MOLECULAR TESTING

Resources & Tools for the Multidisciplinary Team

Virtual Tumor Boards:

Key Concepts and Future Directions in Molecular Testing and Care Delivery

July 19, 2017

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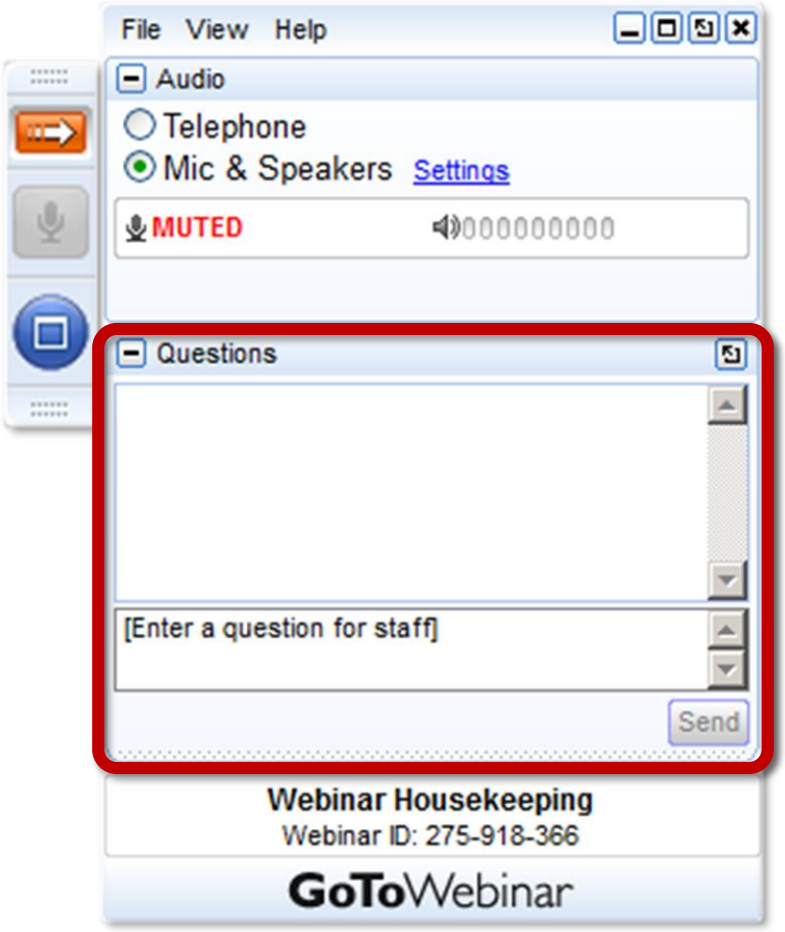
This project is sponsored by Genentech.
ACCC is solely responsible for content.

Webinar Instructions



Your Participation

- Please submit your text questions and comments using the Questions panel

Note: Today's presentation is being recorded and will be provided at a future date.

Project Background and Goal

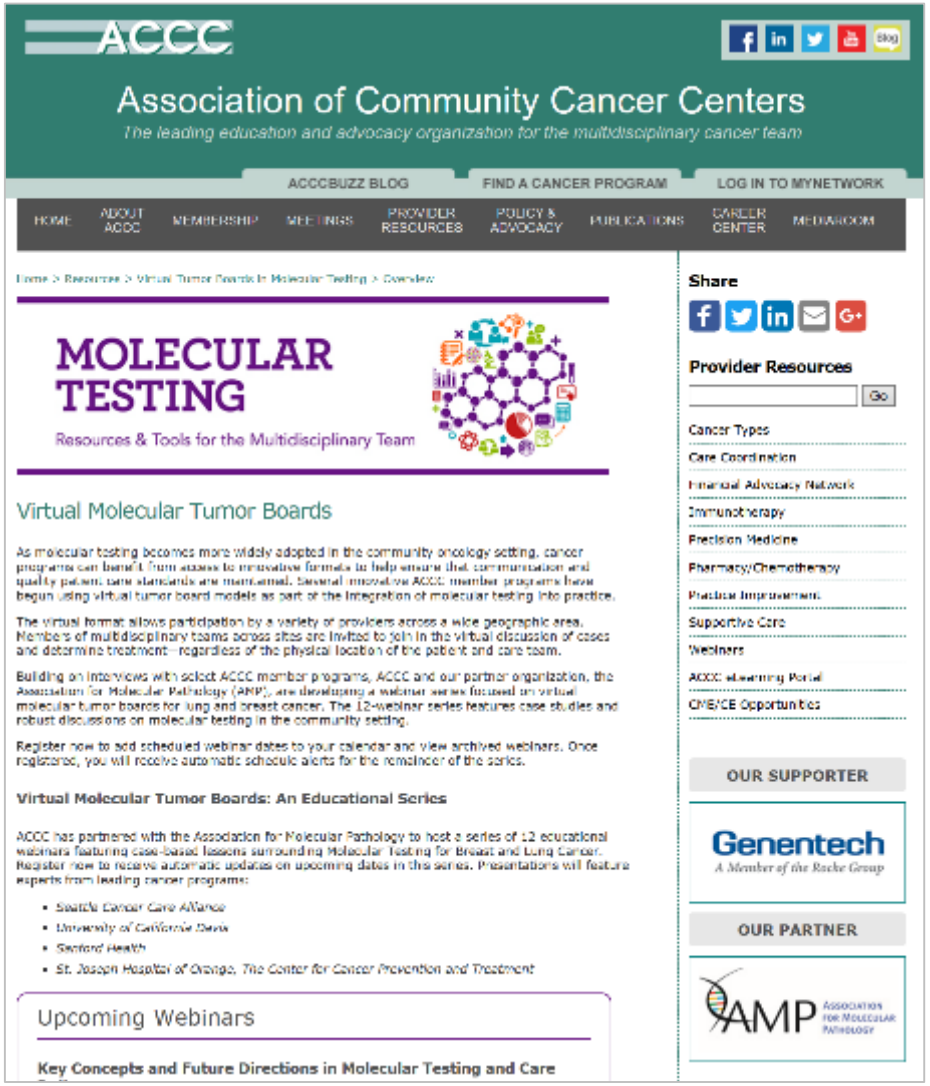
Cancer programs can form valuable relationships with research centers to access experts who can translate molecular and genomic science into individual patient treatment recommendations.

- Compare various tumor board models, partnership benefits, and effective practices
- Identify areas for potential multidisciplinary interactions and collaboration to improve patient care

AMP Association for Molecular Pathology

Project Overview

- Seattle Cancer Care Alliance
- University of California Davis
- Sanford Health
- St. Joseph Hospital of Orange, The Center for Cancer Prevention and Treatment



http://accc-cancer.org/resources/virtual-tumor-boards.asp

Webinars

Title	Presenters
Using Virtual Molecular Tumor Boards to Access the Experts	**Annie Chapman, MPH**, Seattle Cancer Care Alliance **Eric Duncavage, MD**, Washington University in St. Louis **Mark S. Soberman, MD, MBA, FACS**, Frederick Regional Health System
Virtual Molecular Tumor Board: Breast Cancer Case Studies	**Arvind Chaudhry, MD, PhD**, Summit Cancer Centers **V.K. Gadi, MD, PhD,** Seattle Cancer Care Alliance
Overview of Genomic Profiling	**Jeffrey Gregg, MD** University of California, Davis Medical Center
Precision Medicine and Personalized Cancer Therapy in Lung Cancer	**Jeffrey Gregg, MD** University of California, Davis Medical Center

http://accc-cancer.org/resources/virtual-tumor-boards.asp

Webinars (cont…)

Title	Presenters
An Ongoing Journey to Advance Molecular Testing in Lung Cancer	**John Maurice, MD** **Enza Esposito-Nguyen, RN, MSN, ANP-BC** **Lavinia Dobrea, RN, MS, OCN** St. Joseph Hospital of Orange, The Center for Cancer Prevention and Treatment
The Role of Genetics Professionals in a Community Cancer Program	**Megan Landsverk, PhD, FACMG** **Patricia Crotwell, PhD, FACMG** Sanford Cancer Center
Clinical Genetics vs. Tumor Genomic Profiling: Relevance in Cancer Care	**Olufunmilayo I. Olopade, MD, FACP** The University of Chicago Medicine

http://accc-cancer.org/resources/virtual-tumor-boards.asp

Webinars (cont…)

Title	Presenters
The New Age of Molecular Testing and Targeted Therapies for Lung Cancer	**Melissa L. Johnson, MD** Sarah Cannon Research Institute, Tennessee Oncology
Engaging Multidisciplinary Clinicians in Genomic Tumor Boards	**Steven Powell, MD** Sanford Cancer Center
Real-World Considerations When Implementing a Genomic Tumor Board Program	**Sharon Hunt, MBA** Sanford Cancer Center

http://accc-cancer.org/resources/virtual-tumor-boards.asp

Upcoming Article: Virtual Molecular Tumor Boards

- Evolving roles
- Ongoing molecular testing issues
- Developing a program
  - Clinical champions
  - Identifying and preparing patient cases
  - Scheduling
  - Genomics expertise
  - Technology
  - Participation and engagement

