Biology and Background for ctDNA; Advantages and Drawbacks of Assays Currently in Use; Knowledge Gaps

Luis Diaz, M.D.

Workshop on Circulating Tumor DNA assays in Clinical Cancer Research

Cancer Diagnosis Program, NCI

September 29, 2016

SWIM ACROSS AMERICA
MAKING WAVES TO FIGHT CANCER

JOHNS HOPKINS MEDICINE
THE SIDNEY KIMMEL COMPREHENSIVE CANCER CENTER

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Disclosure Information

I have the following financial relationships to disclose:

Founder and shareholder in Pagerbox, Papgene and Personal Genome Diagnostics, Inc.

Consultant for Merck, Illumina, PGDx and Cell Design Labs

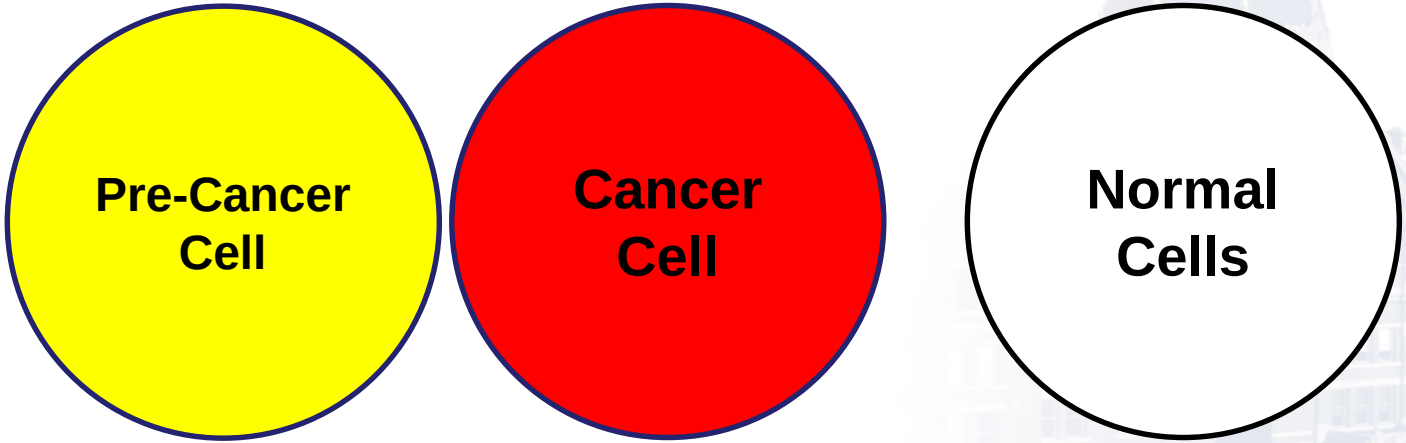
PapGene and Personal Genome Diagnostics (PGDx) s, as well as other companies, have licensed technologies from Johns Hopkins University, on which LD is an inventor. These licenses and relationships are associated with equity or royalty payments. The terms of these arrangements are being managed by Johns Hopkins University in accordance with its conflict of interest policies.

Clinical Application of Cancer Genetics

Mutations as Biomarkers

Clinical Application of Cancer Genetics

Mutations are highly specific



Mutations

No Mutations

Access to Somatic Mutations

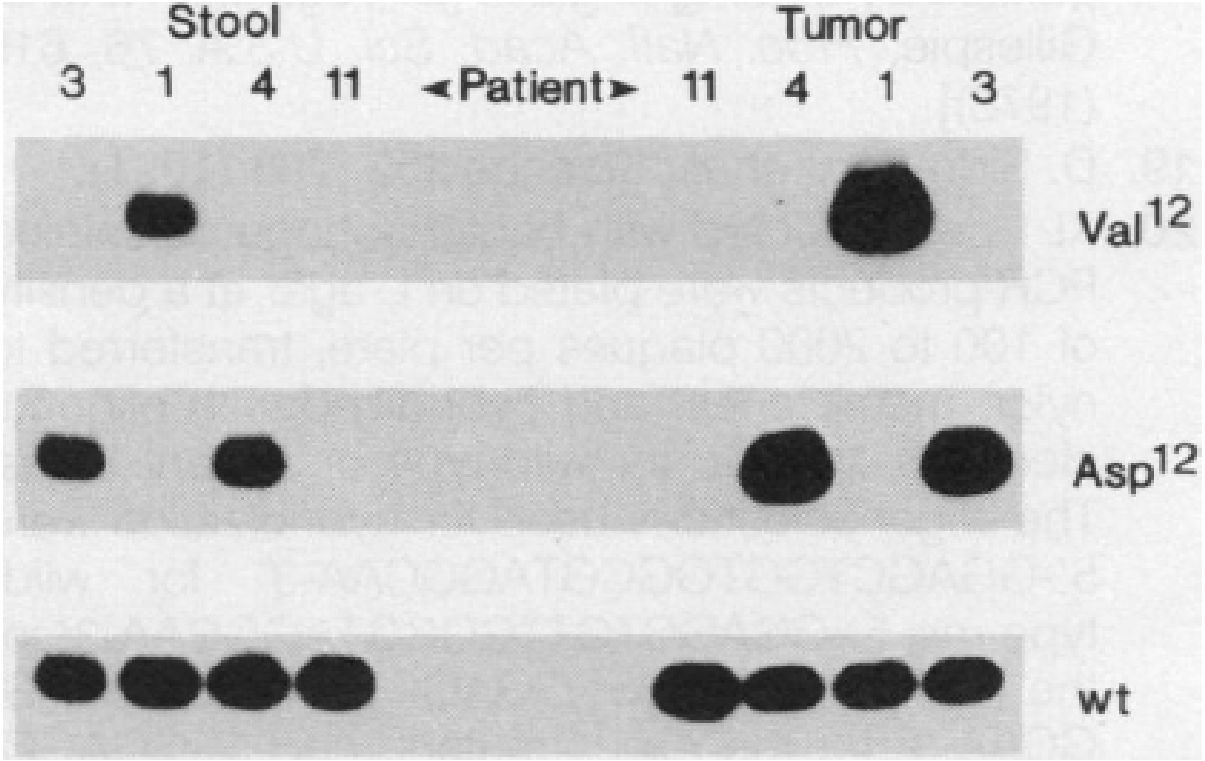
Tumor Tissue

- FFPE
- Frozen tissue

Blood & other bodily fluids

- Cell-free DNA
- Circulating tumor cells (CTCs)

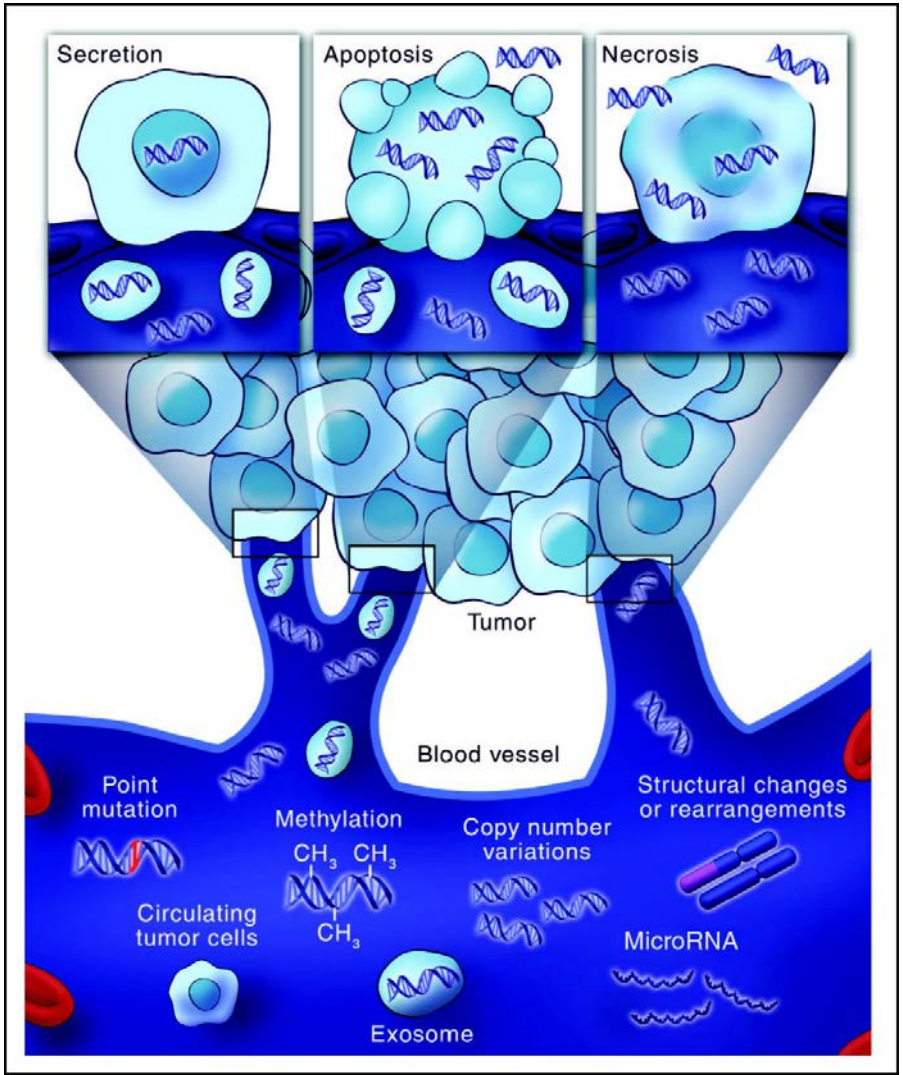
Mutant *RAS* in stool from patients with CRC



