American Association for Cancer Research

FINDING CURES TOGETHER℠

Liquid Biopsies in Oncology Drug and Device Development Workshop

July 19, 2016

Walter E. Washington Convention Center

Washington, DC

American Association
for Cancer Research

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During audience Q&A sessions, webcast participants may send questions and comments to

policyquestion@aacr.org

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Welcome and Workshop Objectives

Workshop Co-Chairs:

Gideon Blumenthal, MD

Reena Philip, PhD

Pasi Jänne, MD, PhD

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Session I

CANCER LIQUID BIOPSIES: STATE OF THE SCIENCE

Chair: Julia Beaver, MD

Speakers:

Howard Scher, MD

Ben Ho Park, MD, PhD

Muneesh Tewari, MD, PhD

Memorial Sloan Kettering Cancer Center™

The Development of an ARv7 Predictive Biomarker Test on CTC from Analytical Validation to the Demonstration of Clinical Utility

Howard I. Scher, M.D.

D. Wayne Calloway Chair in Urologic Oncology
Chief, Genitourinary Oncology Service
Department of Medicine

July 19, 2016

Memorial Sloan Kettering Cancer Center™

Disclosures

I have the following financial relationships to disclose:

Consultant: Medivation (U), Janssen Research (U), Janssen (U), Sanofi (C)

Grant/Research support: Medivation, Janssen Research

Collaboration: EPIC Sciences

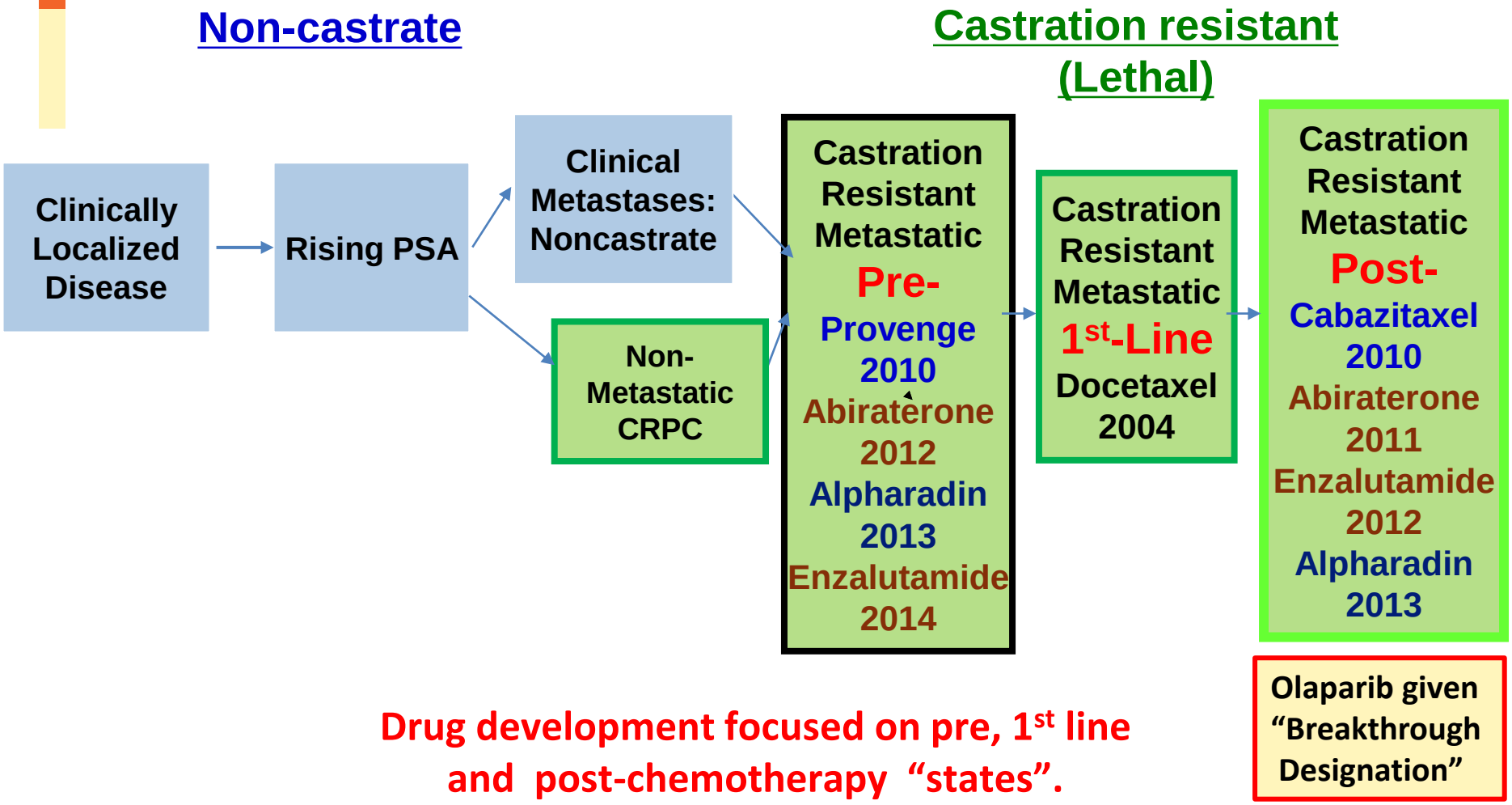
I will discuss the investigational use in my presentation of:

Galeterone

An ARv7 Predictive Biomarker Test

1. **A disease and treatment context.**
2. ARV7 mRNA in CTC predicts outcomes to AR directed therapy.
3. A nuclear localized (NL) single cell ARv7 assay: analytics.
4. Clinical testing of the NL single cell ArV7 assay.

Six Life Prolonging Therapies Are Now FDA Approved For the Management of Metastatic Castration Resistant Prostate Cancer (CRPC)



Drug development focused on pre, 1st line and post-chemotherapy "states".

Modified from Scher and Heller. *Urology*. 2000.

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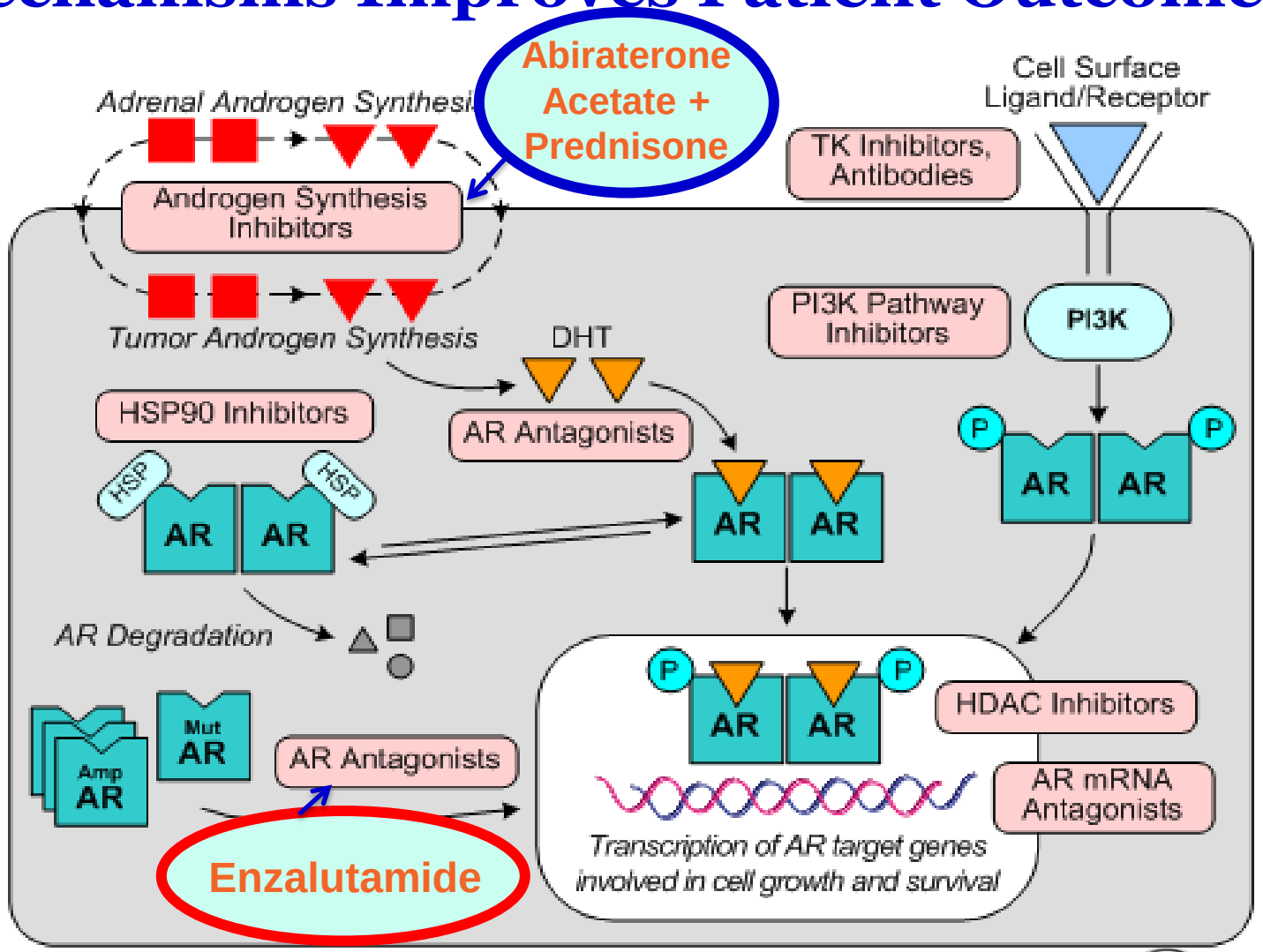
Included Are Two Hormonal Agents Showing That Understanding and Targeting Resistance Mechanisms Improves Patient Outcomes



Increased androgen biosynthesis and AR overexpression.

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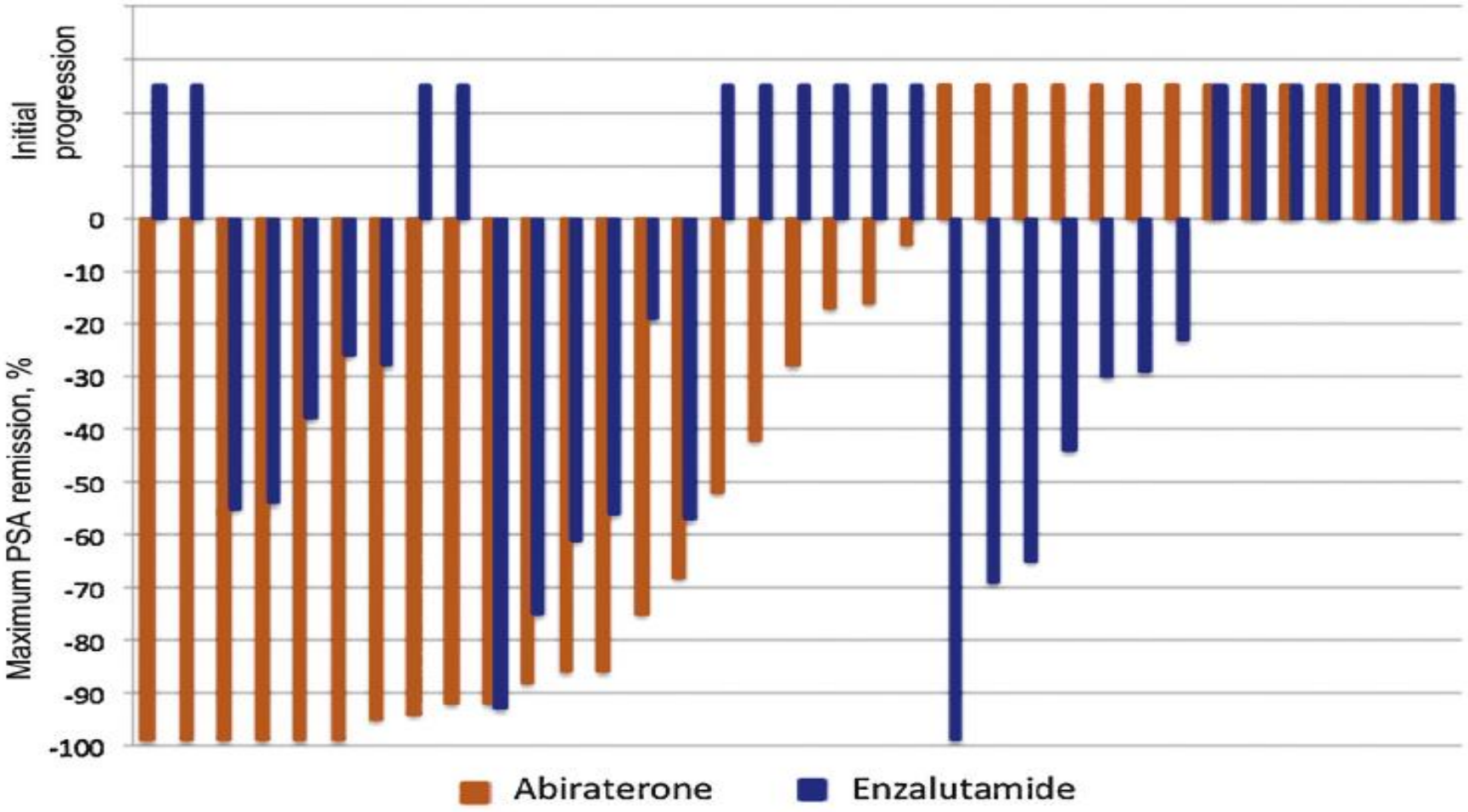
It was assumed that the hormonal agents - abiraterone and enzalutamide **(less toxic oral therapies)** would be *effective in sequence* and that taxane based chemotherapy **(more toxic IV therapy)** would be given later.

One example of many trials, most retrospective . . . . shows this is not the case..

Prior Response to Abiraterone DOES NOT Predict Sensitivity to Enzalutamide and the Overall Response Rate is Lower in the 2nd Line Setting



The question of when to use a taxane remains.

Schrader et al. Eur Urol 65:30, 2014.

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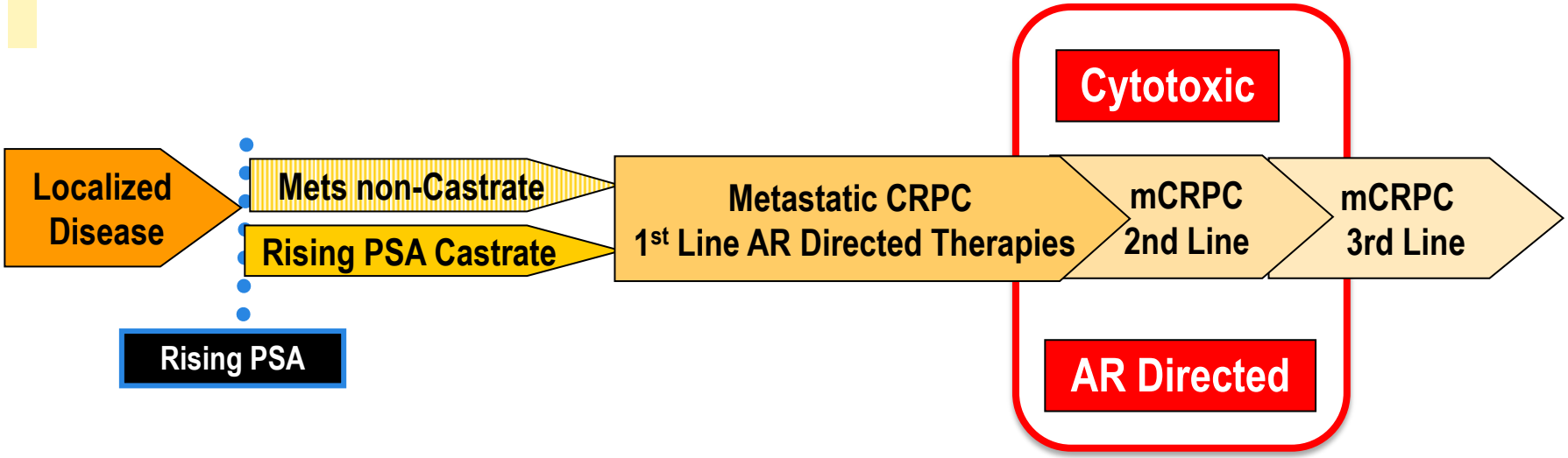
Our Focus is to Develop Predictive Biomarkers in Blood to Inform Treatment Selection When a Change in Therapy is Needed

1. The choice of which life-prolonging therapy to use and when is largely empiric.

2. Given in sequence, the response to **E** after **A**, or **A** after **E** is less frequent and of shorter duration. Whether **T** would be more beneficial than a second AR Tx is a key question.

3. Selecting the ***right treatment*** at the ***right time*** is essential to ***improve individual patient outcomes***.

In Practice, it is the 2nd Line Treatment Decision That is Proving to be the Most Difficult and Most Critical



Our Focus is to Develop Predictive Biomarkers in Blood to Inform Treatment Selection When a Change in Therapy is Needed

1.

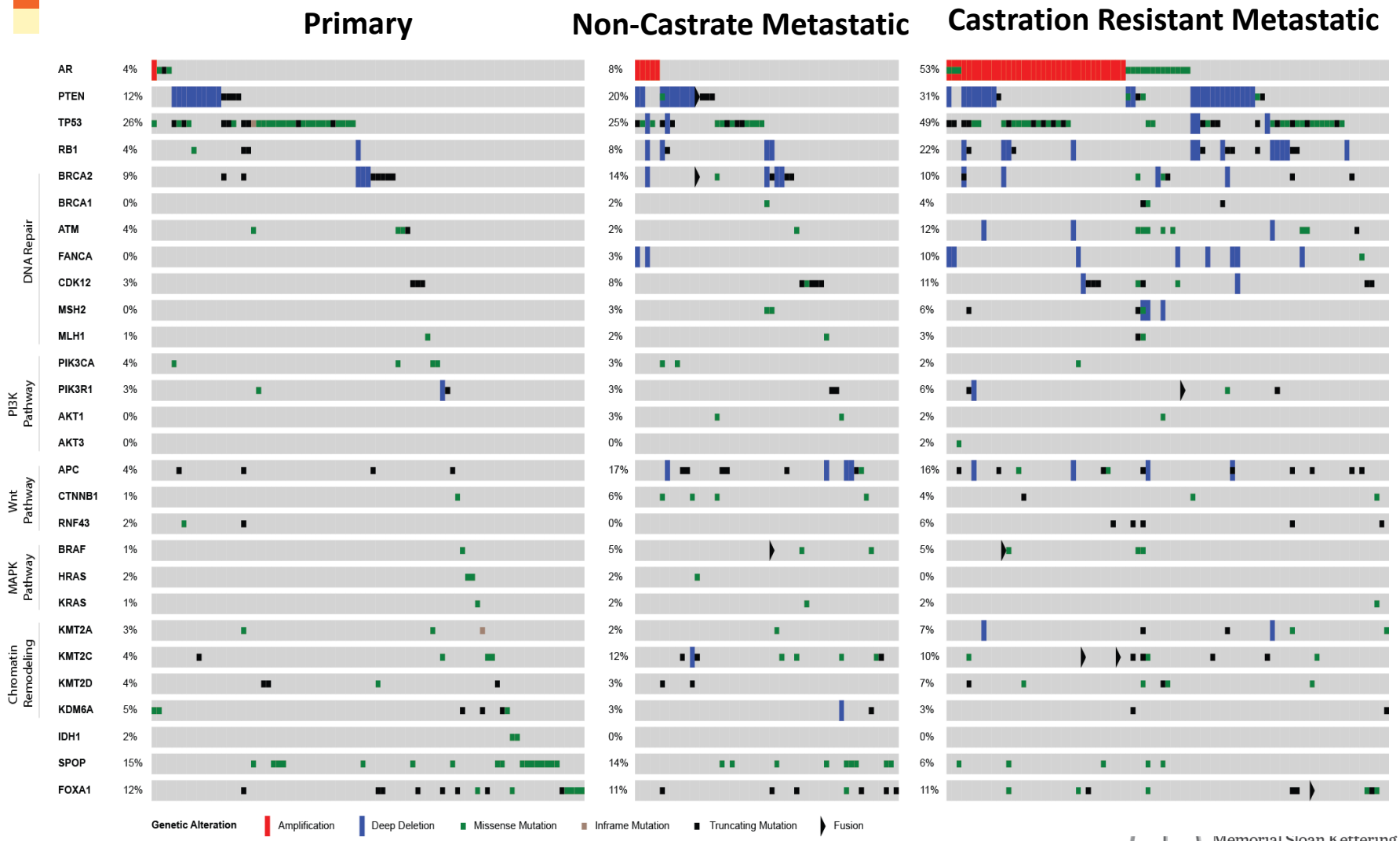
2.

3.

4. To do so requires the direct characterization of an individual patient's tumor –

   but, what material – tissue, CTC cfDNA?

MSKCC IMPACT ONCOPRINT: Targeted Sequencing of 400+ Genes in Tissue Shows That the Disease At Diagnosis Differs from Lethal mCRPC.



Abida W et al., MSKCC (Submitted).

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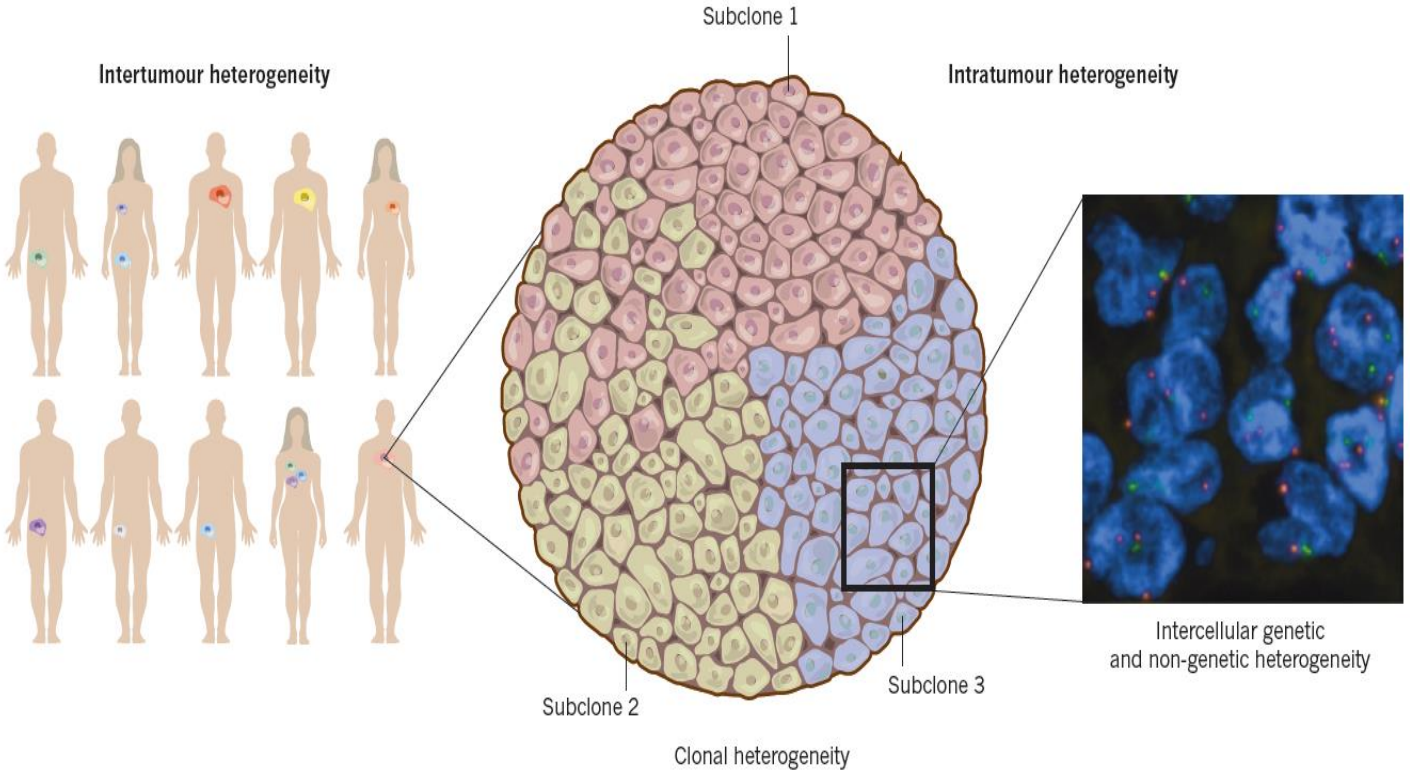
CTCs May Better Reflect Disease Biology Because Cancer is Not a Disease of Averages, but a Disease of Multiple Clonal Species



Burrell, et al. Nature 501:338, 2013

An ARv7 Predictive Biomarker Test

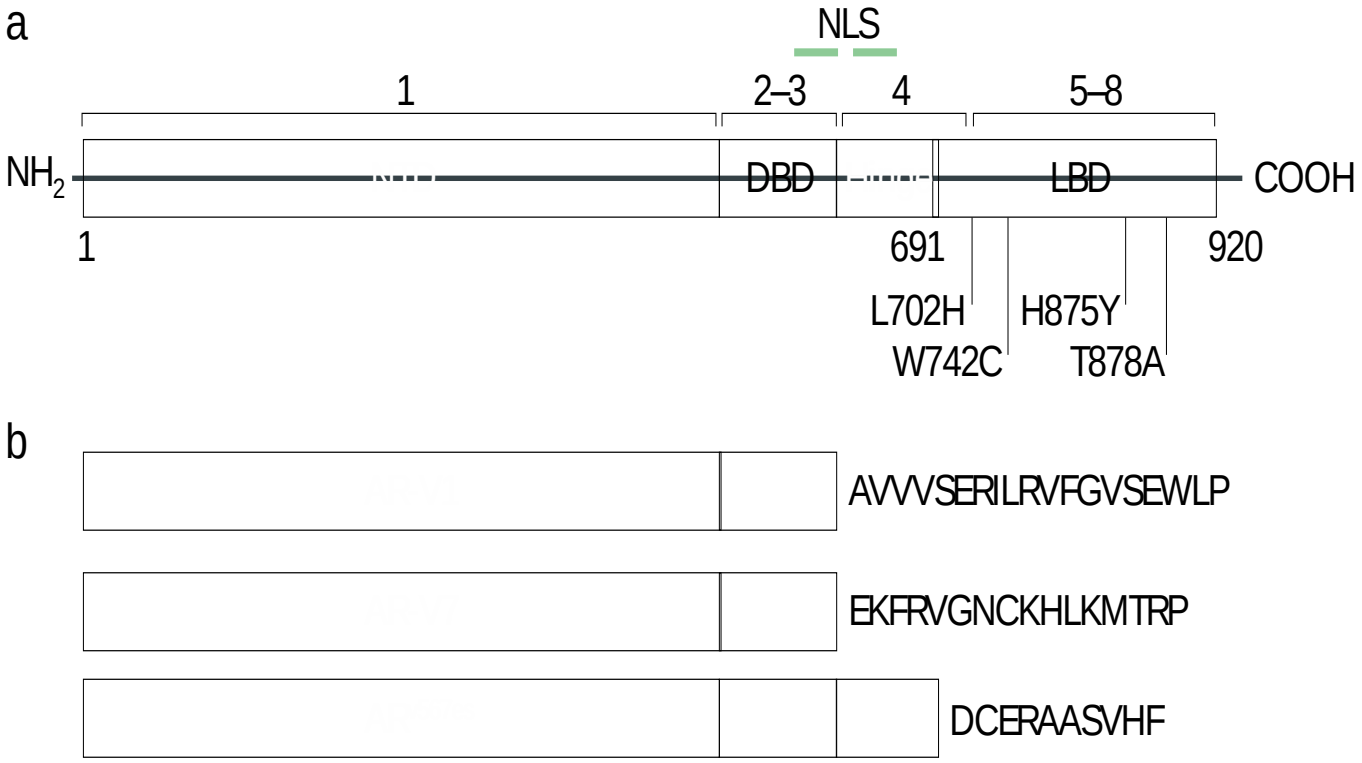
1. A disease and treatment context.
2. **ARV7 mRNA in CTC predicts outcomes to AR directed therapy.**
3. A nuclear localized (NL) single cell ARv7 assay: analytics.
4. Clinical testing of the NL single cell ArV7 assay.

An Overview of Resistance Mechanisms to Next-Generation AR-Targeted Therapies

Restored AR signaling:	AR activating mutations
	AR splice variants
	AR overexpression
	Intratumoral DHT synthesis
AR bypass signaling:	GR upregulation.
Complete androgen independence:	RB1 deletion,
	TP53 deletion, mutation
	MYCN gain
	AURKA gain

Watson et al., Nat Rev Cancer 15:701, 2015

Domain Structure of the AR, Cancer Associated Missense Mutations (a) and Splice Variants (b)



AR splice variants with the in-frame variant specific amino acids derived from the alternative splicing events which can be detected with antibodies..

Watson et al., Nat Rev Cancer 15:701, 2015

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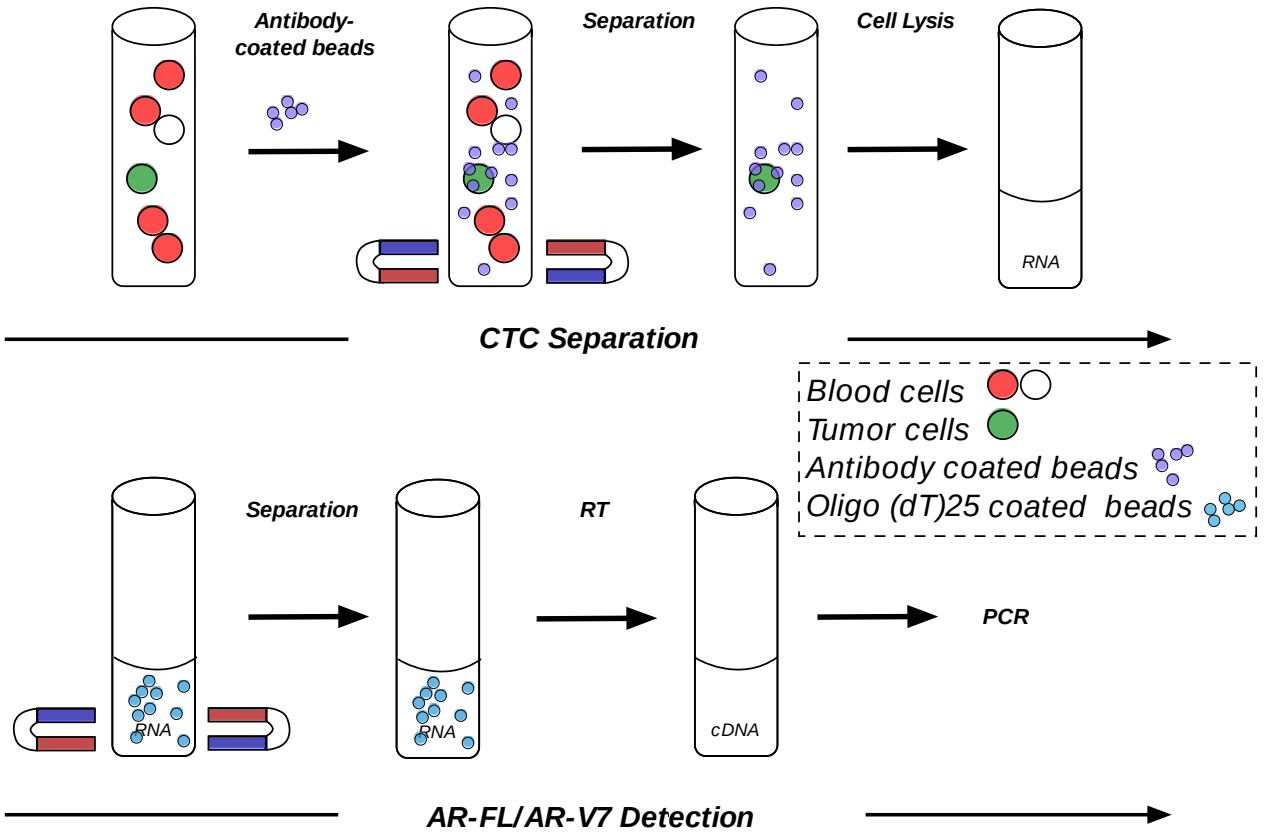
An ARv7 mRNA Assay Performed on CTC in Whole Blood Captured Using the AdnaTest Kit Was Developed by Dr. Jun Luo at Johns Hopkins



Minimal hands-on time, requires no major instrumentation, but Samples must be processed within 4 hours.

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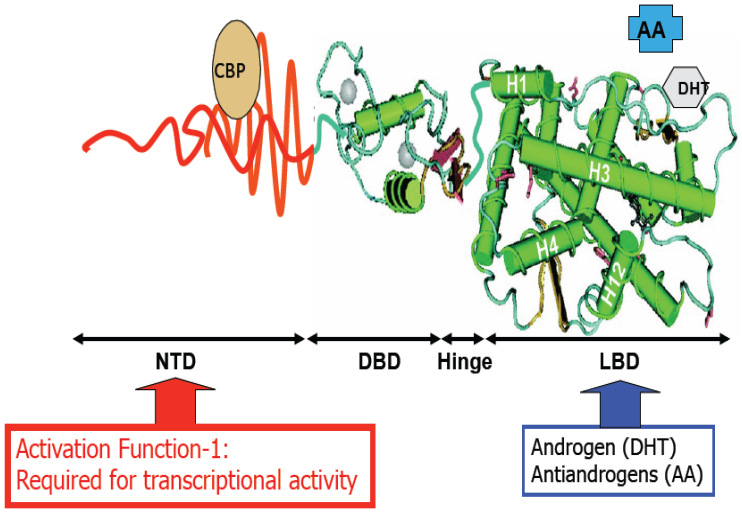
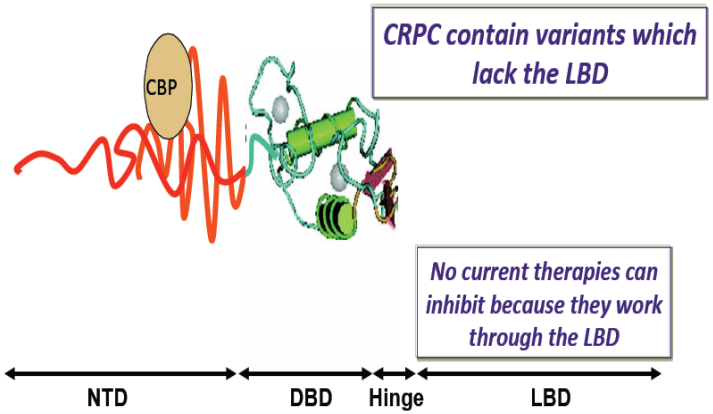
The Presence of the AR V7 Splice Variant Predicts for Sensitivity to Abiraterone and Enzalutamide





Antonarakis et al., ASCO, 2014 - NEJM 371:1028, 2014

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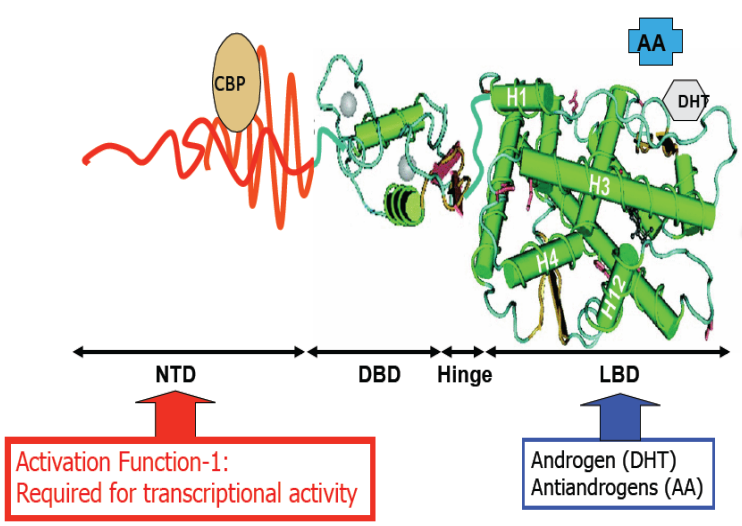

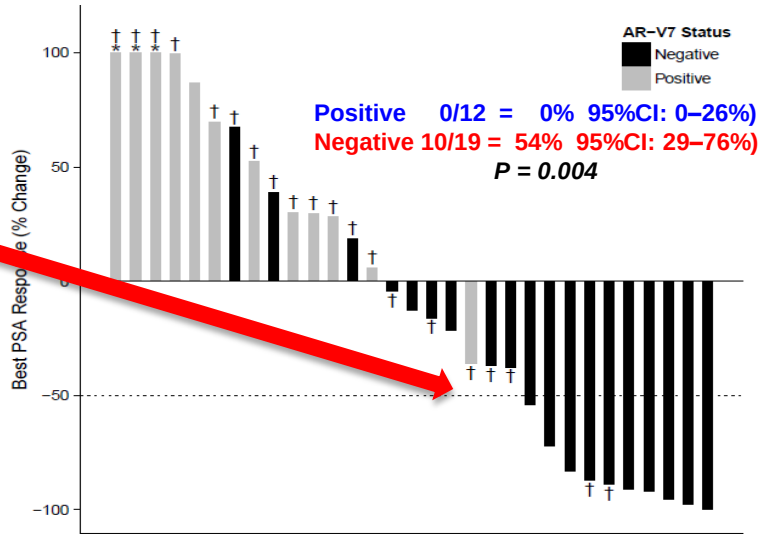

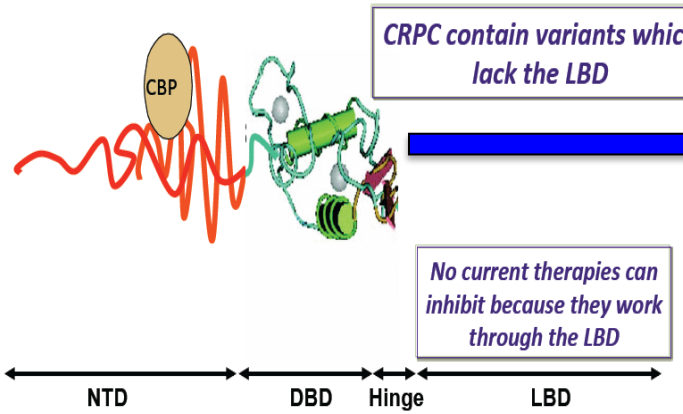

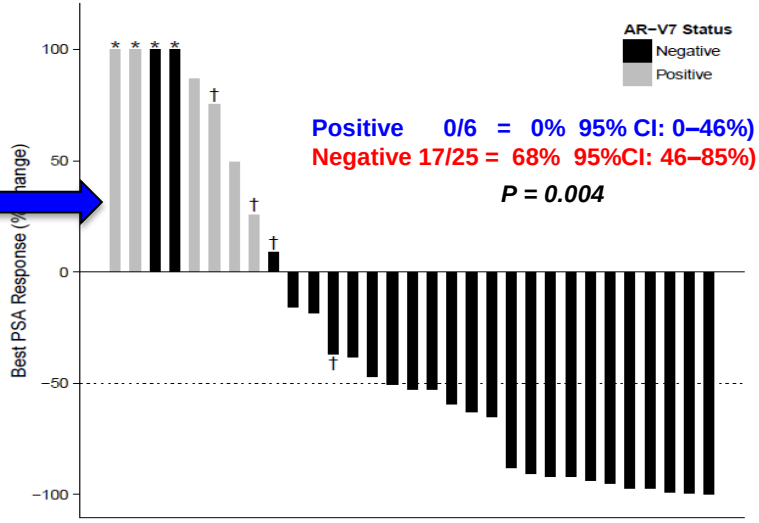

Antonarakis et al., ASCO, 2014 - NEJM 371:1028, 2014

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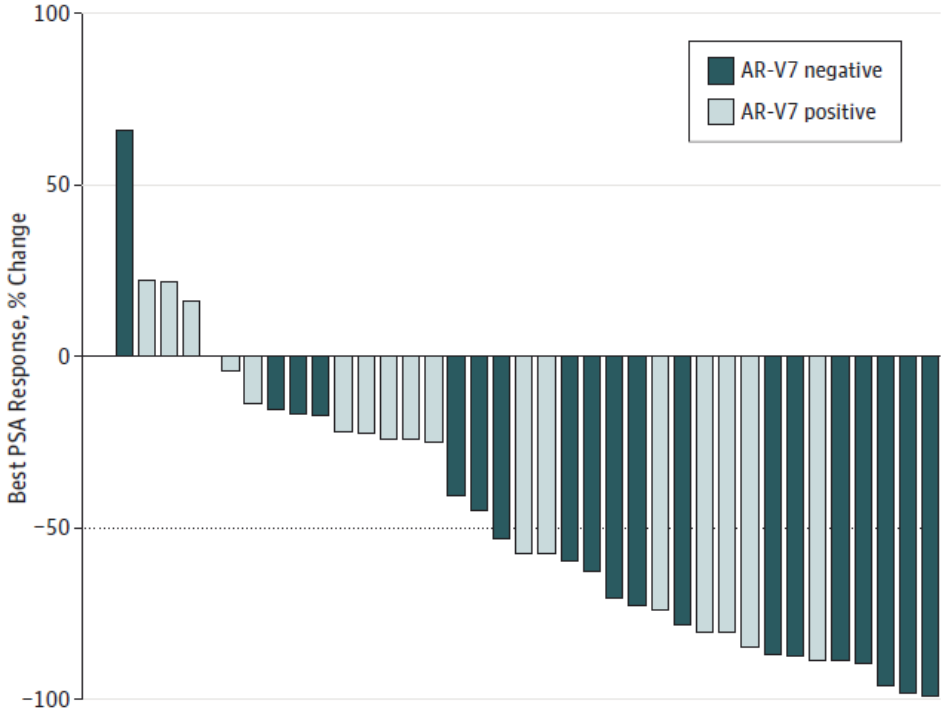
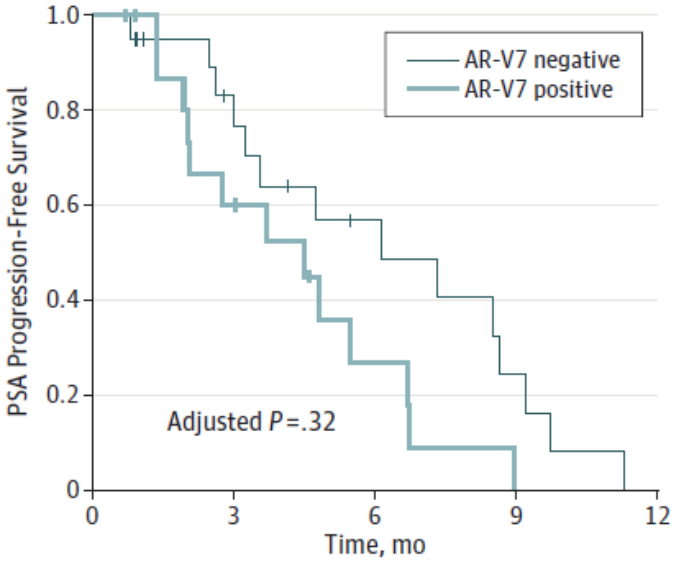
The Same Group Later Reported That ARv7 Status *Does Not* Predict For Taxane Resistance in a Pilot Study





Antonarakis ES et al., JAMA Oncol 1:592, 2015

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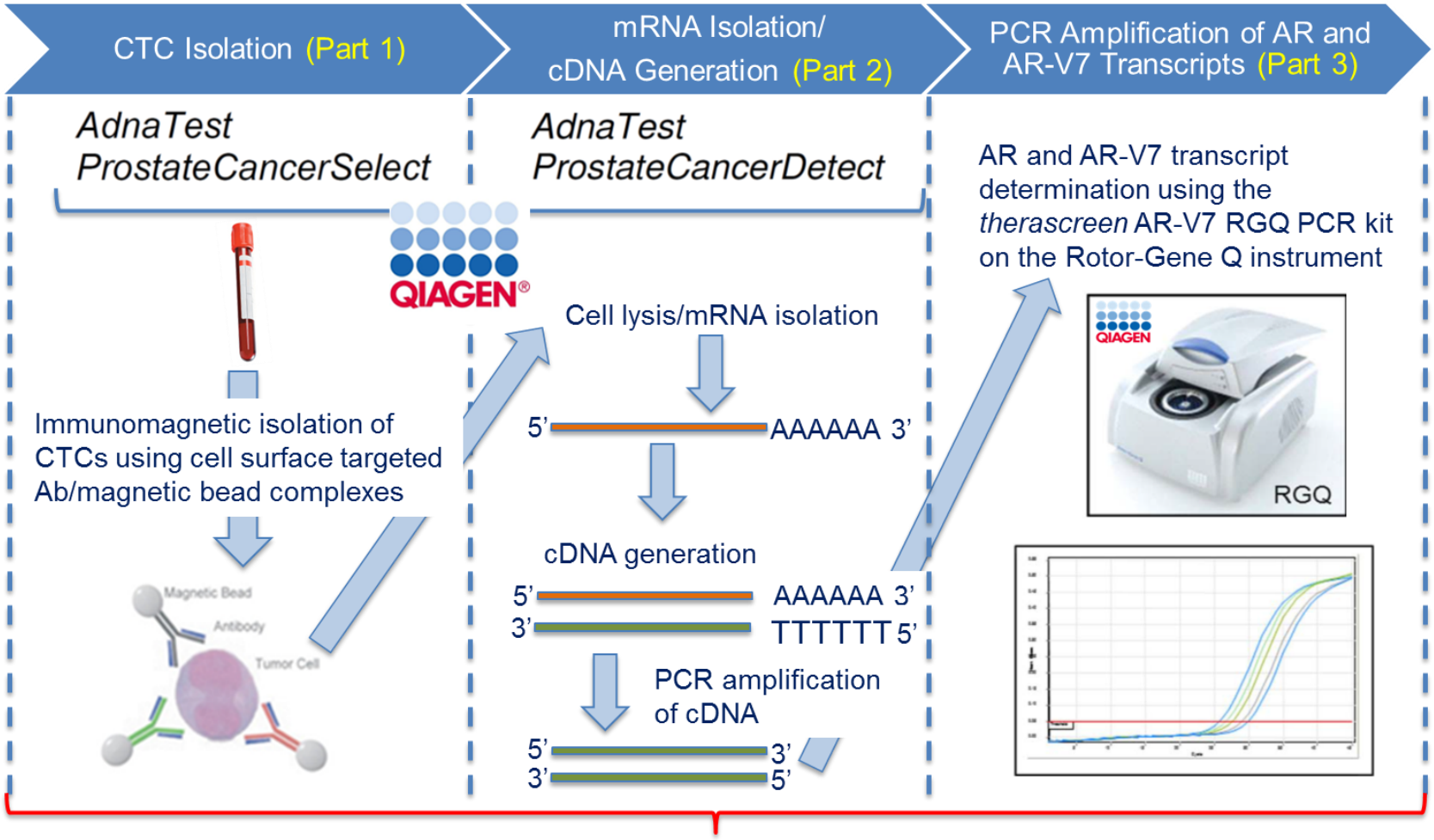
The JHU v7 Assay Was Licensed To Qiagen® Who Developed and Validated a Positive Selection Circulating Tumor Cell Assay



Courtesy of Karen Ferrante

ARMOR3-SV: First Precision Medicine Prostate Cancer Registration Trial; More Than 10 Patients Are Screened to Get 1 Potentially Eligible

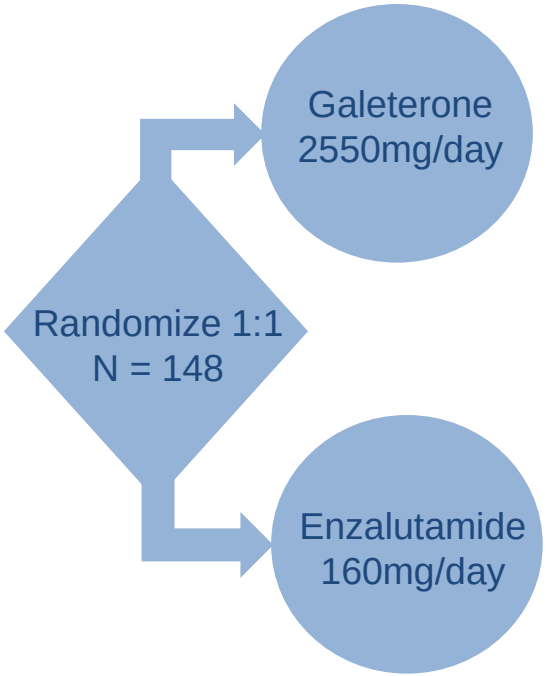
Unique trial design finalized in consultation with FDA and EMA

Key Inclusion:

- Progressive metastatic (M1) disease on androgen deprivation therapy based on PCWG2
- **Detectable AR-V7 from CTCs**
- ECOG 0 or 1

Key Exclusion:

- Prior treatment with second generation anti-androgens (e.g. Zytiga, Xtandi)
- Prior treatment with chemotherapy for CRPC



Primary Endpoint:

- Radiographic Progression Free Survival (rPFS)

Secondary Endpoints:

- Time to cytotoxic therapy
- Overall Survival (OS)
- PSA Changes
- Safety

- Independent Data Monitoring Committee
- Powered to detect an **82% increase in median rPFS** (blinded central radiographic review)

tōkai

Mary Ellen-Taplin, DFCI and Bruce Montgomery, U Wash, P.I.s

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3. **A nuclear localized (NL) single cell ARv7 assay: analytics.**
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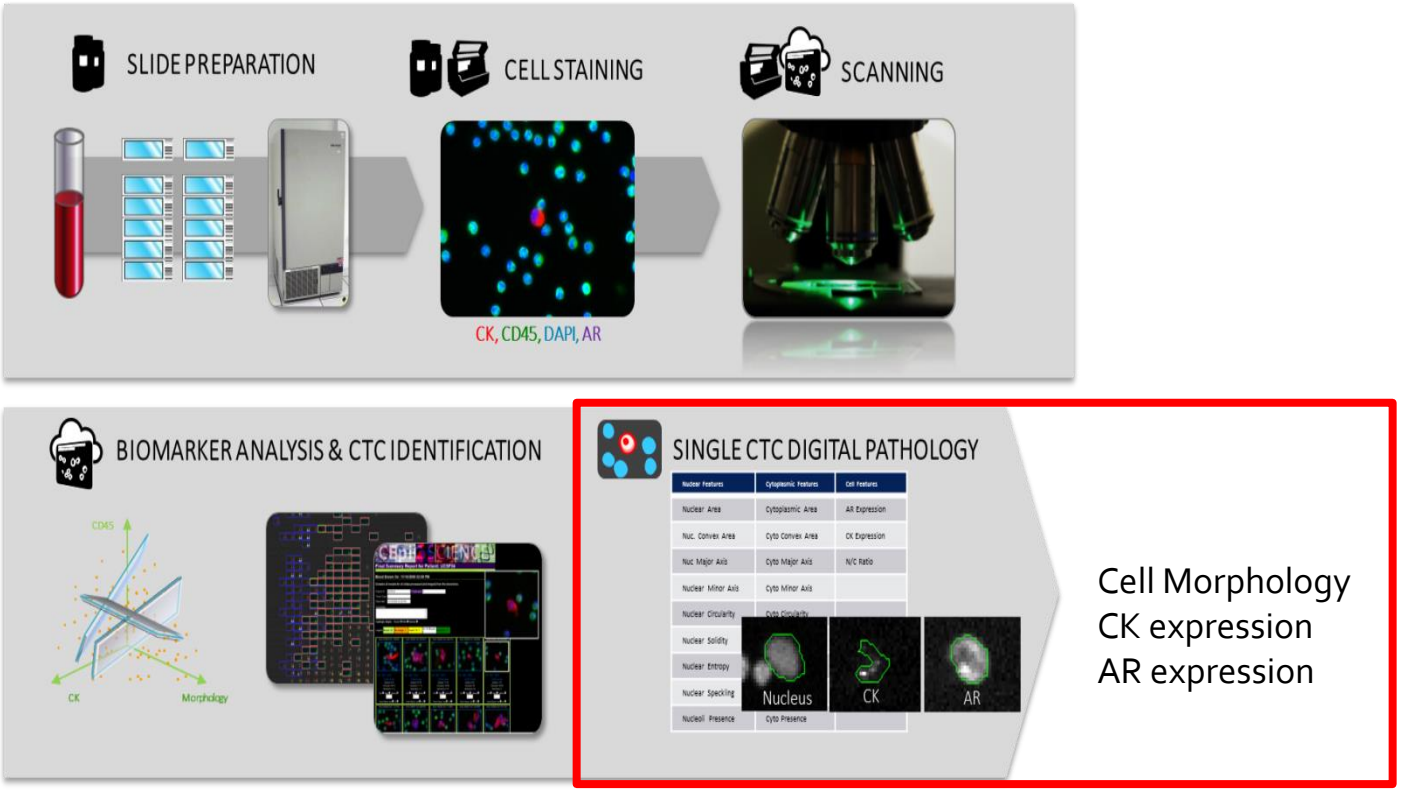
- We are using a non-selection CTC assay that enables the identification of a range of cell types …

- evaluating samples obtained at decision points in management.

With The EPIC Sciences No Selection Platform the Buffy Coat is Deposited, Stained and Analyzed on a Cell by Cell Basis



No cell selection enables characterization of any cell type present: inclusive of CK-, small, apoptotic and CTC clusters.

