Molecular Diagnostics for Lung Cancer

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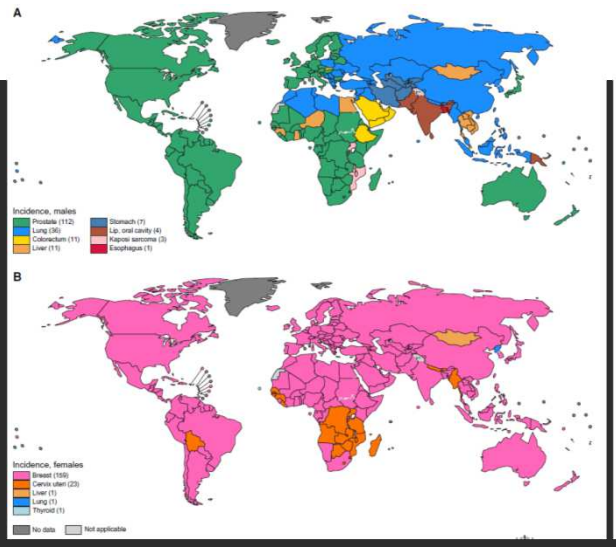
Disclosures

Consultant- GV20 Therapeutics

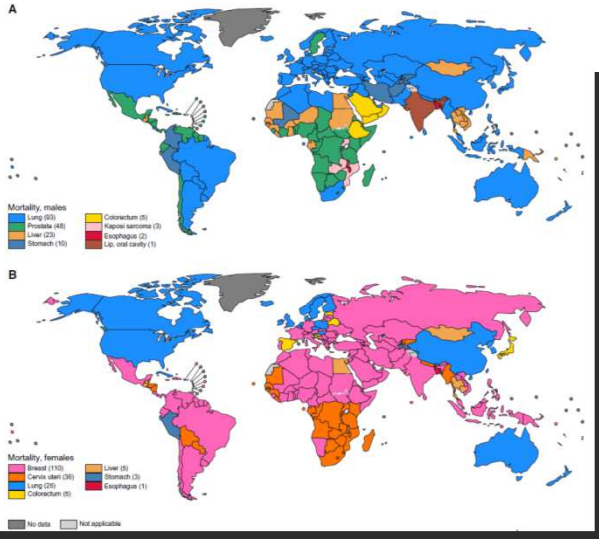
Consulting fees (to my institution) – Genentech, Lilly

Research funding (to my institution) –Genentech

Lung Cancer Incidence

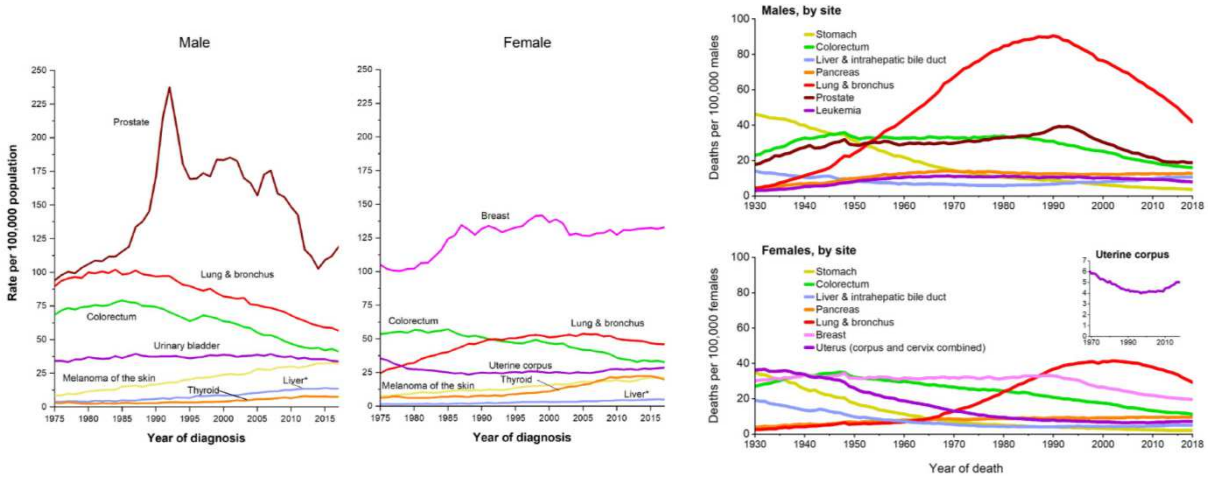


Lung Cancer Mortality



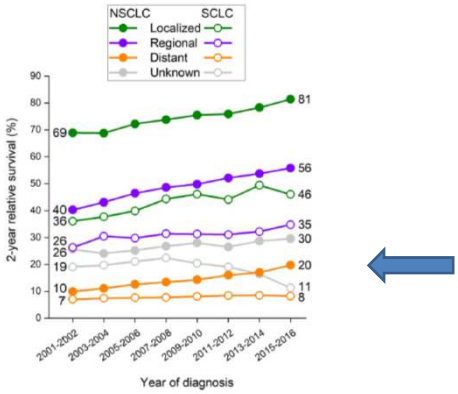
Sung H et al. CA Cancer J Clin. 2021 Feb 4.

