Circulating Tumor Marker Assays: "Liquid Biopsies"

An Old Tool with New Twists

Daniel F. Hayes, M.D., FASCO, FACP

University of Michigan Comprehensive Cancer Center

ROGEL CANCER CENTER

MICHIGAN MEDICINE

DISCLOSURE

- **Circulating Tumor Cells**
  - **CellSearch**
    - **Laboratory and Clinical research funding from Veridex/Janssen Diagnostics/Menarini Silicon BioSystems (MSB)**
    - **Patent regarding circulating tumor cells licensed to MSB**
- **Other**
  - **Stock Options: InBiomotion**
  - **Consultant: Agendia, Cellworks, Cepheid, CVS Caremark, EPIC Sciences, Freenome, Lexent, Salutogenic Innovations, L-Nutra**
  - **Sponsored Clin Research: Merrimack Pharmaceuticals, Eli Lilly, Menarini/Silicon BioSystems, Puma Biotechnology, Pfizer, Astra Zeneca**
  - **Collaborated with GHI, manufacturer of 21-gene RS**

(no financial support or conflict)

2010 - Term "Liquid Biopsy" Was Coined

"Research groups are focusing on the clinical value of CTC analyses….
Although promising data from patients with advanced disease demonstrate the value of CTC analysis as 'liquid biopsy', studies on cancer patients at earlier stages are hampered by low CTC counts..."

Pantel and Alix-Panabieres
Trend in Molecular Medicine, 2010

Circulating Tumor Markers in Br Ca:
"Liquid Biopsies"

- **Proteins**
  - ***MUC1 (CA15-3, CA27.29)***
  - ***CEA***
  - ***Proteomics***
- **Nucleic Acids**
  - ***ctDNA***
  - ***miRNA***
- **Tumor cells (CTC)**

Many assays for each;
They are very different

Circulating "Liquid" vs. Tissue Biopsy

	Circulating	*Tissue*
Logistics	Easy to draw	Invasive, more difficult to obtain
	Phlebotomy – inexpensive	Intervent Radiology, pathology $$$
	Permits easy serial testing	Serial testing difficult
Pre-analytical	Easier to control (fixat, anti-coagul, etc in vacutainer tube)	Processing (response gene activation, time to fixation, type of fixation, etc)
Sensitivity	CTC rare events (n=1-1000/10 cc tube) ptDNA low abundance	10^6-10^8 cells/biopsy
Biology	? "entire organism" – not 1 site May NOT represent biology of tissue-based CA	Only represents 1 site Represents tissue biology at least at THAT site

Circulating Tumor Biomarker Tests: Liquid Biopsies

- **History**
  - **Proteins**
    - **CEA (Colorectal)**

Circulating Tumor Markers: History (CEA)

DEMONSTRATION OF TUMOR-SPECIFIC ANTIGENS IN HUMAN COLONIC CARCINOMATA BY IMMUNOLOGICAL TOLERANCE AND ABSORPTION TECHNIQUES*

By PHIL GOLD,‡ M.D., and SAMUEL O. FREEDMAN, M.D.

(*From the McGill University Medical Clinic, Montreal General Hospital, and the Department of Physiology, McGill University, Montreal, Canada*)

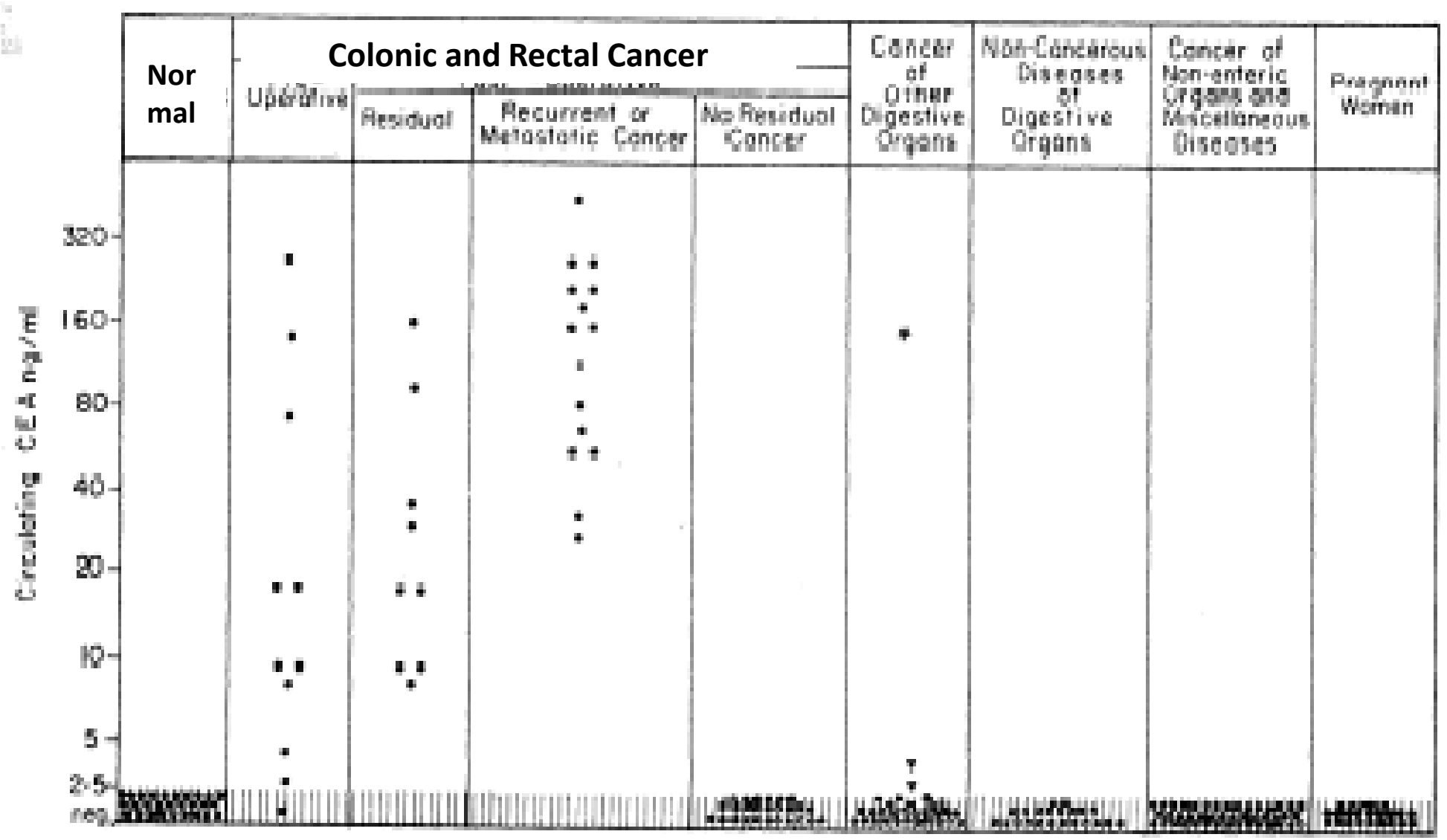
Plates 35 to 39

(Received for publication, November 16, 1964)

Numerous attempts have been made by previous workers to demonstrate the presence of tumor-specific antibodies in sera obtained from animals immunized with preparations of human cancers (1–8). Such demonstrations, if consistently reproducible, would indicate the existence in human cancer tissue of unique homologous antigens not present in normal tissue, and might thus lead to a better understanding of the nature of the neoplastic process.

Journal of Experimental Medicine, 121:439-462, 1965

Circulating Tumor Markers: CEA



Thomson, et al, Gold P., Proc Nat Acad Sciences (USA) 64:161-167, 1969

nature medicine

ARTICLES

https://doi.org/10.1038/s41591-018-0134-3

Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab

David R. Gandara[1,7]*, Sarah M. Paul[2,7], Marcin Kowanetz[2,7], Erica Schleifman[2,7], Wei Zou[2,7], Yan Li[2], Achim Rittmeyer[3], Louis Fehrenbacher[4], Geoff Otto[5], Christine Malboeuf[5], Daniel S. Lieber[5], Doron Lipson[5], Jacob Silterra[5], Lukas Amler[2], Todd Riehl[2], Craig A. Cummings[2], Priti S. Hegde[2], Alan Sandler[2], Marcus Ballinger[2], David Fabrizio[5], Tony Mok[6]* and David S. Shames[2]*

VIEWPOINT

TECHNOLOGY SPOTLIGHT

Genomic Analysis of Plasma Cell-Free DNA in Patients With Cancer

Geoffrey R. Oxnard, MD
Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts; and Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.

Cloud P. Paweletz, PhD
Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, Massachusetts.

Lynette M. Sholl, MD
Department of Pathology, Brigham and Women's Hospital,

Technology

The increased importance of cancer genotyping in guiding cancer treatment has created a need for efficient methods for genomic analysis of patients' cancers. This increased dependence on DNA-based tumor genotyping assays (eg, sequencing, polymerase chain reaction [PCR], fluorescence in situ hybridization [FISH]) has triggered a growing interest in the analysis of free-floating DNA present in the blood of patients with cancer—plasma cell-free DNA (cfDNA). Sensitive PCR techniques together with high-throughput next-generation sequencing (NGS) technologies have evolved to a point where genetic analysis of cfDNA is possible.

Strengths

Genotyping of plasma cfDNA is compelling for a number of reasons.[1] Most importantly, it can noninvasively provide clinically-relevant genomic information that is usually only available after an invasive tumor biopsy

Data Generated

Most cfDNA genotyping assays are designed to be highly sensitive to overcome the challenge of low levels of cancer-derived DNA within plasma. Some cancers may shed very little DNA into circulation because of small size, limited metastatic spread, or other biological factors. As a result, the clinical sensitivity of plasma genotyping (compared with tumor genotyping) has been reported in the range of 60% to 80% in patients with advanced cancer.[2,4,5] Specificity is also very high for most plasma genotyping assays, which is critical because even low false-positive rates can be problematic when testing for relatively rare molecular alterations. If a mutation is present in 5% of patients tested and a test has a 5% false-positive rate (95% specificity), then half of all positive results will be erroneous (50% positive predictive value). It is therefore essential that the expected level of background "noise" is clearly established during assay validation to minimize the risk of false-positive results and maximize specificity. When the false-positive rate ap-

Liquid biopsies come of age: towards implementation of circulating tumour DNA

Jonathan C. M. Wan[1,2], Charles Massie[1,2], Javier Garcia-Corbacho[3], Floren
James D. Brenton[1,2], Carlos Caldas[1,2,4], Simon Pacey[2,4], Richard Baird[2,4] and Nitzan Rosenfeld[1,2]**

Medical News & Perspectives

Going With the Flow: The Promise and Challenge of Liquid Biopsies

M.J. Friedrich

s Top Ten
or 2017,
otentially

1869 in the peripheral circulation of a patient with metastatic cancer (Ashworth TR. *Australian Med J.* 1869;14:146-147)

sue difficult or impossible to obtain, veins are easily accessible. And unlike tumor samples, liquid biopsies can capture how a

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

FRONTIERS IN MEDICINE

Application of Cell-free DNA Analysis to Cancer Treatment

Ryan B. Corcoran, M.D., Ph.D., and Bruce A. Chabner, M.D.

TUMOR BIOPSIES REPRESENT THE STANDARD FOR CANCER DIAGNOSIS and the primary method for molecular testing to guide the selection of precision therapies. Liquid biopsies, particularly those involving cell-free DNA (cfDNA) from plasma, are rapidly emerging as an important and minimally

Mol Diagn Ther (2016) 20:231–240
DOI 10.1007/s40291-016-0193-4

CrossMark

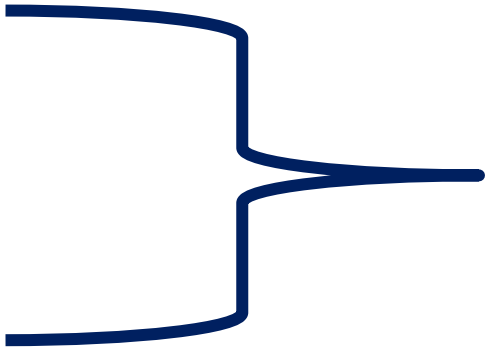
ORIGINAL RESEARCH ARTICLE

Novel Approach for Clinical Validation of the cobas KRAS Mutation Test in Advanced Colorectal Cancer

Abha Sharma[1] · Guili Zhang[1] · Shagufta Aslam[1] · Karen Yu[1] · Melody Chee[1] · John F. Palma[1]

Circulating Tumor Markers in Br Ca:
"Liquid Biopsies"-More than Protein, but Not just ctDNA

- Proteins
  - *MUC1 (CA15-3, CA27.29)*
  - *CEA*
  - *Proteomics*
- **Nucleic Acids**
  - ***ctDNA***
  - ***miRNA***
- **Tumor cells (CTC)**



Many assays for each;
They are very different

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JOURNAL OF CLINICAL ONCOLOGY
36:1631-1641, 2018

ASCO SPECIAL ARTICLE

Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar, Neal Lindeman, Christina M. Lockwood, Alex J. Rai, Richard L. Schilsky, Apostolia M. Tsimberidou, Patricia Vasalos, Brooke L. Billman, Thomas K. Oliver, Suanna S. Bruinooge, Daniel F. Hayes, and Nicholas C. Turner

Analytical Validity: Different ctDNA Assays May Give Different Results

RESEARCH LETTER

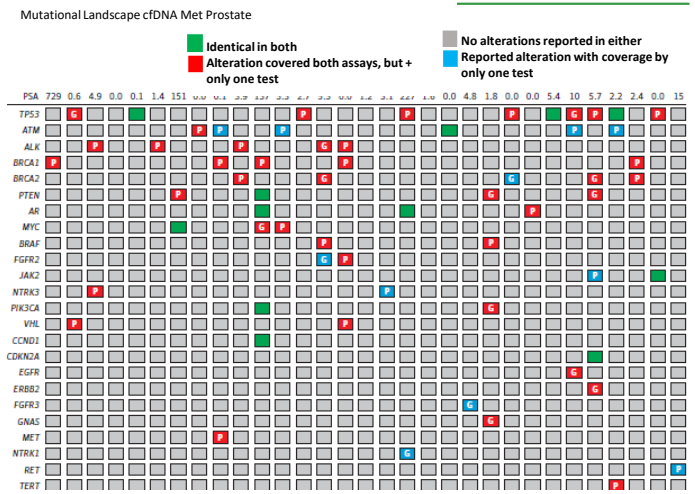
Patient Paired Sample Congruence Between 2 Commercial Liquid Biopsy Tests in Prostate Cancer

Torga, G, et al., JAMA Oncol 2018

Related article page 838

liquid biopsy to be commercially available in the clinical setting.[2,3] The goal of this study was to determine the reliability and potential utility of this technology in the clinical treatment of patients with metastatic prostate cancer, investigating following the instructions of each vendor.

