

Multidisciplinary Approaches to Cancer Symposium

Who, How, When?

Personalized Pathology for Genomics Classification in Lung Cancer

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City of Hope Comprehensive Cancer Center

Disclosures

• I do not have any relevant financial relationships.

This presentation and/or comments will provide a balanced, non-promotional, and evidence-based approach to all diagnostic, therapeutic and/or research related content.

Cultural Linguistic Competency (CLC) & Implicit Bias (IB)

STATE LAW:

The California legislature has passed <u>Assembly Bill (AB) 1195</u>, which states that as of July 1, 2006, all Category 1 CME activities that relate to patient care must include a cultural diversity/linguistics component. It has also passed <u>AB 241</u>, which states that as of January 1, 2022, all continuing education courses for a physician and surgeon **must** contain curriculum that includes specified instruction in the understanding of implicit bias in medical treatment.

The cultural and linguistic competency (CLC) and implicit bias (IB) definitions reiterate how patients' diverse backgrounds may impact their access to care.

EXEMPTION:

Business and Professions Code 2190.1 exempts activities which are dedicated solely to research or other issues that do not contain a direct patient care component.

This presentation is dedicated solely to research or other issues that do not contain a direct patient care component.

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What's biomarker testing

Molecular genomic and cytogenomics analysis for specific gene changes in the cancer cells that could mean certain targeted drugs might help treat the cancer.

- Immunohistochemistry study
- Cytogenetics and FISH analysis. (FISH is a technique that allows DNA sequences to be detected on metaphase chromosome or interphase nuclei from fixed cytogenetics samples)
- Targeted molecular assay (NGS, sanger, RT-PCR or PCR)
- Whole Genome array (OncoScan & CytoScan)
- Whole Genome Sequencing

The American Cancer Society's estimates for cancer in the United States for 2025

2025 Estimated New Cancer Cases

Cases by Cancer Type

Lung & bronchus

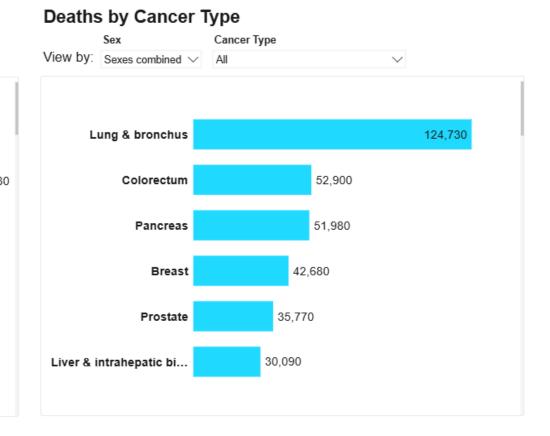
Skin (excluding basal ...

Colorectum

Colon

View by: Sexes combined ✓ All ✓ Breast 319,750 Prostate 313,780

2025 Estimated Cancer Deaths



https://www.cancer.org/cancer/lung-cancer/about/key-statistics.html

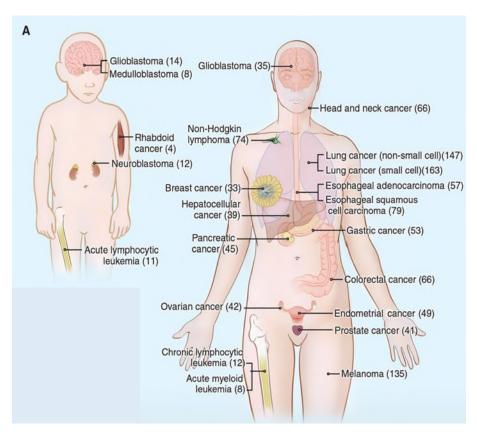
112,690

107,320

226,650

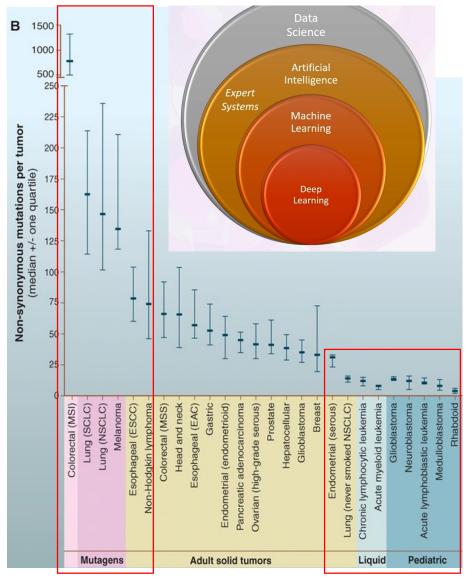
154,270

Genomic Landscapes of Common Human Cancers



Average 33-66 genes mutated

- >90% missense mutations
- 5-8% nonsense changes
- <2-5% splice site or UTR'or fusions

