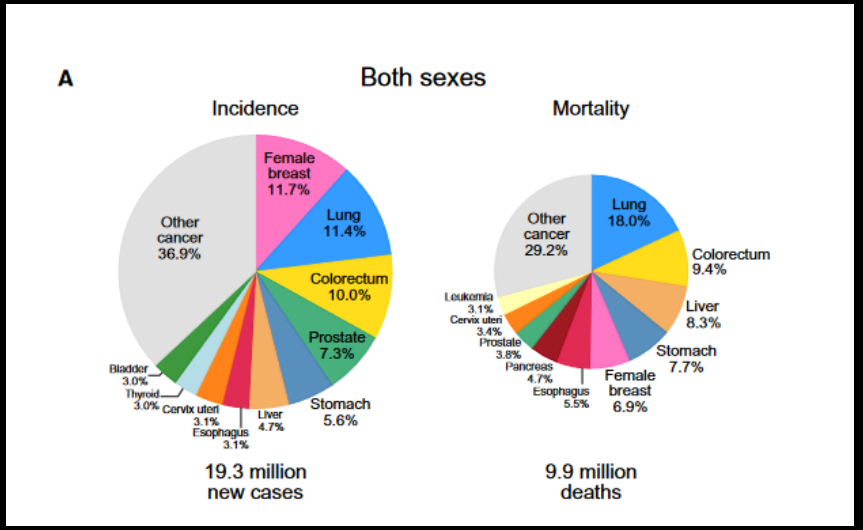
http://whyquit.com

Lung Cancer is Bad for society



It is getting better…slightly



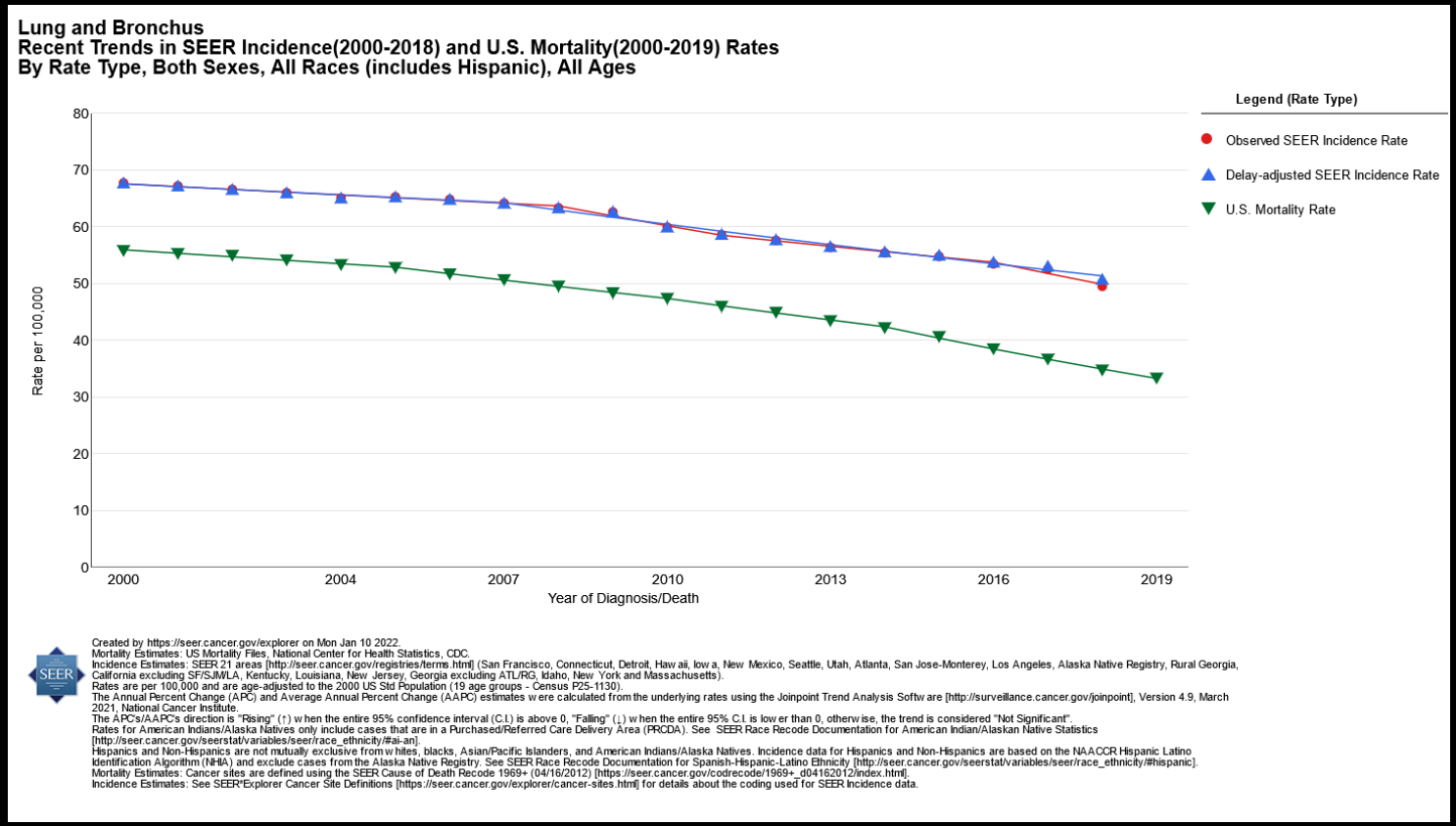


Sung, et al., CA Cancer J Clin, 2021

2017: 1.88 M deaths

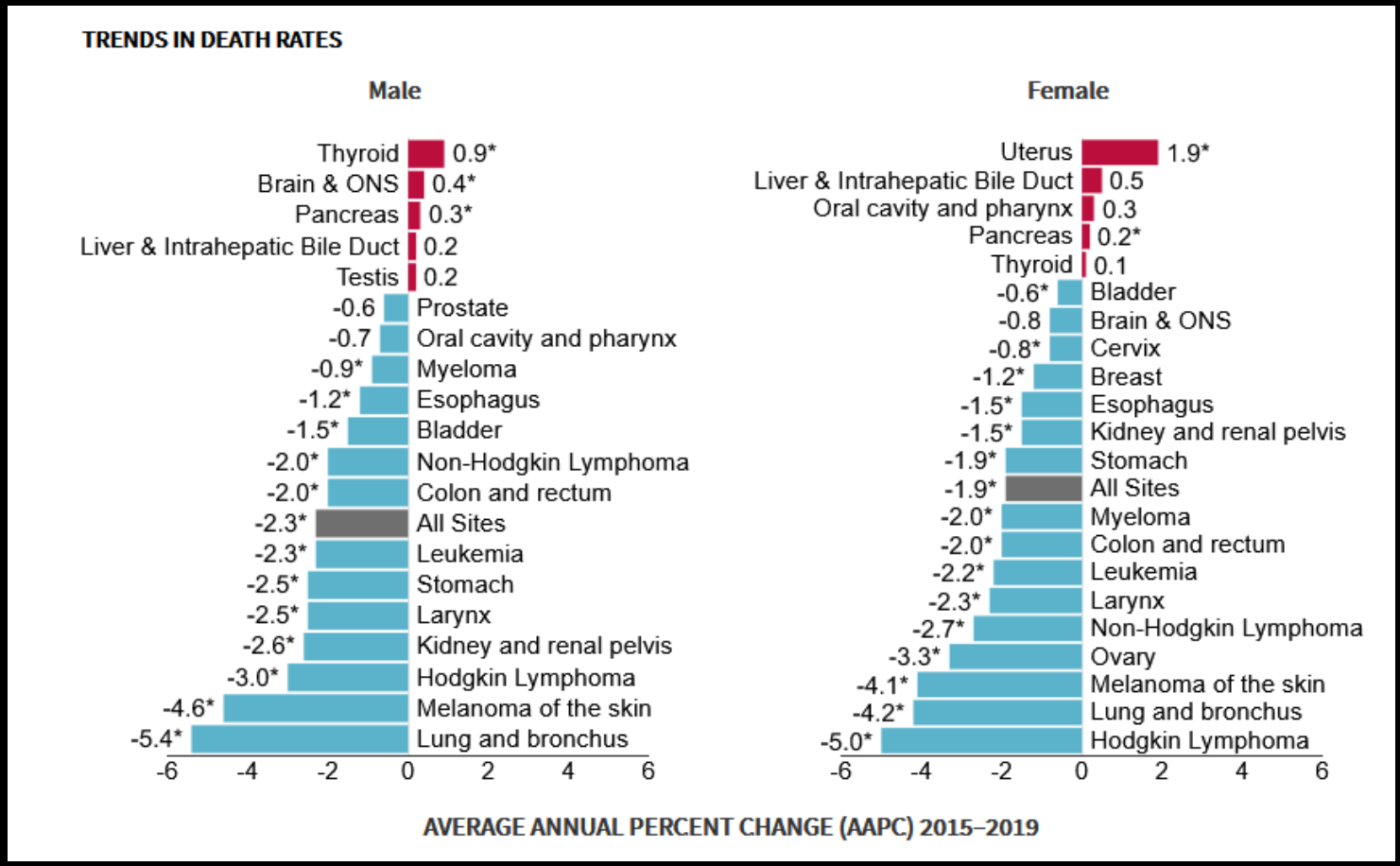
2020: 1.79 M deaths

It is getting better



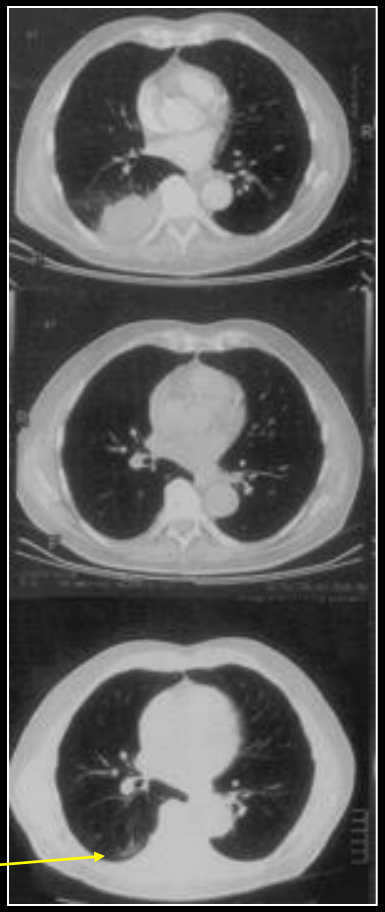
SEER, NIH website, accessed 1/10/22

It is getting better



Return to Case:

- History:
  - **Never smoked**
  - Course:
  - 4/01: Carboplatin-Paclitaxel
    - Response for 6 wks
    - Progression by 12/01
  - 12/01: Cetuximab (Erbitux)
    - Stabilization for 4 mos
    - Progression by 7/02
  - **8/02: Gefitinib (Iressa)**
    - **Sustained response for 30 mos**
    - **Relapse in 2/05**

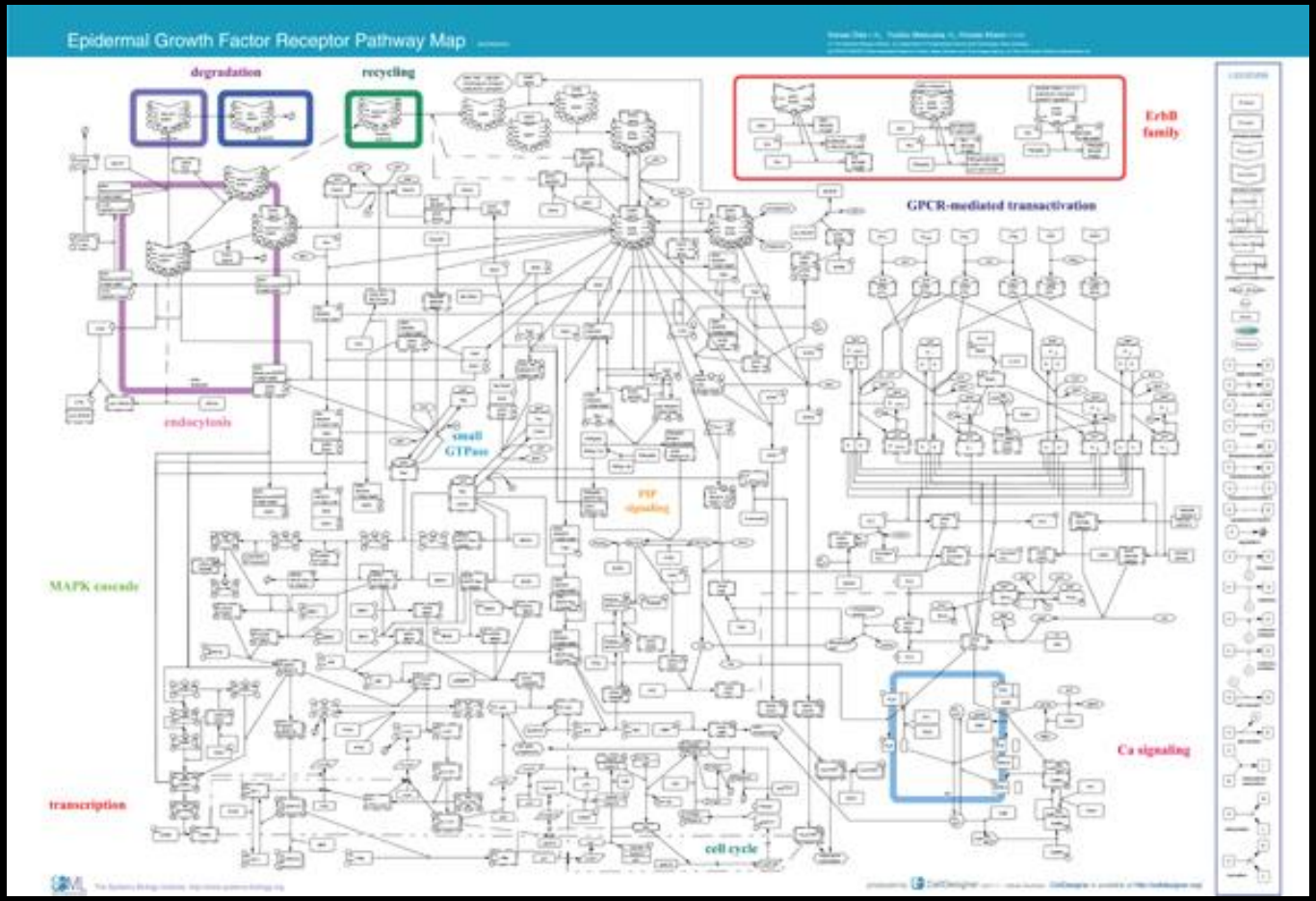


8/02

5/03

2/05

What is EGFR and why target it?