Guardant360 (Guardant Health, Inc) panel includes 73 genes with complete exon sequencing for 19 cancer genes, critical exons in 54 genes and amplifications (18 genes),



EDITORIAL

Precision Medicine and Testing for Tumor Biomarkers–Are All Tests Born Equal?

Daniel F. Hayes, MD

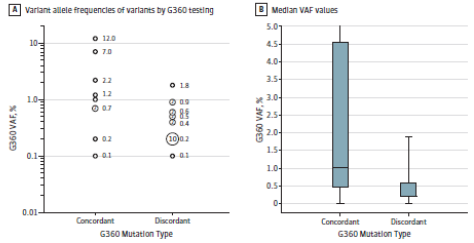
Letters

Kuderer, NM, et al., JAMA Oncol 3:996-998, 2017

RESEARCH LETTER

Comparison of 2 Commercially Available Next-Generation Sequencing Platforms in Oncology

Figure. Association Between Variant Allele Frequencies (VAFs) of Variants Identified by G360 and Concordance With FI Testing

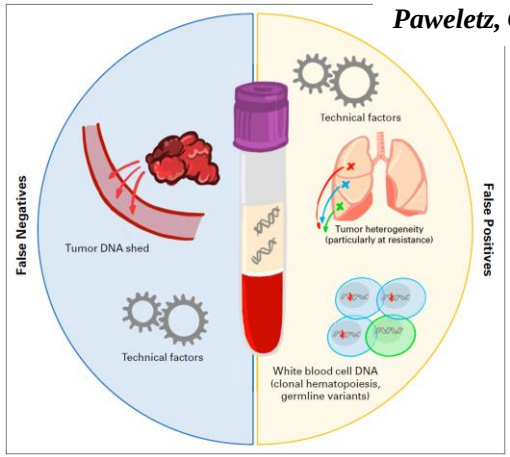


editorial

Does Testing Error Underlie Liquid Biopsy Discordance?

Cloud P. Paweletz, PhD[1]; Christie J. Lau[1]; and Geoffrey R. Oxnard, MD[1]

Paweletz, CP, et al., JCO Prec Oncol 2019



Precision Medicine and Imaging

Clinical Cancer Research

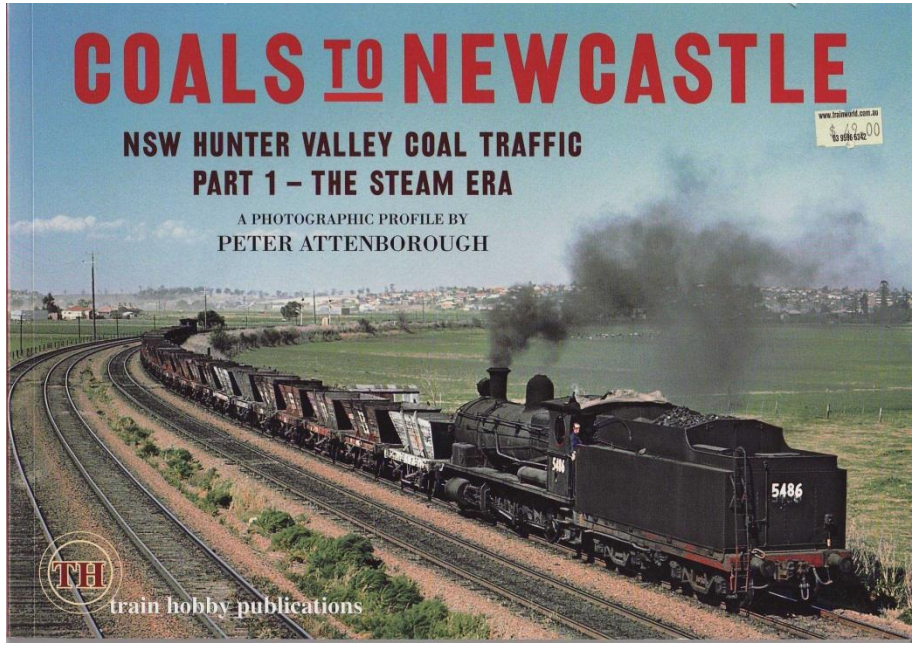
False-Positive Plasma Genotyping Due to Clonal Hematopoiesis

Yuebi Hu[1], Bryan C. Ulrich[2], Julianna Supplee[2], Yanan Kuang[2], Patrick H. Lizotte[2], Nora B. Feeney[2], Nicolas M. Guibert[1,2], Mark M. Awad[1], Kwok-Kin Wong[1], Pasi A. Jänne[1,2], Cloud P. Paweletz[2], and Geoffrey R. Oxnard[1]

Check for updates

Why and When to Use Tumor Biomarkers?

- **Risk assessment**
- **Screening**
- **Differential diagnosis**
- **Prognosis**
- **Prediction**
- **Monitoring disease state**



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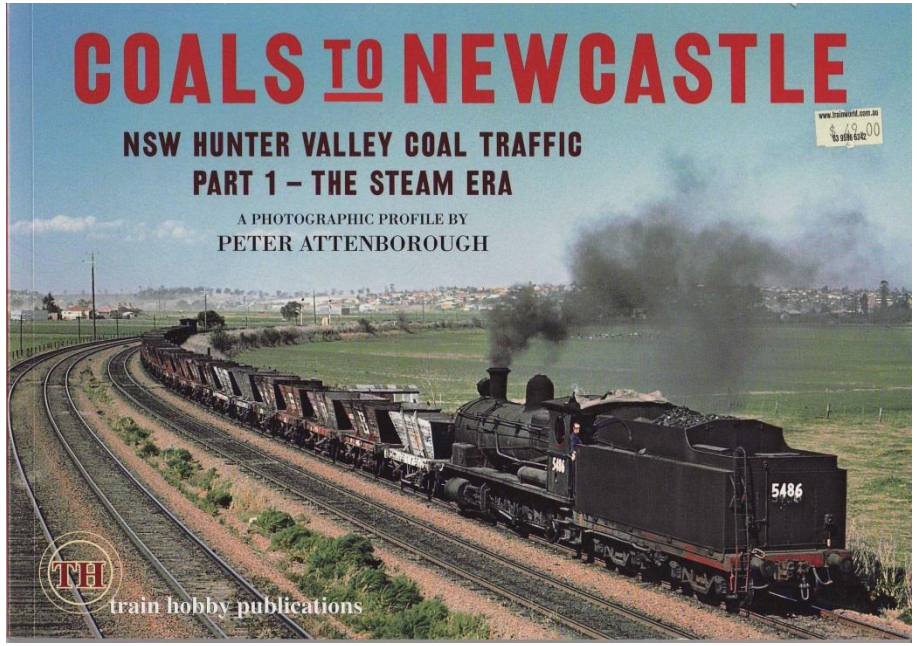


Liquid Biopsies (ctDNA): Screening for Br Ca

Science

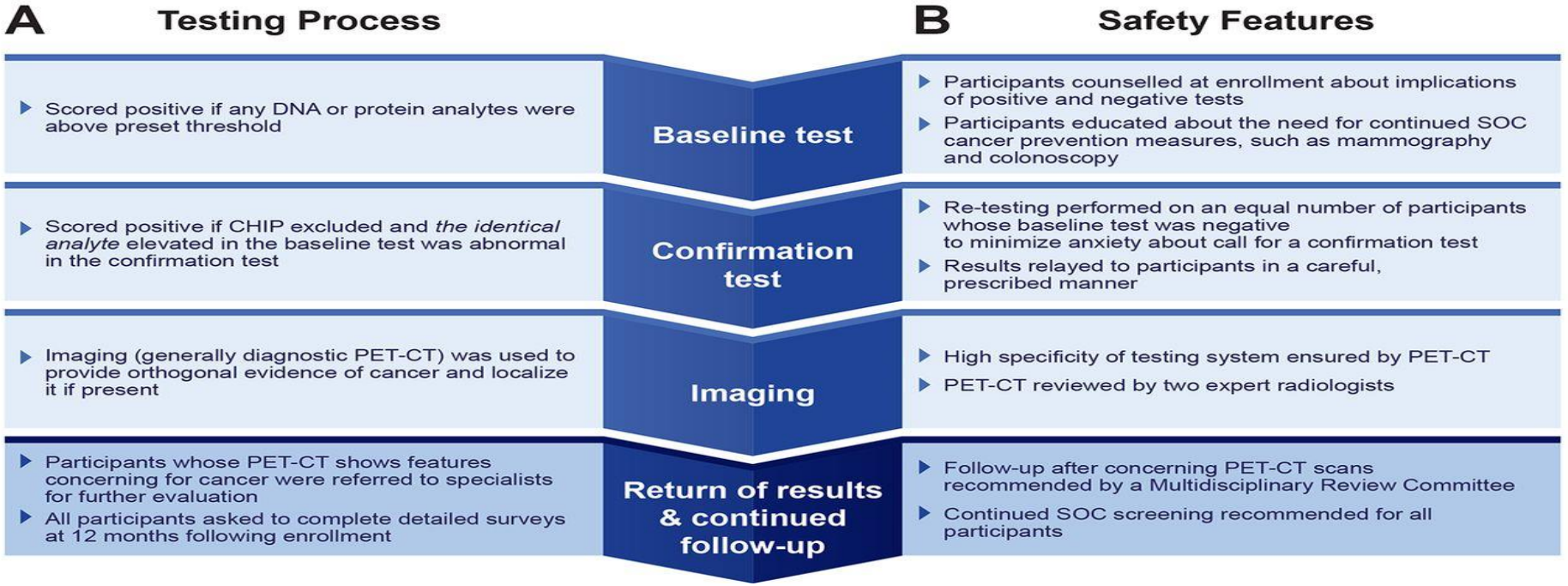
RESEARCH ARTICLES

Cite as: A. M. Lennon *et al.*, *Science* 10.1126/science.abb9601 (2020).

Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention

Anne Marie Lennon[1,4,10*], Adam H. Buchanan[11*], Isaac Kinde[12*], Andrew Warren[12,13*], Ashley Honushefsky[11*], Ariella T. Cohain[12], David H. Ledbetter[11], Fred Sanfilippo[14], Kathleen Sheridan[11], Dillenia Rosica[11], Christian S. Adonizio[11,16], Hee Jung Hwang[12], Kamel Lahouel[1,6], Joshua D. Cohen[1,2,3,4,5], Christopher Douville[1,5], Aalpen A. Patel[11], Leonardo N. Hagmann[12], David D. Rolston[11], Nirav Malani[12], Shibin Zhou[1,3,4], Chetan Bettegowda[1,3,8], David L. Diehl[11], Bobbi Urban[12], Christopher D. Still[11], Lisa Kann[12], Julie I. Woods[11], Zachary M. Salvati[11], Joseph Vadakara[11], Rosemary Leeming[11], Prianka Bhattacharya[11], Carroll Walter[11], Alex Parker[12], Christoph Lengauer[12,13], Alison Klein[1,4,15], Cristian Tomasetti[1,6,7], Elliot K. Fishman[1,4,10], Ralph H. Hruban[1,4,9], Kenneth W. Kinzler[1,3,4†], Bert Vogelstein[1,2,3,4†], Nickolas Papadopoulos[1,3,4,9†]

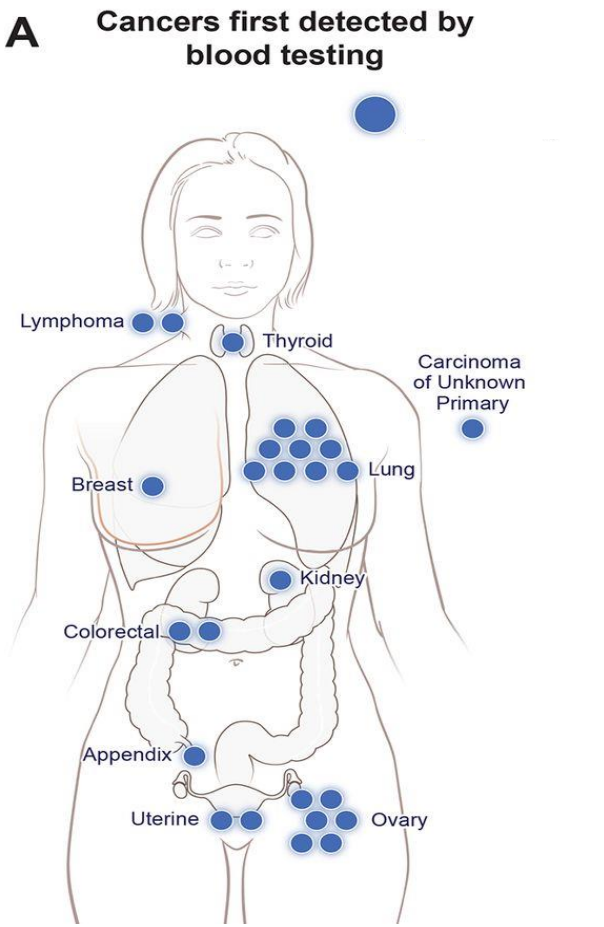
Detecting cancers **E**arlier **T**hrough **E**lective mutation-based blood **C**ollection and **T**esting
(DETECT-A)
(10,000 Women Age 65-75 years, No History Cancer)