Sidransky et. als., Science. VOL 256 3 APRL 1992

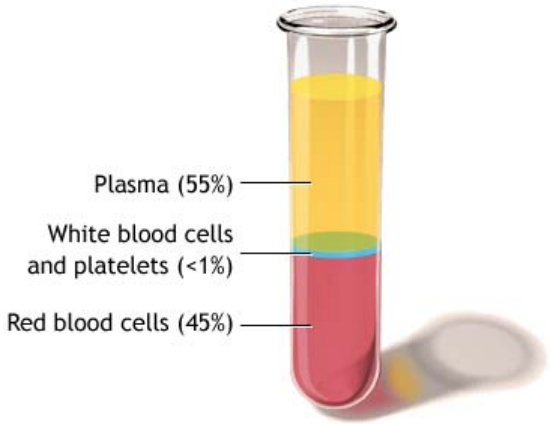
Clinical Application of Cancer Genetics

Liquid Biopsies



- DNA fragments of 120-200bp with half life of ~2 hours
- 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”
- Specific to tumor

Liquid Biopsy



Plasma

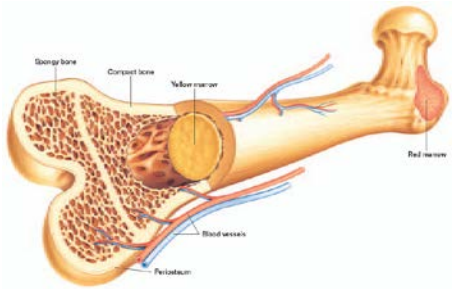
Water 91%
Proteins 7%
Metabolites (trace)
Cell-free DNA (trace)

Cellular Components

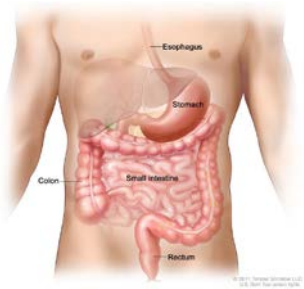
White Blood Cells 2-3%
Platelets 2-3%
Red Blood Cells 90%
Circulating tumor cells (trace)

Source circulating cell-free DNA

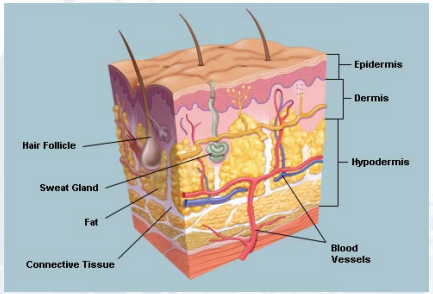
Bone Marrow



GI Tract

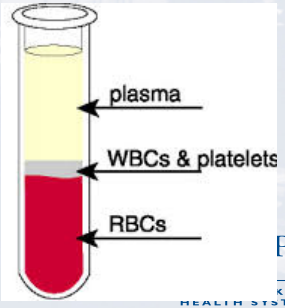


Skin



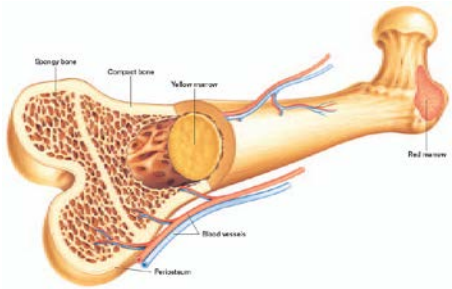
Fetal DNA

Pool of
Cell-free DNA

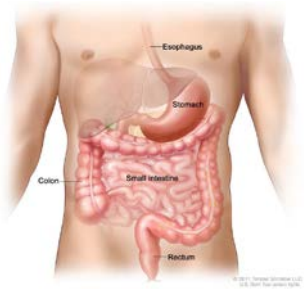


Source circulating cell-free DNA

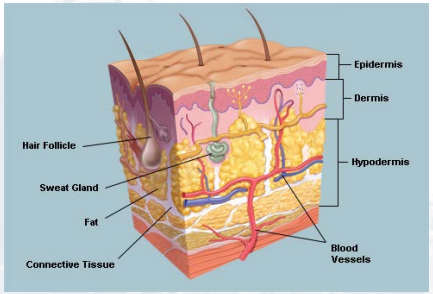
Bone Marrow



GI Tract

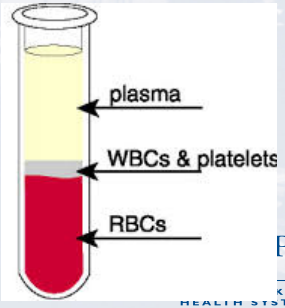


Skin



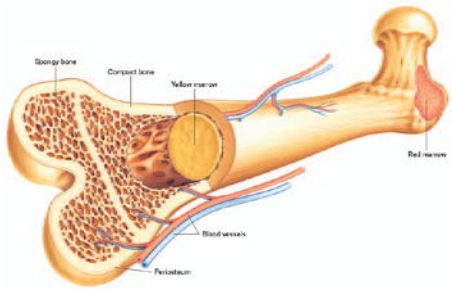
Transplanted Tissue DNA

Pool of
Cell-free DNA



Source circulating cell-free DNA

Bone Marrow



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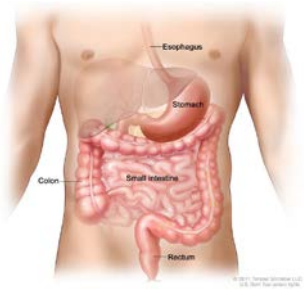
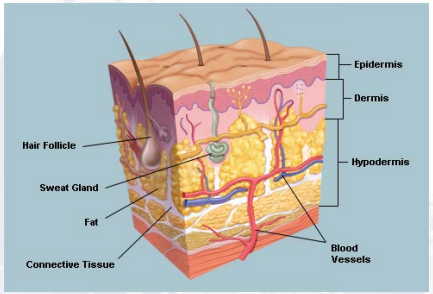
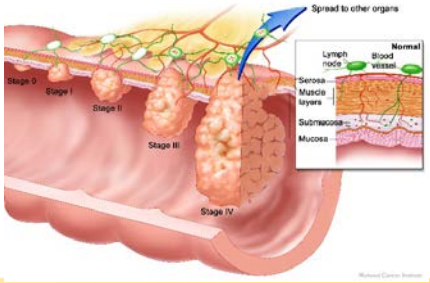