North America: Declining lung cancer incidence and death rates



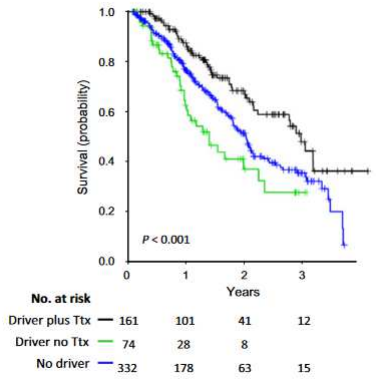
Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021 Jan;71(1):7-33.

Modest improvements in survival for patients with distant spread



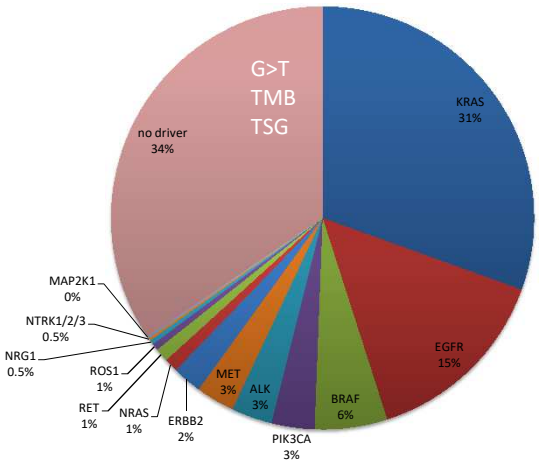
Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021 Jan;71(1):7-33.

Use of appropriate targeted therapy improves survival in metastatic NSCLC



Aisner DL, Sholl LM, Berry LD, et al. Clin Cancer Res. 2018 Mar 1;24(5):1038-1047.

Biomarker-driven Classification:
Non squamous non-small cell carcinoma
(adenocarcinoma + NSCLC, not otherwise specified)

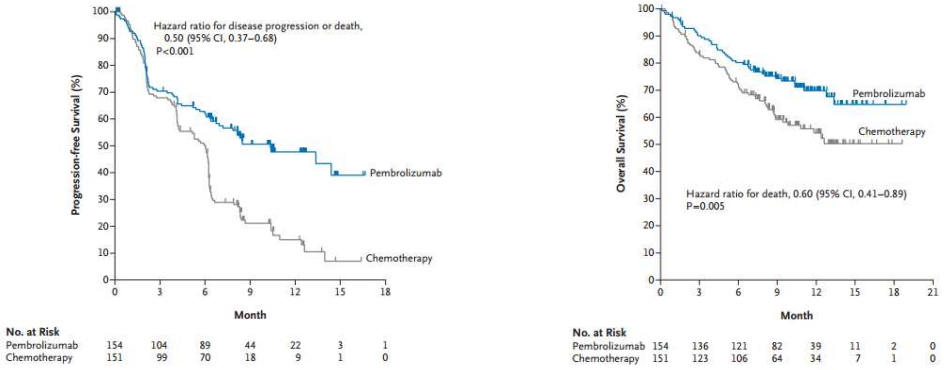


Guide to BIOMARKER-DRIVEN therapies for NSCLC in 2021

Biomarker	Essential	Emerging	Therapies	Recent FDA approvals
EGFR mutations	X		Osimertinib, 1st gen TKIs, Amivantimab*	2020, 2021-breakthrough*
ALK rearrangement	X		Alectinib, Brigatinib, Lorlatinib	2021
ROS1 rearrangement	X		Entrectinib, Crizotinib	2019
RET rearrangement	X		Selpercatinib, Pralesetinib	2020
MET splice mutation	X		Capmatinib, Tepotinib, Crizotinib	2020
BRAF V600E	X		Dabrafanib + Trametinib	2017
NTRK1-3 rearrangement	X		Entrectinib, Larotrectinib	2019
KRAS G12C mutation	X		Adagrasib, Sotorasib*	2021*
PD-L1 expression	X		Pembro., Atezo., Ipilimumab + Nivolumab, Cemiplimab	2021
ERBB2 exon 20 mutation		X	Trastuzumab-deruxtecan	2020-Breakthrough
Tumor mutation burden		X	Pembrolizumab	2020