Oda, et al., Molec Systems Biol, 2005

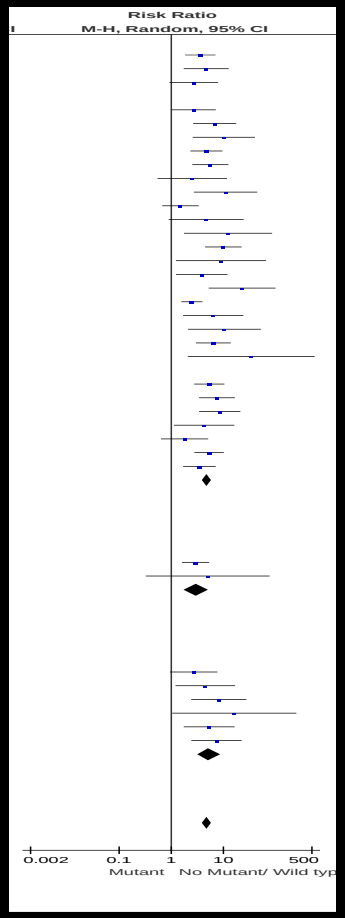
EGFR signaling simplified:

- Ligand binds
- Receptor Dimerization
- Phosphorylation
- Downstream signaling
  - RAS→RAF→ERK
  - JAK→STAT
  - PIK3CA→AKT→mTOR
- Cells proliferate, survive

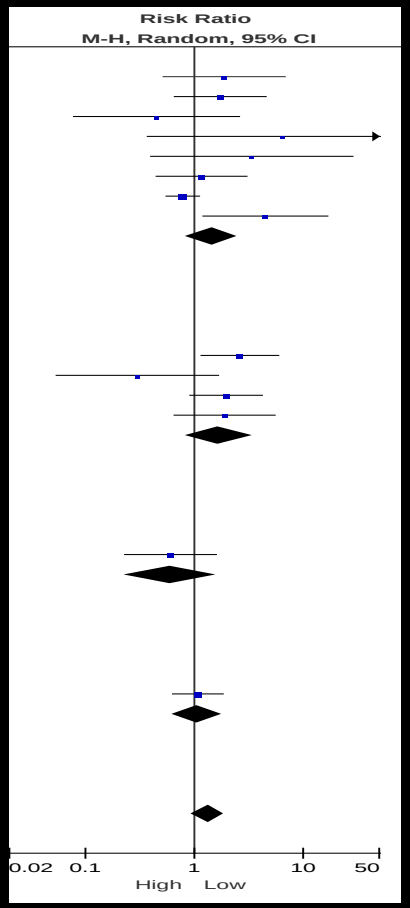
Very commonly overexpressed in human carcinomas

How to test for EGFR? Molecular Diagnosis!

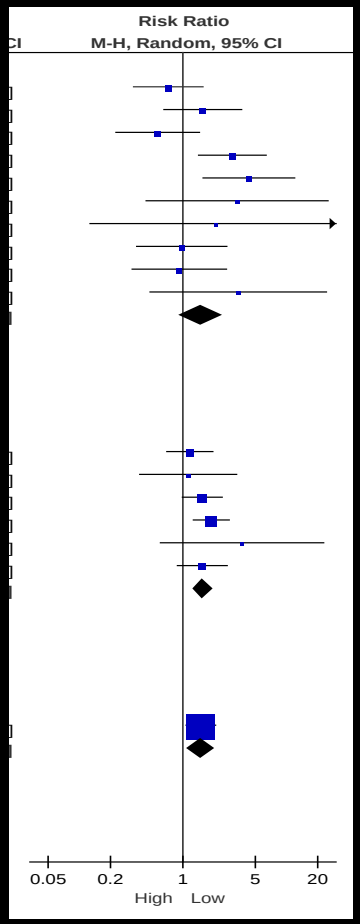
Mol Dx



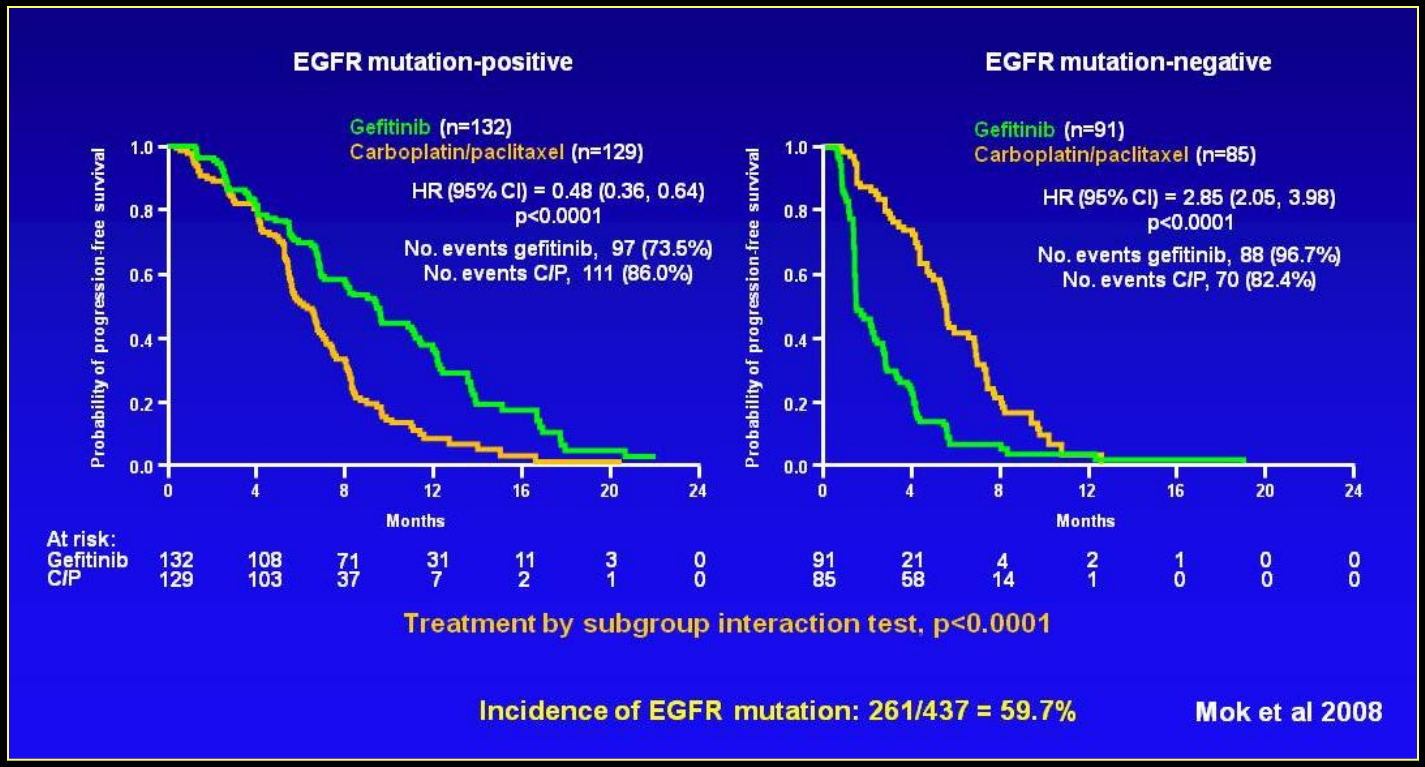
FISH



IHC

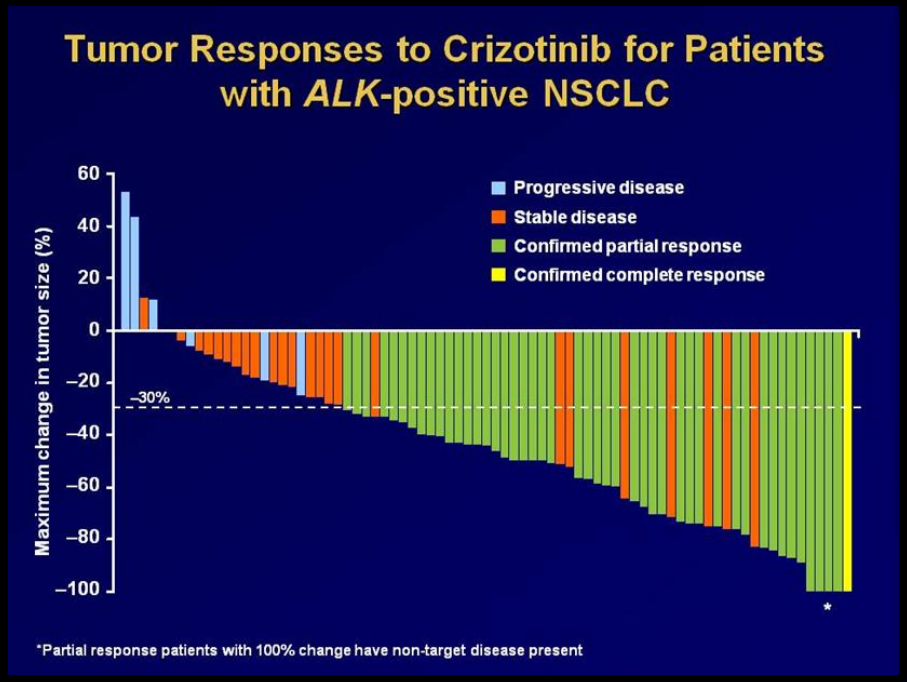


Why test at all?
Empiric therapy is not harmless



But wait…

… there's MORE!