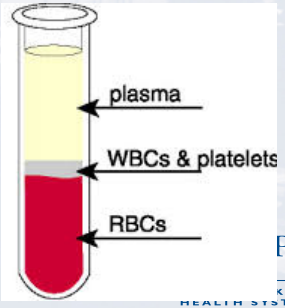
Skin



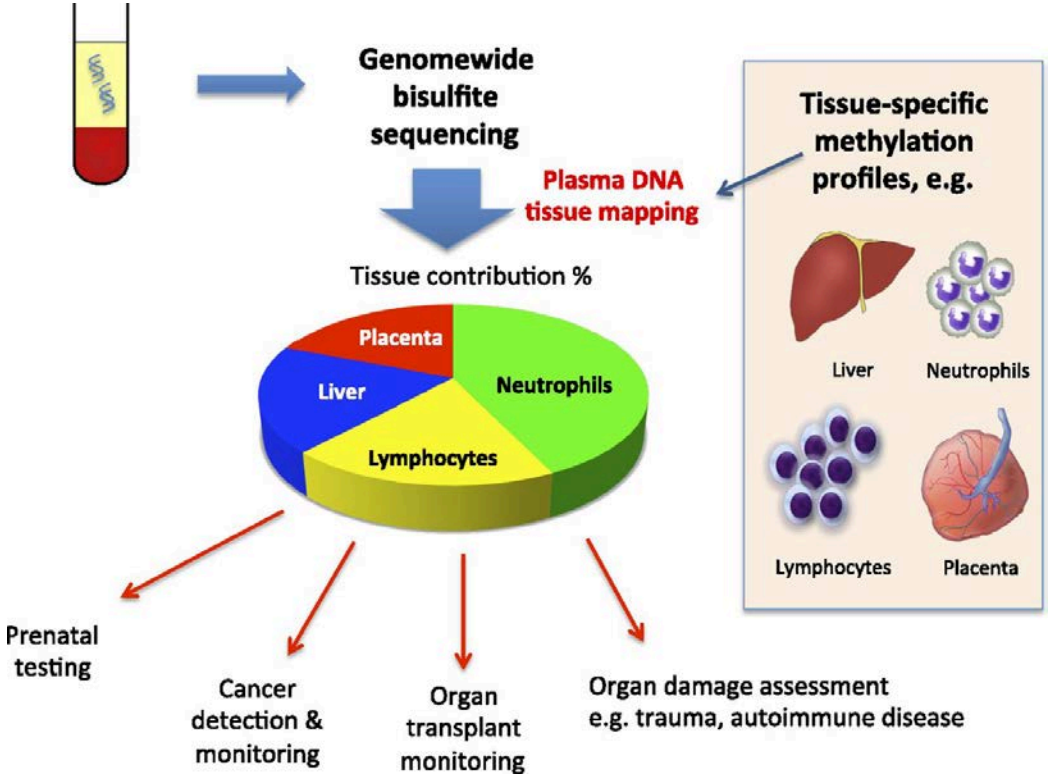
Tumor DNA



Pool of
Cell-free DNA

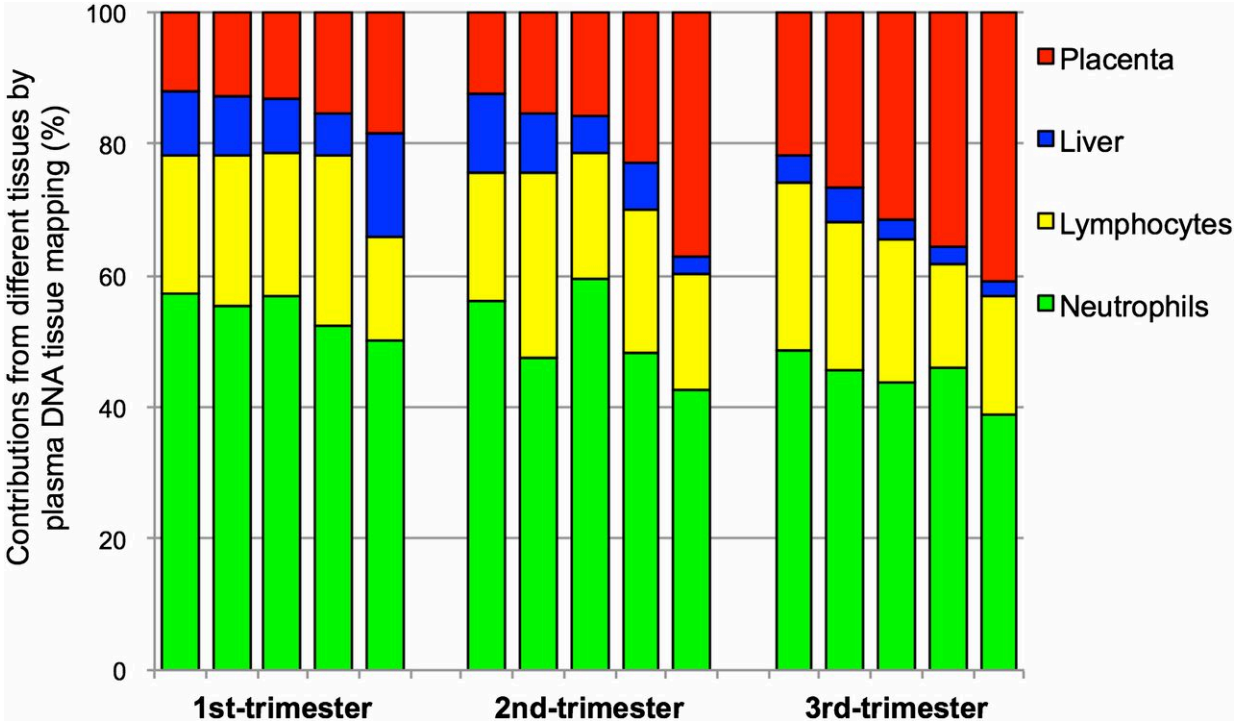


Schematic illustration of the principle of plasma DNA tissue mapping by genome-wide methylation sequencing and its applications.



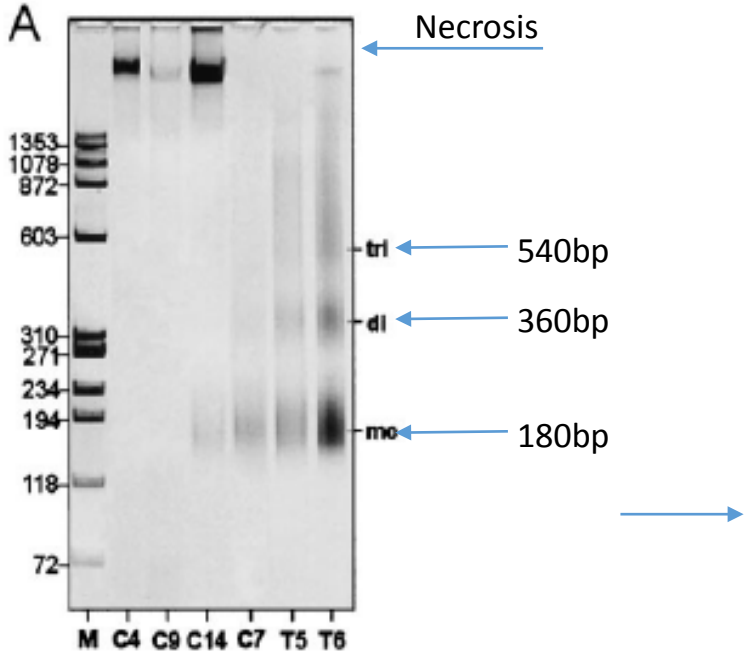
Kun Sun et al. PNAS 2015;112:E5503-E5512

Percentage contributions of different tissues to plasma DNA for 15 pregnant women.