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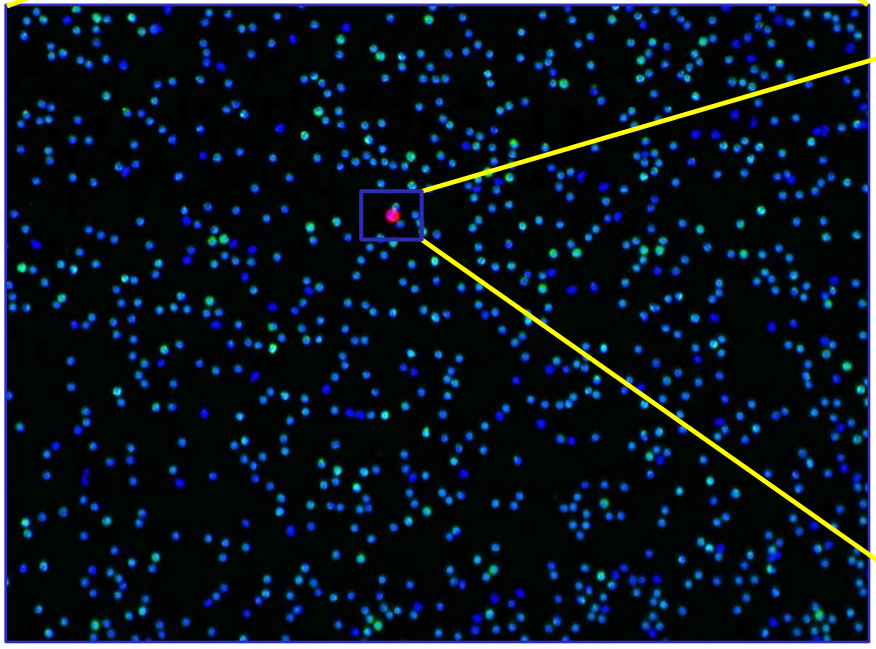
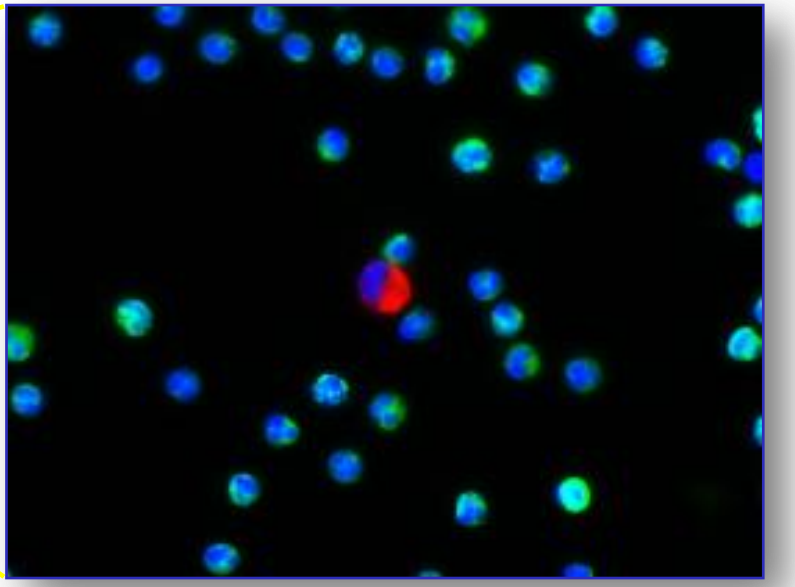
The Scanning Platform is Trained to Recognize Specific Phenotypic and Fluorescence Based Features –Below is a "Traditional" CTC

Cytokeratin+
DAPI+
CD45neg
AR

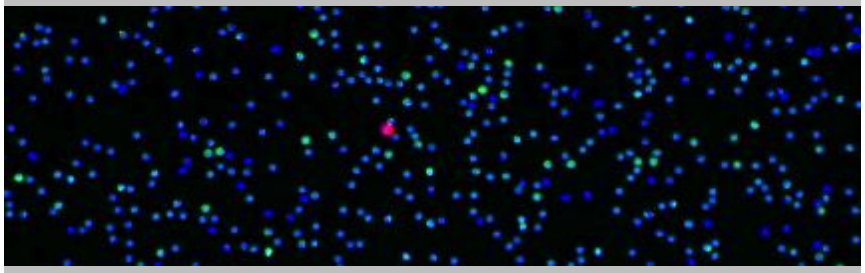
From slide to CTC in *High Definition*



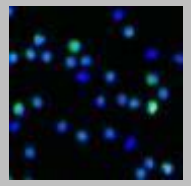


Several Distinct Cell Types Are Identified in mCRPC Patients in Addition to Traditional CTC: Many are Missed by EpCAM Based Capture Methods

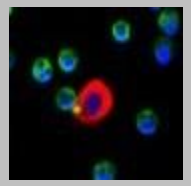
Cells we expected to find

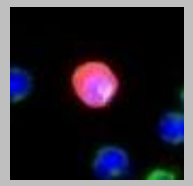


White blood cells



Traditional Epithelial (CK+) CTCs

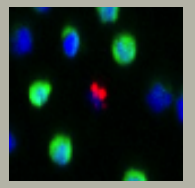




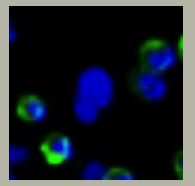
Non-Traditional CTCs

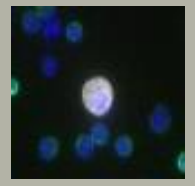
Small CTC

Apoptotic CTC



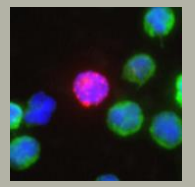
CK- CTC

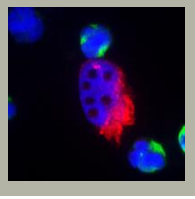




Speckled CK CTC

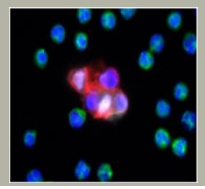
Macro Nucleoli CTC

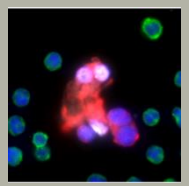




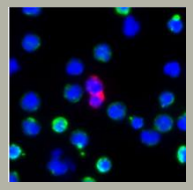
CTC Clusters

Epithelial

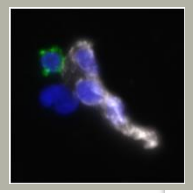




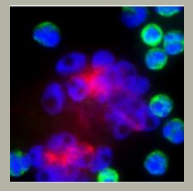
Small



CK-

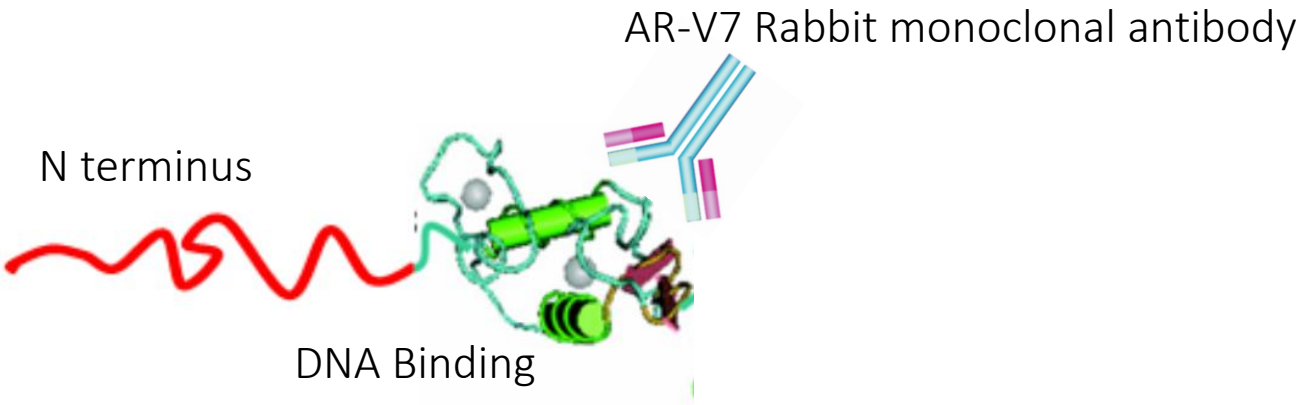


Macro Nucleoli



The Detection and Measurement of AR-V7 Using an Exon Specific Antibody

- Utilization of a rabbit monoclonal antibody (EPR15656, Abcam, Burlingame, CA)
  - Targets the cryptic exon of AR exposed only in presence of AR-V7 splice variant
  - Scored by expression level and nuclear localization



Scher et al. JAMA Oncology 2016 [Epub: June 4]

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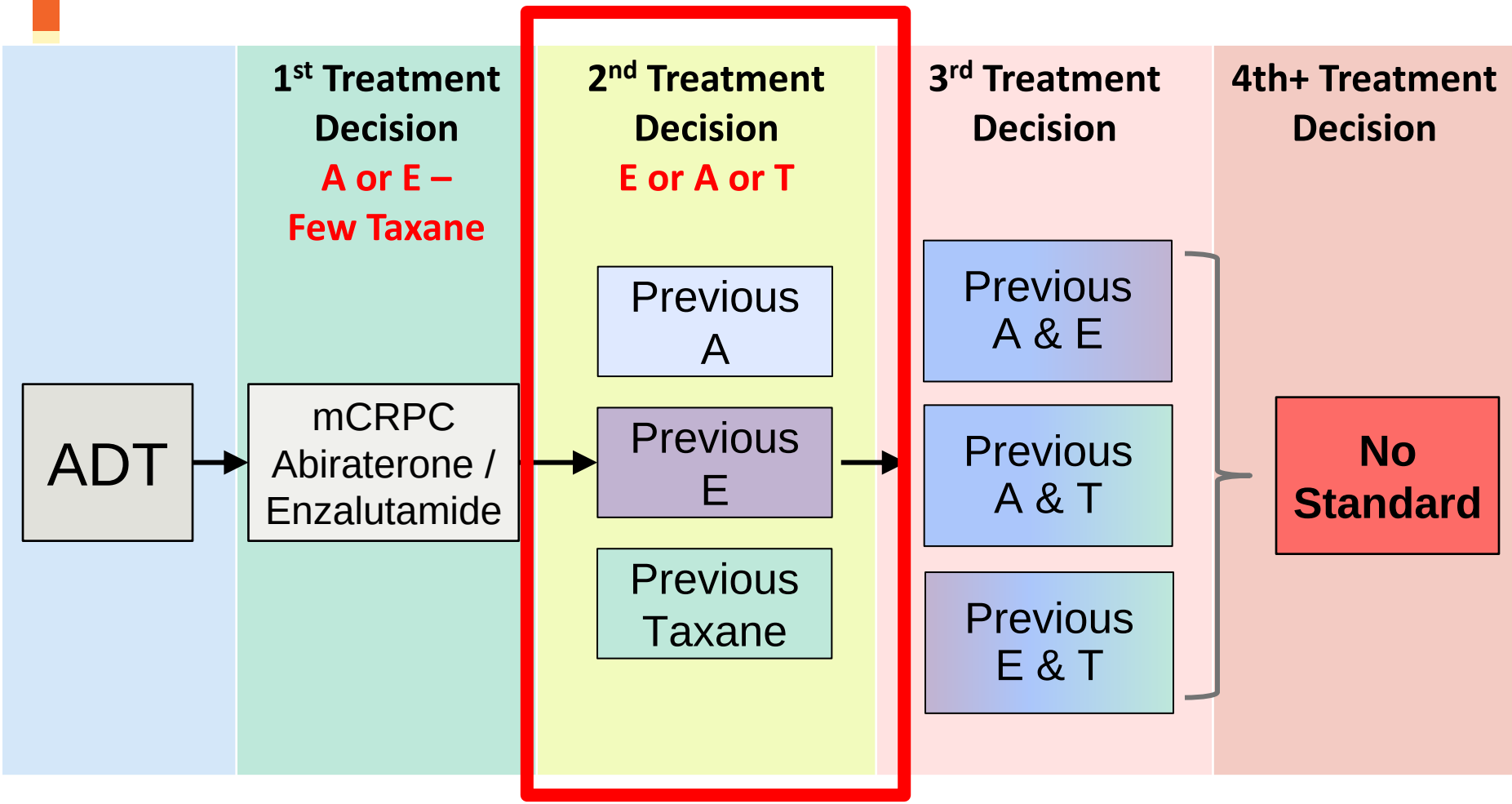
4. **Clinical testing of the NL single cell ArV7 assay.**

Clinical Validation Focused on Decision Points Where Approved Agents Were Prescribed at the Discretion of the Treating Physician to Inform Clinical Utility



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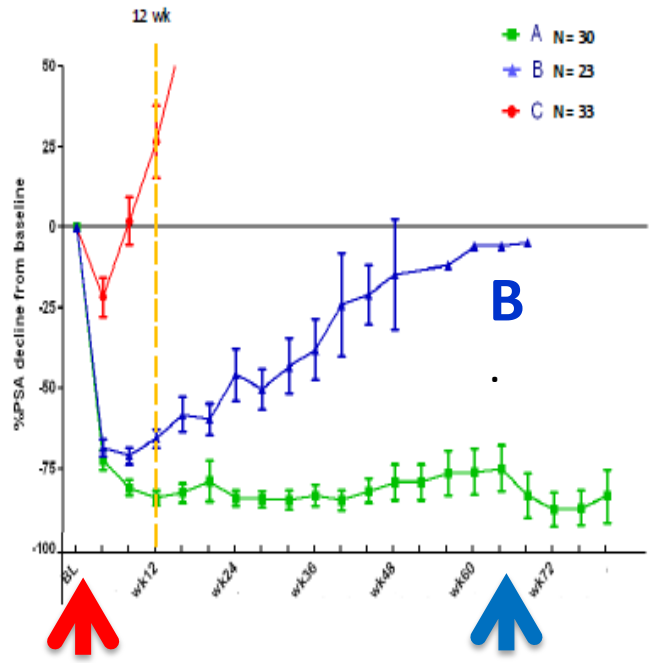
Sensitivity to Therapy Was Categorized by Changes in PSA: *Early* - Resistant or Sensitive and *Later* - Acquired Resistance or Sensitive



Characteristic	All Samples
Number of Baseline Samples	193
Age, years	68 (45 – 91)
Prior Hormone Therapies*	
1 - 2 lines	67 (34.7%)
3 lines	50 (25%)
≥ 4 lines	76 (39%)
Chemotherapy Status	
Chemo-naïve	120 (62%)
Chemo-exposed	73 (38%)
Metastatic Disease	
Bone Only	58 (30%)
Lymph Node (LN) Only**	23 (12%)
Bone & LN	69 (36%)
Bone & Visceral ± LN**	43 (22%)
Laboratory Measures	
PSA, ng/mL	37.7 (0.1 – 3728.2)
Hgb, (g/dl)	12.1 (7.0 – 15.0)
ALK, (unit/L)	111 (25 – 2170)
LDH, (unit/L)	222.5 (123 – 1293)
ALB, (g/dl)	4.2 (3.1 – 4.9)

Importantly, the biology of the disease at Point A will differ from Point B.

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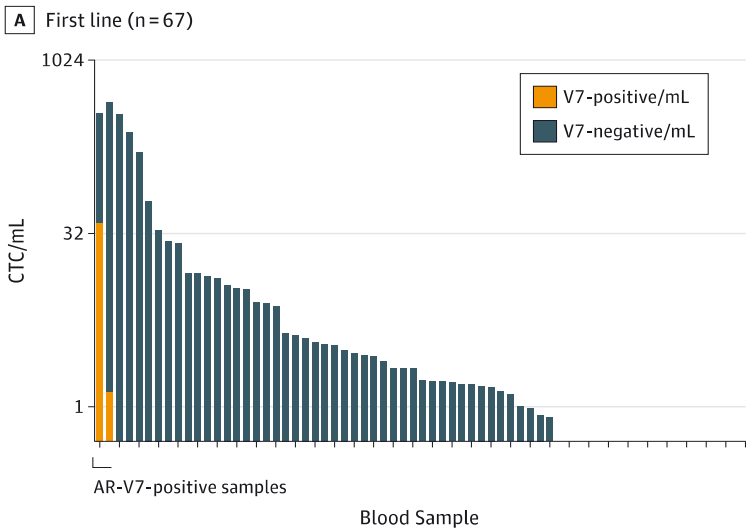
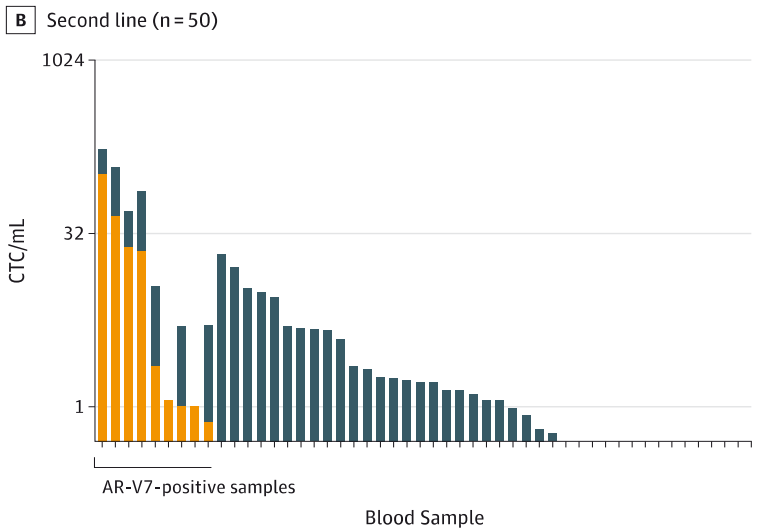
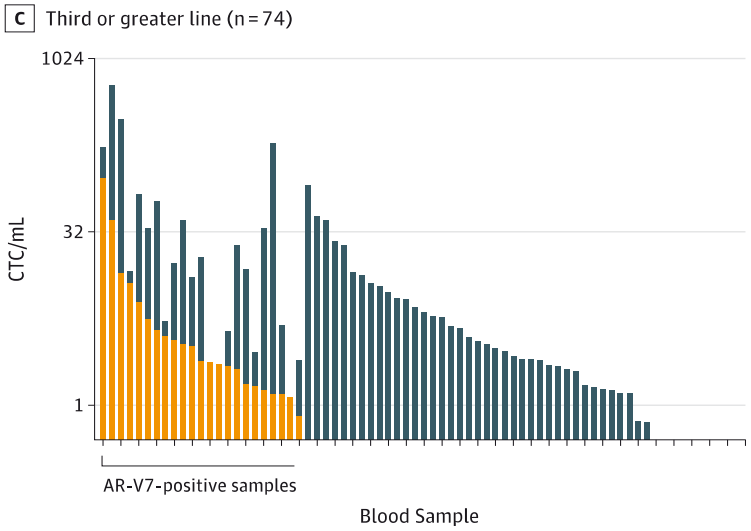
The Frequency of Detection and the Prevalence of the AR-v7 Protein In Individual Patient CTCs Increased by Line of Therapy







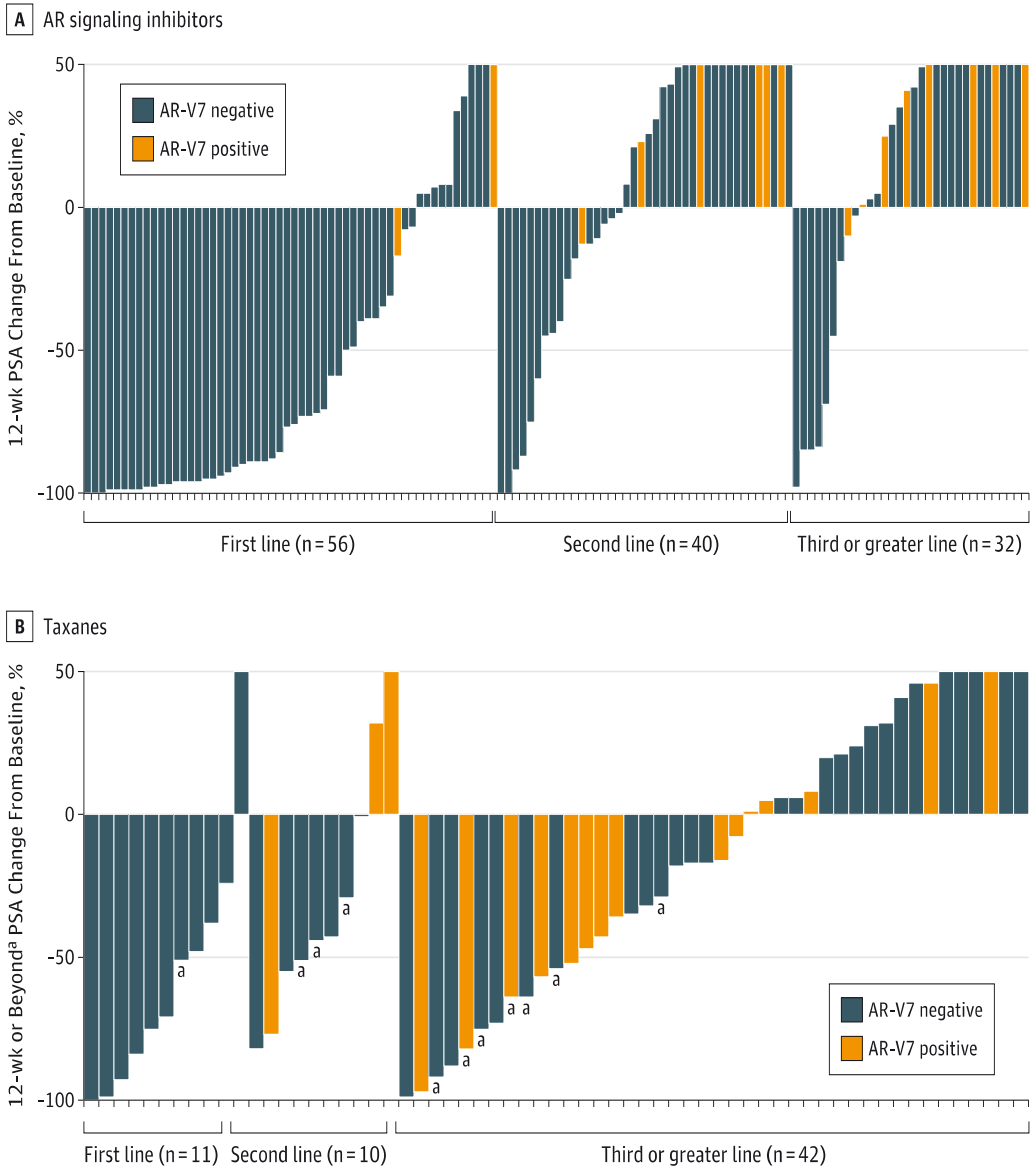
D Incidence and subclonal contribution of AR-V7-positive CTCs by line of therapy

Line of Tx in mCRPC setting (n samples)	First (n = 67)	Second (n = 50)	Third or greater (n = 74)
Samples with AR-V7-positive CTCs	2 (3%)	9 (18%)	23 (31%)
AR-V7-positive CTCs in samples with AR-V7-positive CTCs, %, median (range)	5.7% (0.3%-11.2%)	38% (14.3%-100%)	21% (0.5%-100%)

Scher et al. JAMA Oncology 2016 [Epub: June 4]

Memorial Sloan Kettering Cancer Center

The Presence of AR-V7(+) CTCs Predicts a Lack of PSA Response to AR Signaling Inhibitors (A) But Not Taxane Therapy (B)



Scher et al. JAMA Oncology 2016 [Epub: June 4]

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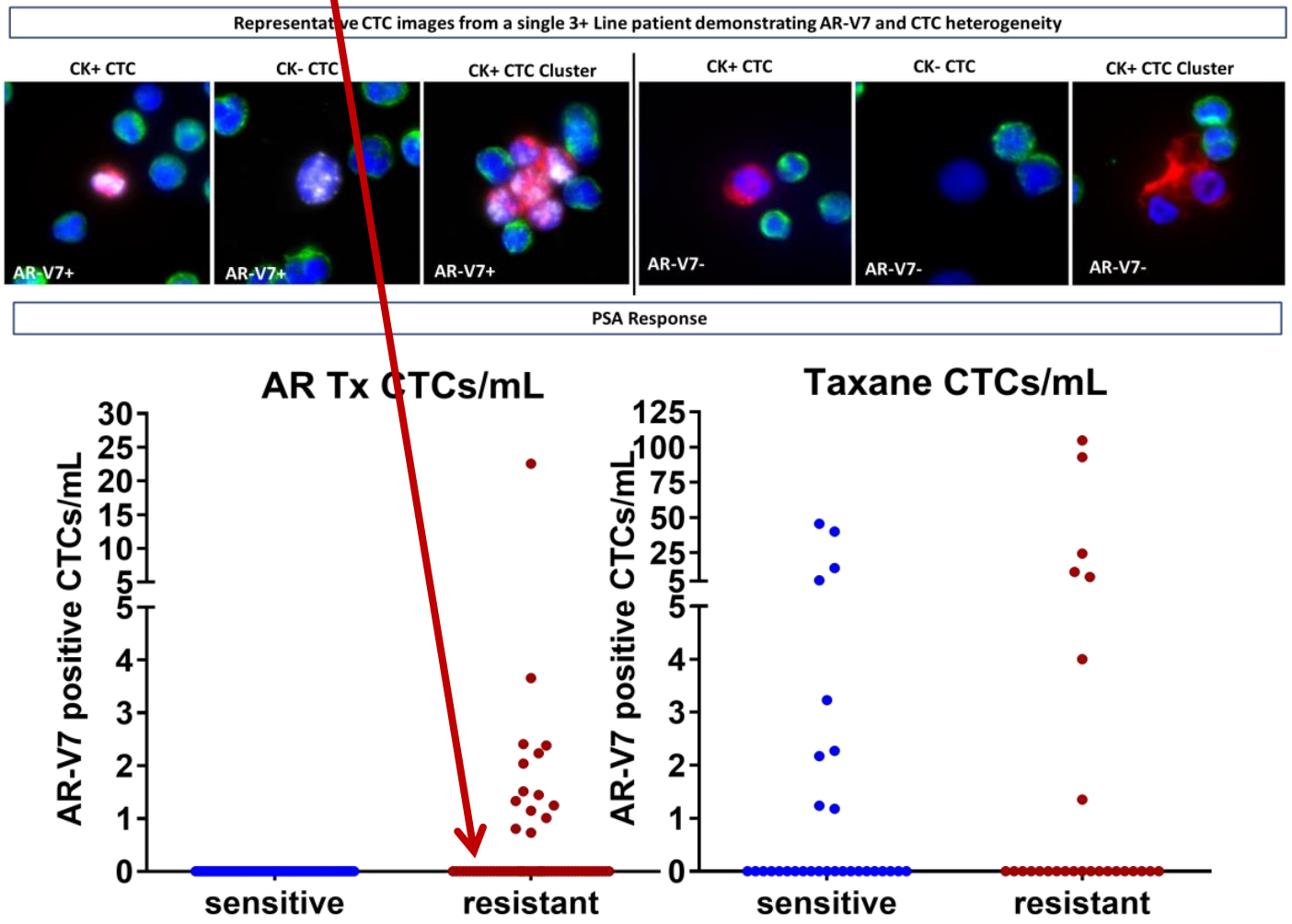
The Presence of AR-V7 Predicted for Resistance to AR Therapies But Many ARv7 Null Patients Did Not Respond: It is Not the Whole Story



Scher et al. JAMA Oncology 2016 [Epub: June 4]

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ARv7 Antibody Staining is Highly *Specific* but Not *Sensitive* for AR Therapy Resistance With No Relation to Taxane Sensitivity

	AR Therapy (N=123)		Taxane Therapy (N=56)	
	PSA Responder	PSA Resistor	PSA Responder	PSA Resistor
AR-v7 Positive	0	15	9	7
AR-v7 Negative	57	51	21	19
Total	57	66	30	26
Performance Estimate	Specificity 57/57 = 100%	Sensitivity 15/66 =22.7%	Specificity 21/30 = 70%	Sensitivity 7/26 = 26.9%
95% CI (Wilson Score Method)	(92.1%, 100%)	(13.7%, 35.0%)	(50.4%, 84.6%)	(12.4%, 48.1%)

Scher et al. JAMA Oncology 2016 [Epub: June 4]

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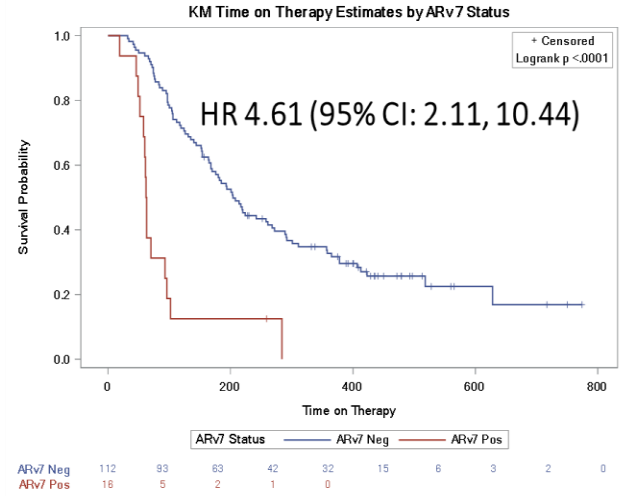
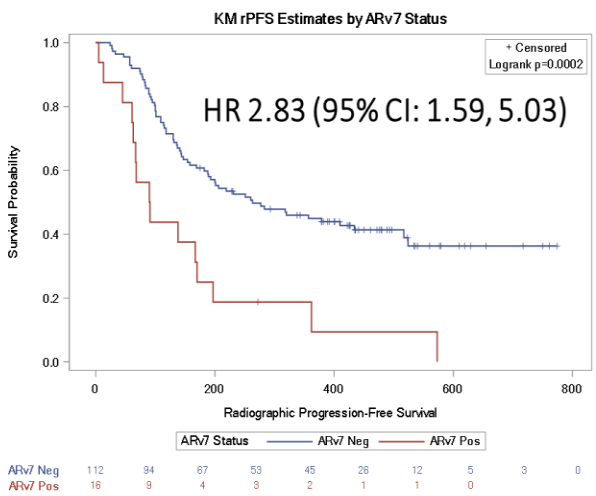
Pre-Therapy AR-V7+ CTCs Predicts for a Shorter Time on Therapy, Radiographic Progression Free Survival and Overall Survival With AR Directed Therapy

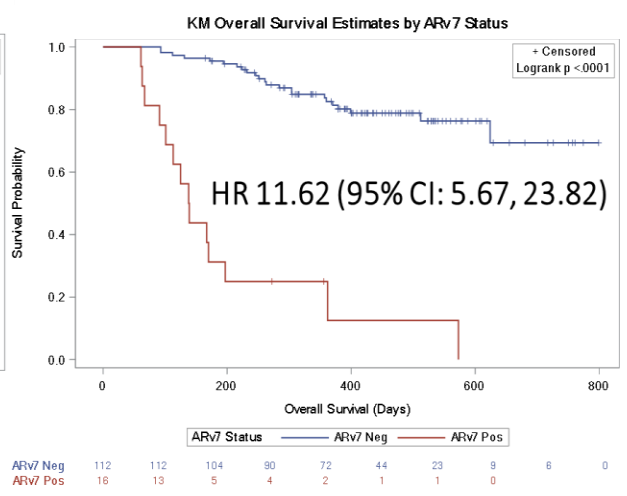
Time on Therapy



Radiographic Progression Free Survival (rPFS)



Overall Survival (OS)



Scher et al. JAMA Oncology 2016 [Epub: June 4]

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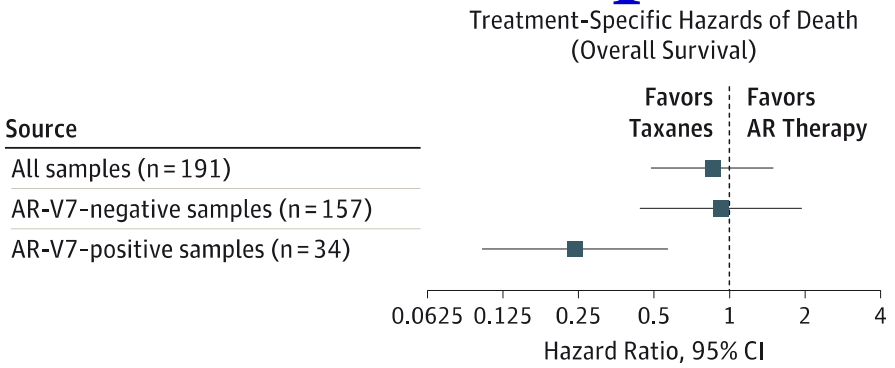
Patients with Pre-Therapy AR-V7(+) CTCs Exhibit Better Overall Survival on Taxanes than AR-Directed Therapies



AR-V7 Therapy Interaction: Multivariable Cox PH Model		
	Comparison	Hazard Ratio (95% CI)
AR-V7 Status and Therapy	AR-V7 positive: Taxane vs AR	0.24 (0.10 to 0.57)
	AR-V7 negative: Taxane vs AR	0.92 (0.44 to 1.95)

Multivariable Cox Proportional Hazard Analysis of Predictors of Overall Survival		
Effect	*P* Value	HR (95% CI)
Line of therapy (3rd or later vs 1st or 2nd)	.16	1.62 (0.83-3.15)
Liver and/or lung metastases pretherapy	.003	2.29 (1.11-4.74)
LDH pretherapy (>250 vs ≤250 U/L)	.006	2.24 (1.26-4.00)
Patient age (>65 vs ≤65 years)	.007	0.48 (0.28-0.83)
Hemoglobin (>12 vs ≤12 g/dL)	.007	0.40 (0.19-0.82)
Therapy (taxane vs AR)	.84	0.86 (0.49-1.50)
AR-V7 status (positive vs negative)	**<.001**	**4.15 (1.76-9.77)**
AR-V7 (positive) taxane interaction	**.035**	**0.24 (0.10-0.57)**

Scher et al. JAMA Oncology 2016 [Epub: June 4]

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ArV7 Biomarker Summary

1. AR-V7 prevalence increases with increased exposure to systemic therapy ($p < 0.0001$) but represents a minority population of total CTCs.

2. AR-V7 expression on CTCs is 100% specific (100% PPV) for *de novo resistance*, shorter time on drug (HR=4.61, $p<0.0001$), shorter rPFS (HR=2.92, $p=0.0002$), and shorter OS (HR=11.44 , $p<0.0001$) even when only 1 cell was detected.

   yet, is only part of the resistance story.

3. A treatment specific effect for taxane therapy was shown when AR-V7 was detected (HR 0.24 (0.10-0.57) but not for AR-V7 negative patients.

4. Assay results are available in a median of 48 hours after receipt of the specimen, in time to inform a treatment decision.

The test is now CLIA certified in California.

A prospective validation trial is in development.

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Gordon Research Conferences
frontiers of science

2016 Gordon Research Conference: Circulating Tumor Cells and other Tumor Products in the Bloodstream

Mount Holyoke College, MA

August 7-12, 2016

Seats still available!

http://www.grc.org/programs.aspx?id=16786

FDA-AACR Workshop on Liquid Biopsies: Session I
Cell-free DNA: Uses and steps needed for clinical utility

Ben Ho Park, M.D., Ph.D.
Department of Oncology
Johns Hopkins University
July 2016

Financial Disclosures

- **I have financial relationships with commercial entities that are relevant to the content of this presentation.**
  - **Scientific Advisory Board Member for Horizon Discovery, LTD**
  - **Royalties from Horizon Discovery, LTD**
  - **Scientific Advisory Board Member for Loxo Oncology**
  - **Ownership interest in Loxo Oncology**
  - **Research contract with Genomic Health, Inc.**
  - **Research contract with and consultant for Foundation Medicine, Inc.**
- **I will not reference unlabeled or unapproved uses of drugs or other products.**

Objectives

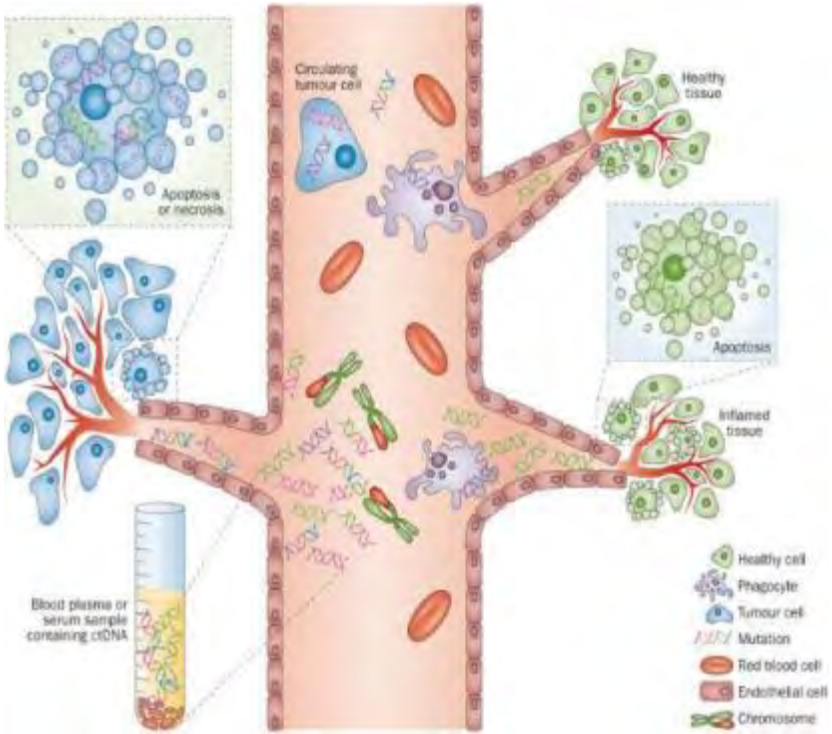
- **Describe the difference between circulating cell-free DNA, circulating tumor DNA and plasma tumor DNA**
- **Describe how circulating plasma tumor DNA can be used as biomarkers to guide individual therapy decisions for clinical oncology**

Overview

- Background on cell-free DNA and technologies to detect this analyte
- Steps needed to adopt cell-free DNA as routine tests for clinical practice
- Studies in breast oncology
- Conclusions

What is circulating cell-free DNA, circulating tumor DNA and plasma tumor DNA??

- Circulating cell-free DNA (ccfDNA or cfDNA) refers to small DNA fragments shed by all cells into the circulation. This includes blood, lymph, urine, saliva, CSF, etc.
- Circulating tumor DNA (ctDNA) refers to DNA shed specifically by tumor cells into the circulation.
- Plasma tumor DNA (ptDNA) refers to the plasma component of ctDNA. Plasma has been shown to be a superior substrate versus serum for ctDNA analysis.



Crowley et al., Nat.Rev. Clin. Onc. 2013

Potential Clinical uses of cfDNA

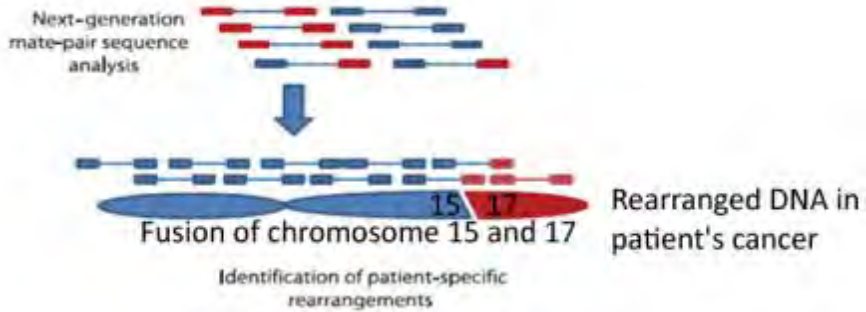
- Screening for genetic alterations in a fetus using maternal blood
  - Already in clinical use
- Biomarkers for inflammatory states
  - Acute coronary events, autoimmune disease
- Solid tumor organ rejection based on DNA differences between donor and recipient
- Biomarkers for graft vs. host disease
- Clinical biomarkers for oncology
  - Assessing response to therapies, identifying drug resistance clones, detecting minimal residual disease

Technical challenges of identifying ptDNA

- The fraction of ptDNA in total plasma DNA is often small
- The quality of plasma DNA is highly dependent on how it is processed.
  - Double spin, within 1 to 2 hours otherwise cellular “bursting” artifactually adds more genomic DNA into the cell-free space
  - May require use of cell stabilizing tubes
- Assaying for rare mutation detection is akin to looking for needles in a haystack

Methods for measuring ptDNA

- Digital PCR
  - BEAMing (Beads, Emulsions, Amplification, Magnetics)
  - Droplet digital PCR (Bio-Rad, RainDance)
  - Microfluidics based digital PCR
- Deep (high coverage/fold) next gen sequencing (an evolution of digital PCR):
    - Tagged Amplicon, SAFE Seqs, Duplex Sequencing, Circle sequencing
- Can measure somatic mutations, structural rearrangements or both



Where is the “mutant” George?

From Matthias Holdhoff, M.D., Ph.D.

Conventional “Analog” Approach

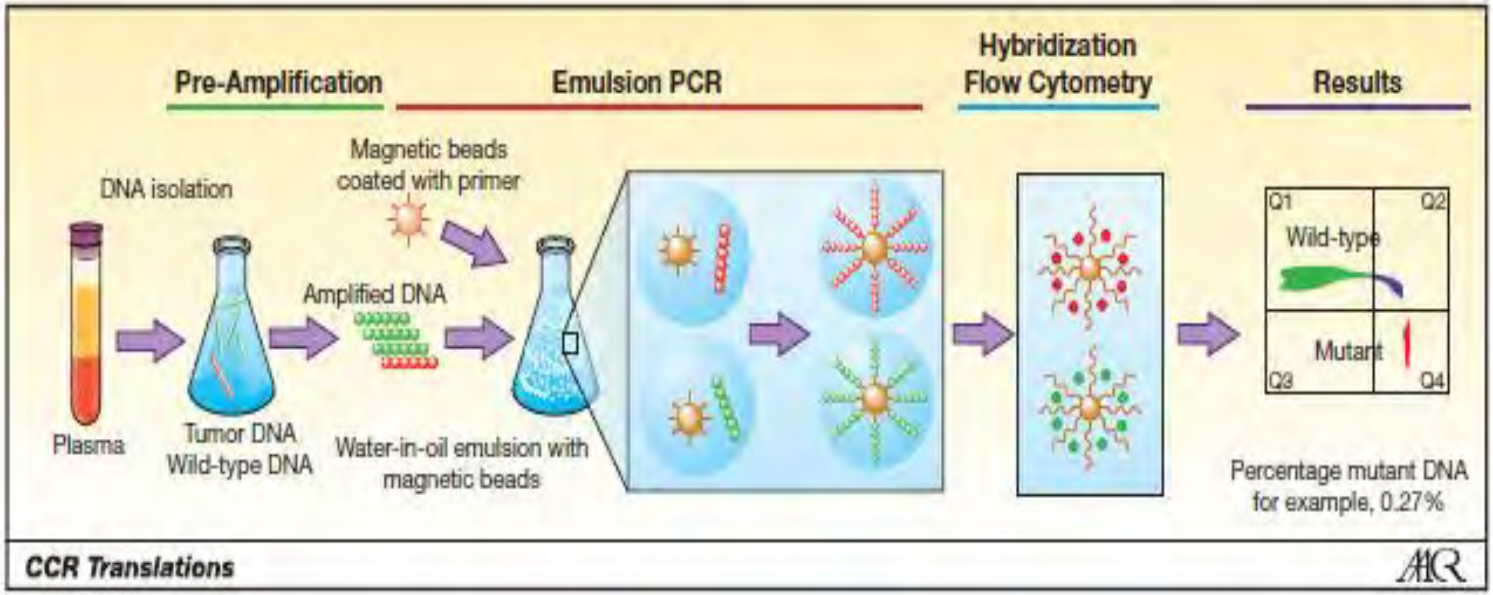
Combine all the pictures into a single mixture

From Matthias Holdhoff, M.D., Ph.D.

From Matthias Holdhoff, M.D., Ph.D.

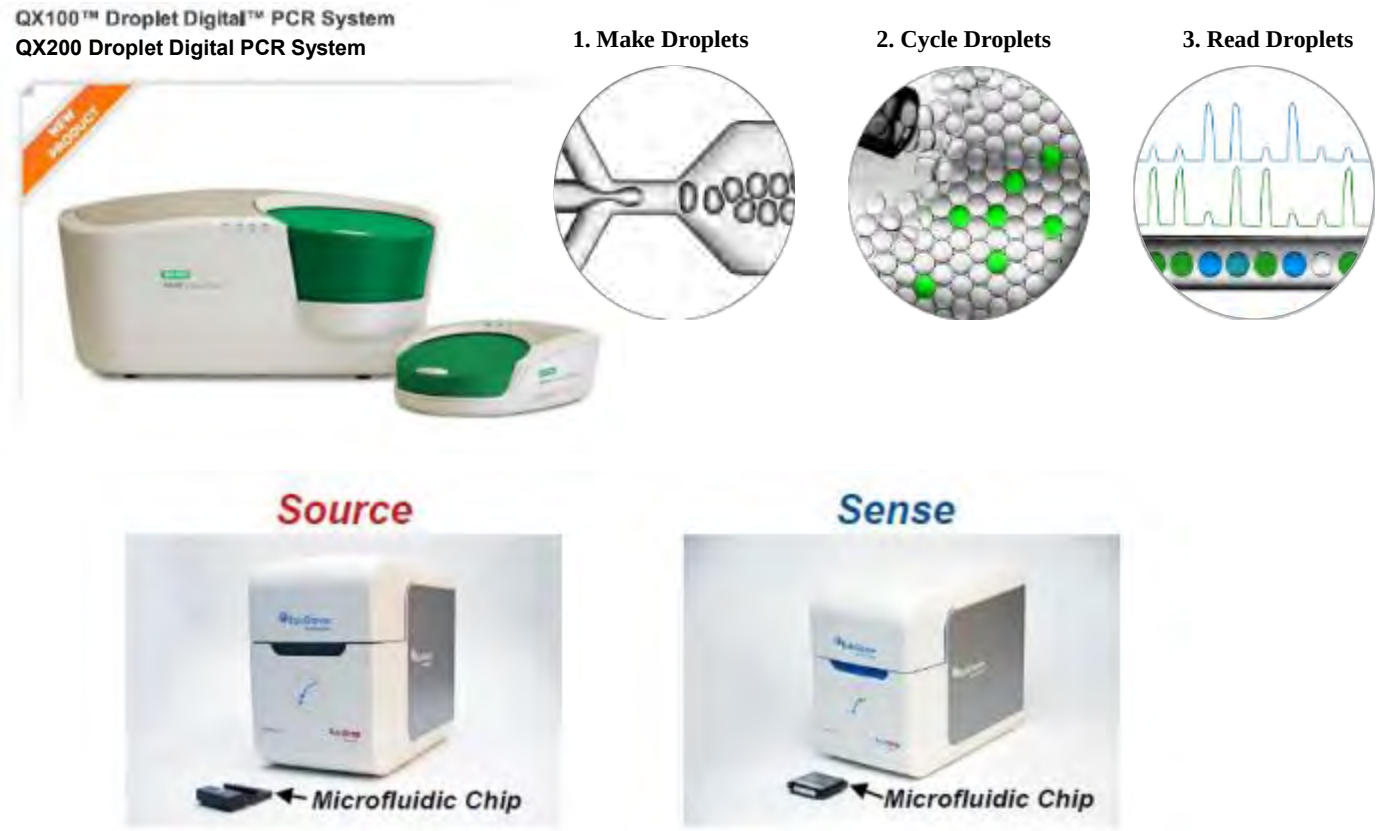
Principle of BEAMing: the first high throughput digital PCR platform

Lauring and Park CCR 2011



A novel and highly sensitive technique developed by the Vogelstein lab at Johns Hopkins, called BEAMing (Beads, Emulsification, Amplification, and Magnetics) can detect mutations quantitatively and qualitatively

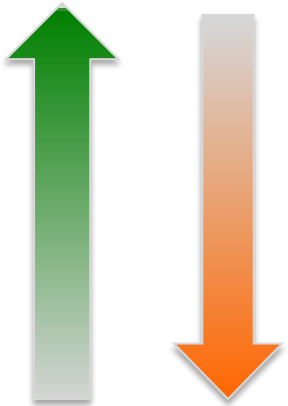
Droplet digital PCR and ptDNA detection



Next Gen Sequencing

- Can assay for single locus/amplicon
- Can use cancer gene panels
- Whole exome sequencing
- Whole genome sequencing

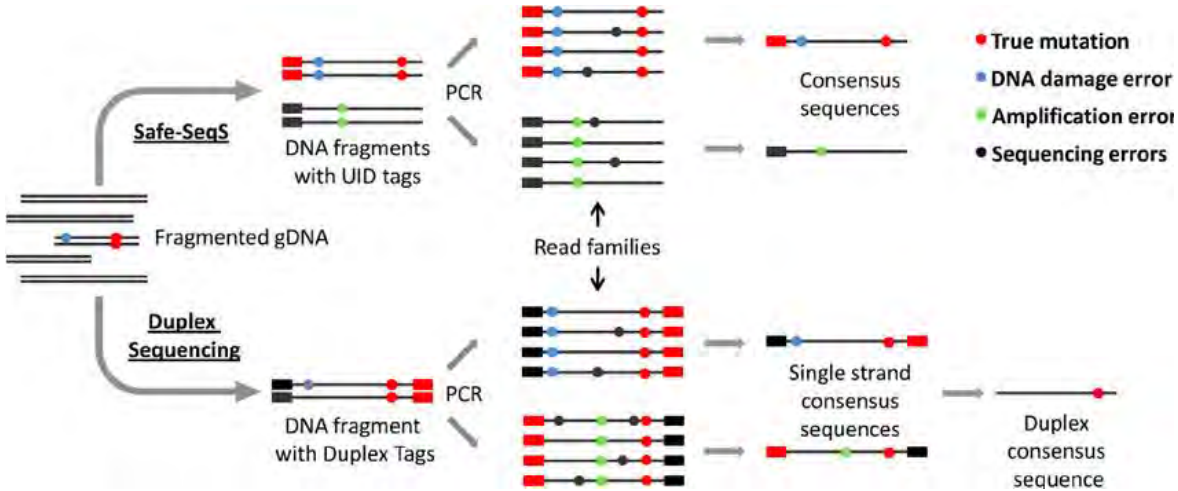
Limit of detection



Number of Genes/Genomes

Ultra-deep sequencing presents bioinformatics challenges

- Often time, ptDNA is present at 0.01% allele frequency
- Inherent “noise” of PCR and NGS makes reliable detection at this low frequency problematic
- “barcoding” individual DNA fragments can help mitigate this problem

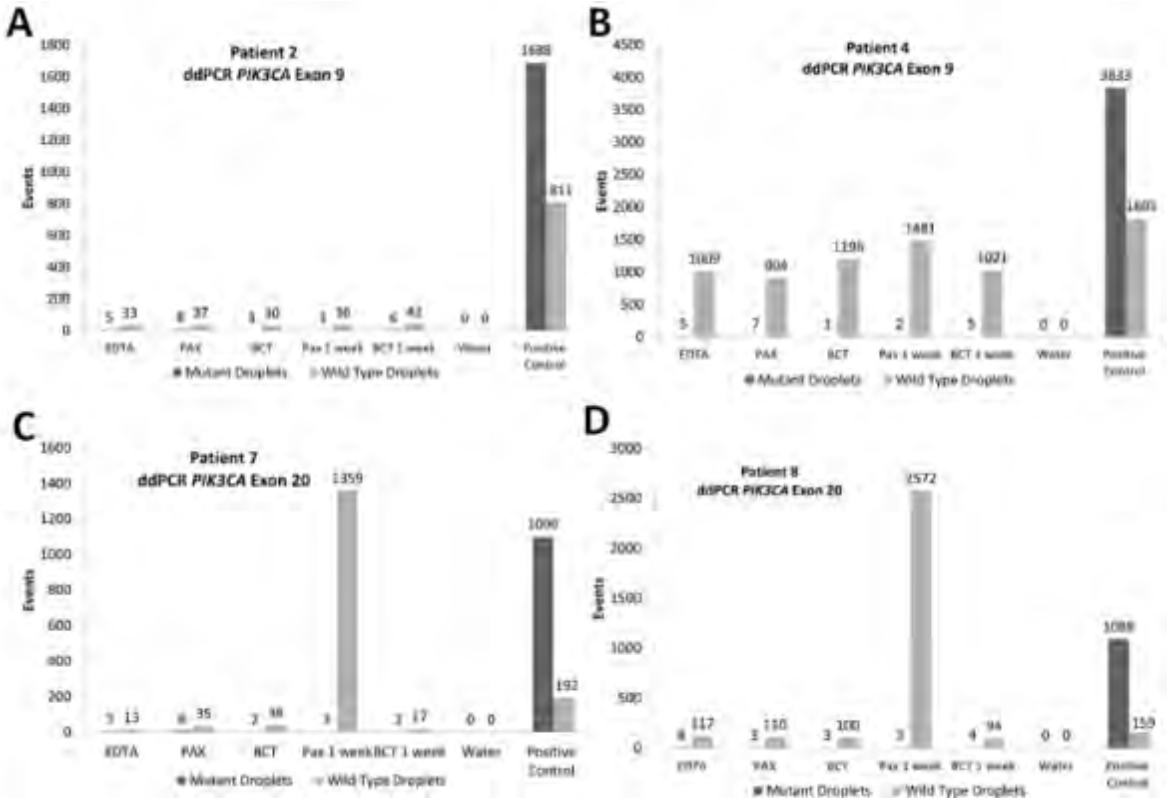


Steps needed for getting these tests to clinical use

- “A bad test can be just as dangerous as a bad drug” Daniel Hayes, M.D., 2016 ASCO President
- Preanalytical/analytic validation: How reproducible is the assay? Have parameters such as analyte preparation and handling been optimized? Correlative studies? etc.
- Clinical validation: Does the assay/biomarker separate out distinct groups of patients? Prognostic value?
- Clinical utility: Does the assay/biomarker actually allow for an intervention that can change outcomes?