Pembrolizumab (PD-1 inhibitor) in the first line treatment of NSCLC

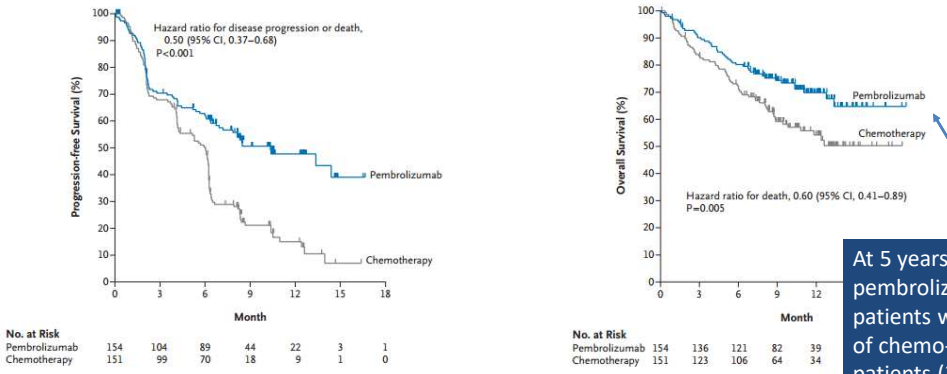
Progression-free and overall survival for tumors with >50% PD-L1 staining



Reck et al. *NEJM* 2016

Pembrolizumab (PD-1 inhibitor) in the first line treatment of NSCLC

Progression-free and overall survival for tumors with >50% PD-L1 staining

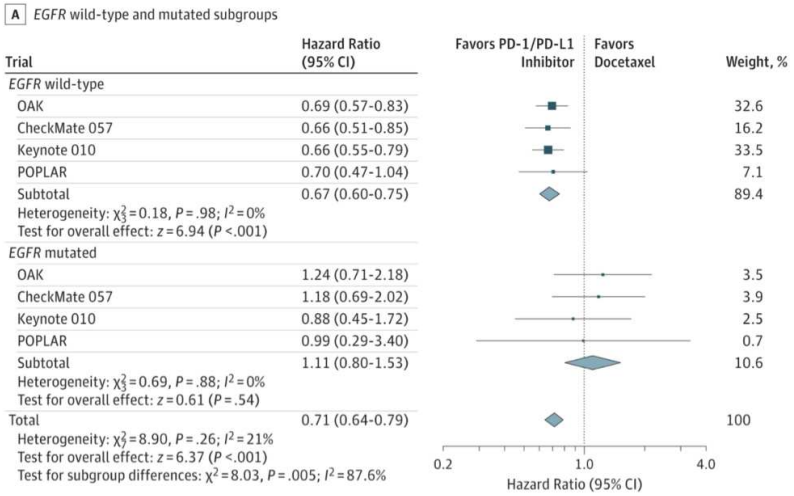


At 5 years, OS rate for pembrolizumab-treated patients was twice that of chemo-treated patients (32 vs 16%).

Reck et al. *NEJM* 2016

Meta-analysis of >2000 clinical trial patients receiving immunotherapy: EGFR-mutant NSCLC do not benefit from these drugs and may be harmed

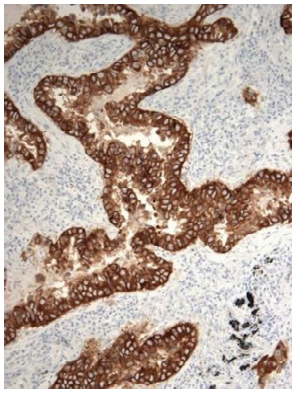
These observations appear to extend to ALK and (maybe) ROS1 patients.



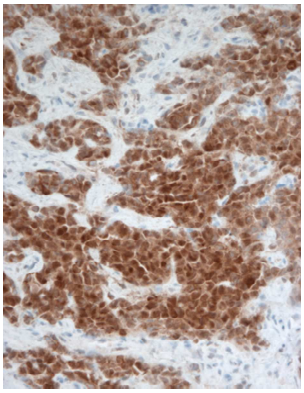
Lee CK, Man J, Lord S, Cooper W, Links M, Gebski V, Herbst RS, Gralla RJ, Mok T, Yang JC. JAMA Oncol. 2018 Feb 1;4(2):210-216.