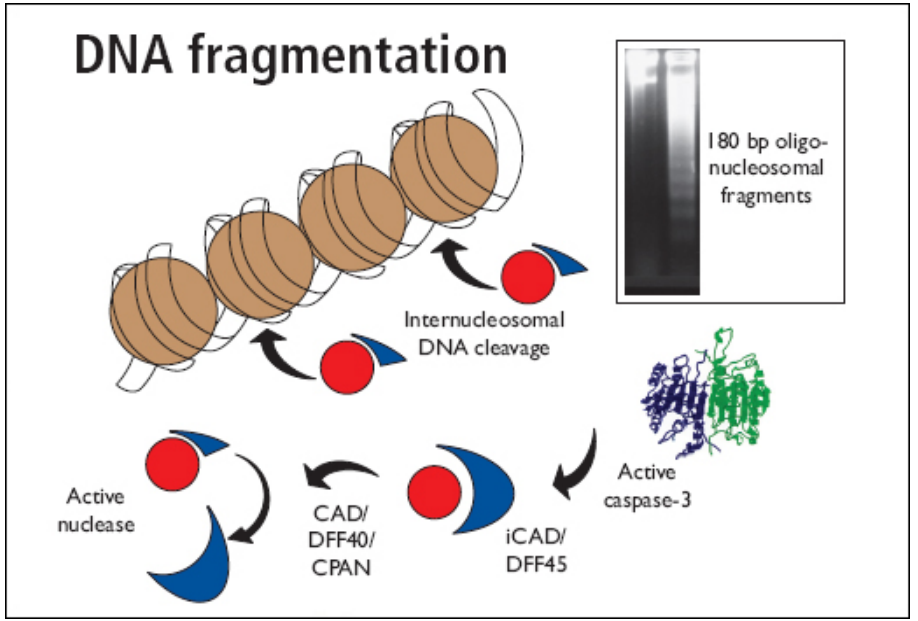


Kun Sun et al. PNAS 2015;112:E5503-E5512

Cell-free DNA – origin

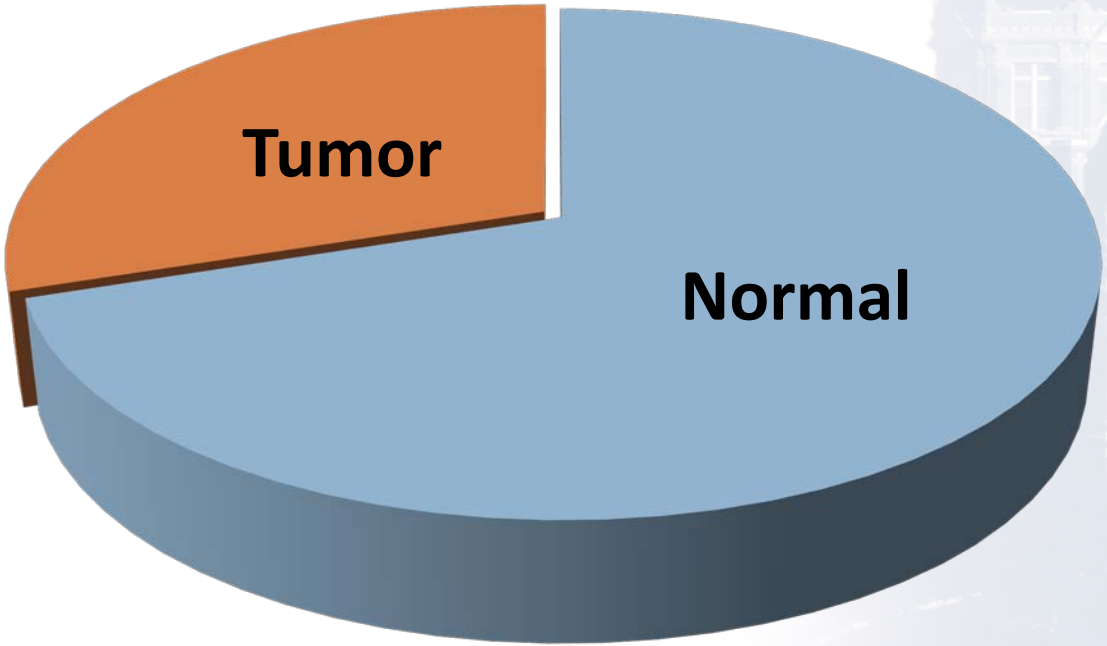


Apoptosis



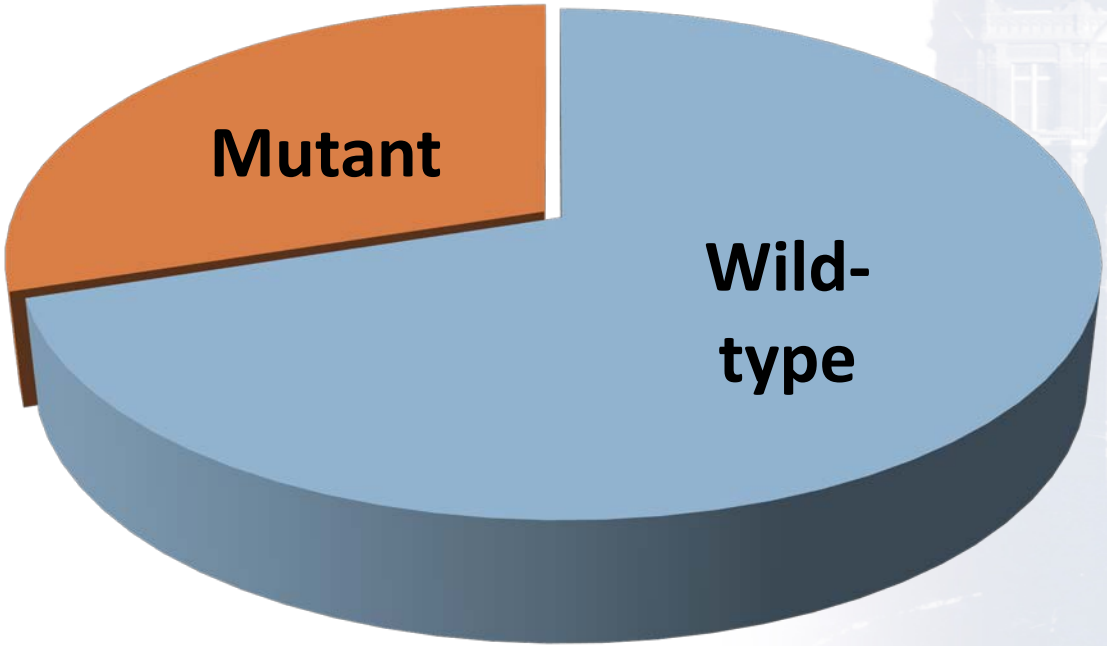
Jahr, S. Cancer Res, 2001

Circulating cell-free DNA in a Cancer Patient



JOHNS HOPKINS MEDICINE
JOHNS HOPKINS HEALTH SYSTEM

Circulating cell-free DNA in a Cancer Patient



Technology to assess circulating tumor DNA

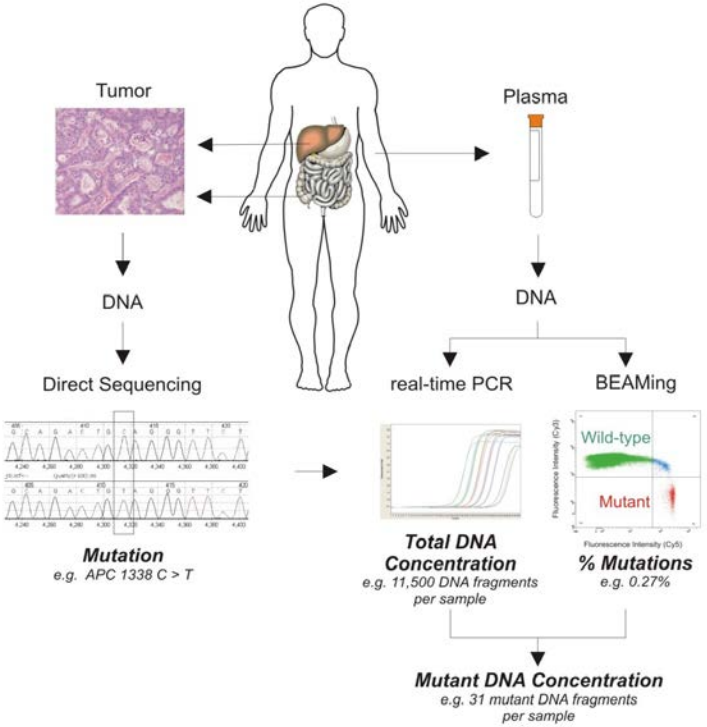
Digital PCR

- Best for individual point mutations but can be used for crude copy number analysis
- Mutation needs to known ahead of time (ie BRAF v600e)
- Sensitivity is dependent on specific mutation and assay optimization
- Multiplexing assay is possible
- Fast and highly reproducible – results in hours
- Minimal bioinformatics needs
- Inexpensive

Next-generation Sequencing

- Evaluates genomic regions of interest using PCR or capture-based methods
- Has been used for point mutations, rearrangements, genomic amplification, aneuploidy, whole-exome and whole genome sequencing
- High false discovery rate that requires pre-sequencing barcoding and post-sequencing bioinformatics for error suppression
- Expensive
- Turnaround time 1-2 days at best

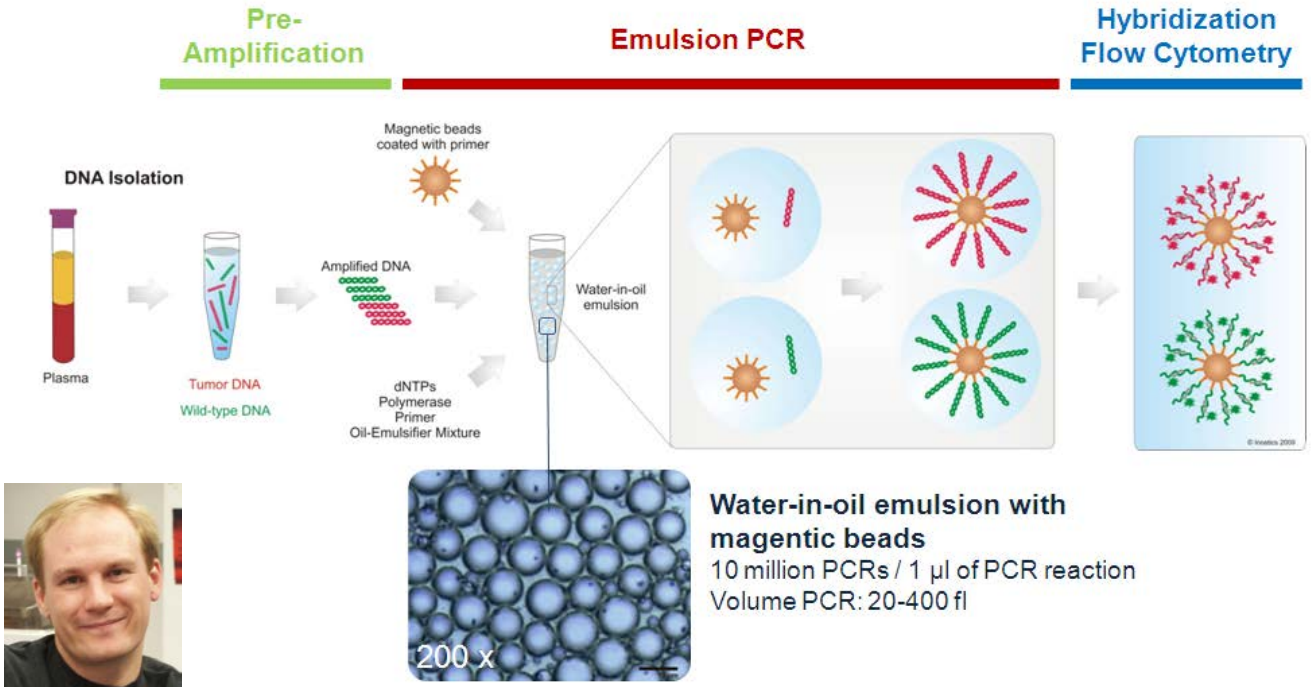
Digital PCR



- **BEAMing – emusion-digital PCR**
- **Sensitivity – 0.01% (depends on mutation)**
- **Mutation to be tested needs to be known ahead of time**