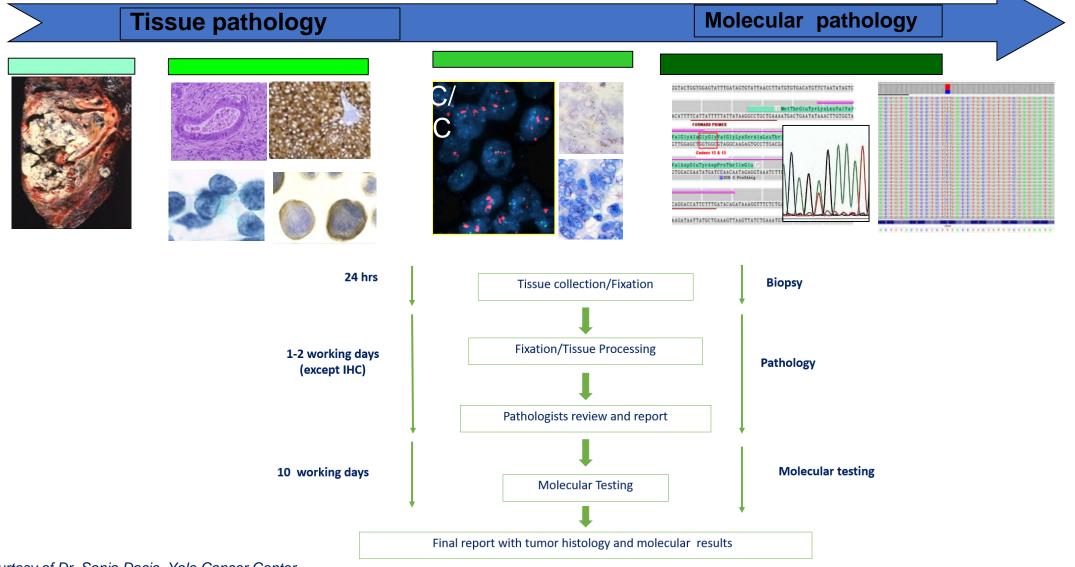


Bert Vogelstein et al. Science 2013;339:1546-1558

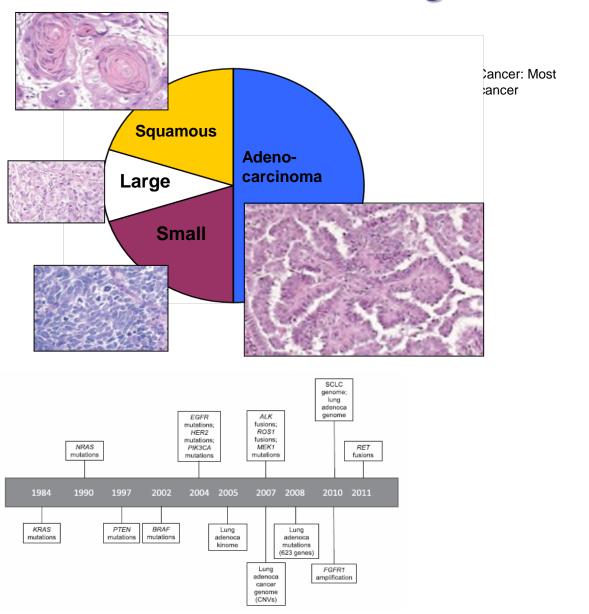
Who Should be tested?

- Advanced stage lung adenocarcinoma (stages IIIB and IV)
- By morphology:
- Adenocarcinoma or any tumors with adenocarcinoma component
- Other biopsies with suspicious of presence of adenocarcinoma component
- Squamous cell carcinoma, when clinically indicated such as younger patient or never smokers who have higher possibility of having oncogenic drivers

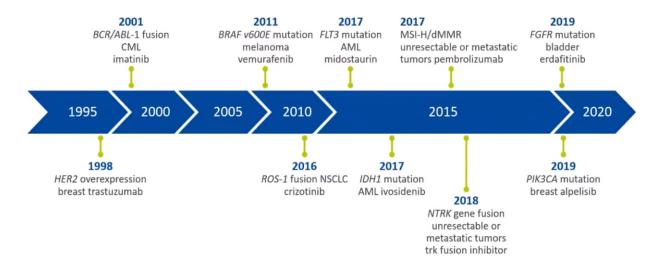
Modern Pathology Phenotype and Genotype are Complementary



Traditional View of Lung Cancers



Evolution of target therapies

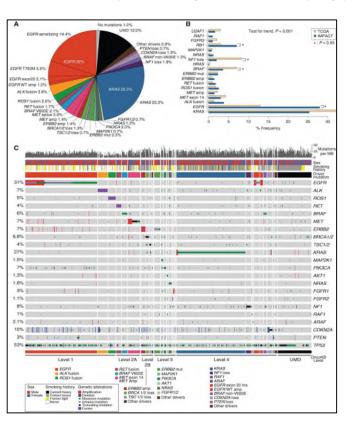


KRAS G12C and EGFR make up ~ 75% of all actionable driver mutations in NSCLC (Analysis of AACR Genie v8 2022)

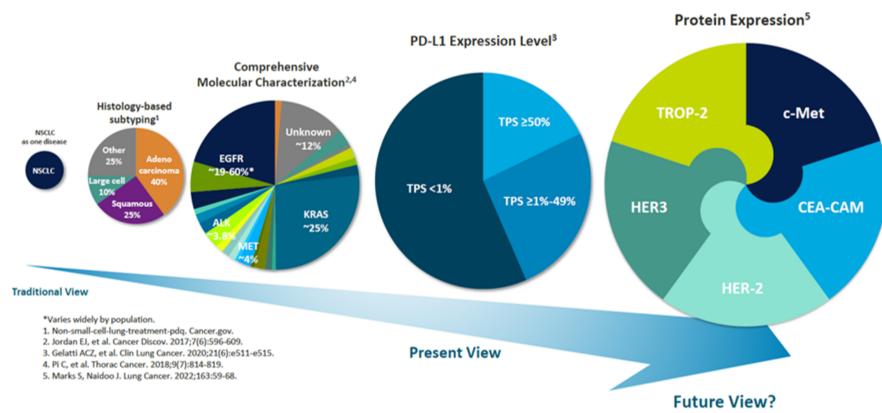
Molecular Classification of Lung Cancers

Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies *⊗*

Emmet J. Jordan; Hyunjae R. Kim; Maria E. Arcila; David Barron; Debyani Chakravarty; JianJiong Gao; Matthew T. Chang; Andy Ni; Ritika Kundra; Philip Jonsson; Gowtham Jayakumaran; Sizhi Paul Gao; Hannah C. Johnsen; Aphrothiti J. Hanrahan; Ahmet Zehir; Natasha Rekhtman; Michelle S. Ginsberg; Bob T. Li; Helena A. Yu; Paul K. Paik; Alexander Drilon; Matthew D. Hellmann, Dalicia N. Reales; Ryma Benayed; Valerie W. Rusch; Mark G. Kris; Jamie E. Chaft; José Baselga; Barry S. Taylor; Nikolaus Schultz; Charles M. Rudin; David M. Hyman; Michael F. Berger; David B. Solit; Marc Ladanyi; Gregory J. Riely ■



Current and Future View of Lung Cancers: Many opportunities for personalized approach in treatment.



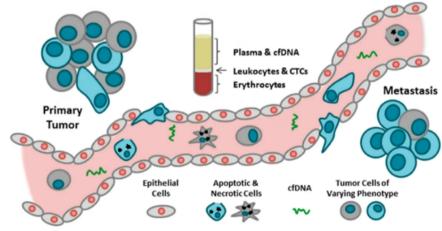
How to Test?

	Tissue-based NGS	ctDNA-based NGS (liquid biopsy)
Nature of procedure	Invasive, requires a biopsy	✓ Minimally invasive
Turnaround time	Longer	✓ Shorter (7 – 14 days)
Source of molecular alterations	Biopsied tissue	✓ Better captures intratumoral heterogeneity and clonal evolution
Sensitivity	✓ Higher sensitivity.	Limited sensitivity, dependent on amount pf DNA shedded into bloodstream Negative results require confirmation by tissue-based testing
Concurrent PD-L1 testing	✓ Concurrent PD-L1 testing possible	Concurrent PD-L1 testing not possible
Testing for acquired resistance mechanism	May document histologic transformation	May detect various resistance mechanisms from multiple clones

- Not all tumors shed ctDNA and may have negative plasma result
 - Tumor size, location
 - Tumor vascularity, metastasis
 - Prior treatment
- How to handle a negative result on liquid biopsy with high suspicion of a resistance mechanism? **Test tissue!**

Blood sample containing cell-free DNA from multiple sources, shed from tumor

1-5 mutant tumor DNA fragments in 10,000+ self DNA fragments*



Lowes. Int J Mol Sci. 2016:17:E1505. Figure 1 of given citation is used in its original form under the terms and conditions of the Creative



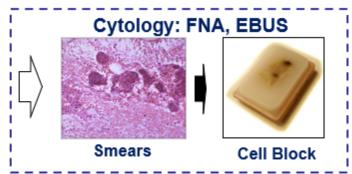
City of Hope Practice: HopeSeq Comprehensive Assays Diagnostic Algorithm for Lung Cancer Diagnosis 2025

FFPE of the cytology cell block or core or resection biopsies or 40 ng of DNA and 40ng of RNA



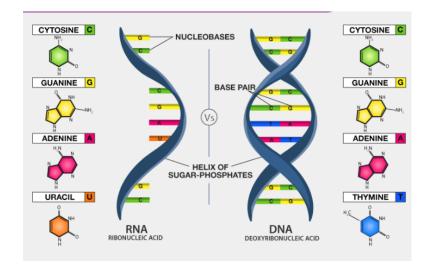








Resection





Comprehensive NGS assay with combined RNA and DNA Seq

- Pre-Analytics Workflow Billing and authorization, Pathologist review, Order and circle best tumor area
- Analytics Workflow (wet lab) RNA and DNA extraction and Sequencing
- Post-Analytics workflow Bioinformatics, Curation, and Pathologists review and sign out