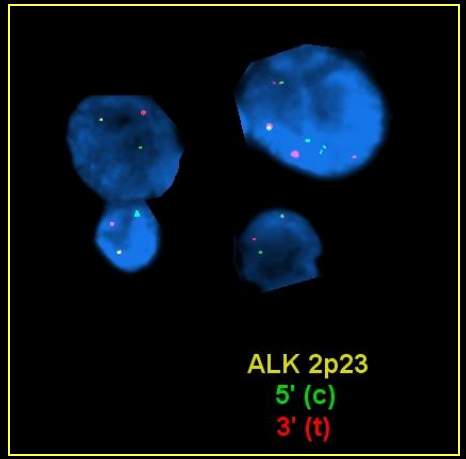


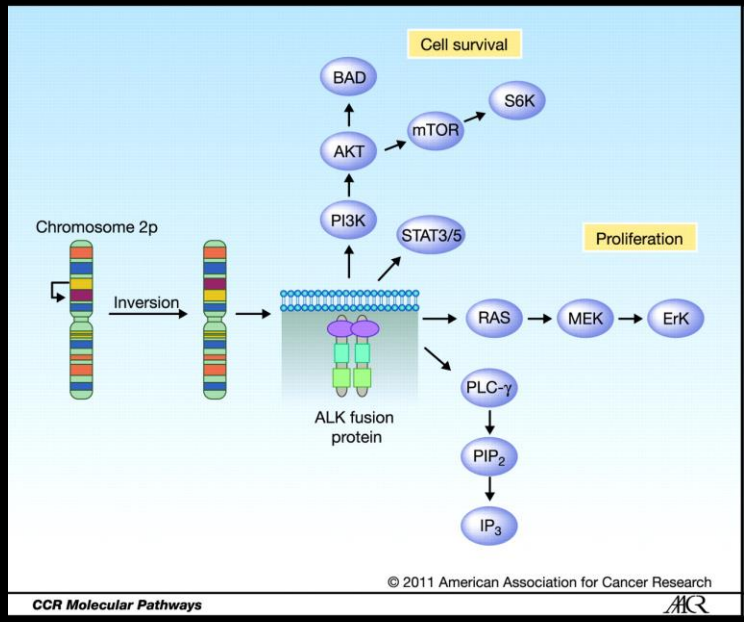
ALK rearrangement predicts crizotinib response

Crizotinib and ALK FISH both approved by FDA, 2011

ALK rearrangement

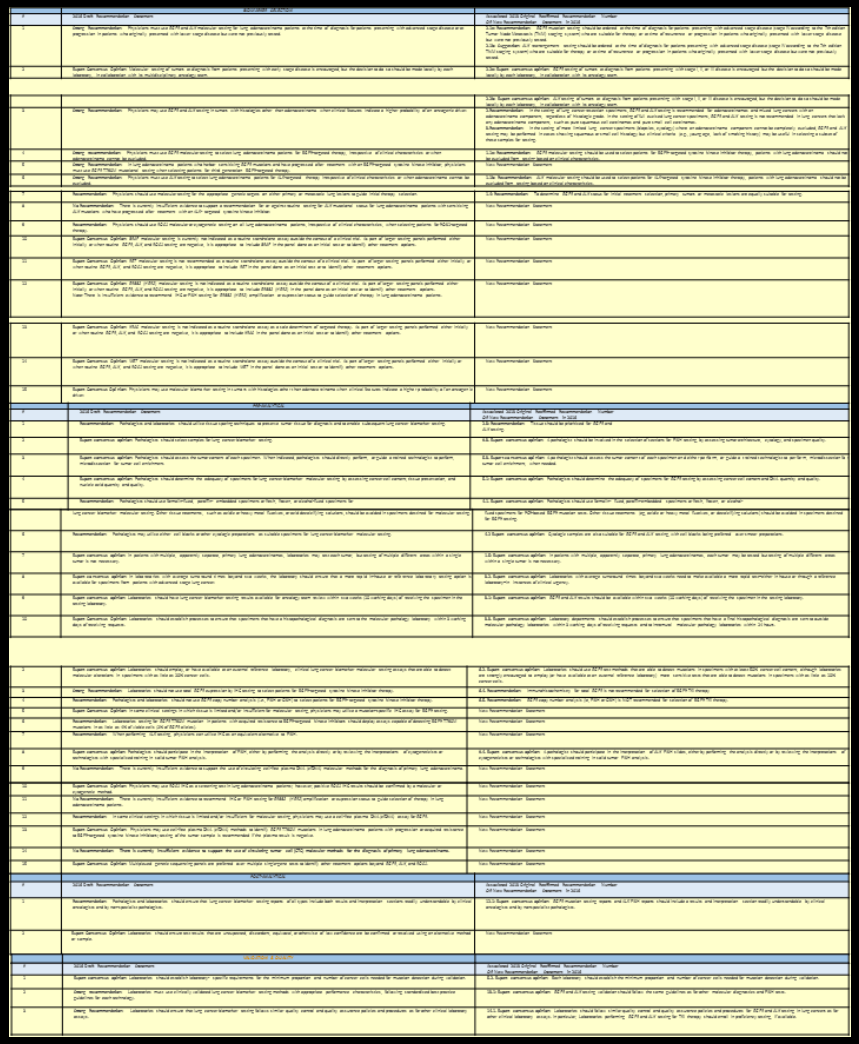
- First report: *Soda, et al, Nature, 2007*
- Typical : Inversion on 2p
  - EML4-ALK fusion
  - Rare Chromosomal variants
    - KIF5B-ALK, TFG-ALK
- Activates ALK kinase
- ~5% of lung adenocarcinomas
- Therapy: crizotinib



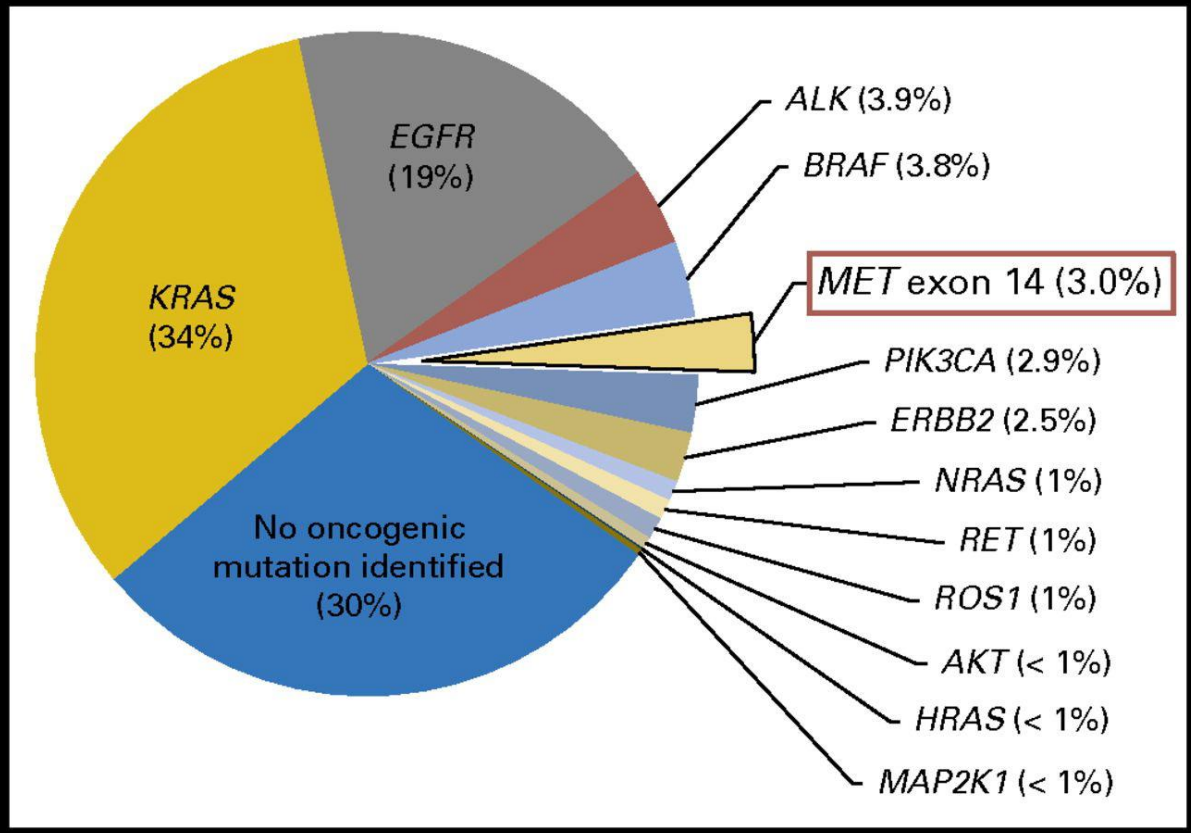


Trivia: More patients have EML4-ALK lung cancer than NPM-ALK lymphoma!

2013: Practice guideline for EGFR and ALK



2017: EGFR/ALK not the whole story



Awad, et al., JCO 2016

COLLEGE of AMERICAN PATHOLOGISTS

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
Conquering Thoracic Cancers Worldwide

AMP ASSOCIATION FOR MOLECULAR PATHOLOGY

CAP Laboratory Improvement Programs

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

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Article in Press

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DOI: https://doi.org/10.1016/j.jtho.2017.12.001

The Journal of Molecular Diagnostics, Vol. 20, No. ■, ■ 2018

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SPECIAL ARTICLE

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What other genes should be tested in lung adenocarcinoma?

ROS1: 1-2% rearrangement

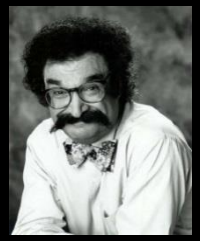
RET: 1-2% rearrangement

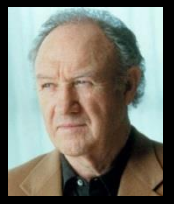
BRAF: 4% mutation half are non-V600E

MET: 3% exon 14 skipping mutations, amplification

ERBB2/ HER2: 2% mutation

KRAS: 30% mutation





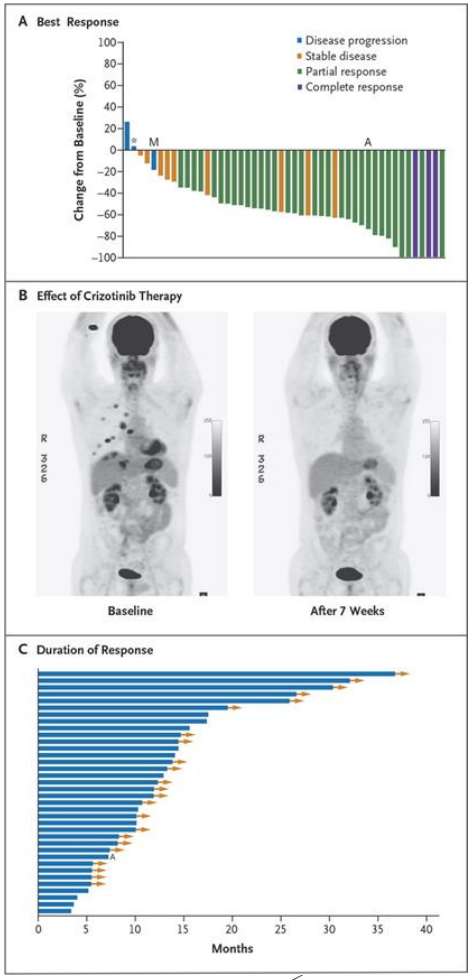
ABRUPT AND JARRING FORMAT CHANGE

Abrupt and jarring format change

AMP Association for Molecular Pathology

New in 2018: *ROS1*

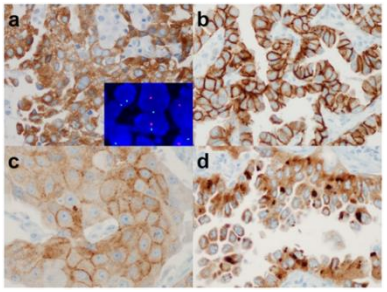
- **Clinical utility**
  - *ROS1*+ tumors respond to crizotinib
  - RR 70-80%
  - Phase I, I/II, II trials
  - No Phase III
- **Oncologists treat with crizotinib**
  - Oncologists use *ROS1* testing
- **Crizotinib approved by FDA**
  - *ALK*+, *ROS1*+ lung cancers

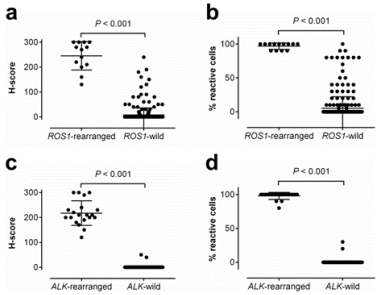


AMP Association for Molecular Pathology

New in 2018: *ROS1*

- **Methodology**
  - Adenocarcinomas, but no sensitive clinical predictors
  - No designated companion diagnostic
  - FISH is predicate method
  - Immunohistochemistry (IHC) for screening; confirm + with FISH
  - RNA methods (RT-PCR, anchored multiplexed PCR)
  - Next generation DNA sequencing





Cha, et al., PLoS One, 2014

Others: *RET, BRAF, ERBB2, KRAS, MET*

- **As with *EGFR, ALK, ROS1*, typically mutually exclusive**
- **Adenocarcinomas, but no sensitive clinical predictors**
- **Clinical trials and/or potential drugs for each:**
  - *RET*: cabozantinib, vandetinib
  - *BRAF*: vemurafenib, dabrafenib, +/- trametinib
  - *ERBB2/HER2*: ?pulsed afatinib?, ?neratinib?, ?dacomitinib?
  - *KRAS*: trametinib, selumetinib
  - *MET*: crizotinib
- **Limited evidence for clinical utility** (Case reports & small series*)

- Not recommended as single tests for lung cancer patients
- If a large panel is being performed, include these
- If *ALK, EGFR, ROS1* all negative, include these

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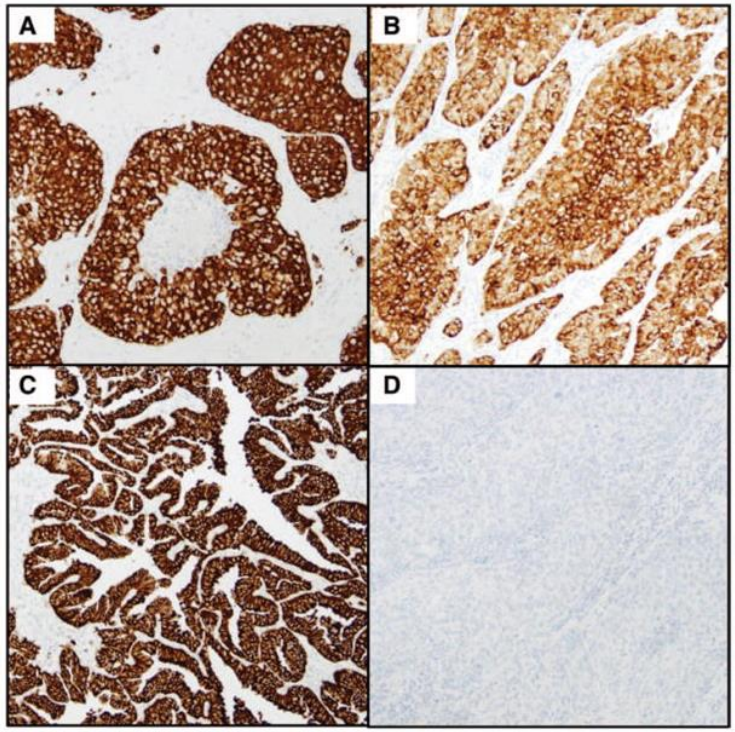
KQ II. Is immunohistochemistry reliable for *ALK* translocations?