Diehl et al Nature Medicine, 2008

BEAMing



Devin
Dressman

Dressman et al. PNAS

Wild-type fragments

Before Surgery
Day 0

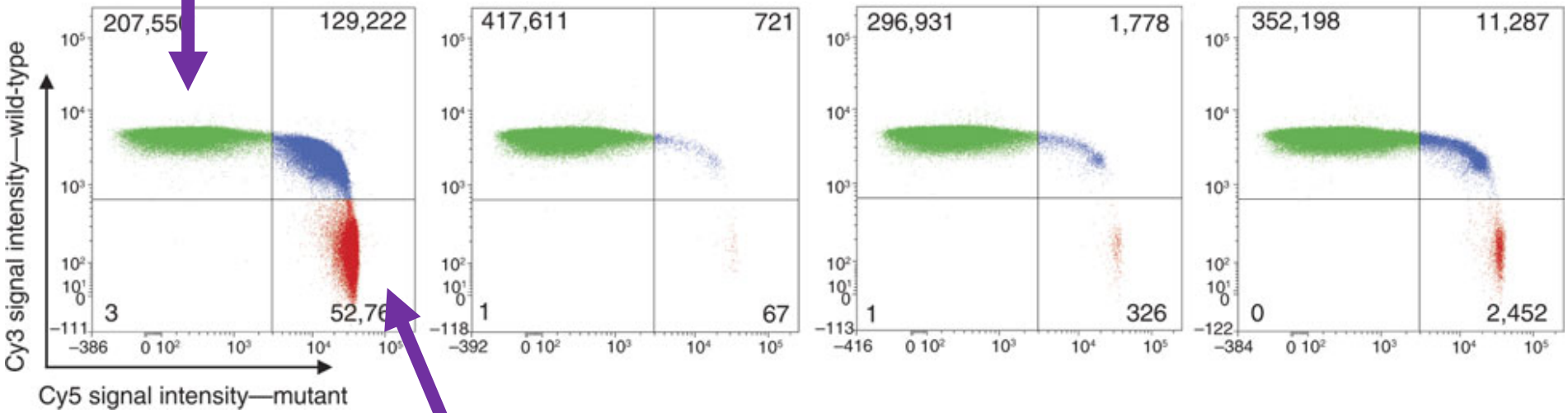
After Surgery
Day 1

CT scan negative

After Surgery
Day 42

CT scan positive

After Surgery
Day 244



13.4 %

0.015 %

0.11 %

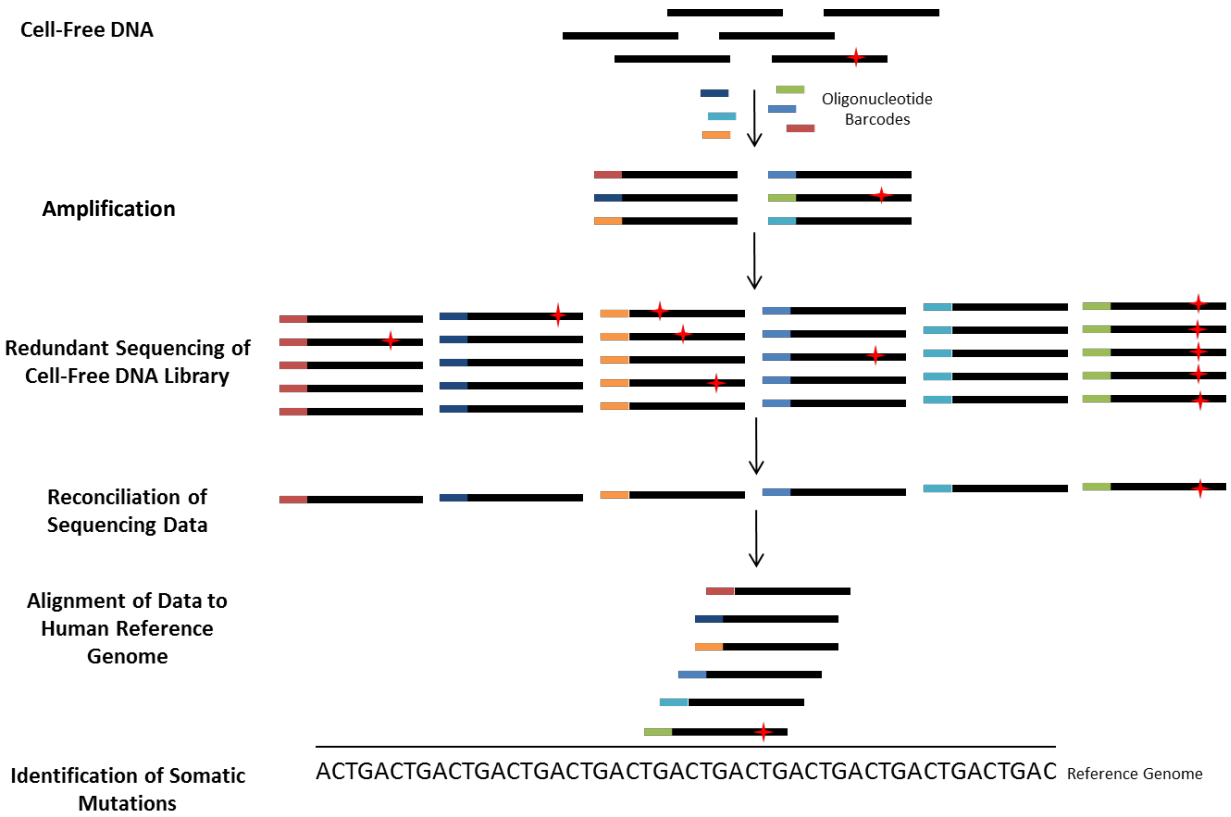
0.66 %

Mutant fragments

Percent Mutant APC

Diehl et al Nature Medicine, 2008

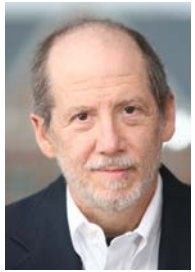
Safe-SeqS – NGS approach to detect ctDNA



Isaac Kinde

N. Papadopoulos

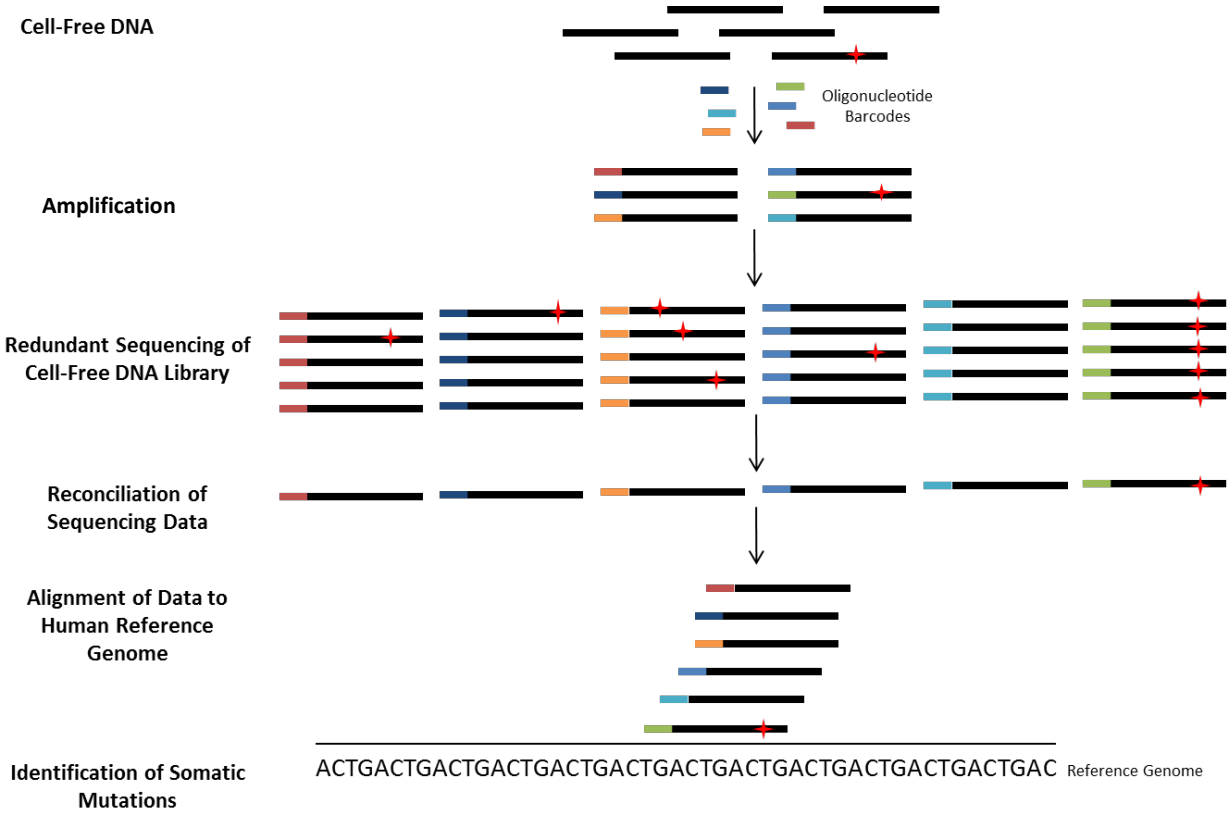
Ken Kinzler



B. Vogelstein

Kinde et al. PNAS

Safe-SeqS – NGS approach to detect ctDNA



- **NGS-based approach**
- **Scans multiple ROIs**
- **Sensitivity – 0.01% (depends on mutation)**
- **Mutation does not need to be known ahead of time**