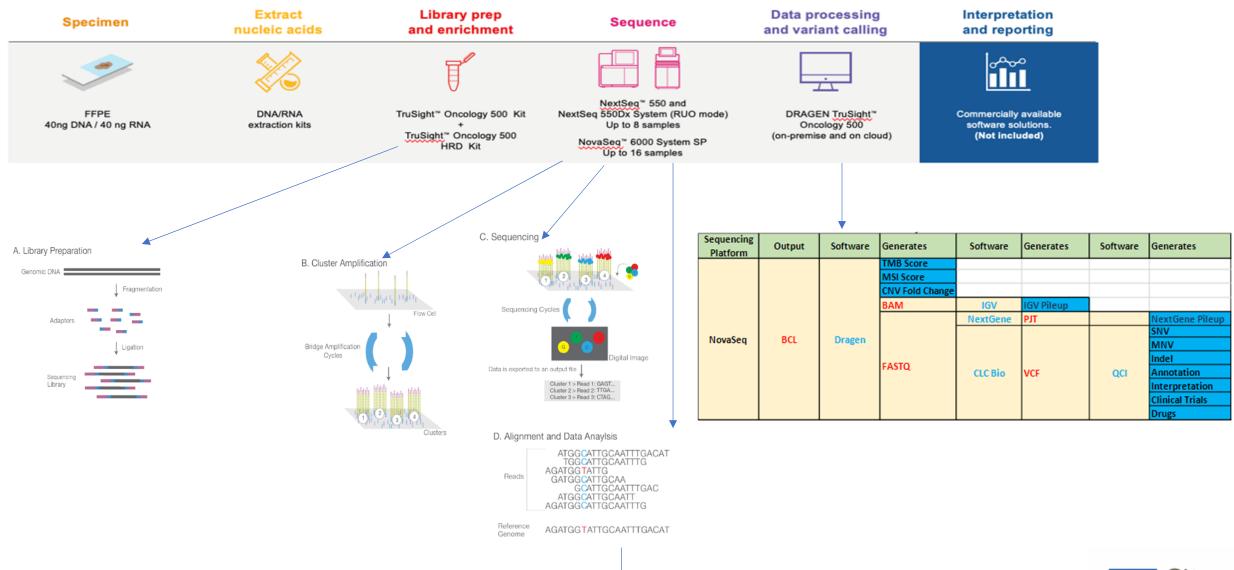
CITY OF HOPE



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HopeSeq Expansion M. Afkhami

HopeSeq Comprehensive NGS assay workflow

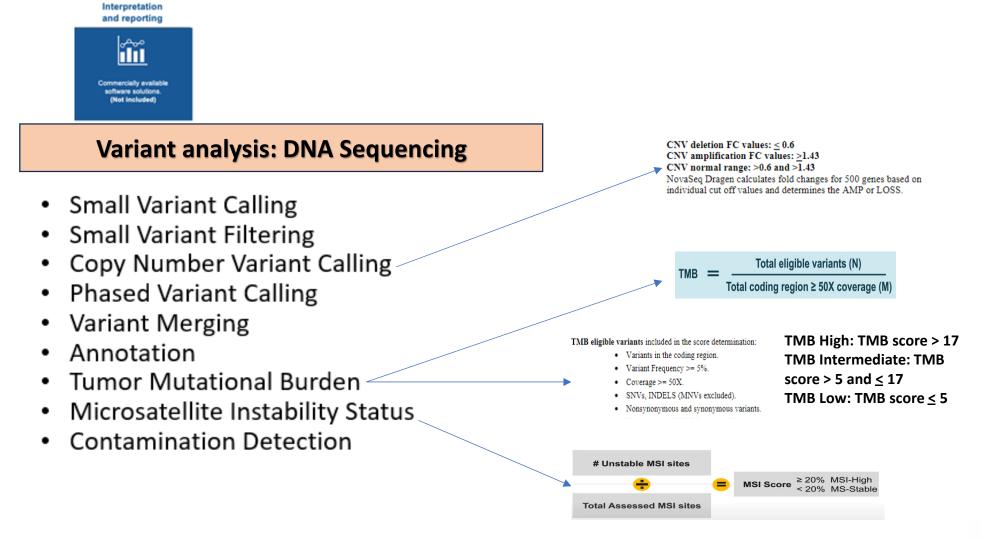


M. Afkhami, MD unpublished

- DNA Alignment and Realignment
- Read Collapsing
- Indel Realignment and Read Stitching

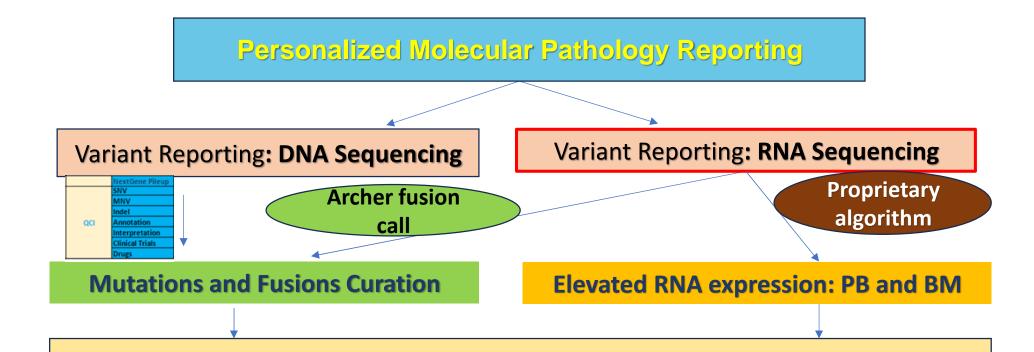


HopeSeq Comprehensive NGS assay-COH





HopeSeq Comprehensive NGS assay-COH



- 1. Variants reviewed after bioinformatics filtering by HopeSeq curation team
- 2. Negative and Positive variants are reviewed by Molecular Pathologists in daily GTB
- 3. Complete chart review is done
- 4. Every Case reviewed by concurrent flow cytometry, BM report, or Surgical path and IHC, liquid biopsy results, and cytogenetics/FISH results
- 5. Every post therapy NGS results compared with previous HopeSeq results