Today's Speakers

Eric Duncavage, MD

Associate Professor of Pathology & Immunology

Washington University School of Medicine

Barnes-Jewish Hospital

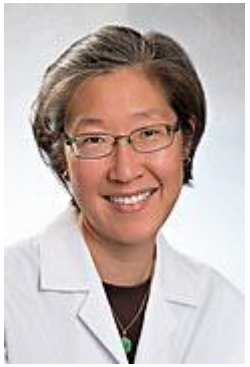
Annette S. Kim, MD, PhD

Associate Professor of Pathology

Harvard Medical School

Associate Pathologist

Brigham And Women's Hospital



Outline

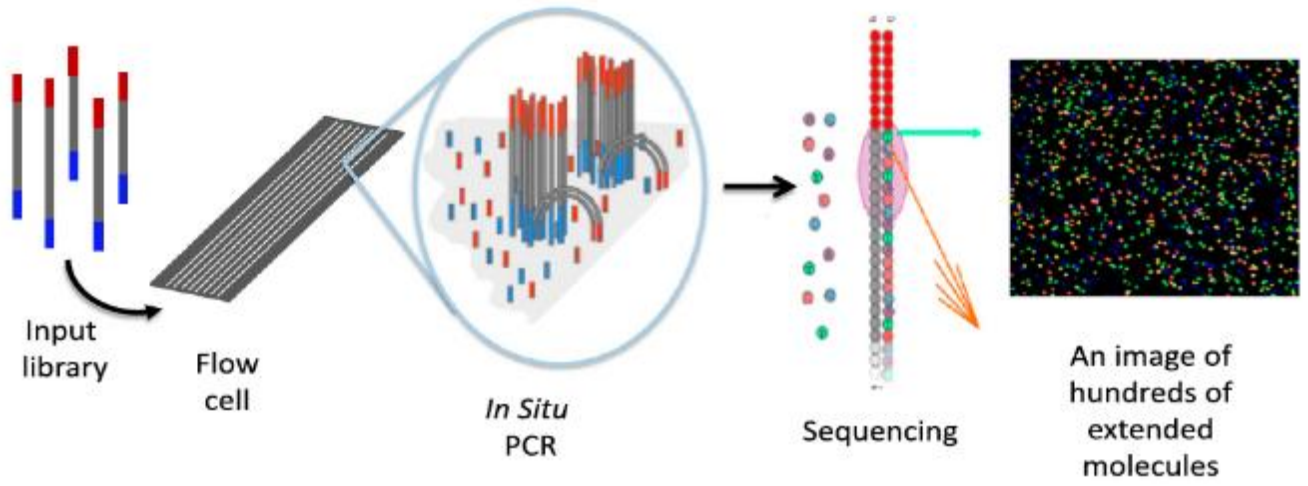
- Brief overview of NGS (Dr. Duncavage)
- Lung Cancer (Dr. Kim)
  - Standard of care (brief)
  - Not standard of care (brief)
  - Current BWH practice of molecular testing
  - Future of molecular testing
  - BWH Tumor Boards
- Breast Cancer (Dr. Kim)
  - Standard of care
  - Not standard of care
  - Current BWH practice of molecular testing
  - Future of molecular testing
  - BWH Tumor Boards
- How to bring the fore-front of molecular testing to the community (Dr. Duncavage)

Sequencing Based Diagnostics

- Next Generation Sequencing (NGS) or Massively Parallel Sequencing encompasses class of new sequencing technologies that for inexpensive sequencing of large regions
- NGS can be used in ‘cancer panels’ to identify clinically important genes mutated in common cancer types.
- NGS may also be used to detect chromosomal rearrangements, gains, and losses

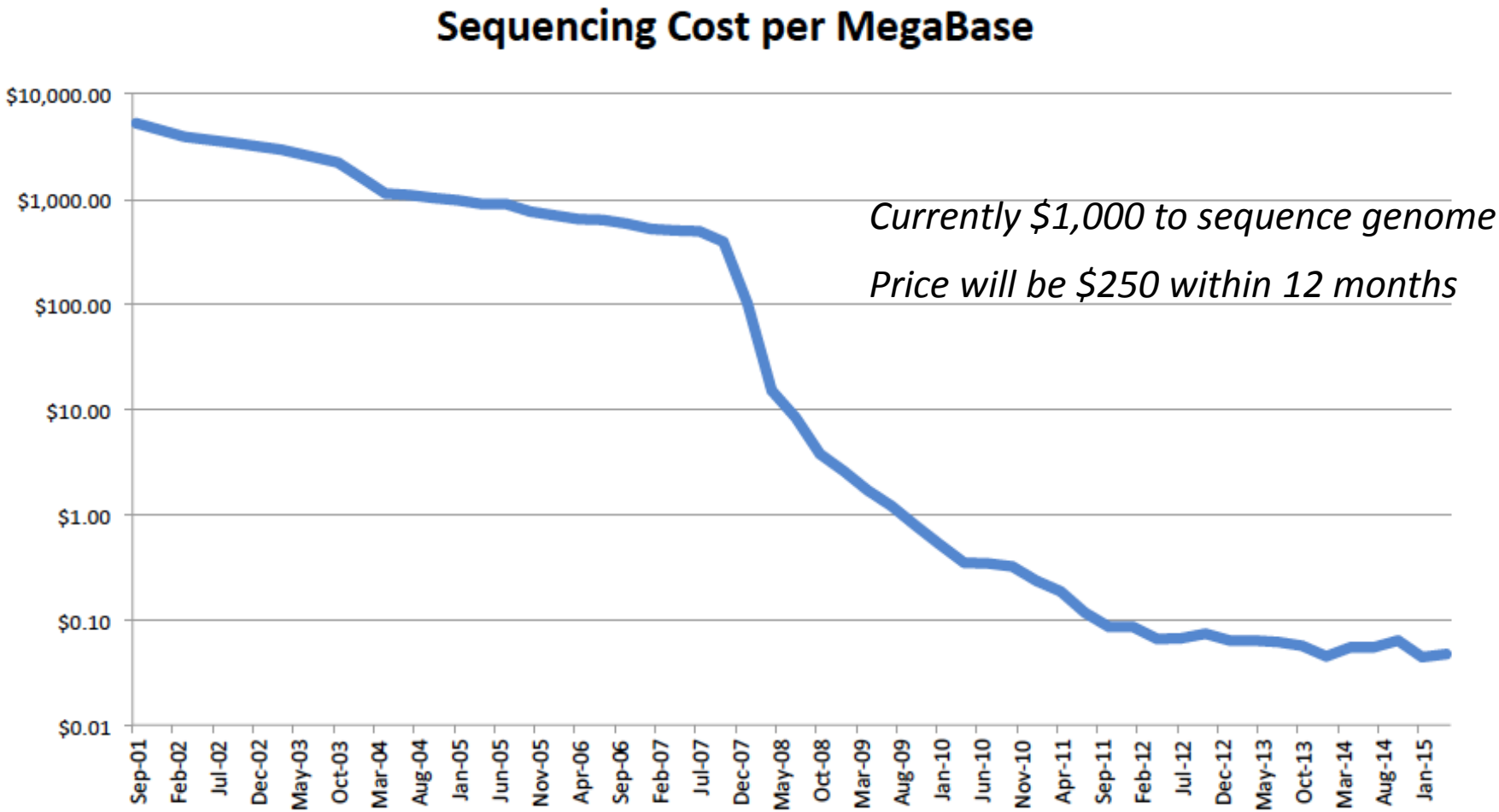
Next Generation Sequencing Methods

- All NGS methods rely on large scale parallelization so that millions of sequencing reactions take place simultaneously.
- Sequence parallelization decreases cost and greatly increases throughput.