Applications of ctDNA

- Genotyping cancer & identify actionable genetic alterations
  - For patients lack tissue for molecular analysis
  - For patients whose tumors have evolved over time and treatment (too risky to perform or after relapse when biopsies are not routine)
    - Discordance between mutations in primary/metastases lesions
    - Acquired resistance (e.g., patients who develop resistance to EGFR blockade)
- Monitoring of tumor burden / response to treatment (vs. CEA or imaging)
- Detection of Occult Disease
  - Minimal Residual Disease
  - Early Detection/Screening

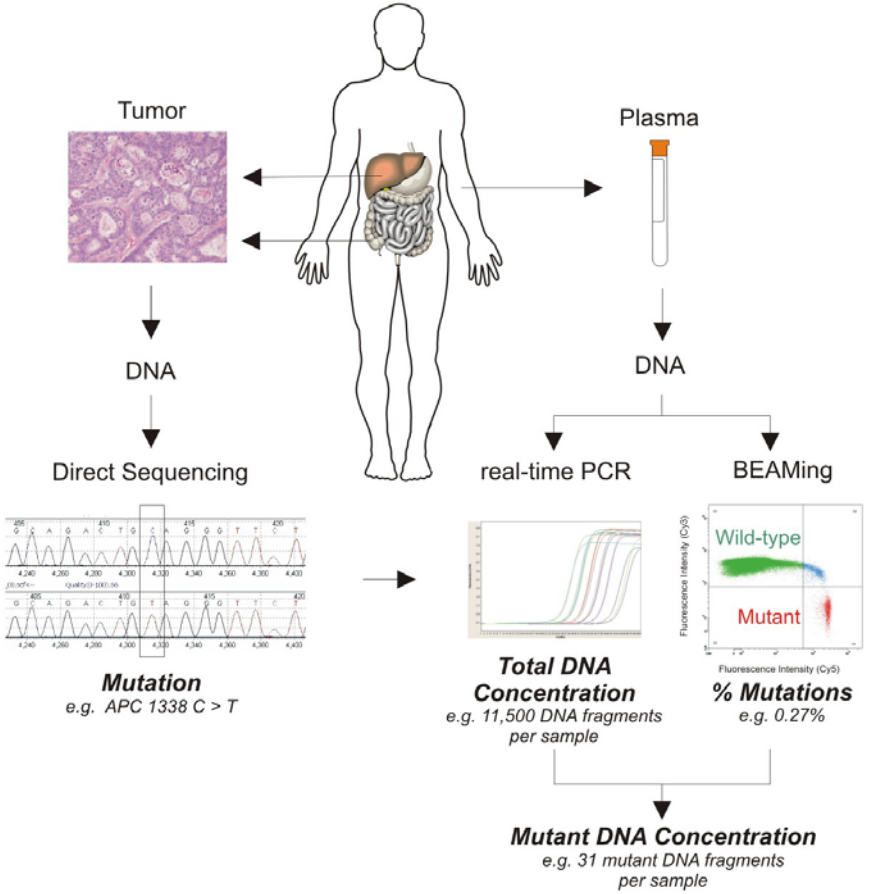
Potential of Liquid Biopsies in Precision Medicine