Wynes, et al., JTO, 2014

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KQ II. Is immunohistochemistry reliable for *ALK* translocations?

YES

AMP Association for Molecular Pathology

KQ II. Is immunohistochemistry reliable for *ALK* translocations?

Table 3.
Comparative FISH/IHC Analysis in 3244 NSCLC Patients

		FISH			
		Positive	Negative	NC	Total
IHC	Positive	80	19	15	114
	Negative	36	2579	435	3050
	NC	0	49	31	80
	Total	116	2647	481	3244

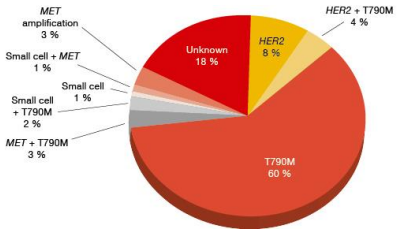
FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NC, noncontributive analysis or technical failure; NSCLC, non–small-cell lung cancer.

- **Numerous studies showed excellent concordance**
- **Discordances seen in both directions**
  - both FISH and IHC can be either false negative or positive
- **No scientific need to perform both methods**
- **Do NOT use the ALK1 antibody developed for Anaplastic Lymphoma**

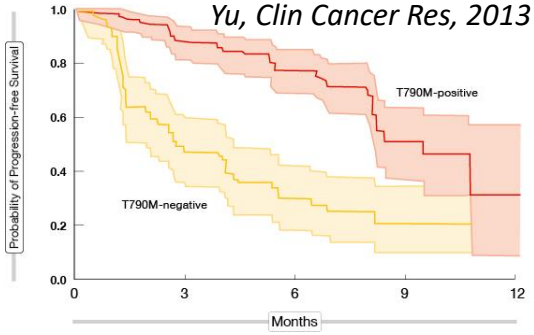
AMP Association for Molecular Pathology

KQIII: Testing in acquired resistance

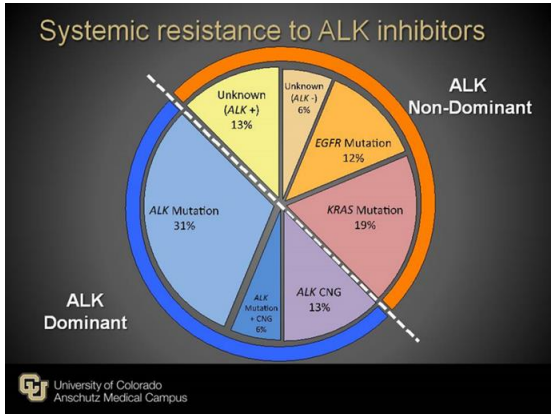
- ***EGFR:*** **all about T790M, and ultrasensivity**
  - T790M responds to specific inhibitor
  - Relapsed specimens are heterogeneous
  - Recommended cutoff is 5% mutant alleles
  - Circulating cell-free DNA may be superior*
- ALK: unclear value of identifying resistance mutations

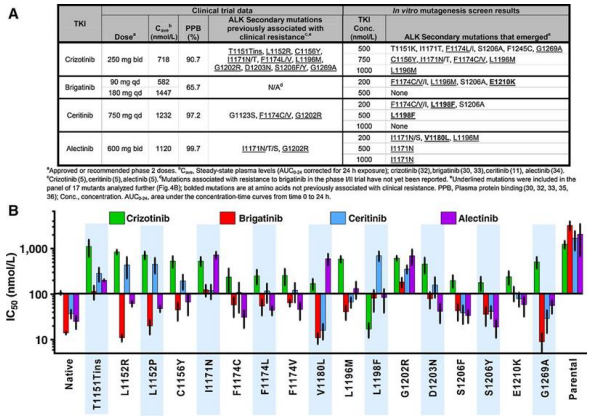


Yu, Clin Cancer Res, 2013



Janne, NEJM, 2015





Zhang, et al., , Clin Cancer Res 2016; 22(22): 5527-38

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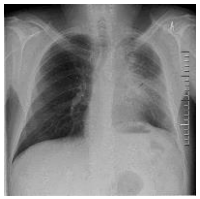
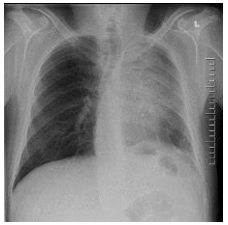
KQ IV. Test squamous or small cell carcinomas?

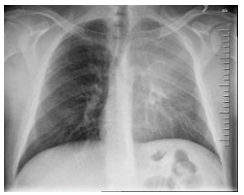
- **Small cell carcinomas: No**
- **Squamous cell carcinomas: Maybe**
  - "Squamous carcinoma genes": *FGFRs*, *DDR2*
    - Insufficient evidence to support for/against testing
  - "Adenocarcinoma genes": *EGFR, ALK, ROS1*
    - If clinical or pathologic features are "high risk"
      - Can't exclude unsampled adenocarcinoma histology
      - Young patient
      - No history of tobacco use
  - Other therapies: EGFR antibodies, immunotherapy



1 cycle gemcitabine/cisplatin



Switch to crizotinib



Images courtesy of Erick Bernicker, MD

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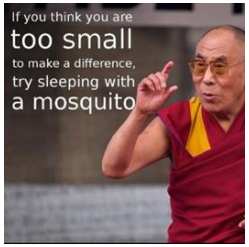
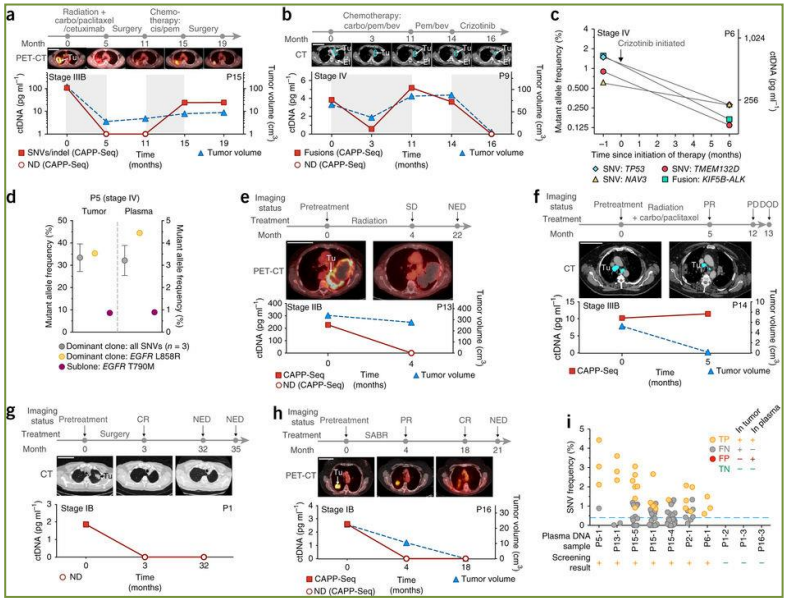
KQ V. What is the role of testing cell-free DNA or circulating tumor cells?

- **Initial diagnosis:**
  - Appropriate when tissue testing unavailable
    - No adequate sample
    - Patient cannot undergo biopsy
- **Monitoring on therapy:**
  - Very exciting and very unproven
  - Cannot recommend at this time
- **Acquired resistance:**
  - Appropriate alternative to tissue testing
    - Sensitivity poor, specificity high
    - Treat if plasma positive
    - Biopsy and test tissue if plasma negative





Newman, et al., Nature Med 2014

AMP Association for Molecular Pathology

KQ VI: What is the role of sequencing panels in lung cancer?

- **2013: insufficient evidence to support NGS panels**
- **2018: NGS panels preferred over single gene tests**
  - Single gene methods still acceptable, provided TAT met
  - Results returned quicker
  - Spares sample, which is often limiting
  - Enables expanded testing beyond *EGFR, ALK, ROS1*
    - Help patients find appropriate clinical trials
- **TAT recommendation: two weeks, for all testing**

AMP Association for Molecular Pathology

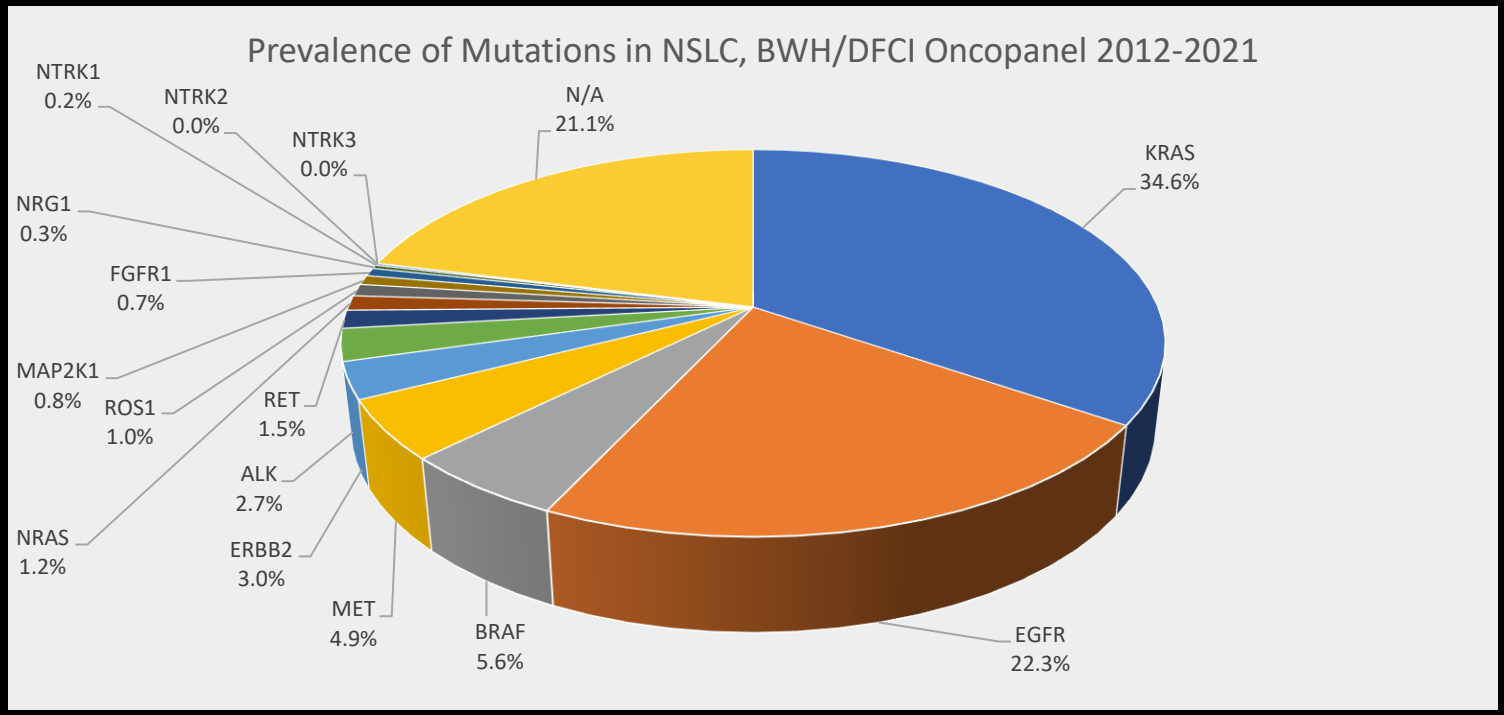
Not a KQ, but should have been: What is the role of PD-1/PD-L1 IHC?