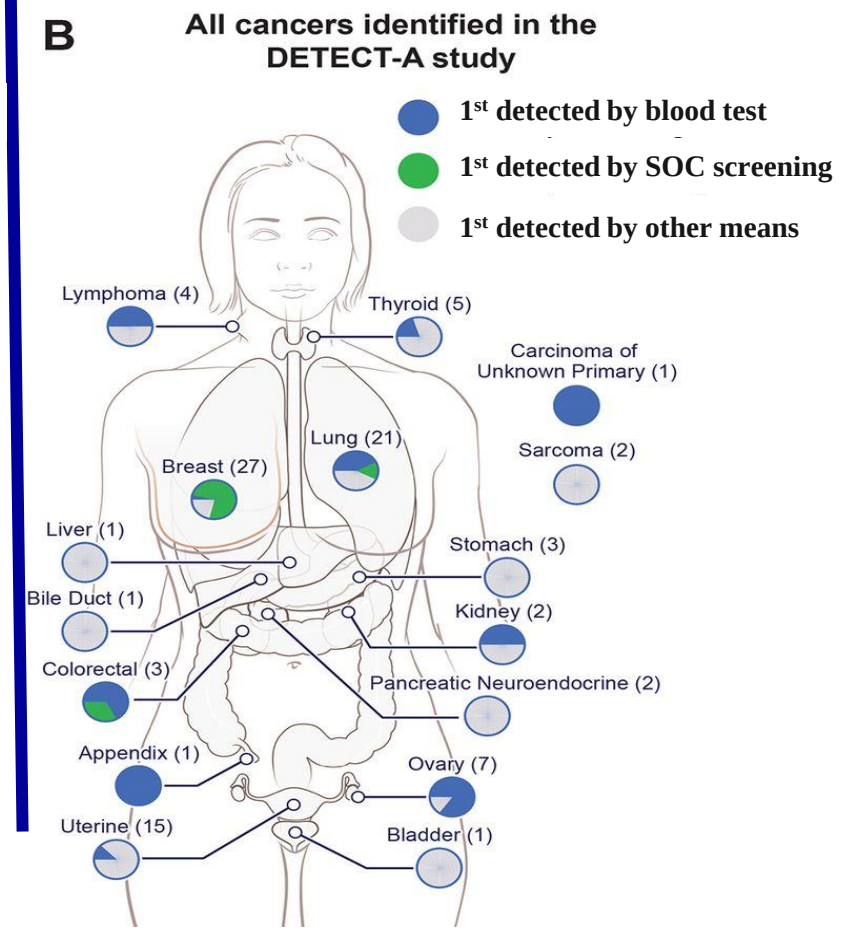
Anne Marie Lennon et al. Science 2020;science.abb9601

Liquid Biopsies (ctDNA): Screening for Br Ca

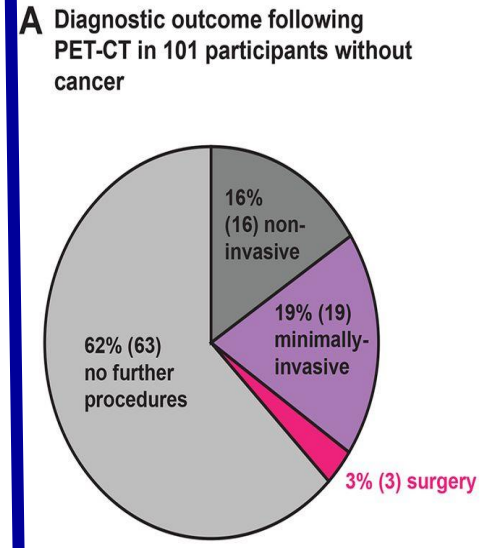
True POSITIVE
(ctDNA POS/Cancer Found)

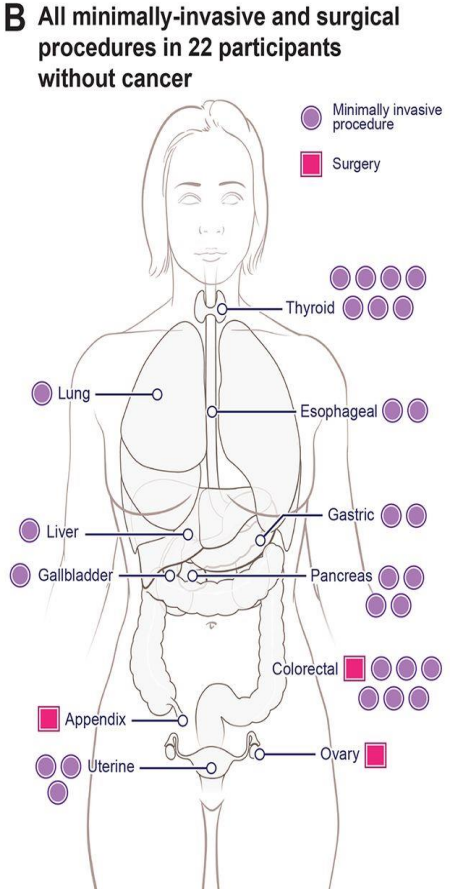


True POSITIVE + False NEGATIVE
(ctDNA NEG/Cancer Found)



False POSITIVE
(ctDNA POS, No Cancer Found)





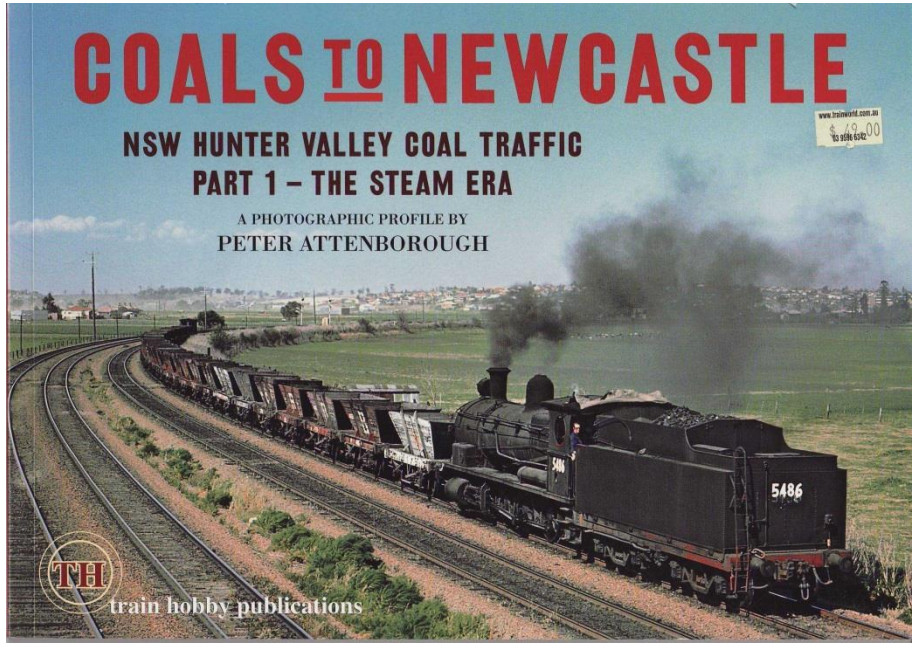
Anne Marie Lennon et al. Science 2020;science.abb9601

Liquid Biopsies (ctDNA): Screening for Br Ca

- **Conclusions**
  - **Authors' Conclusions** *Anne Marie Lennon et al. Science 2020;science.abb9601*
    - **Able to address fundamental issues *but not designed for regulatory approval***
    - **Larger trials necessary**
    - **Not certain that the blood test *helped any participant***
      - **Not randomized**
      - **May have led to over diagnosis**
    - **Will facilitate future randomized, interventional trials to assess the ability of blood tests to improve cancer screening**
  - **MY CONCLUSIONS:**
    - **Intriguing Preliminary data- but required 10,000 participants!**
    - ***NOT READY FOR ROUTINE PRACTICE***

Why and When to Use Tumor Biomarkers?

- Risk assessment
- Screening
- Differential diagnosis
- Selection of Therapy
  - Prognosis
  - Prediction



- **Monitoring disease state**
  - **For recurrence if patient is apparently free of disease**
  - **If patient has documented metastases**
    - **Evidence of progression**
    - **Selection of therapy**

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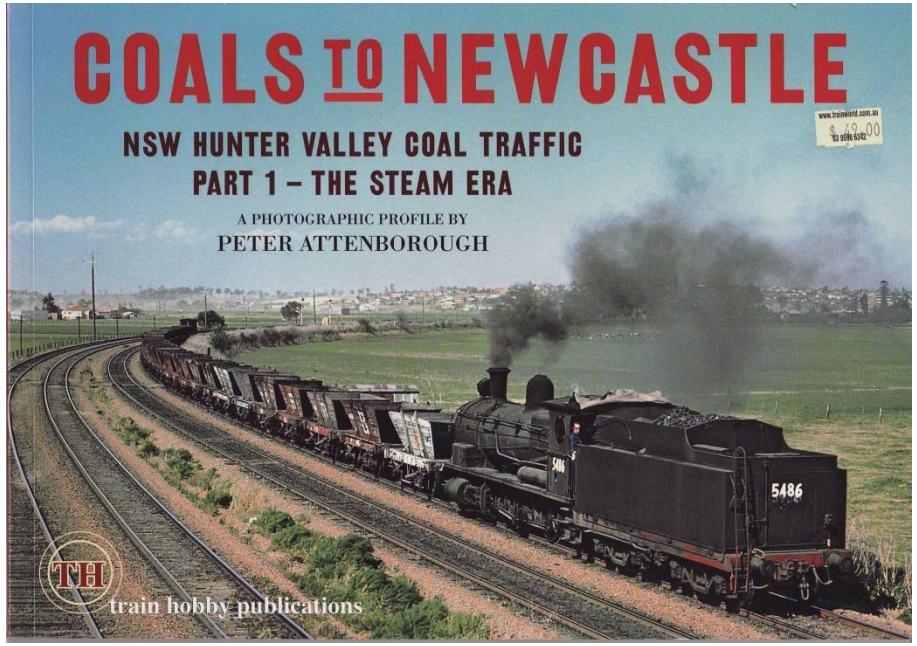


Cancers for Which Circulating Protein TMs Are Used

Cancer	Circ Tumor Marker
Breast	MUC1 (CA15-3, 27.29)
Gastrointestinal and Pancreas	CEA, CA19-9
Ovarian	CA125
Prostate	PSA

Circulating Tumor Markers to Detect Occult Recurrence Solid Tumors

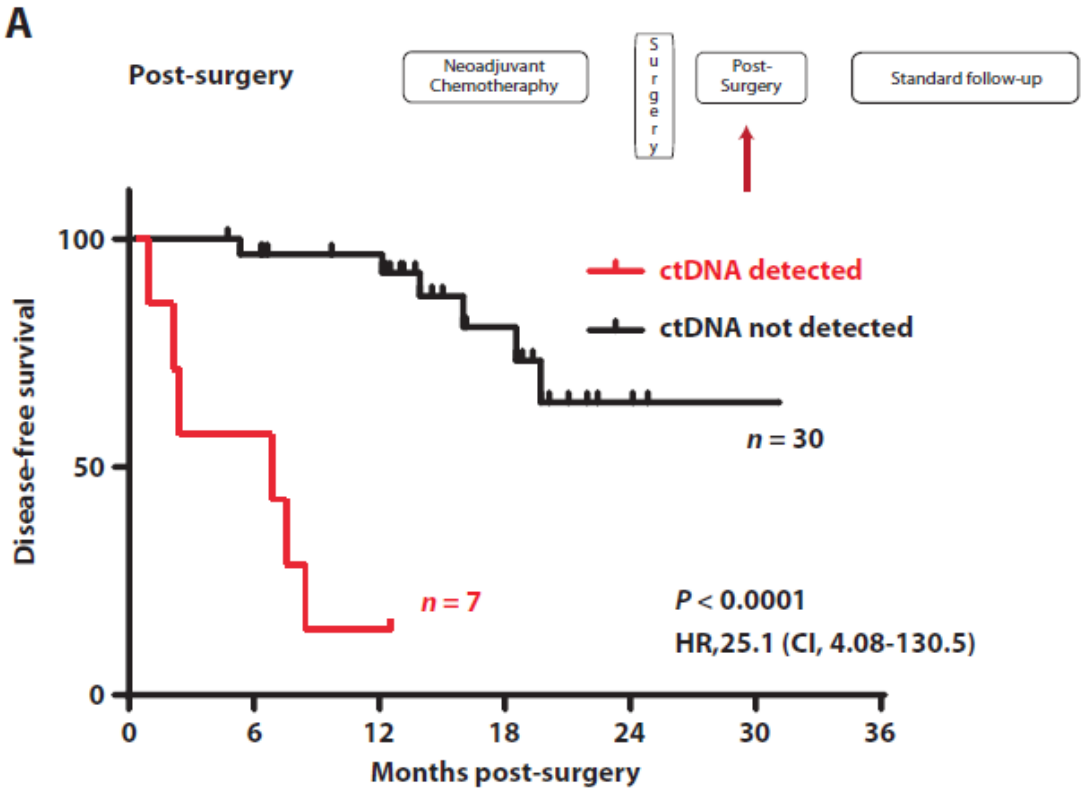
- **Breast** — **No Evidence Clinical Utility**
- **GI** — **CEA to detect and remove isolated hepatic met**
- **Ovarian** — **PRCT shows no Clinical Utility, often done anyway**
- **Prostate** — **Little or no data to determine, often done anyway**

How About Other Liquid Biopsy Assays for this Use?