Monitoring tumor dynamics

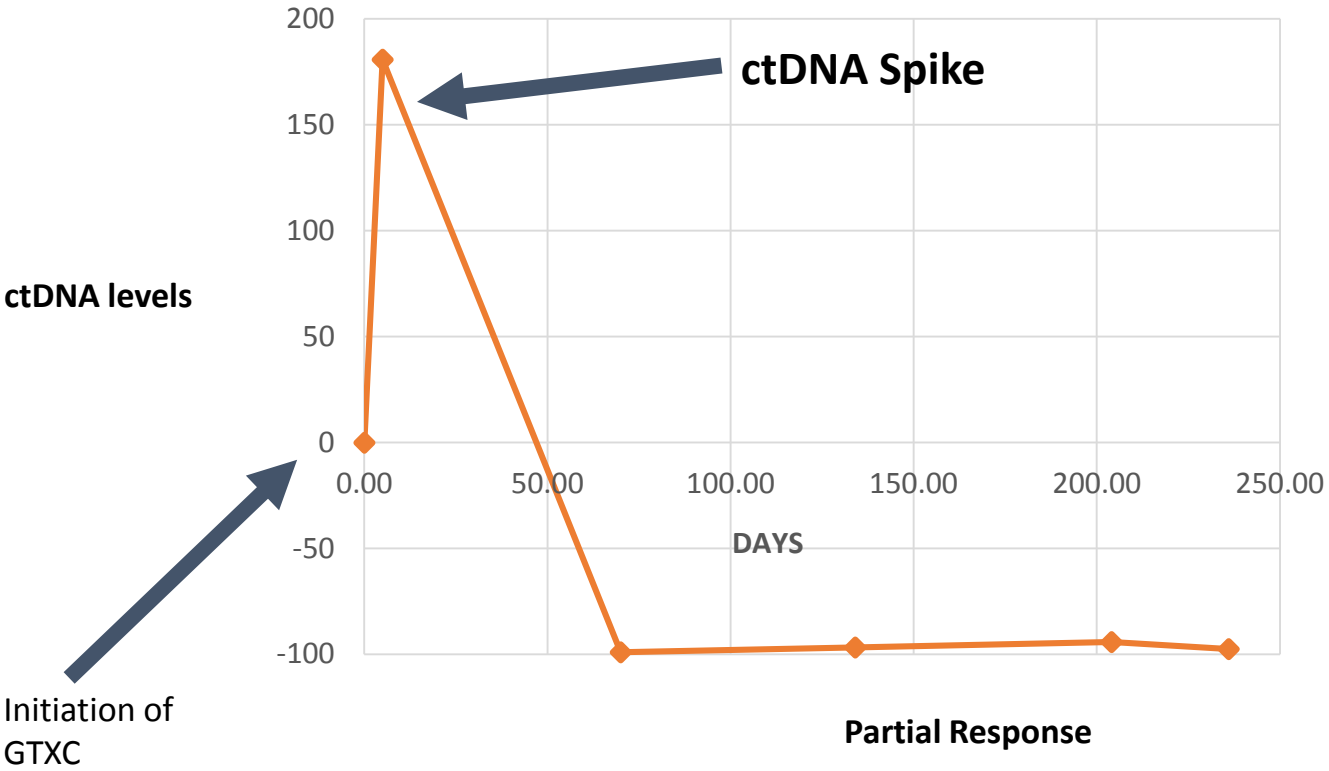
Molecular Analysis

Kerstin
Schmidt

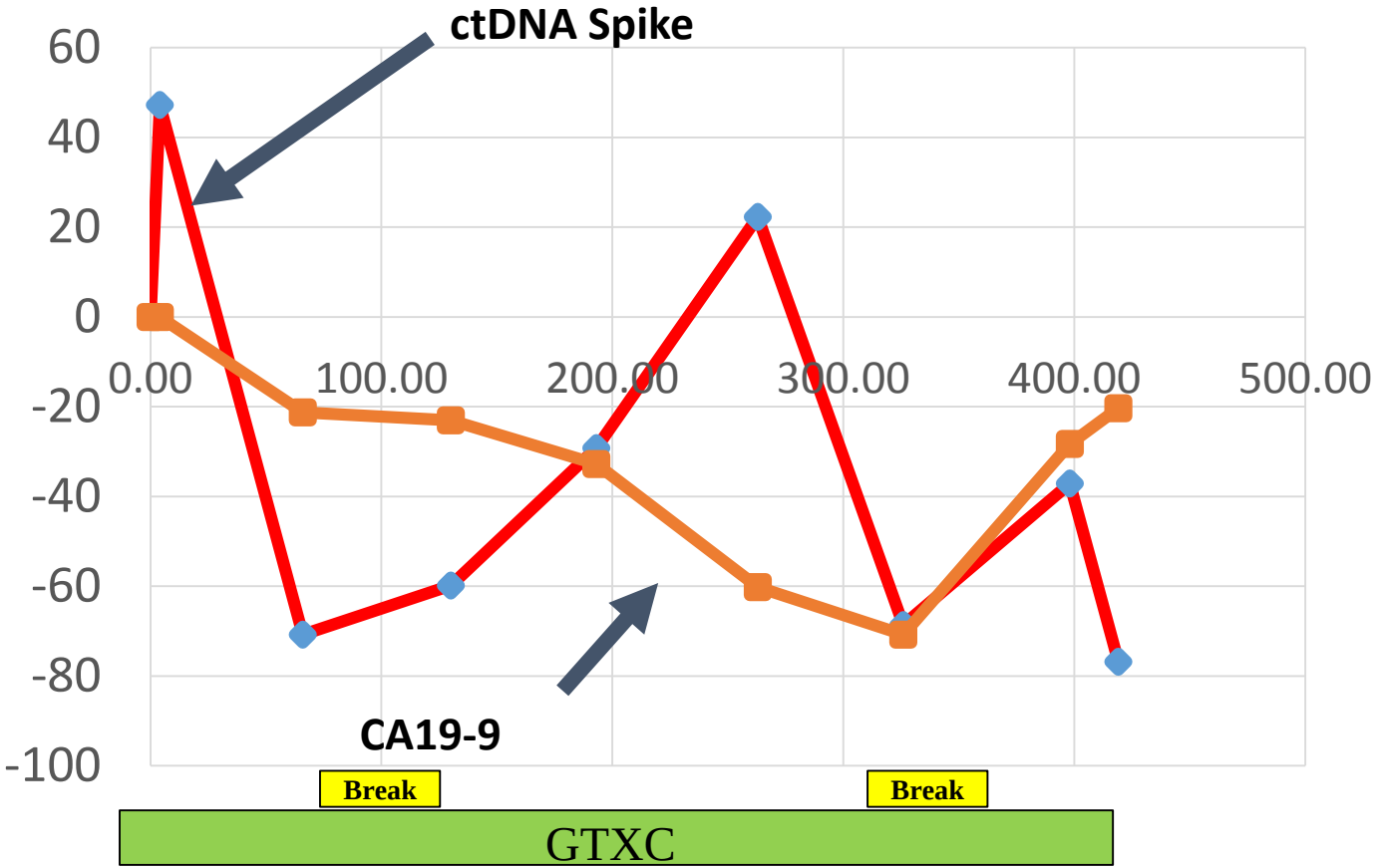
Frank
Diehl



Circulating tumor DNA is a rapidly dynamic biomarker

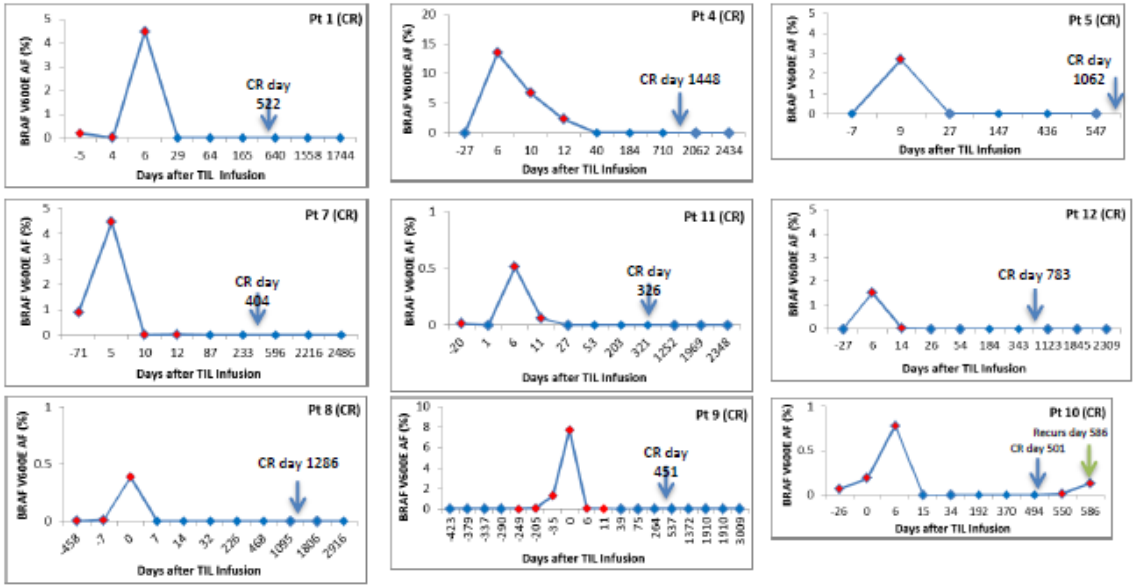


CTDNA VS CA19-9



Circulating Tumor DNA as an Early Indicator of Response to T-Cell Transfer Immunotherapy 2 in Metastatic Melanoma

Patients with CR

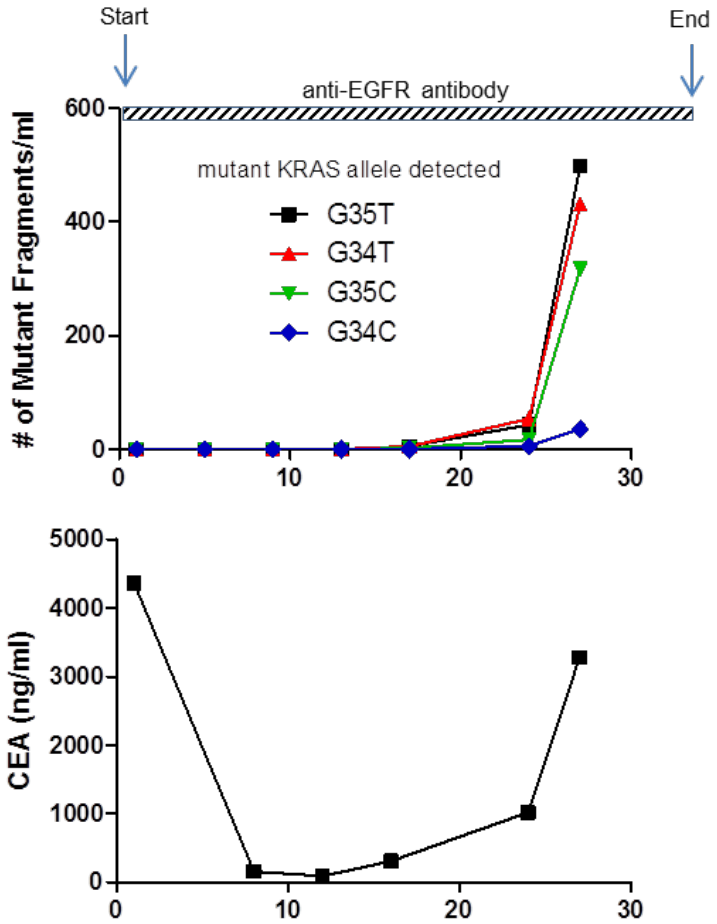


Clinical Application of Cancer Genetics

Tracking Resistance

Tracking Resistance

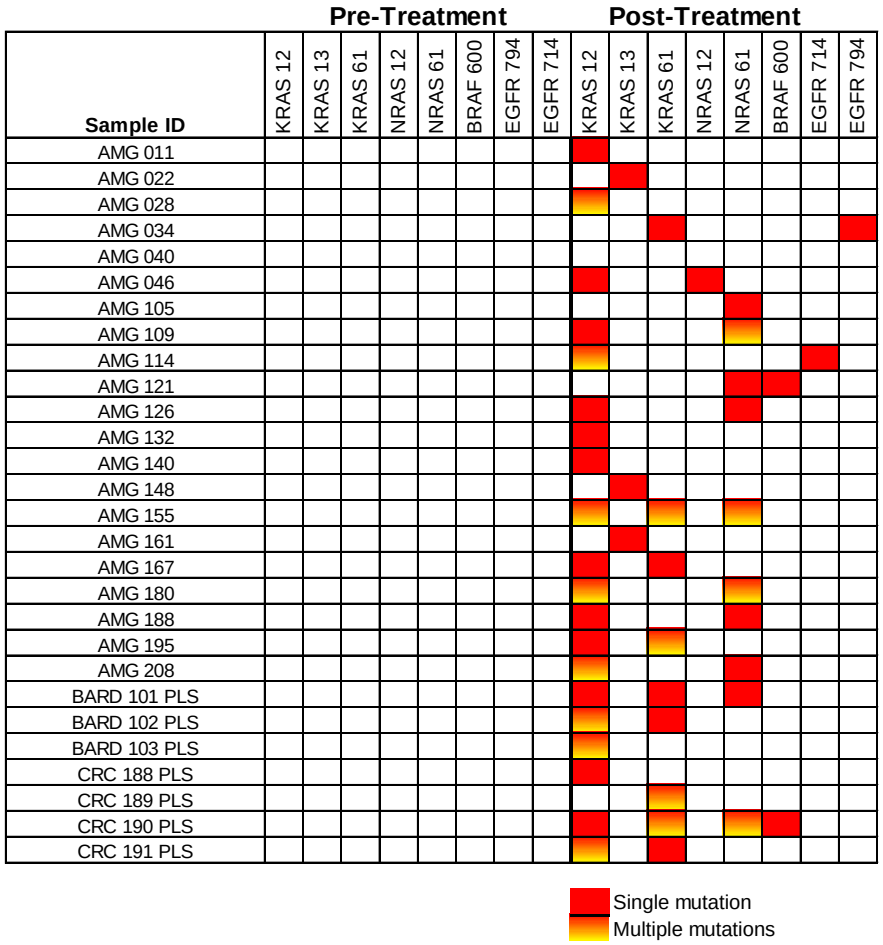
- Understand the molecular resistance to EGFR blockade in colorectal cancer
- Patients are KRAS WT prior to initiate therapy
- Monitoring the emergence of resistant mutations in KRAS WT patients treated with EGFR blockade