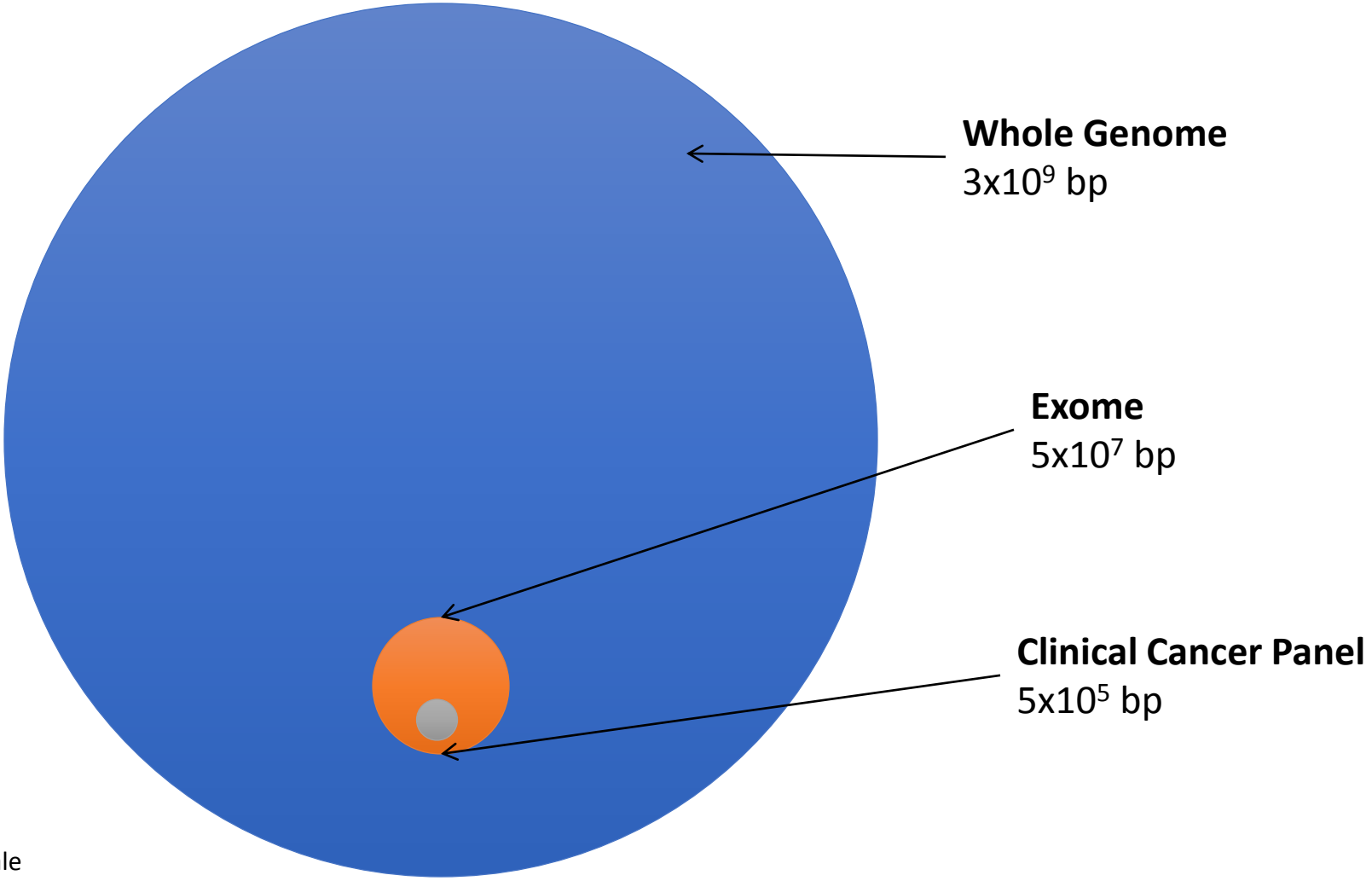
Johnsen, Blood, 2013

Cost of Sequencing



Source: genome.gov/sequencingcosts

Types of NGS Panels

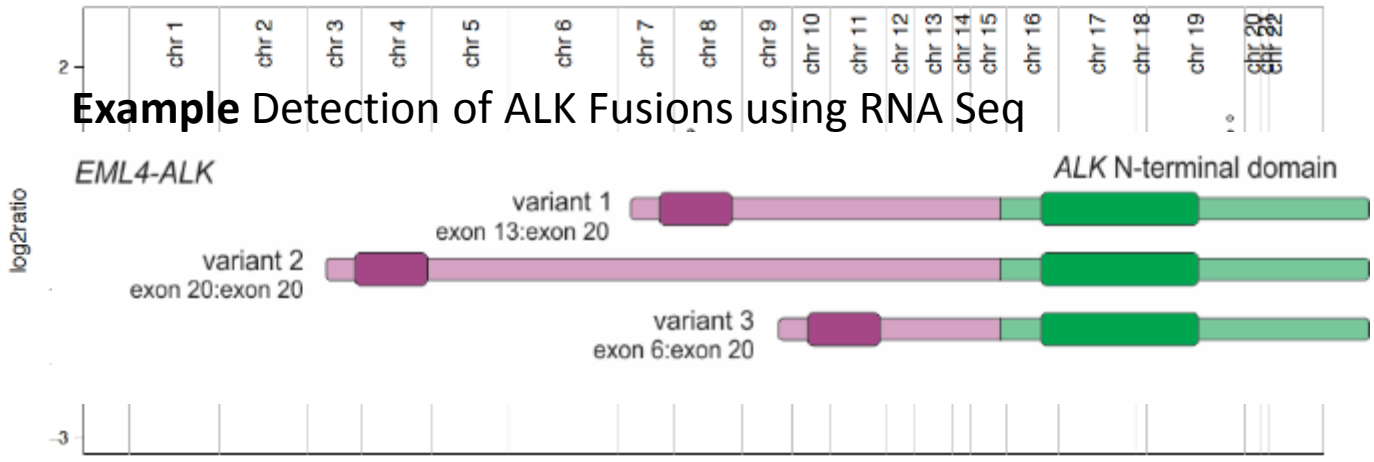


* Not to scale

NGS Can More Than DNA Mutations

- NGS can identify chromosomal alterations and could potentially replace routine cytogenetics.
- RNA sequencing can be used to detect gene fusions and gene expression

Example Copy Number Calling with Panel Based NGS



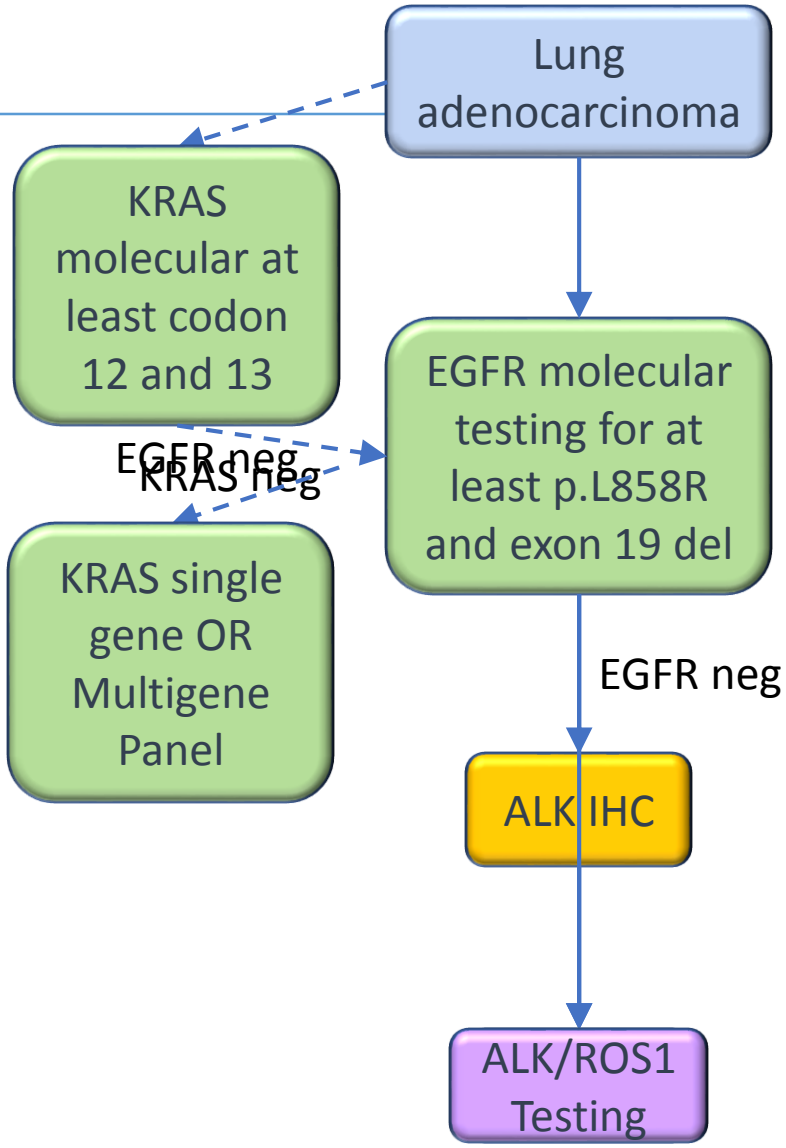
Example Detection of ALK Fusions using RNA Seq

Rosenbaum et al

Lung

Standard of Care

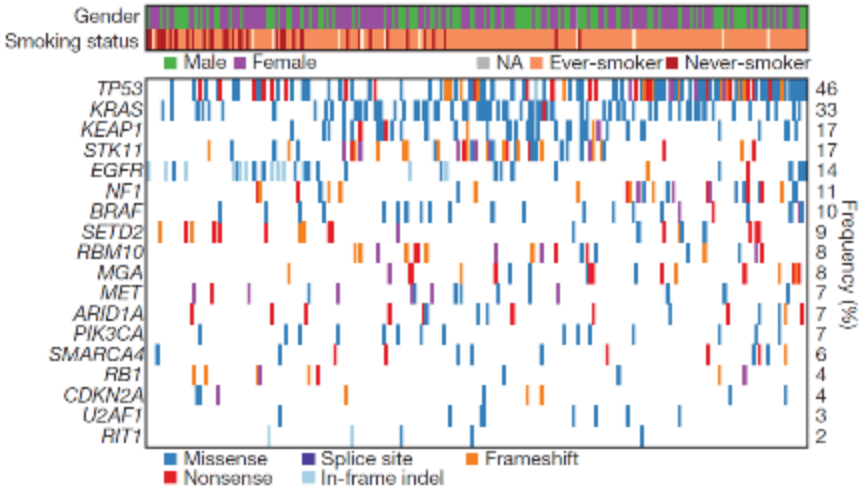
- Revised Lung Cancer Guidelines pending (first version Lindeman *et al. J Thorac Oncol.* 2013;8:823-859.)
  - Required testing: EGFR, ALK, ROS1 (new)
    - EGFR = Molecular
    - ALK/ROS1: FISH or IHC (or molecular if intronic breakpoint regions sufficiently covered)
  - Possible Testing (new):
    - ERBB2, BRAF, MET, RET, KRAS (all panel)
    - Just KRAS if only single gene assays are available and EGFR mutated
  - Who to test? (new recommendations)
    - All stage 3B/4 with adenocarcinoma component
    - Non-Adenocarcinoma NSCLC, <50 yo
    - Non-Adeno NSCLC, any age non-smoker



NOT Standard of Care

Lung adeno

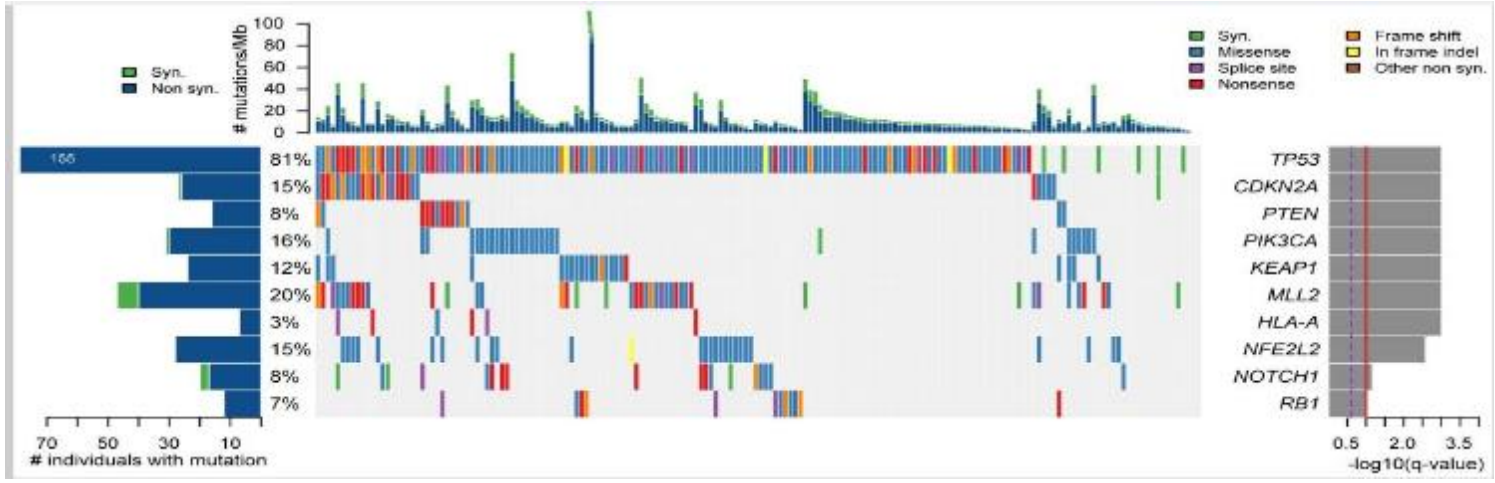
Nature. 2014 Jul 31;511(7511):543-50.