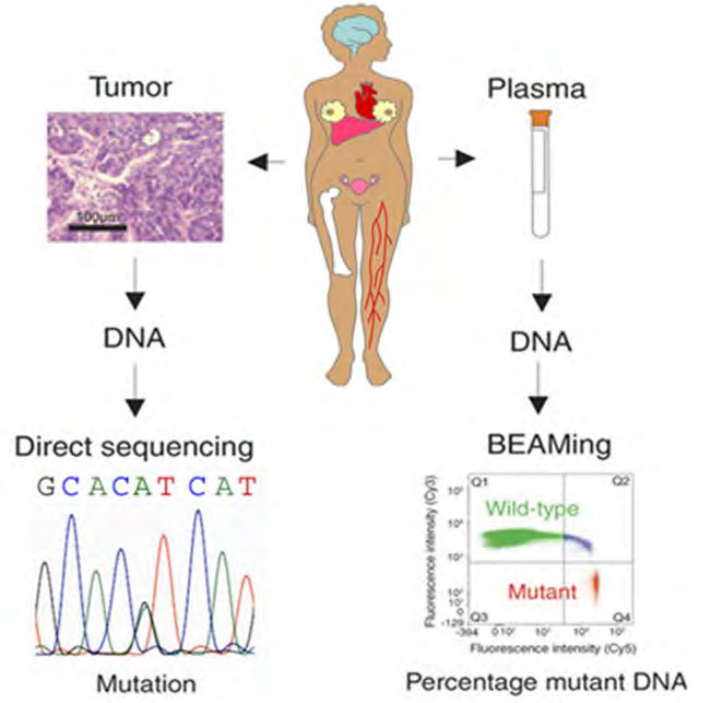
Preanalytical/analytical validation

- Can cell-stabilizing tubes preserve plasma DNA integrity? Yes.



Analytical validation: Concordance study in metastatic breast cancer patients

- Retrospective and prospective cohort of metastatic breast cancer patients comparing tumor tissue with ptDNA
- BEAMing employed for these initial studies

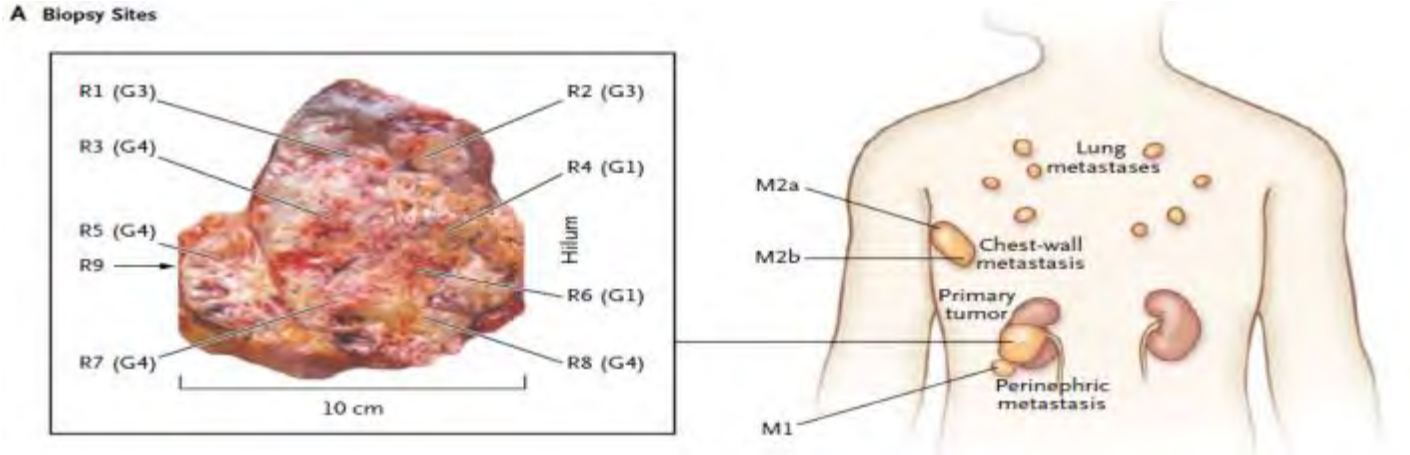


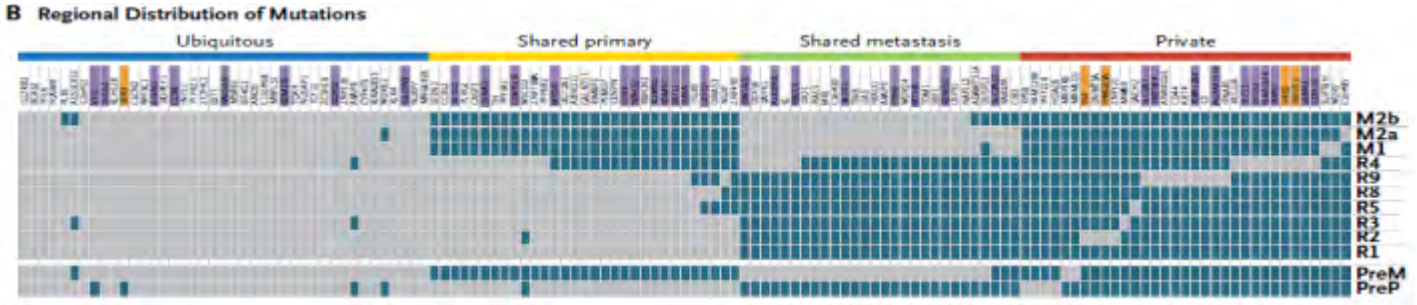
Higgins et al., ClinCanRes, 2012

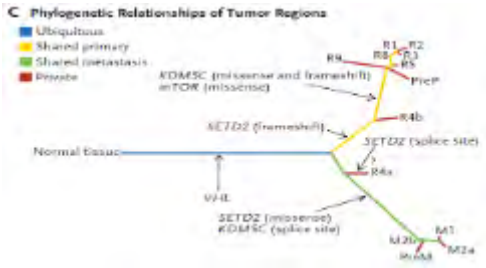
Timing matters

- Discordant results ***only*** in patients with greater than 3 years between archival tissue and blood draw in prospective study
- 100% concordance in paired samples when biopsy and blood draw taken concurrently (retrospective analysis)
- 100% concordance when BEAMing and sequencing used on the same DNA
- PIK3CA mutational status can change or “convert” with disease recurrence; tumor heterogeneity

Tumor Mutational Heterogeneity







Gerlinger et al, NEJM 366:883, 2012

ptDNA as a liquid biopsy

- In metastatic breast cancer, mutations can be reliably detected within ptDNA
- Tumor heterogeneity may lead to clonal evolution making metastatic biopsies necessary for current and future targeted therapies (e.g. recent studies of *ESR1* mutations)
- This further supports the clinical importance and utility of ptDNA mutation detection

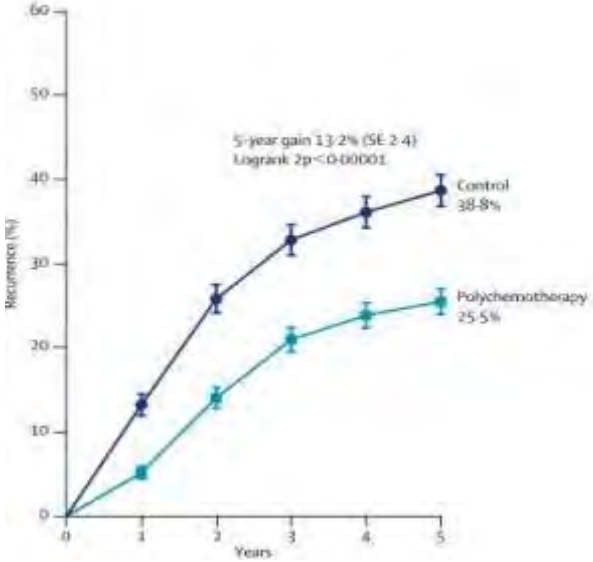
Metastatic vs. early stage breast cancer

- Levels of ptDNA and circulating free DNA (cfDNA) are increased in metastatic patients making detection of ptDNA relatively easy
- Early stage (non-metastatic) cancer patients have less cfDNA and ptDNA but the ability to detect ptDNA in these patients has great clinical utility

Goals of systemic therapies

- Metastatic – Prolong life and treat symptoms
- Adjuvant – generally therapies given after primary modality (surgery) to eliminate ***micrometastatic*** disease and improve cure rates, i.e. goal is *curative.*
  - Each year more than 210,000 cases of breast cancer are diagnosed in the United States
  - Of these patients 150,000 or more are **candidates** for adjuvant therapies

Adjuvant systemic therapy for breast cancer



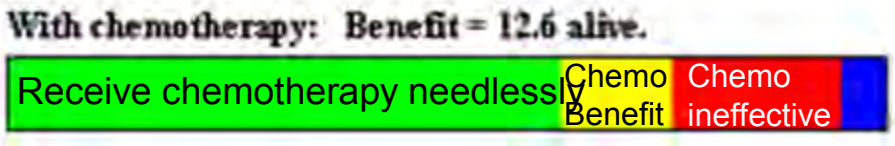
Lancet, 2005

Adjuvant! Online

Decisions to treat are based on results from clinical trials

- Large randomized multi-institutional trials comparing adjuvant therapy vs. controls
- Takes years to prove effectiveness
- A consequence of this type of study is that it leads to “overtreatment” and toxicity

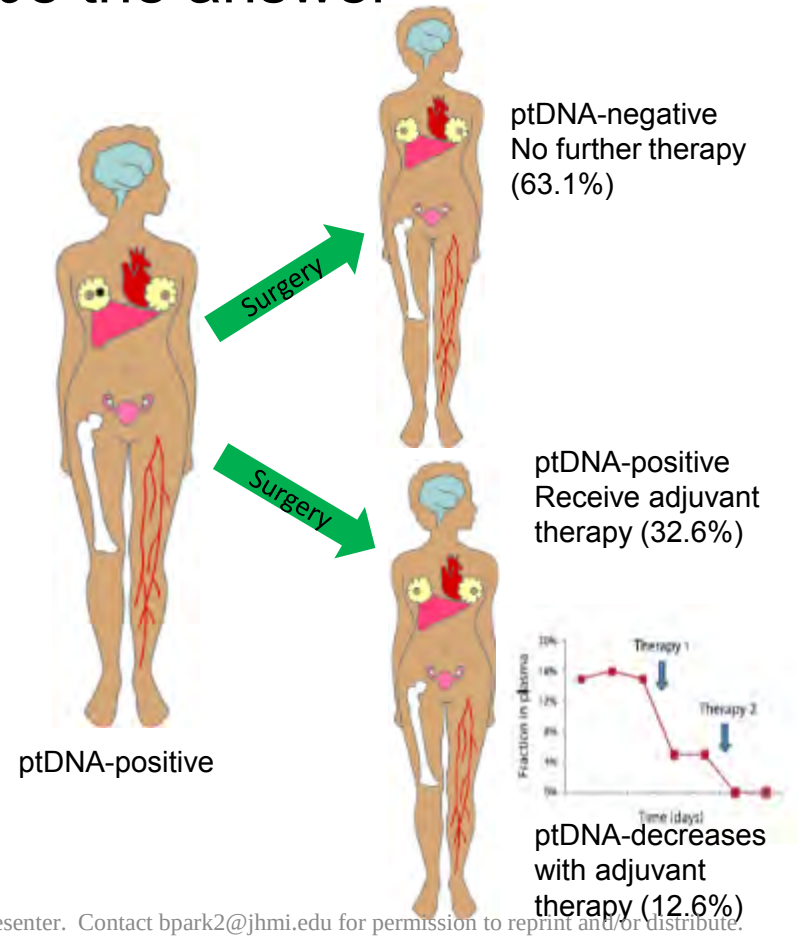
Current problems with the adjuvant paradigm



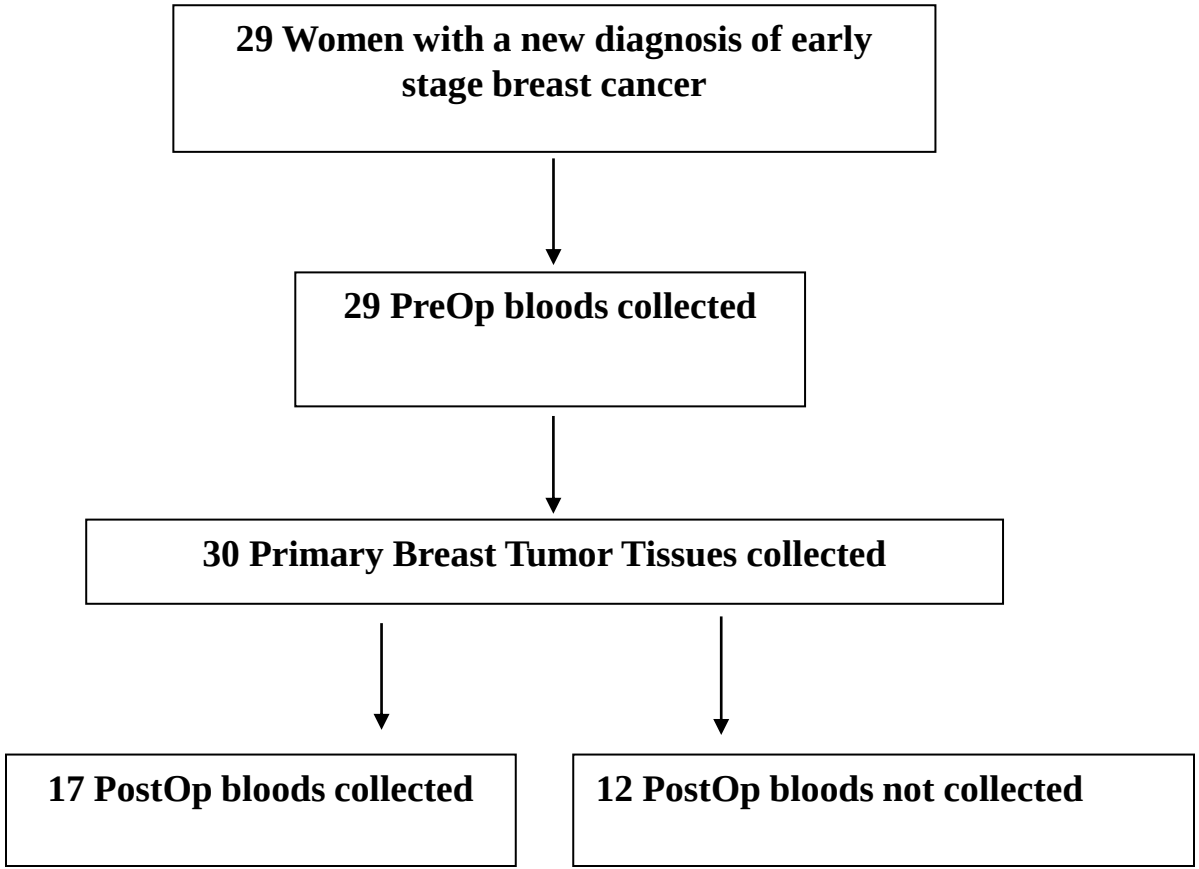
- How can we determine on an individual level who truly needs additional therapies, i.e. has microscopic metastatic disease?
- How can we determine in patients who need adjuvant therapies whether systemic treatment is working?
- Can we accelerate the pace of clinical trials and drug approval with new clinical endpoints? Enroll only patients with residual disease/highest risk for recurrence?

Measuring microscopic tumor burden could be the answer

- Circulating ptDNA could be a marker of whether *micrometastatic* disease is present
- Somatic mutations/alterations are 100% specific for cancer DNA



Preop PIK3CA study



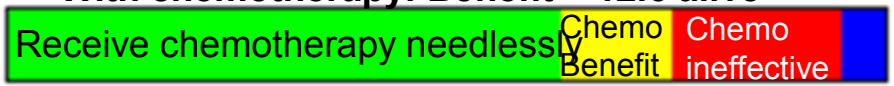
PIK3CA mutation detection in early stage breast cancer- Summary

- Twenty-nine patients prospectively enrolled
- Obtained preop blood (29), cancer tissue (30) and post op blood (17)
- Detected 15 *PIK3CA* mutations in 14 patients
- Detected 14 of 15 mutations in preop blood (93.3%)
- All wild type tissues samples were wild type in preop blood (100% specificity)
- Of 10 *PIK3CA* mutation positive patient samples (11 mutations), 5 had persistent postop ptDNA

Potential Impact

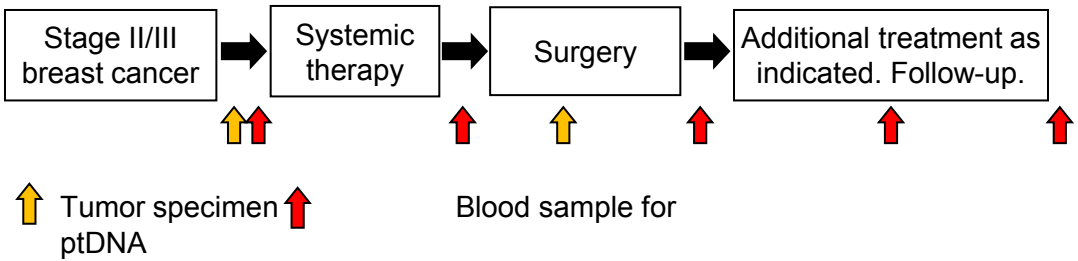
- Will allow for a rapid non-invasive assay of residual disease and response to therapies
- May avoid needless therapies (over treatment) in the neoadjuvant and adjuvant settings
  - Optimally, quantify residual risk and likelihood of cure
- May allow for individualized decisions about changing therapy strategies in patients based on actual residual risk of recurrence
- May serve as useful intermediate endpoints and improve efficiency of clinical trials; concept may also apply to other tumor types

With chemotherapy: Benefit = 12.6 alive



Clinical Validity: New prospective trial (TBCRC 040)

- Neoadjuvant: Assess ptDNA prior to chemo and at time of surgery (before surgery): Defining “liquid pCR”.
  - Can we avoid surgery for breast cancer?



Conclusions

- ptDNA detection can change the paradigm and rationale for how we administer systemic therapies in early stage breast and other solid cancers
- Can avoid overtreatment and undertreatment
- Allows for individualized precision medicine; treat patients with exactly what they need; not too little, not too much
- To get there, we will need the same level of evidence required for new drug approval, i.e. prospective randomized trials demonstrating clinical utility

Acknowledgments

- Park Lab
  - Heather Parsons, M.D., M.P.H.
  - Julia Beaver, M.D.
  - David Chu
  - Dan Zabransky
  - Patricia Toro Valda
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  - Karen Cravero
  - Brian Dalton M.D., Ph.D.
  - Berry Button
  - Kelly Kyker-Snowman
  - Ian Waters
  - Arielle Medford, M.D.
  - Justin Lee
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  - Marc Rosen
- Pedram Argani, M.D., Ashley Cimino-Mathews M.D.
- Vered Stearns M.D., Antonio Wolff, M.D.
  - Dustin VanDenBerg, Jill Kessler, Liz Yakim, Stacie Jeter
- Leslie Cope, Ph.D., Gary Rosner, Ph.D. and Rob Scharpf, Ph.D.
- Paula J. Hurley Ph.D. and Josh Lauring, M.D., Ph.D.

Avon Foundation
Breast Cancer Research Foundation
NIH/NCI
FAMRI
DOD
Commonwealth Foundation
Santa Fe Foundation
Eddie and Sandy Garcia Foundation
Diane and Mike Canney Foundation
Lebor Foundation
ME Foundation
WTFC Foundation
Golde Fund

Extracellular RNA: a next frontier for liquid biopsy biomarkers

Muneesh Tewari, MD, PhD

University of Michigan

Departments of Internal Medicine and Biomedical Engineering

Center for Computational Medicine and Bioinformatics

Biointerfaces Institute

UM Comprehensive Cancer Center

FDA-AACR Liquid Biopsies in Oncology Drug and Device Development Conference

Walter E. Washington Convention Center Washington, D.C.

July 19, 2016

Disclosures:

M. Tewari is a paid Advisor for Miroculus, Inc.

- *A startup company in San Francisco developing a new instrument for microRNA analysis.*

- *Not directly related to my presentation. Mentioned in interest of full disclosure.*

Many types of blood analytes could serve as biomarkers

- Proteins:
  e.g., PSA (prostate ca), troponin (myocardial infarction)
- Lipids:
  e.g., triglycerides
- Nucleic Acid:

  (i) Extracellular DNA

  e.g., mutant DNA for cancer

  (ii) Extracellular RNA

  → microRNA

  → messenger RNA, lncRNA, other noncoding RNAs

- Cells: e.g., circulating tumor cells

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Outline: Extracellular RNAs (exRNAs) as liquid biopsy biomarkers

I. Circulating microRNAs in plasma/serum

- past, present and what's next

II. Circulating exRNA: going beyond plasma/serum microRNAs

- exosomes/extracellular vesicles and exRNA

- next generation sequencing for exRNA biomarker discovery

→ the number of exRNA biomarkers is likely to expand soon

III. Looking into the future:

The missing element for liquid biopsy biomarkers to have a "transformative" positive impact on healthcare

In 2005, idea of RNA as a blood-based biomarker was dubious....

- some suggestion in 1980's/1990's of potential of circulating, cell-free RNA as biomarkers, ***but not much traction initially...***

- results variable

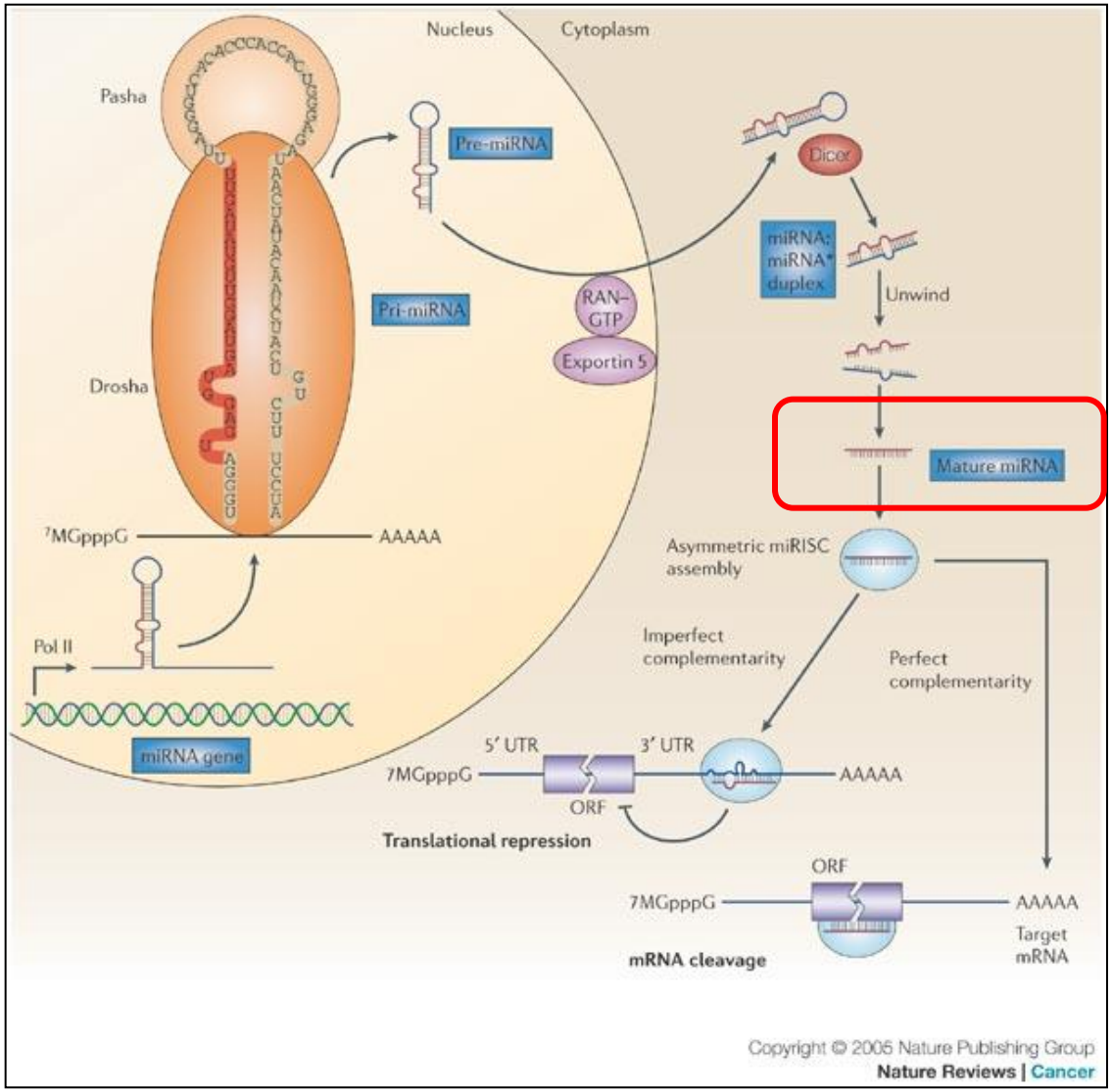
- high ribonuclease activity in blood

- ExRNA believed to be unstable in the circulation

Extracellular (micro)RNAs in the circulation?

MicroRNAs

- short (~22 nt) noncoding RNAs that silence expression of specific mRNA targets
- >1000 miRNAs currently known to be encoded by human genome
- wide-ranging biological roles, especially in development/differentiation



Could miRNAs released from cancer cells be useful biomarkers in blood as well?

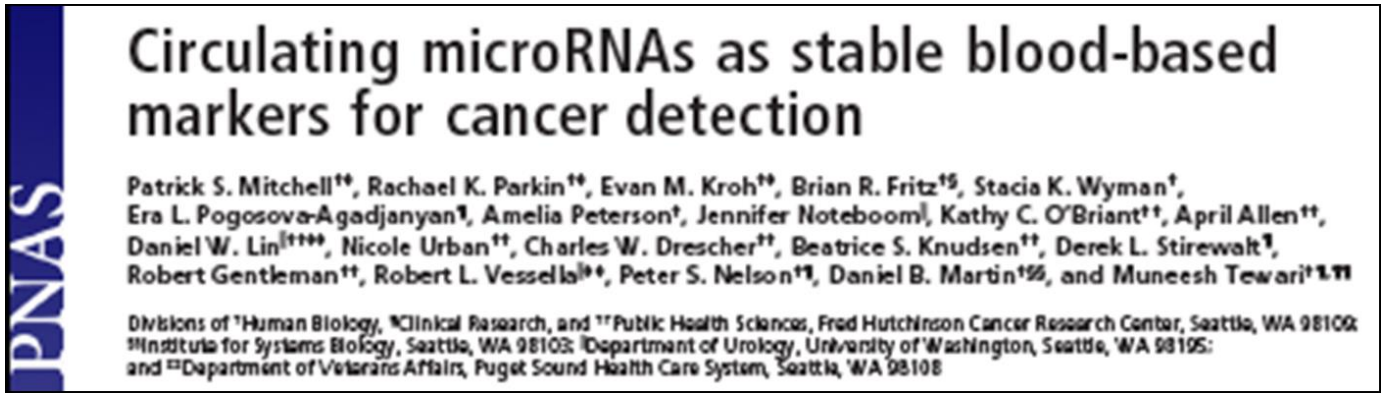
Potential advantages:

- miRNA signatures in tissues valuable as biomarkers
- many copies per cell (hundreds to tens of thousands of copies)
- advantages of being a nucleic acid biomarker
  - sensitive, robust assays (e.g., qRT-PCR)

Could miRNAs released from cancer cells be useful biomarkers in blood as well?

Key questions:

- Can miRNAs be found in the blood?
- Are they stable?
- Can cancer-derived miRNAs get into the blood?
- Can cancer-derived miRNAs in the blood distinguish individuals with and without prostate cancer?



PNAS

Circulating microRNAs as stable blood-based markers for cancer detection

Patrick S. Mitchell[†¶], Rachael K. Parkin[†¶], Evan M. Kroh[†¶], Brian R. Fritz[†§], Stacia K. Wyman[†], Era L. Pogosova-Agadjanyan[¶], Amelia Peterson[†], Jennifer Noteboom[‖], Kathy C. O'Briant[†¶], April Allen[†¶], Daniel W. Lin[‖†**], Nicole Urban[†¶], Charles W. Drescher[†¶], Beatrice S. Knudsen[†¶], Derek L. Stirewalt[¶], Robert Gentleman[†¶], Robert L. Vessella[‖¶], Peter S. Nelson[†¶], Daniel B. Martin[†§§], and Muneesh Tewari[†¶,¶¶]

Divisions of [†]Human Biology, [§]Clinical Research, and [††]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; [¶]Institute for Systems Biology, Seattle, WA 98103; [‖]Department of Urology, University of Washington, Seattle, WA 98195; and [§§]Department of Veterans Affairs, Puget Sound Health Care System, Seattle, WA 98108

(Mitchell et al, PNAS, 2008)

Key questions:

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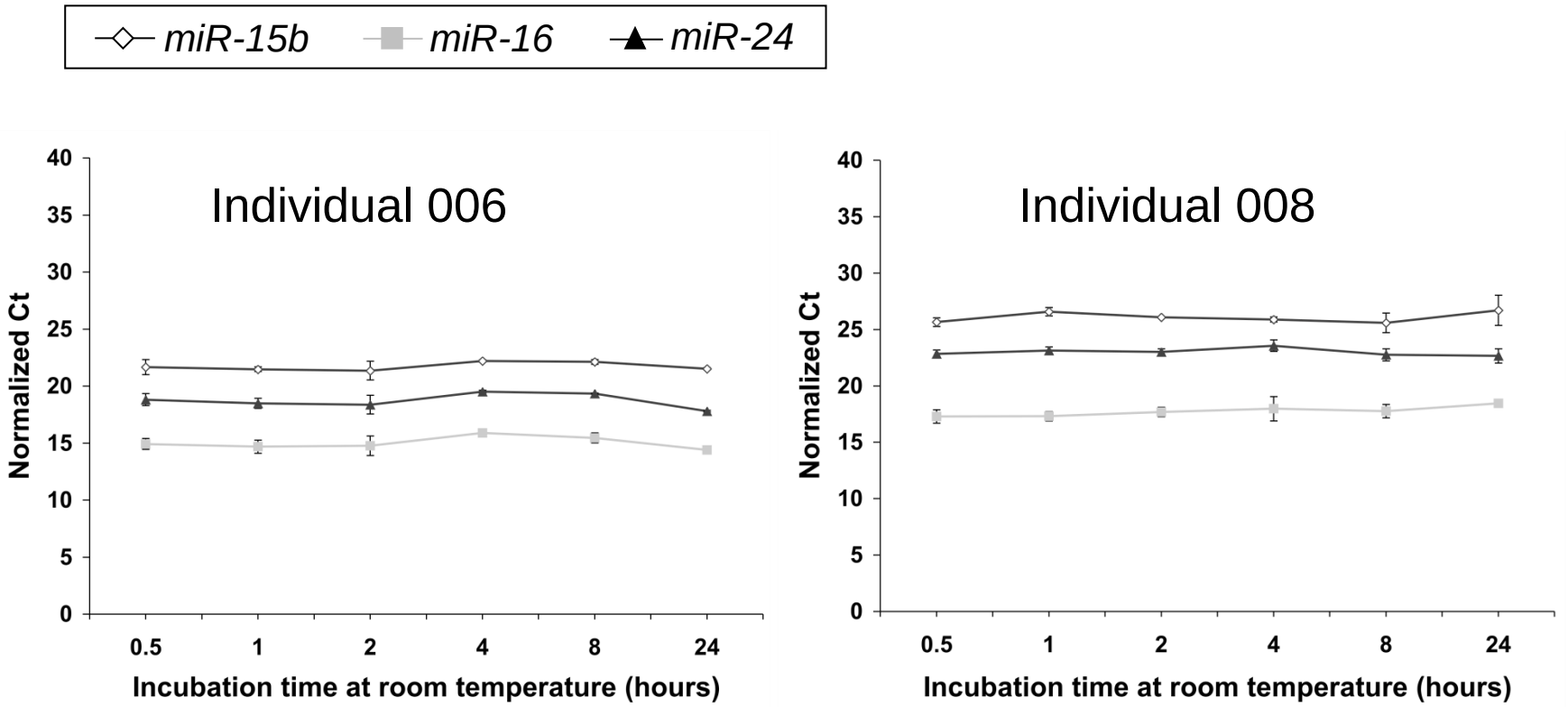
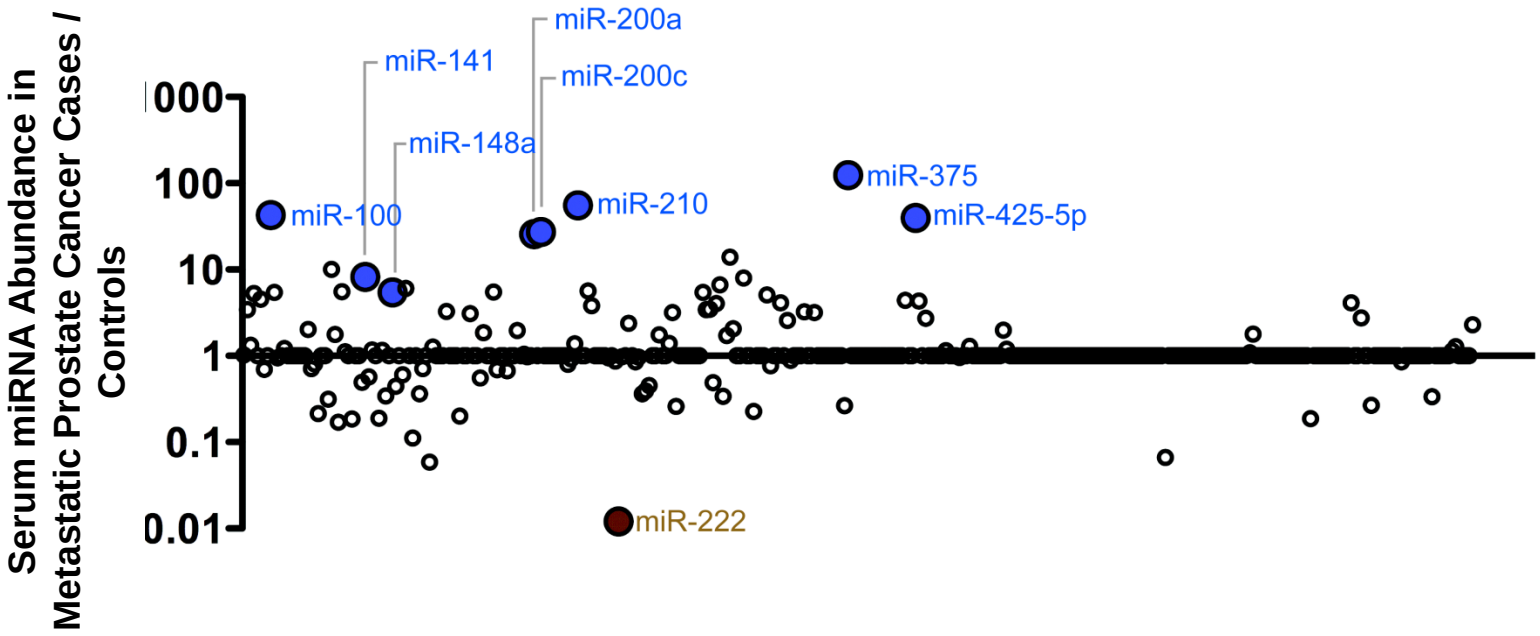
Endogenous miRNAs in plasma are highly stable

Room temperature incubation of plasma up to 24 hours



Global serum miRNA profiling identifies prostate cancer circulating miRNA markers



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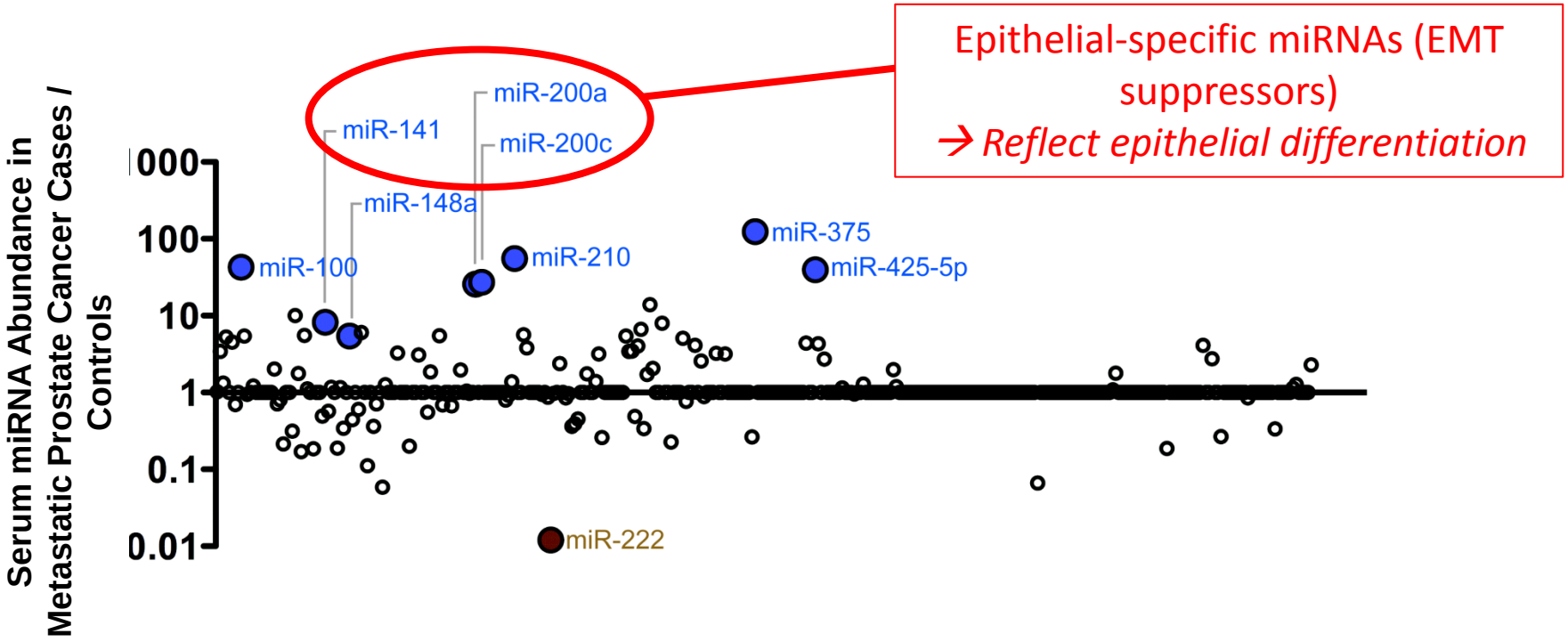
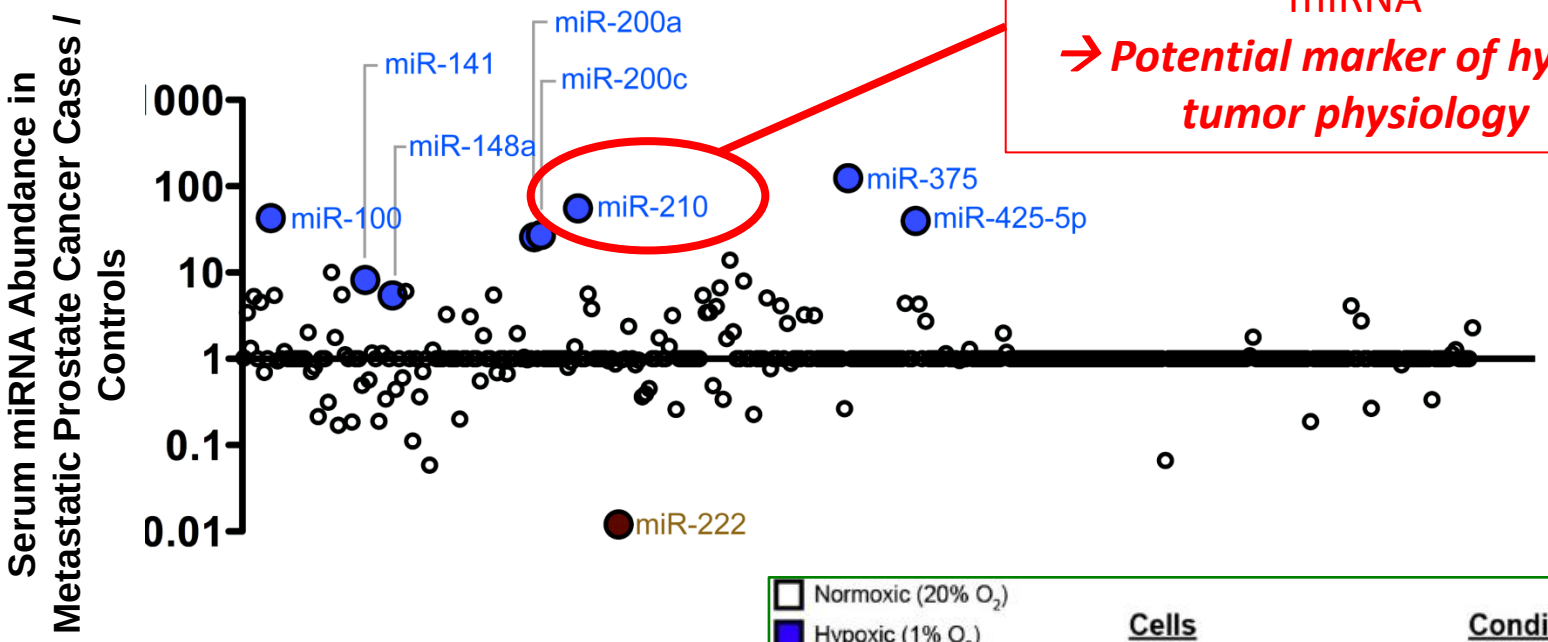


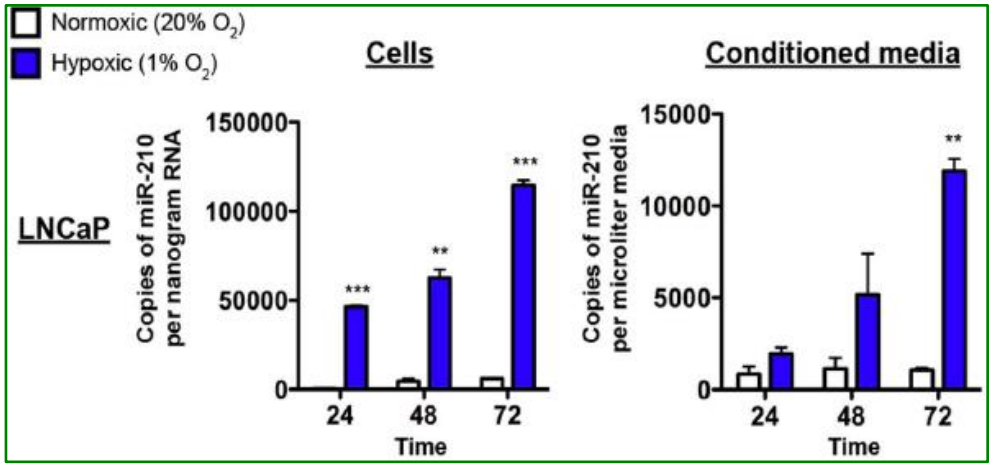
Global serum miRNA profiling identifies prostate cancer circulating miRNA markers

miR-210: hypoxia-inducible miRNA

→ ***Potential marker of hypoxic tumor physiology***



Hypoxia induces transcription and release of miR-210 from prostate cancer cells



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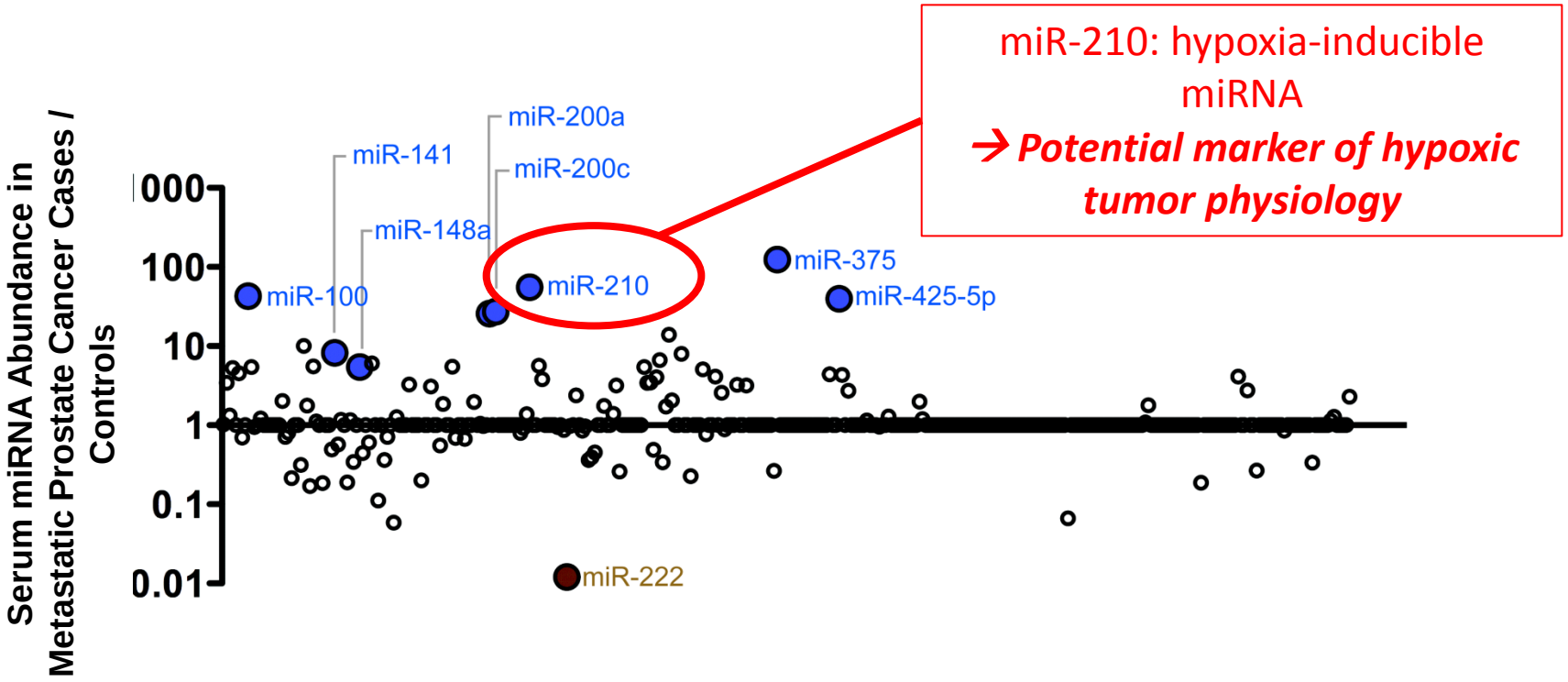


Tumor hypoxia is known to be associated with <u>treatment resistance</u>

→ across a range of cancers and types of treatment

miR-210 may predict treatment response

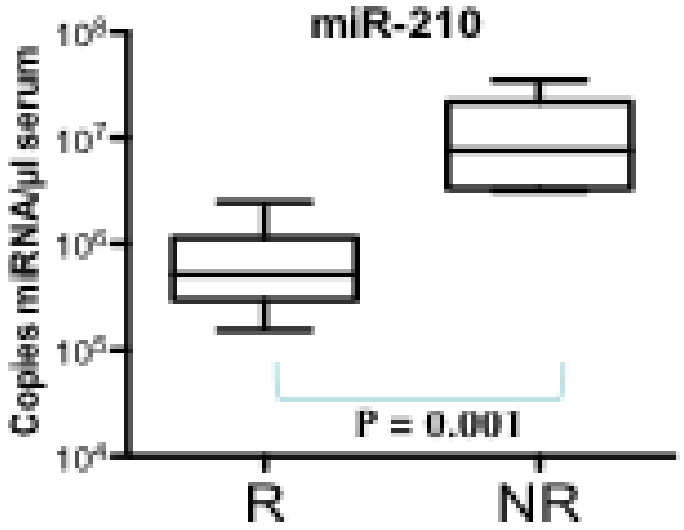
OPEN ACCESS Freely available online

PLOS ONE

Circulating microRNA Profiling Identifies a Subset of Metastatic Prostate Cancer Patients with Evidence of Cancer-Associated Hypoxia

Heather H. Cheng[1,2☯], Patrick S. Mitchell[3☯¤a], Evan M. Kroh[3], Alexander E. Dowell[4], Lisly Chéry[4], Javed Siddiqui[5,6], Peter S. Nelson[1,2,3], Robert L. Vessella[4,7], Beatrice S. Knudsen[8¤b], Arul M. Chinnaiyan[5,9,10], Kenneth J. Pienta[11], Colm Morrissey[4], Muneesh Tewari[1,2,3,8*]

(*PLoS One*, *2013*)



Baseline miR-210 levels are higher in patients who had resistance to subsequent therapy

Conclusions Thus Far:

- **Cell-free miRNAs exist in a highly stable form in the circulation**

- **Cancer-derived miRNAs reach the circulation**

- **Circulating miRNAs may be informative regarding:**
  - ***Cell/Tissue Origin*** *(e.g., epithelial differentiation)*
  - ***Cell/Tissue State*** *(e.g., hypoxia pathway activation)*

ExDNA vs. ExRNA: Different Kinds of Information

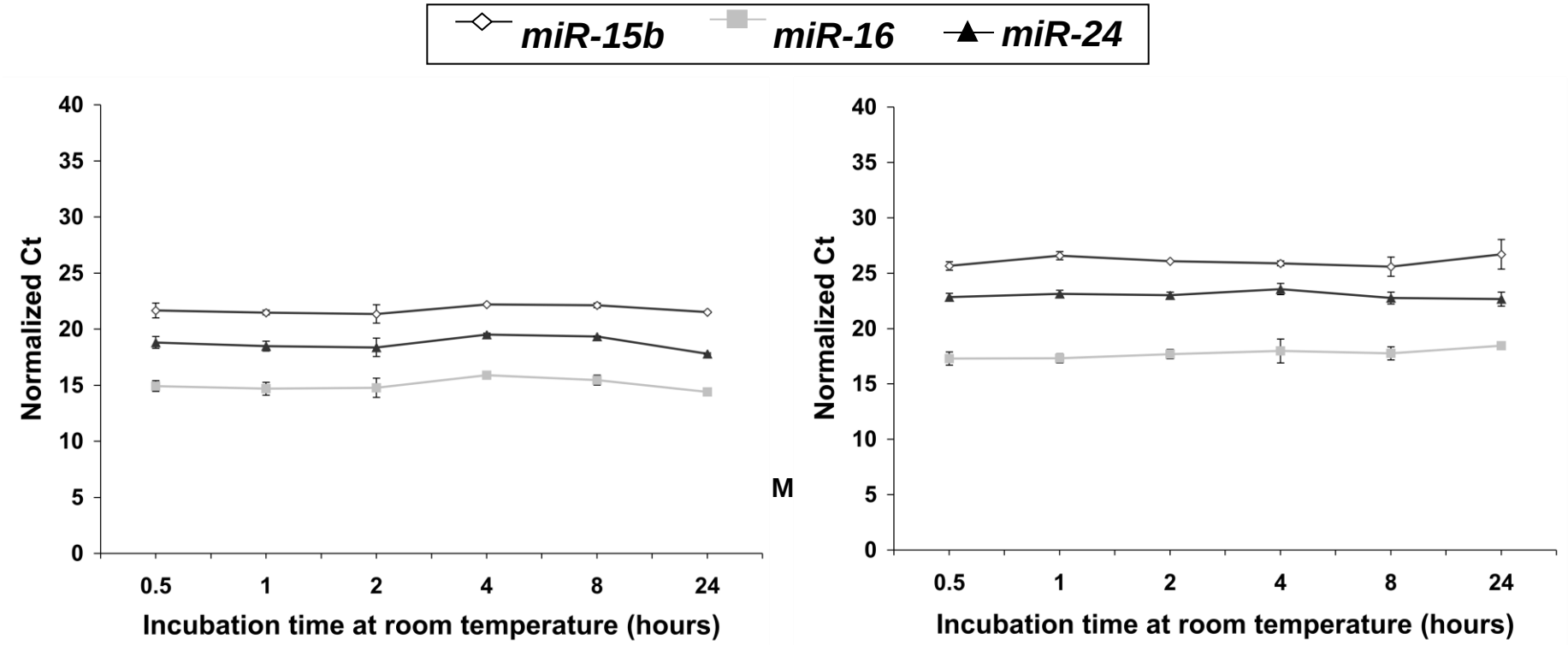
- ExDNA: - *highly specific* (e.g. mutant DNA)
  - generally "hard-wired", *less dynamic*
  - 1 or 2 copies per cell (for single copy gene)

ExDNA vs. ExRNA: Different Kinds of Information

- ExDNA: - *highly specific* (e.g. mutant DNA)
  - generally "hard-wired", *less dynamic*
  - 1 or 2 copies per cell (for single copy gene)

- ExRNA: - *relative specificity*, based on expression pattern
  (potential to detect some mutations if transcribed)
  - *more dynamic* due to transcriptional regulation
  - potential to inform re: *Cell Type*, *Cell State*?
  - 100's-1000's of copies per cell…more sensitive?