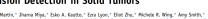


How to Test? Predictive IHCs

Targets	Clones	Sensitivity	Specificity	Recommendation
EGFR	EGFR-L858R EGFR-E746	70-90%	95-97%	Not recommended
ALK	₽D5F3	97-99%	98-100%	FDA-approved CDx assay
ROS1	D4D6	100%	92%	Useful for screening
BRAF1	VE1	90-97%	99-100%	May be useful
Pan-NTRK	EPR17341	95-97%	98-100%	More studies needed
MET (for MET ex14)	SP44	64-90%	47-83%	Not recommended
RET	EPR2871	87%	82%	More studies needed

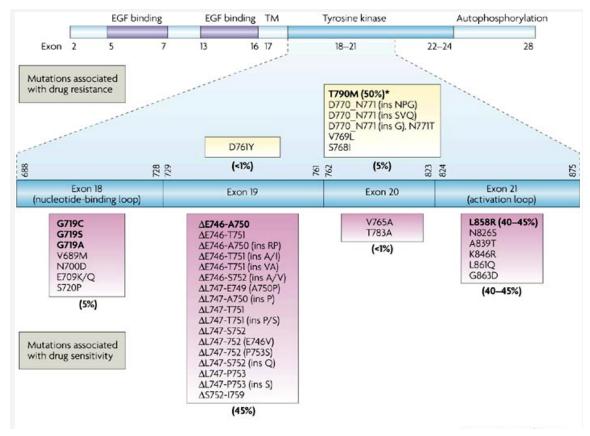
	NGS ²⁻⁸	DNA FISH ^{5,9-14}	IHC ^{4-6,8}	
Strengths {	 Allows for efficient multiplex testing with the ability to find NTRK gene fusions and identify multiple genomic targets Comprehensive: Can identify the exact fusion partner 	 Well suited to identifying fusions There is utility in using FISH in diseases such as IFS, where the predominant driver for IFS is ETV6-NTRK3 	 IHC used in a majority of labs Research-use-only TRK antibodies Ventana is commercially available; pan-TRK in vitro diagnostic under development 	
Considerations 	 Large tissue sample required Review and perform bioinformatic data analyses of prior NGS results to uncover NTRK gene fusions (provided panel included coverage) 	 Not designed for multiplexing In order to detect fusions at multiple locations (such as the 3 NTRK genes), multiple FISH tests would be needed Must use break apart methodology to identify novel fusions 	 Single-plex (additional genetic targets require additional samples) Protein expression may not always indicate a fusion; confirmation of NTRK gene fusion via additional testing is required treatment 	
Turnaround Time	14 days (for the majority of labs)	1-14 days	1-14 days	

Validation of a Targeted RNA Sequencing Assay for Kinase Fusion Detection in Solid Tumors





EGFR mutations



Testing for *EGFR* Mutations

- EGFR Tyrosine Kinase domain mutations (exons 18 to 21) = most reliable predictors of response to EGFR TKI
- Must be able to detect mutations in samples with 20% tumor purity
- NGS, Sanger, PCR all acceptable
- Methods not recommended:
 - FISH for EGFR amplification
 - Total EGFR expression by IHC
 - EGFR mutation-specific IHC



EGFR codon			Approximate % of
	(amino acid)	substitutions	all EGFR mutations
E709	E709K	c.2125G>A	1%
	E709A	c.2126A>C	
	E709G	c.2126A>G	
	E709V	c.2126A>T	
	E709D	c.2127A>C, c.2127A>T	
	E709Q	c.2125G>C	
G719	G7195	c.2155G>A	2-5%
	G719A	c.2156G>C	
	G719C	c.2155G>T	
	G719D	c-2156G>A	
K739, I740, P741,	Insertions		1%
V742, A743, 1744	18 bp ins	_	
E746, L747, R748,	Deletions		45%
E749, A750, T751,	15bp del		
5752, P753	18bp del		
	9 bp del		
	24bp del		
	12bp del		
A763, A767, S768,	Insertions		5-10%
V769, D770, N771,	3 bp ins		
P772, H773, V774	6 bp Ins		
	9 bp ins		
	12 bp ins		
\$768	S768I		1-2%
T790	T790M	c.2369C>T	25/9
L858	L858R	c.2573T>G	40%
	L858M	c.2572C>A (rare)	
L861	L861Q	c.2582T>A,	2-5%
	L861R	c.2582T>G	
	E709 E736, 1740, P741, V742, A743, 1744 E746, 1747, R748, E749, A750, 1751, S752, P753 A763, A767, S766, V770, M771, P772, H773, V774 S766 LE58	(emino acid)	Common acids

EGFR mutations enriched in Women

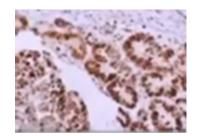
East Asians

Never smokers



Fusion testing

- Immunohistochemistry study
 - May be used as screening tool prior to confirming positive testing
 - Fast TAT
 - Low cost
 - Not sensitive or specific for most of the NTRK and RET fusions



- DNA Fluorescence in situ hybridization (FISH)
 - Not available for all partner detection
 - Costly
 - TAT 5-7 days
- Next Generation Sequencing (NGS)
 - NGS is the preferable method in detection of fusions as it identifies the causative driver and partner in a single test saving in both TAT and cost