- Other recurrent variants in lung cancer- most of the genes on our Oncopanel

Lung squamous cell

Nature. 2012 Sep 27;489(7417):519-25



- We have clinical Oncopanel that specifically is reimbursed for EGFR, KRAS, BRAF hotspot variants
- We have a research oncopanel (DFCI grant)

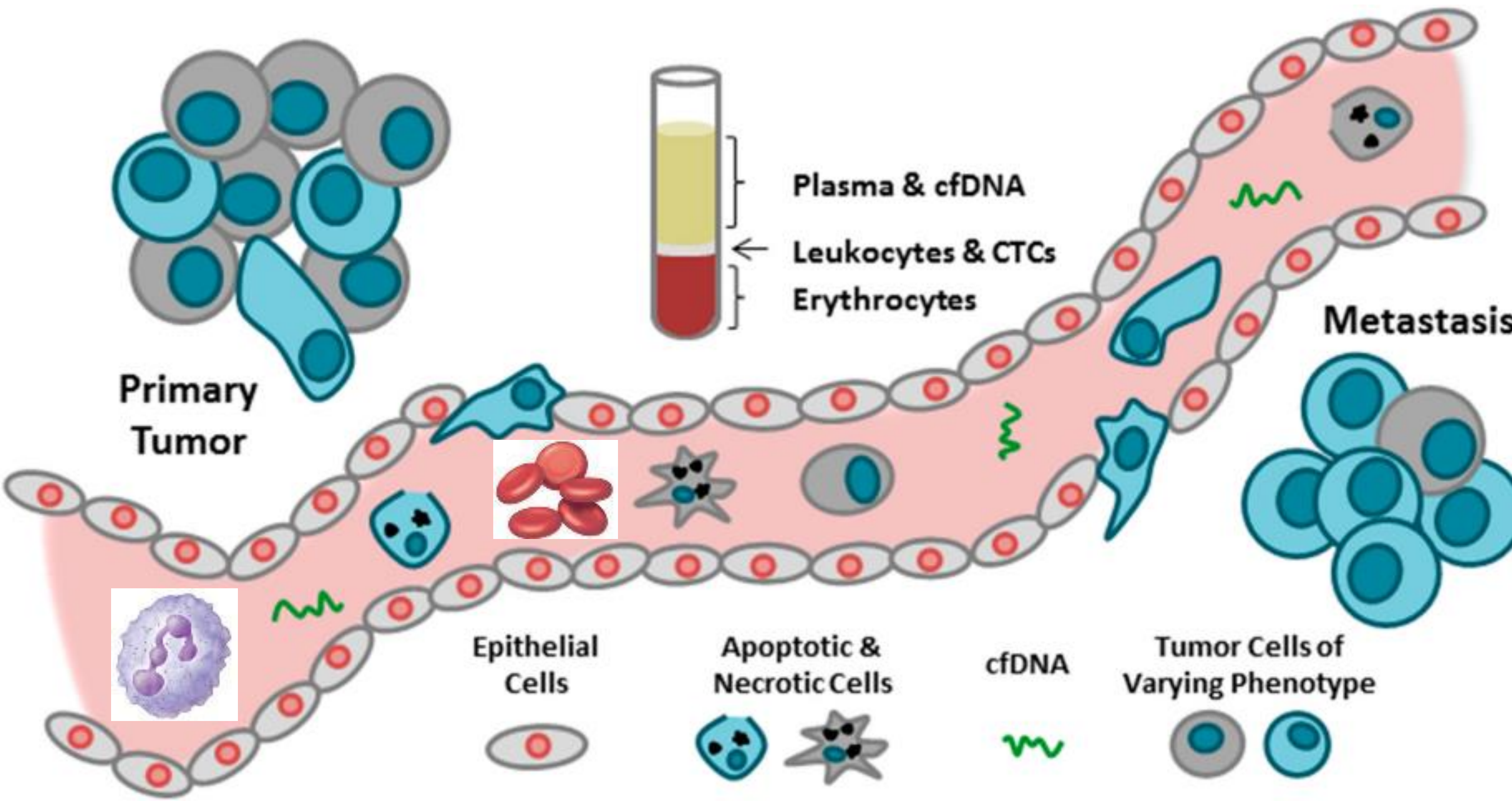
Current use of Molecular Testing

- ddPCR (tumor testing, new diagnosis)
  - EGFR p.L858R (43% of EGFR mutations in lung cancer)
  - EGFR exon 19 deletion (48% of EGFR mutations)
- ddPCR (liquid biopsy, progression or new diagnosis)
  - EGFR p.L858R/EGFR exon 19 deletion (to determine if ctDNA is present)
  - EGFR p.T790M (to assess for secondary resistance)
- Oncopanel (NGS 447-gene panel)

What is in a name?

[illegible]
urine) to try to assess various species of
molecules and cells that come from solid
tissues (typically tumors) that may save the
patient a more invasive tissue biopsy

[illegible] biopsy methods



Lowes, LE *et al. Int. J. Mol. Sci.* **2016**, *17*, 1505.

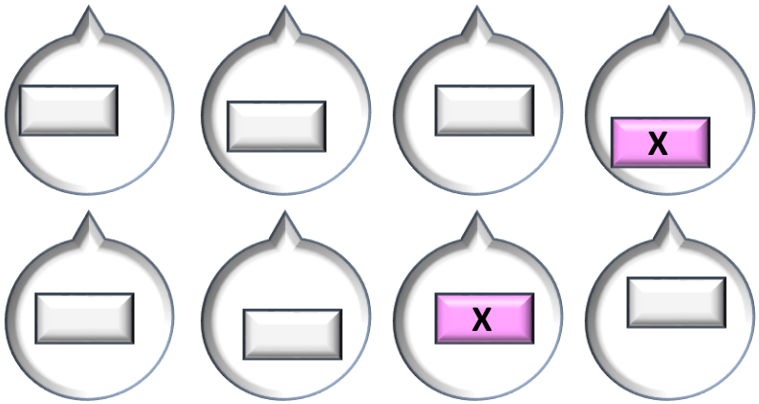
Liquid Biopsies (cfDNA)

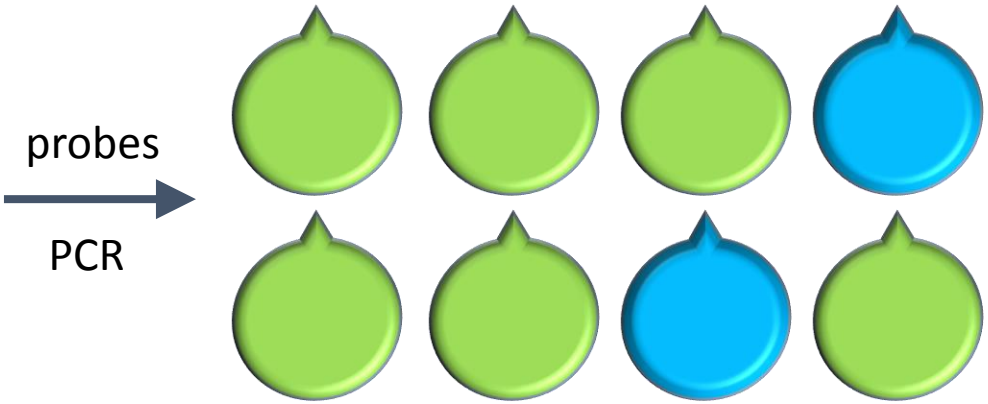
- Requirements:
  - Samples: How do you preserve the cfDNA?
    - Peripheral blood in EDTA tube
    - Must be spun within 4 hours to separate the cfDNA in the plasma from the white blood cells (esp. PMNs) that can degrade the DNA

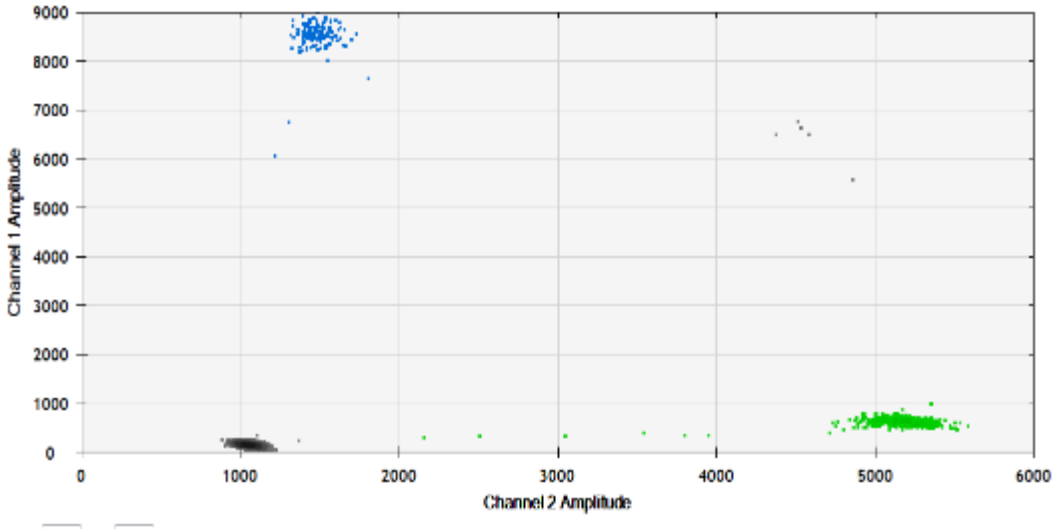
      OR
    - Specialized tubes designed to preserve cfDNA (or cfRNA), e.g., Streck tubes
  - Methodology: Is your assay sufficiently sensitive?
    - ddPCR methods can detect as low as 3 droplets as definitively positive, with the denominator as high as you can go (>300, but often much higher)
    - Digital sequencing can identify down to 0.25% VAF with 99.6% PPV for SNVs
  - Interpretation: How do you know you are testing the tumor (i.e., you have ctDNA rather than just cfDNA)?
    - Must have sufficient clinical sensitivity- this is why we test for *EGFR* p.L858R/exon 19 deletions to assure us that we are looking at tumor-derived DNA

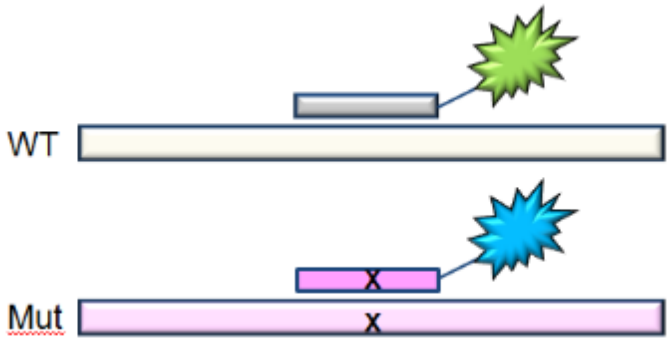
Liquid Biopsies (cfDNA)