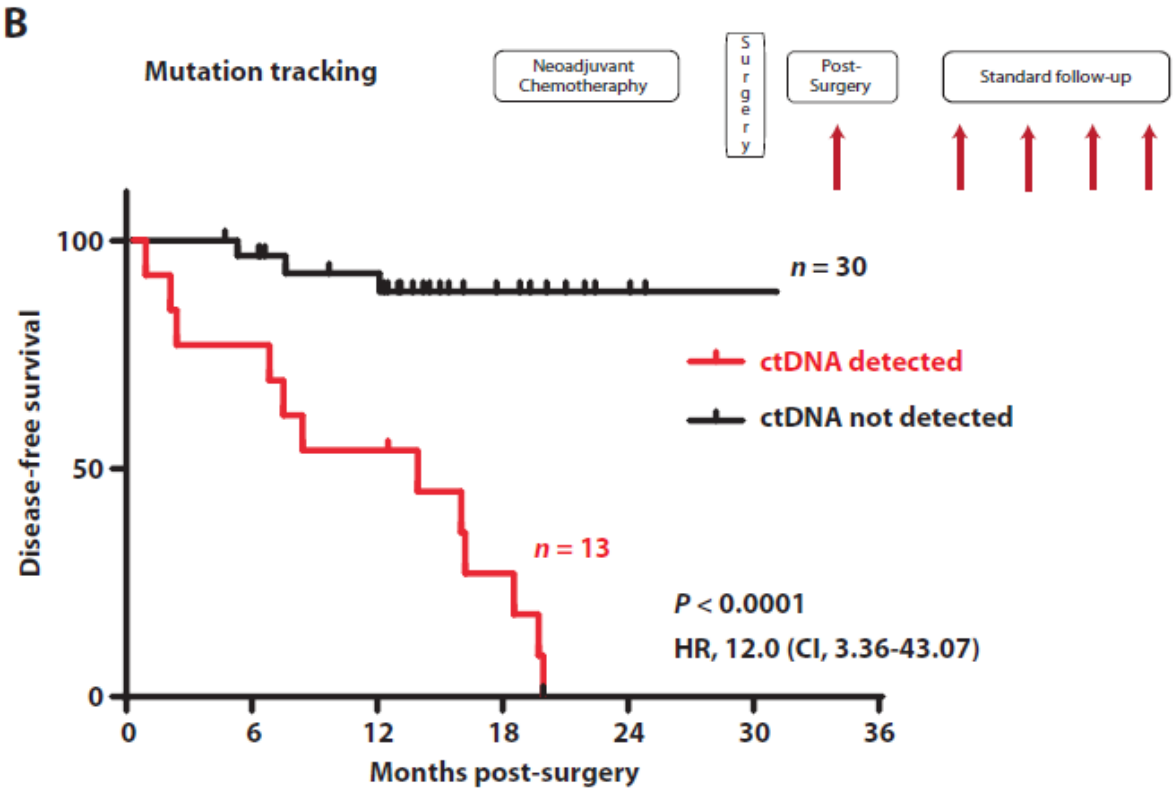
- **None with proven clinical utility**
- **Interesting preliminary data in Breast, Colon, Lung and other Cancers for CTC and ctDNA**

ctDNA is Prognostic in Patients Who Are NED

Post Surgery; one time point

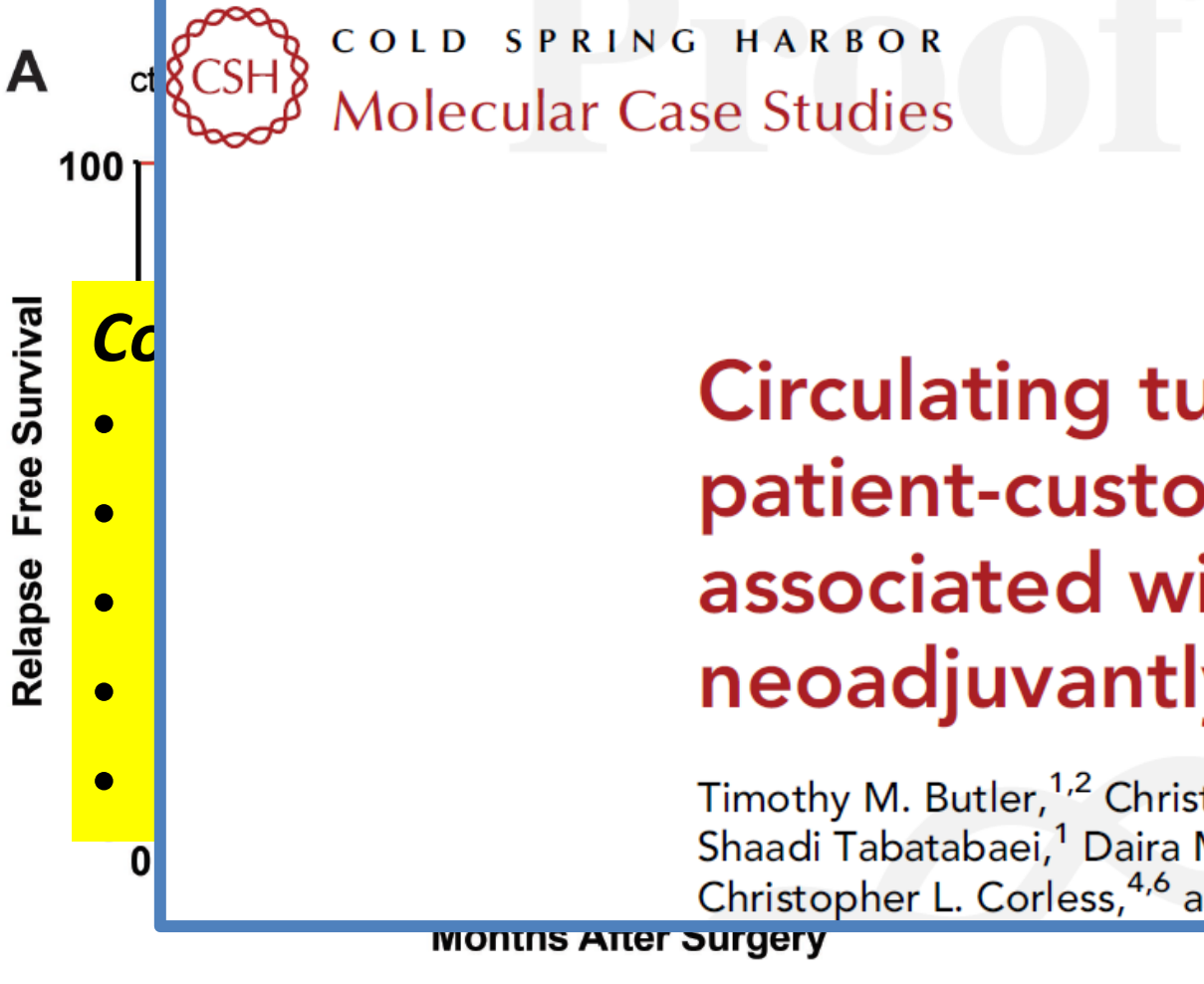


Post Surgery; serial time points



Garcia-Murillas I. Sci Transl Med. 2015;7(302):302

Personalized ctDNA to Detect Occult Recurrences



COLD SPRING HARBOR
Molecular Case Studies
RESEARCH REPORT

Circulating tumor DNA dynamics using patient-customized assays are associated with outcome in neoadjuvantly treated breast cancer

Timothy M. Butler,[1,2] Christopher T. Boniface,[1] Katie Johnson-Camacho,[1] Shaadi Tabatabaei,[1] Daira Melendez,[1] Taylor Kelley,[1] Joe Gray,[3,4,5] Christopher L. Corless,[4,6] and Paul T. Spellman[1,4,5]

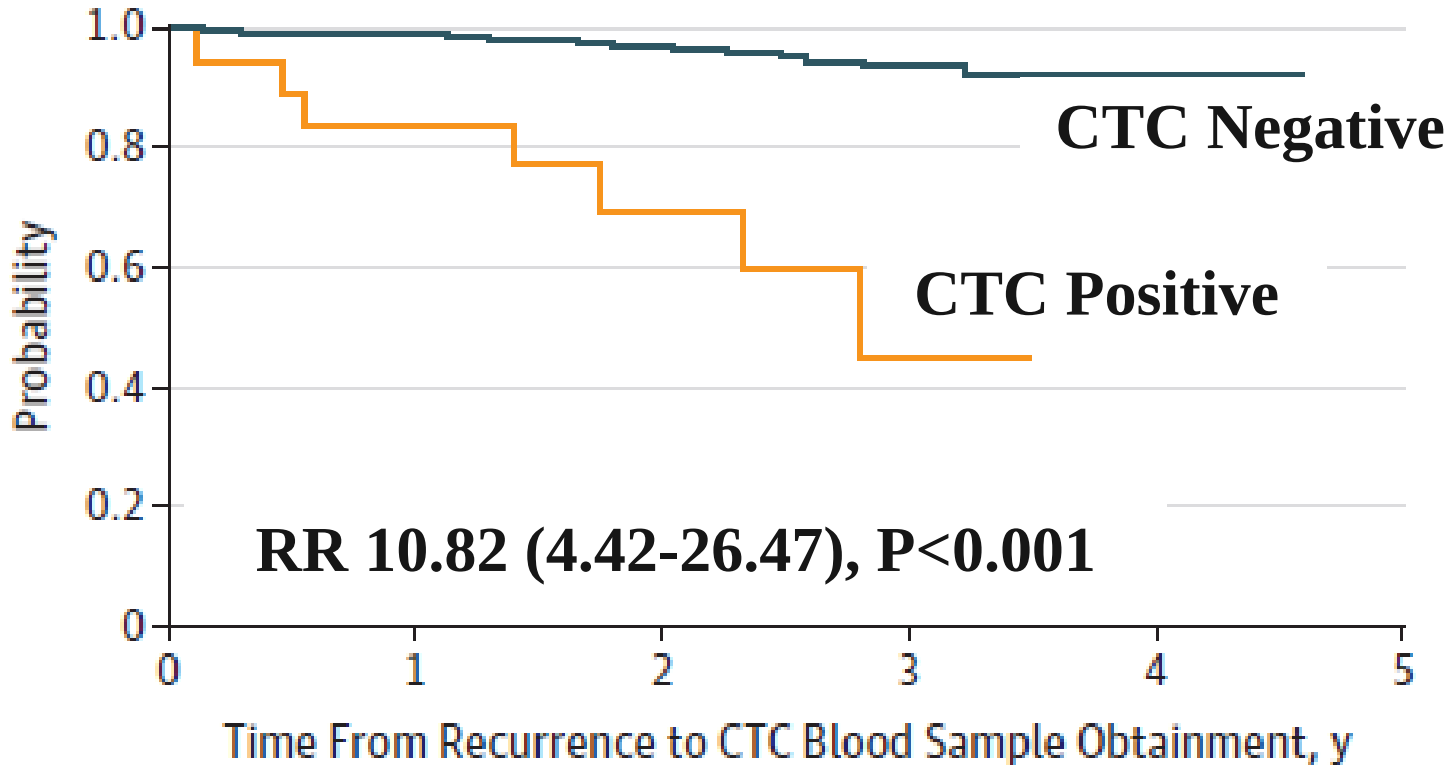
Months After Surgery

Coombes, RC, et al., Clin Cancer Res 2019

ER Positive, Early Br CA, Free of Detectable Disease

~ 5 Years After Diagnosis

Risk of Recurrence According to CTC at ~ 5 Years



No. at risk						
CTC negative	335	306	211	102	16	0
CTC positive	18	13	7	3	0	0

Sparano, et al., JAMA Oncol 2018

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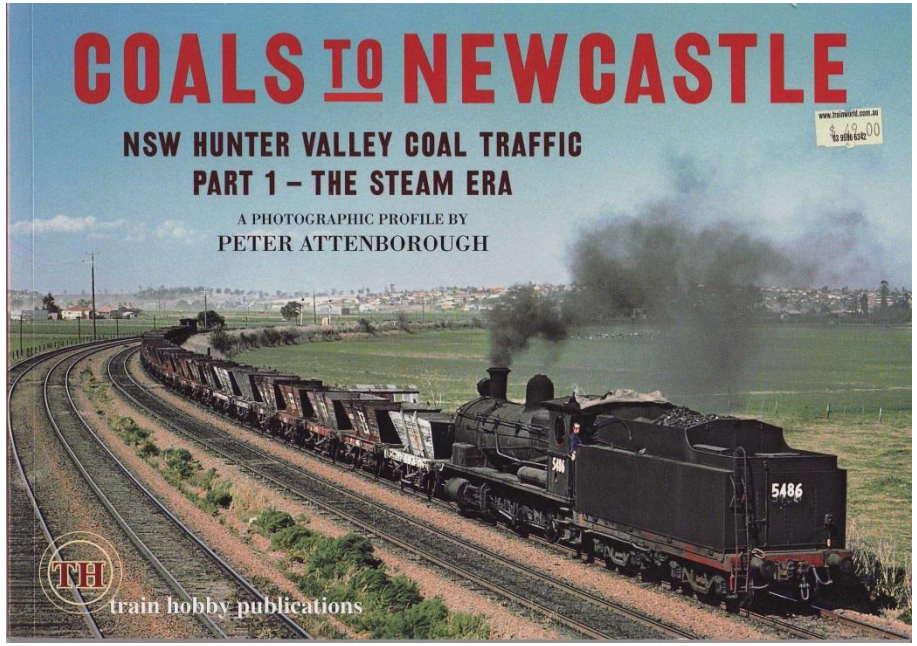