	IHC	FISH	DNA NGS	RNA NGS
Pros	✓ Potential local implementation	✓ Potential local implementation	✓ Simultaneously get mutation information	✓ Obtain fusion partner information without intron coverage issues directly assess fusion expression
Cons	 Significant false neg, pos except for ALK 	False neg, posTissue utilization	 Poor coverage of some intronic areas due to repetitive sequences leads to reduced sensitivity 	





	ALK	ROS1	RET	NTRK1,2,3	NRG1	FGFR1,2,3	ALK Fusions
Prevalence	~5%	~1%	1-2%			<1%	 First generation Crizotinib Second generation CeritinibAlectinibBrigatinib Third generation
Clinical features	Young, never to light smokers	Young, never to light smokers	Young, never to light smokers				
Common	RNAseq/	RNA	RNA	RNA seq/NGS,	RNA	RNA	 Lorlatinib Solomon, JCO 202
Testing Methods	NGS, FISH,	seq/NGS, FISH	seq/NGS, FISH	FISH, Pan TRK IHC	seq/NGS , FISH	seq/NGS, FISH	ROS1 Fusions
	ALK- D5F3 IHC				,		First generationCrizotinibEntrectinib
FDA approved Therapies	Yes	Yes	Yes	Yes	Yes	Yes	Second generation Repotrectinib
resistance r mechanism s	stance mutation mutation		Point	NK	NK	NK	RET Fusions
		mutations, MET				Efficacy of Selpercatinib in RET Fusion-Positive Non-Small-Cell Lung Cancer. Drilon A et al. N Engl J PMID: Med. 2020 32846060	
		n amplifica	amplificatio n				Selpercatinib in Patients With RET Fusion-Positive Non-Small-Cell Lung Cancer: Updated Safety and Efficacy
ouMIRACLE USCIENCE OSSOUL	From the Registrational LIBRETTO-001 Phase I/II Trial. Orilon A et al. J Clin Oncol. PMID:						

- Pralsetinib

- RR 84% vs 65%
- PFS 24.8m vs 11.2m (HR 0.46)
- 2023

Solomon, JCO 2024

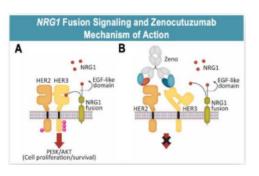
36122315

NTRK1,2,3 Fusions

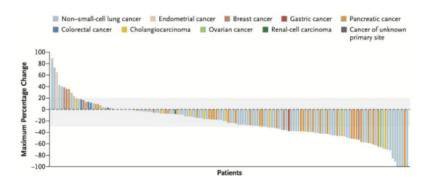
- <1% of lung adenocarcinoma</p>
- Common testing methods
 - NGS and FISH
- FDA approvals for all solid tumors harboring NTRK gene fusions
 - Larotrectinib (Nov 2018)
 - Entrectinib (Aug 2019)
- Second generation
 - Repotrectinib

NRG1 Fusions

- NRG fusions present in <1% of NSCLC
- RNA-sequencing
- First approved agent
 - Zenocutuzumab



- Phase II eNRGy1 trial
 - Zenocutuzumab in NRG1+ tumors
 - RR 29%, DOR 11.1m, PFS 6.8m



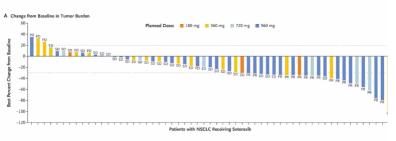


KRAS G12C Mutation

- 13% of lung adenocarcinoma
 - More common in former/current smokers
- Testing methods:
 - NGS, PCR
- Targeted therapies:
 - Sotorasib (FDA approval June 2021)
 - Adagrasib (FDA breakthrough Designation)

KRAS^{G12C} Inhibition with Sotorasib in Advanced Solid Tumors

D.S. Hong, M.G. Fakih, J.H. Strickler, J. Desai, G.A. Durm, G.I. Shapiro, G.S. Falchook, T.J. Price, A. Sacher, C.S. Denlinger, Y.-J. Bang, G.K. Dy, J.C. Krauss, Y. Kuboki, J.C. Kuo, A.L. Coveler, K. Park, T.W. Kim, F. Barlesi, P.N. Munster, S.S. Ramalingam, T.F. Burns, F. Meric-Bernstam, H. Henary, J. Ngang, G. Ngarmchamnanrith, J. Kim, B.E. Houk, J. Canon, J.R. Lipford, G. Friberg, P. Lito, R. Govindan, and B.T. Li



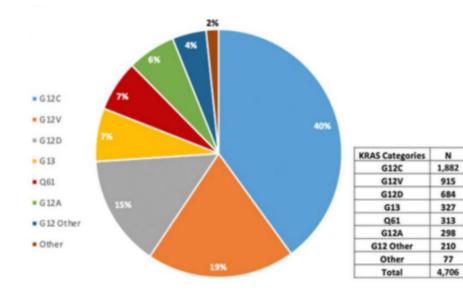
Hong et al. NEJM. 2020.