Gene	Variant type	NGS (DNA)		RNA sequencing	(RT) PCR	IHC	FISH
		Hybrid capture	Amplicon seq				
PD-L1	**expression**					✓	
EGFR	**Mutation, indel**	✓	✓		✓	✓*	
ALK	**Fusion, mutation**	✓		✓	✓	✓	✓
ROS1	**Fusion, mutation**	✓		✓	✓	✓	✓
BRAF	**Substitution**	✓	✓		✓	✓	
MET	**Mutation, indel, amplification**	✓	✓	✓	✓		✓
RET	**Fusion**	✓		✓	✓		✓
TRK	**Fusion, mutation**	✓		✓	✓		✓
KRAS	**G12C mutation**	✓	✓		✓		
ERBB2	**Mutation*, amplification, overexpression**	✓	✓		✓	✓	✓

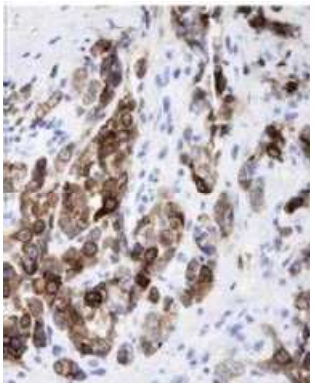
Predictive Immunohistochemistry



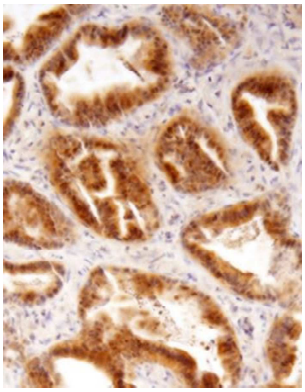
EGFR L858R IHC



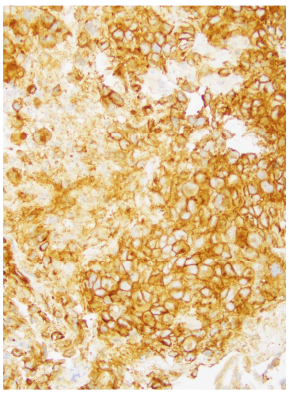
ALK IHC



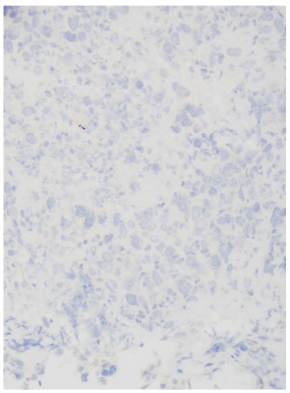
ROS1 IHC



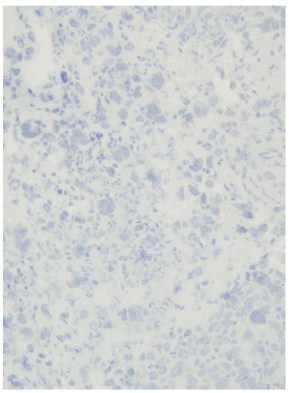
BRAF V600E IHC



PD-L1

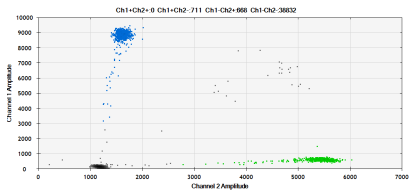


ALK



ROS1

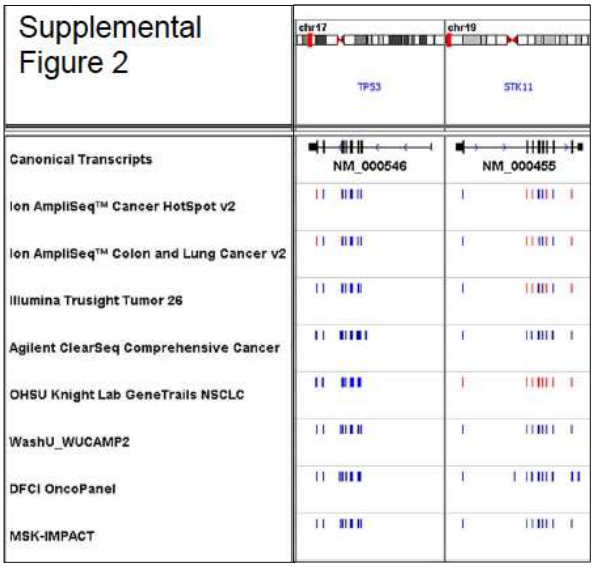
NSCLC biomarker testing→ tricky timing



EGFR mutation

- **1-2 days:**
  - **PD-L1 immunohistochemistry**
  - **ALK, ROS1 immunohistochemistry**
- **< week:**
  - **Rapid EGFR, BRAF mutational profiling**
  - **ALK, ROS1, RET, NTRK FISH**
- **2+ weeks:**
  - **Other molecular biomarkers**

Not all NGS is Created Equal



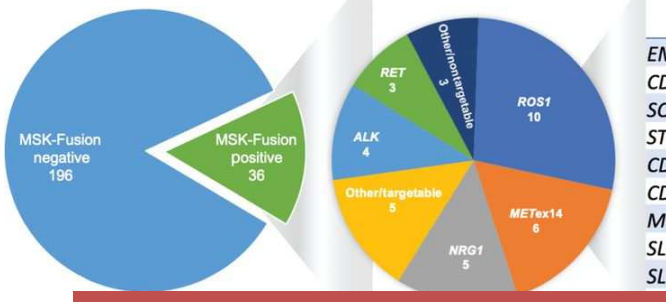
- Focused panels may still have limited gene coverage
- Tumor suppressor genes and genes with diverse mechanisms of activation (e.g. MET) may be under-covered

Aisner DL et al. *Clin Cancer Res.* 2018;24(5):1038-1047.