- **Pulling up lame: Out of scope for us**
  - Wrong panel constituency to assess this properly
  - Not included in initial search or data evaluation tools
  - Incorporating it would entail a near-restart and a 1+ yr delay
  - Another project is addressing this in a broader disease context
- **Non-evidence based opinion**
  - Immune checkpoint therapies are proven effective in lung cancer
  - Test methods, in a global sense, are not yet established
    - IHC: PD-1, PD-L1, CTLA-4, with multiple different antibodies
    - Tumor lymphocytes, mutational load, neoantigen expression
- **Some agents require specific tests to determine eligibility**
- **Opinion: for now, use the tests required for the agents being considered**

AMP Association for Molecular Pathology

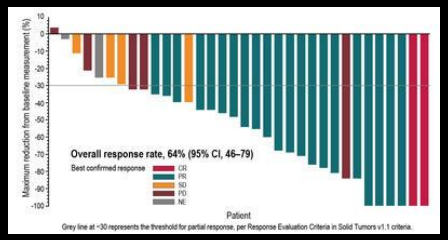
2021: Time for a new guideline

Landscape evolution

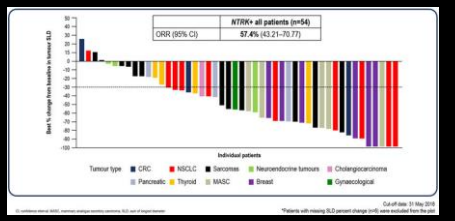


Treatment evolution:

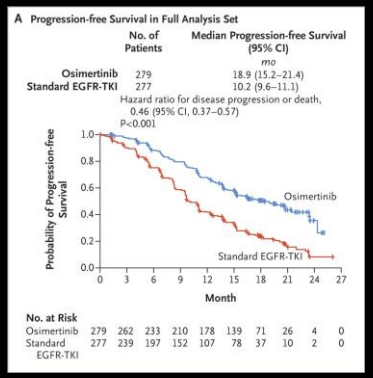
- BRAF inhibitor approved
- NTRK inhibitor approved
- T790M inhibitor now first line
- RET inhibitor approved
- MET inhibitor approved
- Inhibitor for EGFR exon 20 insertions
- Immune therapies exploded
- KRAS G12C inhibitors
- Coming: ERBB2 conjugates



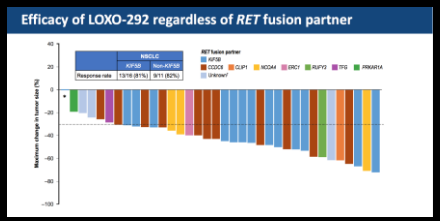
Planchard, ESMO, 2017



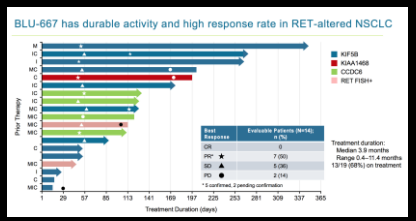
Demetri, ESMO 2018



Soria, et al., NEJM, 2018



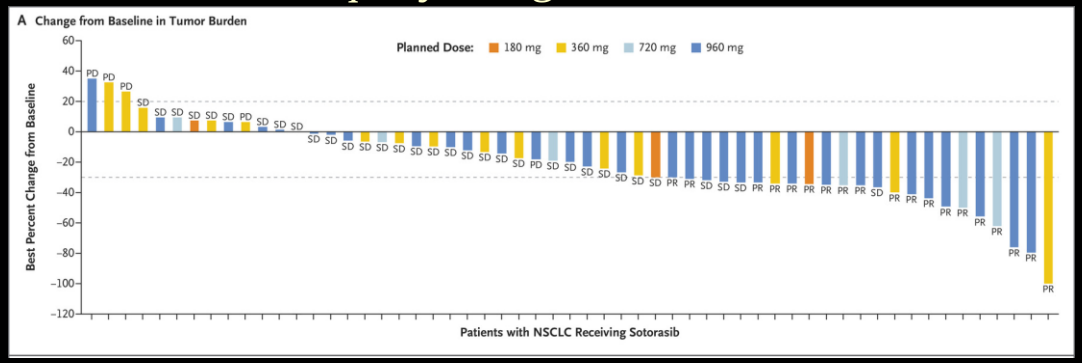
Koski, 2018



Soria, et al., NEJM, 2018

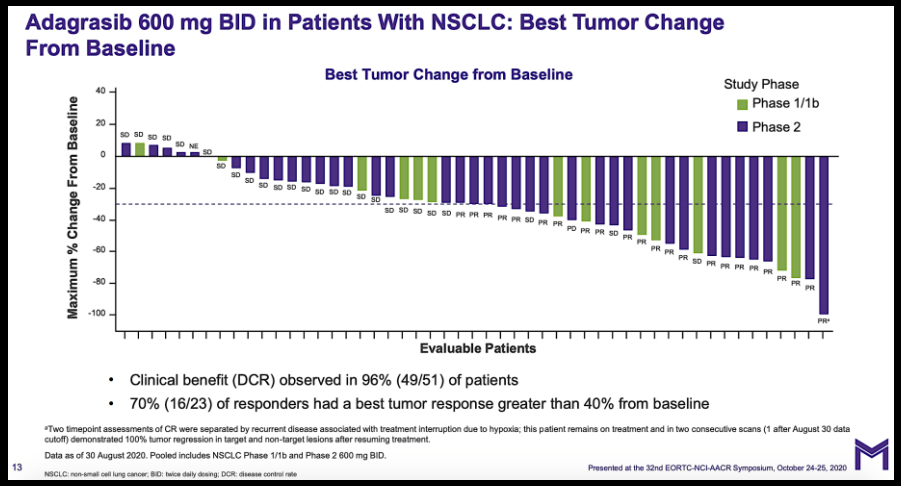
KRAS G12C in NSCLC

- **Creates a novel binding pocket**
- **Clinical utility: G12C mutation and sotorasib, adagrasib**
  - Inhibitors uniquely designed for this exact mutation



sotorasib:
response rate 32%
disease control 88%

-Hong, et al., NEJM, 2020



KRAS Mutations in NSCLC

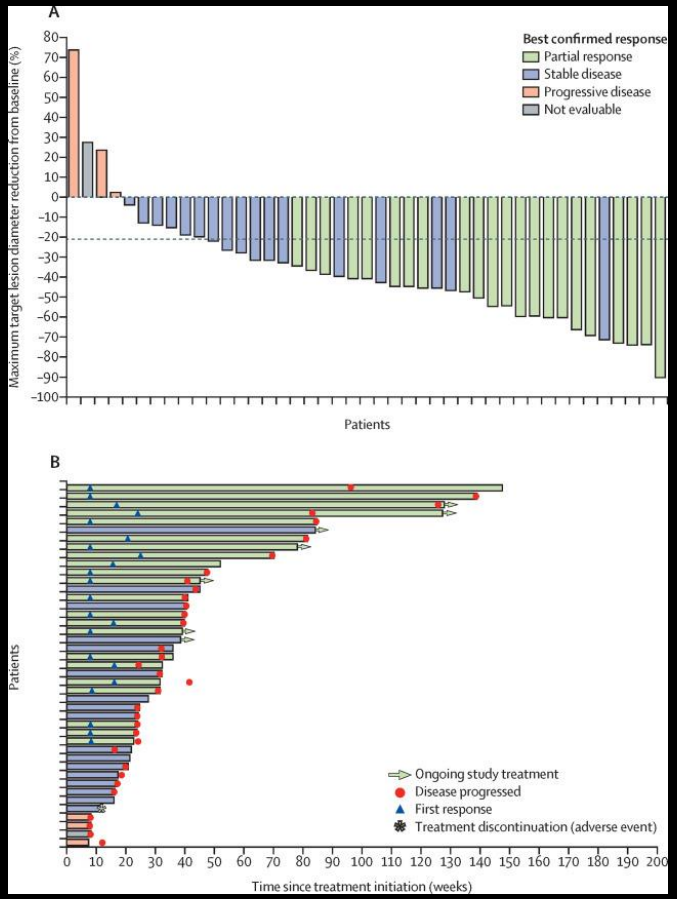
- **Useful to "rule out" other less common alterations**

A	B	Neither	A Not B	B Not A	Both	p-Value	Tendency
KRAS	EGFR	2225	1431	1032	56	<0.001	Mutual exclusivity
KRAS	MET	2929	1435	328	52	<0.001	Mutual exclusivity
KRAS	ERBB2	3050	1446	207	41	<0.001	Mutual exclusivity
BRAF	EGFR	3379	277	1046	42	<0.001	Mutual exclusivity
ALK	EGFR	3282	374	1022	66	<0.001	Mutual exclusivity
KRAS	BRAF	3005	1420	252	67	<0.001	Mutual exclusivity
KRAS	ROS1	2987	1407	270	80	<0.001	Mutual exclusivity
MET	EGFR	3340	316	1024	64	0.002	Mutual exclusivity
ERBB2	EGFR	3450	206	1046	42	0.011	Mutual exclusivity
KRAS	ALK	2935	1369	322	118	0.017	Mutual exclusivity

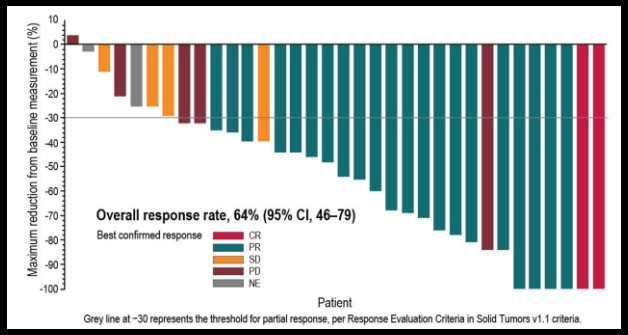
  - Mutually exclusive with other driver alterations
  - ~30% of lung adenocarcinomas
  - Simple and widely available single gene assays
    - **Many cannot distinguish G12C from others, however**

BRAF V600E mutations

- **Clinical utility: response to BRAF+MEK inhibition**

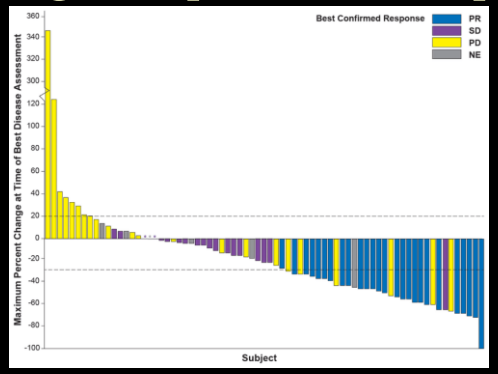


Subbiah, et al., Lancet Oncol, 2020



Planchard, et al., Lancet Onc, 2017

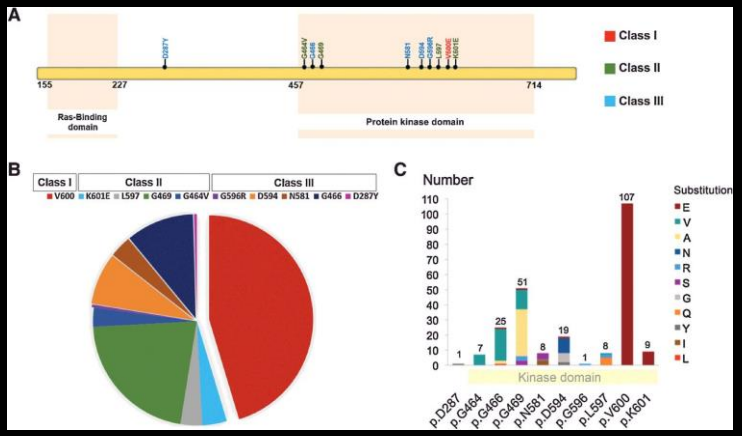
Single target response less impressive

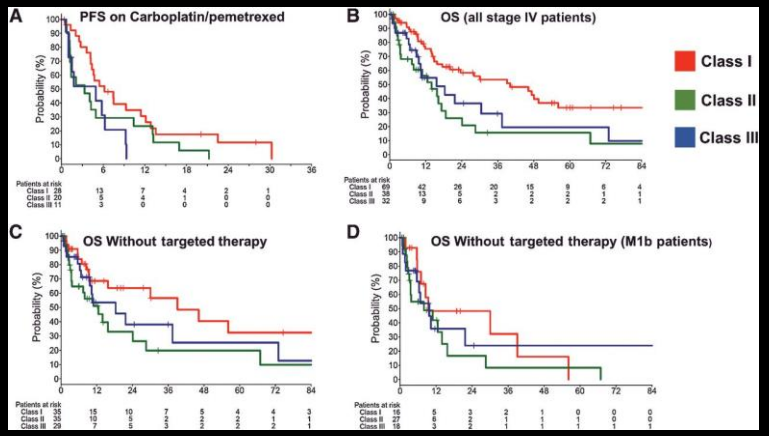


Planchard, et al., Lancet Onc, 2016

Other BRAF mutations

- Class I: activating, as monomers → readily inhibited
- Class II: activating, require dimerization
- Class III: kinase null, but activate through other mechanisms
  - Typically co-occur with RAS activating alterations





Dagogo-Jack, et al., Clin Cancer Res, 2019

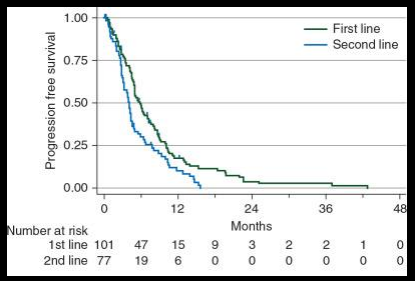
ERBB2 (HER2)