Circulating Tumor Markers to Monitor Metastatic Disease Solid Tumors

- **Breast** **No High Level Evidence Clinical Utility, done anyway**
- **GI** **Same**
- **Ovarian** **Same**
- **Prostate** **Same**

Circulating Plasma Cell Free Tumor DNA in Breast Cancer

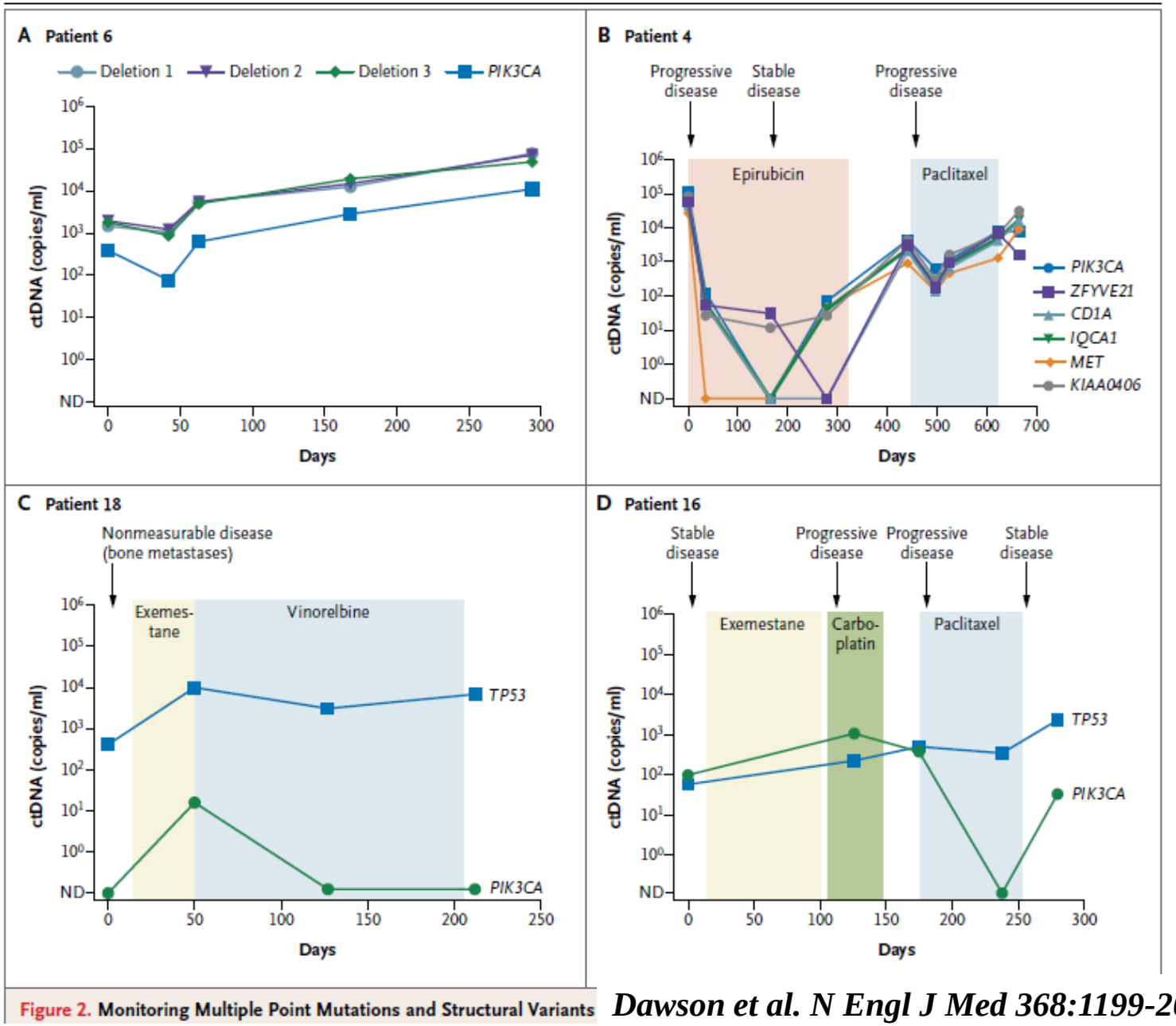


Figure 2. Monitoring Multiple Point Mutations and Structural Variants

Dawson et al. N Engl J Med 368:1199-209, 2013

Monitorina

USUALLY HAVE TO WAIT 3-4 CYCLES (9-12 WEEKS) TO DETERMINE IF PATIENT….

HAS RESPONSIVE/STABLE DISEASE = “CLINICAL BENEFIT”

Continue Current Regimen

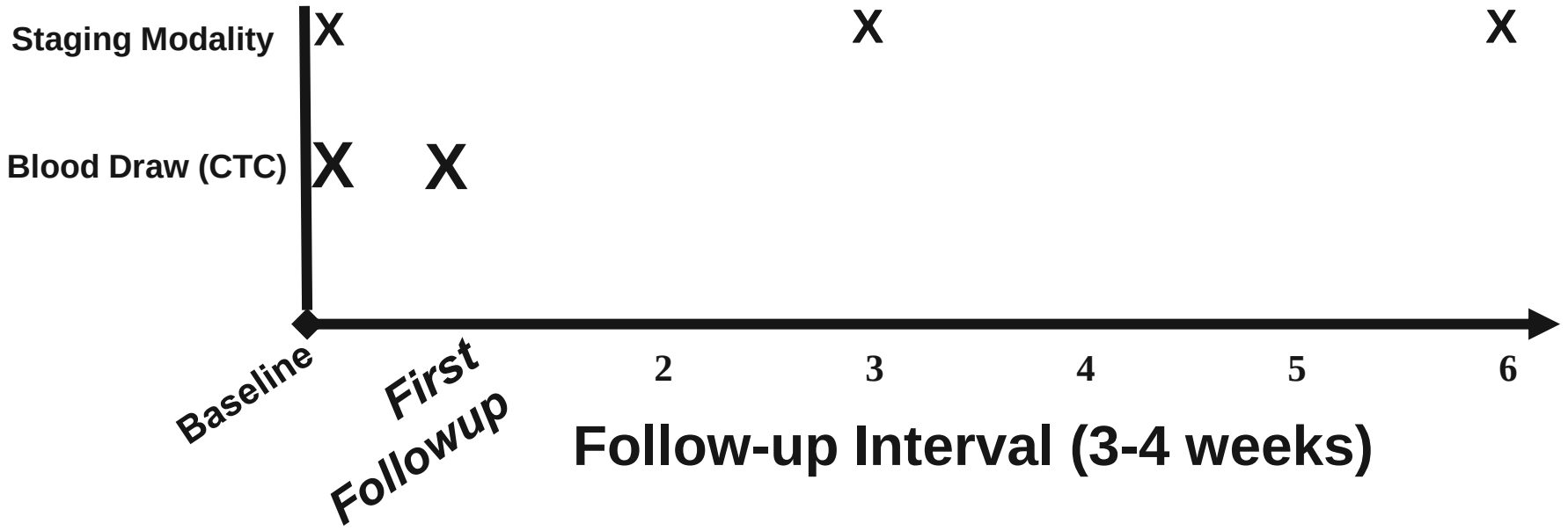
OR

IS PROGRESSING

Change Therapy

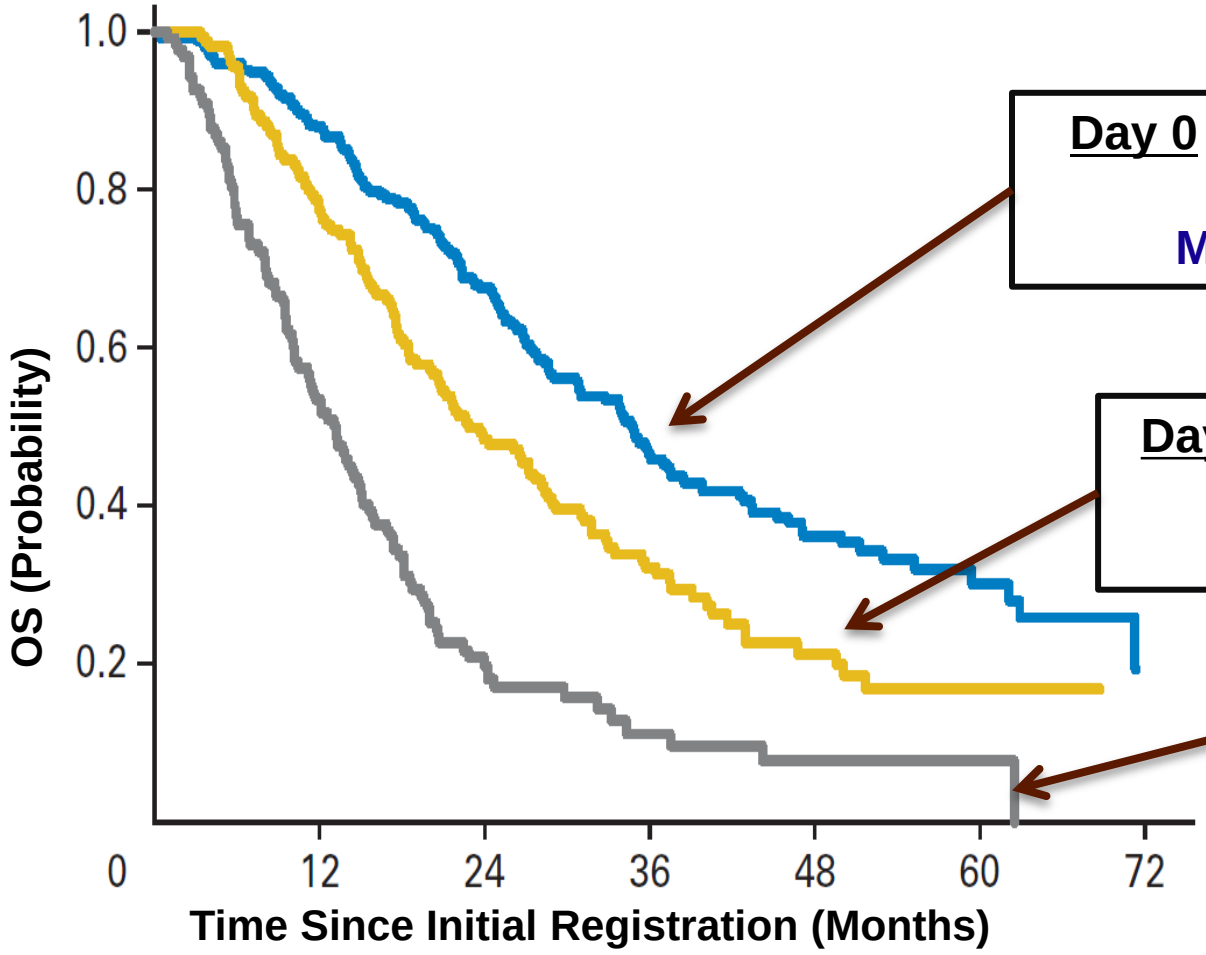
•Radiographs

CTC at 1st Follow-up Predict OS (SWOG S0500)



- **Sample collected at first follow-up visit**
  - **usually 3-4 weeks**

SWOG 0500:
Lack of a CTC "Response" at 1st Followup in Met Br Ca Receiving 1st Line CTX



MBC starting 1st line chemotherapy
N = 624

Day 0 CTC < 5/7.5 ml WB
ARM A
MED OS = 36m

Day 0 CTC ≥ 5 ml WB
N = 319

CHEMO

Day 21 CTC < 5 ml WB
ARM B
MED OS = 24m

Day 21 CTC ≥ 5 ml WB
ARM C
MED OS = 13m

ARM C
50% Mortality at 13 months
75% Mortality at 18 months
= ChemoResistance

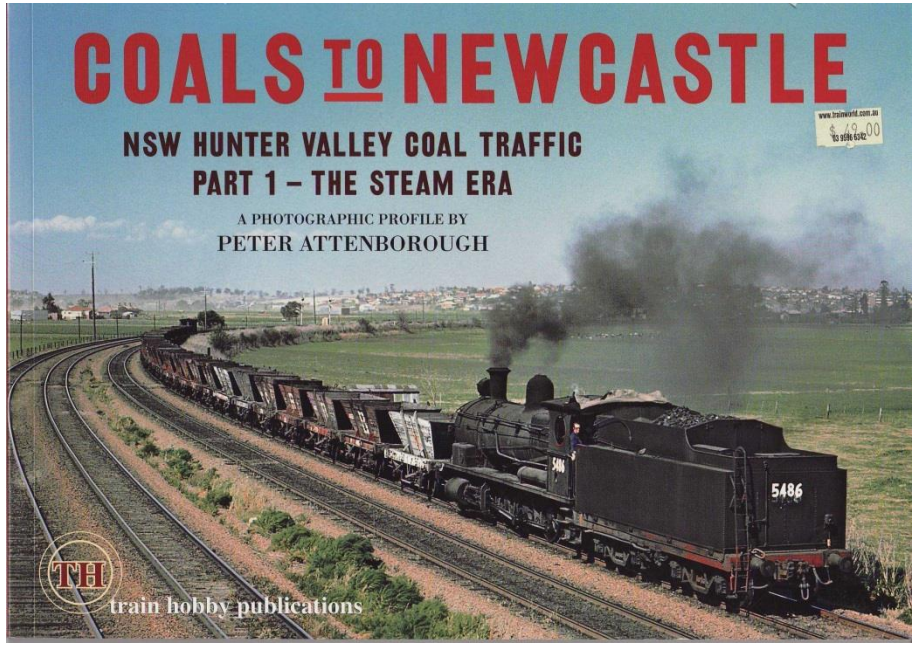
Smerage, JB, et al., J Clin Oncol 32:3483-9, 2014