- Digital Droplet PCR









Clinical scenarios for liquid biopsies

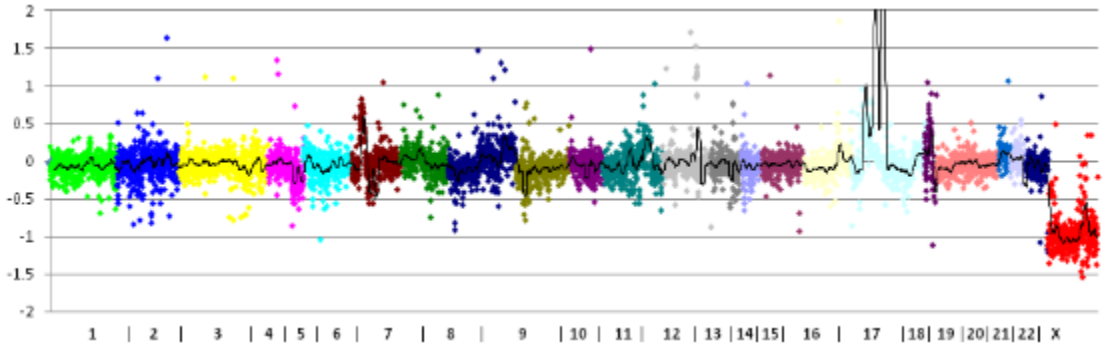
- Background:
  - Tumors with larger volume and increased Ki67 (proliferation)
  - High stage disease (3B-4)
  - Tumor shedding of ctDNA: squamous > adenocarcinoma
- Current Clinical Applications
  - At progression (ideally with known tumor variant(s))
  - At diagnosis and tissue not sufficient or not attainable
- Future Clinical Applications
  - At diagnosis in lieu of a tissue biopsy even in early stage dz
  - For serial monitoring of minimal residual dz

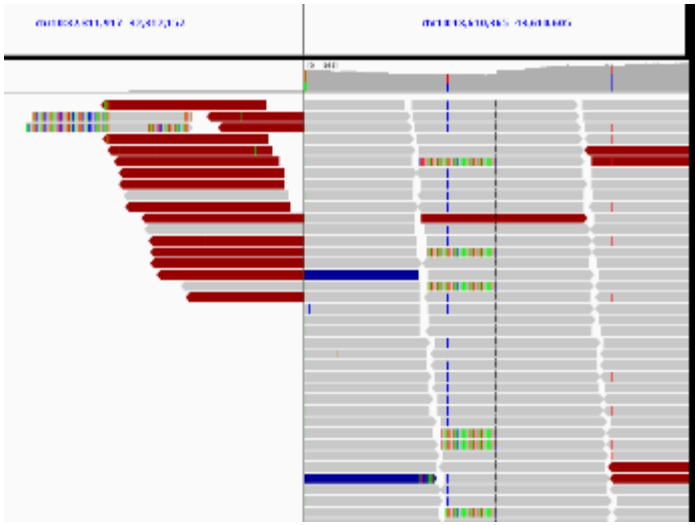
Current use of Molecular Testing

- ddPCR (tumor testing, new diagnosis)
- ddPCR (liquid biopsy in patients with a diagnosis)
- Oncopanel (NGS 447-gene panel)
  - Assess for other SNVs (e.g., EGFR, KRAS, BRAF and others)
  - Assess for amplification (e.g., MET, ERBB2, FGFR1)
  - Assess for translocations (e.g., ALK, ROS1, RET)
    - All common break regions for ALK, ROS1 (except for ROS1 intron 31), and RET
  - **NEW** Assess for a mismatch repair deficient signature (for immunotherapy purposes)
    - Based upon small indels in homopolymer regions as a percentage of all MB covered by the assay
  - Tissue Type: FFPE, Fresh tissue, cytology smears (we use cut-off of >500 cells) – even better than cores since there are no fixation issues

Current use of Molecular Testing

	Chr	position	Gene	DNA	AA	AF	Cov	Canonical
IGV	1	155874257	RIT1	c.274G>T	p.A92S	8	235	Missense
IGV	10	76719815	KAT6B	c.709T>C	p.C237R	7	197	Missense
IGV	10	76719828	KAT6B	c.722G>C	p.G241A	6	180	Missense
IGV	11	118344991	KMT2A	c.3117G>C	p.K1039N	53	195	Missense
IGV	11	119142516	CBL	c.515G>T	p.G172V	5	184	Missense
IGV	17	7577535	TP53	c.746G>T	p.R249M	5	173	Missense
IGV	18	42531736	SETBP1	c.2431A>T	p.I811F	8	274	Missense
IGV	12	25398284	KRAS	c.35G>C	p.G12A	28	262	Missense





Mutational Burden:

Tumor Mutational Burden/Megabase: 12.167
This is higher than 73% of all Non-Small Cell Lung Cancer cancers sequenced by this version of OncoPanel.
This is higher than 87% of all Profile cases sequenced by this version of OncoPanel.

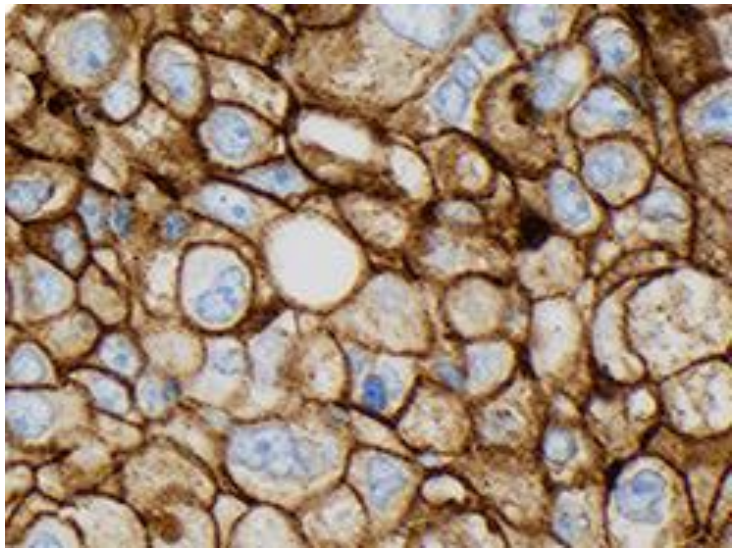
Mutational Signatures:

MMR-Status: Proficient (MMR-P / MSS)

Future of Molecular Testing

- Use of RNA to detect fusions is becoming routine
- Expanded use of liquid biopsies with NGS
  - At diagnosis
  - For minimal residual disease tracking
- Immunotherapy
  - We already routinely do PDL1 staining for all advanced stage NSCLC
  - Pembrolizumab approved for multiple tumor types:
    - Melanoma, NSCLC, HNSCC, cHL, urothelial cancer
    - Any tumor with MSI-H or MMR defects (method not specified)

Mouse monoclonal anti-PDL1 clone 22C3



www.dako.com

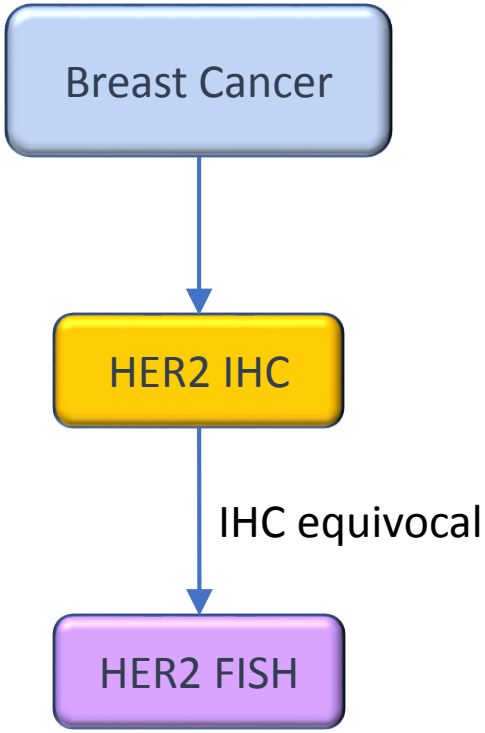
Tumor Boards

- Monthly “Precision Medicine Tumor Board”
  - Tumor focus varies from month to month
  - 3-5 cases that illustrate the impact of NGS panel testing on patient care
- Biweekly Thoracic Oncology Program Meeting
  - 2-3 active cases reviewed with clinical, pathology, radiology teams
  - Moderator calls on individuals for optimal interactions and educational benefit
  - 20-30 minute didactic on a chosen topic
- DFCI faculty have outlying hospital appointments/practices
- Molecular Tumor Boards currently focused on cases within the BWH/DFCI/BCH community

Breast

Standard of Care

- CAP Biomarkers guidelines
  - New Focused update of guidelines in progress
- ER/PR by IHC
- HER2 IHC, reflex to FISH if equivocal

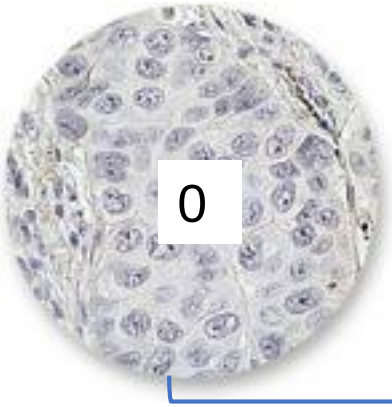


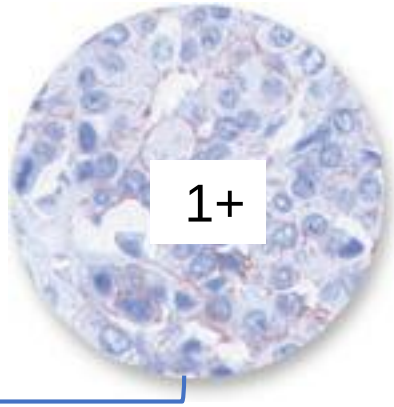
COLLEGE of AMERICAN PATHOLOGISTS

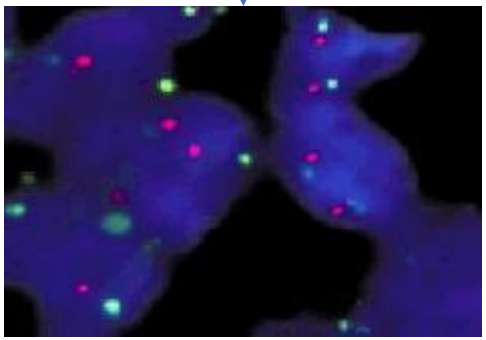
Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Breast

http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/cp-breast-biomarker-template-14.pdf

HER2 IHC-FISH Correlation



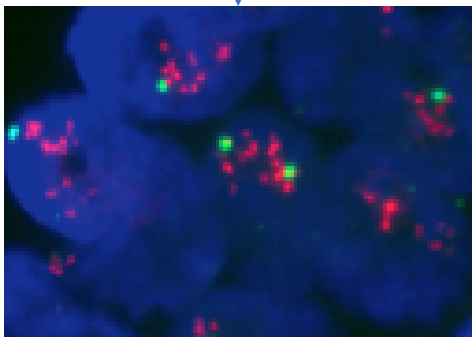




>95% negative

What about these 30-40% of cases?