- **Critical gene in breast cancer**
  - Amplification (FISH) or overexpression (IHC)
  - Responds to treatment with trastuzumab
- **Different role in lung cancer**
  - Mutations, typically insertions in exon 20
    - Amplifications do occur as well
  - FISH, IHC are not useful for lung cancer
  -

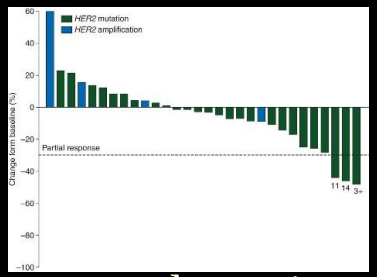
ERBB2 (HER2)

Clinical Utility

- Do not respond to therapeutic antibodies
  - Treatment with TKIs disappointing



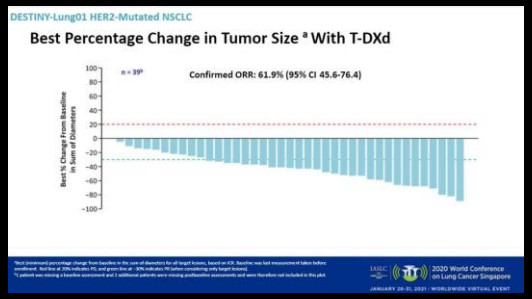
Mazieres., Ann Oncol, 2016



Response rate: 12%

Kris, et al., Ann Oncol, 2015

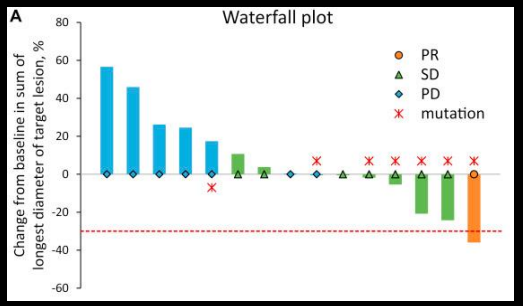
  - Trastuzumab conjugates (toxic payload) maybe promising



TopoI inhibitor

Response rate: 61%

Smit. et al., World Lung, 2021



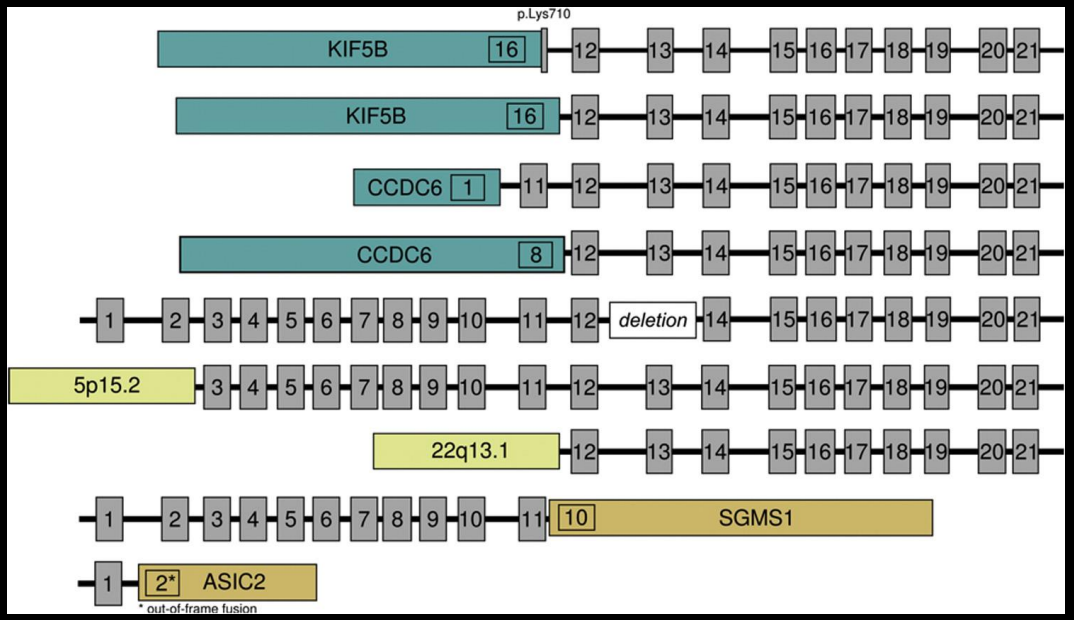
Microtubule inhibitor

Response rate: 7%

Hotta, et al., JTO, 2018

RET

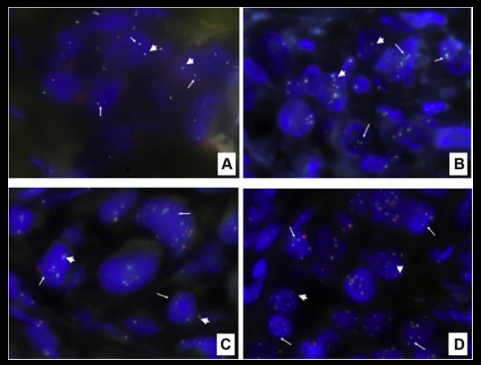
- **Characteristic mutations in thyroid cancer**
  - Mutations in MEN II syndrome, medullary carcinoma
  - *RET/PTC* fusions in papillary carcinoma
- **Overlapping alterations in lung cancer**
  - Multiple fusions, including *KIF5B*, *CCD6*, *NCOA4*

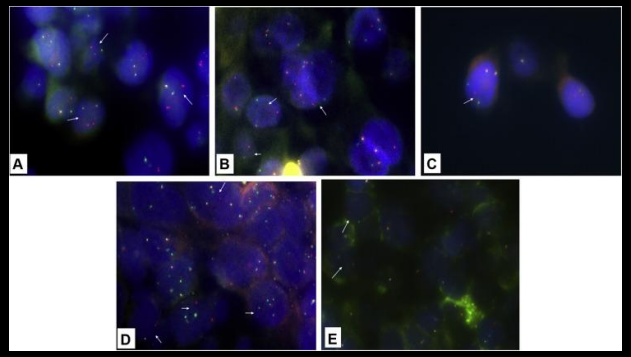


Radonic, JTO, 2021

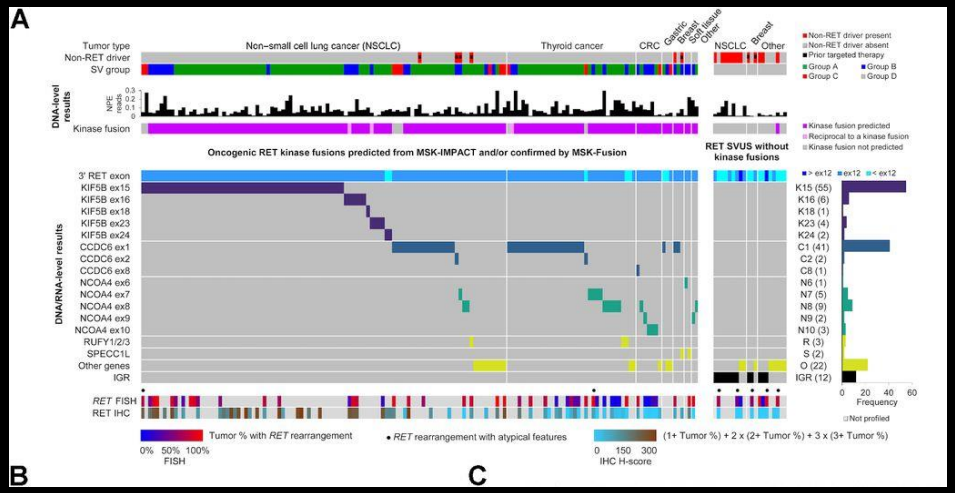
RET methodology

- **Challenging!**
  - Discovered by DNA NGS
  - FISH and IHC can be challenging
  - RT-PCR may be best





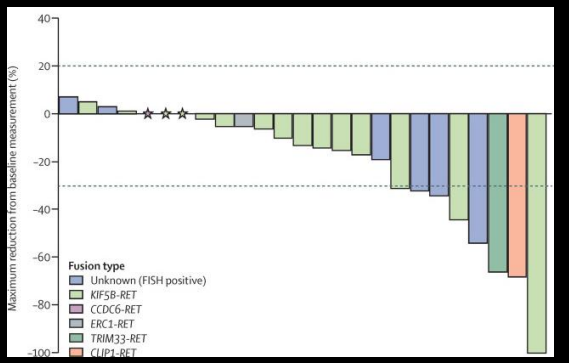
Radonic, JTO, 2021



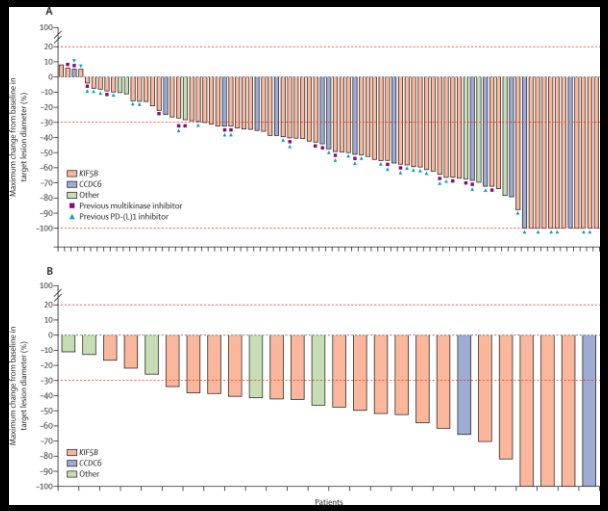
Yang, Clin Cancer Res, 2021

RET

- **Response to TKIs:**
  - Broad TKIs: ~30-50% ORR, PFS 5-8 mos
  - Selective RET TKIs: 50-70% ORR, PFS 18.4mos

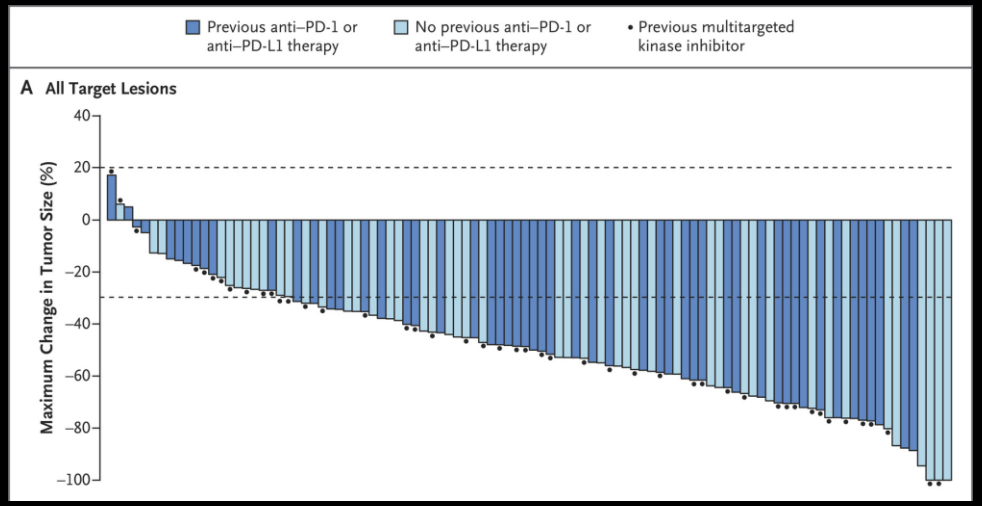


Drilon Lancet Onc, 2016



Pralsetinib: 61% response

Gainor, Lancet Onc, 2021

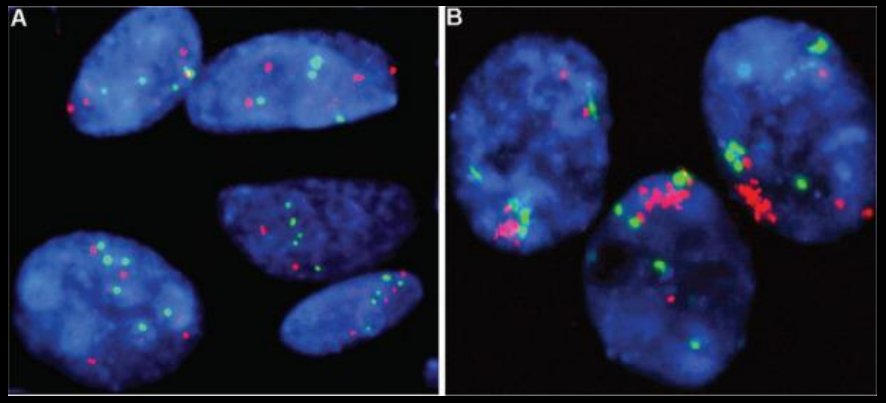


Selpercatinib: 64% response

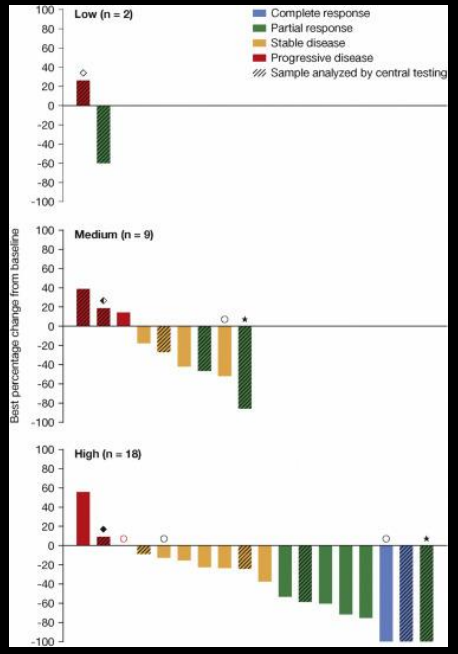
Drilon, NEJM, 2020

MET

- **Clinical utility - a complicated story…**
  - *MET* copy gain first seen in who relapsed on anti-EGFR therapy
    - Confusion : “copy gain” vs “amplification”
    - Can co-exist with other oncogene mutations