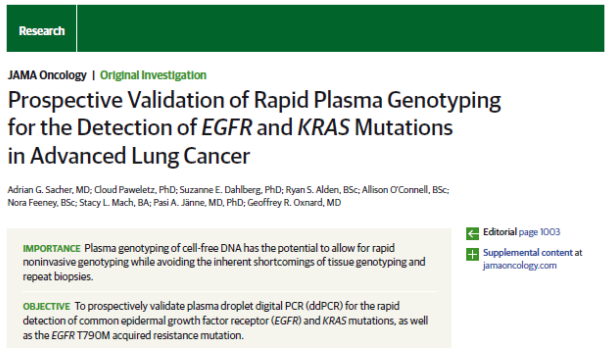
S0500: Conclusions

- **Lack of a CTC "Response" after 1 cycle of first line chemotherapy = Very high likelihood of complete chemotherapy resistance.**
- **Giving these patients more chemotherapy (even if different) is unlikely to be of any value!**
- **We need serial real-time evaluation of tumor molecular status**
  - **Liquid biopsy**

Why and When to Use Tumor Biomarkers?

- Risk assessment
- Screening
- Differential diagnosis
- Selection of Therapy
  - Prognosis
  - Prediction
- Monitoring disease state
  - For recurrence if patient is apparently free of disease
  - If patient has documented metastases
    - Evidence of progression
    - Selection of therapy





Research

JAMA Oncology | Original Investigation

Prospective Validation of Rapid Plasma Genotyping for the Detection of *EGFR* and *KRAS* Mutations in Advanced Lung Cancer

Adrian G. Sacher, MD; Cloud Paweletz, PhD; Suzanne E. Dahlberg, PhD; Ryan S. Alden, BSc; Allison O'Connell, BSc; Nora Feeney, BSc; Stacy L. Mach, BA; Pasi A. Jänne, MD, PhD; Geoffrey R. Oxnard, MD

Editorial page 1003

Supplemental content at jamaoncology.com

IMPORTANCE Plasma genotyping of cell-free DNA has the potential to allow for rapid noninvasive genotyping while avoiding the inherent shortcomings of tissue genotyping and repeat biopsies.

OBJECTIVE To prospectively validate plasma droplet digital PCR (ddPCR) for the rapid detection of common epidermal growth factor receptor (*EGFR*) and *KRAS* mutations, as well as the *EGFR* T790M acquired resistance mutation.

Circulating EGFR mutation assay (Cobas) Approved by U.S. FDA (*2016, 2018)*

EGFR mutations for patients with metastatic lung cancer-Selection of anti-EGFR Therapy

- *If **POS**:*

Drug	Mutations
Erlotinib	Exon 19 deletion, L858R
Osimertinib	Exon 19 deletion, T790M
Gefitinib	Exon 19 deletion, L858R

- *If **NEG**: reflex to tissue testing*

Sacher, AG, et al., JAMA Oncol 2:1014-22, 2016
Allegra, CJ, et al., J Clin Oncol 34:179-85, 2016

CTC - AR-V7 Fusion Predicts Resistance to anti-Androgen but Not Taxane Therapy in Prostate CA

Using EPIC CTC Assay

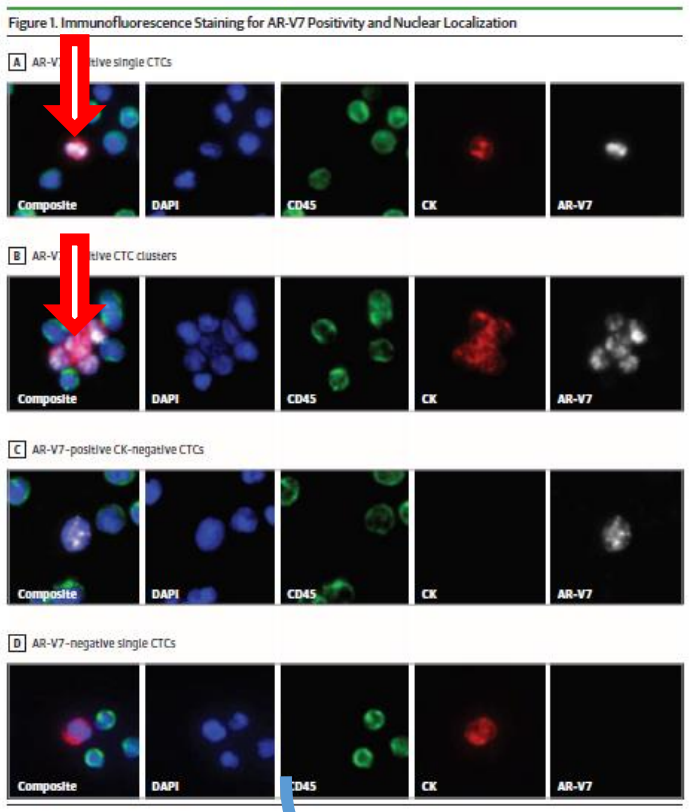
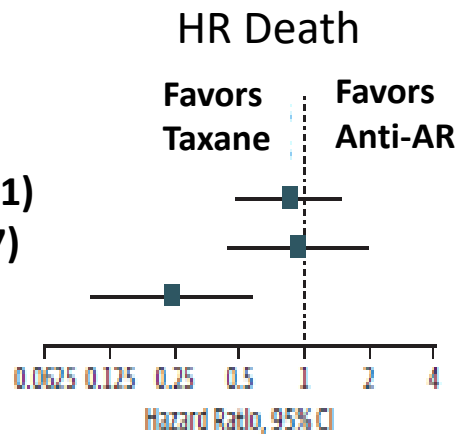


Figure 4. Patients With Pretherapy AR-V7-Positive CTCs and Overall Survival on Taxanes and/or AR Signaling Inhibitors.



Source:
All Samples (n=191)
AR-V7 Neg (n=157)
AR-V7 Pos (n=34)

Scher, HI, et al., JAMA Oncol 2:1441-1449, 2016

Compare ADNA/Johns Hopkins vs. Epic CTC Assay

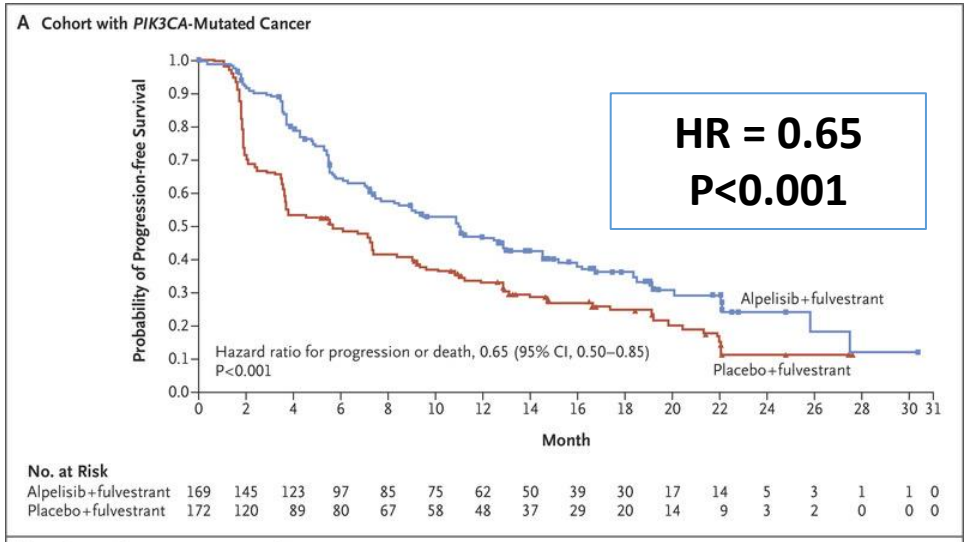
Outcome	JHU AR-V7 (n = 116)*	Epic AR-V7 (n = 107)†
PFS		
Median PFS, months		
Positive	3.1	3.1
Negative	6.9	6.1
P‡	.032	.020
HR (95% CI)	2.4 (1.5 to 3.7)	2.5 (1.3 to 4.7)
HR‡ (95% CI)	1.9 (1.1 to 3.3)	2.4 (1.1 to 5.1)
OS		
Median OS, months		
Positive	10.8	8.4
Negative	27.2	25.5
HR (95% CI)	3.9 (2.2 to 6.9)	3.4 (1.6 to 7.0)
HR‡ (95% CI)	4.2 (2.1 to 8.5)	3.5 (1.6 to 8.1)
≥ 50% confirmed PSA decline, %		
Positive	11	0
Negative	28	26

Armstrong, AJ, et al., J Clin Oncol JCO1801731, 2019

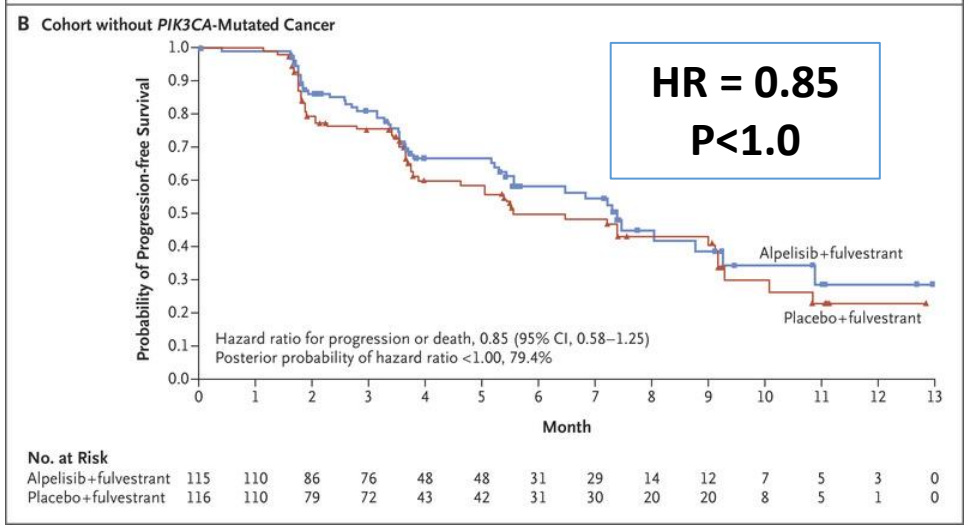
Alpelisib (plus fulvestrant) Is Active in Mutated PIK3CA but NOT Wild Type ER POS Metastastic Breast Cancer

PFS

PIK3CA Mutated



PIK3CA Wild Type



Alpelisib
Placebo

The FDA concurrently approved the companion diagnostic test, Therascreen PIK3CA RGQ PCR Kit, to select patients who have *PIK3CA* mutations in tumor tissue specimens **and/or in circulating tumor DNA (ctDNA) isolated from plasma specimens.**

If the test is negative for *PIK3CA* mutations in plasma, patients should undergo testing for *PIK3CA* mutations in tumor tissue.

Andre, F, et al., N Engl J Med 380:1929-1940, 2019

nature genetics

ESR1 ligand-binding domain mutations in hormone-

Cell PRESS Open ACCESS

Cell Reports
Article

Endocrine-Therapy-Resistant *ESR1* Variants Revealed by Genomic Characterization

Clinical Cancer Research

AACR

Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor positive breast cancer

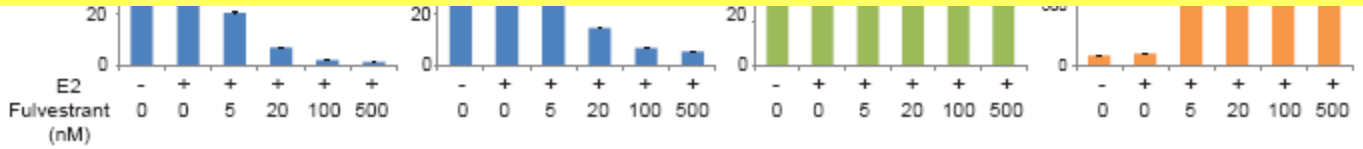
Rinath Jeselsohn, Roman Yelensky, Gilles Buchwalter, et al.

Clin Cancer Res Published OnlineFirst January 7, 2014.