RNA-Based Amplicon Sequencing to Identify Fusions Missed by DNA Panel NGS

MSK-IMPACT negative (*n* = 232)

MSK-Fusion positive (*n* = 36)



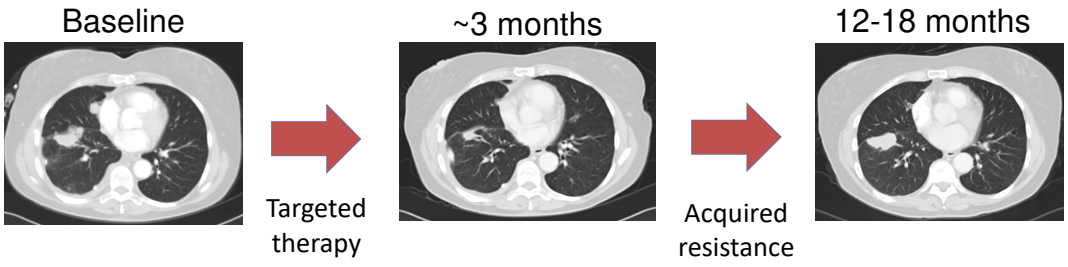
Clinical benefit of matched targeted therapy (*n* = 10)

Rearrangement	Matched therapy	Best response*
EML4-ALK	Alectinib	SD
CD74-ROS1	Entrectinib	SD
SQSTM1-NTRK3	Larotrectinib	PR**
STRN-NTRK2	Larotrectinib	SD
CD74-ROS1	Entrectinib	PR**
CD74-NRG1	Afatanib	SD
MET Exon14 Skipping	Crizotinib	SD
SLC34A2-ROS1	Crizotinib	PD
SLC34A2-ROS1	Crizotinib	SD

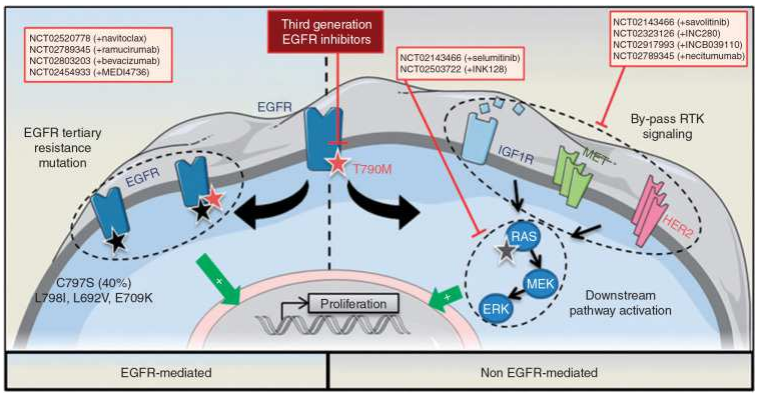
15% of driver-negative NSCLC cases by DNA NGS have fusion detectable by RNAseq.

Benayed R et al. *Clin Cancer Res.* 2019;25(15):4712-4722.

Targeted therapy resistance



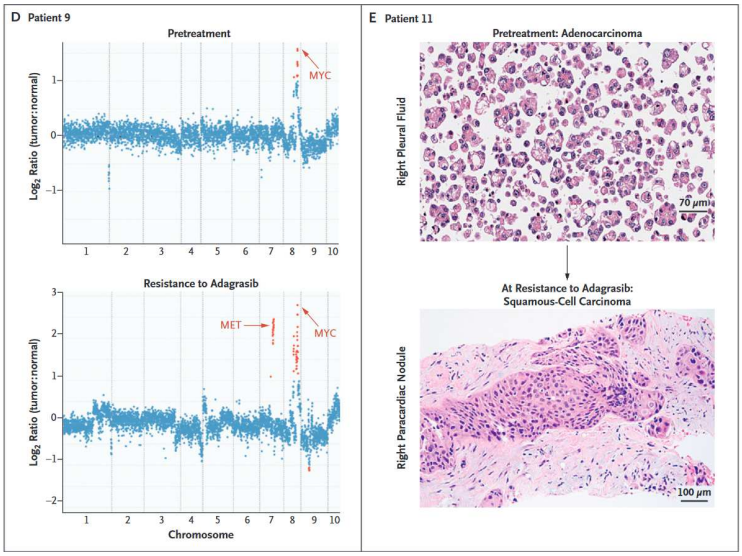
ON Target vs OFF target resistance



Ricordel et al. Ann Oncol. 2019 May 1;30(5):858.