Cappuzzo, Ann Onc, 2009

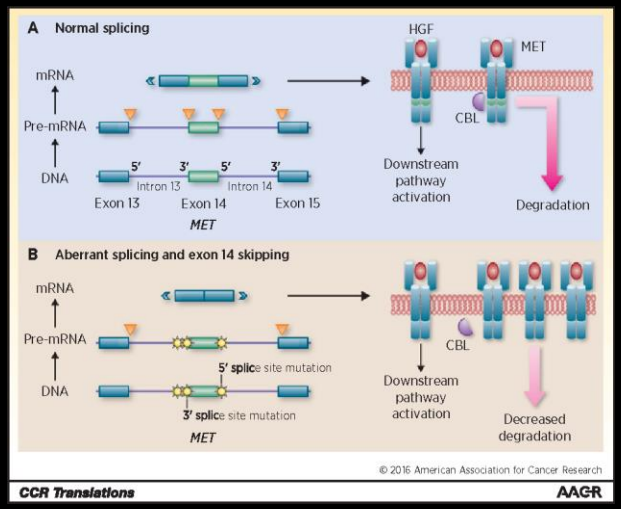


Camidge, JTO, 2021

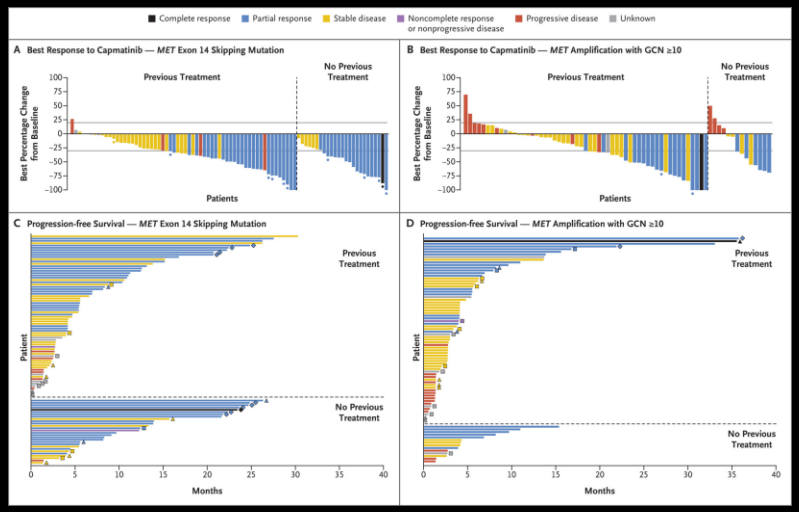
  - MET inhibitors were developed (crizotinib), but didn’t work in general
    - Rare “true” MET amplification does respond to crizotinib (~40% rr)

MET

- **Clinical utility – fast forward…**
  - NGS discovers mutations affecting splicing of exon 14
    - Common: ~3% of lung cancers
    - exon 14 skipping mutations do respond to targeted inhibitors



Drilon, Clin Cancer Res, 2016

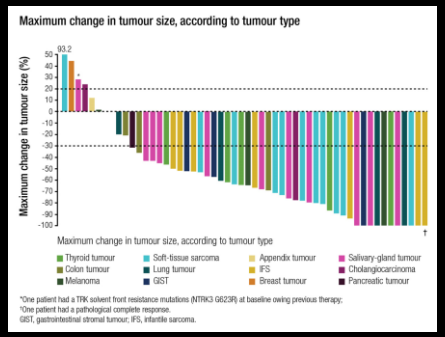


Wolf, NEJM, 2020

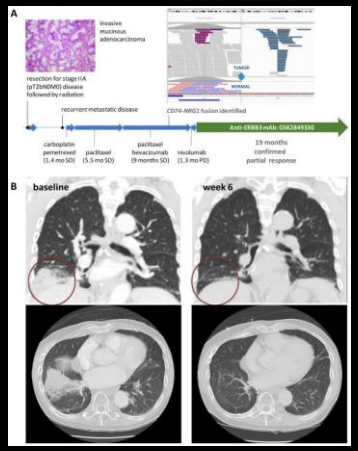
- Methodology: many choices still, but RNA favored

Rare mutations

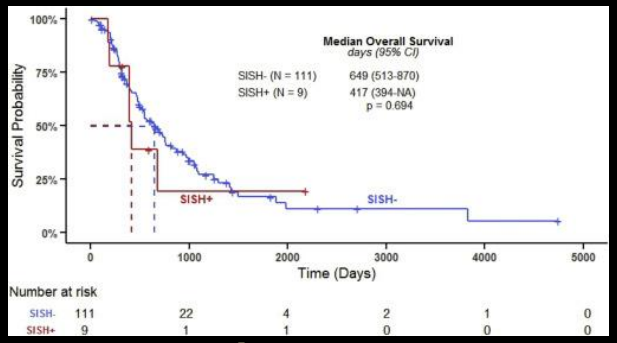
- **Insufficient evidence for review in 2018:**
  - NTRK1,2,3 fusions in all solid tumors
    - Specific Trk inhibitors
  - NRG1 fusions
    - ~30% in mucinous tumors
    - Ligand for HER3
    - Anti-HER3 anitbodies?
  - FGFR1,2 amplification
    - disappointing



Drilon, NEJM, 2018



Drilon, Cancer Disc, 2018



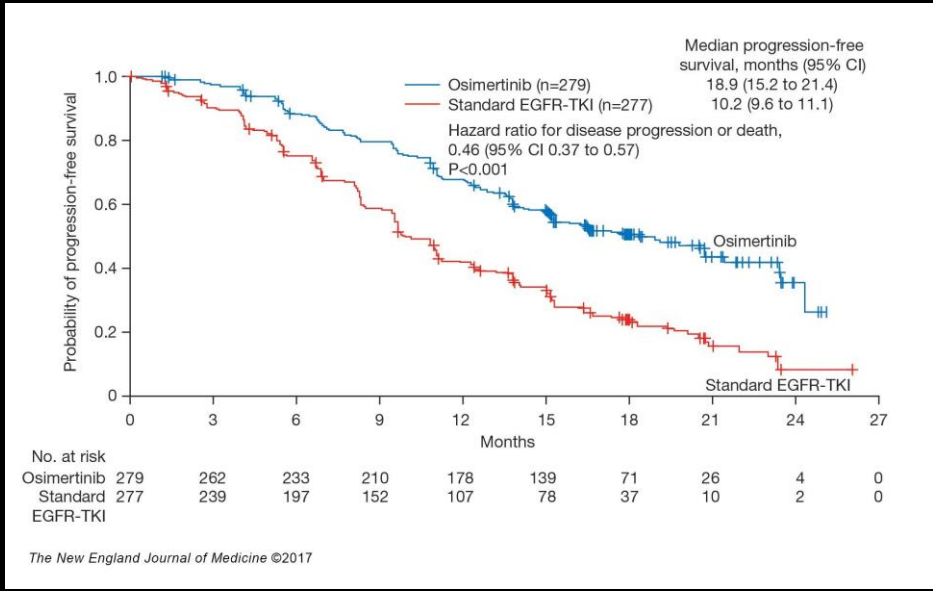
Ng, Clin Lung Cancer, 2018

New issues with old genes

- EGFR
  - 3rd generation inhibitors in first line
  - Novel resistance mechanisms
  - Early stage disease
  - Exon 20 insertion therapies
- ALK
  - Secondary resistance

New issues with old genes

- EGFR
  - Third generation inhibitors now used in first line
    - Covalently binds EGFR, rather than competing with ATP