These may be truly biologically equivocal, or may be due to suboptimal IHC.

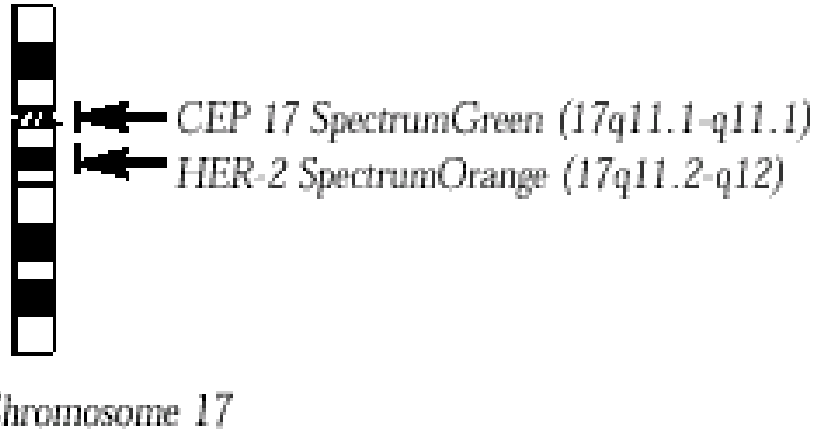


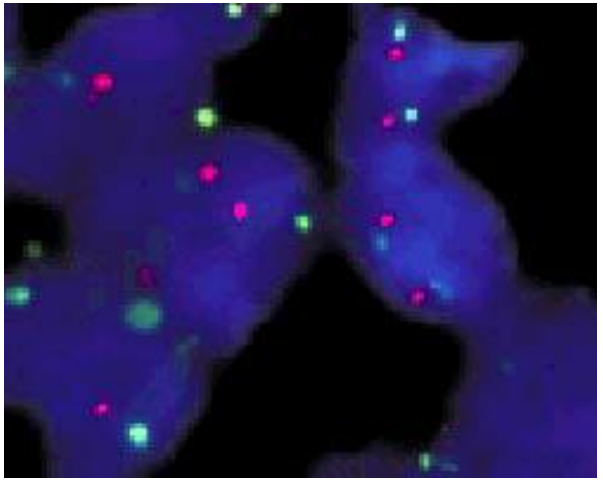
>95% positive

Images courtesy of Dr. Deborah Dillon

HER2 FISH

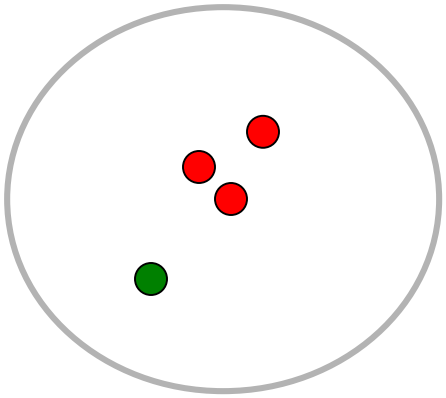
Result	Criteria
Negative (not amplified)	*HER2/CEP17* ratio <2.0 AND average HER2 copy number <4.0 signals/cell
Equivocal*	*HER2/CEP17* ratio <2.0 AND average HER2 copy number ≥4.0 but <6.0 signals/cell†
Positive (amplified)	*HER2/CEP17* ratio ≥2.0† (regardless of average HER2 copy number) *or* Average HER2 copy number ≥6.0 signals/cell† (regardless of ratio)



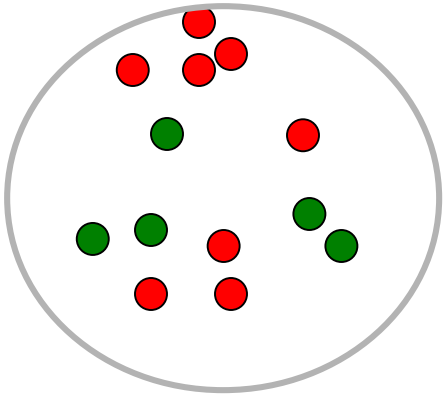


Images courtesy of Dr. Deborah Dillon
CAP Breast Caner Biomarker Guidelines

HER2 FISH Caveats



Technically positive, but due to loss of CEP17, not significant amplification of HER2



Technically negative, even though HER2 increased by copy number but not by ratio due to polysomy or centromere amplification

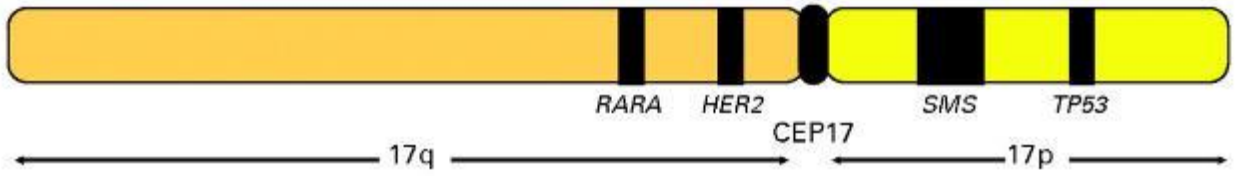
Marchio *et al, J Pathol* 2009; 219:16.
Yeh *et al, Mod Pathol* 2009; 22:1169.
Hofmann *et al, J Clin Pathol* 2008;61:89.

Images courtesy of Dr. Deborah Dillon

Other Molecular Testing Options

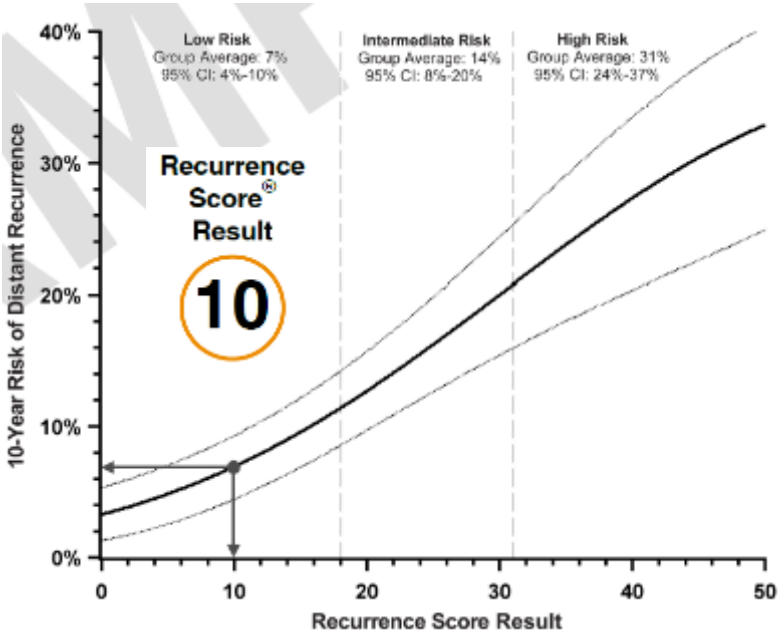
1. Alternative probes for FISH

Marchio, C. *et al. J Pathol* . 2009;219:16.



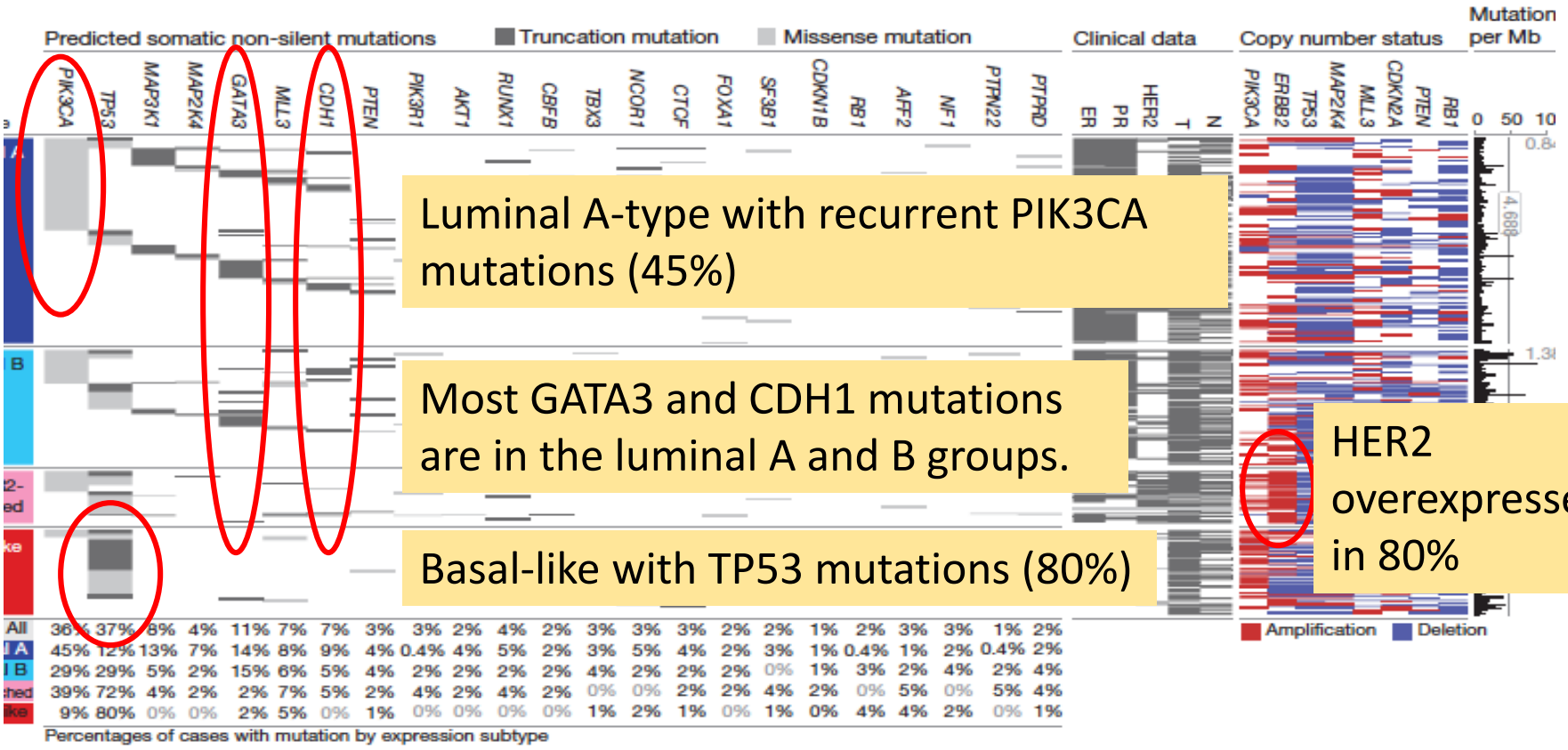
2. Multigene expression assays

- Makes the oncologist/patient feel better about not using chemo when the result comes back low risk…
- Makes the oncologist/patient feel better about using chemo when the result comes back high risk…



- MAJORITY OF CASES ARE INTERMEDIATE RISK!