ESR1 Mutants Maintain Sensitivity to Fulvestrant

Luciferase Activity in HEK-293T human embryonic kidney cells transfected with ESR (WT or Mutant)

- **ESR1 mutations in ER Positive MET Breast Cancer**
- **Rare or never seen in primary cancer**
- **~ 20% in metastatic cancer**
- **In theory, Predicts for**
  - **Resistance to E2 depletion**
  - **Not for resistance to SERM or SERD**



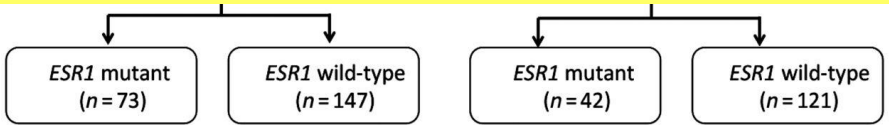
Robinson, et al., Nat Genet 2013

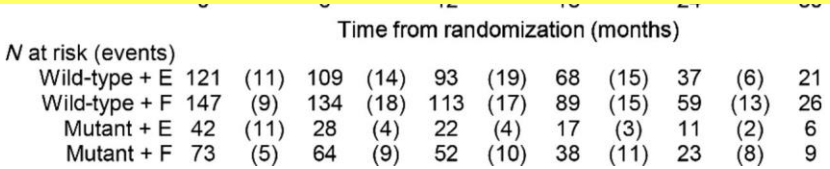
Tissue ESR1m and Relative Benefit SERD (Fulvestrant) vs. AI (Exemestane)

Published OnlineFirst June 16, 2020; DOI: 10.1158/1078-0432.CCR-20-0224

- **ESR1 mutations in ER Positive MET Breast Cancer**
- **Rare or never seen in primary cancer**
- **~ 20% in metastatic cancer**
- **In theory, Predicts for**
  - **Resistance to E2 depletion**
  - **Not for resistance to SERM or SERD**

- **Intriguing data, but needs confirmation before ET is chosen based on ESR1 mutation**



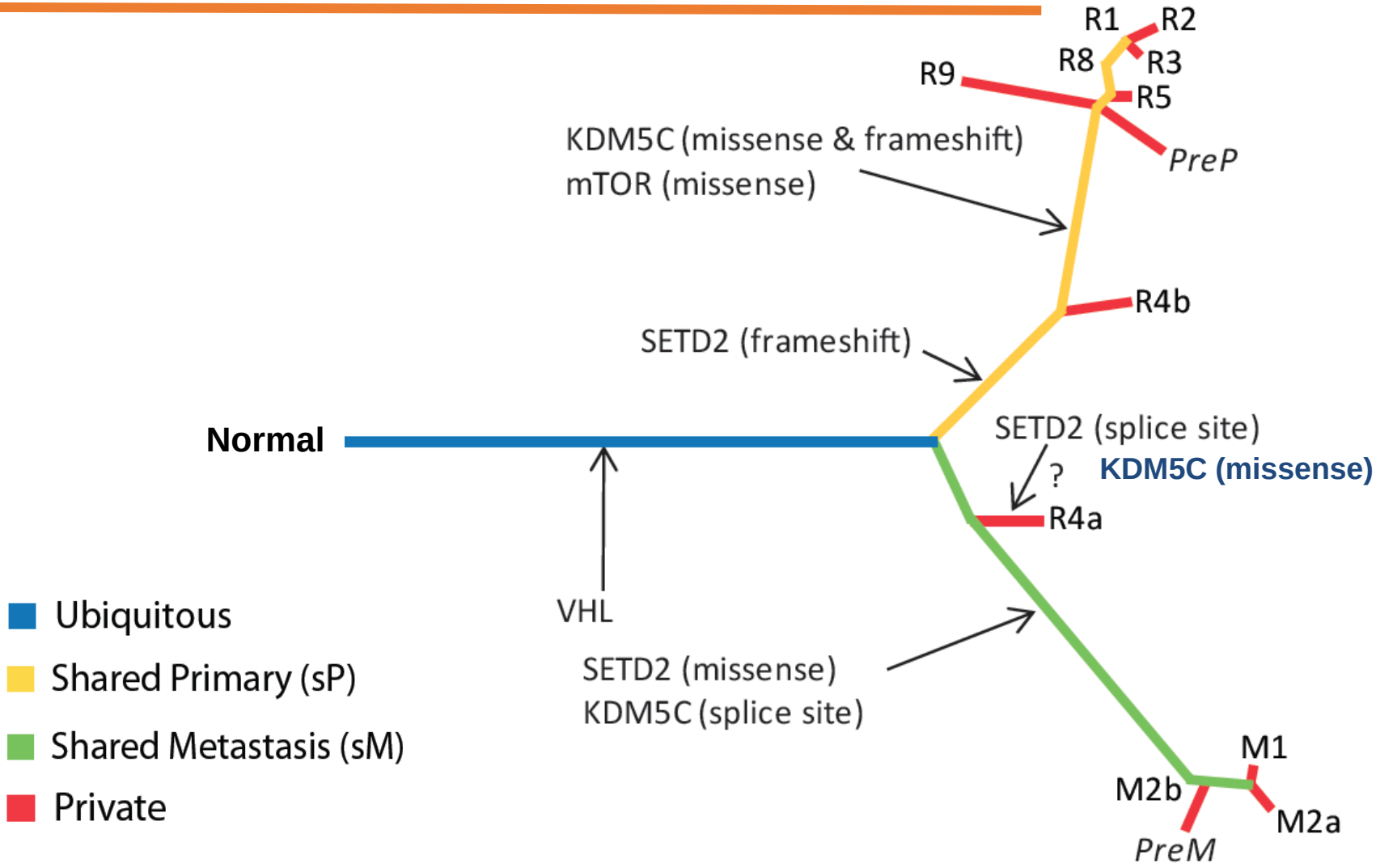


Potential Uses of Liquid Biopsies in Selection of Next Therapy

- Exploratory
  - Resistance mechanisms
  - New targets

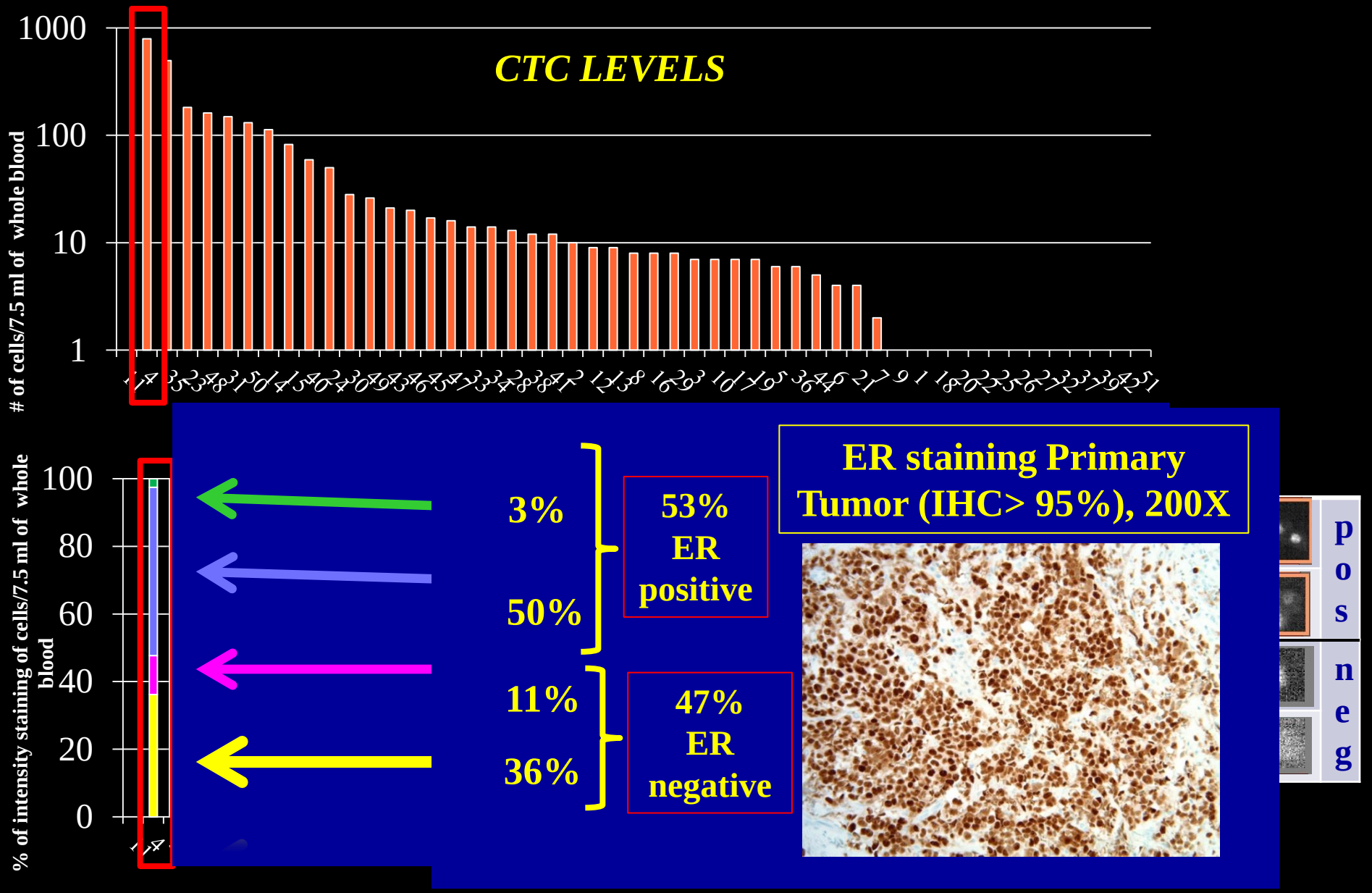
Heterogeneity

Tumour Phylogenetic Evolution (Renal Cell Cancer)



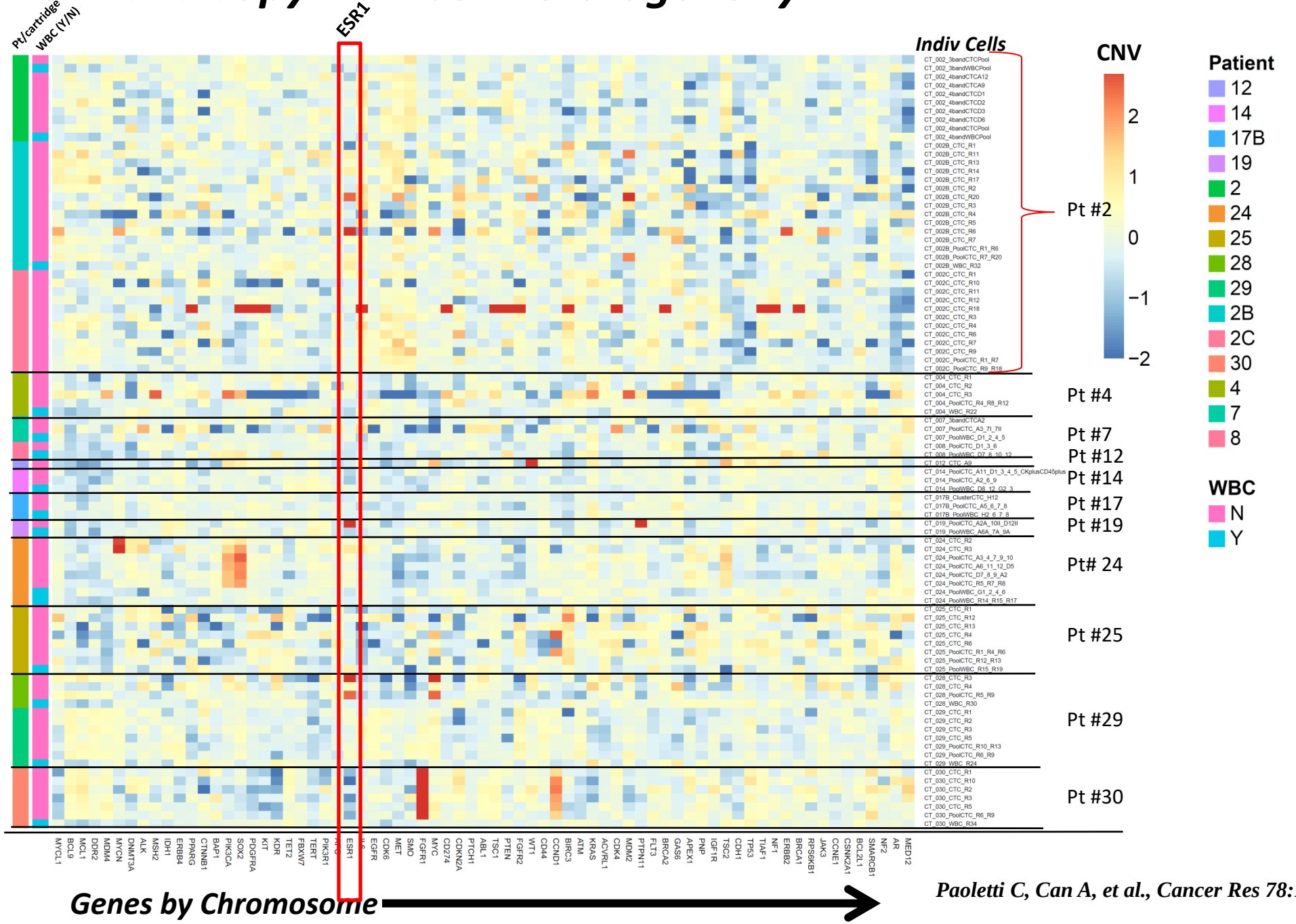
Gerlinger, M., et al.; N Engl J Med; 2012

CTC-ER ENUMERATION AND STAINING INTENSITY FOR EACH PT

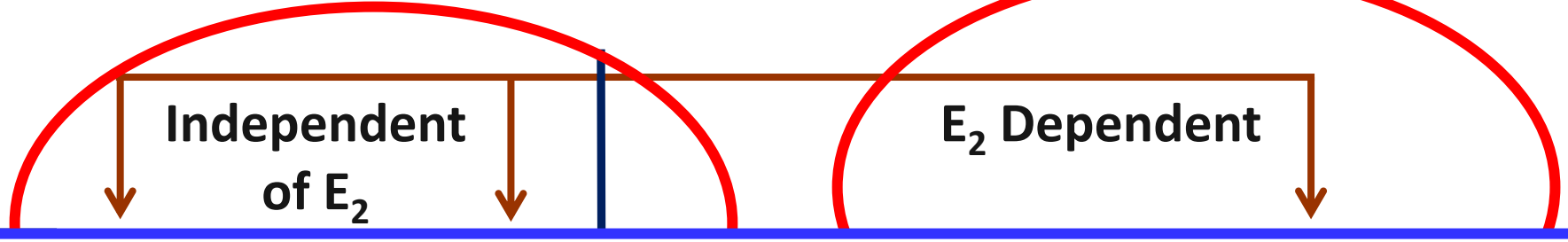


Paoletti, C, et al., Clin Cancer Res 21:2487-98, 2015

Intra-Patient CTC Copy Number Heterogeneity



Paoletti C, Can A, et al., Cancer Res 78:1110-1122, 2018



***Heterogeneity*:**

- **In a *Single Patient*, we can see MULTIPLE mechanisms of resistance to ET:**
  - **ER Negative CTC**
  - **ESR1 mutated with different mutations in different CTC**
  - **WT ESR1 CTC, but different genes mutated/CNVs**
- **Taken together these data suggest we need to return to concept of combination therapies**

Other?
Pharmogenetics/kinetics

Return to Combination Therapy in MBC?