Circulating miRNAs are highly stable despite high RNase activity in plasma (and serum)

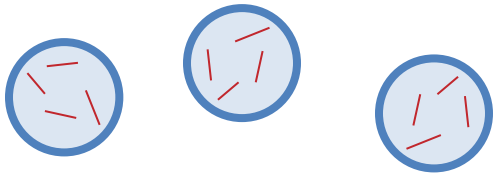


What mechanism(s) stabilize circulating miRNAs?

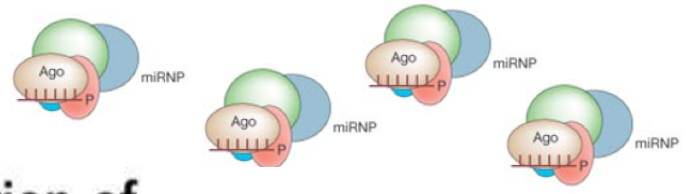
Mechanisms of circulating miRNA stability

- Two, somewhat overlapping, populations of circulating miRNA:

"Exosomes"-associated (~2%)



Argonaute/Protein-associated (~98%)



PNAS

Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma

Jason D. Arroyo[a], John R. Chevillet[a], Evan M. Kroh[a], Ingrid K. Ruf[a], Colin C. Pritchard[b], Donald F. Gibson[b], Patrick S. Mitchell[a,1], Christopher F. Bennett[a,c], Era L. Pogosova-Agadjanyan[d], Derek L. Stirewalt[d], Jonathan F. Tait[b], and Muneesh Tewari[a,d,e,2]

[a]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024; [b]Department of Laboratory Medicine, University of

(*PNAS*, *2011*)

(*PNAS*, *2014*)

PNAS

Quantitative and stoichiometric analysis of the microRNA content of exosomes

John R. Chevillet[a], Qing Kang[a,b], Ingrid K. Ruf[a,1], Hilary A. Briggs[a,1], Lucia N. Vojtech[c,1], Sean M. Hughes[c,1], Heather H. Cheng[a,d], Jason D. Arroyo[a], Emily K. Meredith[a], Emily N. Gallichotte[a], Era L. Pogosova-Agadjanyan[e], Colm Morrissey[f], Derek L. Stirewalt[e], Florian Hladik[c,d,g], Evan Y. Yu[d], Celestia S. Higano[d,e,f], and Muneesh Tewari[a,b,e,h,i,j,k,2]

Divisions of [a]Human Biology, [e]Clinical Research, [g]Vaccine and Infectious Disease, and [i]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; Departments of [b]Internal Medicine and [h]Biomedical Engineering, [j]Biointerfaces Institute, and [k]Center for Computational Medicine, University of Michigan, Ann Arbor, MI 48109; and Departments of [c]Obstetrics and Gynecology, [d]Medicine, and [f]Urology, Division of Oncology, University of Washington, Seattle, WA 98195

Extracellular microRNA research has grown into a new field

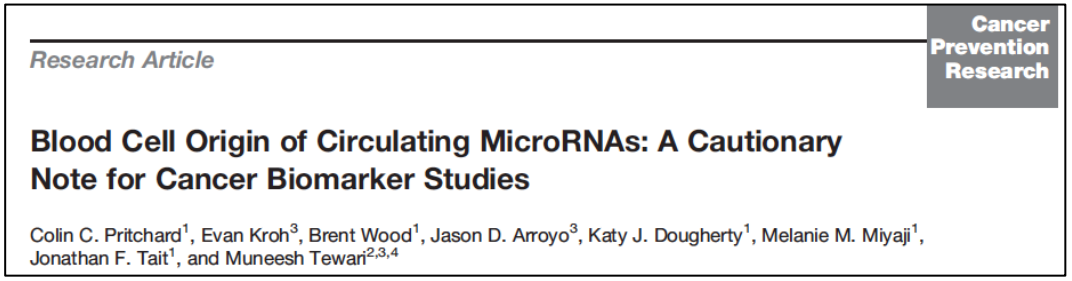
- **Over 3,000 papers published on extracellular microRNAs, mostly for biomarker applications in various conditions**

Examples of applications being developed:

- cancer prognostication and prediction
- cancer early detection
- monitoring drug toxicity (e.g., liver toxicity, acetaminophen overdose)
- radiation exposure monitoring
- other diseases (e.g., muscular dystrophy, cardiac injury)
- forensics

Extracellular microRNA research has grown into a new field

- **Many pre-analytic and analytic challenges identified and overcome**



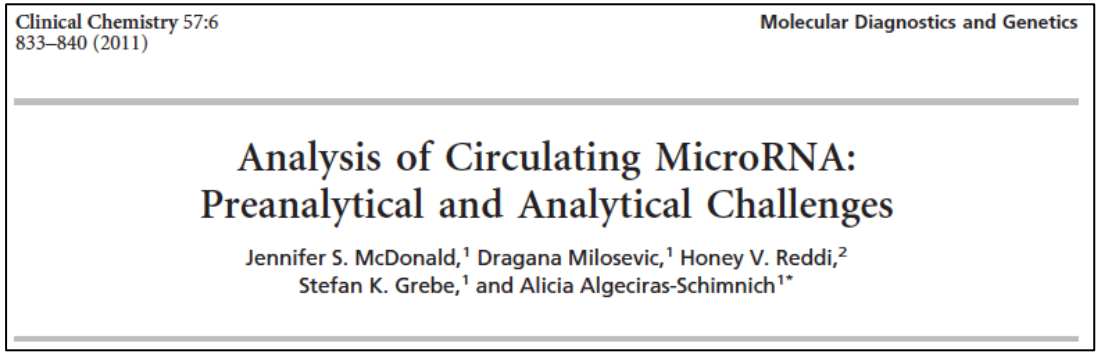
Research Article

Cancer Prevention Research

Blood Cell Origin of Circulating MicroRNAs: A Cautionary Note for Cancer Biomarker Studies

Colin C. Pritchard[1], Evan Kroh[3], Brent Wood[1], Jason D. Arroyo[3], Katy J. Dougherty[1], Melanie M. Miyaji[1], Jonathan F. Tait[1], and Muneesh Tewari[2,3,4]

- Blood cell origin of most plasma miRNAs



Clinical Chemistry 57:6
833–840 (2011)

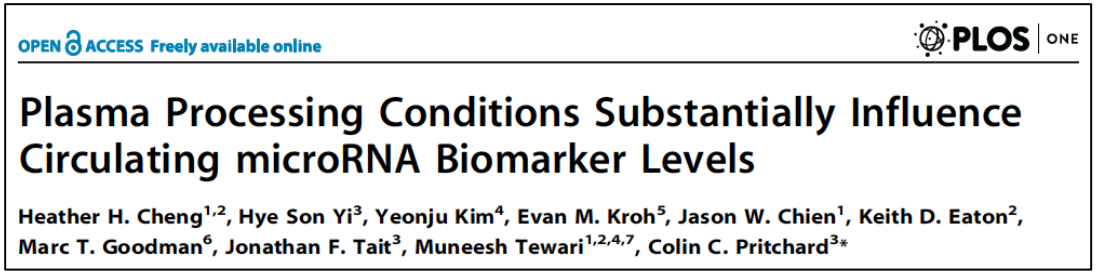
Molecular Diagnostics and Genetics

Analysis of Circulating MicroRNA: Preanalytical and Analytical Challenges

Jennifer S. McDonald,[1] Dragana Milosevic,[1] Honey V. Reddi,[2] Stefan K. Grebe,[1] and Alicia Algeciras-Schimnich[1*]

- Effects of Hemolysis

- Effects of Platelet contamination and its mitigation



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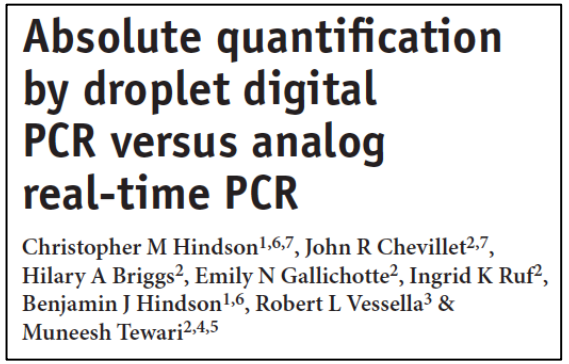
PLOS ONE

Plasma Processing Conditions Substantially Influence Circulating microRNA Biomarker Levels

Heather H. Cheng[1,2], Hye Son Yi[3], Yeonju Kim[4], Evan M. Kroh[5], Jason W. Chien[1], Keith D. Eaton[2], Marc T. Goodman[6], Jonathan F. Tait[3], Muneesh Tewari[1,2,4,7], Colin C. Pritchard[3]*

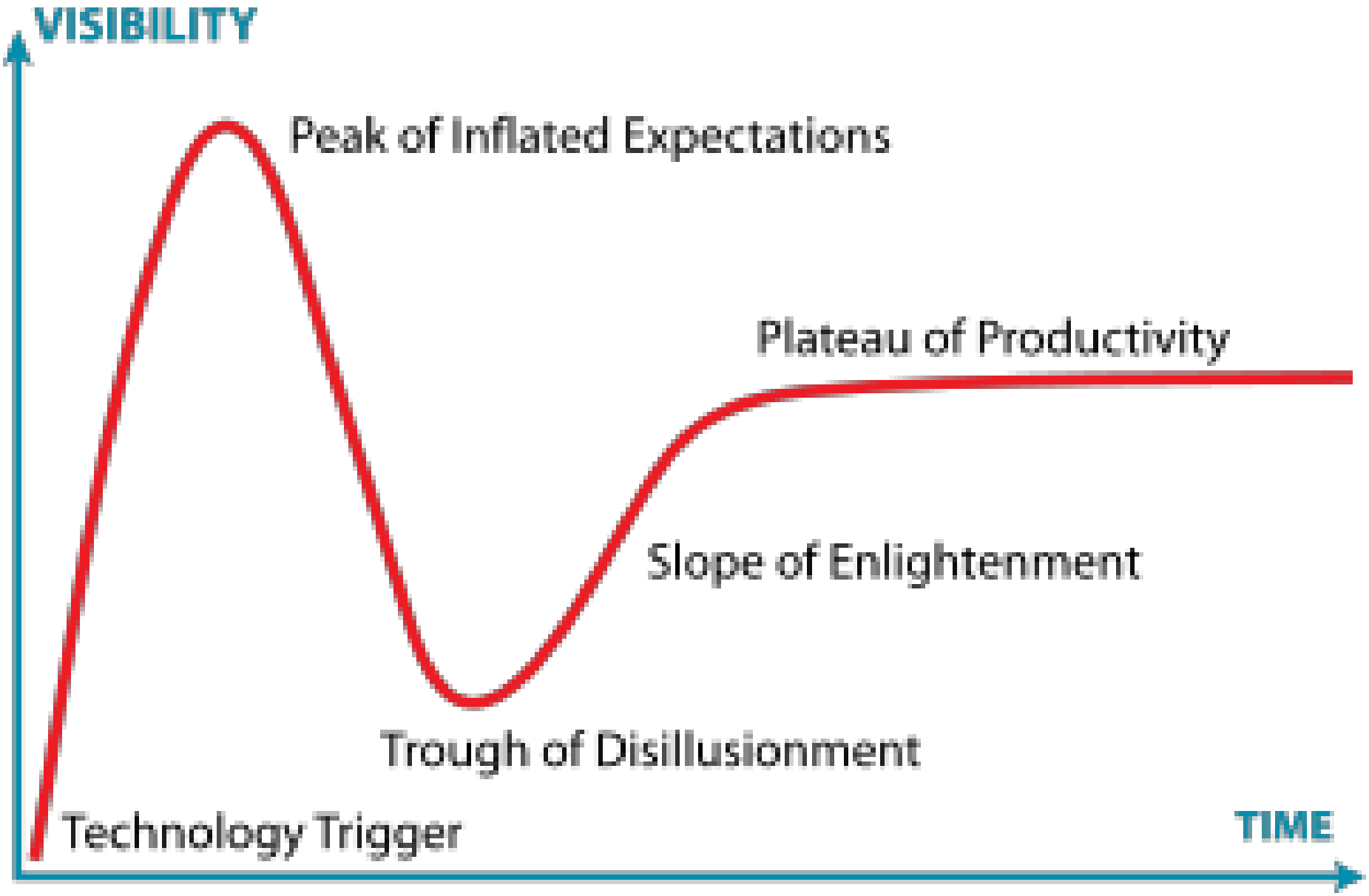
- Digital PCR to overcome day-to-day analytic variation



Absolute quantification by droplet digital PCR versus analog real-time PCR

Christopher M Hindson[1,6,7], John R Chevillet[2,7], Hilary A Briggs[2], Emily N Gallichotte[2], Ingrid K Ruf[2], Benjamin J Hindson[1,6], Robert L Vessella[3] & Muneesh Tewari[2,4,5]

Gartner Innovation Cycle – A Repeating Cycle, in fact



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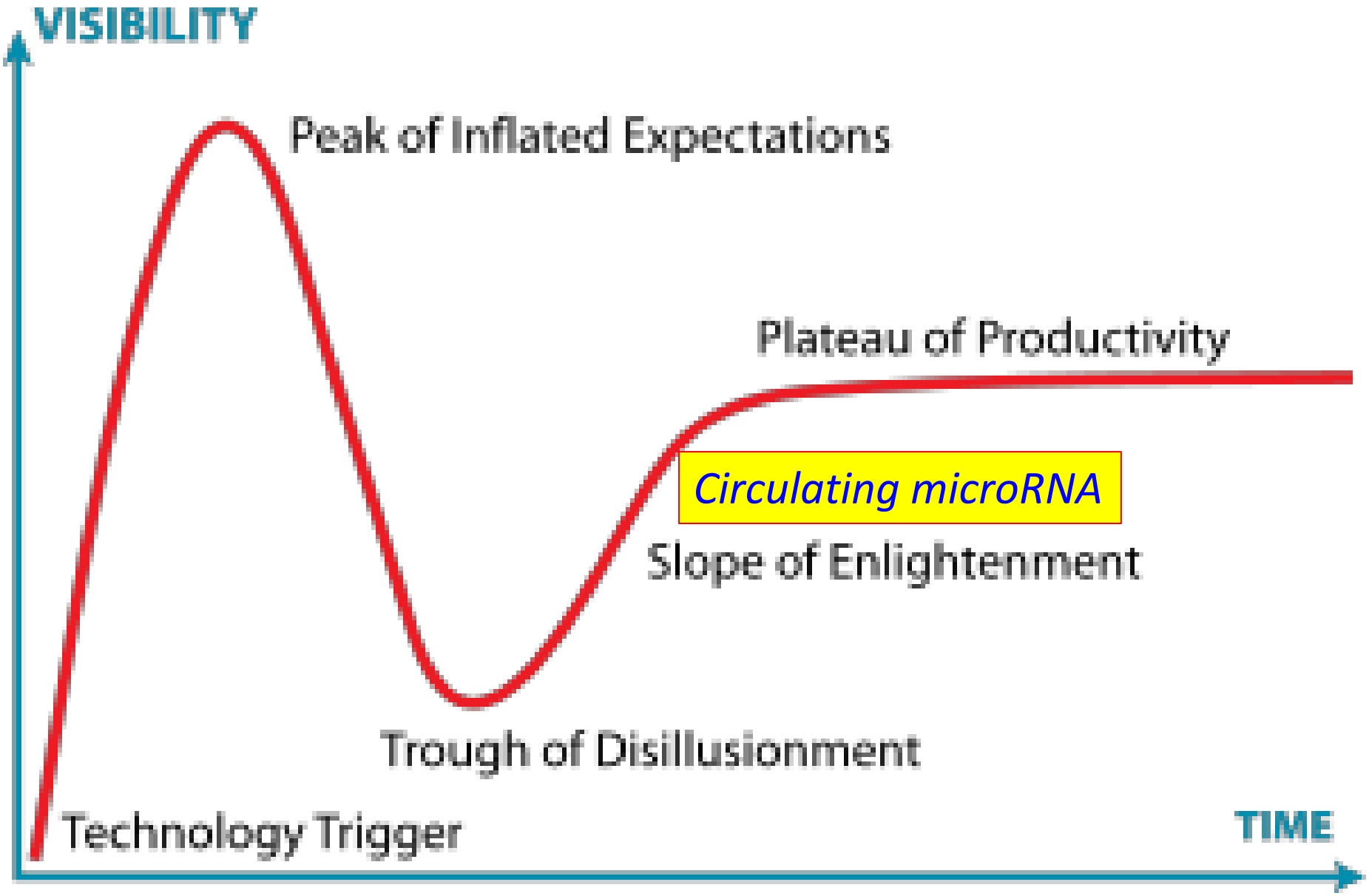
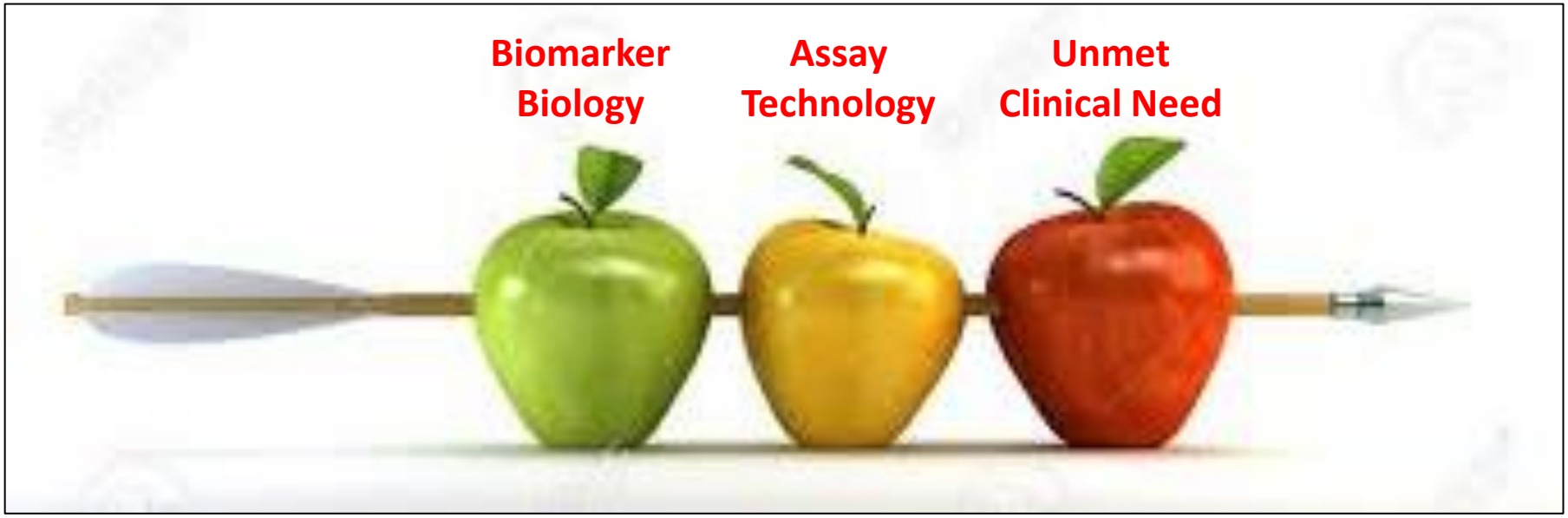


Biomarker development: an alignment challenge



For Circulating microRNAs:

✔ Analytic Validation → ✔ Clinical Validation → *Under Study* Clinical Utility

Outline: Extracellular RNAs (exRNAs) as liquid biopsy biomarkers

I. Circulating microRNAs in plasma/serum

- past, present and what's next

II. Circulating exRNA: going beyond plasma/serum microRNAs

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The missing element for liquid biopsy biomarkers to have a "transformative" positive impact on healthcare

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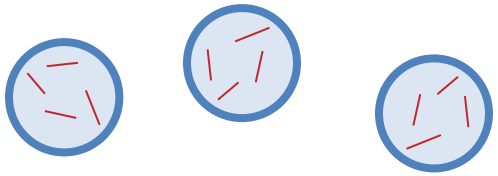

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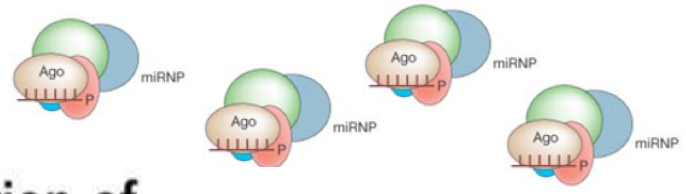

PNAS

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(*PNAS*, *2011*)

(*PNAS*, *2014*)

PNAS

Quantitative and stoichiometric analysis of the microRNA content of exosomes

John R. Chevillet[a], Qing Kang[a,b], Ingrid K. Ruf[a,1], Hilary A. Briggs[a,1], Lucia N. Vojtech[c,1], Sean M. Hughes[c,1], Heather H. Cheng[a,d], Jason D. Arroyo[a], Emily K. Meredith[a], Emily N. Gallichotte[a], Era L. Pogosova-Agadjanyan[e], Colm Morrissey[f], Derek L. Stirewalt[e], Florian Hladik[c,d,g], Evan Y. Yu[d], Celestia S. Higano[d,e,f], and Muneesh Tewari[a,b,e,h,i,j,k,2]

Divisions of [a]Human Biology, [e]Clinical Research, [g]Vaccine and Infectious Disease, and [i]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; Departments of [b]Internal Medicine and [h]Biomedical Engineering, [j]Biointerfaces Institute, and [k]Center for Computational Medicine, University of Michigan, Ann Arbor, MI 48109; and Departments of [c]Obstetrics and Gynecology, [d]Medicine, and [f]Urology, Division of Oncology, University of Washington, Seattle, WA 98195

A brief discussion of exosomes and exRNA diagnostics

Are exosome-associated exRNA biomarkers less promising, given that most plasma miRNA is not in exosomes?

A brief discussion of exosomes and exRNA diagnostics

Are exosome-associated exRNA biomarkers less promising, given that most plasma miRNA is not in exosomes?

- **Not necessarily!**

- **Depends on the specific miRNA marker(s) and disease**
  - needs to be determined on a case-by-case basis for different miRNA biomarkers in different clinical contexts

A brief discussion of exosomes and exRNA diagnostics

Are exosome-associated exRNA biomarkers less promising, given that most plasma miRNA is not in exosomes?

- **Not necessarily!**

NATURE CELL BIOLOGY VOLUME 10 | NUMBER 12 | DECEMBER 2008

Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers

Johan Skog[1], Tom Würdinger[1,2], Sjoerd van Rijn[1], Dimphna H. Meijer[1], Laura Gainche[1], Miguel Sena-Esteves[1], William T. Curry, Jr.[3], Bob S. Carter[3], Anna M. Krichevsky[4] and Xandra O. Breakefield[1,5]

EGFRvIII messenger RNA detectable in serum using a PCR-based assay

Table 1 RNA in glioblastoma microvesicles can be used as a sensitive biomarker

Patient number	Time of serum collection (days post-surgery)	Serum volume (ml)	Biopsy *EGFRvIII*	Serum microvesicle *EGFRvIII*
1	0	3	D	D
2	0	2	ND	ND
3	0	2.5	ND	ND
4	0	1	D	ND
5	0	1	D	ND
6	0	1	ND	ND

A brief discussion of exosomes and exRNA diagnostics

Some under-appreciated facts about exosomes that have implications for interpreting results:

- Exosomes are one of multiple classes of Extracellular Vesicles (EVs)

A brief discussion of exosomes/EVs and exRNA diagnostics

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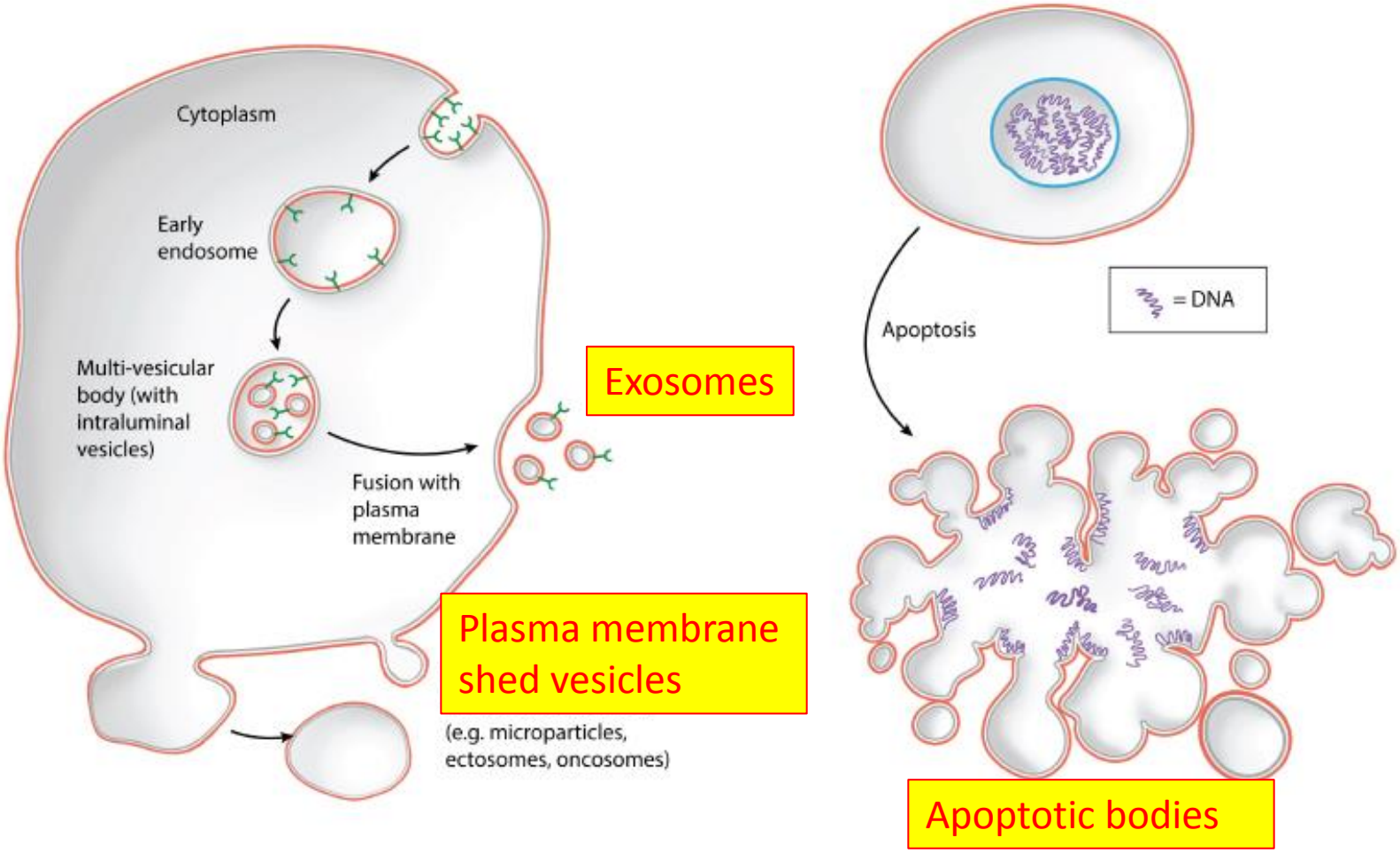

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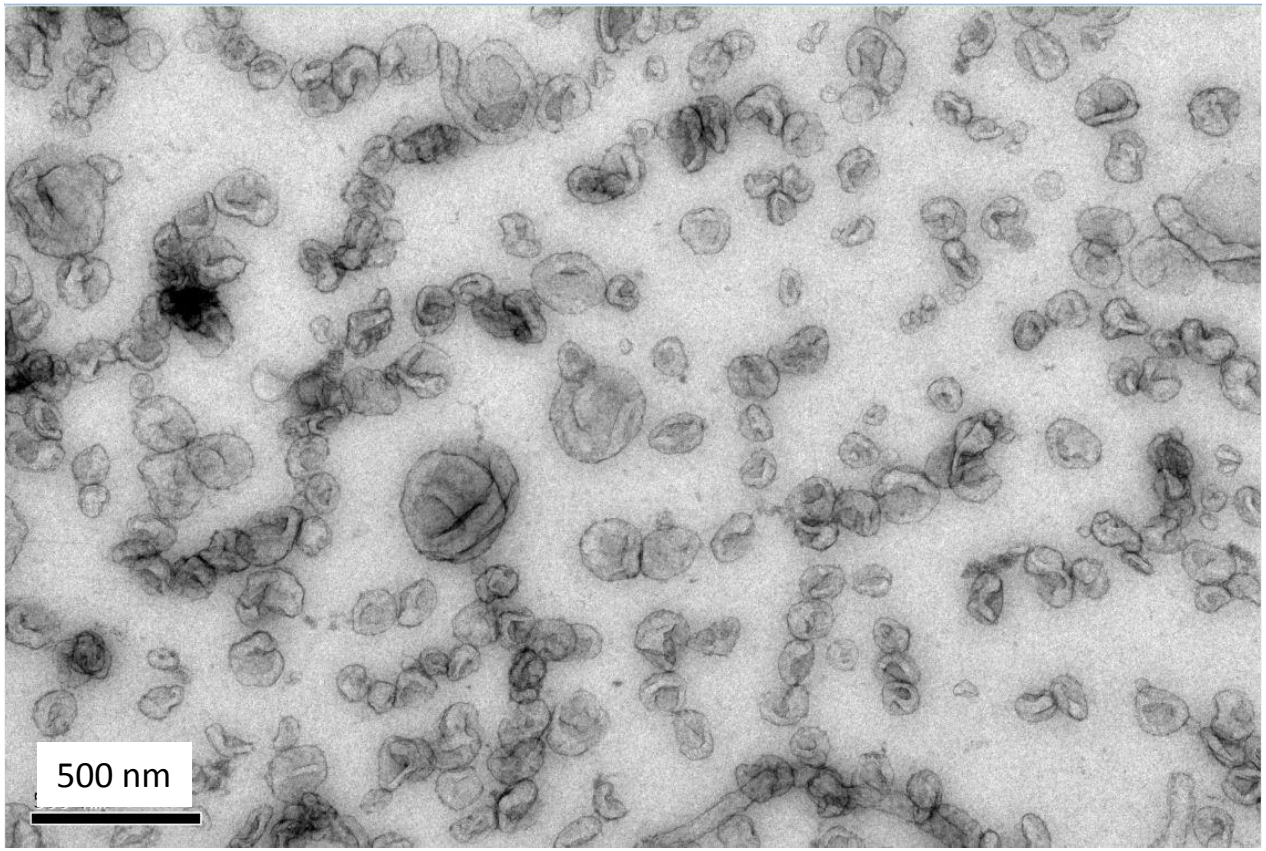
- All EV purification methods purify multiple types of vesicles and also likely co-purify ribonucleoprotein complexes to varying degrees



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Some under-appreciated facts about exosomes that have implications for interpreting results:

- Exosomes are one of multiple classes of Extracellular Vesicles (EVs)
- All EV purification methods purify multiple types of vesicles and also likely co-purify ribonucleoprotein complexes to varying degrees
- This likely contributes to conflicting results in the literature about exosomes/extracellular vesicles and RNA

However efforts are now underway to tease out the biology and define better standards for EV purification and QC

→ International Society for Extracellular Vesicles

→ NIH ERCC, other programs including Internationally

→ **will help advance EV-associated RNA biomarker research**

- improved reproducibility, potentially better performance with more pure preparations of the most relevant particles

Outline: Extracellular RNAs (exRNAs) as liquid biopsy biomarkers

I. Circulating microRNAs in plasma/serum

- past, present and what's next

II. Circulating exRNA: going beyond plasma/serum microRNAs

- exosomes/extracellular vesicles and exRNA

- next generation sequencing for exRNA biomarker discovery

→ the number of exRNA biomarkers is likely to expand soon

III. Looking into the future:

The missing element for liquid biopsy biomarkers to have a "transformative" positive impact on healthcare

Extracellular RNA and the NIH: Common Fund Program

- **ExRNA Discovery by Next Gen Sequencing (i.e., exRNAseq)**

Institution	Contact PI	Location
University of Michigan	Muneesh Tewari	Ann Arbor, MI
Pacific Northwest Research Institute	David Galas	Seattle, WA
UCSD	Louise Laurent	San Diego, CA
UCSF	Prescott Woodruff/David Erle	San Francisco, CA
Beth Israel Deaconess Medical Center	Ionita Ghiran	Boston, MA
University of	Jane Freedman	Worcester, MA

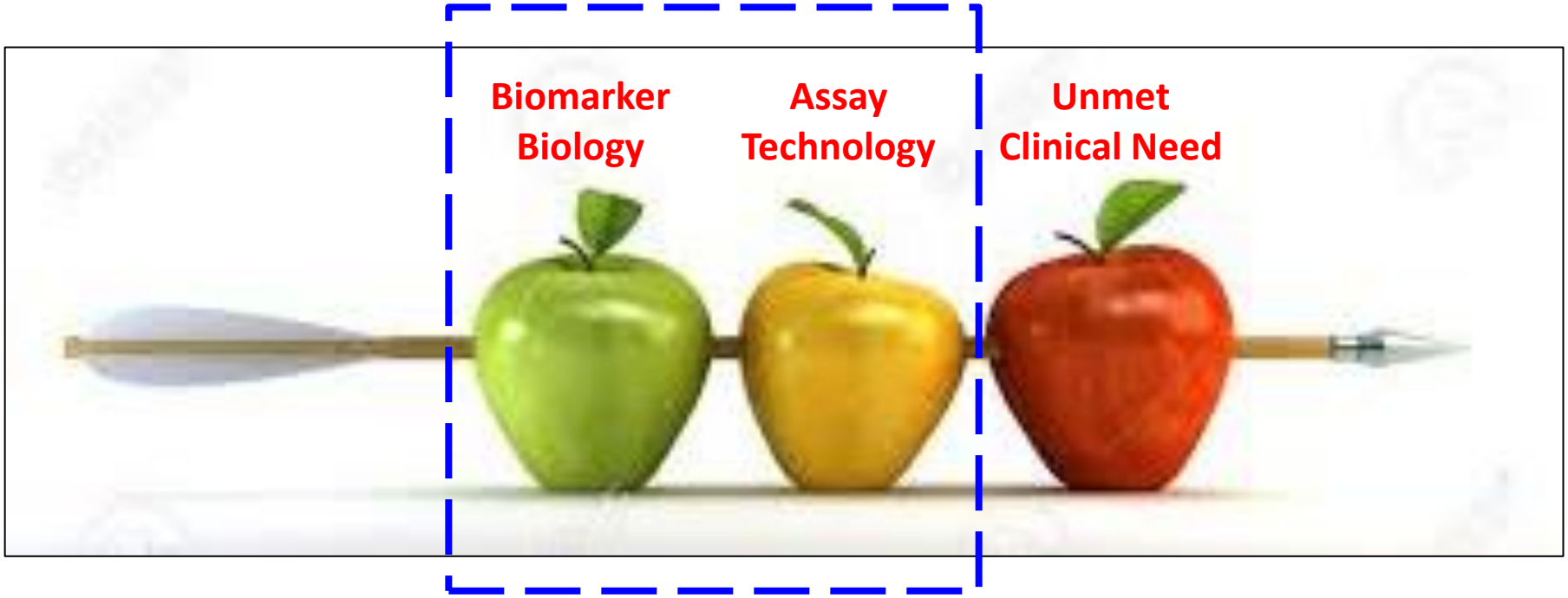
NIH U01-funded ExRNA Reference Profiling teams are:

- *developing methods for comprehensive, reproducible exRNAseq*
- *characterizing "baseline" exRNA profiles in biofluids from healthy control individuals*

Extracellular RNA and the NIH: Common Fund Program

- **ExRNA Discovery by Next Gen Sequencing (i.e., exRNAseq)**
  - Technology challenges being overcome to enable more comprehensive exRNAseq:
    - *extremely low RNA input, degraded RNA, extreme cloning bias*
    - *need better QC and normalization strategies*
  - However even with current technology, a range of RNA classes appear to be detectable in plasma, though studies are early

Biomarker development: an alignment challenge



For Circulating Extracellular RNA Beyond microRNA:

Under Study	*Under Study*	*Under Study*
Analytic Validation	→ Clinical Validation	→ Clinical Utility

“Take-home” points

- **Stable, circulating tumor-derived extracellular RNAs exist**
  - miRNA, EGFR mRNA, potentially other RNAs as well

- **ExRNA could be complementary to ctDNA as a biomarker for precision medicine**
  - dynamic information – e.g., Cell Type(s) and Cell State(s)

- **ExRNA is a frontier for precision oncology biomarker development**

  Need:
  - more biological knowledge, especially for non-miRNA ExRNAs (e.g., what types of vesicle(s), other forms, etc.)
  - technology development for better exRNAseq, better assays
  - optimization of pre-analytic and analytic factors for non-miRNAs
  - progress on pathway toward clinical validation and utility

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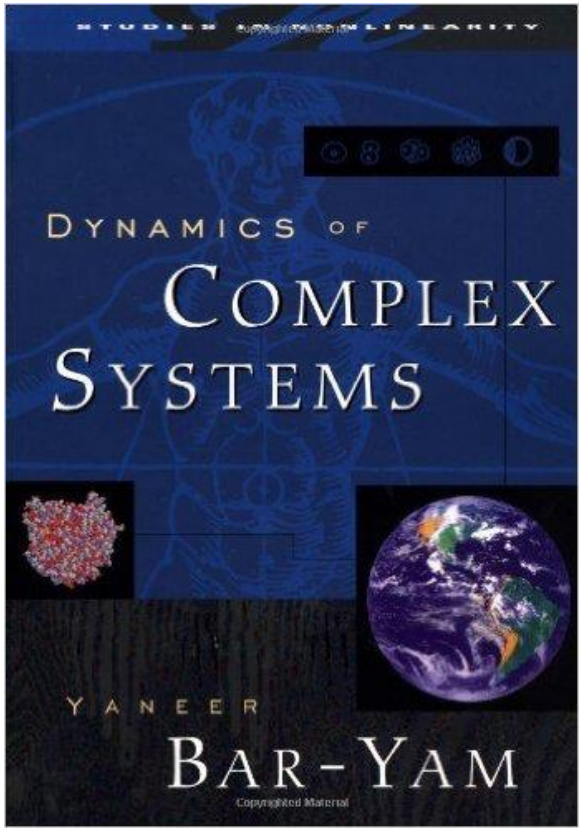


Humans are Complex Systems:

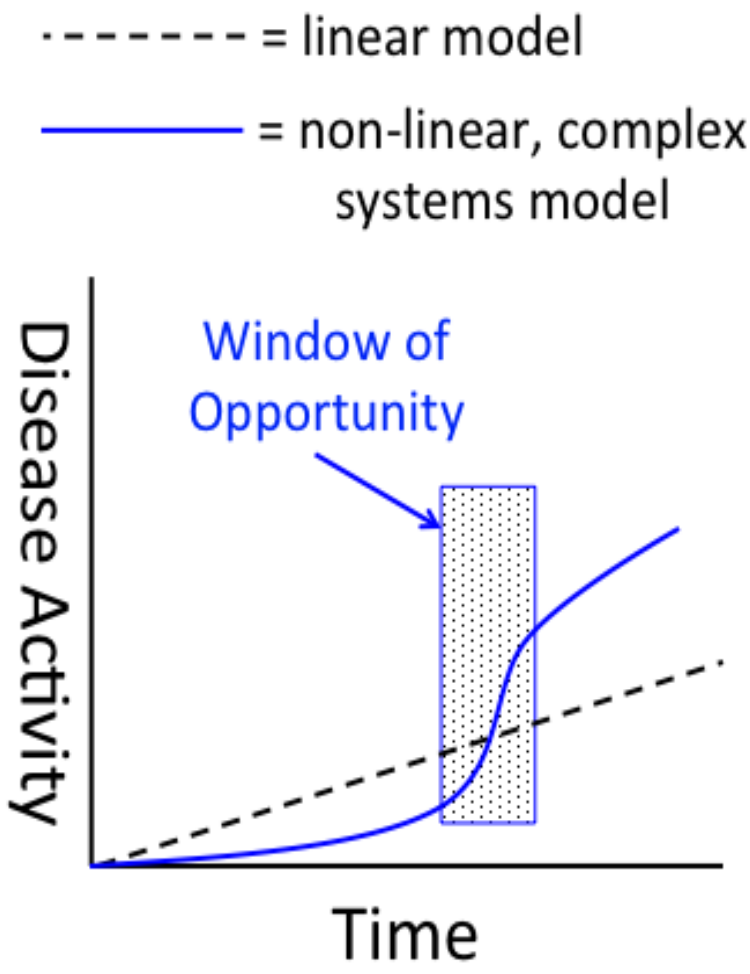
The Fantasy of Prediction in Complex Systems

- Precise, longer-term prediction often not possible in complex systems

- **Short-term** predictions can be much more accurate (e.g. weather forecast)
→ *require ongoing, frequent monitoring*

Implications of complexity science for disease biomarkers

Figure 1

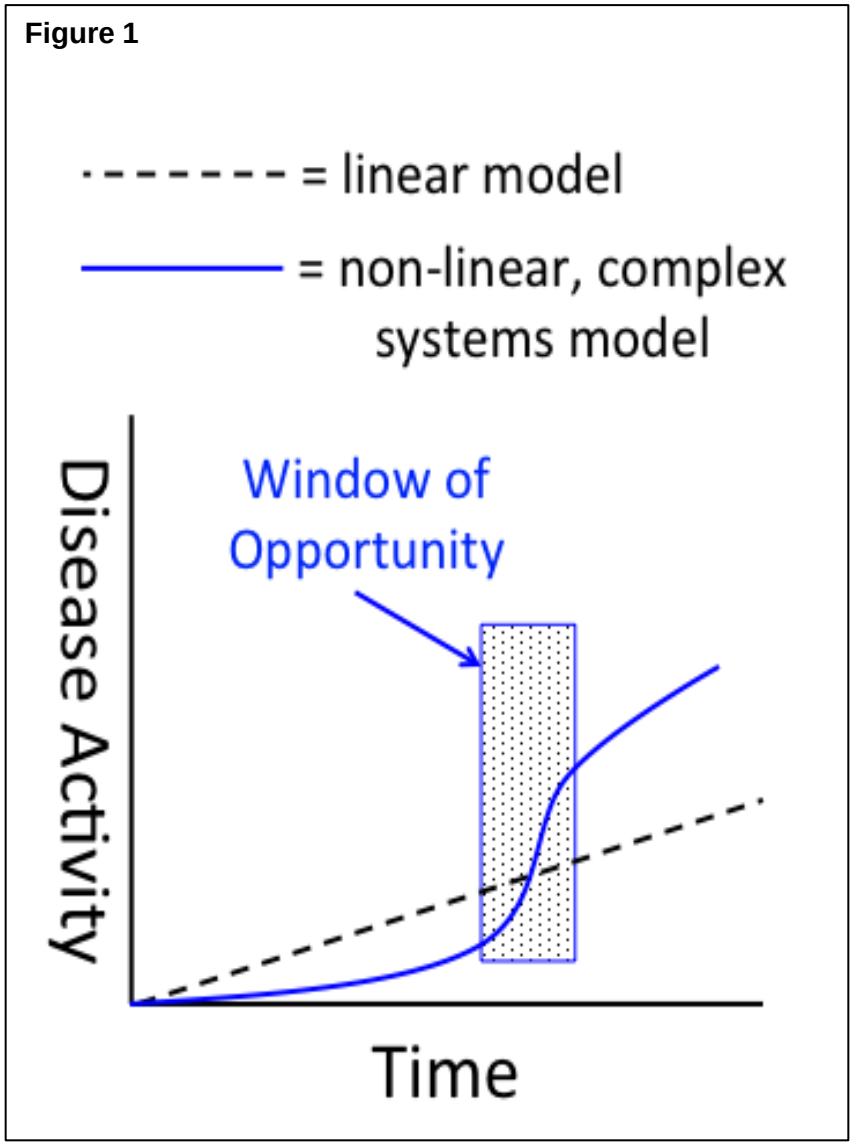


- **Non-linear disease dynamics**

→ *"window-of-opportunity"*

e.g., HIV post-exposure prophylaxis

Implications of complexity science for disease biomarkers



- **Non-linear disease dynamics**

→ *"window-of-opportunity"*

e.g., HIV post-exposure prophylaxis

- **Current, "snapshot"-based biomarker strategies *largely ignore the Time dimension***

(or at best sample sparsely over time)

Current "Snapshot" Paradigm with Reference Ranges

- **"Population-based Reference ranges"**

  → biomarker level interpreted based on population of controls (e.g., rx responders, cancer-free controls, etc.)

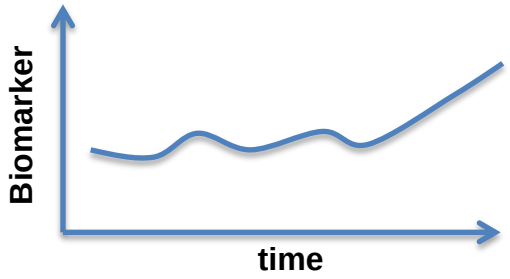
- **Single, "snapshot" measurement**

  → Time dimension is usually missing or very low time resolution

- **Day-to-day and person-to-person variation is likely huge and definitely understudied**

The alternative: Serial, high time-resolution measurements – *Temporal Pattern as the Biomarker*

- Patterns of variation over time, not single time-point cutoffs
- Overcomes between-person variation
- **High time-resolution** (i.e., many more time points, much more sampling) **may sort out many confounding factors**



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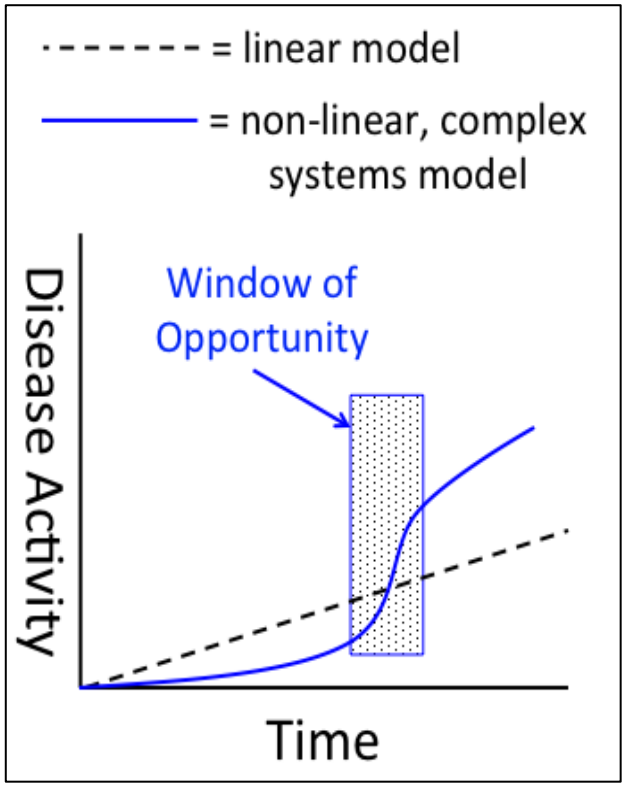
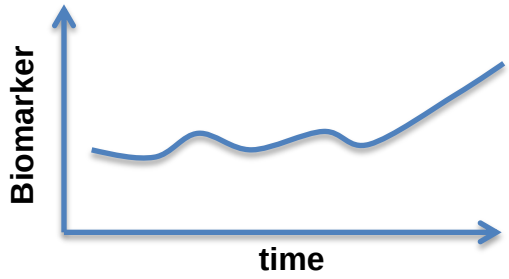


*Aligned with **non-linear** nature of disease progression, to be expected in complex systems*



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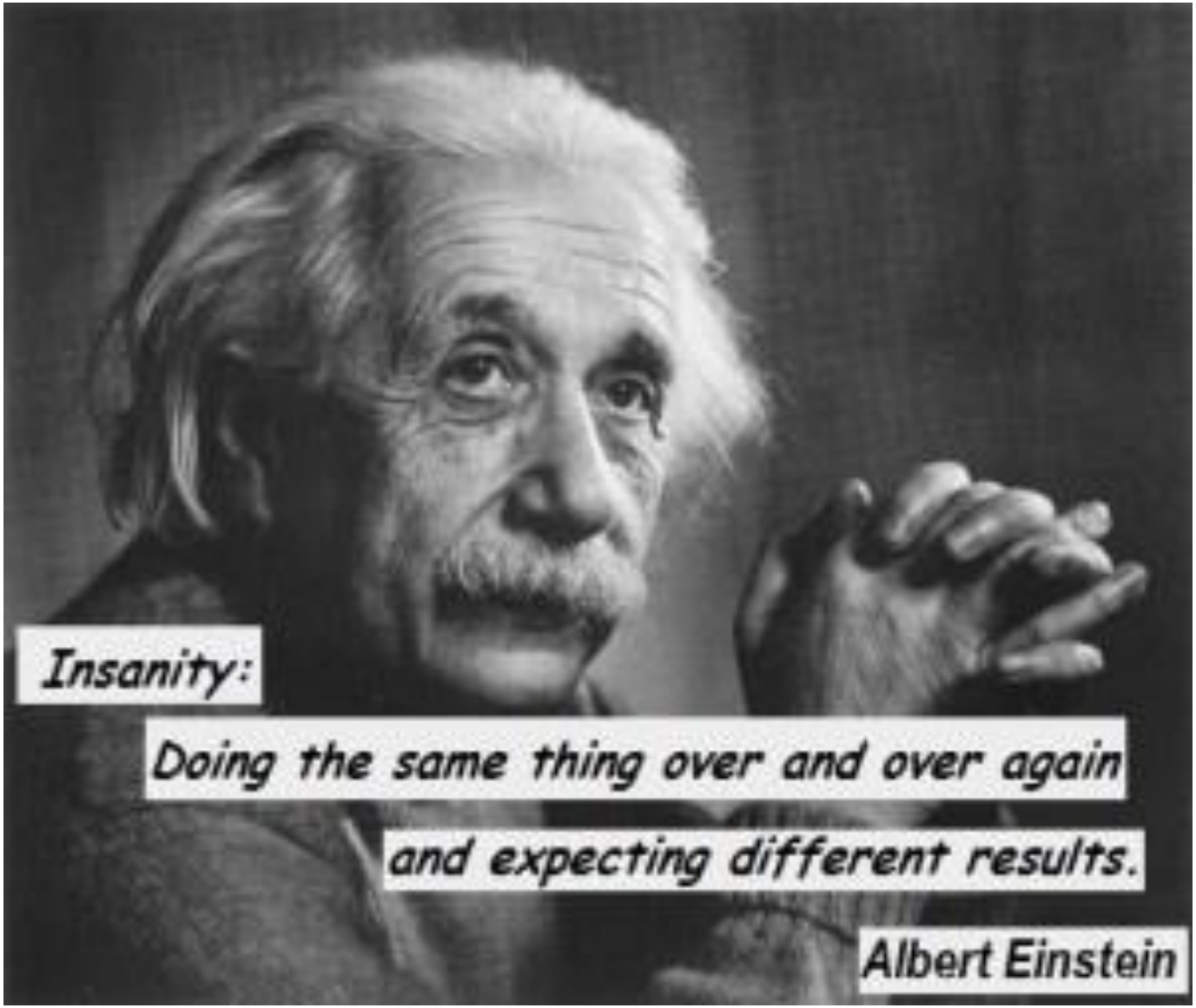
The Dimension of TIME

Why do we need to try a different way?



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Arul Chinnaiyan
Yashar Niknafs
Hui Jiang

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FHCRC
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Evan M. Kroh
Rachael K. Parkin
Patrick S. Mitchell
Hilary Briggs
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Emily Gallichotte
Jamie Guenthoer
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Derek L. Stirewalt

Johanna Lampe
Florian Hladik
Julie McElrath

Peter S. Nelson

University of Washington
Jennifer Noteboom
Robert L. Vessella
Colm Morrissey
Colin Pritchard
Jonathan Tait
Daniel W. Lin

FUNDING
NIH
DOD
Damon Runyon-Rachleff
Innovation Award
Prostate Cancer Foundation
Canary Foundation
Stand Up To Cancer Foundation

American Association
for Cancer Research

FINDING CURES TOGETHER℠

Break

9:50 – 10:10 a.m.