KRAS G12C inhibitors for previously treated NSCLC

First generation

- Sotorasib
- Adagrasib

- Phase III CodeBreaK200
 - Sotorasib vs 2L docetaxel
 - PFS 5.6m vs 4.5m, HR 0.66
 - No significant difference in OS



Second generation inhibitor

- Olomorasib, LOXO-RAS-20001 ph 1/2 trial
- NSCLC previously treated with KRAS G12Ci

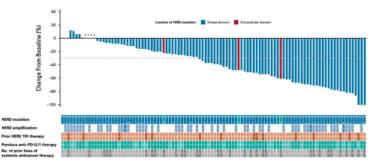
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MET exon 14 Mutations

- 3-4% of NSCLC
 - · Adenocarcinoma,
 - Squamous cell carcinoma (rarely),
 - Sarcomatoid/pleomorphic carcinoma (9-22%)
- FDA-approved therapies:
 - Capmatinib (May 2020)
 - Tepotinib (Feb 2021)
- Testing methods:
 - DNA sequencing
 - RNA sequencing (confirms absence of exon 14)
 - MET IHC is not recommended

HER2 Mutations

- HER2 mutations present in ~2% of NSCLC
- First approved agent
- Phase II DESTINY-Lung0.
- Trastuzumab deruxtecan
- T-DXd at 5.4mg/kg q3w
- RR 49%, mDOR 16.8m

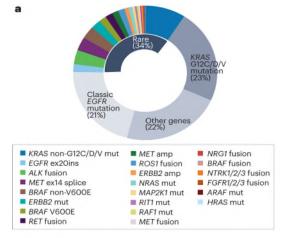


BRAF V600E Mutation

- BRAF mutations seen in 2-3% of NSCLC
 - BRAF V600E seen in 1-2%
 - · Strong association with smoking
- Dabrafenib + trametinib FDA approved for NSCLC harboring BRAF V600E (June 2017)
- Phase II PHAROS trial
 - Encorafenib + binimetinib

Less commonly recognized subtypes:

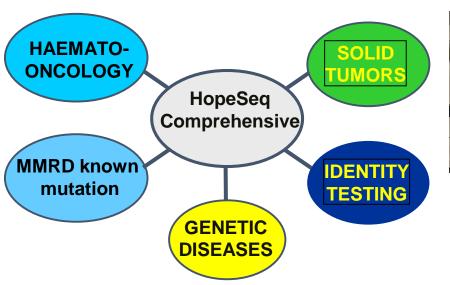
• KRAS non-G12C, HRAS, NRAS, DDR2, LTK, RIT1, ARAF, non-BRAF^{V600E}, RAF1, and MAP2K1.





Molecular Pathology and Therapy Biomarker Division Technologies:

Clinical Molecular Genomics and Cytogenomics Laboratory with Annual volume 2024 of 35,000 Molecular and 15,000 Cytogenomics Tests















What's distinct <u>City of hope NGS approach</u> from others

- Personalized Genomic Report
- Daily Genomic Tumor Board (GTB)
- Accessibility to molecular and other pathologists by Text, EPIC, and Email
- Communication of plan for treatment or next testing plan during disease team and GTB
- Rapid Heme and Solid tumor panel with same DNA/RNA extraction
- Low tumor cellularity requirements for both DNA and RNA sequencing for Comprehensive NGS
- Fusion detection of genes included with any known and novel partners,~ >5000 rearrangements
- Immunohistochemistry (IHC) studies addition to the test, PD-L1, HER2, FOLR1, cMET, MTAP, CLND18, etc.
- High depth of coverage: Minimal residual disease detection
- Fast Turn Around Time



Daily

GTB

Phenotype



When to test?

- At the time of diagnosis for advance stage adenocarcinoma
- At time of recurrence or progression
- Testing of stages I,II,IIIA is encouraged (eg. ADURA trials: adjuvant Osimertinib following resection of EGFR-mutant early-stage tumors)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