Recurrent off-target mechanisms of resistance

MET amplification

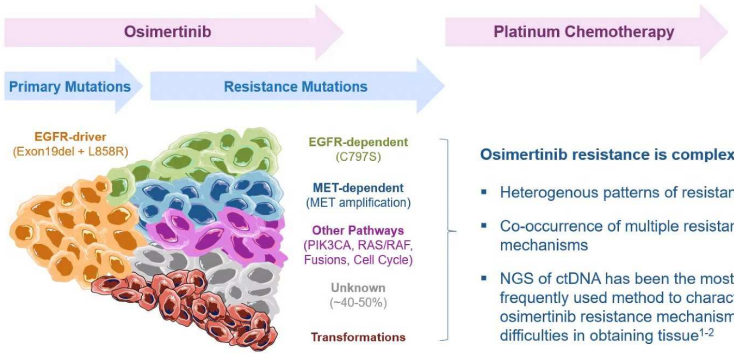


Histologic transformation

Awad et al. N Engl J Med. 2021 Jun 24;384(25):2382-2393.

Molecular profiling to define therapy in the resistance setting

Acquired Resistance to Osimertinib in EGFRm NSCLC



Osimertinib resistance is complex

- Heterogenous patterns of resistance
- Co-occurrence of multiple resistance mechanisms
- NGS of ctDNA has been the most frequently used method to characterize osimertinib resistance mechanisms due to difficulties in obtaining tissue[1-2]

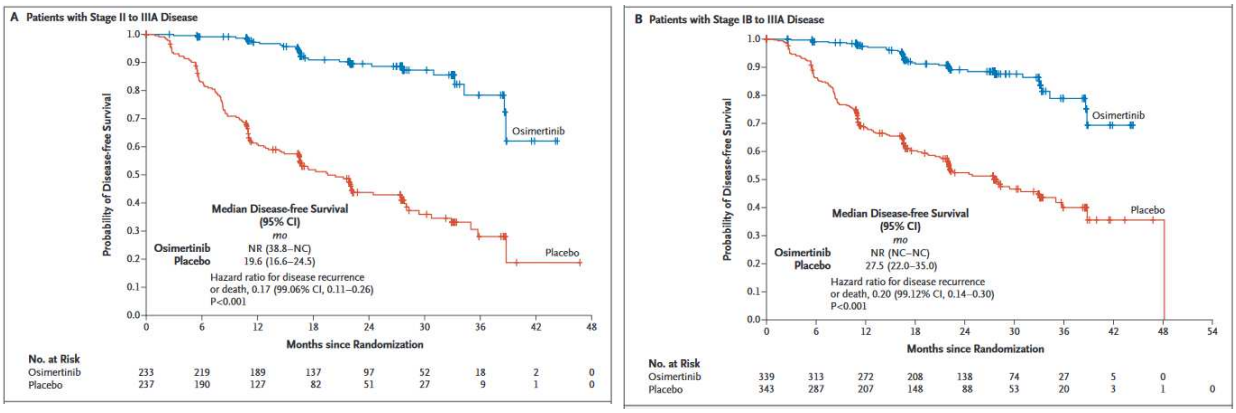
Cho BC. 2021 ASCO Annual Meeting

Biomarker testing in early stage NSCLC?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Osimertinib in Resected *EGFR*-Mutated Non–Small-Cell Lung Cancer



Led to 2020 FDA approval of Osimertinib in the adjuvant setting for clinical stage IB-IIIA NSCLC with EGFR L858R or ex19del mutations.

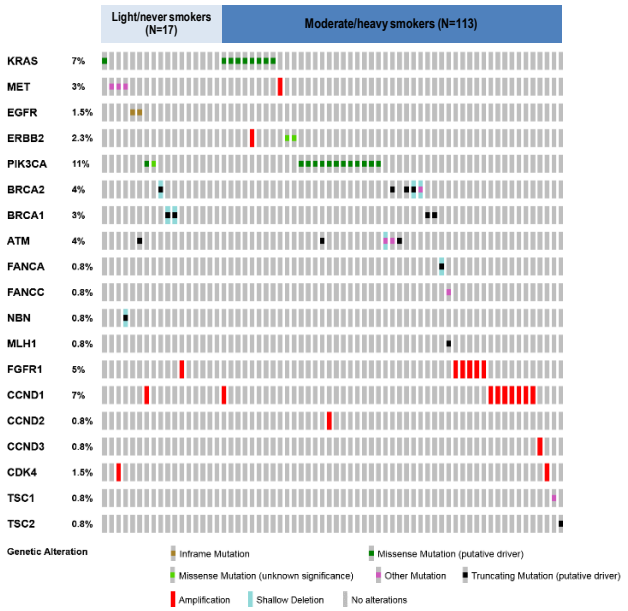
Role for biomarker testing outside of lung adenocarcinoma and NSCLC-NOS?