American Association for Cancer Research

FINDING CURES TOGETHER℠

Session II

Liquid Biopsies in Lung Cancer Drug Development and Clinical Use

Chair: Pasi Jänne, MD, PhD

Speakers:

Geoffrey Oxnard, MD

Lecia Sequist, MD, MPH

Panelists:

David Shames, PhD

Kenneth Thress, PhD

Victoria Zazulina, MD

Plasma Genotyping for Treatment Selection in Advanced NSCLC

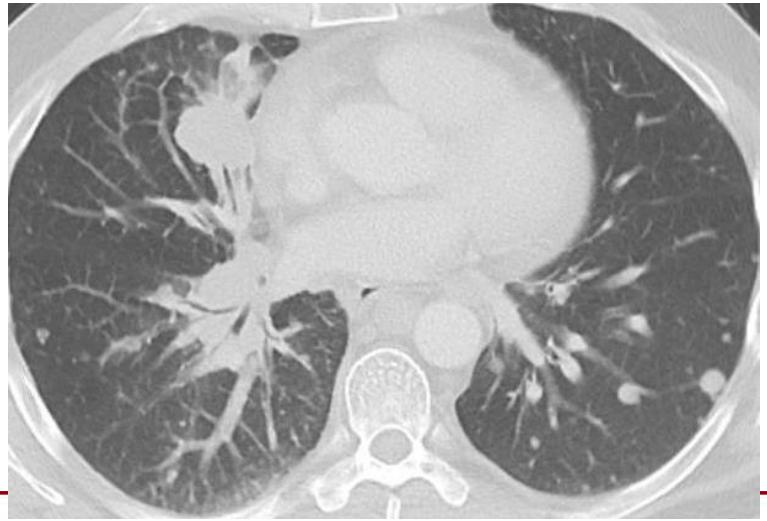
Geoffrey R. Oxnard, MD
Assistant Professor, Dana-Farber Cancer Institute
Damon Runyon Clinical Investigator

Outline

- Three cases of plasma genotyping for NSCLC:
  1. Newly diagnosed NSCLC, genotype unknown
  2. Acquired resistance to EGFR TKI, resistance mechanism unknown
  3. Suspected recurrence of NSCLC

Case #1

- 49 yo M never-smoker p/w several weeks of cough, headache
  - Chest CT shows adenopathy, pulm nodules



Case #1

- 49 yo M never-smoker p/w several weeks of cough, headache
  - Chest CT shows adenopathy, pulm nodules
  - Brain MRI with 8mm cerebellar lesion, cannot rule out lepto
  - Supraclav biopsy shows NSCLC
- Presents to oncology 4 days post-biopsy
  - Path not yet finalized, genomics not started

EGFR genotyping in cfDNA

- Tumor biopsies involve multiple steps:

- Liquid biopsies involve fewer steps:

Sacher et al, JAMA Onc, 2016

EGFR genotyping in cfDNA

- Tumor biopsies involve multiple steps:

- Liquid biopsies involve fewer steps:

- Is plasma testing faster than tumor testing?
  - Plasma ddPCR: median TAT until result = 3 days
  - Resistance biopsy: median TAT until result = 27 days

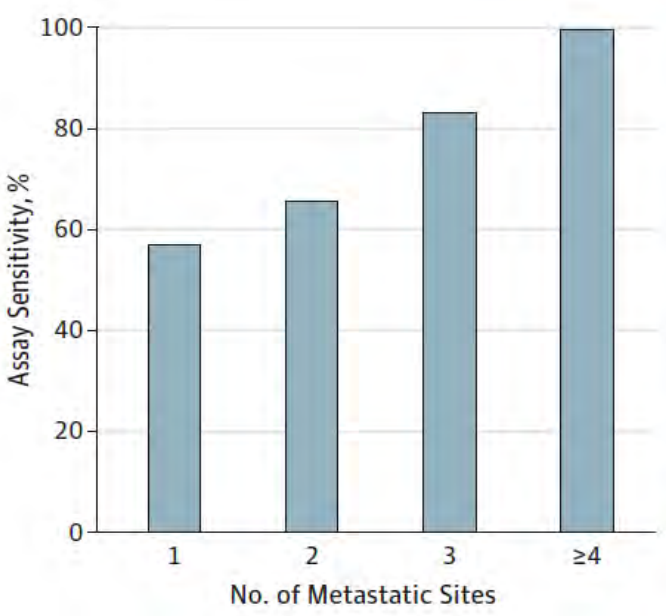
Sacher et al, JAMA Onc, 2016

Case #1

- 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
  - Tumor genotyping pending
- Plasma genotyping of EGFR ordered
  - Seen on a Monday, blood drawn that day
  - Results reported on Wednesday
  - EGFR L858R detected at 34% AF

EGFR genotyping in cfDNA

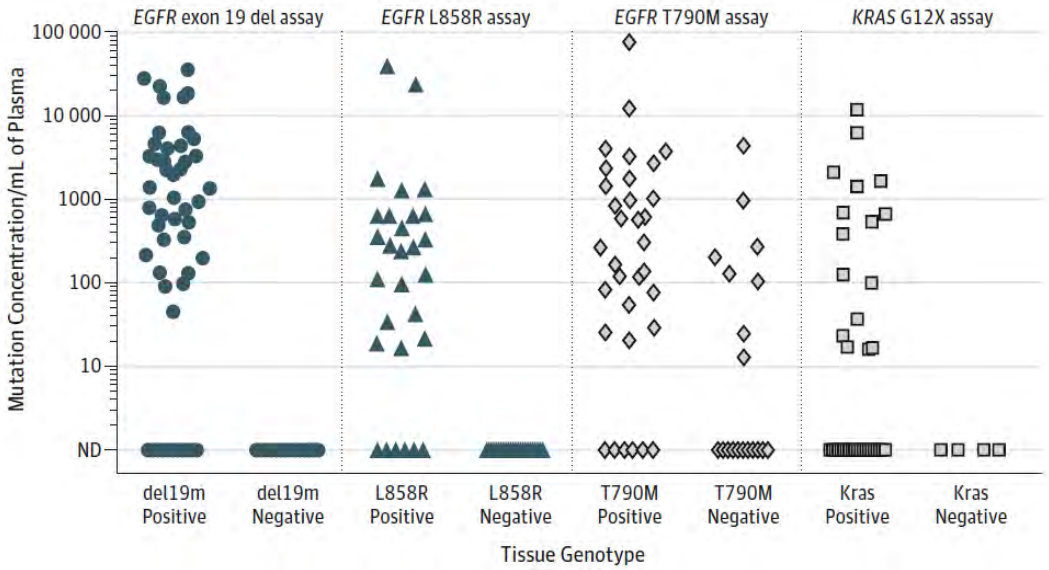
- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - Tested for key mutations in EGFR and KRAS
  - Overall sensitivity of 64-82% for detection of known tumor genotype
  - Rate of detection increases with increased tumor burden



Sacher et al, JAMA Onc, 2016

EGFR genotyping in cfDNA

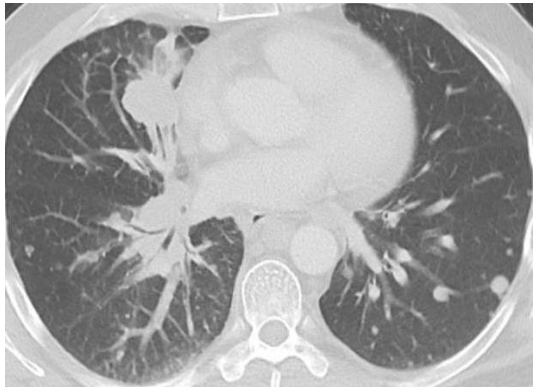
- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - 100% specificity for driver mutations (0% FPR)
  - 63% specificity for T790M resistance mutation



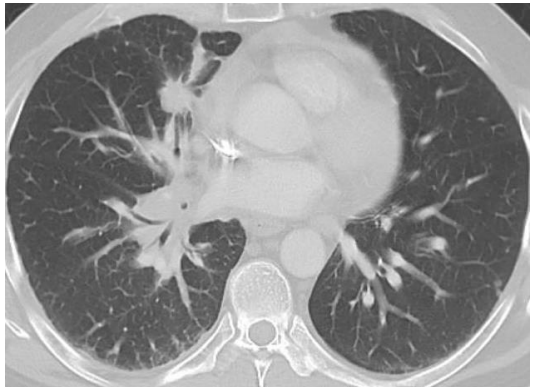
Sacher et al, JAMA Onc, 2016

Case #1

- 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
- Erlotinib initiated, patient symptoms rapidly improve



Baseline



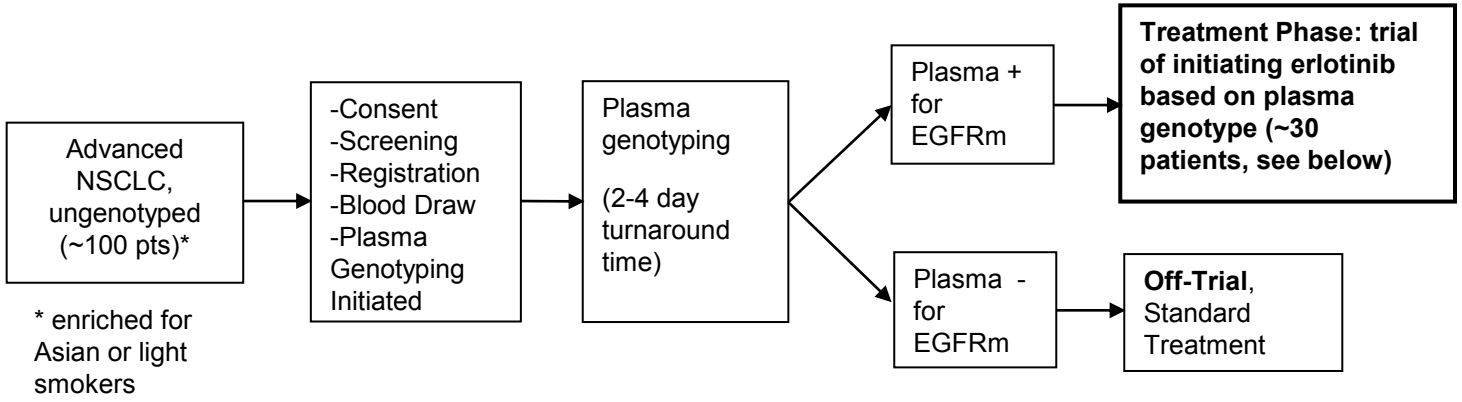
2 months

Conclusions #1

- There are practical / logistical challenges to widespread use to tumor genotyping
- Plasma genotyping can allow rapid testing and initiation of targeted therapy
- High positive predictive value means a positive result can be trusted
- However, sensitivity of ~80% so false negatives could be misinterpreted

Next steps #1

- DFCI now launching a clinical trial of plasma EGFR genotyping for rapid initiation of erlotinib (NCT02770014)



  - Will use our validated plasma ddPCR which is now offered as a CLIA test at BWH

Case #2

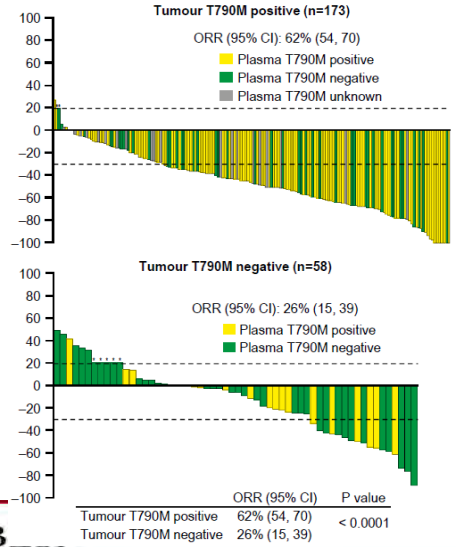
- 73 yo F with EGFR-mutant NSCLC presents on erlotinib x2 years
  - CT shows increased para-aortic mass (4 → 5cm) and increased subcm LN
  - She is asymptomatic
- Is it now standard of care to get a biopsy to molecularly characterize resistance?

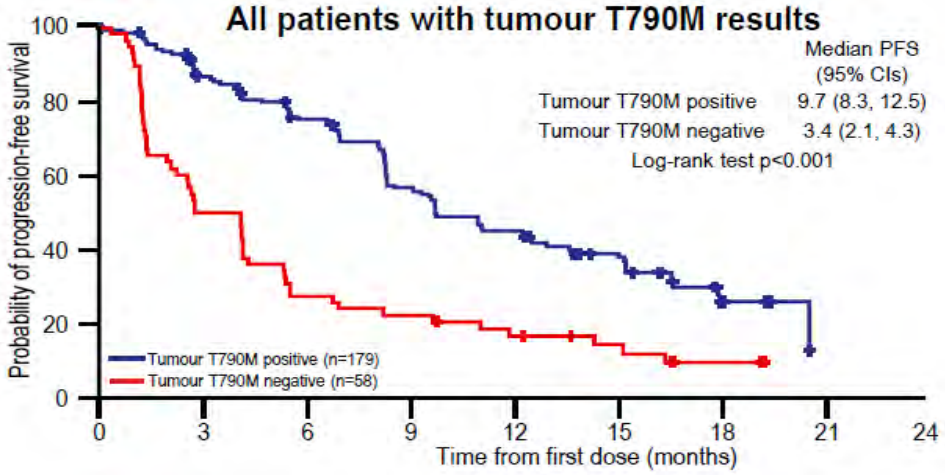
Osimertinib & tumor T790M

- Osimertinib (AZD9291) approved in multiple countries for T790M+ resistance
  - T790M status is associated with outcome

T790M+ in tumor: 62% RR

T790M- in tumor 26% RR

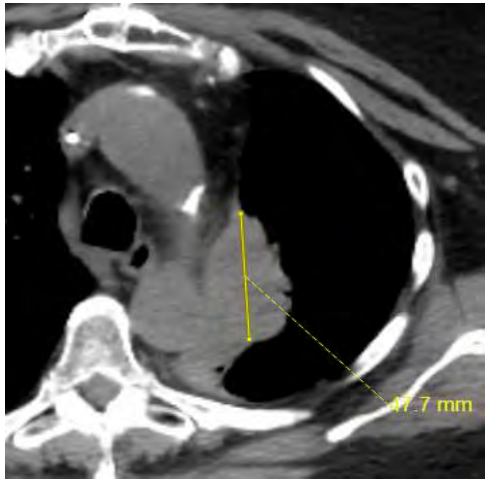




Oxnard et al, JCO, 2016

Case #2

- 73 yo F with EGFR-mutant NSCLC p/w acquired resistance to erlotinib
- Tumor biopsy proposed to test for T790M and evaluate for osimertinib
  - Biopsy would be risky and technically challenging

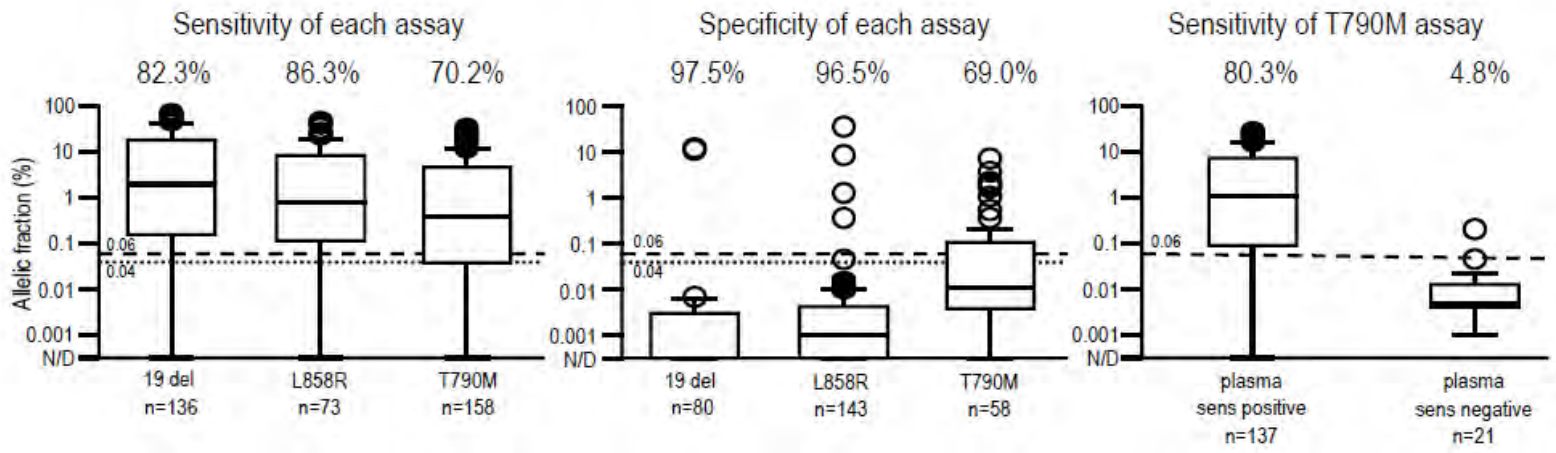


Case #2

- 73 yo F with EGFR-mutant NSCLC p/w acquired resistance to erlotinib
- Tumor biopsy proposed to test for T790M and evaluate for osimertinib
- Genotyping of cfDNA sent instead:
  - EGFR L858R “detected” at <1% AF
  - EGFR T790M not detected

Osimertinib & plasma T790M

- Plasma from AURA trial sent for BEAMing
  - Similarly found that sensitivity was 70%-86%
  - Similarly found a high specificity (>95%) for driver EGFR mutations but only 69% specificity for T790M



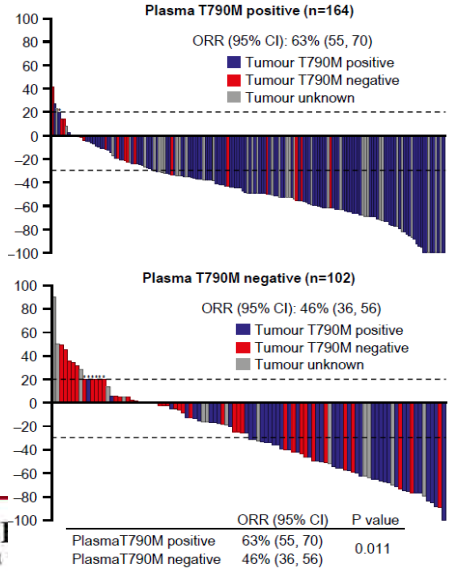
Oxnard et al, JCO, 2016

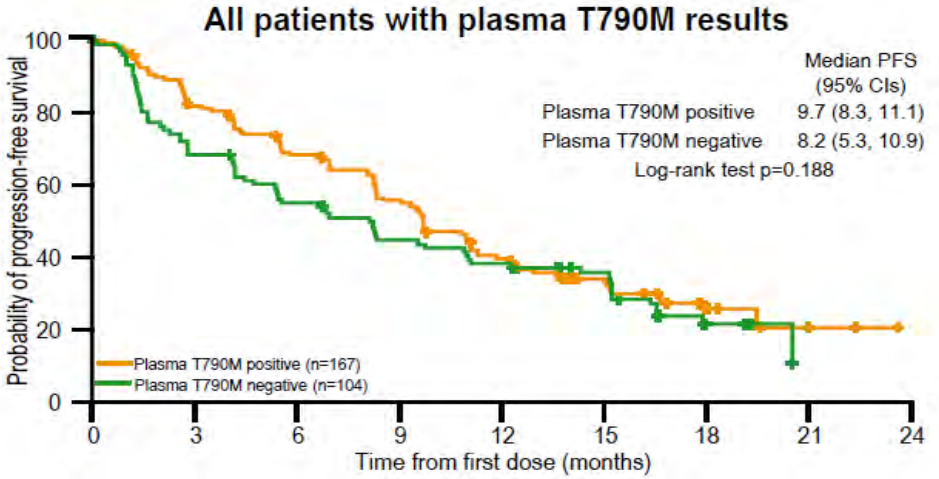
Osimertinib & plasma T790M

- Plasma from AURA trial sent for BEAMing
  - Despite the false positives, plasma T790M+ cases do well, like tumor T790M+
  - But plasma T790M- cases do better than expected

T790M+ in plasma: 63% RR

T790M- in plasma 46% RR





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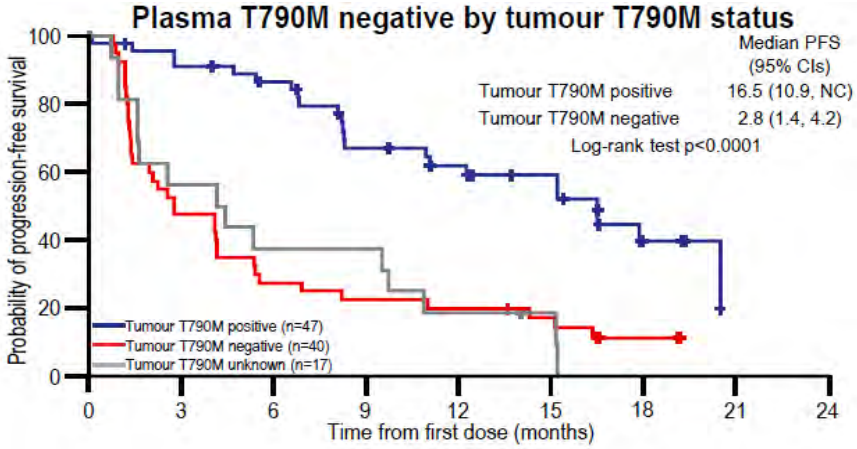
Osimertinib & plasma T790M

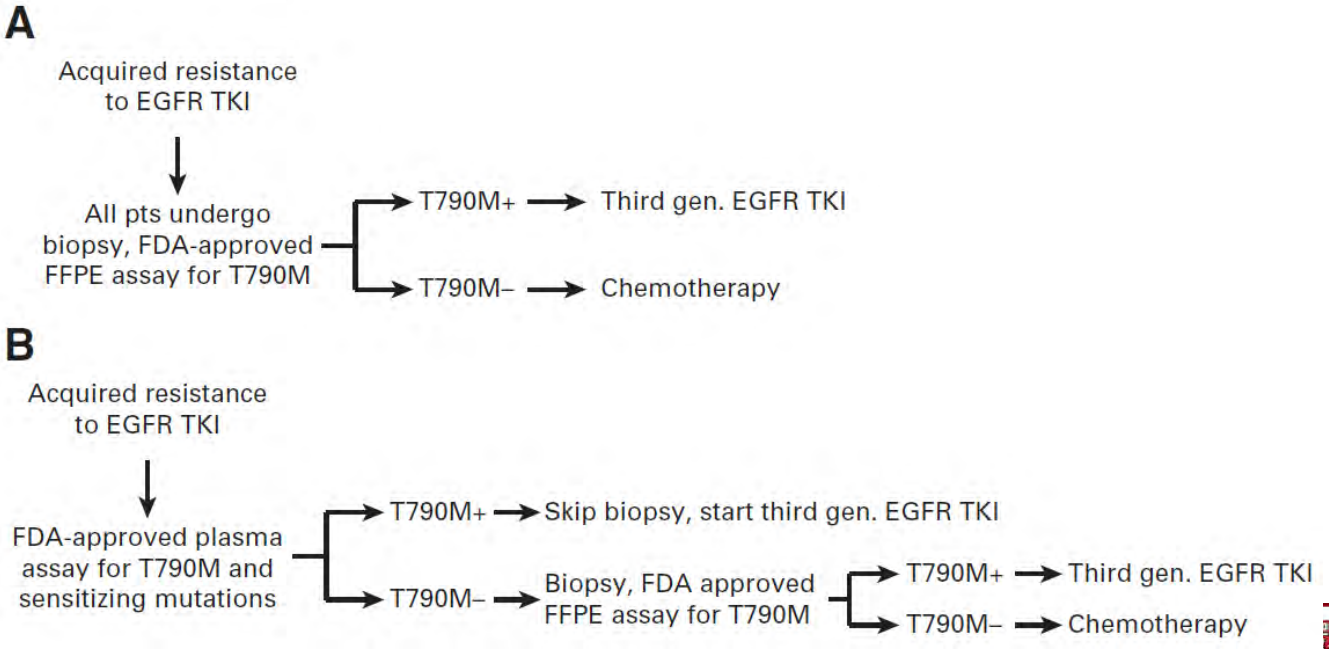
- Plasma from AURA trial sent for BEAMing
  - Despite the false positives, plasma T790M+ cases do well, like tumor T790M+
  - But plasma T790M- cases do better than expected
    - Tumor genotyping can clarify which plasma T790M- patients do better or worse on osimertinib



Oxnard et al, JCO, 2016

Osimertinib & plasma T790M

- Proposed algorithm where plasma genotyping is a screening test prior to biopsy:



Oxnard et al, JCO, 2016

Case #2

- 73 yo F with EGFR-mutant NSCLC p/w acquired resistance to erlotinib
- Tumor biopsy proposed to test for T790M and evaluate for osimertinib
- Genotyping of cfDNA sent instead:
  - EGFR L858R “detected” at 0-1% AF
  - EGFR T790M not detected
- Elected to continue erlotinib and repeat scans in 2 months

Case #2

- Patient is hospitalized 6 weeks later with hypoxia, PE, and progression in lung
  - Too ill for a biopsy
  - Needs anticoagulation
- Repeat plasma genotyping is sent:
  - EGFR L858R positive at 2% AF
  - EGFR T790M negative**

Case #2

- Patient is hospitalized 6 weeks later with hypoxia, PE, and progression in lung
  - Too ill for a biopsy
  - Needs anticoagulation
- Repeat plasma genotyping is sent:
  - EGFR L858R positive at 2% AF
  - Note that, in contrast to other "low positive" results, in fact in this case the overall number of droplets was rather high - 16 of 2757 droplets at codon 790 showed the T790M mutation. This is an overall percentage below our validated threshold for reporting a positive result, so under CLIA we must report it as negative, but most of the false positive or indeterminate results that were the basis for this cutoff had overall lower numbers of droplets.

    In this context, I am concerned that there is, in fact, a T790M mutation being shed into the plasma at a low rate. If this information is necessary for clinical management, I recommend repeat analysis - either after a brief period of close clinical observation or after a biopsy is performed to obtain tissue directly from the lesion in question.

Case #2

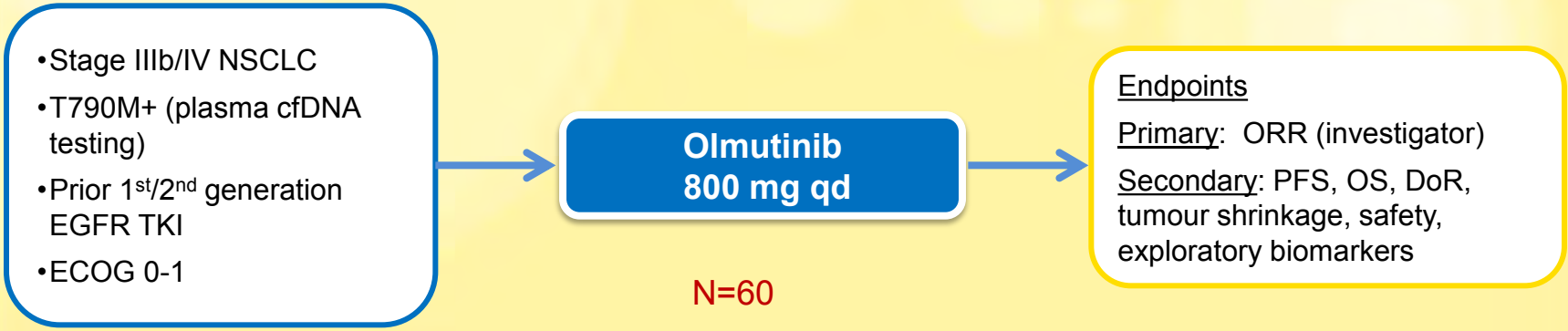
- Patient is hospitalized 6 weeks later with hypoxia, PE, and progression in lung
  - Too ill for a biopsy
  - Needs anticoagulation
- Repeat plasma genotyping is sent:
  - EGFR L858R positive at 2% AF
  - EGFR T790M negative**
- She is started on off-label osimertinib

Conclusions #2

- There is clear clinical need for plasma genotyping to aid the management of drug resistance
- Unclear “reference standard” for resistance mutations given genomic heterogeneity across disease sites
- Many of the available assays have not been optimized / validated to meet clinical need

ELUXA 6: Phase II study olmutinib (BI 1482694) in T790M+ NSCLC

In preparation



Study trial country: USA

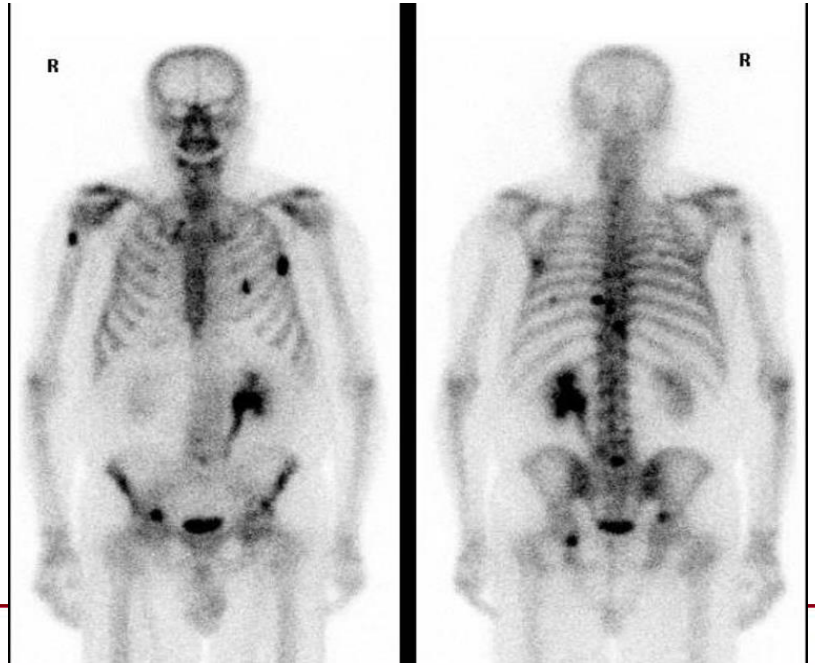
Study initiation: Q3' 2016

Case #3

- 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions
  - Stage II adenocarcinoma resected 3 years prior, followed by adjuvant chemo
  - Surveillance CT shows new sclerotic lesions in bilateral ribs
  - Bone scan confirms abnormal uptake in rubs, spine, pelvis suspicious for a metastatic process

Case #3

- 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions



Case #3

- 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence
- Bone biopsy felt to be technically challenging
- Plasma genotyping for EGFR & KRAS
  - Positive for EGFR L858R, 3.5% AF
- Does this confirm recurrence of his NSCLC?

Plasma genotyping for cancer diagnosis

- No clear data available on the use of plasma genomics to make a diagnosis
- However, tumor NGS is increasingly informing standard pathologic eval:
  1. Neuroendocrine carcinoma → Ewings sarcoma
  2. Esophageal cancer → ALK+ NSCLC
  3. Poorly differentiated NSCLC → mesothelioma
- Alternatively, some mutations (e.g. KRAS) offer little insight into primary

Case #3

- 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence
- Plasma genotyping positive for EGFR L868R
- Core biopsy of R hip lesion positive for lung adenocarcinoma
  - Tumor genotyping: EGFR L858R
- Patient is started on erlotinib

Conclusions #3

- Unclear whether a biopsy for cancer diagnosis or staging can really be replaced by plasma genotyping
- But perhaps when combined with other tools like CTC analysis, this could be possible in the future?

Acknowledgements

- Lowe Center for Thoracic Oncology, DFCI
  - Pasi Jänne, Ryan Alden, Adrian Sacher
- Translational Research Lab / Belfer Center, DFCI
  - Cloud Paweletz, Yanan Kuang, Nora Feeney
- CAMD at Brigham & Women's Hospital
  - Lynette Sholl, Neal Lindeman
- Industry Collaborators
  - AstraZeneca, Boehringer-Ingelheim, Astellas, etc.
  - Resolution Bioscience, Guardant, Illumina, etc.
- Funding
  - Damon Runyon Cancer Research Foundation, NCI, DOD, Conquer Cancer Foundation of ASCO, Stading-Younger Cancer Research Foundation

Looking to the future: Liquid biopsies for treatment monitoring, risk stratification, and early detection

Lecia V. Sequist, MD, MPH

Massachusetts General Hospital Cancer Center

Associate Professor of Medicine, Harvard Medical School

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COI

- Uncompensated consulting for Boehringer Ingelheim, Novartis, Merrimack, Clovis
- Consulting fees from AstraZeneca, Ariad, Genentech/Roche

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Where are we today with respect to being able to….

- Use liquid biopsies to meaningfully monitor response to treatment?
- Use liquid biopsies as a method to risk stratify patients?
- Incorporate liquid biopsies into early cancer detection research?

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Monitoring Response to Treatment

Traditionally we start a new therapy and wait ~ 8 weeks to determine tumor response via CT scan

Can liquid biopsies be used to give an earlier clue?

Could they warn us about emerging resistance?

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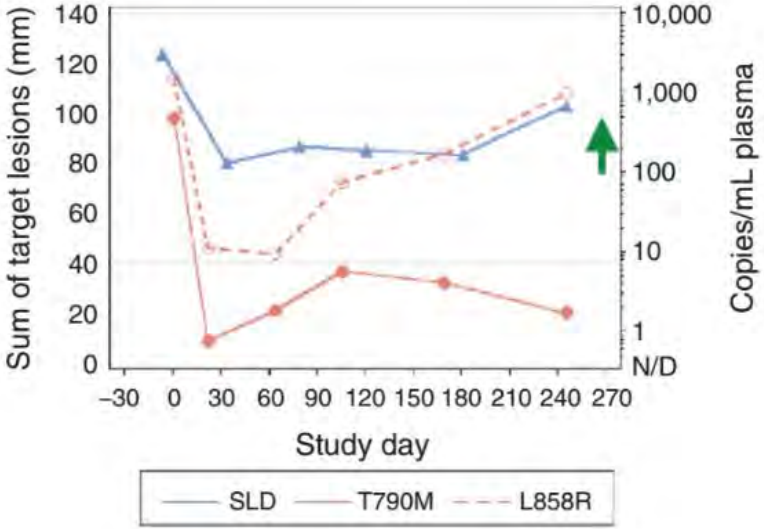
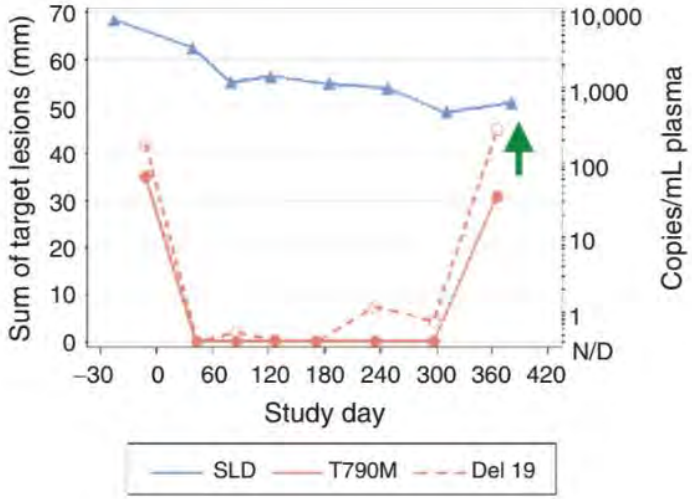
Serial ctDNA can correlate with outcomes





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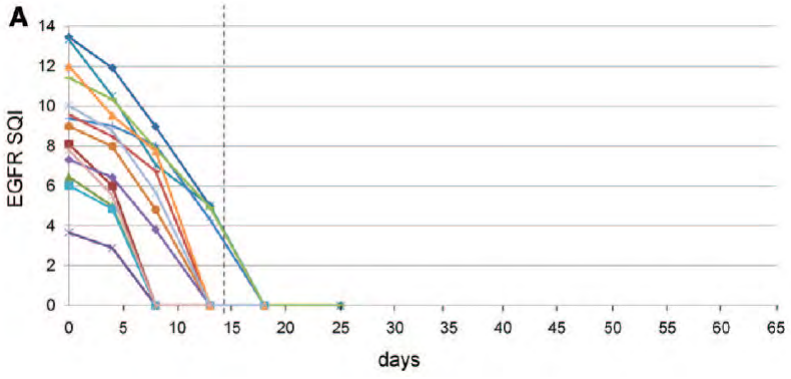
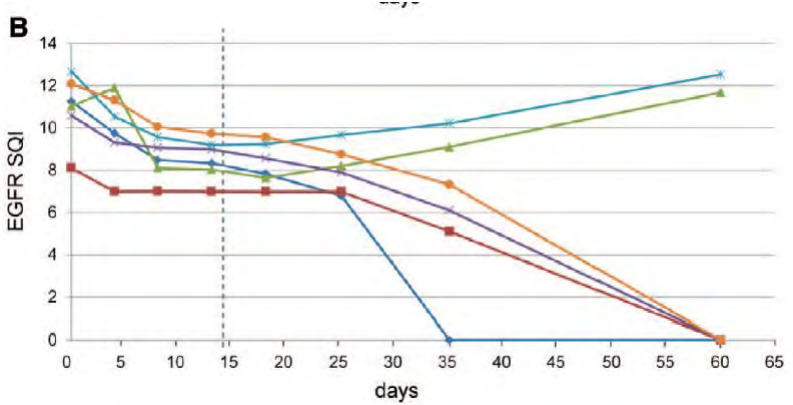
Piotrowska, et al. Cancer Disc 2015

BEAMing assay for ctDNA

Serial ctDNA can correlate with outcomes



“Fast” Responders
(N=14)
Median percent tumor shrinkage = 59%



“Slow” Responders
(N=6)
Median percent tumor shrinkage = 18%

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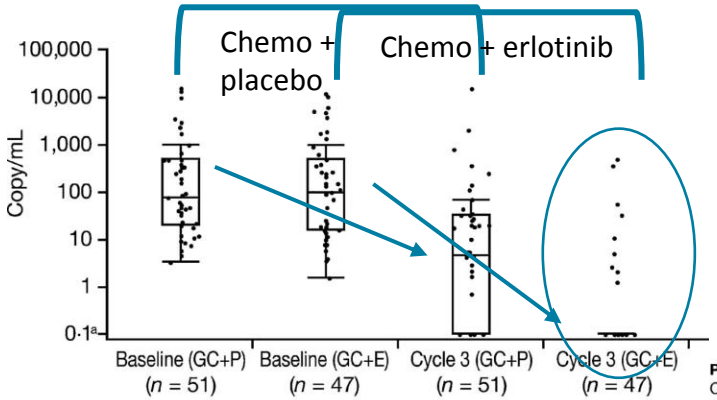
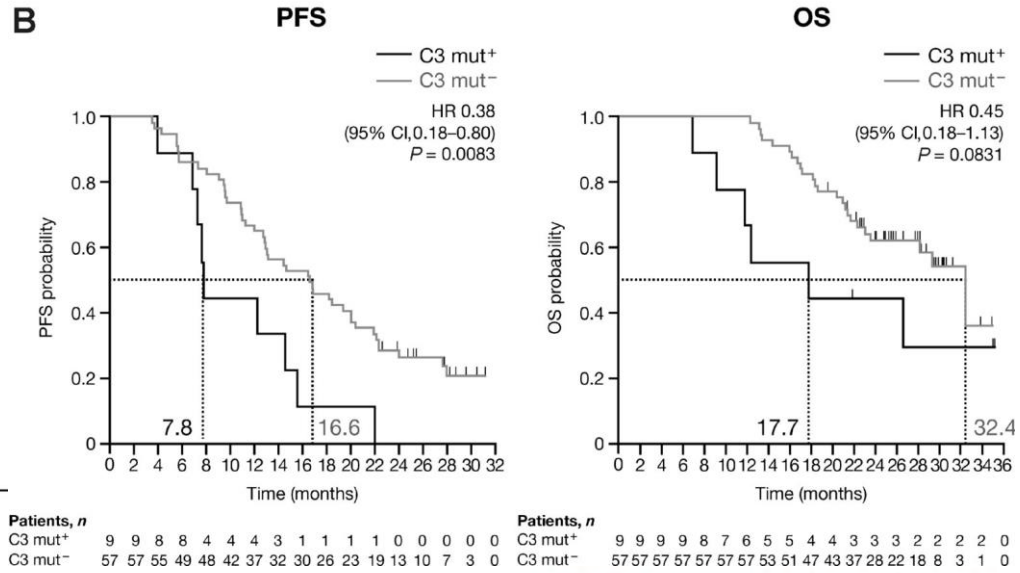
Marchetti et al. 2015 J Thorac Oncol
Semiquantitative index (SQI) derived using Cobas EGFR PCR test (Roche).

Serial ctDNA can correlate with outcomes

FAST-ACT 2 trial: Chemo with intercalated Erlotinib or Placebo: Subset of EGFR+ patients with detectable cfDNA at baseline





Median *EGFR* mut+ cfDNA (copy/mL of blood)	GC+P	GC+E
Baseline	78	94
C3	5	0
PD	83	6

Mok et al. 2015 Clin Cancer Res

Cobas EGFR PCR test (Roche).

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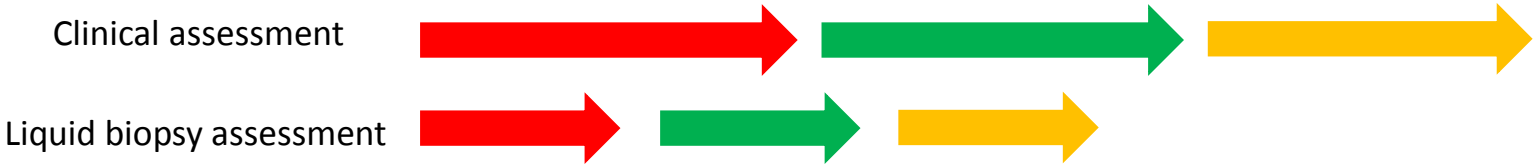
However, what to do with this information?

- No clear evidence in lung cancer that *early* therapy switch prior to clinical progression improves outcomes.



- There is ample data on maintenance therapy, however this is conceptually distinct with a focus on non-progressors and pivotal comparison to holiday from all therapy
- In EGFR disease specifically, much more data exists supporting treatment beyond progression

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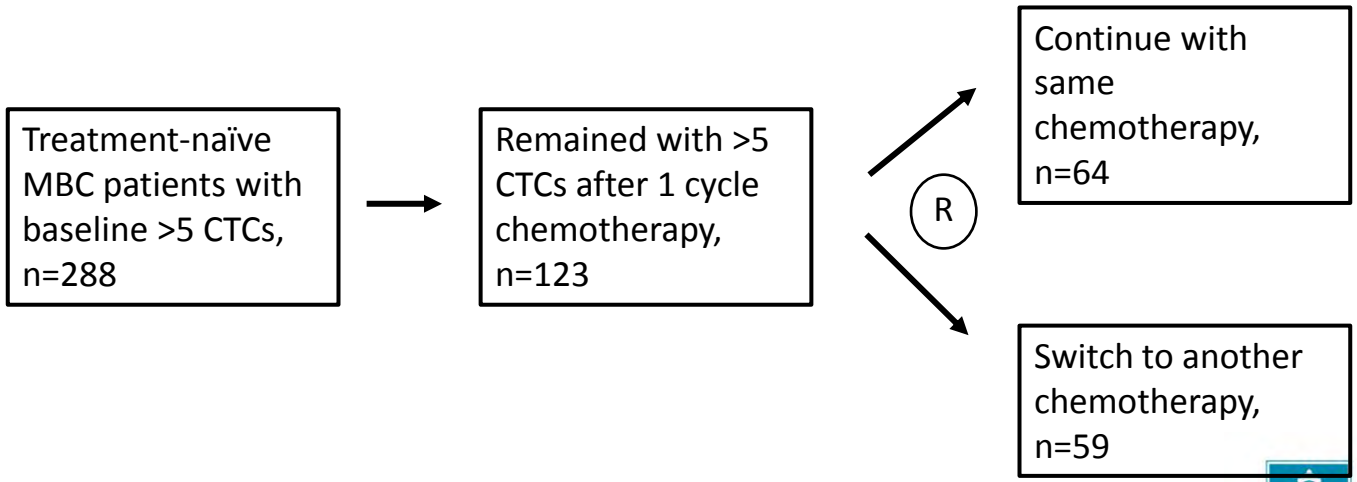
Breast cancer S0500 Study



Primary outcome = OS

Smerage et al, JCO 2014
Cellsearch CTC assay

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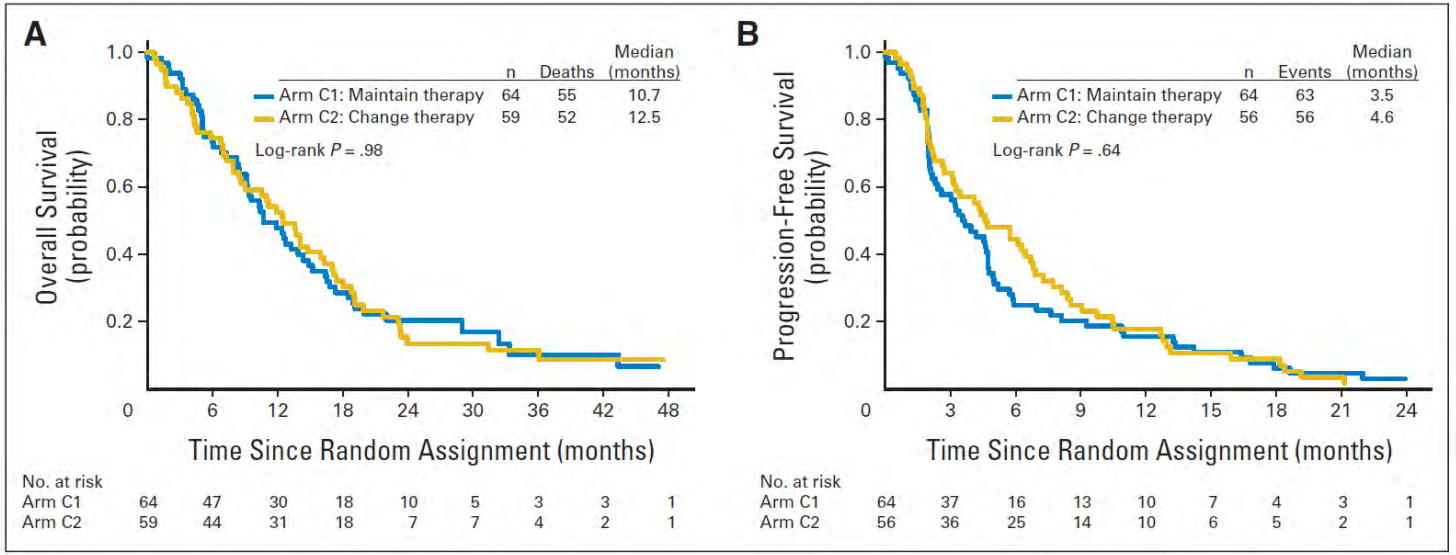
Breast cancer S0500 study



Conclusion: Switching therapy early for "failure to respond" did not impact overall survival, ie did not improve treatment outcome

Smerage et al, JCO 2014
Cellsearch CTC assay

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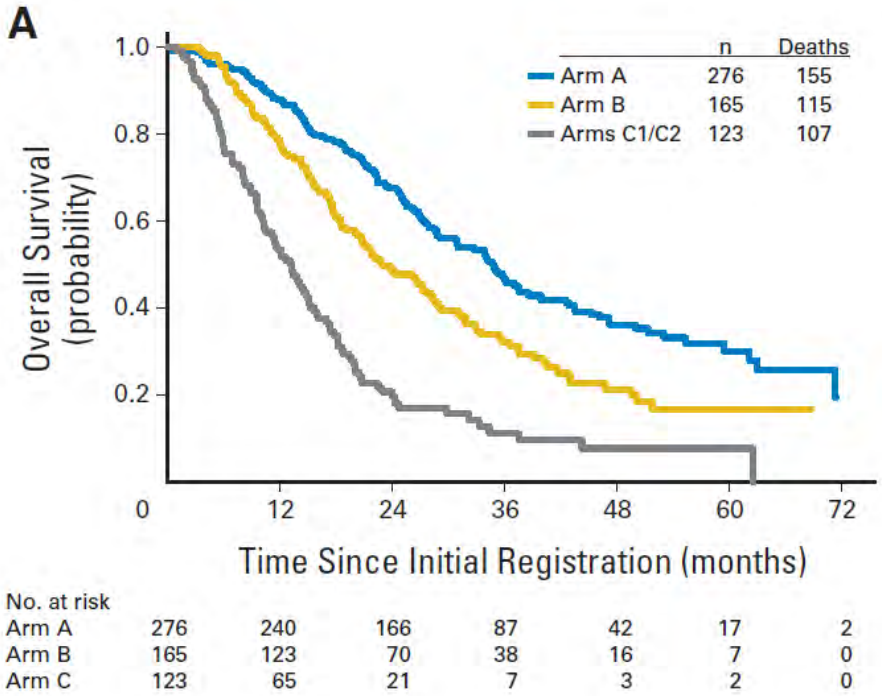
Breast cancer S0500 Study



A = no baseline elevated CTCs
B = CTCs fell < 5 with 1 cycle chemo
C = randomized group w/ ongoing elevated CTCs

Conclusion: CTC assessment of "failure to respond" was prognostic, but intervention did not salvage pts. So…..risk stratification not necessarily helpful unless you can act to modify the risk

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Smerage JCO 2014
Cellsearch CTC assay

So……Is there a situation where monitoring makes sense in clinical lung cancer care today?

- No evidence basis for doing this
- However, the wide availability of reliable ctDNA-based mutation monitoring makes it appealing for patients and doctors alike in those with genotype-defined cancers like EGFR
- We should be cautious about using such monitoring to drive treatment decisions until data is generated that this improves outcomes

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And now for a horse of a different color….

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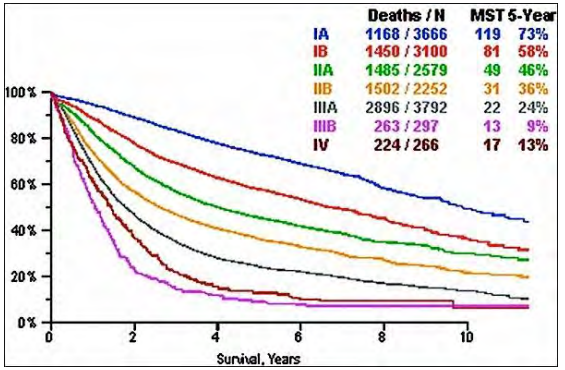
Screening for early stage lung cancer

- 226,000 new cases and 158,000 deaths in the US from lung cancer in 2015 (equivalent to 433 deaths per day)
- Early stage lung cancer is curable
- 5-year survival can be up to 70%
- Most cases present at a late stage when detected by symptoms and carry a poor prognosis of 5-year overall survival < 5%



Goldstraw, et al, JTO 2007

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NLST: National Lung Screening Trial

- Eligibility criteria:
  - 55-74 years old, no signs or symptoms of lung cancer
  - Active or former smoker with a 30 pack year history
  - If a former smoker, must have quit within 15 years
- Exclusions:
  - Requirement for home oxygen, prior history of lung cancer
- Compared low-dose (LDCT) yearly (baseline, 1 year, 2 years) to CXR
- Results: 20% reduction in lung cancer-specific mortality and 7% reduction in all cause mortality
- LDCT screening now recommended by USPSTF

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Aberle et al, NEJM 2011

Issues surrounding NLST and LC screening

- 24% of participants had a nodule detected but 96% of these were not cancer after further work-up (ie, very high false positive rate)
- ~8.6 million Americans would qualify for screening by NLST criteria. If LDCT scan is $200, then this will cost $1.72 billion per year for the first scan only, not including the subsequent work-up for nodules
- Other than cost, concerns include over-diagnosis, medical complications, radiation exposure and screening for those who don't meet NLST criteria (lower pre-test probability)
- Great interest in a companion test that could improve specificity

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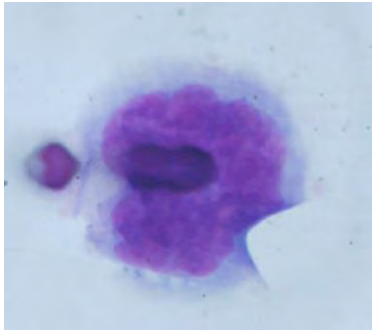
CTCs in early detection of NSCLC

- Ilie et al used the ISET filtration technique in 168 pts with COPD but no significant lung nodules. All pts also underwent baseline + annual CTs
- 5 out of 168 patients had CTCs detected at baseline
- Amazingly, all 5 went on to develop lung cancer at a median time of 3.2 years after the initial blood draw while none of the other 163 patients without CTCs had CT scan positive for cancer after a median follow-up of 60 months
- All 5 lung cancer patients had IA disease and underwent surgical resections



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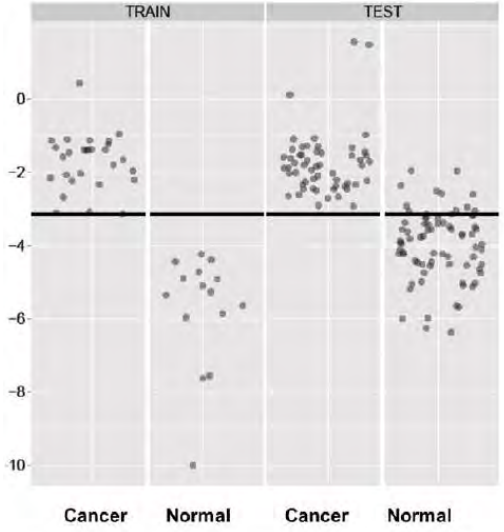
Ilie et al, PLOS 2014

MicroRNAs in early detection of NSCLC

- Hennessey et al examined plasma for microRNAs in n=50 training set and n=130 validation set of patients with and without NSCLC (cancers were mostly early stage)

miRNA-15b/miR-27b	Training set	
	Lung Cancer	Normal
Predicted Lung Cancer	30	0
Predicted normal	0	20

Sensitivity	100%
Specificity	100%
PPV	100%
NPV	100%



miRNA-15b/miR-27b	Test Set	
	Lung Cancer	Normal
Predicted Lung Cancer	55	12
Predicted normal	0	63

		95% CI
Sensitivity	100%	0.93-1.0
Specificity	84%	0.73-0.91
PPV	82%	0.70-0.90
NPV	100%	0.94-1.0

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Hennessey et al, PLOS 2014

MicroRNAs in early detection of lung cancer

- Italian group used microRNAs in a cohort of over 900 pts participating in 2 randomized LC screening trials
- LDCT alone had a false positive rate of 19% but combined with their miRNA signature classifier, false positive rate fell to 4%

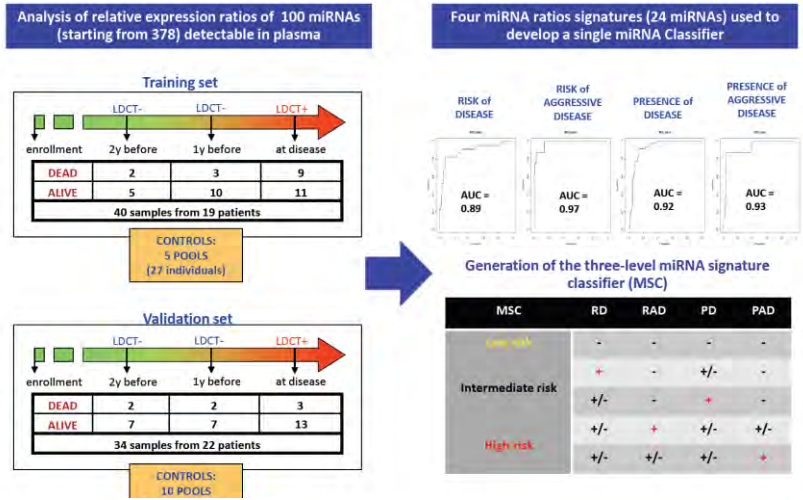

Table 3 Overall diagnostic performance of MSC

	Total	MSC (risk of lung cancer)		
		High (%)	Intermediate (%)	Low (%)
All subjects	939	63 (6.7)	159 (16.9)	717 (76.4)
No lung cancer	870	32 (3.7)	130 (14.9)	708 (81.4)
Lung cancer	69	31 (44.9)	29 (42.0)	9 (13.0)

MSC, miRNA signature classifier.