- **Caveats:**
  - **Difficult trial design required to show true clinical benefit**
  - **Possible antagonism**
    - **Biologic**
    - **Pharmacokinetic**
  - **Additive TOXICITY, TOXICITY, TOXICITY!!!**
  - **Multiple drugs/multiple companies**
    - **Who gets credit if success?**
    - **Who gets blame if toxic**
    - **Good news: Several companies have multiple drugs now**
  - **$$$**

Are CTC the Buggy Whip of Liquid Biopsies?

I Do Not Think So

Tissue, CTC, & ctDNA May Be Complementary

	Tissue (NGS)	CTC	ptDNA
Mutations/Genetic Abnormalities	All (100s-1000s)	Candidate (10-100s)	Selected 1-10s
Phenotype	Yes	Yes	No
Total Body	No	Yes	Yes
Represents Tissue Biology	Yes	Unknown	Unknown
Represents Live Cells	Yes	Yes	Unknown (? Dead cells or secreted exosomes?)
Serial	Difficult	Yes	Yes

Incidence of Elevated CTC and ctDNA is complementary

ESR1$_{LBD}$*m* status at baseline	CTC at baseline		
	<5 CTC/7.5 mL WB	≥5 CTC/7.5 mL WB	Total
ESR1$_{LBD}$*m*+ ctDNA	8	4	12
ESR1$_{LBD}$*m*- ctDNA	24	7	31
Total	32	11	43[a]

Legend: CTC: circulating tumor cells; ctDNA: circulating tumor DNA; *ESR1*$_{LBD}$*m*+ : *ESR1* mutation detected; *ESR1*$_{LBD}$*m*- : *ESR1* mutation "not detected"; LBD: ligand-binding domain; WB: whole blood; [a]43/45 patients had both CTC and ctDNA at baseline (2 patients only had ctDNA, but not CTC assessed).

23 pts (53.5%) had either *ESR1*$_{LBD}$*m*+ or elevated CTC

Only 4 pts (9%) had both

8/32 (25%) pts with <5 CTC, had elevated *ESR1*$_{LBD}$*m*+ ctDNA

7/31 (23%) pts with *ESR1*$_{LBD}$*m* *not detected* ctDNA had elevated CTC

Paoletti C*, Schiavon G*. et al Clin Ca Research 18: 1569, 2018

Summary: Liquid Biopsies

- **Offer Potential Advantages Over Tissue Biopsy**
  - Convenience, Safety, ? Cost
  - Biological
- **Not just ctDNA**
  - Proteins, CTC, other Nucleic Acids
- **May be Complementary, Not Mutually Exclusive**
- **Challenges**
  - **Analytical** (Pre-analytical and Analytical)
  - **Demonstration (NOT Assumption) of Clinical Utility**

The Hayes Laboratory

Dafydd Thomas, MD, PhD

Costanza Paoletti, MD

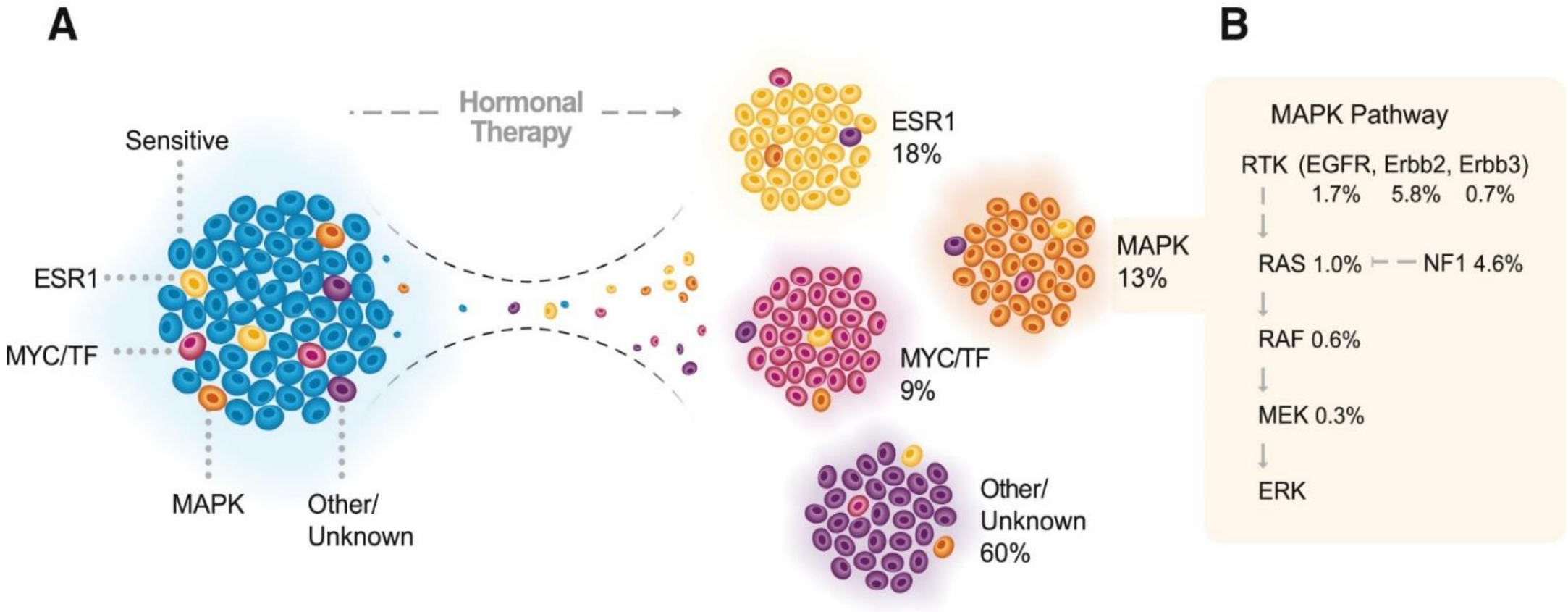
Emily Dolce, BS

Elizabeth Darga, MS

Marty Brown, BS

UM Dept Pathology
Scott Tomlins, MD, PhD
Andi Cani (now post-doc in my lab)

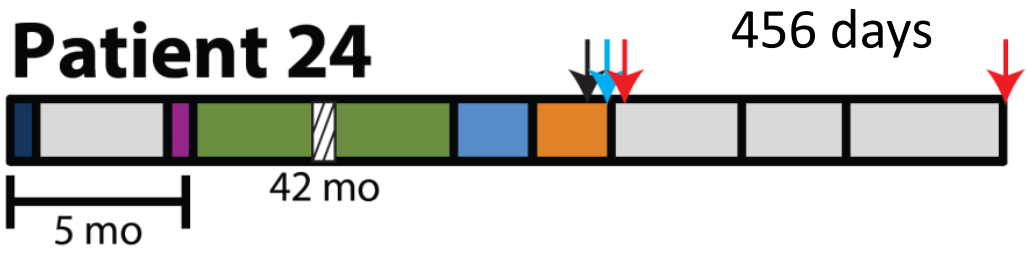
Several different mechanisms of resistance

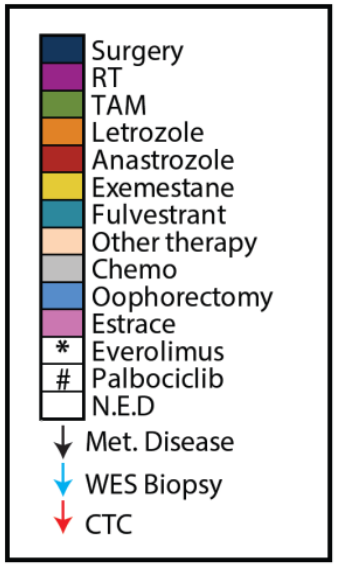


Each of these mechanisms may result in absolute endocrine independence or, if cancer remains endocrine dependent, resistance to specific therapies directed toward ER pathway.

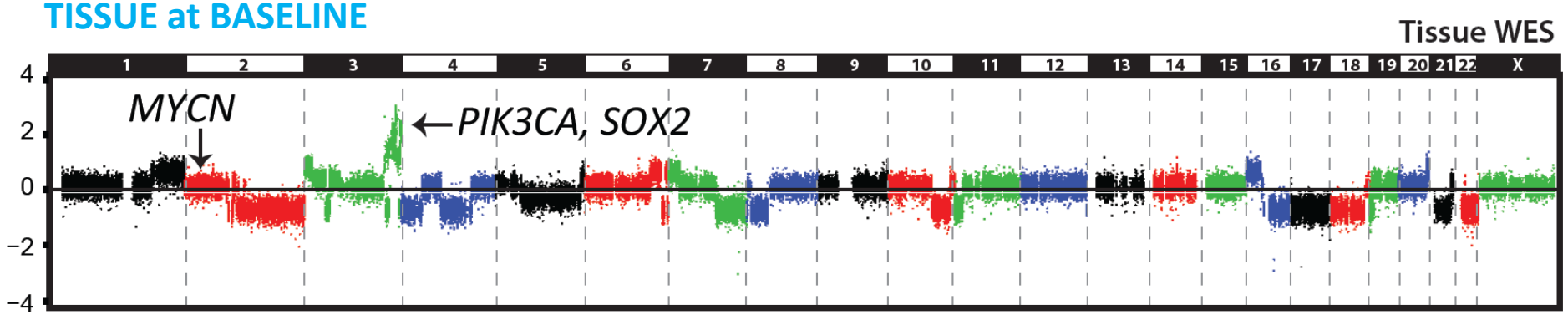
Razavi et al, 2018

Emergence of CTC genomic alterations over time



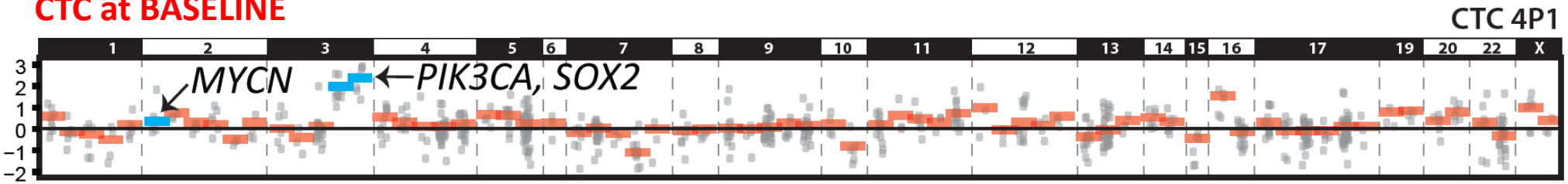


TISSUE at BASELINE



Mutations: *CDH1 p.I584fs, TP53 p.152_156fs*

CTC at BASELINE



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CTC at PROGRESSION

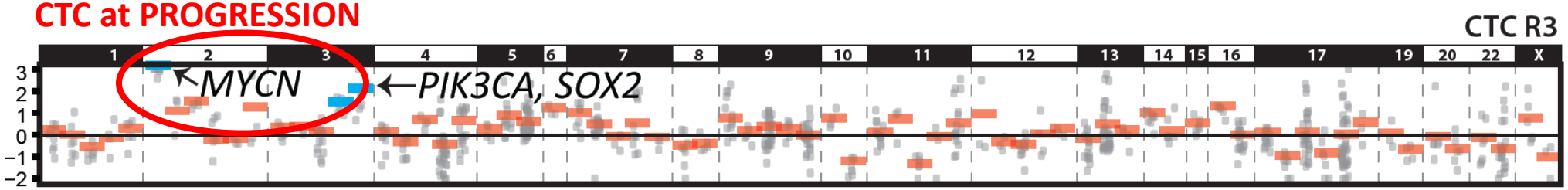

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Table showing CTC enumeration and $ESR1_{LBD}m$ status in 43 patients who had both CTC and ctDNA assessed at baseline

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Paoletti C*, Schiavon G*. et al CCR DOI: 10.1158/1078-0432.CCR-18-1569

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