NOT SOC: Other Molecular Testing



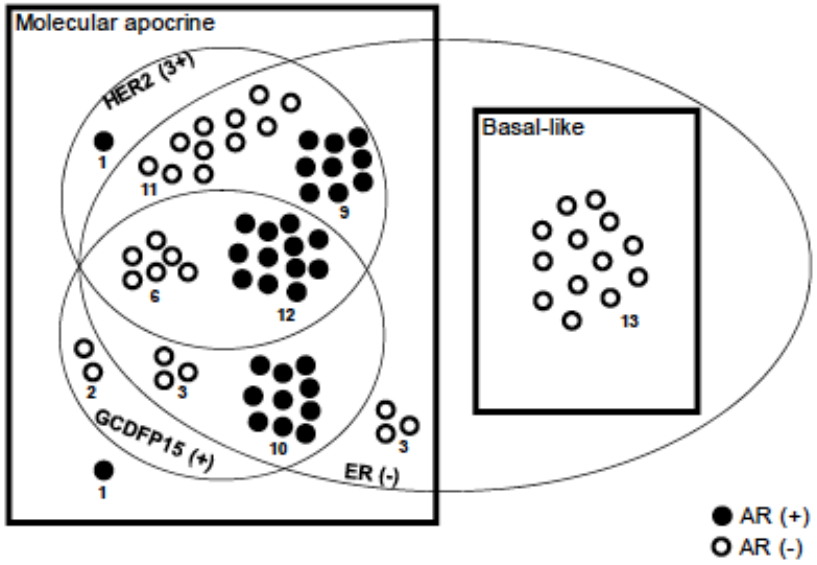
TP53, PIK3CA, ERBB2, MYC, FGFR1, GATA3, CCND1 = 58% of driver mutations

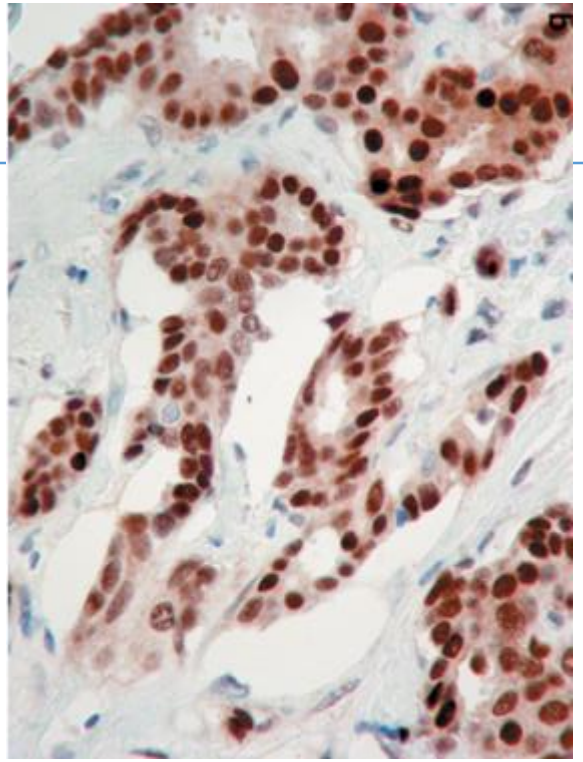
Nature. 2012 Oct 4;490:61-70.

Also NOT SOC

- Androgen Receptor IHC
- ESR1 mutations – only in mets of patients treated with aromatase inhibitors
- ERBB2 mutations- in not amplified cases
  - Better response to neratinib

Androgen Receptor IHC





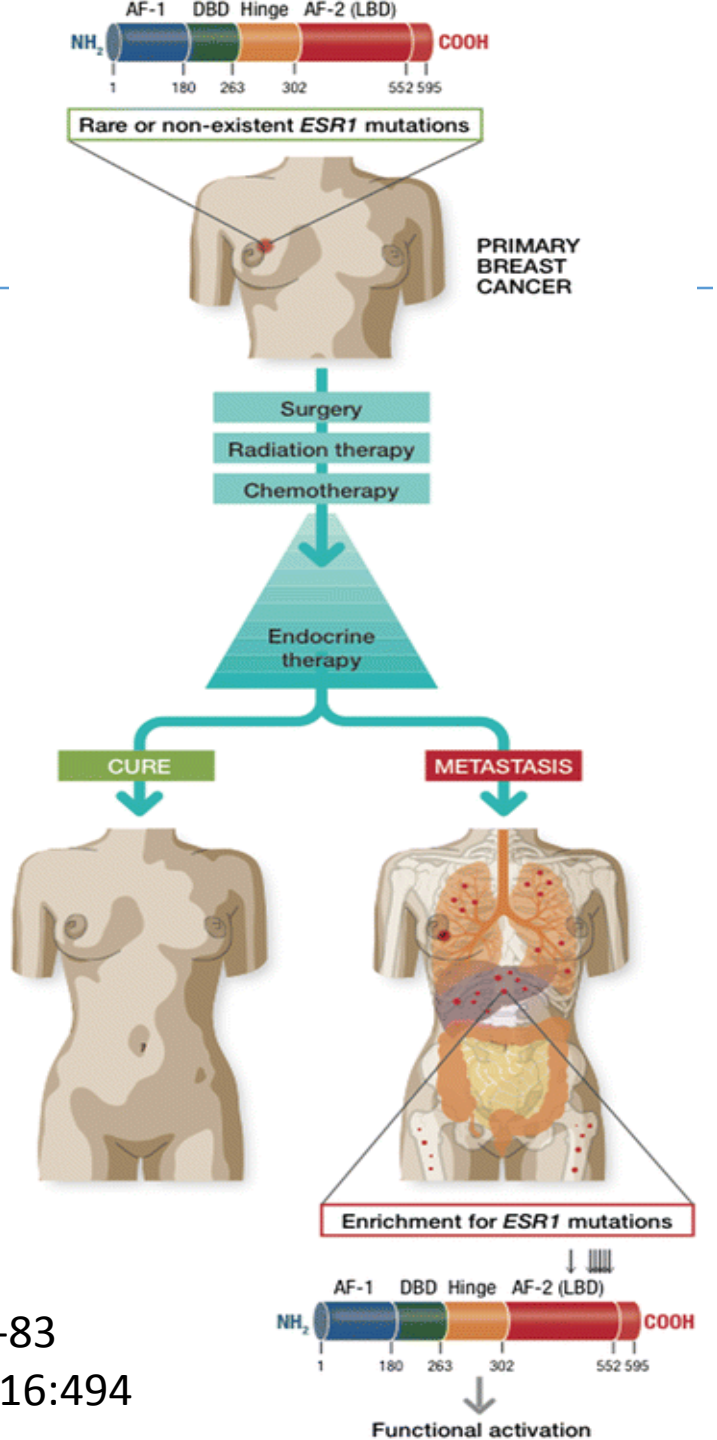
- Of ER neg AND HER2 pos and/or GCDFP15 pos cases, >50% are AR positive by IHC (>10%)
- Of ER-/PR- mBC, 12% AR positive
- 19% achieve clinical benefit from AR targeting (bicalutamide)

Lehmann-Che et al, Breast Ca Res 2013 15:R37

Images courtesy of Dr. Deborah Dillon

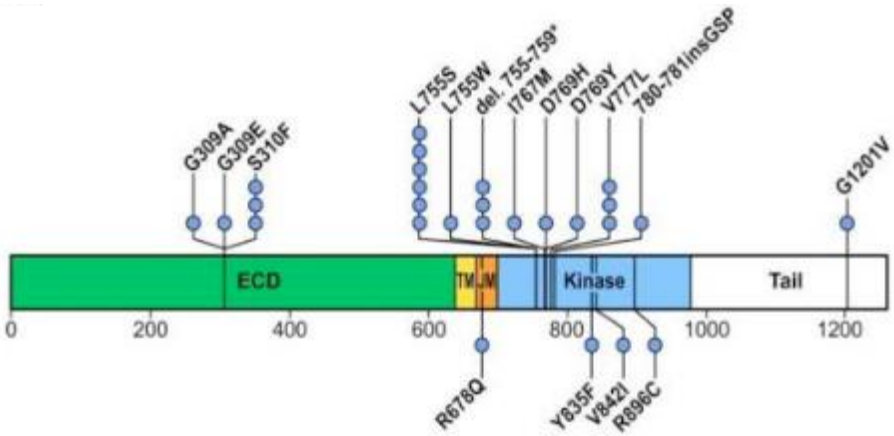
ESR1 Mutations

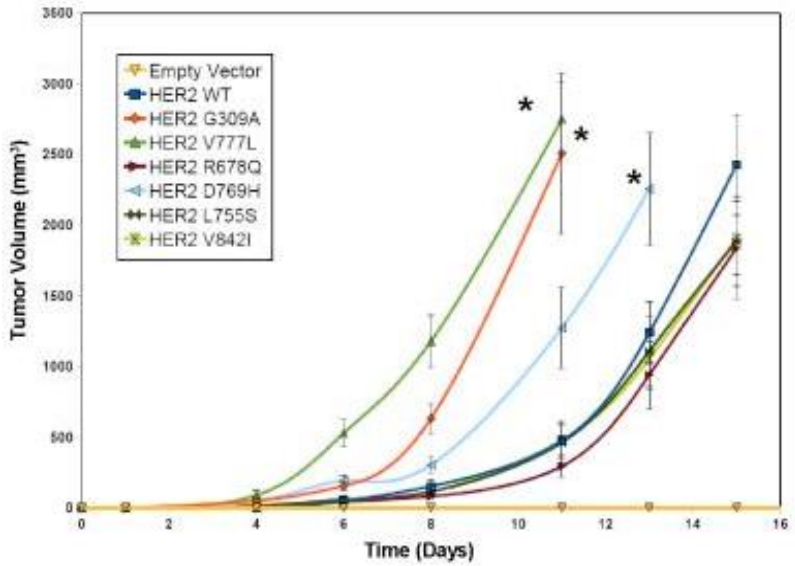
- Rare in primary tumors
- Present in 15-20% of patients with metastatic ER+ disease who received endocrine therapy
- Mutations are clustered in the ligand-binding domain of the ER and lead to constitutive ER activity and acquired endocrine resistance