Biomarker testing in squamous cell carcinoma patients?

Among light/never smokers:
47% have a targetable biomarker

18% have BRCA1/2 loss of function
- At least 2 patients with germline BRCA mutations

35% have a revised diagnosis
- Metastases from skin, mesothelioma



Sands et al. *Lung Cancer.* 2020;140:35-41.

Biomarker testing in small cell lung carcinoma?

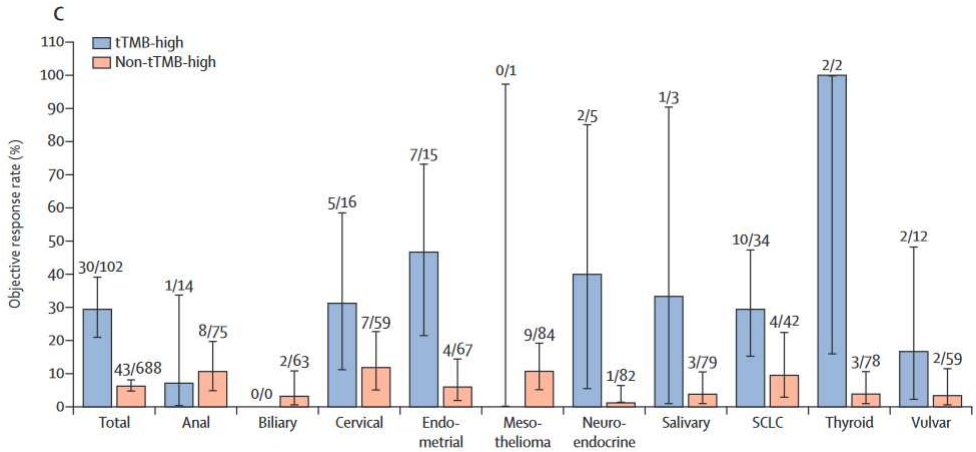
Patient	Age	Sex	Smoking (pack-years)	Original diagnosis	Key genomic findings	RB1/TP53 status	Revised diagnosis
1	68	F	0	SCLC	ATM mutation	RB1 WT/TP53 WT	Combined LCNEC and SCLC
2	50	F	0	SCLC	MET amplification	RB1 WT/TP53 WT	Combined LCNEC and SCLC
3	60	M	0	SCLC	BRCA1 mutation	RB1 WT/TP53 WT	Neuroendocrine tumor grade 2/atypical carcinoid, pancreas or lung primary
4	52	F	0	SCLC	KRAS mutation	RB1 mt/TP53 mt	Small cell of unknown primary hepatobiliary origin suspected
5	70	M	0	SCLC		RB1 mt/TP53 mt	SCLC
6	59	F	0	SCLC		RB1 WT/TP53 WT	SCLC
7	50	M	0	Poorly differentiated carcinoma, favor SCLC	NRAS mutation	RB1 WT/TP53 WT	Poorly differentiated carcinoma
8	47	F	2	SCLC	EGFR mutation	RB1 WT/TP53 WT	NSCLC undergoing de novo SCLC transformation
9	51	F	4	SCLC		RB1 mt/TP53 mt	SCLC
10	62	F	7	SCLC	EGFR mutation	RB1 WT/TP53 mt	NSCLC undergoing de novo SCLC transformation
11	76	M	10	SCLC	TMPRSS2-ERG fusion	RB1 mt/TP53 mt	Metastatic prostate cancer, with small-cell transformation

Ogino et al. Mol Oncol. 2021 Jan;15(1):27-42.

Genomic biomarkers of response to immunotherapy

- Tumor mutation burden
- Co-mutations
  - STK11
  - KEAP1
  - SWI/SNF complex members
    - SMARCA4
    - ARID genes

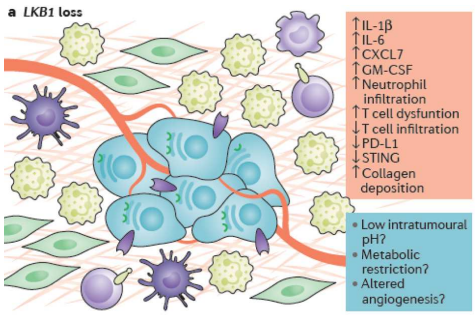
Keynote -158: basis of pan-cancer FDA approval of TMB as a biomarker for pembrolizumab therapy

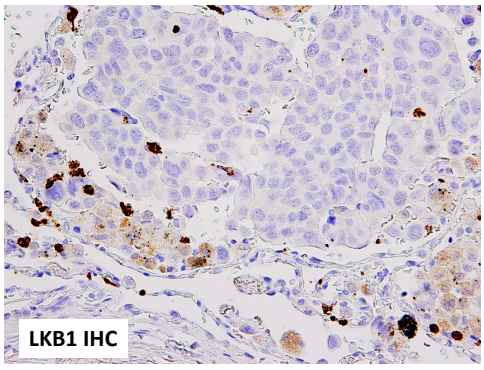


Marabelle A et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020 Oct;21(10):1353-1365.