Boeri et al, PNAS 2011
Sozzi, et al, JCO 2014

What will the next few years bring?

Technology explosion with new platforms / liquid biopsies

+

Broad scale implementation of LDCT screening programs

=

Amazing opportunity to study novel technologies along with LDCT screening

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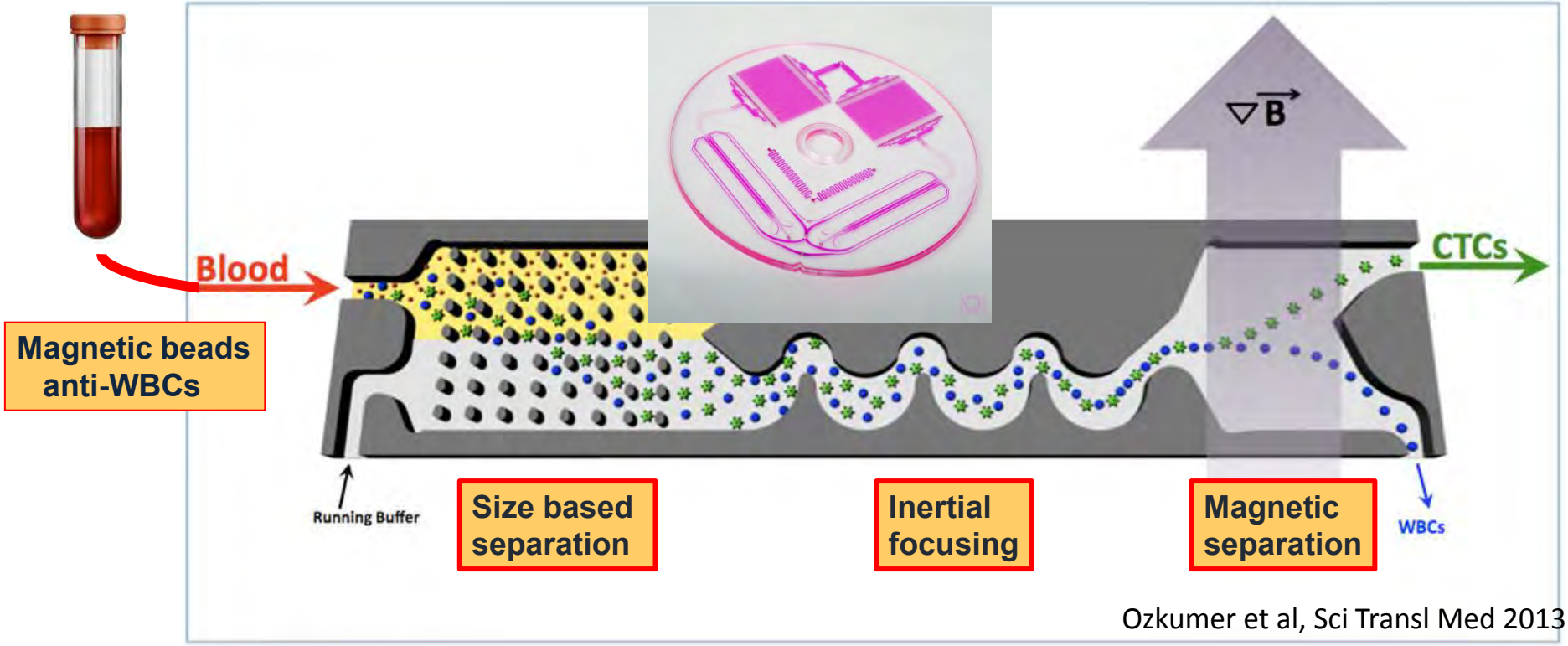
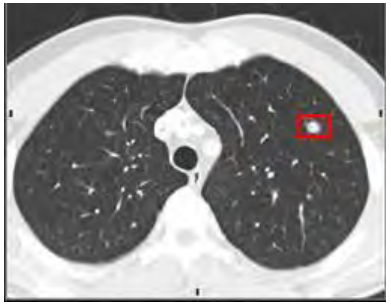
Ongoing research at MGH: the iChip for CTCs



Ozkumer et al, Sci Transl Med 2013

Early stage lung cancer ctDNA collection, MGH



Pre-op
Intra-op
Post-op

ctDNA collection

Zosia Piotrowska and Mike Lanuti, Guardant LUNAR study

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Summary

- Most data to date on disease monitoring is using ctDNA to detect specific mutations like EGFR
- Though appealing, disease monitoring and risk stratification are tricky without evidence of an action to take for poor risk patients that can improve outcome
- CTCs, miRNA, ctDNA, exosomes, others could be used in addition to LDCT screening to help improve specificity of lung nodules on CT scan. Many studies are ongoing in this arena

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American Association for Cancer Research

FINDING CURES TOGETHER℠

Session II Panel Discussion

Liquid Biopsies in Lung Cancer Drug Development and Clinical Use

Chair: Pasi Jänne, MD, PhD

Speakers:

Geoffrey Oxnard, MD

Lecia Sequist, MD, MPH

Panelists:

David Shames, PhD

Kenneth Thress, PhD

Victoria Zazulina, MD

American Association
for Cancer Research

FINDING CURES TOGETHER℠

During audience Q&A sessions, webcast participants may send questions and comments to

policyquestion@aacr.org

B-FAST (Blood-Fast Assay Screening Trial)

NGS-selected umbrella NSCLC trial platform

David S. Shames, PhD
Principal Scientist
Genentech Inc.,
FDA-AACR Workshop on Liquid Biopsies, July 19th
Washington D.C.

Genentech
A Member of the Roche Group

Initially, B-FAST will allocate patients with EGFR, ALK and RET mutations to treatment

Current TKIs

Oncogenic driver mutations

- EGFR → Multiple options being considered
- ALK → Alectinib
- RET → Alectinib

Future Cohorts TKIs and Combos

Oncogenic driver mutations

- Rare Mutations → BRAF, HER2, MET, FGFR, etc.
- Combinations → ALK/MEK, BRAF/MEK/aPDL1, etc.

1L Non-SCC, SCC NSCLC
PS 0-2
20 ml mandatory blood-draws at screening, enrollment, on-study, PD

FOUNDATIONACT COVERS THE 62 MOST DRUGGABLE GENES

Full Gene List				
ABL1	CDK6	FGFR3	KRAS	NRAS
AKT1	CDKN2A	FLT3	MAP2K1	PDCD1LG2
ALK	CRKL	FOXL2	MAP2K2	PDGFRA
ARAF	CTNNB1	GNA11	MDM2	PDGFRB
BRAF	DDR2	GNAQ	MET	PIK3CA
BRCA1	EGFR	GNAS	MPL	PTEN
BRCA2	ERBB2	HRAS	MTOR	PTPN11
BTK	ERRFI1	IDH1	MYC	RAF1
CCND1	ESR1	IDH2	MYCN	RET
CD274	EZH2	JAK2	MYD88	SMO
CDH1	FGFR1	JAK3	NF1	TERT
CDK4	FGFR2	KIT	NPM1	TP53
				VEGFA

Fusions
ALK
EGFR
FGFR3
PDGFRA
RET
ROS1

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Session II Panel Discussion

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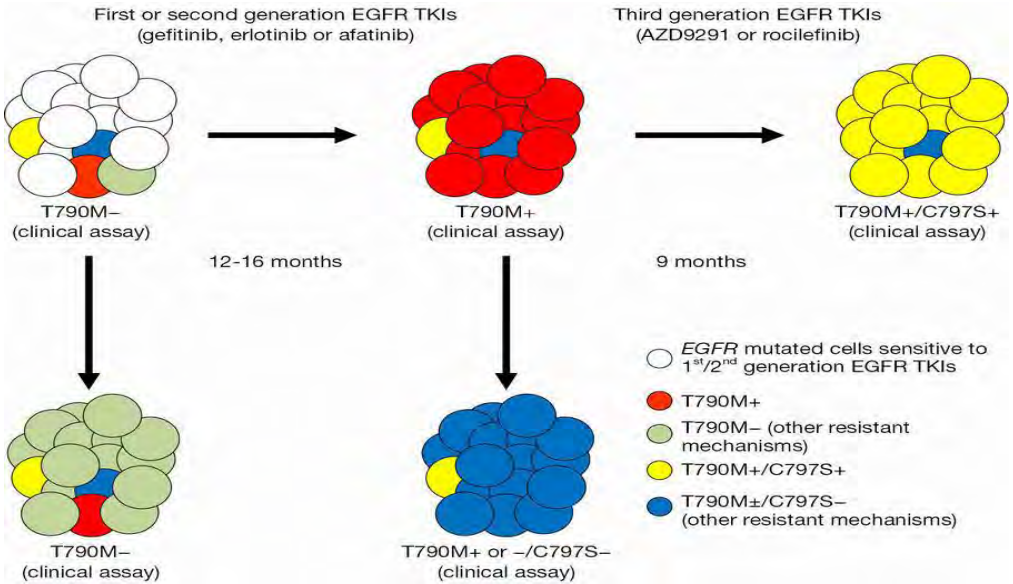
Patient selection with cfDNA

- C797S-mediated acquired resistance to 3rd generation EGFR-TKIs has been reported for osimertinib, rociletinib, and olumutinib (HM61713)*
- Predictable: C797 of the EGFR protein is the covalent binding site for irreversible 3rd gen inhibitors

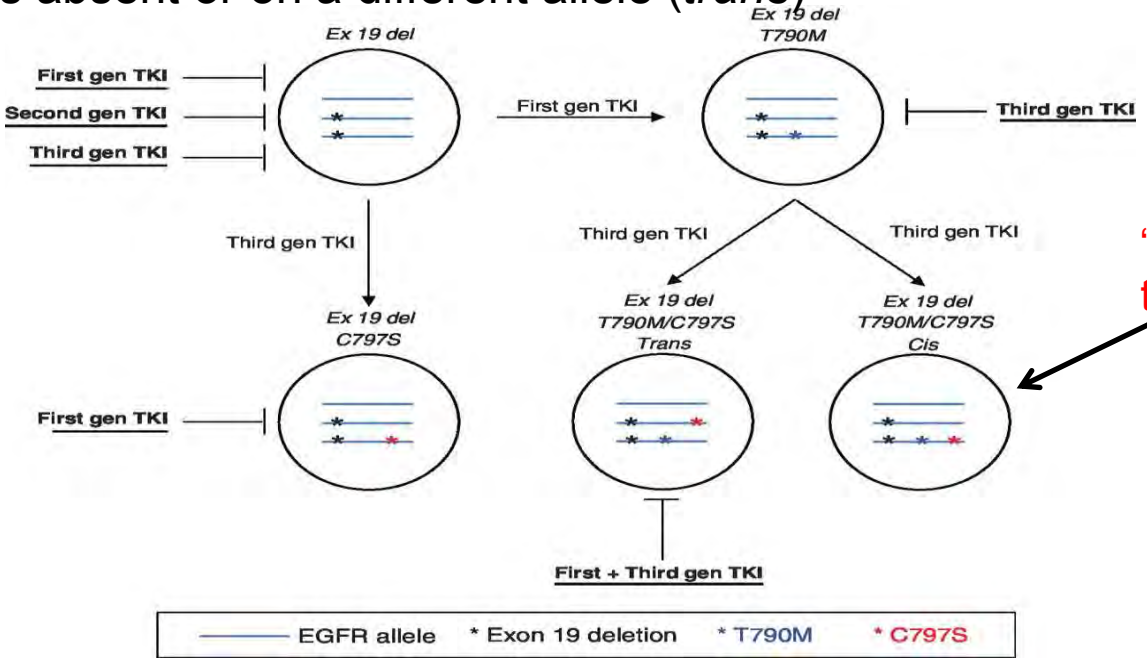


Costa and Kobayashi, *TLCR*; 2015

*Thress et al *Nat Med*; 2015; Song et al *JTO*, 2015; Chabon et al *Nature Comm* 2016;

Allelic context of C797S (may) matter

- *In vitro* cell line work has shown that 1st generation EGFR-TKIs (erlotinib, gefitinib, etc) retain activity against the C797S mutation…BUT only when T790M is absent or on a different allele (*trans*)

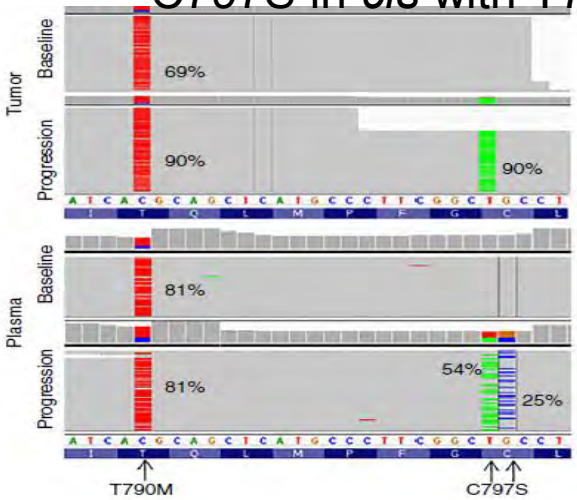


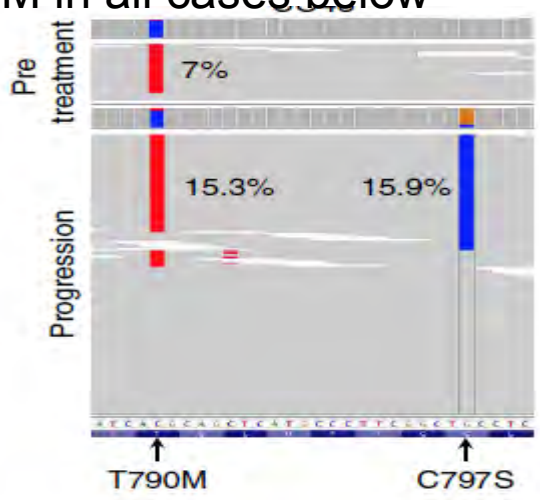
"Triple" mutants are insensitive to any EGFR-TKI combo

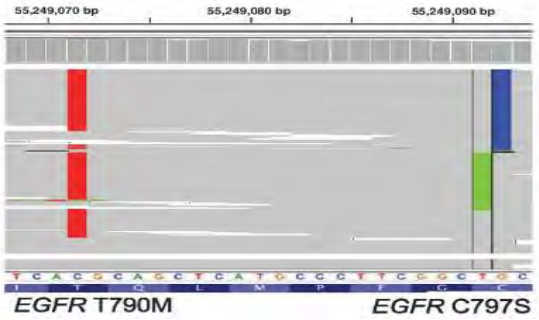
Niederst et al *CCR*; Sept 2015

NGS reveals that C797S is complex

- Different reports using plasma NGS detail complex nature of the acquired C797S mutation (di-nucleotide changes)
- C797S in *cis* with T790M in all cases below







Plasma & tumor NGS
Osimertinib resistance

Plasma NGS
Rociletinib resistance

Plasma NGS
Osimertinib resistance

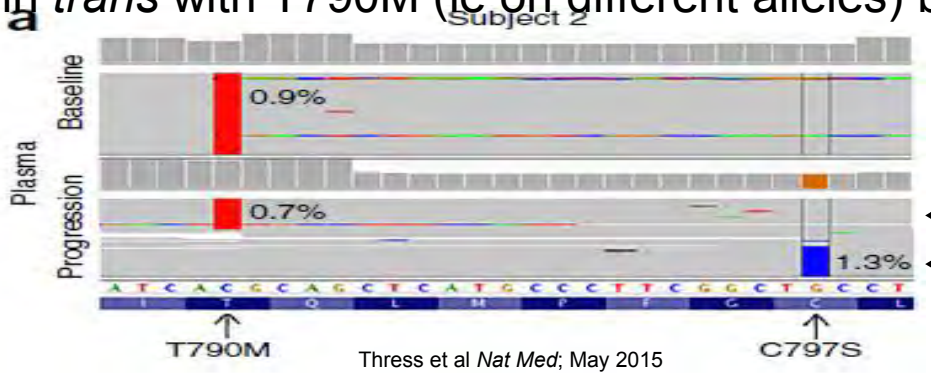
Thress et al *Nat Med*; May 2015

Chabon et al *Nature Comm* 2016

Paweltez et al, *CCR* 2016

C797S can be in *trans* with T790M

- Single reported case (out of 8 in literature) where C797S has been identified in *trans* with T790M (ie on different alleles) by NGS.



Thress et al *Nat Med*; May 2015

Would such a patient benefit from the addition of a 1st gen EGFR-TKI?

Different alleles

Allelic context (via plasma NGS) may be critical to identify the appropriate patients for the right anti-C797S treatment(s)

LETTER

doi:10.1038/nature17960

Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors

Jia et al, *Nature* June 2016

Confidentiality Notice

American Association for Cancer Research

FINDING CURES TOGETHER℠

Session II Panel Discussion

Liquid Biopsies in Lung Cancer Drug Development and Clinical Use

Chair: Pasi Jänne, MD, PhD

Speakers:

Geoffrey Oxnard, MD

Lecia Sequist, MD, MPH

Panelists:

David Shames, PhD

Kenneth Thress, PhD

Victoria Zazulina, MD

American Association
for Cancer Research

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During audience Q&A sessions, webcast participants may send questions and comments to

policyquestion@aacr.org

American Association
for Cancer Research

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Lunch Break

11:45 a.m. – 12:00 p.m.

American Association for Cancer Research

FINDING CURES TOGETHER℠

Case Study

cobas® EGFR Mutation Test v2, a blood-based companion diagnostic for the cancer drug Tarceva (erlotinib)

Speakers:

Reena Philip, PhD

David Shames, PhD

Karen Bijwaard, MS, RAC,MB(ASCP)

Walter Koch, PhD

Erin Larkins, CDR, USPHS

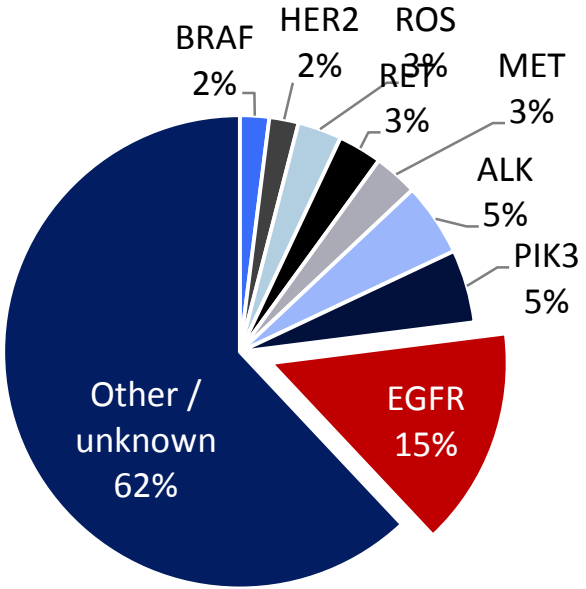
Blood Assay Development in Lung Cancer

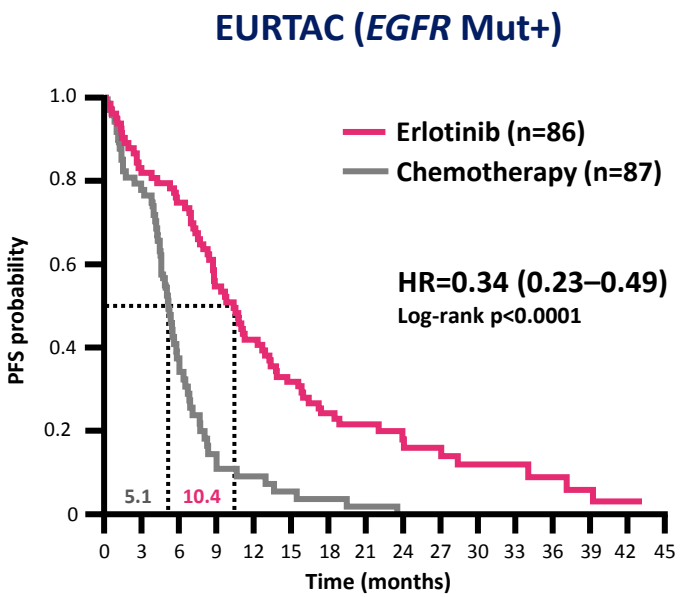
David S. Shames, PhD
Principal Scientist
Oncology Biomarker Development

FDA-AACR Workshop on Liquid Biopsies, July 19th
Washington D.C.

Genentech
A Member of the Roche Group

Recent progress in translational and clinical science has defined several actionable alterations in NSCLC



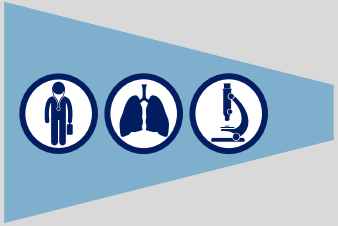


Tarceva is indicated for the first-line treatment of patients with metastatic NSCLC whose tumours have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test

Barlesi, et al. ASCO 2013; NCCN Guidelines – NSCLC v4 2016; Tarceva US PI

Why is blood testing important in metastatic lung cancer?

Even today, fewer than half of patients are treated

Incident patients	Patient diagnosis	Treated patients
		
US: 123,000 EU: 106,000 China: 208,000	GP, Specialist, Oncologist, Pathologist etc.	US: 55% EU: 48% China: <20%
Late presentation or lack of awareness	*Delays, referrals, inconsistent or incomplete use of diagnostics, long turnaround*	*Motivation, understanding, adherence*

As more targeted therapies are approved, molecular testing in alternative substrates will be essential to maximize testing rates for rarer mutations

Tumour tissue sample analyses

8–10 biopsy slides

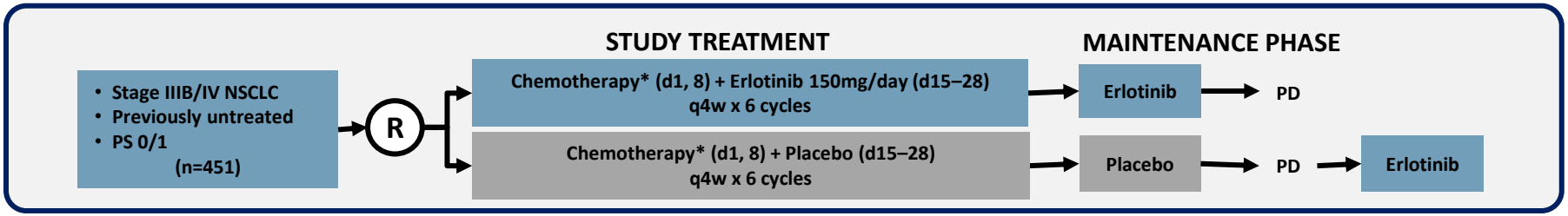
NCCN guidelines

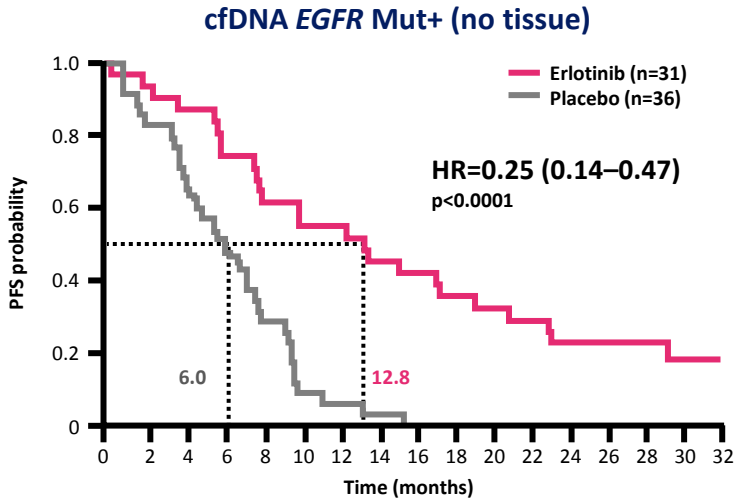
- **H&E + squamous / non-squamous**
- ***EGFR* testing (60% in US)**
- ***ALK* testing (45% in US)**
- **PD-L1 testing**

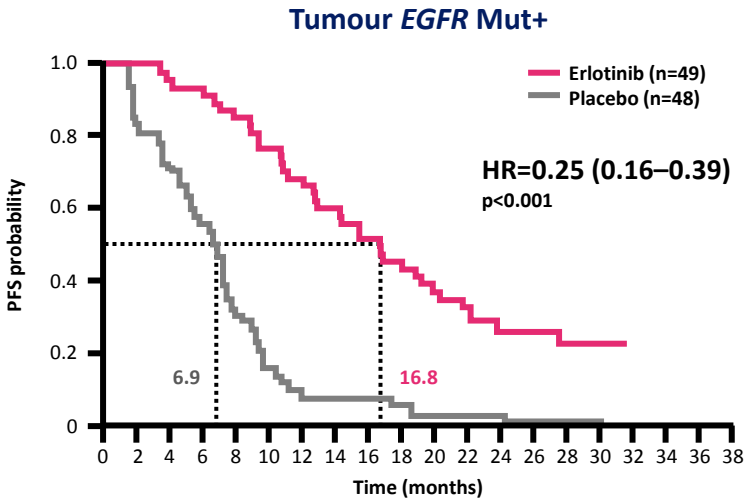
Plasma sample analyses

- ***EGFR* (15%)**
- ***HER2* (1%)**
- ***BRAF* (2%)**
- ***ALK* (5%)**
- ***ROS1* (2%)**
- ***RET* (1.7%)**
- ***MET* (3%)**

FASTACT2 demonstrated the clinical feasibility and utility of plasma-based EGFR testing



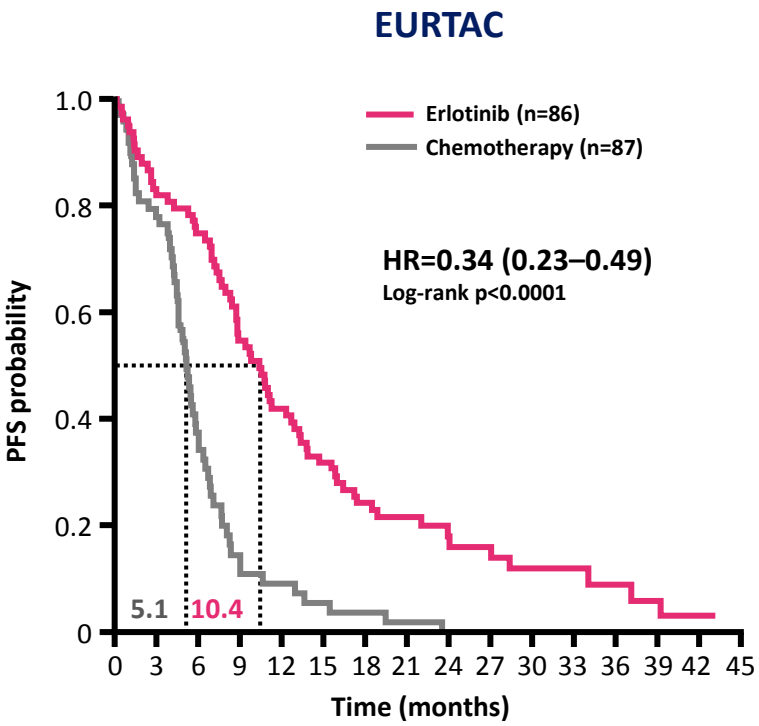


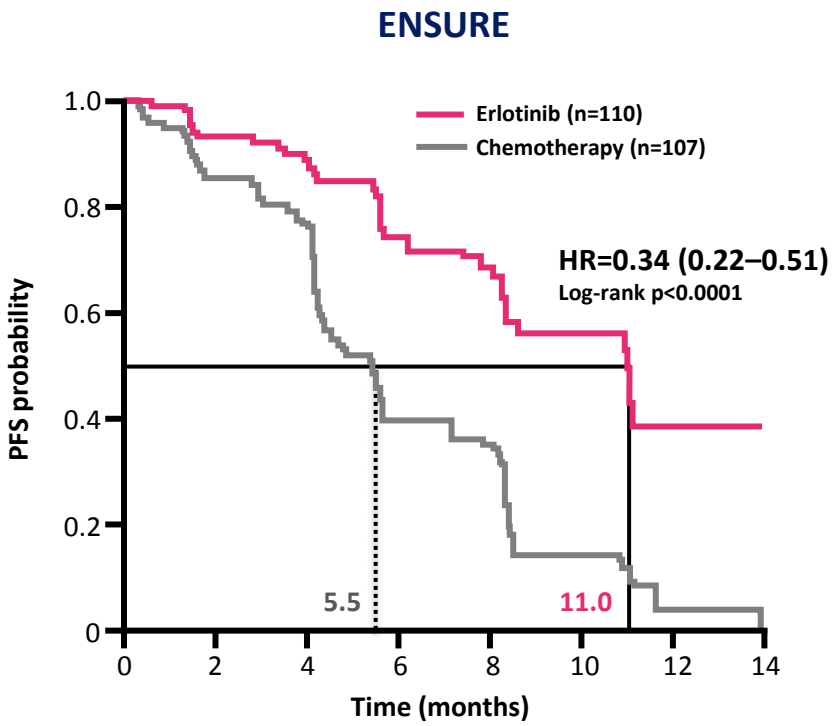


*Chemotherapy = Gemcitabine 1,250mg/m^2 (d1, 8)
+ carboplatin AUC=5 or cisplatin 75mg/m^2 (d1)

Wu, et al. Lancet Oncol 2013; Mok, et al. Clin Cancer Res 2015

ENSURE and EURTAC: PFS with first-line erlotinib vs chemotherapy in *EGFR* Mut+ NSCLC





Wu, et al. Ann Oncol 2015; Tarceva US PI

Plasma testing addresses the limitations of tissue testing

Plasma testing...

…offers a less-invasive alternative to biopsy for *EGFR* mutation analysis in patients with advanced NSCLC for improved and timely patient testing

…can be performed in patients who are unsuitable for biopsy due to their health status

…provides an alternative for patients for whom a biopsy did not yield a definitive result

…spares tissue samples for additional biomarker testing

cobas® EGFR Mutation Test v2 Case Study

Karen Bijwaard, MS, RAC
Lead Reviewer, CDRH/OIR/DMGP/MPCB

cobas® EGFR Mutation Test v2 Case Study

- First ctDNA test for NSCLC
- P150047 - Approved on June 1, 2016
- Intended Use
  - Due to lower agreement between plasma and tissue reflex language

Intended Use

The **cobas**® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) patients. **Defined EGFR mutations are detected using DNA isolated from** formalin-fixed paraffin-embedded tumor tissue (FFPET) ***or circulating-free tumor DNA (cfDNA) from plasma derived from EDTA anti-coagulated peripheral whole blood.***

The test is indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 below in accordance with the approved therapeutic product labeling:

Table 1

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	Exon 19 deletions and L858R	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M	

Patients with positive **cobas**® EGFR Mutation Test v2 test results using plasma specimens for the presence of EGFR exon 19 deletions or L858R mutations are eligible for treatment with TARCEVA® (erlotinib). ***Patients who are negative for these mutations by this test should be reflexed to routine biopsy and testing for EGFR mutations with the FFPET sample type.***

Intended Use – Analytical claims

Drug safety and efficacy have not been established for the following EGFR mutations listed in Table 2 below that are also detected by the **cobas**® EGFR Mutation Test v2:

Table 2

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q	G719X, exon 20 insertions, T790M, S768I and L861Q
TAGRISSO™ osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q	G719X, exon 19 deletions, L858R, exon 20 insertions, T790M, S768I, and L861Q

For manual sample preparation, FFPET specimens are processed using the **cobas**® DNA Sample Preparation Kit and plasma specimens are processed using the **cobas**® cfDNA Sample Preparation Kit. The **cobas** z 480 analyzer is used for automated amplification and detection.

Regulatory History

- RMS first contacted FDA regarding plasma testing – May 2014
  - Pre-submission in response to publication of EAP program draft guidance
    - Unmet medical need
    - Breakthrough technology that provides a clinically meaningful advantage
    - Pre-market/Post-market considerations
  - CDRH requested consults with CDER OHOP/DOP2
  - Multiple interactions regarding clinical and analytical study designs and statistical analyses
  - Modular PMA submission

Discussion Points & Regulatory Considerations:

- Use of contrived specimens in some analytical studies
- FDA request for a commutability study comparing contrived specimen make-up
  - Intact vs. sheared cell line DNA in Healthy Donor vs. NSCLC EGFR wild-type (WT) plasma
- Establishing efficacy based on clinical studies enrolled based on FFPET specimens
- Preanalytic effects and need for well controlled protocols

Studies Conducted in Support of PMA

Analytical Performance Studies	
Limit of Detection (LoD): Predominant Mutations	Commutability/Contrived Sample Comparison Study
LoD Verification of Rare Mutations	Clinical Specimen Confirmation of LoD
Reproducibility *(with EGFR WT NSCLC plasma)*	Precision (Repeatability)
Robustness	Reference Method Validation
Analytical Accuracy	Reagent Lot Interchangeability
Potentially Interfering Substances[1]	
Sample Preparation Kit Reagent Stability[1]	Prepared Specimen Stability[1]
cobas EGFR Mutation Test v2 Reagent Stability[1]	Activated MMx Stability[1]
cfDNA Kit Open Container Stability[1]	Prepared Specimen/Activated MMx Stability[1]
cobas EGFR Mutation Test v2 Kit Open Container Stability[1]	Plasma Specimen Handling Stability[1,2]

[1]preliminary data provide; [2]post-market committment

cobas EGFR Mutation Test v2 Case Study

FDA-AACR: Liquid Biopsies in Oncology Drug and Device Development
Washington, D.C. July 19, 2016

Walter H. Koch, PhD

Head of Research, Roche Molecular Systems, Inc.

EGFR testing is not performed for ~20% of eligible NSCLC patients – *Survey of 562 oncologists around the world*

Roche

Testing requested in 81% cases

- Results obtained before first-line therapy was initiated in 77% of cases (51% in France to 89% in Japan)
- Some suitable patients not tested at all for tumor EGFR mutations
- Some patients tested, but 1L treatment decision was taken without the result

Challenges with tissue biopsies:

- Biopsy ineligible patients
- Clinical complications: ~15-18% rate of adverse events (eg pneumothorax)
- Inadequate biopsy sample
- Tumor heterogeneity
- Tissue preservation (FFPE) process
- Delays in time to test results

Ann Intern Med. 2011 Aug 2; 155(3): 137–144.

http://www.medscape.com/viewarticle/843332?src=emailthis

2015 European Lung Cancer Conference (ELCC): Abstract LBA2_PR. Presented April 17, 2015

A few points to consider when developing a plasma-based test for tumor mutations

Customer and Product Requirements

- Physician and patient needs – Fast TAT, high accuracy
- Analytical and Clinical Sensitivity and Specificity required to be clinically useful
- Robust, consistent, reliable, cost-effective

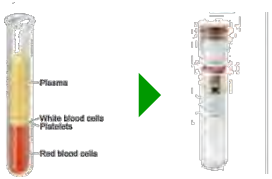
Regulatory requirements

- Collaboration with FDA for novel diagnostic indication and claims, as well as Global Registration and Use
- Specimen acquisition for Development and Technical Performance Verification – Adequate real world sample availability? Alternatives?
- Reference Methods – How is truth established with regard to tissue, and plasma results?

cobas® EGFR Mutation Test v2

Plasma Workflow: 2mL = 1 test result in a standard work day

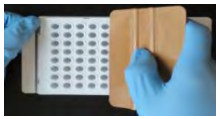
Step 1
Blood Draw



2 mL
Plasma

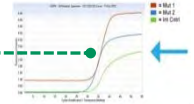
Step 2
cobas® cfDNA
Sample Prep

Step 3
PCR Setup



Step 4
Reporting

cobas® 4800
System v2.1 or
higher



Automated
Analysis

Automated
Reporting

<4 hours

Plasma and Tissue Specimen Acquisition/Use

Impractical to Use Clinical Specimens for all Development and TPV Studies

Practical considerations

- Prospective collection and time to screen samples = very expensive!
  - ~$2 million spent on cell line DNA and specimens
- Large volumes needed for non-clinical performance study panels
- Low mutation prevalence and low cfDNA concentrations when present
  - Purchased ~1000 specimens (commercial vendors) to find ~80 specimens with detectable mutations
  - Most had EGFR mutation cfDNA concentrations < 200 copies/mL

Ethical considerations

- HIPAA, IRB, ICF
- Patient population is very ill
- IRB typically permits 30 – 40mL whole blood to be drawn (15 – 20mL plasma)

Drives need for constructing appropriate contrived samples to evaluate analytical test performance

Plasma cfDNA Reference Methods

The Challenge of defining "Truth"

How to define biological truth?

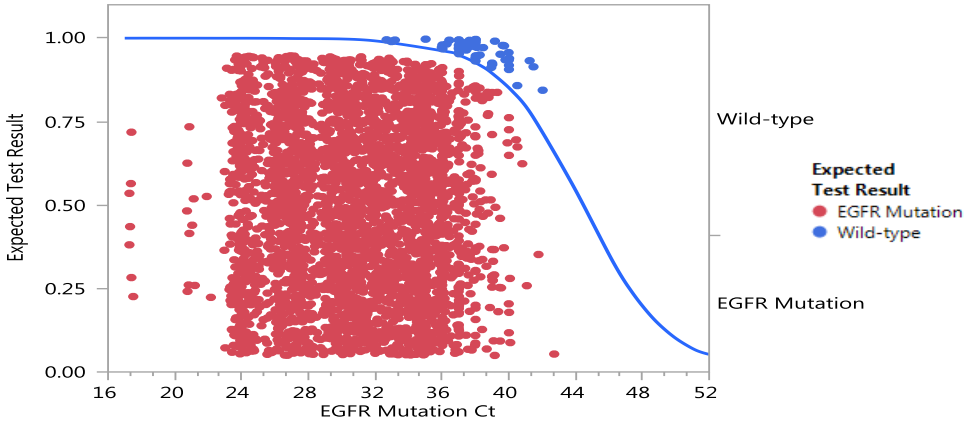
- No validated reference methods readily available
- No international standards
- Partially dependent on ***concurrent*** tissue and plasma collection
- Dependent on tumor heterogeneity, disease stage and metastatic progression
- Clinical drug response the ultimate indicator

Quantitative reference methods as options

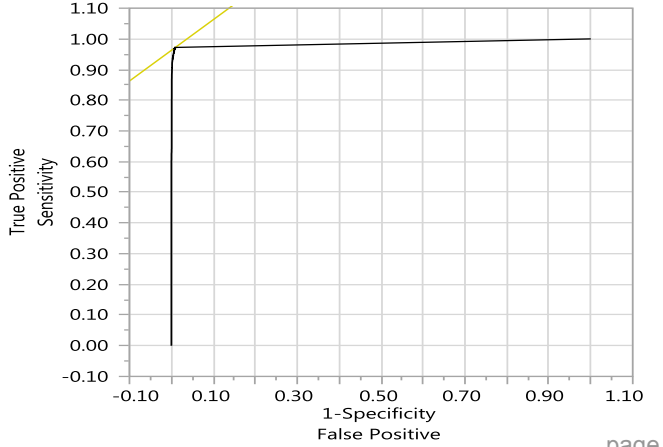
- Must validate sensitivity, specificity, reportable range of reference methods
- Most methods to detect rare mutations are affected by the amount of background, *normal* cfDNA present (eg from lymphocyte lysis)
- ddPCR sensitivity is not easily multiplexed, FDA recommended using a non-PCR method
  - **cobas®** EGFR Mutation Test v2 detects at least **42** mutations
- NGS sensitivity depends on ***absolute*** and ***relative*** mutant cfDNA concentration
  - Must increase read depth to detect low mutant cfDNA concentrations

Receiver Operator Characteristic (RoC) Curve Analysis to Optimize Sensitivity and Specificity

- **cobas®** EGFR Mutation Test v2: allele-specific PCR test that uses an adjacent *wild-type* EGFR gene sequence as internal control.
- Evaluated ***hundreds*** of NSCLC patient and healthy-donor samples with and without EGFR mutations over ***several thousand***



- Balance sensitivity against specificity for each mutation type reported by the test
  - *Wild-type* Ct values represent 'breakthrough', used to set cutoff thresholds
  - EGFR Mutation Ct values represent true positive results for RoC analysis



Non-Clinical Performance Studies

Clinical and Contrived Plasma Samples

Contrived sample performance directly traceable to clinical specimen results

- Determined ***Limit of Detection (LoD)*** with contrived samples consisting of sheared cell line DNA containing EGFR mutations diluted in healthy donor (HD) K2-EDTA plasma

- Demonstrated ***commutability:*** sheared cell line DNA diluted in HD plasma or NSCLC K2-EDTA plasma yield equivalent results at concentrations near LoD

- Confirmed LoD in clinical setting using NSCLC plasma panels

Non-Clinical Performance Studies

LoD Study Results (according to CLSI EP17-A2)

EGFR Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	LoD (copies/mL)	COSMIC ID
18	G719X	2156 G>C	**100**	6239
19	Exon 19 Deletion	2235_2249del15	**75**	6223
20	T790M	2369 C>T	**100**	6240
	S768I	2303 G>T	**25**	6241
	Exon 20 Insertion	2307_2308insGCCAGCGTG	**25**	12376
21	L858R	2573 T>G	**100**	6224
	L861Q	2582T>A	**30**	6213

Notes:

- **LoD study tested 72 replicates across 3 lots per level in healthy donor plasma**
- Limit of Blank (LoB) determined to be zero for all mutations reported by the test (N=198 across 3 lots for each of 33 Healthy Donor samples)
- Samples used in this study had a *wild-type* DNA background of approximately 100,000 copies/mL **(~0.025-0.1% mutation)**

Non-Clinical Performance Studies

Contrived Sample Commutability

Study Design

- **Demonstrate test results equivalent in healthy donor (HD) and NSCLC plasma**
- Diluted sheared cell line DNA spiked into HD plasma and NSCLC plasma
  - Focused on Exon 19 Deletion, L858R, and T790M mutations
  - 8 mutant levels from 0.03x -10xLoD, 20 replicates per panel

Results

- Hit rates similar between plasma backgrounds for each level of mutation
- p-values for regression and intercept t-tests are not significant when compared against NSCLC plasma as a background
- Bias between observed Ct and regression predicted Ct values were within test variability

Conclusion

- FDA accepted the contrived samples as a substitute for NSCLC patient specimens

Non-Clinical Performance Studies

LoD Confirmation in Clinical Setting

Study Objective: Confirm the LoD of EGFR mutations in NSCLC patient plasma

- **Panel Design:**
  - NSCLC clinical specimens with known EGFR mutations diluted into NSCLC EGFR *wild-type* plasma
  - **11 member panel (1X LoD & 2X LoD):**
    - Three most prevalent exon 19 deletion mutations
    - One L858R mutation sample
    - One T790M mutation sample
    - One *non-mutant EGFR normal* sample
- **Test plan**
  - Three testing sites (two external and one internal, two operators per site),
  - Three reagent lots (two non-identical lots per site)
  - Two non-consecutive testing days
  - Two replicates per panel member per run

Non-Clinical Performance Studies

LoD Confirmation in Clinical Setting

Summary Results:

- **Hit rates for 10 of 11 panel members, including two levels each for Exon 19 Deletion and L858R, were 100% (23/23 or 24/24) with 95% Confidence intervals (85.2, 100.0%)**
- **Hit rate for Exon 20 T790M at 1 X LOD was 95.8% (23/24) with 95% Confidence intervals (78.9, 99.9%)**
- **Mutations detected at abundance of approximately 0.3% in wild-type background.** (Clinical specimens used in this study had a wild-type DNA background of approximately 24,000 copies/mL)

Clinical Reproducibility Study

Plasma

Study Objective: Evaluate the **reproducibility** for the detection of mutations in exons 18, 20, and 21 of the EGFR gene across the following factors:

- 3 manufactured lots of reagents, 2 non-identical lots per site
- 3 **cobas z** 480 instruments, 1 per site
- 3 sites, 2 operators per site
- 3 non-consecutive days

Specimen panel design: 648 total replicates

- **9 panel members**: 7 different mutations - each panel member tested in **duplicate**
- For each mutation: **100 and 300 copies/ml**

Cell-Line DNA Combination 1	Cell-Line DNA Combination 2	Cell-Line DNA Combination 3	Cell-Line DNA Combination 4
Exon 19 Del	L858R	S768I	L861Q
T790M	T790M	G719A	Exon20 ins

Note: The samples used in this study consisted of cell line DNA mechanically sheared to an average size of 220bp and had a WT DNA background of approximately 12,000 copies/mL.