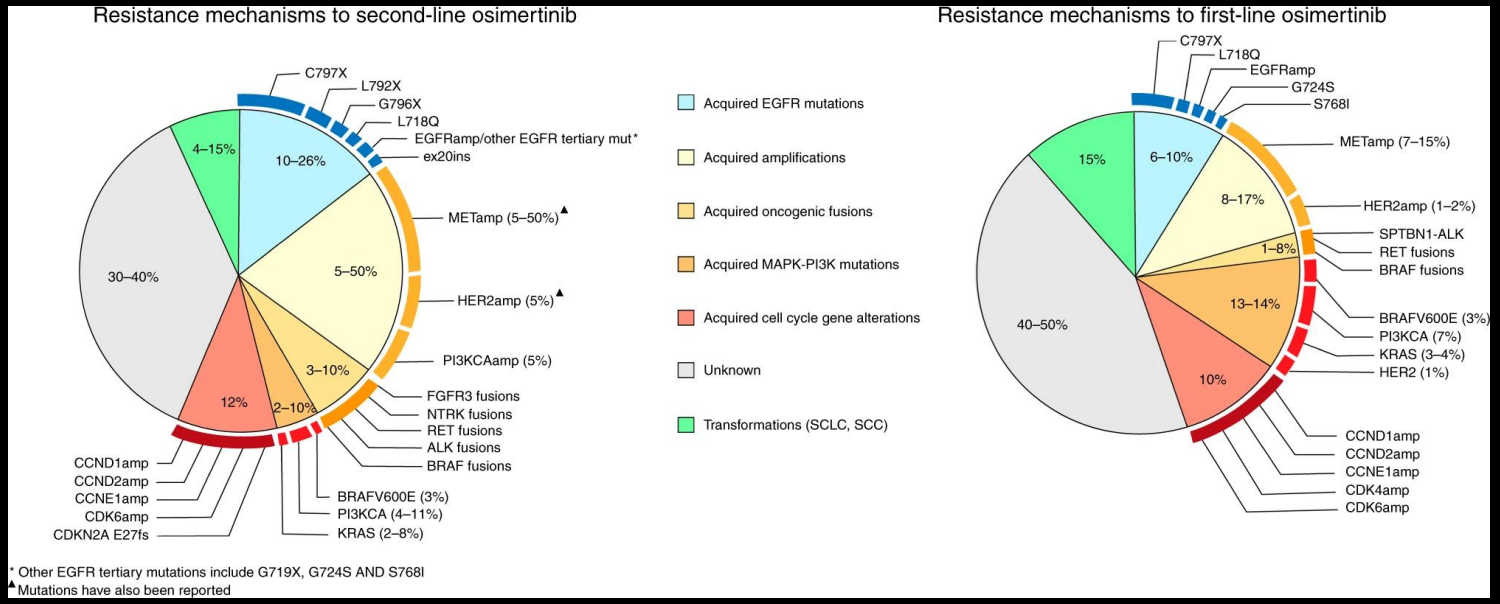
Ramalingam, NEJM, 2020

Overall survival
1st gen: 31.8 mos
3rd gen: 38.6 mos

Recall slide 7:
placebo: 6 mos

New issues with old genes

- 3rd Gen EGFR inhibitors in first line
  - New profile for secondary resistance

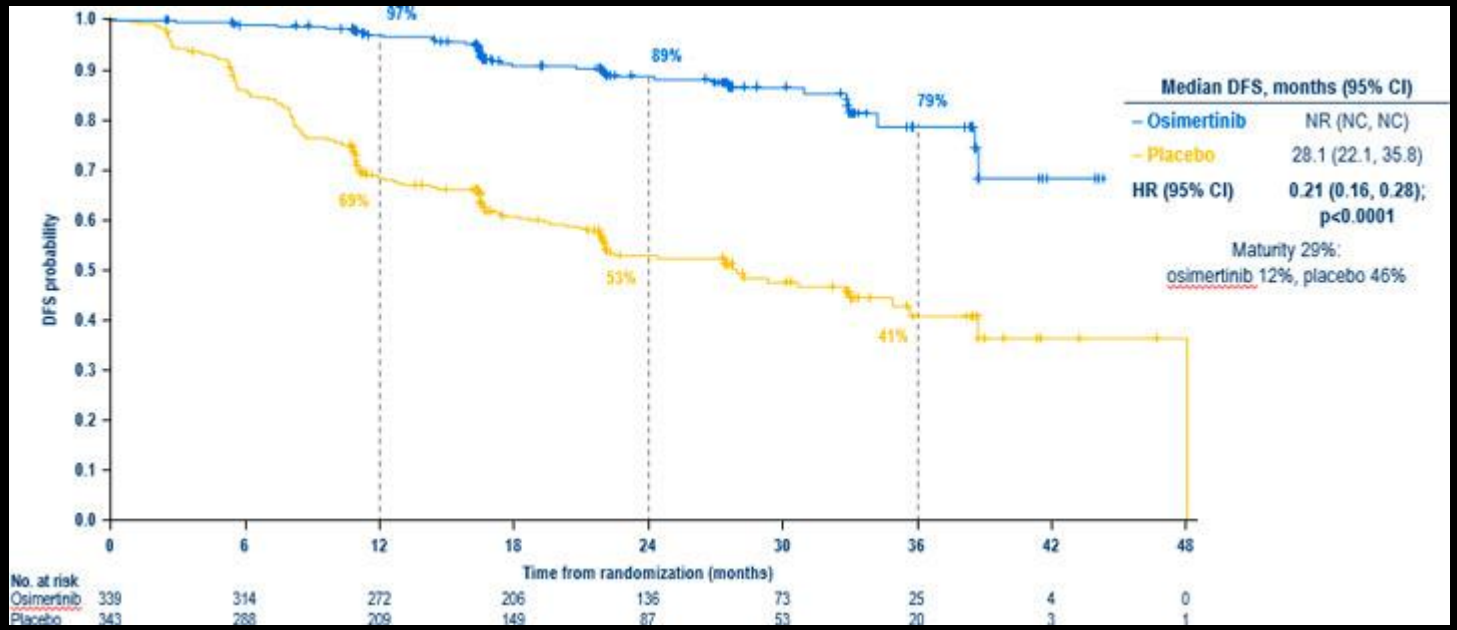


Leonetti, BJC, 2019

- Requires broad-spectrum technology, or multiple assays

New issues with old genes

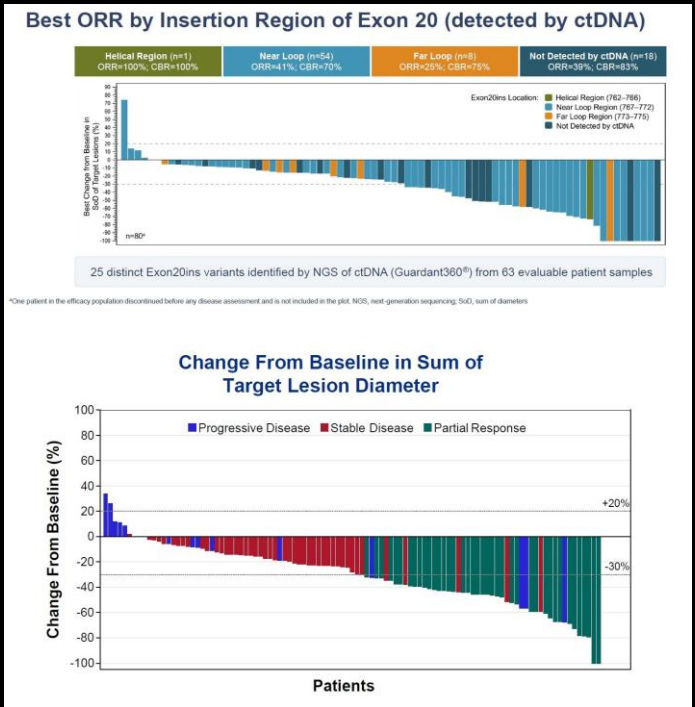
- EGFR inhibition in early stage disease



Herbst, ASCO, 2020

New issues with old genes

- EGFR exon 20 insertions
  - Resistant to first, second, and third generation inhibitors
  - New drugs:



Plieth, World Lung, 2021

Amivantamab:
bispecific antibody (EGFR-MET)
ORR 40%, PFS 8.3 mos

Mobocertinib:
specialized TKI
ORR 26%, PFS 7.3 mos

New issues with old genes

- ALK resistance mutations
  - Different inhibitors have different sensitivity profiles
  - More analogous to BCR-ABL than to EGFR

Table 2

Pharmacologic properties of ALK inhibitors approved by the US FDA or in clinical testing.

ALK TKI	Crizotinib (PF-02341066)	Ceritinib (LDK378)	Alectinib (RO/CH5424802)	Brigatinib (AP26113)	Lorlatinib (PF-06463922)	Entrectinib (RXDX-101)	Ensartinib (X-396)
Manufacturer	Pfizer	Novartis	Genentech	Ariad	Pfizer	Ignyta	Xcovery
Targets other than *ALK*	*ROS1MET*	*ROS1* *IGF-1R* *IR*	*GAK* *LTK* *RET*	*ROS1*	*ROS1*	*NTRK1* *NTRK2* *NTRK3* *ROS1*	*ROS1* *MET* *AXL*
Resistance mutations known to be targeted by TKI	L1198F	I1171T/N L1196M S1206C/Y G1269A/S	L1152P/R C1156Y/T F1174C/L/V L1196M S1206C/Y G1269A/S	I1151Tins L1152P/R C1156Y/T F1174C/L/V L1196M **G1202R**[a] G1269A/S	I1151Tins L1152P/R C1156Y/T I1171T/N/S F1174C/L/V L1196M **G1202R**[b] S1206C/Y E1210K G1269A/S	C1156Y/T L1196M	C1156Y/T L1196M
Reported resistance mutations to the TKI	I1151Tins L1152P/R C1156Y/T I1171T/N/S F1174C/L/V V1180L L1196M G1202R S1206C/Y E1210K G1269A/S	I1151Tins L1152P/R C1156Y/T F1174C/L/V G1202R	I1171T/N/S V1180L G1202R	**G1202R**[a] E1210K + S1206C E1210K + D1203N	**L1198F +** **C1156Y**[c]	G1202R	N.D.

Lin, Europe PMC, 2017

Notable mutations

L1196M "gatekeeper"
blocks sterically likeT790M

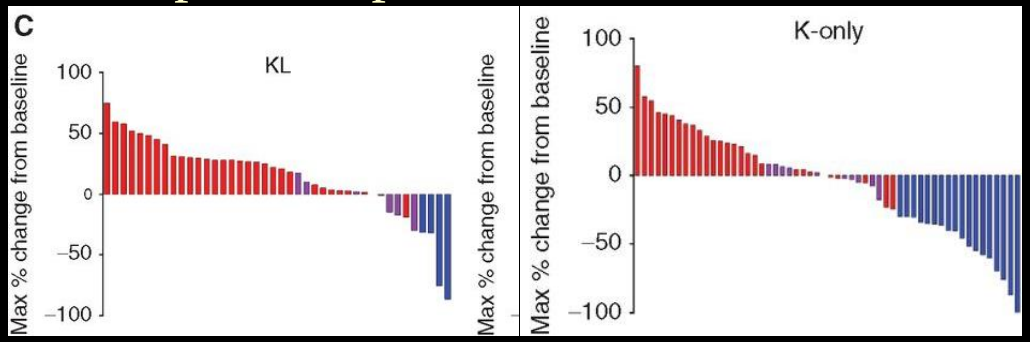
G1269A
also steric hindrance

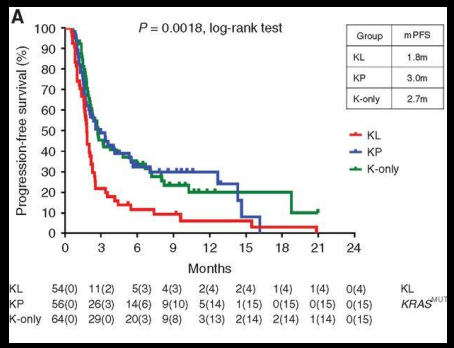
G1202R "solvent front"
adjacent to binding site
alter binding affinity

- Also: ALK amplification, bypass, epigenetics

Influential passengers

- STK11 (aka LKB1):
  - causes Peutz-Jeghers in germline
  - Impairs response to PD1 blockade in KRAS mutant cancer



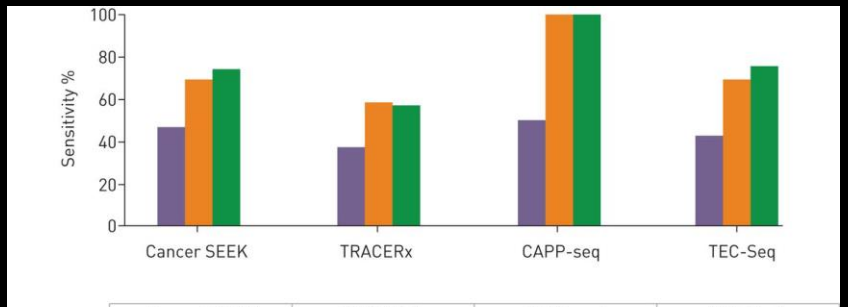


Sikouldis, Cancer Disc, 2018

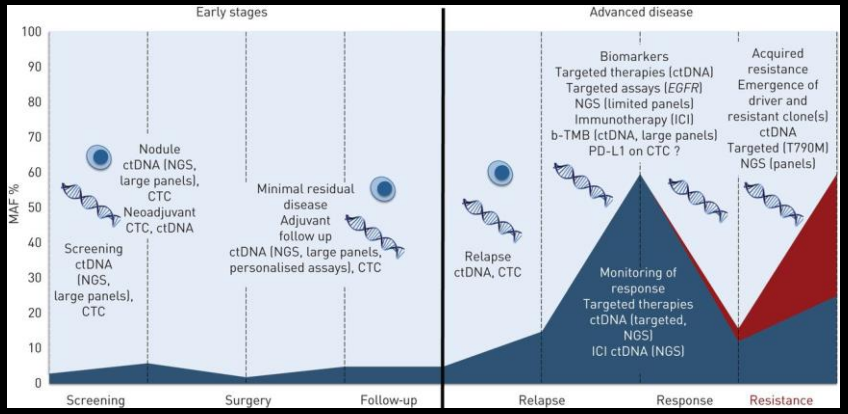
- PIK3CA
  - Often co-exist with other targetable mutations
  - Treatment under investigation

Expansion of "liquid biopsy"

- Early detection
  - Screening?
  - Avoid biopsy?
- Monitoring
  - Indication of response?
  - Resistance mechasnisms?



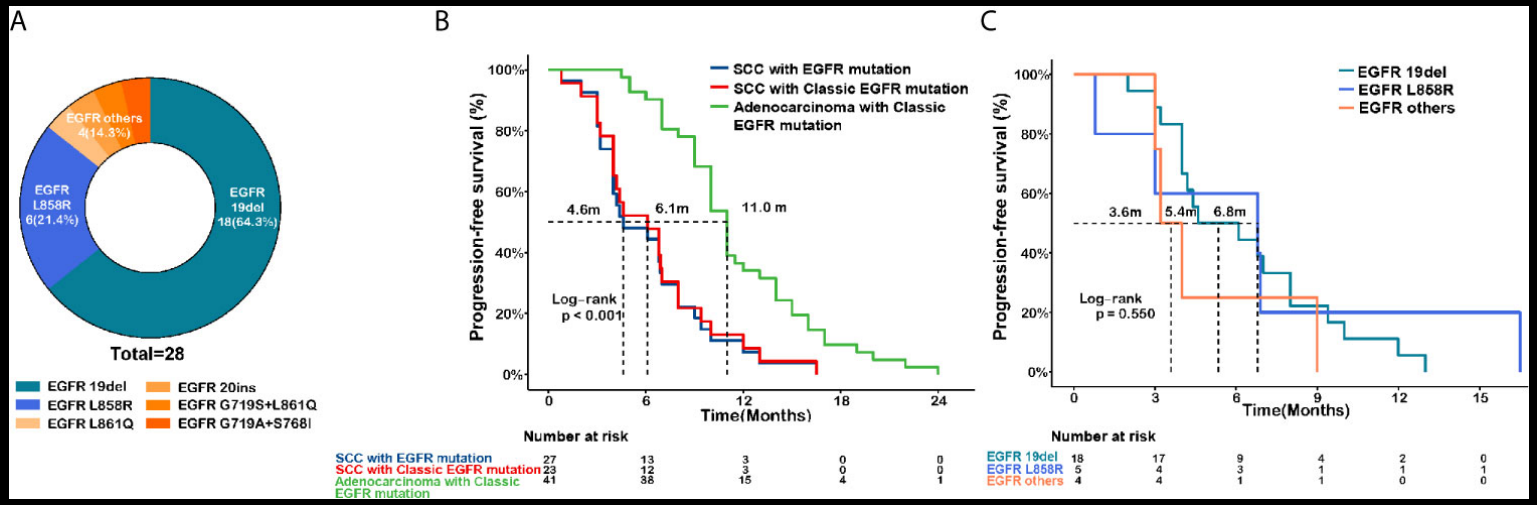
	Cancer SEEK#	TRACERx¶	CAPP-seq*	TEC-Seq§
Stage I	43	37	50	45
Stage II	69	59	100	72
Stage III	74	57	100	75



Guibert, Eur Resp Rev, 2020

Other cancers

- We test for variants in <1% of adenocarcinomas
- EGFR mutation is seen in ~5% of squamous carcinomas
- Not great evidence about treatment response yet



Jin, Front Oncol, 2021