STK11 (LKB1) and *KEAP1* mutations

- In *KRAS*-mutated NSCLC, *STK11* co-mutation correlates with poor response rates following immunotherapy
- Possibly due to exclusion of effector T cells from tumor compartment
- Multicohort retrospective analyses, no prospective confirmation to date
- *KEAP1* mutations are poor prognostic factors- not clearly independently predictive

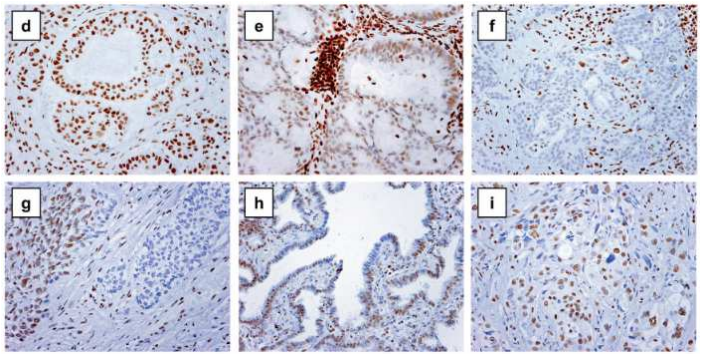




Skoulidis and Heymach. Nat Rev Cancer. 2019 Sep; 19(9): 495–509.
Skoulidis et al. Cancer Discov. 2018 Jul;8(7):822-835
Mitchell KG et al. Ann Thorac Surg. 2020 Oct;110(4):1131-1138.
Singh et al. Clin Canc Res. 2021;27(3):1362-1368.

ARID1A alterations – Biomarkers of immunotherapy response?

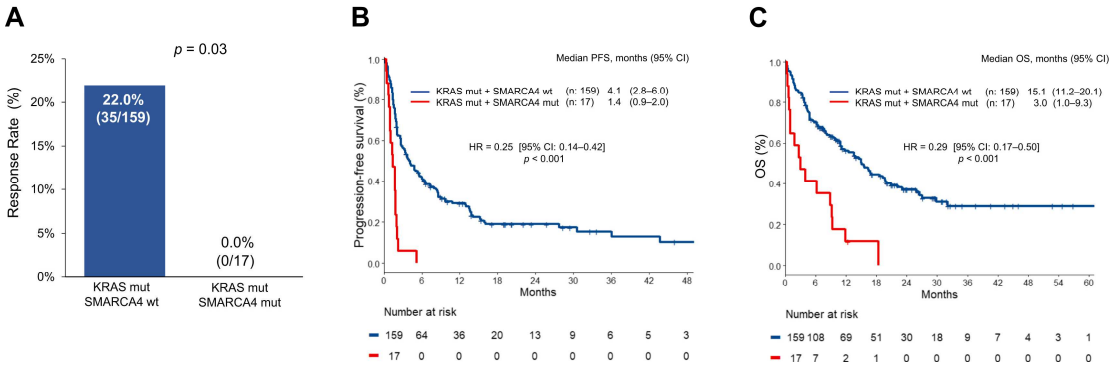
- An independent predictor of response to PD-1/PD-L1 inhibitor therapy?
- Mechanistically, ARID1A deficiency may contribute to impaired mismatch repair and mutator phenotype
- However, many ARID1A mutations detected by sequencing do not lead to protein deficiency
- ARID1A expression can be heterogeneous even in the setting of loss of function alterations
- In one study, ARID1A mutations predicted increased response rates, but no PFS or OS benefit.



Okamura R et al. J ImmunoTher Cancer. 2020;8:e000438
Shen et al. Nat Med. May 2018;24:556-562.
Anagnostou V. Nat Cancer. 2020 Jan;1(1):99-111.
Alessi et al. J Thorac Oncol. 2021 Jul;16(7):1176-1187.

Hung YP et al. Mod Pathol. 2020 Nov;33(11):2256-2268.

SMARCA4 co-mutation is a negative predictor of immunotherapy response in KRAS+ NSCLC



Alessi et al. J Thorac Oncol. 2021 Jul;16(7):1176-1187.

Take home points

- Declines in lung cancer deaths over the last decade likely multifactorial→ decreased smoking, improved screening, improved treatments for patients with advanced disease
- For patients with metastatic NSCLC, a therapeutic dichotomy?
  - Immunotherapy vs genomic biomarker-driven targeted therapy
- Access to rapid and comprehensive testing strategies is key to treatment selection in first line and recurrence setting