Jeselsohn R, Nat Rev Clin Oncol. 2015 Oct;12(10):573-83
Alluri PG (Chinnaiyan). Breast Cancer Research 2014, 16:494

ERBB2 (HER2) Mutations





- Prevalence 2.4% in mBC, most in tumors without HER2 amplification, and somewhat more common in relapsed ILC
- Kinase domain mutations are activating in preclinical models (alternate way to activate HER2)
- Appear to be LESS sensitive to trastuzumab and apatinib, but SENSITIVE to neratinib (15% response rate- MSKCC data)

MSKCC. SABCS 2015

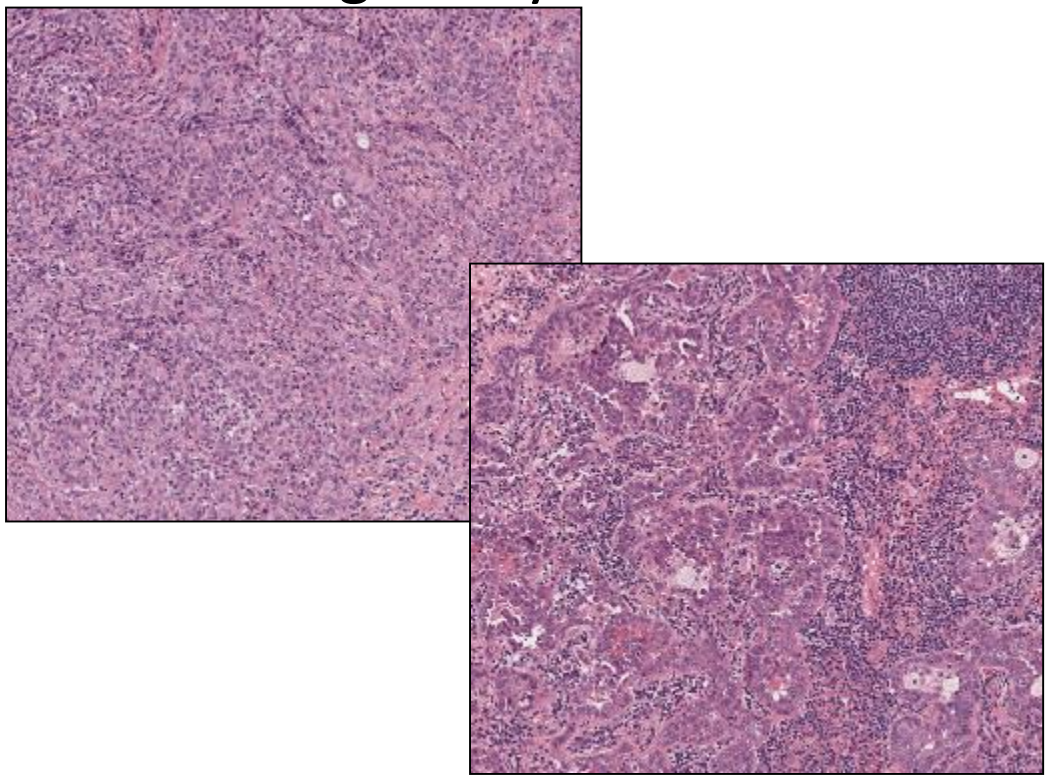
Bose F (Ellis M). Cancer Discov. 2013 Feb;3(2):224.

Current Use of Molecular Testing

- Still for research purposes
- All cases of metastatic disease go through our Oncopanel (do mets preferentially to primary tissue if tissues/resources are limited)
- Considerations of molecular testing:
  - Tissue has to get to testing site in a timely manner or the results will be too late (current TAT of our Oncopanel is <3 weeks)
  - *Community pathology site MUST RETAIN tissue*
    - Often pathology sites do not retain blocks beyond the minimal requirement
    - Minimum of 1 good block of primary tumor and 1 good block of each metastatic tumor

Future of Molecular Testing

- Liquid Biopsy (cell free DNA (cfDNA), circulating tumor DNA (ctDNA))
- Sequential testing to look for changes as you treat
- Immunotherapy
  - Expression of PDL1 by IHC (not yet by copy number assessment)
  - Tumor infiltrating lymphocytes (TILs)



Tumor Boards

- Monthly “Precision Medicine Tumor Board”
  - Tumor focus varies from month to month
  - 3-5 cases that illustrate the impact of NGS panel testing on patient care
- Weekly Breast Tumor Board (BWH/DFCI)
  - Covers all aspects (histology, FISH, molecular, clinical)- not dedicated molecular
  - SOC markers as well as availability of clinical trials
  - Includes other BWH affiliates
- Eastern Maine Medical Center Tumor Boards
  - BWH/DFCI faculty present there as well
  - Real-time networked Tumor Board with 8-10 outlying hospitals
  - Face-to-face interactions help develop relationships

Making Treatment Decisions

- How do community physicians access new diagnostic technologies such as sequencing based diagnostics?
- How do we integrate complicated molecular testing in to patient care?
- How can the community physician get help interpreting data?

Ordering Molecular Testing

- In-house molecular testing
  - Ideal, but now always available
- Reference lab model (Mayo, Quest, ARUP, etc)
  - Return results with interpretation
  - May not integrate external clinical or pathological findings
- Technical only molecular services (PierianDx Gateway, others)
  - Sequencing performed at large center and results interpreted at local center
- ‘Expert Diagnostic’ model (PrecipoDx)
  - Cases tested at company and signed out by experts at local academic centers

How to Choose the Right Molecular Assay

- Does the panel have the correct genes for the cancer type?
  - Pan-cancer panels vs. disease-specific panels
- Number of Genes
  - More genes on the panel is not necessary better
- Mutation Spectrum Identified
  - Does the assay detect larger insertions/deletions?
  - Will it detect chromosomal rearrangements
- Turn around time
  - Generally 2-3 weeks
- Reimbursement
  - Will insurance cover the the assay?

How to Make Clinical Sense of Molecular Testing Data—Tumor Boards

- Tumor boards are a great venue to discuss molecular findings
- Many institutions have organ-system based tumor boards where molecular data is discussed along with other clinical findings
- Molecular only tumor boards are generally focused on interesting molecular findings or the application of new techniques

Finding Help

- Call your local pathologist
- Community oncologists can connect to larger centers through **virtual tumor boards**
  - Experts from a larger center provide opinions in real time
  - Offered by several academic centers as well as private companies
- Pathology Consults
  - Patient materials including the results of molecular testing can be sent to an academic for review and interpretation
- Patient Consults
  - Patient may be seen at a center with more expertise in the desired area

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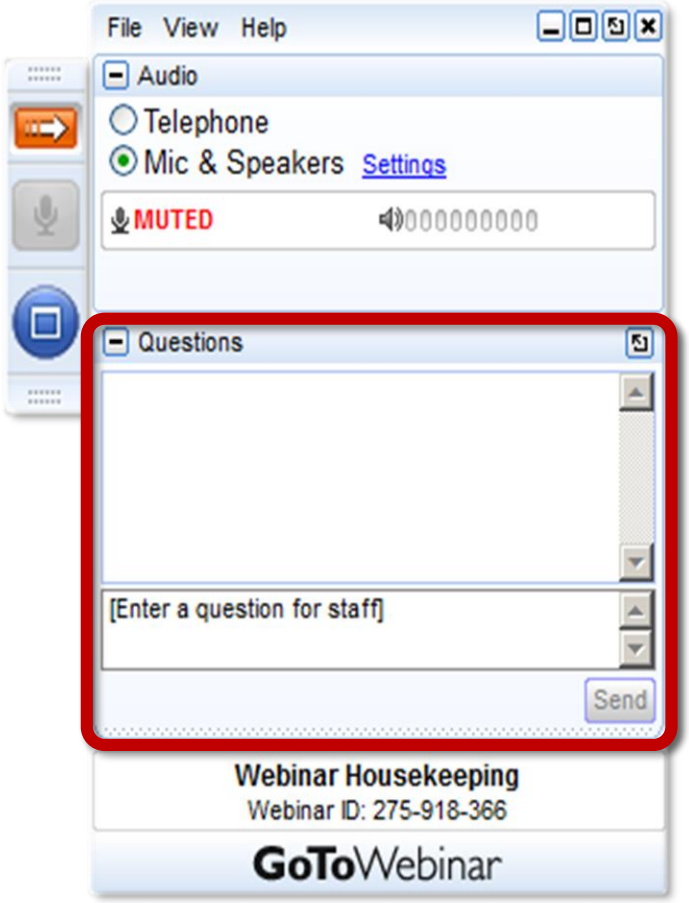
Q/A



Submit Questions

- Please submit your text questions and comments using the Questions panel

LEARN MORE
View archived webinars

http://accc-cancer.org/resources/virtual-tumor-boards.asp

MOLECULAR TESTING

Resources & Tools for the Multidisciplinary Team

Precision Medicine: Strategies for Improving Cancer Team Communication

In 2016, ACCC conducted four focus groups at ACCC member programs on the state of their breast and non-small cell lung cancer molecular testing programs.

An easy-to-use assessment tool designed to help programs identify potential gaps in patient identification, diagnosis, test selection, tissue preparation, and test results.

accc-cancer.org/MolecularTestingCommunication