Clinical Reproducibility Study

Plasma Results

Invalid rate = 0.33% (4/1224 tests)

100 copies/ml:

- **100 %** hit rate for 4 mutations (ex19del, ex20ins, S768I, L861Q)
- **98.6%** for L858R
- **97.2%** for T790M
- **90.3%** for G719A

300 copies/ml:

- **100 %** hit rate for 4 mutations (ex19del, ex20ins, S768I, L861Q)
- **98.6%** for 3 mutations (L858R, T790M, G719A)

WT had a **100%** hit rate; No false positive results for WT specimens

Roche

cobas® Life needs answers

Clinical Studies

Correlation to NGS, FFPET; Clinical Outcome

Analytical Accuracy: Correlation to NGS

Samples: 128

Correlation of plasma to NGS using phase III plasma samples from ASPIRATION, MetLung, MetMab cohorts

Clinical Outcome

Samples: 180

PFS improved with erlotinib versus chemotherapy when EGFR activating mutations present in plasma

Correlation between Plasma and FFPET

Samples: 431 (paired valid results)

Correlation of plasma to FFPET using phase III FFPET and plasma samples from the ENSURE cohort

NGS Reference Method Validation

Setting Cutoffs for Plasma mutation detection

- Two-tube amplicon assay run on Illumina MiSeq
- 25 uL eluent input (*same input as for the cobas test*)
- cfDNA from healthy-donor plasma and NSCLC patient plasma without mutation (~ 300 individual samples)
- *Wild-type* DNA pools and cell-line DNA blends ranging from 0.03% to 1.0% mutation
- ≥8 replicates for each mutation at each of 5 DNA input levels (2 ng – 500 ng)

Roche

cobas® Life needs answers

NGS Reference Method Validation

Verification and Validation of Plasma mutation assay

- *Wild-type* cfDNA from 3 individual normal human donor plasma (***24 replicates each***)
- Mutant cell line DNA spiked into healthy-donor plasma from 20 copies/mL to 1000 copies/mL (resulting mutant % as low as 0.076%), ***24 replicates per mutation at each of four levels***
- After initial validation, each Miseq run included a set of amplicons either as *wild-type* or with known mutations at various levels as ***run controls***
- System and software were validated similarly to a Class III IVD system, including verification of requirements, and installation and operational qualifications

Roche

cobas® Life needs answers

Analytical Accuracy: Correlation between cobas® EGFR Test and Validated NGS method in Plasma

*ASPIRATION cohort**

- Evaluated analytical accuracy for detection of Exon 19 Deletion and L858R
- Total of 128 plasma samples with valid paired results from both the **cobas®** EGFR Test in plasma and the NGS method
  - A total of 32 samples had Mutation Detected (MD) and 95 had No Mutation Detected (NMD) results by NGS

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	87.5% (28/32)	71.9%, 95.0%
Negative Percent Agreement (NPA)	96.8% (92/95)	91.1%, 98.9%

ASPIRATION cohort used due to insufficient plasma sample volumes from clinical utility study (ENSURE) for testing with reference method

* Includes minority samples from *MetMab, and MetLung* clinical trials

Clinical Studies

Correlation to NGS, FFPET; Clinical Outcome

Analytical Accuracy: Correlation to NGS

Samples: 128

Correlation of plasma to NGS using phase III plasma samples from ASPIRATION cohort

Clinical Outcome

Samples: 180

PFS improved with erlotinib versus chemotherapy when EGFR activating mutations present in plasma

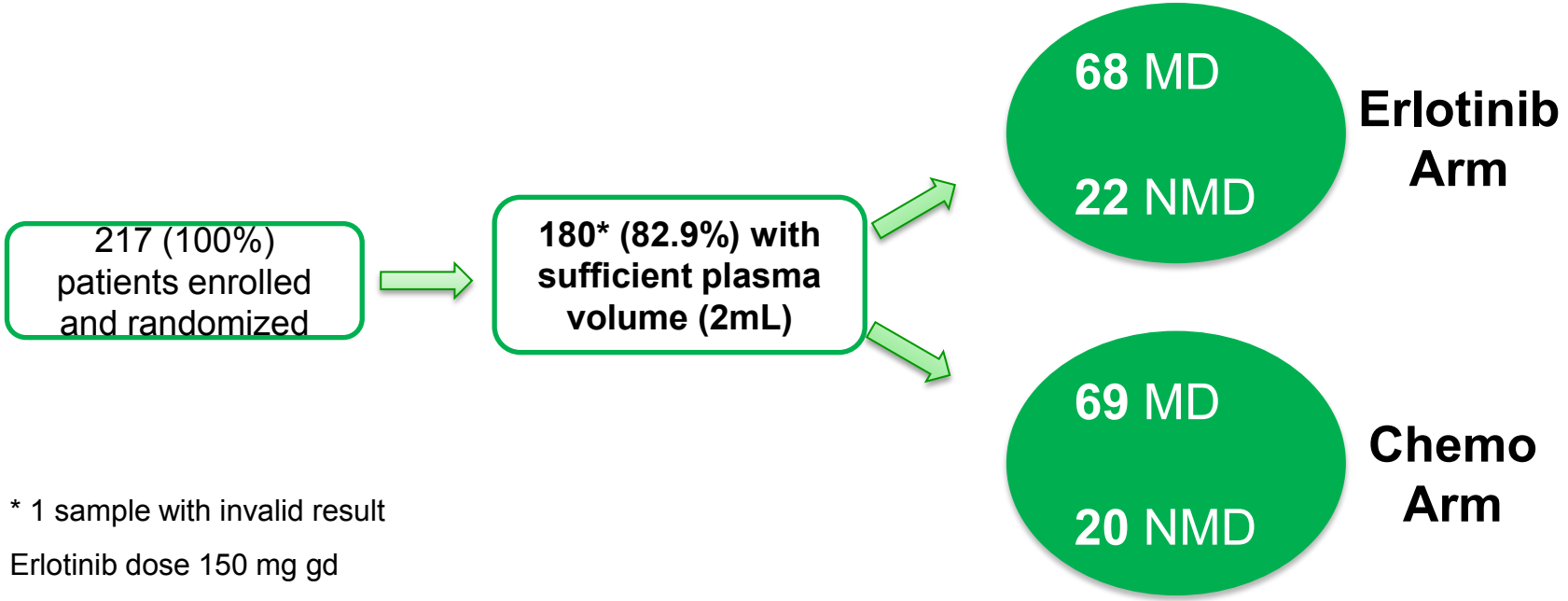
Correlation between Plasma and FFPET

Samples: 431 (paired valid results)

Correlation of plasma to FFPET using phase III FFPET and plasma samples from the ENSURE cohort

ENSURE: A Study of First-Line Therapy with Tarceva in Patients with NSCLC

Clinical Outcome



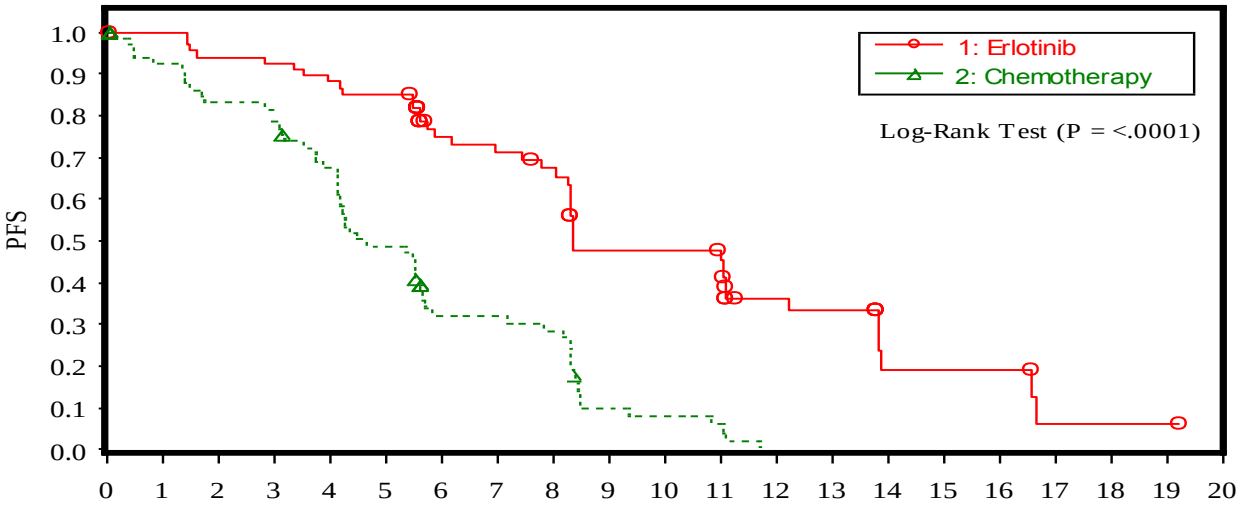
* 1 sample with invalid result

Erlotinib dose 150 mg gd

Chemo: Gemcitabine (d1, 8) + carboplatin or cisplatin (d1); q4w x 4 cycles GC-placebo

ENSURE Clinical Trial

Kaplan-Meier Curve of Investigator-Assessed PFS

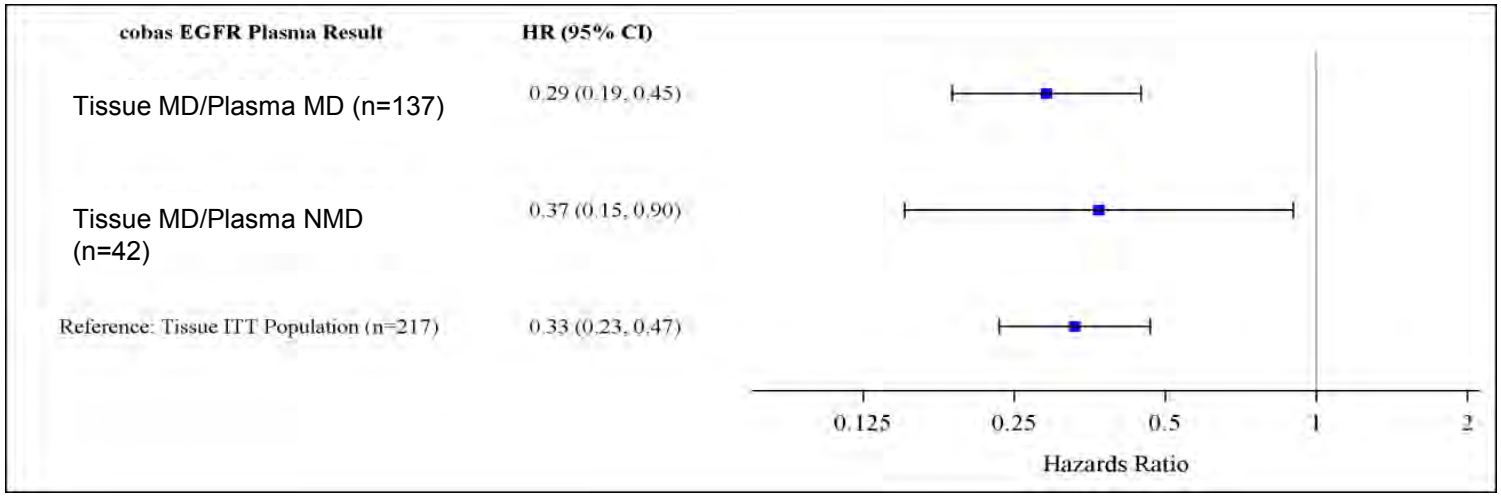


N at Risk

1	68	67	63	62	59	57	40	38	35	23	23	22	12	11	4	4	4	1	1	1	0
2	69	60	54	51	43	31	18	18	16	5	4	3	0								

ENSURE Clinical Trial

Forest Plot of HRs for PFS by investigator assessment



Clinical Studies

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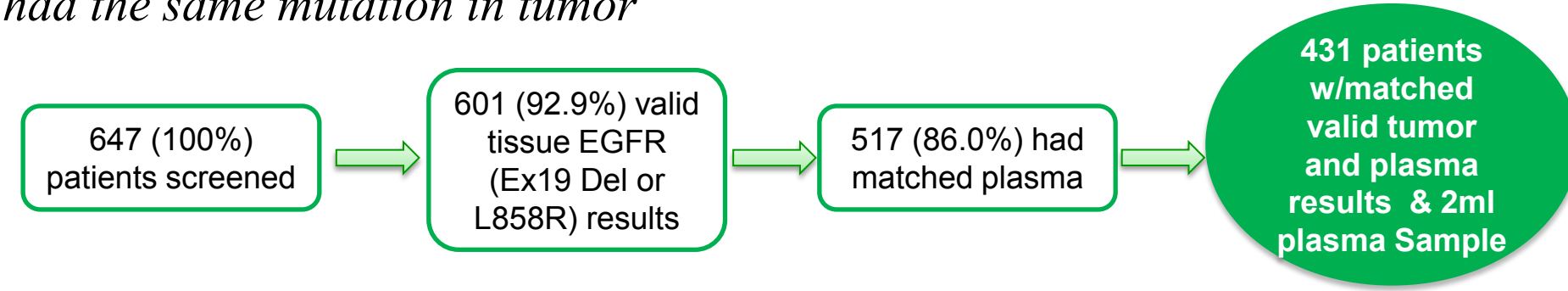
Correlation Between Plasma and Tissue ENSURE Clinical Trial Specimens

EGFR mutations detected in plasma of 3 out of 4 patients who had the same mutation in tumor

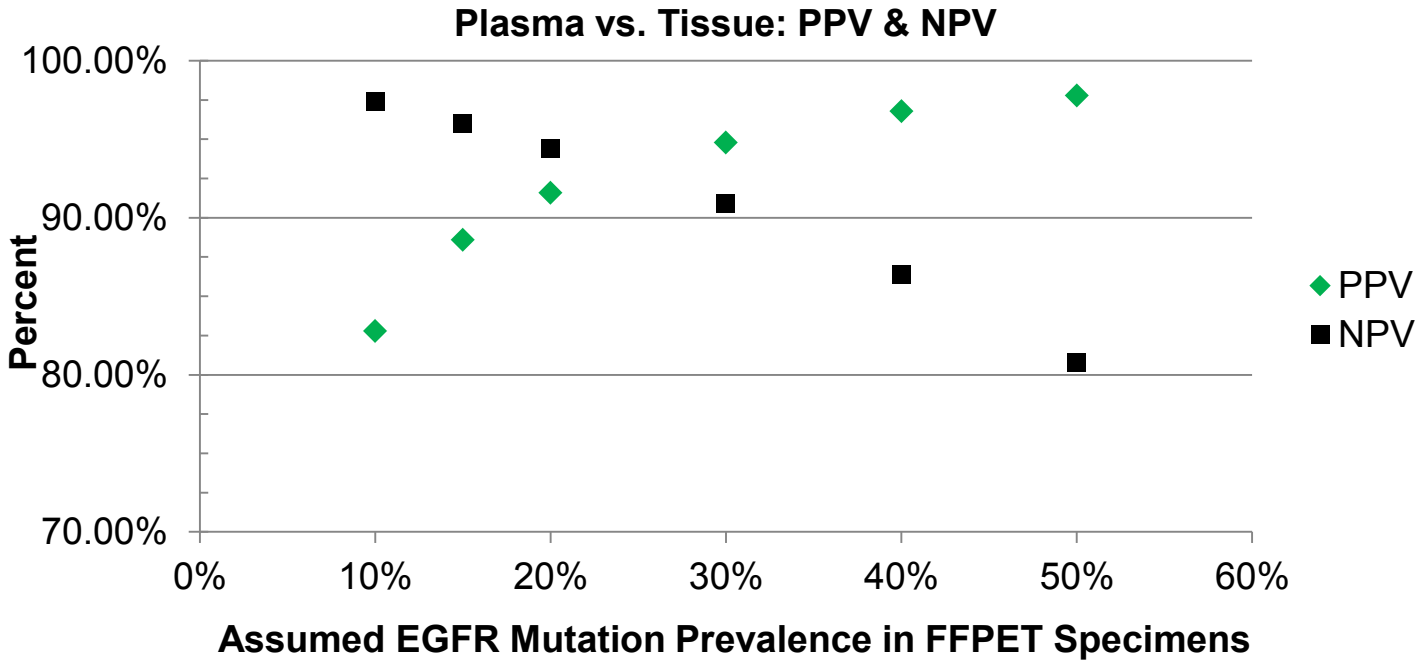


- 431 patients had both tumor and baseline plasma samples (2mL) with available *EGFR* mutation analysis results
- Aggregate analysis results
  - Sensitivity: **77%** (161/210)
  - Specificity: **98%** (217/221)
  - Overall concordance: **88%** (378/431)

ENSURE Clinical Trial

Estimated PPV and NPV based on assumed EGFR Prevalence

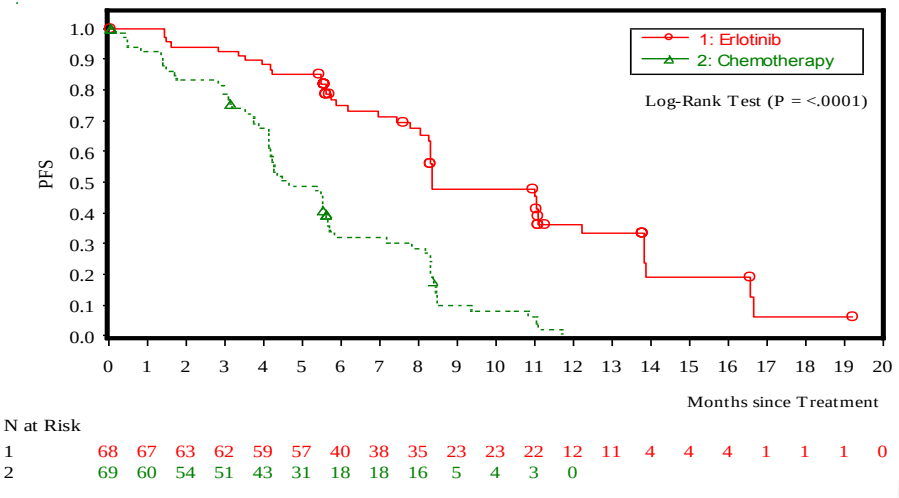
Analyzed PPV and NPV in aggregate with plasma as reference using bootstrap method, based on the different population tissue prevalence



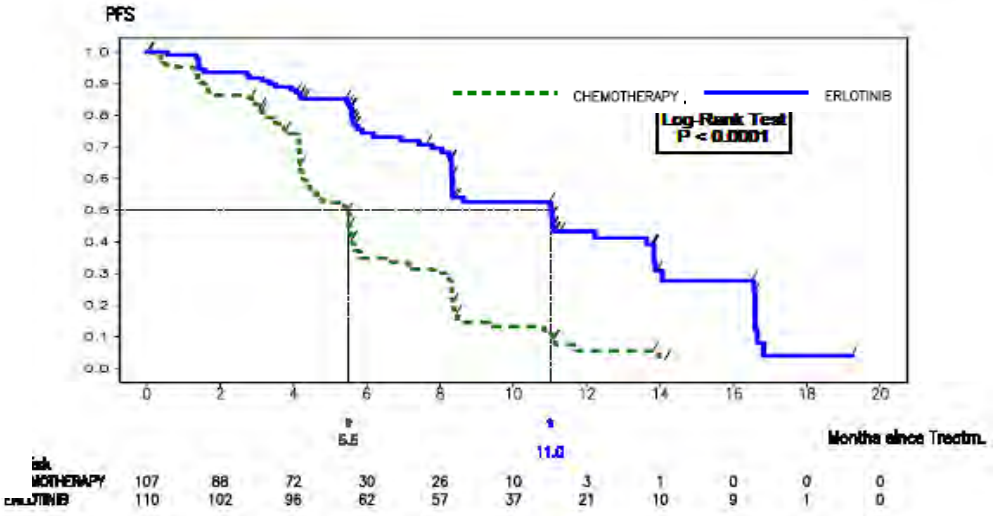
ENSURE Clinical Trial

Comparison of PFS Based on Plasma or Tissue Results

Plasma



Tissue



Doing now what patients need next

Roche

cobas®
Life needs answers

American Association for Cancer Research

FINDING CURES TOGETHER℠

Case Study

cobas® EGFR Mutation Test v2, a blood-based companion diagnostic for the cancer drug Tarceva (erlotinib)

Speakers:

Reena Philip, PhD

David Shames, PhD

Karen Bijwaard, MS, RAC,MB(ASCP)

Walter Koch, PhD

Erin Larkins, CDR, USPHS

American Association for Cancer Research

FINDING CURES TOGETHER℠

Session III

Liquid Biopsy Test Development

Chair: Reena Philip, PhD

Speakers:

Abraham Tzou, MD

Phil Stephens, PhD

Panelists:

Tera Eerkes, PhD

AmirAli Talasaz, PhD

Walter Koch, PhD

Mark Lee, MD, PhD

Mark Sausen, PhD

CDRH Considerations for Liquid Biopsy Diagnostic Development

Abraham Tzou
Division of Molecular Genetics and Pathology
FDA/CDRH/OIR

Liquid Biopsies in Oncology Drug and Device Development
July 19, 2016

Outline

- Liquid Biopsy Scenario
- General Principles
- Example Considerations
  - Orthogonal method
  - Contrived specimens
  - Tissue concordance

Scenario

- Oncology (patients diagnosed with cancer)
- Liquid biopsy (LB) compared to tissue
- LB results to select treatment?

Issues

- Differences in evaluating LB analytical status
- Insufficient clinical specimens for analytical validation
- Treatment outcomes based on tissue, not LB

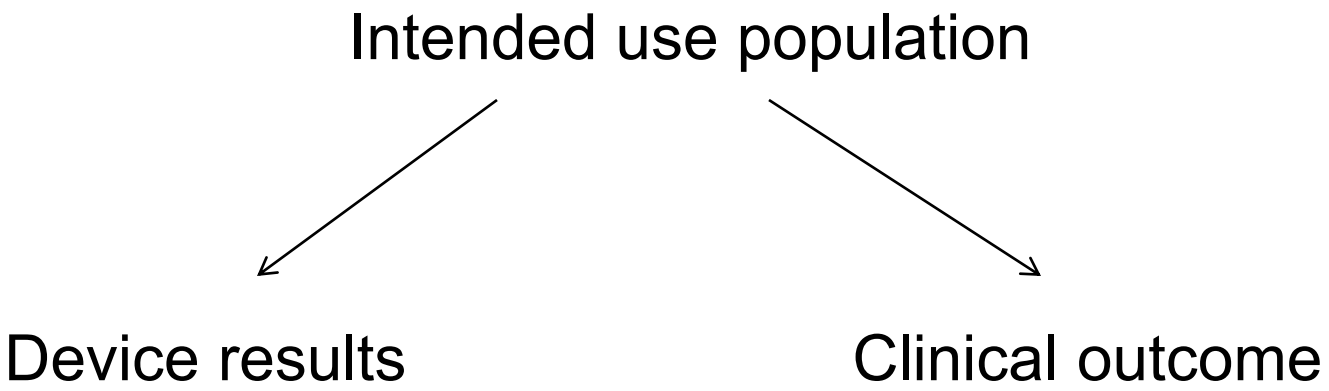
Outline

- Liquid Biopsy Scenario
- General Principles
- Example Considerations
  - Orthogonal method
  - Contrived specimens
  - Tissue concordance

Themes

- Represent the intended use patient population, not just available specimens
- Performance for clinical specimens, not just contrived samples
- Relevance to clinical outcomes, not just analytical status

Study Design Fundamentals



- Validation study independent from training, development

Liquid Biopsy Study Ideal

Intended use LB

Consistent, comparable, uniform LB results

Clinical outcome based on LB

Liquid Biopsy Study Issues

Available, contrived LB

LB device ↔ orthogonal method results *not consistent, comparable, and/or uniform*

Tissue with(out) outcome

Outline

- Liquid Biopsy Scenario
- General Principles
- Example Considerations
  - Orthogonal method
  - Contrived specimens
  - Tissue concordance

Analytical Performance

Available, contrived LB

LB device ↔ orthogonal method results *not consistent, comparable, and/or uniform*

Tissue with(out) outcome

LB/Orthogonal Analytical Issues

- Inconsistent call (e.g., cutoff below limit of detection)
- Non-comparable range (e.g., allele frequencies)
- Non-uniform across scope of analytes (e.g., variant types, genomic context)

Contrived Specimens

Available, contrived LB

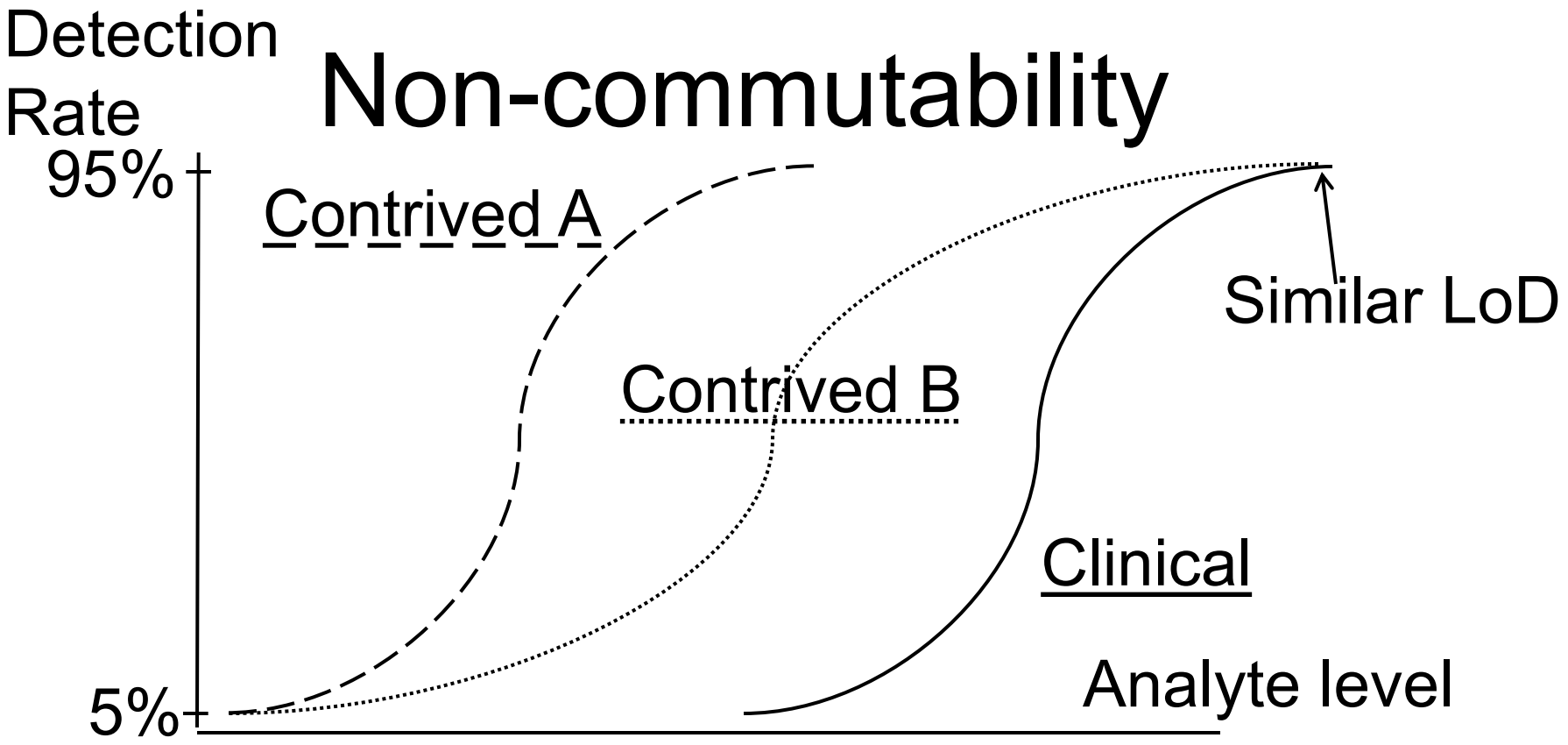
LB device ↔ orthogonal method results *not consistent, comparable, and/or uniform*

Tissue with(out) outcome

Contrived = Clinical?

- Include, verify performance in clinical
- Contrive similar to clinical
- Nature of analytes of interest (e.g., sheared DNA)
- Background (e.g., circulating DNA milieu)

Non-commutability



LB ↔ Tissue Comparison

Available, contrived LB

LB device ↔ orthogonal method results *not consistent, comparable, and/or uniform*

Tissue with(out) outcome

LB ↔ Tissue Concordance

- Specimens with clinical outcome preferred
- Differences in trials (e.g., line of therapy, treatment resistance)
- Matched sampling – avoid discordance due to heterogeneity, evolution

LB ↔ Tissue Discordance

- LB-/Tissue+ → reflex to tissue
- LB+/Tissue- → less likely to be referred, underestimated in tissue+ trials?
- Impact of selecting altered intended use population, especially LB+/tissue-

Procured LB ↔ Tissue?

- Key factors impacting circulating vs. tissue status (e.g., tumor type, stage) observed in cohorts outside of therapeutic trials
- Potential inflated concordance if “easier” distribution and/or frequency of analytes, in specimen cohort

Some LB Study Options

Available clinical [representative of intended use population], supplement contrived [commutable] LB

LB device ↔ orthogonal method results *[address consistency, comparability, uniformity]*

[Matched] LB/tissue, *[LB-/tissue+ reflex, address impact of LB+/tissue-]* for clinical outcome

Thank You

abraham.tzou@fda.hhs.gov

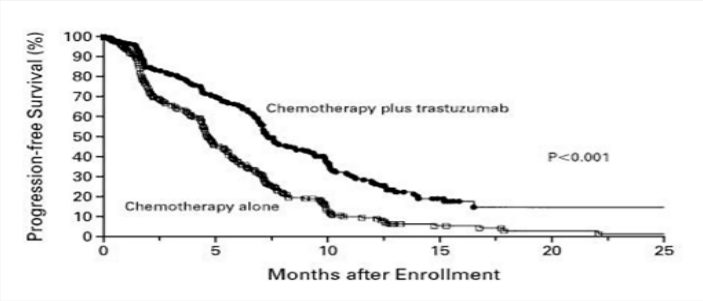
Analytical and clinical validation of liquid biopsy tests

Phil Stephens, Foundation Medicine Inc,

FOUNDATION MEDICINE®

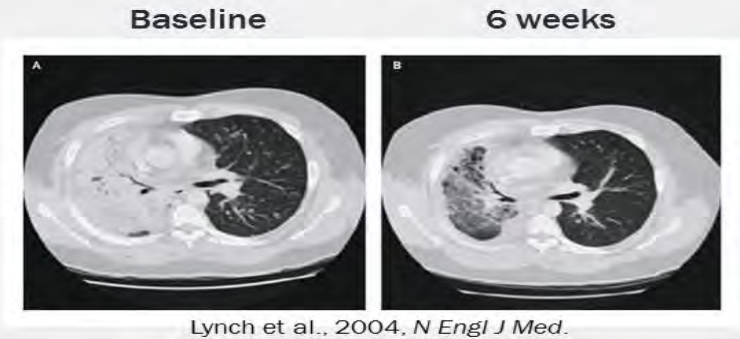
Molecularly Targeted Therapy Is Evolving

Trastuzumab in HER2 positive breast cancer



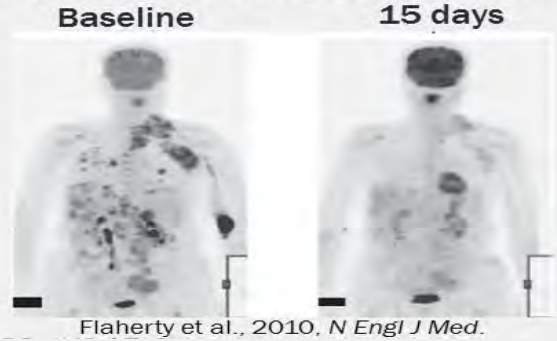
Slamon et al., 2001, *N Engl J Med*.

Gefitinib in *EGFR*-mutated NSCLC



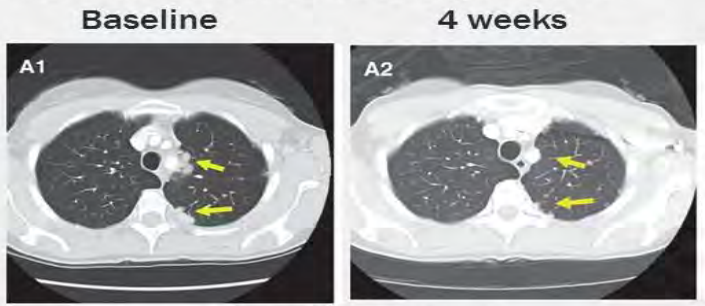
Lynch et al., 2004, *N Engl J Med*.

Vemurafenib in *BRAF* V600E mutated melanoma



Flaherty et al., 2010, *N Engl J Med*.

Cabozantinib in *RET*-rearranged NSCLC



Drilon et al., 2013, *Cancer Discov*.

From Discovery To Clinic In ~1 Year

From Test (2/2012)…

nature medicine

Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies

Doron Lipson[1,9], Marzia Capelletti[2,9], Roman Yelensky[1], Geoff Otto[1], Alex Parker[1], Mirna Jarosz[1], John A Curran[1], Sohail Balasubramanian[1], Troy Bloom[1], Kristina W Brennan[1], Amy Donahue[1], Sean R Downing[1], Garrett M Frampton[1], Lazaro Garcia[1], Frank Juhn[1], Kathy C Mitchell[1], Emily White[1], Jared White[1], Zac Zwirko[1], Tamar Peretz[3], Hovav Nechushtan[3], Lior Soussan-Gutman[4], Jhingook Kim[5], Hidefumi Sasaki[6], Hyeong Ryul Kim[7], Seung-il Park[7], Dalia Ercan[2], Christine E Sheehan[8], Jeffrey S Ross[1,8], Maureen T Cronin[1], Pasi A Jänne[2] & Philip J Stephens[1]

Applying a next-generation sequencing assay targeting 145 cancer-relevant genes in 40 colorectal cancer and 24 non–small cell lung cancer formalin-fixed paraffin-embedded tissue specimens identified at least one clinically relevant genomic alteration in 59% of the samples and revealed two gene fusions, *C2orf44-ALK* in a colorectal cancer sample and *KIF5B-RET* in a lung adenocarcinoma. Further screening of 561 lung adenocarcinomas identified 11 additional tumors with *KIF5B-RET* gene fusions (2.0%; 95% CI 0.8–3.1%). Cells expressing oncogenic *KIF5B-RET* are sensitive to multi-kinase inhibitors that inhibit RET.

… To Treatment (3/2014)

CANCER DISCOVERY

RESEARCH BRIEF

Response to Cabozantinib in Patients with *RET* Fusion-Positive Lung Adenocarcinomas

Alexander Drilon[1], Lu Wang[2], Adnan Hasanovic[5], Yoshiyuki Suehara[4], Doron Lipson[6], Phil Stephens[6], Jeffrey Ross[6], Vincent Miller[6], Michelle Ginsberg[3], Maureen F. Zakowski[2], Mark G. Kris[1], Marc Ladanyi[4], and Naiyer Rizvi[1]

ABSTRACT The discovery of *RET* fusions in lung cancers has uncovered a new therapeutic target for patients whose tumors harbor these changes. In an unselected population of non–small cell lung carcinomas (NSCLCs), *RET* fusions are present in 1% to 2% of cases. This incidence increases substantially, however, in never-smokers with lung adenocarcinomas that lack other known driver oncogenes. Although preclinical data provide experimental support for the use of RET inhibitors in the treatment of *RET* fusion-positive tumors, clinical data on response are lacking. We report preliminary data for the first three patients treated with the RET inhibitor cabozantinib on a prospective phase II trial for patients with *RET* fusion-positive NSCLCs (NCT01639508). Confirmed partial responses were observed in 2 patients, including one harboring a novel *TRIM33–RET* fusion. A third patient with a *KIF5B–RET* fusion has had prolonged stable disease approaching 8 months (31 weeks). All three patients remain progression-free on treatment.

SIGNIFICANCE: Driver oncogene discovery in lung cancers has dramatically changed today's therapeutic landscape. This report of the activity of cabozantinib in *RET* fusion-positive disease provides early clinical validation of *RET* fusions as drivers in lung cancers and suggests that RET inhibition may represent a new treatment paradigm in this molecular cohort. *Cancer Discov; 3(6); 630–5. ©2013 AACR.*

Matching the correct targeted therapy to the correct patient is diagnostically challenging as the number of "clinically relevant" genomic alterations increases

All classes of genomic alteration need to be accurately identified to maximize patient care

Clinically relevant genes in non-small cell lung cancer (NSCLC)

Base Substitution: *ALK, AKT1, AKT2, AKT3, ATM, BRAF, BRCA1, BRCA2, CDKN2A, EGFR, ERBB2, FGFR1, FGFR2, GNA11, GNAS, KRAS, MAP2K1, MAP2K2, MET, NF1, NOTCH1, NRAS, PIK3CA, PTCH, PTEN, STK11, TSC1, TSC2*

Short Insertion/Deletion: *ATM, BRCA1, BRCA2, EGFR, ERBB2, MET, NF1, NOTCH1, PTCH, PTEN, STK11, TSC1, TSC2*

Focal Amplification: *AKT1, AKT2, AKT3, CDK4, CCND1, CCND2, CCNE1, EGFR, ERBB2, FGFR1, FGFR2, KRAS, MDM2, MET*

Homozygous Deletion: *BRCA1, BRCA2, NF1, NOTCH1, PTCH, PTEN, STK11, TSC1, TSC2*

Gene Fusion: ***ALK, RET, ROS1, NTRK1, FGFR1***

FOne detects clinically relevant alterations in 65% of patients with pan-negative NSCLC tested at MSKCC

MSKCC testing: *EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, AKT1, ALK, ROS1 & RET*

- **65% of patients harbored a clinically relevant alteration by FoundationOne**

  26% of patients: targeted therapy in NCCN guidelines

  39% of patients: Approved therapy or active MSKCC clinical trial

MSKCC and FMI published results in CCR

~¼ EGFR mutations & ~1/3 of ALK fusions missed by current testing

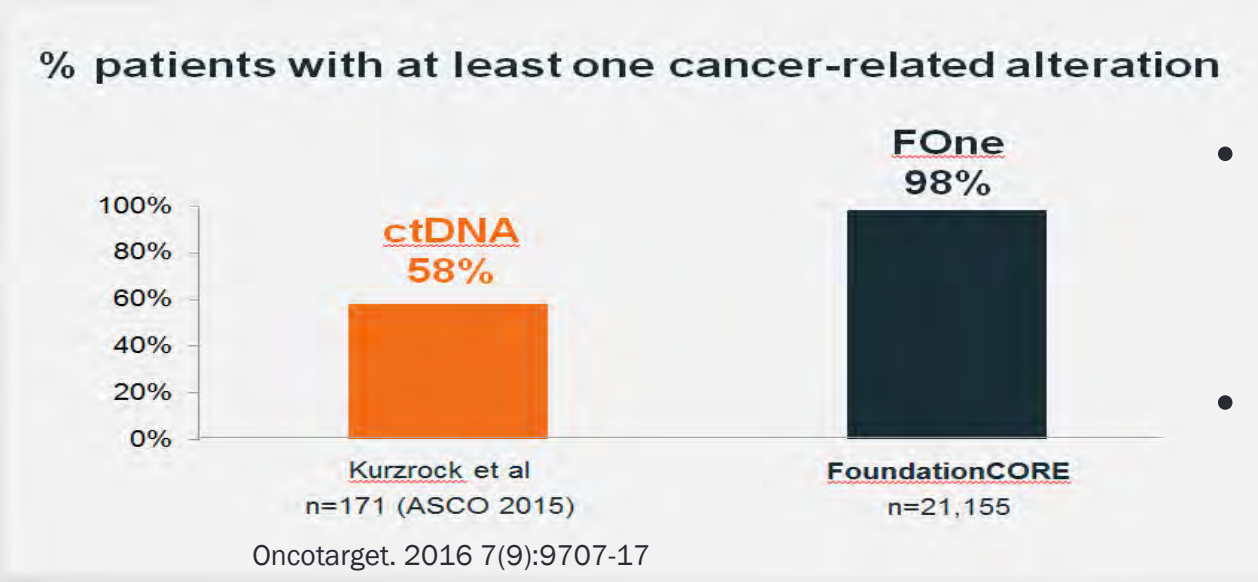
Clinical Cancer Research Dec 2014: Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in "driver-negative" lung adenocarcinomas. 158/1078-0432.CCR-14-2683

FOUNDATION MEDICINE®

Genomic profiling is orders of magnitude difficult to accurately perform from the blood

ctDNA is present at <1% purity in many cancer patients

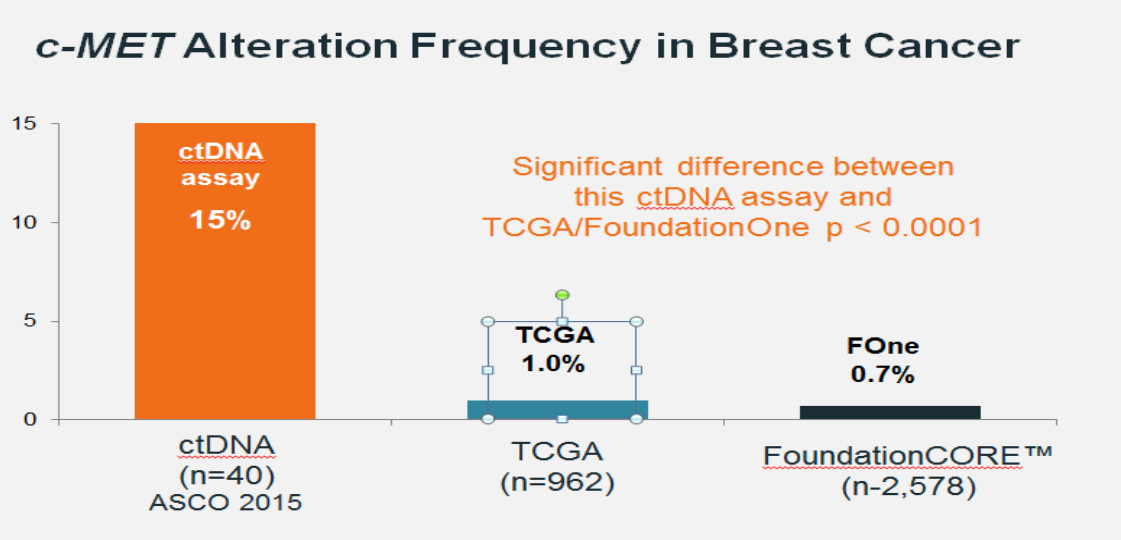
Not all patients with advanced cancer shed sufficient ctDNA into the circulation



Oncotarget. 2016 7(9):9707-17

- 42% of patients shed insufficient ctDNA into bloodstream.
- In these patients, standard of care alterations (e.g. *EGFR*) would be missed.

Low Levels of ctDNA Challenge the Accuracy of Current Commercial Assays



Oncotarget. 2016;7(9):9707-17

For every fifteen patients in whom this ctDNA assay recommends targeted c-MET therapy (e.g - crizotinib).

Approximately fourteen of those patients ***will not*** have a druggable c-MET alteration.

FOUNDATION MEDICINE®

Potential Treatment Ramifications of Poorly Validated Assay Development

36 y/o female: Stage IV lung adenocarcinoma

FOUNDATIONONE

Patient Name | Report Date | Diagnosis: Lung adenocarcinoma

Date of Birth	Not Given	Client	Cancer Center	Specimen Received	Not Given
Gender	Female	Ordering Physician	Doctor, Denise	Specimen Site	Lymph Node
FMI Case #	SRF000005	Additional Recipient	Not Given	Date of Collection	Not Given
Medical Record #		FMI Client #	-1	Specimen Type	Block
Specimen ID	Not Given	Pathologist	Not Given		

ABOUT THE TEST:
FoundationOne™ is a next-generation sequencing (NGS) based assay which identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- 4 genomic alterations
- 3 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 6 clinical trials

TUMOR TYPE: LUNG ADENOCARCINOMA

Genomic Alterations Identified†

CCDC6-RET fusion
TP53 V143M
CDKN2A/B loss
ATM A2707-2A>C
KRAS

Additional Disease-relevant Genes with No Reportable Alteration Detected

ALK, BRAF, ERBB2, EGFR, MET, NRAS, PIK3CA, RET, ROS1

†For a complete list of the genes assayed, please refer to the Appendix

THERAPEUTIC IMPLICATIONS

ALK EML4-ALK fusion	Crizotinib	None	None	Yes, see clinical trials section
TP53 L194R	None	None	None	None

Note: Genomic alterations detected may be associated with activity of certain FDA approved drugs; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type. Neither the therapeutic agents nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type.

***CCDC6-RET* fusion**

Patient received and responded to a RET inhibitor

- ctDNA assay (from another commercial entity) failed to identify any alterations revealed by FoundationOne.
- Instead, the assay identified a false positive *EGFR* alteration.

FOUNDATION MEDICINE®

Foundation Medicine's ctDNA Assay Optimized to Address the Extreme Challenges of ctDNA Diagnostics

Genes interrogated				
ABL1	CDKN2A	FOXL2	MDM2	PIK3CA
AKT1	CRKL	GNA11	MET	PTEN
ALK**	CTNNB1	GNAQ	MPL	PTPN11
ARAF	DDR2	GNAS	MTOR	RAF1
BRAF	EGFR**	HRAS	MYC	RET**
BRCA1	ERBB2	IDH1	MYD88	ROS1**
BRCA2	ERRFI1	IDH2	NF1	SMO
BTK	ESR1	JAK2	NMYC	TERT
CCND1	EZH2	JAK3	NPM1	TP53
CD274	FGFR1	KIT	NRAS	VEGFA
CDH1	FGFR2	KRAS	PDCD1LG2	
CDK4	FGFR3**	MAP2K1	PDGFRA**	
CDK6	FLT3	MAP2K2	PDGFRB	

** Assayed for gene fusions

- **ctDNA assay optimized for both sensitivity and specificity for all alteration classes**
- Base substitutions, short INDELs, copy number changes and fusions
- >5,000x coverage
- ~150,000 bps targeted

FOUNDATION MEDICINE®

Extensive Analytic Validation of ctDNA Assay is Based upon Established Best Practices

- **Accuracy**: sensitivity and specificity (PPV) for base substitutions, indels, gene fusions and CNA amplifications
  - Mixtures of cancer reference cell-lines and synthetic DNA spike-ins titrated to low minor allele frequencies
- **Workflow**: compatibility with routine clinical (prospective and retrospective) samples
  - Demonstrate contamination control and detection
- **Precision:** high intra- and inter-run concordance using clinical samples
- **Concordance**: orthogonal validation of genomic alterations detected by ctDNA assay

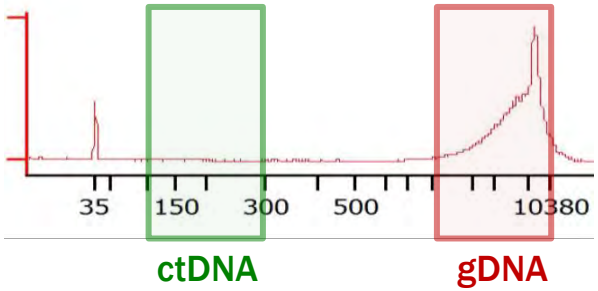
Improper Sample Collection Compromises Assay Performance: Case Study in *KRAS*+ Pancreatic Cancer

Improperly prepared plasma

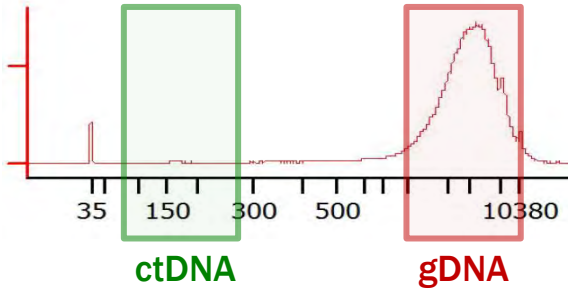
Prolonged storage in EDTA

Incomplete removal of buffy coat

***KRAS*: 0%**



***KRAS*: 0%**

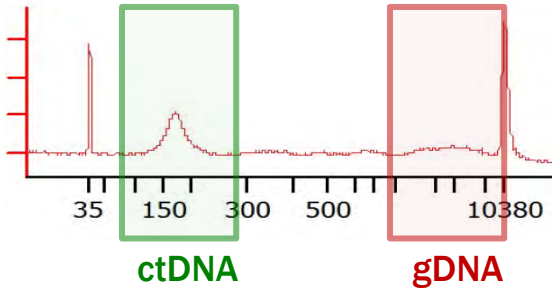


Quality plasma

Streck

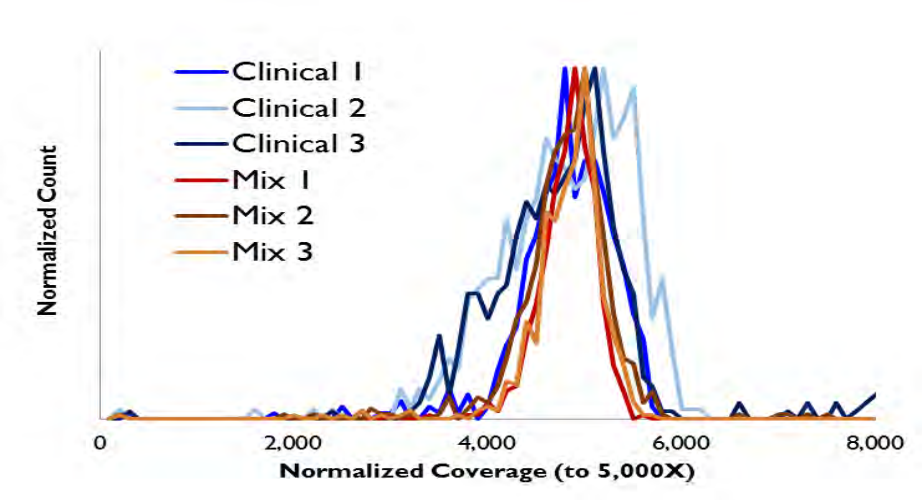
Double-spin prep

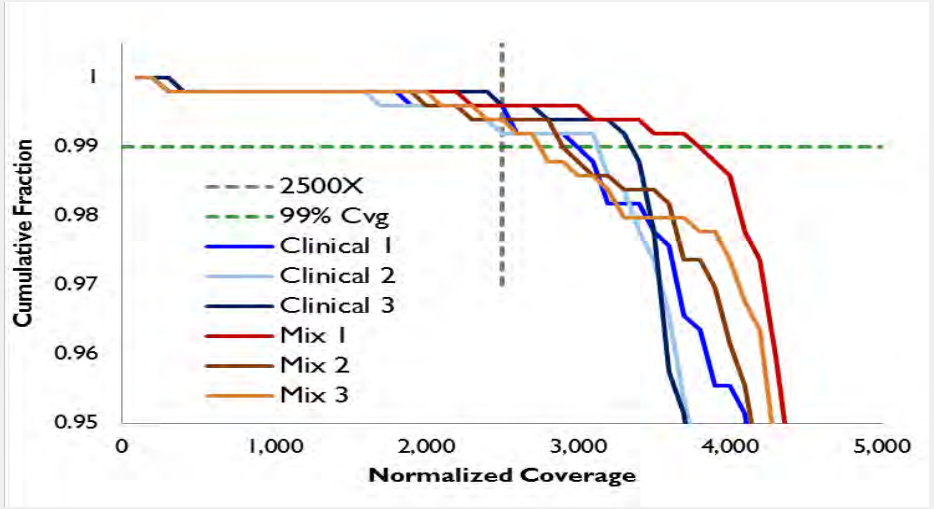
***KRAS*: 26%**



- Detection of *KRAS* base substitution compromised due to improper preparation of plasma due to contamination of ctDNA with genomic DNA (gDNA) from lysed PBMCs

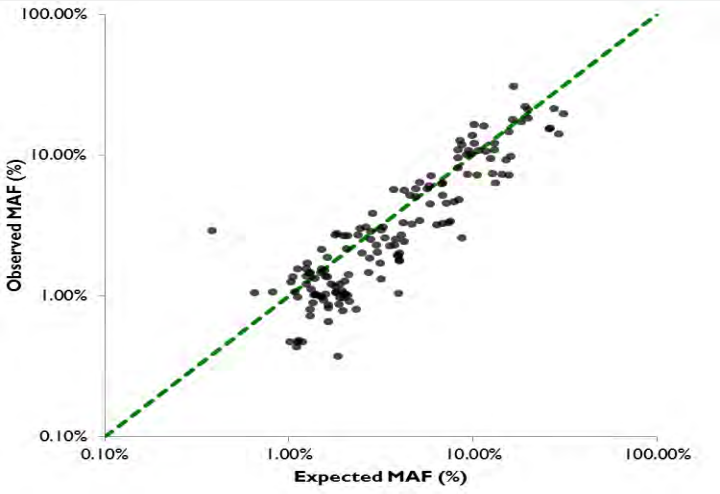
Deep Coverage on Clinical Samples is Highly Uniform

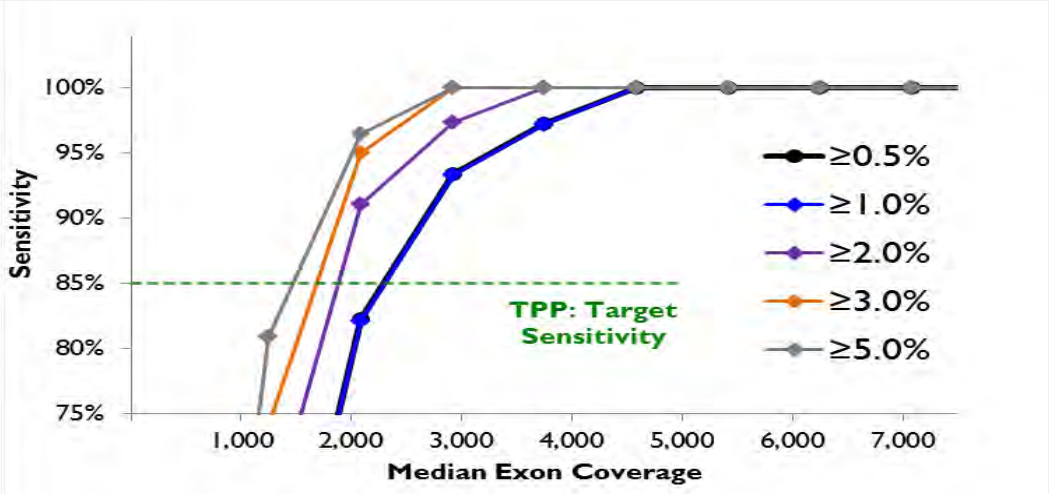




- At 5,000X unique median exon coverage, >99% of exons covered at >2,500X across most clinical and cell-line model samples

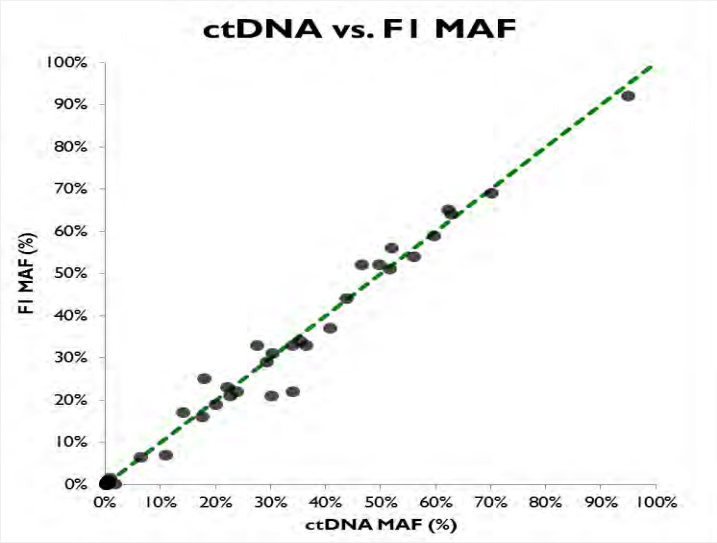
Quantitative Detection of base subs and Indels with High Sensitivity and PPV at Low Allele Frequencies

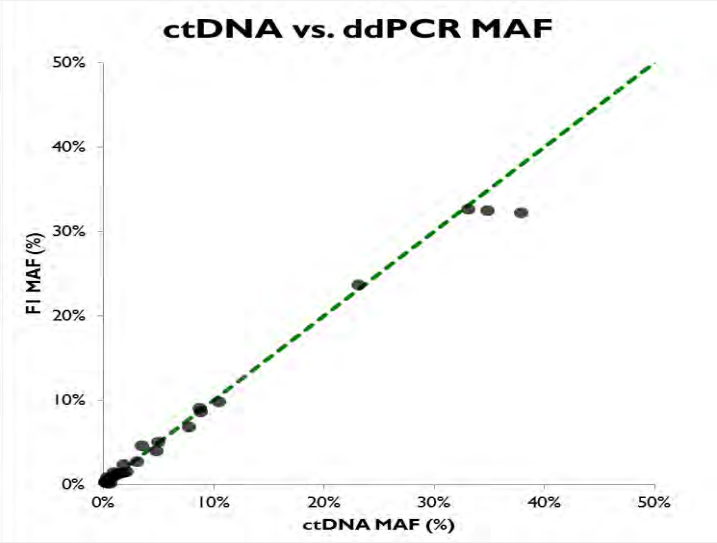




Indels	True Positives	False Positives	False Negatives	Sensitivity	PPV
Total	168	2	0	100.0%	98.8%

Observed 100% Concordance During Orthogonal Validation of ctDNA Assay with FOne or ddPCR





- 87 of 87 somatic alterations (47 at <5% MAF) called in clinical ctDNA were confirmed by F1 or ddPCR

ctDNA Assay Performance Surpasses Specifications

Criteria	Validated Results at >5,000X			
Recommended ctDNA Input	Passed	≥25 ng ctDNA		
Performance Specifications:		MAF	Sensitivity	PPV
Base Subs	Passed	0.5%	99%	99.9%
Indels	Passed	1.0%	>99%	98.8%
Gene Fusions	Passed	1.0%	>99%	98.0%
Focal Amplifications	Passed	High ctDNA %	93%	97.5%

- Fully automated ctDNA workflow met or exceeded all of the target specifications as demonstrated by results from over 400 model and clinical samples

FOUNDATION MEDICINE®

Summary

- THERE IS NO ROOM FOR ERROR or OMISSION in the care of patients with cancer
- Many cancer patient's tumors shed insufficient tumor DNA and a ctDNA assay will always be negative
- Diagnostic assays require robust validation for all alteration classes to allow physicians to make INFORMED choices, not guesses
- Patients are being denied standard of care, on label therapies and clinical trial options due to inaccurate, poorly validated diagnostics

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Session III Panel Discussion

Liquid Biopsy Test Development

Chair: Reena Philip, PhD

Speakers:

Abraham Tzou, MD

Phil Stephens, PhD

Panelists:

Tera Eerkes, PhD

AmirAli Talasaz, PhD

Walter Koch, PhD

Mark Lee, MD, PhD

Mark Sausen, PhD

American Association
for Cancer Research

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During audience Q&A sessions, webcast participants may send questions and comments to

policyquestion@aacr.org

Plasma to Tissue Concordance

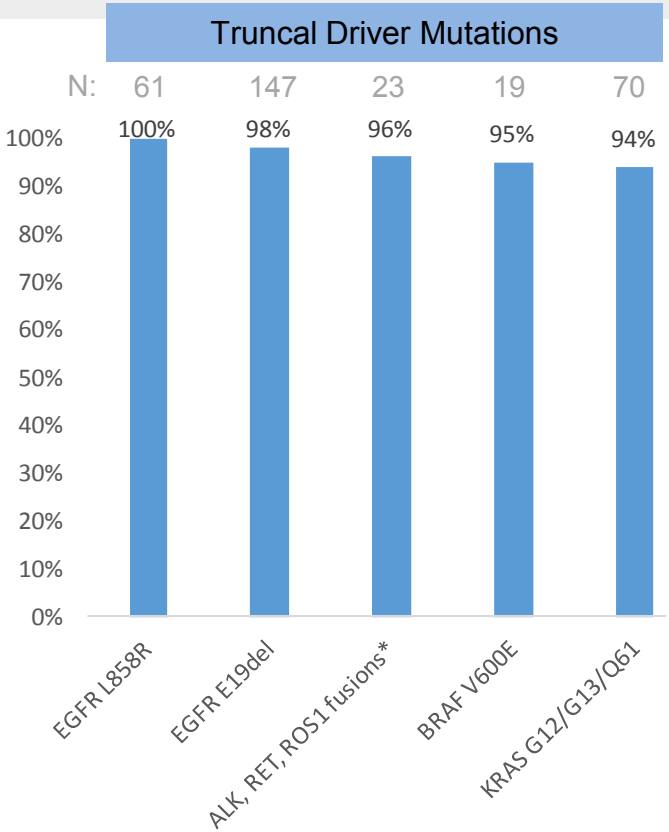
Why would some plasma to tissue concordance studies be more/less informative? Comment on:

- Assessing the relevance of specimens with/without clinical outcomes (e.g., comparison to trial clinicopathologic characteristics)
- Caveats of conducting and interpreting retrospective studies (e.g., disease burden, treatment resistance, matched sampling)
- Appropriate clinical use (e.g., reflex to tissue testing if plasma negative, impact of plasma positive/tissue negative)

ctDNA cohort: 15,000+ pts, advanced stage

Cohort (Data Source)	ctDNA (Guardant Health)	Tissue (TCGA)
Number of patients	15,191	9,077
Number of samples	17,628	9,077
Patients with alterations	12,664 (83.4%)	8,492 (94%)
Number of cancer types	50+	27
Stage	III, IV	I, II, III, IV
Alterations per sample	3 (median); 0-166 (range)	2 (median); 0-110
Treated	Generally 2nd line or higher	Pre-treatment
Diagnosis to blood draw	748 (342) days (mean/median)	--
FDA-approved therapy	7,499 (49%)	--
Gender	59% Female, 41% Male	58% Female, 42% Male
Age	63 (median); 23-91 (range)	56 (median); 10-90 (range)

ctDNA PPV *vs* tissue biopsy (NSCLC, CRC, Breast, Others)



- Truncal driver mutations
  - PPV remained high (94.5%, n=71) for low MAF truncal mutations (<0.5%)
  - * The single ctDNA-positive, tissue-negative ALK fusion responded to crizotinib

Subclonal mutations indicate likely resistance

- Discordant resistance cases likely reflect evolution on therapy after initial tissue biopsy

NEXT-2: A Prospective Clinical Study

Interim Analysis

	Gastric Cancer (N = 78)	NSCLC (N = 72)
% 2nd Line and above	63%	71%
ctDNA matched Therapies (n)	10	17
Therapeutic Targets	• *ERBB2* amplification (6) • *PIK3CA* mutation (2) • *FGFR2* amplification (1) • *MET* amplification (1)	• *EGFR* mutation (8) • *EGFR* T790M mutation (8) • *EML4-ALK* fusion (1)
Results	• 1 PD, 1 CR, 5 PR, 3 SD	• 1 PD, 1 SD, 15 PR
Response Rate (PR+CR)	**60%**	**88%**

ASCO Abstract June, 2016

Plasma to Tissue Concordance

Relevance of specimens with/without clinical outcomes

- Clinical outcomes are necessary in establishing novel clinical applications; however, extending existing clinical applications should not require repeating these studies
- ctDNA and tissue-derived genomic DNA are two manifestations of the same biological entity and may be expected to have similar implications
- Method comparison to the original FDA approved CDx method, using clinical samples that are representative of patients in the pivotal drug trial
- Clinical outcomes may still be required to explore novel applications or variants

Plasma to Tissue Concordance

Retrospective study caveats (e.g., disease burden, resistance, matched sampling)

ctDNA and tissue-derived genomic DNA represent the same biological entity but often differ due to biological, clinical, and technical reasons in retrospective studies

ctDNA and tissue DNA are generally sampled at different times—ctDNA is most often taken at progression, while tissue DNA is obtained at diagnosis

- intervening time, treatment, disease evolution, and disease heterogeneity means genomic differences, including more resistance mutations and genomic diversity
- truncal drivers usually remain but may be suppressed by effective treatment

Orthogonal reference methods may enable blood-to-blood comparison to adjudicate tissue-plasma discordances

Plasma to Tissue Concordance

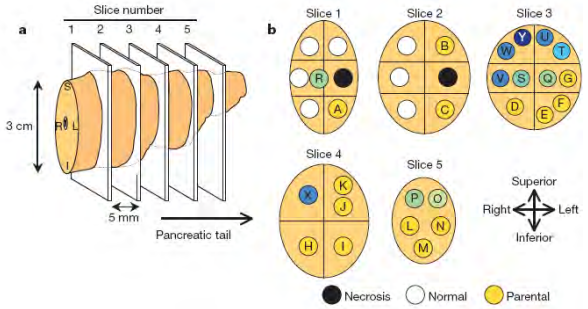
Appropriate claim (e.g., reflex to tissue testing if plasma negative)

- ctDNA levels vary greatly due to disease burden, variable non-tumor shedding (e.g. surgery, BMI), clearance (e.g. renal function), etc.
- Due to this biological variability and its attendant negative affect on PPA, ctDNA testing is inappropriate for definitive “rule-out” use at this time
- A “variant not detected” ctDNA result should reflex to tissue-based testing when biopsy/rebiopsy is possible
- Variants detected in ctDNA but not tissue should be considered equally actionable as concordant calls (Karachaliou 2014, Thress 2015, Sacher 2016)

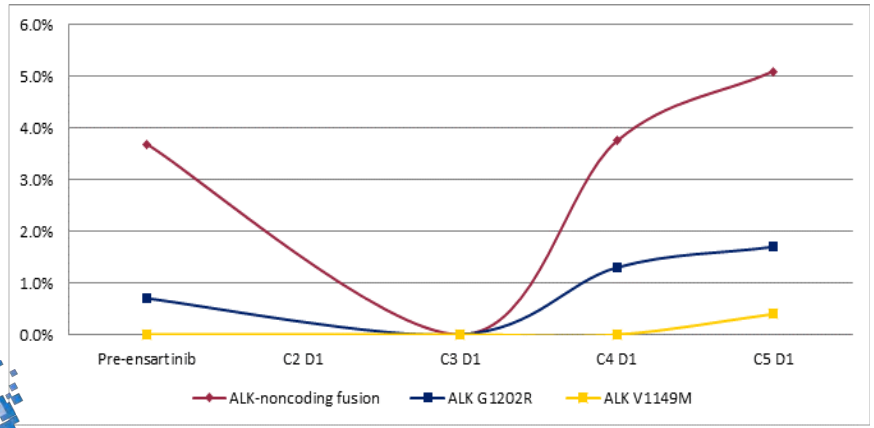
Plasma to tissue concordance

Tissue to plasma concordance - no better than tissue to tissue

- Potential heterogeneity within/between tumors
- Temporal discrepancies between tumor and plasma collections
- Processing differences between assays/analytes



Reference:
Yachida S, et al.
Nature 2010;
467:1114-1117



Reference: Horn, L. et. al., presented at ASCO 2016, J. Clin. Oncol. 34, (suppl: abstr. 9056)

Alternative metrics for concordance calculation

- Plasma-to-plasma, same-patient/time concordance is comprehensive verification
- When possible, clinical response rates comparable to standard of care in matched cohorts
- Appropriate submission claims may include reflex to tissue

Plasma to Tissue Concordance

Why would some plasma to tissue concordance studies be more/less informative? Comment on:

- Assessing the relevance of specimens with/without clinical outcomes (e.g., comparison to trial clinicopathologic characteristics)
- Caveats of conducting and interpreting retrospective studies (e.g., disease burden, treatment resistance, matched sampling)
- Appropriate clinical use (e.g., reflex to tissue testing if plasma negative, impact of plasma positive/tissue negative)

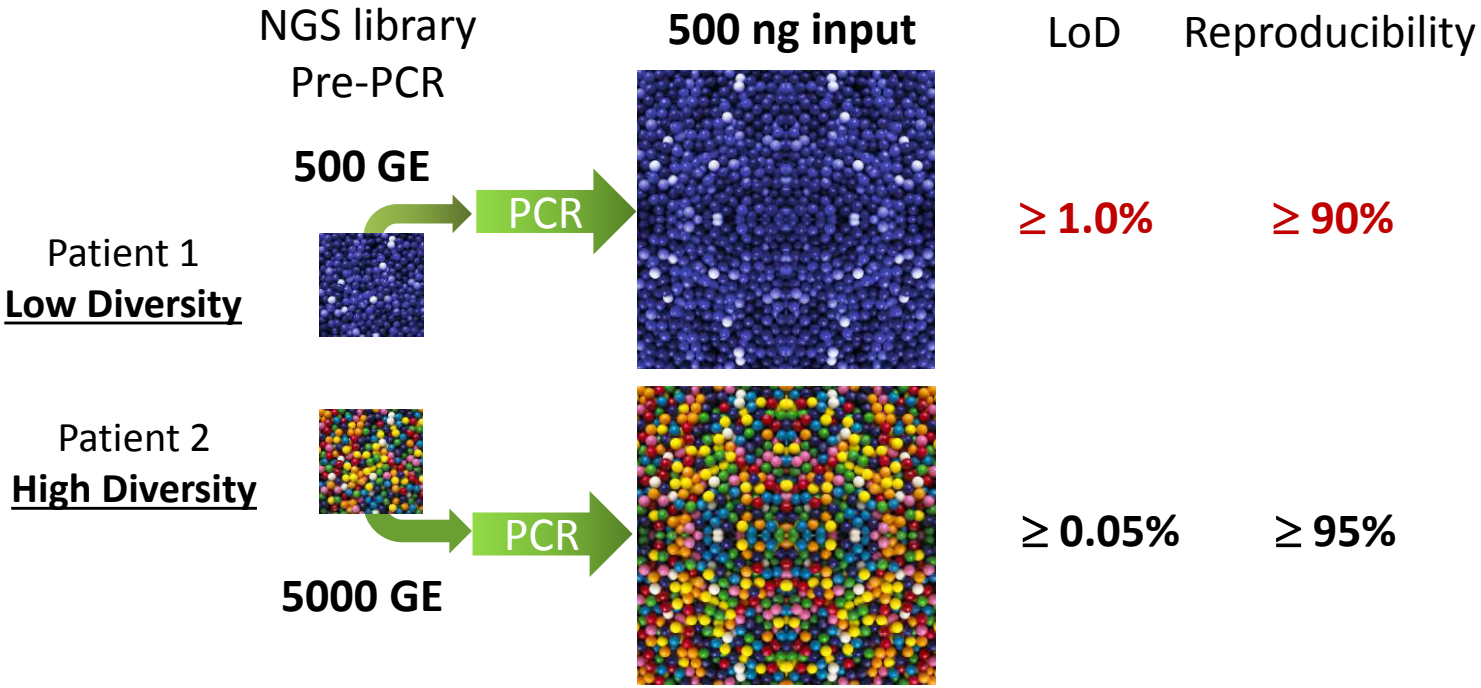
Analytical Performance Characteristics

What are important considerations for establishing analytical performance characteristics? Discuss:

- Appropriate orthogonal method validation (e.g., limit of detection (LoD), reproducibility, accuracy)
- Potential of low level calls and selection of cutoff (e.g., signal vs. error vs. contamination)
- Evaluating consistency, comparability, uniformity (e.g., across/within variant types)

Analytical Performance I

Performance metrics are meaningful only in context of library diversity



Library diversity and assay conditions critically influence validation metrics

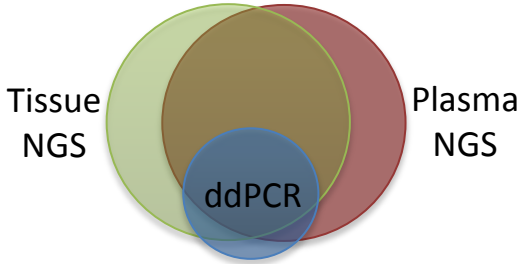
Genome Equivalents: The depth of clone coverage, measured as the number of ***unique*** genomic fragments, detected at target sites in an NGS assay

Analytical Performance II

Orthogonal comparators

- No perfect comparator exists using the same analyte and methodology
- ddPCR and tissue NGS are equally complex methodologies requiring their own validation
- Comparator may not detect all types of variants or detect with same sensitivity

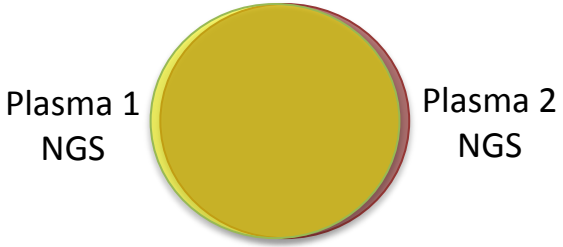
Limited concordance expected

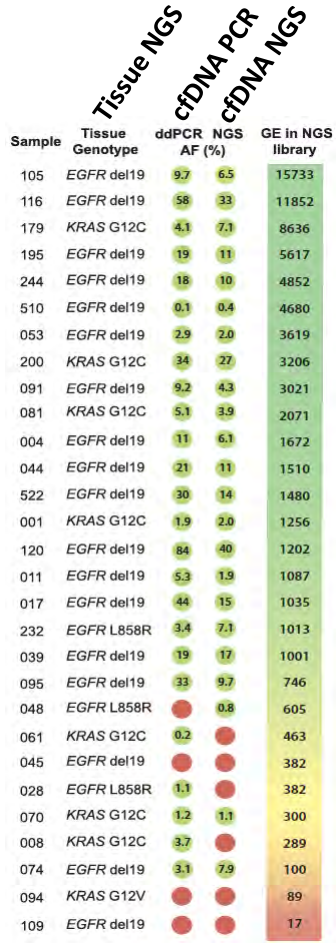


Alternative approach

- Plasma NGS to Plasma NGS, intra- and/or inter-lab comparison using same assay methodology

Comprehensive concordance expected





Paweletz et al. Clin Cancer Res October 2015

Analytical Performance Characteristics

Orthogonal comparator (e.g. LoD, reproducibility, accuracy)

– Orthogonal comparator essential for comparability and accuracy

– Traditional NGS methods, Digital PCR, BEAMing, qClamp, etc. all have distinct qualitative and quantitative error modes that must be acknowledged and managed (orthogonal ≠ truth)

– Quantitative accuracy and reproducibility particularly important for LoD study verification

– Qualitative accuracy and sensitivity particularly important for accuracy studies, especially those at low MAFs

Analytical Performance Characteristics-

Low level detection, cutoff (e.g., signal vs. error vs. contamination)

Probability of detection is independent of accuracy of detection

– e.g. LoD95 is defined as the MAF at which the probability of detecting a variant is ≥95%; below this MAF, however, the probability is not zero, and these calls may be both accurate and clinically useful

Sub-LoD calls must be subject to accuracy studies independent of near- and above-LoD accuracy studies

– Accuracy studies that lump together all MAFs can mask poor performance at low MAFs

Accuracy studies should be supported by independent reference methods

Analytical Performance Characteristics-

Consistency, Comparability, Uniformity (e.g. across/within variant types)

Rigorous development using normal and clinical sample sets required

Validation using standardized reference materials and reference methods

Complete and rigorous precision/reproducibility studies across all variant types and contexts

Analytical Performance Characteristics

What are important considerations for establishing analytical performance characteristics? Discuss:

- Appropriate orthogonal method validation (e.g., limit of detection (LoD), reproducibility, accuracy)
- Potential of low level calls and selection of cutoff (e.g., signal vs. error vs. contamination)
- Evaluating consistency, comparability, uniformity (e.g., across/within variant types)

Use of Contrived Specimens

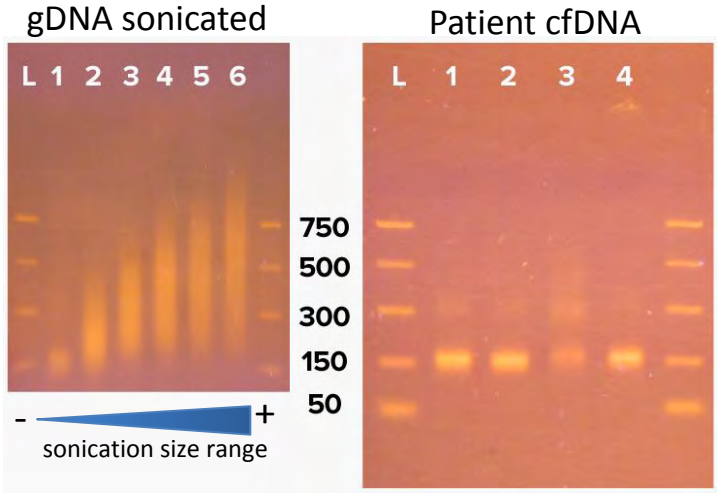
When are contrived specimens more/less suitable for analytical validation? Consider:

- Studies where clinical specimens have priority use (e.g., LoD confirmation)
- Methods of contrived specimen composition (e.g., DNA fragmentation, background plasma DNA)
- Evidence supporting commutability (e.g., checking contrived detection rate below clinical LoD)

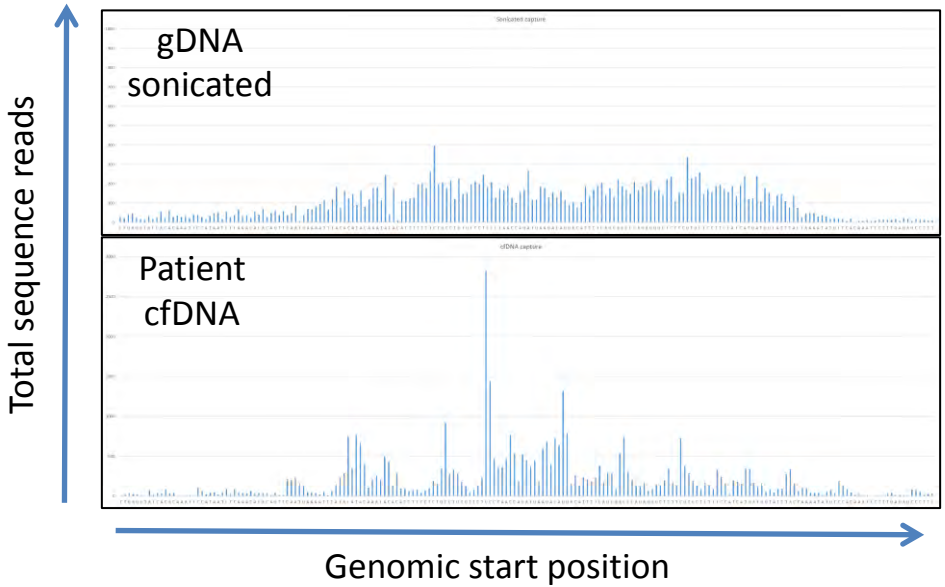
Contrived Specimens I

Sonicated gDNA ≠ cfDNA

Divergent size profiles



Divergent start-site distributions



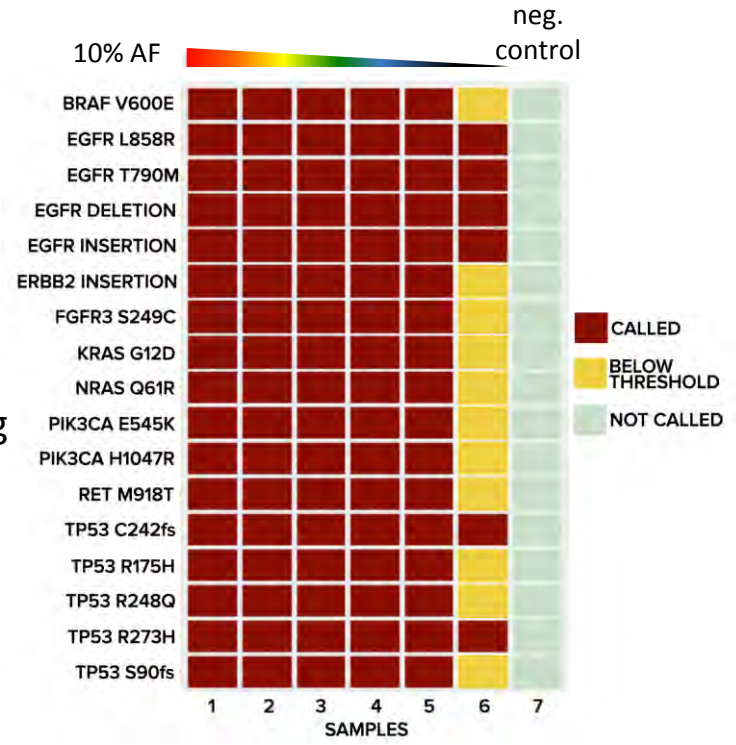
Contrived Specimens II

Needed: well-characterized, renewable, analyte-positive resource

Specifications for consideration:

- Include SNVs, indels, fusions and CNVs
- Range of allelic frequencies and mutations to evaluate diverse gene panels and clinical conditions
- Easily differentiate contrived and clinical calls
- Utility for assay controls, performance and proficiency testing

Use of contrived specimens is current best-practice for analytical validation



Many thanks to MoCha lab
Sims et al. (2016) JMD 18:336

Contrived Specimens

Need for clinical specimens (e.g. LoD confirmation)

Contrived materials necessary for:

- high degree of characterization
- facile manipulability
- representation/creation of rare/novel variants
- ease of reproducible material generation in large quantities

Must be carefully controlled to ensure commutability

Must be explicitly verified with clinical specimens to establish commutability

Contrived Specimens

Contrived specimen composition (e.g. cell line, fragmentation)

- Many types of contrived material available; differ substantially in the fidelity of human cfDNA simulation
- Commutability of cell line-derived material must be rigorously demonstrated at low MAFs
- Blends of tumor cell line-derived cfDNA containing known variants spiked into cfDNA from a healthy donor are better suited as contrived material
- Sheared genomic DNA simulates cfDNA poorly; Synthetic materials (e.g. oligonucleotides) simulate cfDNA poorly
- Since only a limited number of indels are available in cell lines, in-silico studies could supplement the analysis

Use of Contrived Specimens

When are contrived specimens more/less suitable for analytical validation? Consider:

- Studies where clinical specimens have priority use (e.g., LoD confirmation)
- Methods of contrived specimen composition (e.g., DNA fragmentation, background plasma DNA)
- Evidence supporting commutability (e.g., checking contrived detection rate below clinical LoD)

Panel Discussion

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policyquestion@aacr.org

Break

3:20 – 3:35 p.m.

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Session IV

Accelerating Liquid Biopsy Applications to Improve Patient Care

Chair: Gideon Blumenthal, MD

Speakers:

Sumimasa Nagai, MD, PhD

Andrea Ferris

Gary Kelloff, MD

Robert McDonough, MD, JD

Panelists:

Julia Beaver, MD

Geoffrey Oxnard, MD

Girish Putcha, MD, PhD

Stakeholder Perspective: Japanese Regulatory Agency (PMDA)

Sumimasa NAGAI, M.D., Ph.D.

Companion Diagnostics Working Group
Pharmaceuticals and Medical Devices Agency
(PMDA)

Companion Diagnostics WG

- Companion diagnostics (CDx) working group in PMDA

One of the projects across multi-offices in PMDA

Founded in April, 2012

HP: http://www.pmda.go.jp/rs-std-jp/standards-development/cross-sectional-project/0013.html

Definition of CDx in Japan

"Notification on Approval Application for *In Vitro* Companion Diagnostics and Corresponding Therapeutic Products"

was released on July 1, 2013.

Main Content

- Specify definition of CDx
- Recommend contemporaneous submission of marketing authorization applications of CDx and the corresponding drug

Definition of CDx in Japan

PMDA: Notification on Approval Application for *In Vitro* Companion Diagnostics and Corresponding Therapeutic Products

CDx is essential for using the pertinent therapeutic product, and corresponds to either of the following (except *in vitro* diagnostic agents or medical devices intended simply for disease diagnosis, etc.) :

- that is used to identify patients who are expected to respond better to a specific therapeutic product
- that is used to identify patients who are likely to be at high risk of developing adverse events associated with a particular therapeutic product
- that is necessary for optimizing the treatment including dose, schedule, and discontinuation of a particular therapeutic product

Technical Guidance in Japan

"Technical Guidance on Development of In Vitro Companion Diagnostics and Corresponding Therapeutic Products"

was released on December 26, 2013.

Main Content

- Biomarker-negative patients in clinical trials
- Retrospective analyses on biomarkers
- Concordance studies

PMDA Workshop

PMDA Workshop on CDx was held on Sep. 1, 2014

More than 400 participants from academia, industries and regulatory agencies

This workshop concluded that stakeholders must cooperate to deal with the following issues:

- What type/amount of clinical data are necessary for approval of follow-on CDx

- How to regulate multiplex diagnostics such as Next Generation Sequencing (NGS)

NGS-based Diagnostic System

"Legislation Notice to Applicants for Marketing Authorization of DNA Sequencers, etc. Utilized for Genetic Testing Systems"

was released on April 28, 2016.

Basic concept

This notification indicates handling of DNA sequencers, etc. manufactured and marketed for the purpose of diagnosing, treating, or preventing diseases in accordance with the Japanese Law on Securing Quality, Efficacy and Sefety of Products Including Pharmaceuticals and Medical Devices.

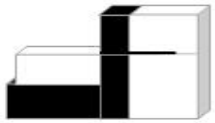
NGS-based Diagnostic System

DNA
RNA

Template DNA preparation reagent

Class III
In vitro diagnostic

DNA Sequencer



+ software for alignment etc.

Sequencing sample preparation reagent

Medical device (not class I)

Medical device (not class I)

Software for bioinformatics analysis

Referring

Database

DNA sequencer diagnostic system

NGS-based Diagnostic System

Handling of Genetic Variations of Uncertain Clinical Significance

- Although detecting genetic variations of uncertain clinical significance is not eligible for marketing authorization, providing the results as reference information is acceptable only when the physician considers it necessary.

- In such circumstances, caution must be exercised so that physicians are appropriately informed that the genetic variations which have not been approved have uncertain clinical significance and unknown analytic validity.

Clinical Evaluation of Equivalency

"Clinical evaluation of equivalency between companion diagnostics using clinical samples"
is now being discussed in a research group composed of academia, industry and regulators.

This is applicable to CDx that

- Detect or measure the same biomarker
- Correspond to the same drug
- Are utilized for patients with the same disease

as the reference CDx.

These CDx may utilize

- Different methods of detection or measurement
- Different object substances (DNA, RNA, Protein, etc.)
- Different specimen types (FFPE, Plasma, etc.)

from the reference CDx.

Clinical Evaluation of Equivalency

"Clinical evaluation of equivalency between companion diagnostics using clinical samples" under discussion in Japan

Principles

- Retrospective studies usually cannot collect data on efficacy and safety of the corresponding drug in patients adjudged to be biomarker-positive by new CDx and biomarker-negative by reference CDx.

↓

- In principle, clinical samples of patients treated with the corresponding drug should be collected prospectively in a new clinical trial in order to evaluate clinical validity.

		New CDx	
		Positive	Negative
Reference CDx	Positive		
	Negative		

Clinical Evaluation of Equivalency

"Clinical evaluation of equivalency between companion diagnostics using clinical samples" under discussion in Japan

- There may be cases where conducting a new prospective clinical study is not necessary for evaluation of clinical validity.

↓

- Examples described in the following slides are now under discussion.

Clinical Evaluation of Equivalency

Potential cases where a prospective clinical study is not necessary for evaluation of clinical validity

Good concordance in analytical performance was demonstrated using samples of patients with the same disease as the indication of the corresponding drug in each case described below:

- New CDx utilizes same components related to methods of detection or measurement, same methods, same object substances, and same specimen types and has same purposes as reference CDx.

- New CDx utilizes different components or methods but same specimen types and detect same point mutation or deletion, etc as reference CDx.

(This is not applicable to cases where clinical cutoff value needs to be specified such as gene amplification)

Clinical Evaluation of Equivalency

Potential cases where a prospective clinical study is not necessary for evaluation of clinical validity

- Cases where very high analytical concordance rate was demonstrated between reference CDx and new CDx

Reason

✓ In such cases, it is impractical to collect clinical samples with discordant results between reference CDx and new CDx even if a new prospective clinical study is conducted.

		New CDx	
		Positive	Negative
Reference CDx	Positive		
	Negative		

Clinical Evaluation of Equivalency

"Clinical evaluation of equivalency between companion diagnostics using clinical samples" under discussion in Japan

- Clinical utility of CDx for plasma samples may have to be taken into account in some cases where tumor tissue biopsy is not feasible or at high risk.

- Equivalency between CDx for plasma samples and CDx for tissue samples is evaluated on a case-by-case basis.

Our Publication

Nature Biotechnology **34**, 141–144 (2016) doi:10.1038/nbt.3478

Published online 05 January 2016

Evolving Japanese regulations on companion diagnostics

To the Editor:

Precision medicine involves the identification of a group of patients on the basis of a biomarker measured via a companion diagnostic who are then treated with a therapeutic targeted against that biomarker. The Japanese regulatory agency responsible for overseeing the regulatory oversight of companion diagnostics is the Pharmaceuticals and Medical Devices Agency (PMDA; Tokyo), which works with the Ministry of Health, Labor and Welfare (MHLW; Tokyo). In September 2014, the PMDA held a workshop on companion diagnostics that brought together >400 participants from academia, industry and regulatory agencies. The workshop identified the evaluation of concordance among different diagnostics targeting the same marker and the assessment of multiplex diagnostics as two key outstanding challenges. In the following correspondence, we briefly introduce PMDA's and MHLW's activities with regard to companion diagnostics, compare key differences among Japanese, US and European Union (EU; Brussels) regulations, and conclude by describing challenges relating to companion diagnostics discussed at the workshop.

The MHLW categorizes *in vitro* diagnostics (IVDs) into class I products (self-certified), class II products (subject to third-party certification) and class III products (subject to PMDA review under marketing authorization applications (MAAs) and approved by the MHLW), mainly depending on risk profile. Class I or II products without existing standards, like class III products, are also subject to PMDA review. Companion diagnostics generally fall under class III and usually require review not only by PMDA officers (standard MAAs) but also by outside advisory experts. In July 2013, the PMDA published its "Notification on approval applications for *in vitro*

Sumimasa Nagai, Masaaki Urata, Hiroyuki Sato, Motoki Mikami, Wataru Kuga, Reiko Yanagihara, Daisei Miyamoto, Yuka Suzuki & Mayumi Shikano

Companion Diagnostics Working Group, Pharmaceuticals and Medical Devices Agency, Tokyo, Japan.

LUNGEVITY

Find it. Treat it. Live.

Liquid Biopsies
A patient perspective

THE PATIENT - IS ALL OF US

LUNGEVITY
Find it. Treat it. Live.

FDA-AACR: Liquid Biopsies in
Oncology Drug and Device Development

Walter E. Washington Convention Center, Washington, DC

July 19, 2016

The Value of Multisector Collaboration

Gary J. Kelloff, MD

National Cancer Institute

Value Proposition/
Benefit for Partners in Public Private Partnership (PPP)

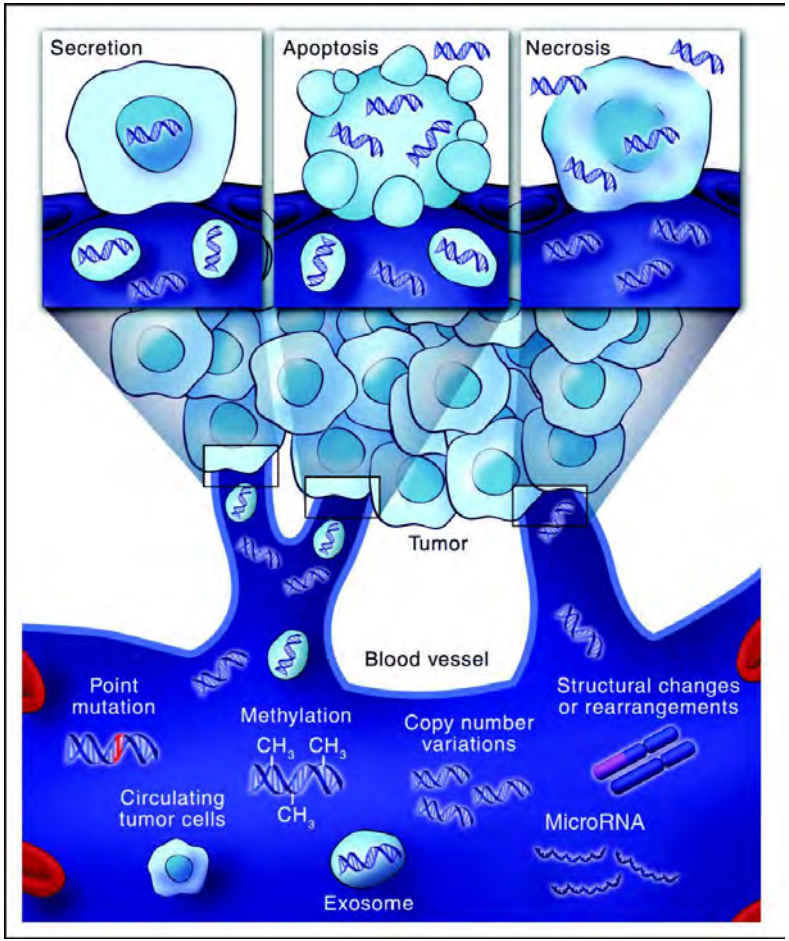
FNIH, FoCR, MMRF	• Nonprofit Convener and Partnership Builder
Diagnostics/ Devices Industry	• Companion Diagnostics • Imaging-based Biomarkers • Improved Business Models
Pharma	• More Efficient Drug Development and Approval Path • Better Early Response Criteria
FDA	• Provides for Evidence-Based Regulatory Policy
Academia, NCI	• Better Clinical Data • More Effective Treatment/Management
Patients	• Opportunity to Drive Path to Personalized Treatment • Potentially More Effective Treatment/Management
Non-Profit Foundations	• Education, Advocacy, Specific Issues, Funding Source
CMS, Payers	• Helps Define Reasonableness and Need

Beyond Cancer Detection: Tissue *vs.* Imaging Biomarkers

Tissue Biomarker	Imaging Biomarker
• **Can probe many features●**	• Probes 1-2 features
• Single location, limited sampling	• **Tissue volume, full tumor burden sampling●**
• **Variable cost●**	• Often expensive
• Invasive (tissue)	• **Non-invasive●**
• Serial assay challenging	• **Serial assay possible●**
• **Widely available — central assay●**	• Less widely available—local assay

- **Serum/plasma biomarkers address many limitations**

Circulating Tumor DNA

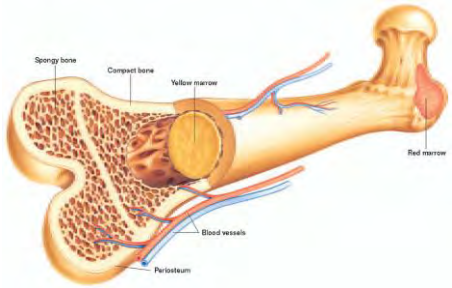


- DNA fragments of 180-200bp with half life of ~2 hours
- Specific to tumor
- Real-time, non-invasive, multi-lesions, potentially cheaper (considering cost of biopsies)
- Often very low amount of ctDNA in the sea of wild type DNA - ”Needle in a farm”

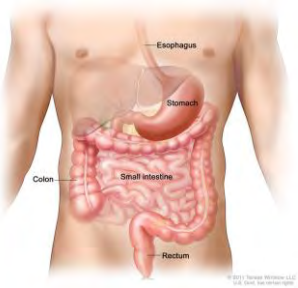
From Diaz LA and Bardelli A J Clin Oncol 2014;32:579-86

Sources of Circulating Cell-Free Tumor DNA

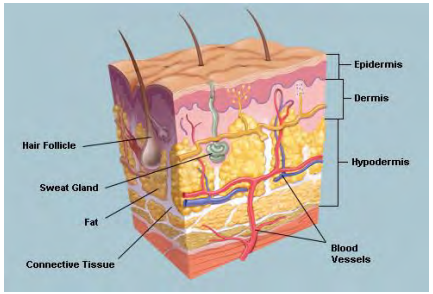
Bone Marrow



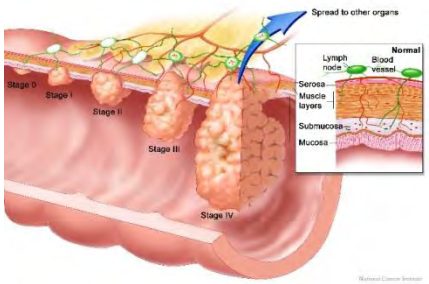
GI Tract



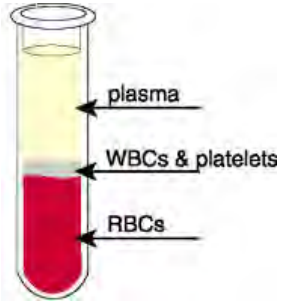
Skin



Tumor DNA

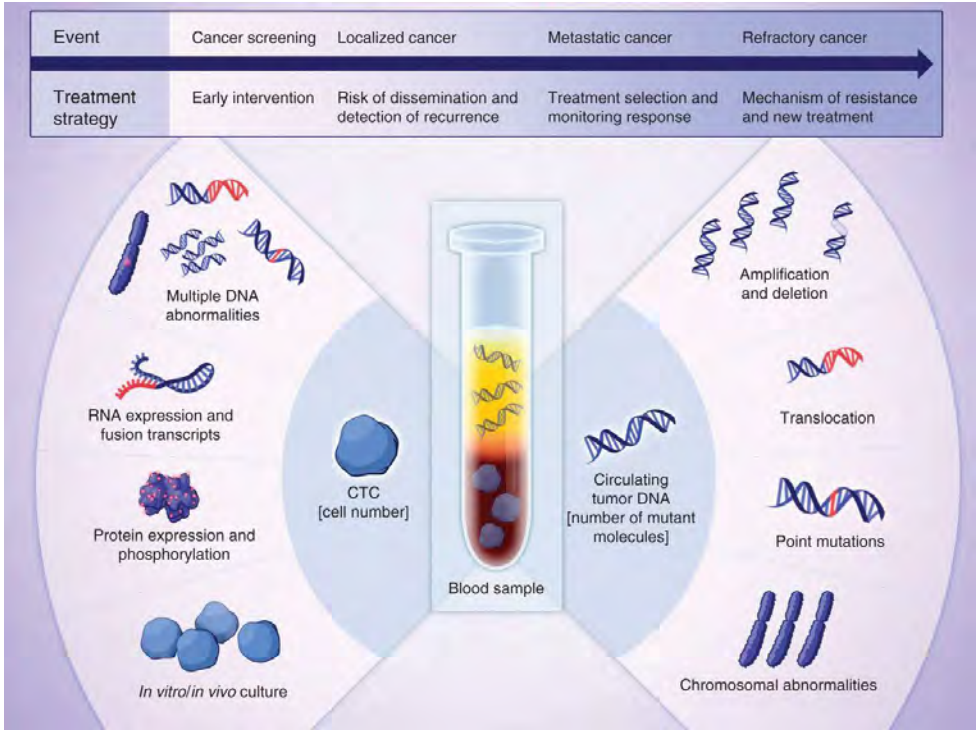


Pool of
Cell-free DNA



From LA Diaz, Presentation at Biomarkers Consortium Cancer Steering Committee Annual Meeting, October 26, 2015

Proving the Liquid Biopsy—Circulating Nucleic Acids



Haber DA and Velculescu VE Cancer Discovery 2014;4:650-661

Considerations In The Development Of Circulating Tumor Cell Technology For Clinical Use

Parkinson DR, Dracopoli N, Petty BG, Compton C, Cristofanilli M, Deisseroth A, Hayes DF, Kapke G, Kumar P, Lee JSh, Liu MC, McCormack R, Mikulski S, Nagahara L, Pantel K, Pearson-White S, Punnoose EA, Roadcap LT, Schade AE, Scher HI, Sigman CC, Kelloff GJ

J Transl Med. 2012 Jul 2;10:138

Evidence Of Clinical Utility: An Unmet Need In Molecular Diagnostics For Patients With Cancer

Parkinson DR, McCormack RT, Keating SM, Gutman SI, Hamilton SR, Mansfield EA, Piper MA, Deverka P, Frueh FW, Jessup JM, McShane LM, Tunis SR, Sigman CC, Kelloff GJ

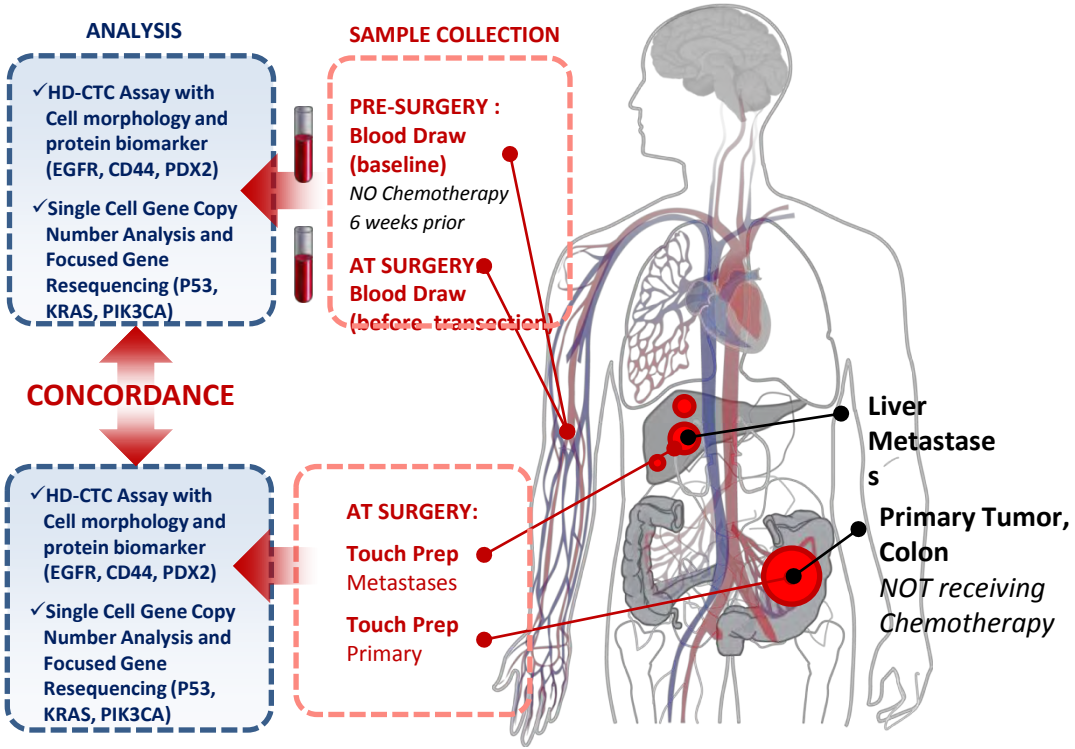
Clin Cancer Res. 2014;20:1428-44.

FNIH Launches New Project to Evaluate the Effectiveness of Liquid Biopsies as Biomarkers in Colorectal Cancer Patients (April 2016)

The Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium is launching a research **partnership** to determine whether **liquid biopsies** can be used instead of traditional solid tumor biopsies for diagnosing and monitoring metastatic colorectal cancer. Metastatic colorectal cancer is the third leading cause of cancer death in the United States according to the American Cancer Society.

Peter Kuhn, PhD, of the University of Southern California (USC), will lead the Project Team for *High Definition Single Cell Analysis (HD-SCA) of Blood and Tissue Biopsies in Patients with Colorectal Cancer Undergoing Hepatic Metastasectomy*. The team is comprised of experts from USC, Scripps Clinic, Baylor College of Medicine, Mayo Clinic, FDA, NCI and four sponsoring pharmaceutical companies (AbbVie, Amgen, Daiichi-Sankyo, and Lilly).

HD-SCA of Blood and Tissue Biopsies in Patients with Colorectal Cancer Undergoing Hepatic Metastasectomy



HD-SCA in Colorectal Cancer: Expected Benefits and Outcomes upon Completion of Stages 1 and 2

Confirmation that liquid biopsies can be correlated with solid biopsies and used for characterizing metastatic states

Relevant applications for stakeholders:

Generation of high-content bio-signatures that may have different intended uses depending on the stakeholder interest (compound and/or target specific)

Full characterization and interrogation of the liquid phase of solid tumors, potentially contributing to understanding therapeutic mechanisms of action, monitoring the effect of therapy in clinical trials, and understanding development of resistance.

Ease *in vitro* diagnostics and drug development by lowering barriers related to the difficulty in obtaining tumor specimens with the more easily obtained liquid biopsies

Exosomal Nucleic Acids

- Microvesicles or exosomes released by tumor cells into the circulation (blood, CSF) or urine may provide an alternative to cfDNA to characterize the genetic status of individual tumors
- Exosomes contact intact protein and RNA (and DNA) in a protected highly stable vesicle
- When the exosomes are released, they have the same orientation as the cell membrane and have been shown to display many of the surface markers from their cell of origin
- Expression analysis using microarrays and ultra-deep sequencing reveals a large number of both coding and non-coding RNA biomarkers available for analysis
- Exosomes have shown promise for early diagnosis of different cancers, including melanoma, ovarian cancer, kidney, and brain tumors
- A number of laboratories offer PCR analysis of exosomes and at least one company is nearing commercialization of specialized technology for evaluation of exosomal RNA

Cancer Molecular Profiling in Exosomes

Blood
- Lung cancer
- Colon cancer
- Melanoma
- Brain tumors

Transcriptome analysis;
Oncogenic mutations, *e.g.*, K-*ras*, B-*raf*

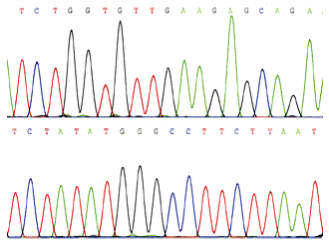
Urine
- Prostate cancer

Gene expression signature for detection of prostate cancer

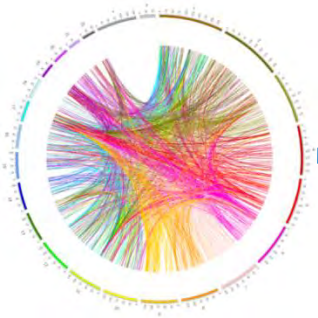
CSF
- Brain cancer

Detection of oncogenic brain tumor mutations, *e.g.*, IDH1, EGFRvIII

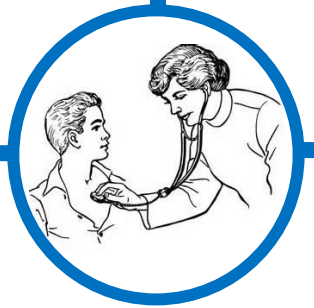
The Domains of Genomics

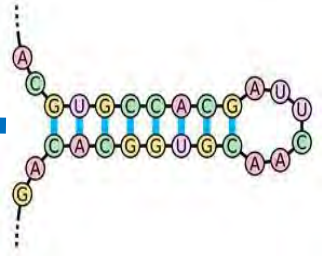


Structural Genomics



Computational Genomics





Functional Genomics

Louis Staudt, December 2, 2014

Complexity of Genomic/Proteomic Analysis

Genes (23,000)

⇓ (3-5x)

Allelic Variants

⇓ (3-5x)

mRNA Splicing Variants

⇓ (3-5x)

Protein Post-translational Modifications

⇓ (>10^6)

Protein-Protein Interactions

Kelloff GK and Sigman CC & Sigman Nat Rev Cancer 2007; 7: 508–518

"There's no standardization for cell-free DNA extraction, preparation, and storage," says Dr. Martin Fleisher. "That's a major failing."

Courtesy of Dr. Robert McCormack

(Im)Proving the Liquid Biopsy: Developing Reference Materials to Accelerate the Clinical Use of ctDNA

Project being developed by a Working Group of the Biomarkers Consortium Cancer Steering Committee led by Drs. Robert McCormack (Janssen) and Mickey Williams (NCI) with members/collaborators from NIST, NCI, FDA, academia, pharmaceutical companies, and diagnostics industry

Project Objectives:

- Develop CONTROL material to be used to assess performance of technology, reagents, and lab practices to assure validity of results
- Intended to be used for basic research, clinical research, clinical use, quality (CLIA), regulatory (FDA), payer needs
- Control will be quantitative and qualitative
- Relevant representatives variants (indels, SNVs, with clinically relevant and known difficult to sequence variants) included
- Context and technology independent

Project Components:

- White paper
- Well-vetted multi-lab validation study
- Downstream clinical trials

Uses of ctDNA in Oncological Drug Development and Patient Care

- Predictive biomarker to indicate whether a patient will benefit from a particular treatment, *e.g.*, companion or complementary diagnostic
- Response biomarker to serve as indicator of drug efficacy and to monitor patient treatment
- Biomarker of emerging resistance
- Early detection biomarker
- Prognostic biomarker to inform treatment selection

Challenges and Approaches for Realizing Precision Medicine in Oncology—2016: Liquid Biopsy Is an Important Advance

Challenge	Research Approach
Cancer Biology: — Tumor Heterogeneity, Clonal Evolution and Selection—Resistance Develops to Initially Effective Targeted Therapy — Normal Genetic Variation, Understanding Precancer and Early Disease — Role of Tumor Microenvironment, Particularly Immune Status	• High Content Screening Assays, Single-Cell Analysis, Next Generation DNA and RNA Sequencing (NGS), Proteomics, Epigenetics, Cytogenetics, Molecular Imaging, Multispectral IHC [Better Understanding of Cancer Progression, Characterization at Diagnosis] • Analysis of Both Cancer and Stromal Cells, Particularly Immune System Components
Requirement for Further Standards Development to Keep Apace of Rapidly Advancing Technologies; FDA Clearance versus CLIA –Facilitated Access, Insurance Coverage Policy	• Analytical and Clinical Validation of Assays for Evaluating Cancer Progression/Response to Therapy • Development of Reference Materials • Promote Coverage with Evidence Development, Increasing Interest and Clarity in Clinical Utility
Integrating Molecular and Target-Organ Based Disease Strategies	• Increased Efficiency and Sophistication of Clinical Trial Designs: Expansion Cohorts, Standard Protocols Across Collaborative Networks, Drug Combinations (e.g., Multiple Molecularly Targeted Drugs, Targeted Drug with Immunomodulator) • Co-Development of Diagnostics and Therapeutics: Complementary and Companion Diagnostics; Use of Diagnostics for Immune System Status • Improved Measurements of Response for Non-Cytotoxic Drugs • Clarification of Regulatory Pathways (Fast Track, Priority Review, Breakthrough Therapies, Accelerated Approval)
Managing and Analyzing High Volume Data, Matching Outcomes to Phenotypic and Genotypic Data, Access to Complete Datasets with Outcomes	• Establish Data Collection Parameters from Clinical Studies and High Content Screening Assays Including NGS and Proteomics • Developing Comprehensive and Collaborative Resources for Collection of High Content Data • Developing Systems for Analyzing Electronic Health Records and Cancer Registries
Precompetitive and Intellectual Property Issues	• Promote Multi-Sector Cooperation (e.g., Public-Private Partnerships)

Kelloff GJ, Sigman CC. DIA Global Forum 2015; 7:28-34 and 2016; 8:84-88

Payer Perspective on Liquid Biopsy

Robert McDonough, M.D.
Senior Director, Clinical Policy Research and Development

aetna SM

Clinical Policy Unit Function

- Aetna's Clinical Policy Unit is responsible for evaluating medical technologies to determine whether they are "experimental and investigational" and "medically necessary" as defined in applicable coverage documents
- Aetna has developed more than 800 Clinical Policy Bulletins (CPBs).
- The goal is to develop objective, clinically supported and defensible determinations.

TEC Criteria

The following criteria are considered in evaluating a medical technology:

- The technology must have final approval from the appropriate governmental regulatory bodies, when required
- The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes
- The technology must improve net health outcome
- The technology must be as beneficial as any established alternatives
- The improvement must be attainable outside investigational settings

Liquid Biopsy: Payer Considerations

- Benefit plan provisions
- Experimental and investigational status
- Medical necessity
- Clinical policy implementation (e.g., claims, precertification)
  - Coding considerations (CPT, HCPCS, ICD-10)
- Laboratory network contracting
  - Quality considerations
  - Reimbursement rates

aetna SM

Questions & Discussion

American Association for Cancer Research

FINDING CURES TOGETHER℠

Session IV Panel Discussion

Accelerating Liquid Biopsy Applications to Improve Patient Care

Chair: Gideon Blumenthal, MD

Speakers:

Sumimasa Nagai, MD, PhD

Andrea Ferris

Gary Kelloff, MD

Robert McDonough, MD, JD

Panelists:

Julia Beaver, MD

Geoffrey Oxnard, MD

Girish Putcha, MD, PhD

American Association
for Cancer Research

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During audience Q&A sessions, webcast participants may send questions and comments to

policyquestion@aacr.org

American Association for Cancer Research

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Wrap Up

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Adjourn

Thank You for Participating!