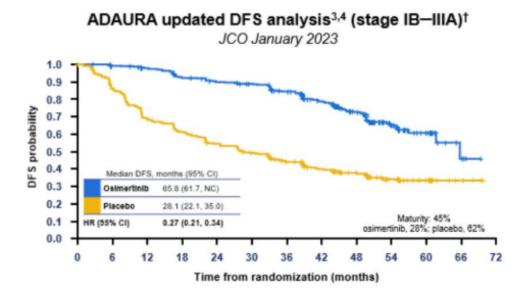
OCTOBER 29, 2020

VOL. 383 NO. 18

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

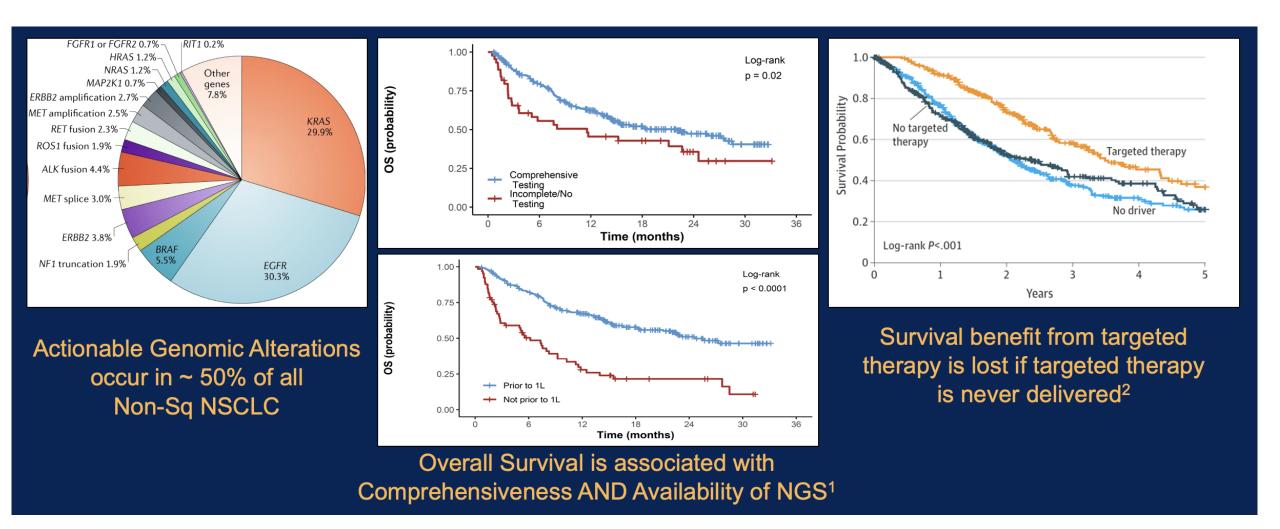
Yi-Long Wu, M.D., Masahiro Tsuboi, M.D., Jie He, M.D., Thomas John, Ph.D., Christian Grohe, M.D., Margarita Majem, M.D., Jonathan W. Goldman, M.D., Konstantin Laktionov, Ph.D., Sang-We Kim, M.D., Ph.D., Terufumi Kato, M.D., Huu-Vinh Vu, M.D., Ph.D., Shun Lu, M.D., Kye-Young Lee, M.D., Ph.D., Charuwan Akewanlop, M.D., Chong-Jen Yu, M.D., Ph.D., Filippo de Marinis, M.D., Laura Bonanno, M.D., Manuel Domine, M.D., Ph.D., Frances A. Shepherd, M.D., Lingmin Zeng, Ph.D., Rachel Hodge, M.Sc., Ajlan Atasoy, M.D., Yuri Rukazenkov, M.D., Ph.D., and Roy S. Herbst, M.D., Ph.D., for the ADAURA Investigators*

Wu et al. NEIM, 2020.





Personalized Therapy Relies on Comprehensive NGS, Timely Testing, and Delivery of Care





^{2.} Singal G et al. *JAMA*. 2019;321(14):1391-1399.

Future Direction: Digital Pathology and AI for Prediction of biomarkers and Therapy

- "third generation sequencing" e.g. oxford nanopore
- Optical genomic mapping e.g. Bionano
- Digital spatial profiling
- Digital imaging and machine learning
- Others...

ARTICLE OPEN

Direct identification of *ALK* and *ROS1* fusions in non-small cell lung cancer from hematoxylin and eosin-stained slides using

deep learning algorithms

Chen Mayer (1) 1,4 H., Efrat Ofek 1,4, Danielle Even Fridrich 1, Yosse Nurit Paz-Yaacov 2 and Iris Barshack 1,3

Review > Ann Oncol. 2024 Jan;35(1):29-65. doi: 10.1016/j.annonc.2023.10.125. Epub 2023 Oct 23.

Check for updates

Artificial intelligence for predictive biomarker discovery in immuno-oncology: a systematic review

A Prelaj ¹, V Miskovic ², M Zanitti ³, F Trovo ⁴, C Genova ⁵, G Viscardi ⁶, S E Rebuzzi ⁷, L Mazzeo ², L Provenzano ⁸, S Kosta ³, M Favali ⁴, A Spagnoletti ⁸, L Castelo-Branco ⁹, J Dolezal ¹⁰, A T Pearson ¹⁰, G Lo Russo ⁸, C Proto ⁸, M Ganzinelli ⁸, C Giani ⁸, E Ambrosini ⁴, S Turajlic ¹¹, L Au ¹², M Koopman ¹³, S Delaloge ¹⁴, J N Kather ¹⁵, F de Braud ⁸, M C Garassino ¹⁰, G Pentheroudakis ¹⁶, C Spencer ¹⁷, A L G Pedrocchi ⁴

Affiliations + expand

PMID: 37879443 DOI: 10.1016/j.annonc.2023.10.125

> Nat Commun. 2021 Mar 12;12(1):1613. doi: 10.1038/s41467-021-21896-9.

Human-interpretable image features derived from densely mapped cancer pathology slides predict diverse molecular phenotypes

```
James A Diao * 1 2, Jason K Wang * 1 2, Wan Fung Chui * 1 2, Victoria Mountain 1, Sai Chowdary Gullapally 1, Ramprakash Srinivasan 1, Richard N Mitchell 2 3, Benjamin Glass 1, Sara Hoffman 1, Sudha K Rao 1, Chirag Maheshwari 1, Abhik Lahiri 1, Aaditya Prakash 1, Ryan McLoughlin 1, Jennifer K Kerner 1, Murray B Resnick 1 4, Michael C Montalto 1, Aditya Khosla 1, Ilan N Wapinski 1, Andrew H Beck * 5, Hunter L Elliott * 1, Amaro Taylor-Weiner * 6
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Affiliations + expand

PMID: 33712588 PMCID: PMC7955068 DOI: 10.1038/s41467-021-21896-9