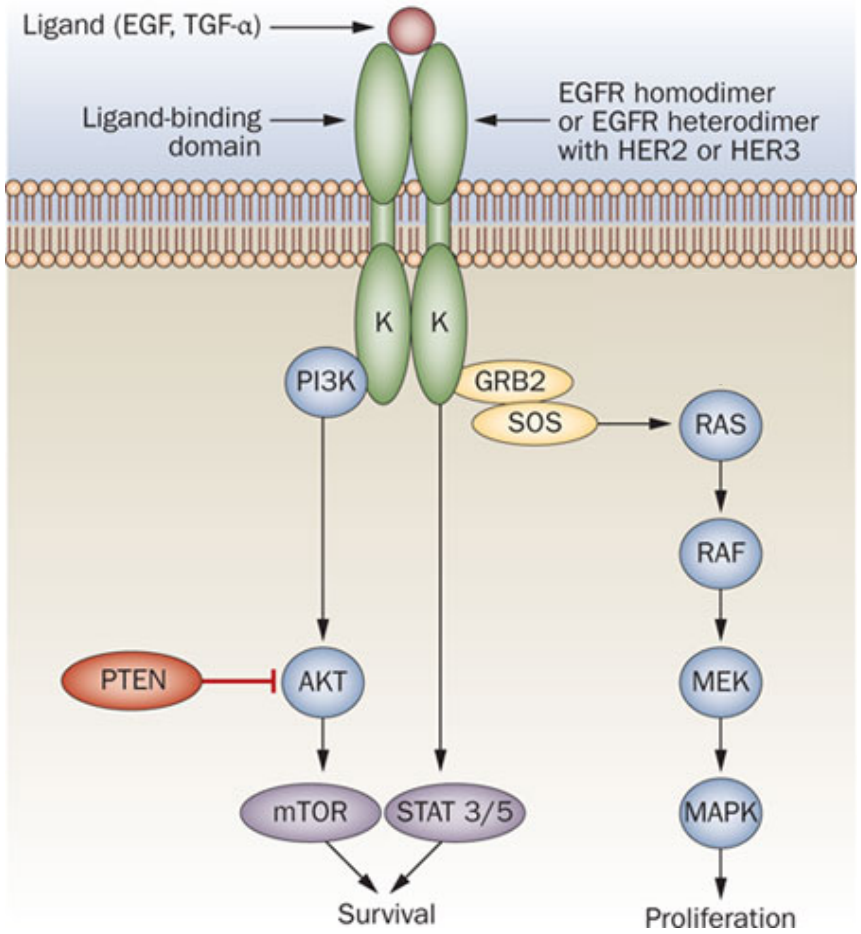
Diaz et al Nature, 2012.

Tracking Resistance

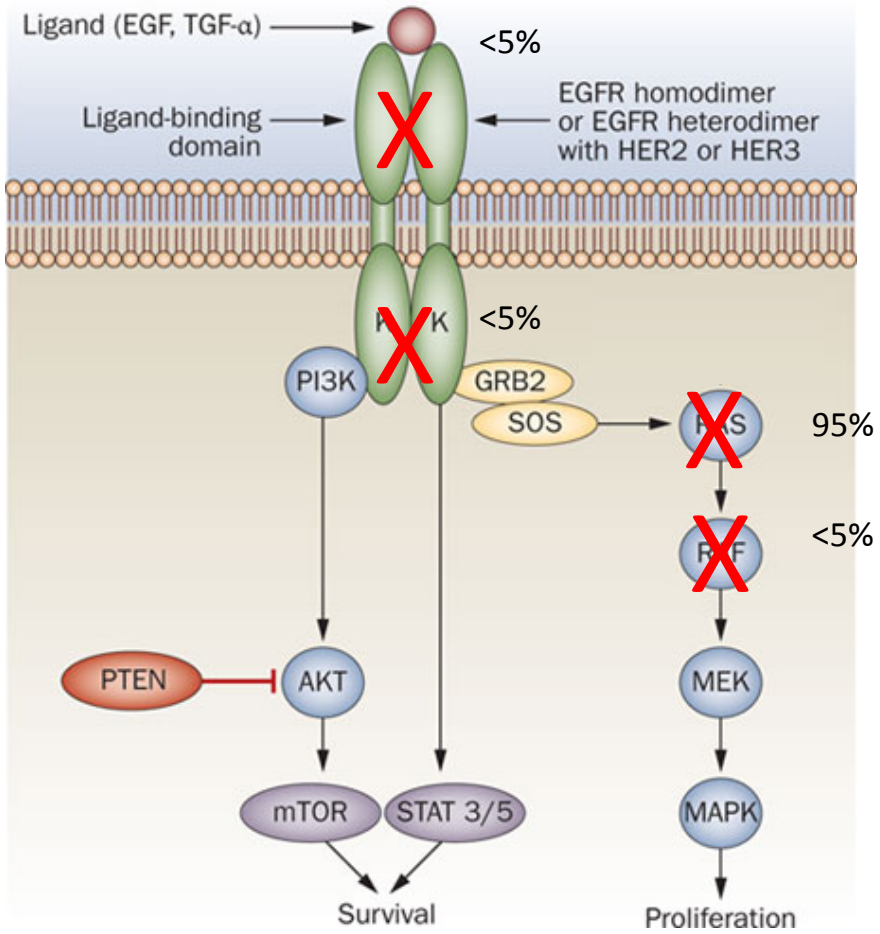
- Interrogated all exons of KRAS, NRAS, BRAF, PIK3CA and EGFR
- 96% of cases had at least 1 mutation KRAS or NRAS



Bettegowda et al, Sci Tran Med 2014



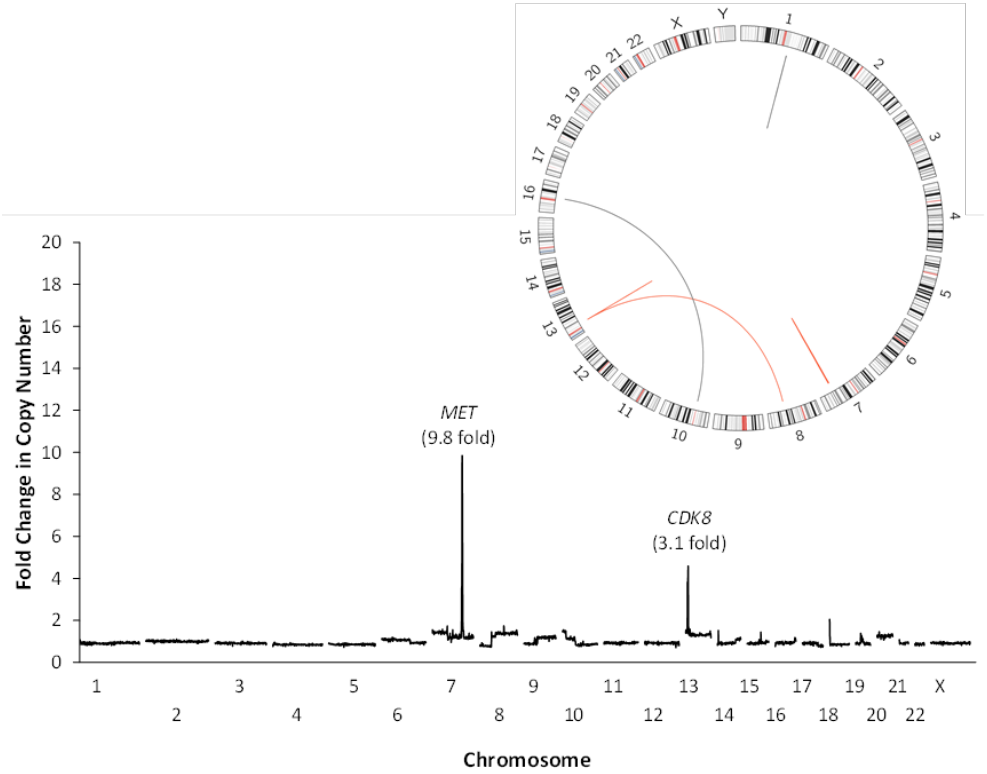
Janku, F. *et al.* (2010) *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2010.64



Janku, F. *et al.* (2010) *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2010.64

WGS of plasma DNA in EGFR resistant CRC patient

High-level Focal Amplification of *MET*



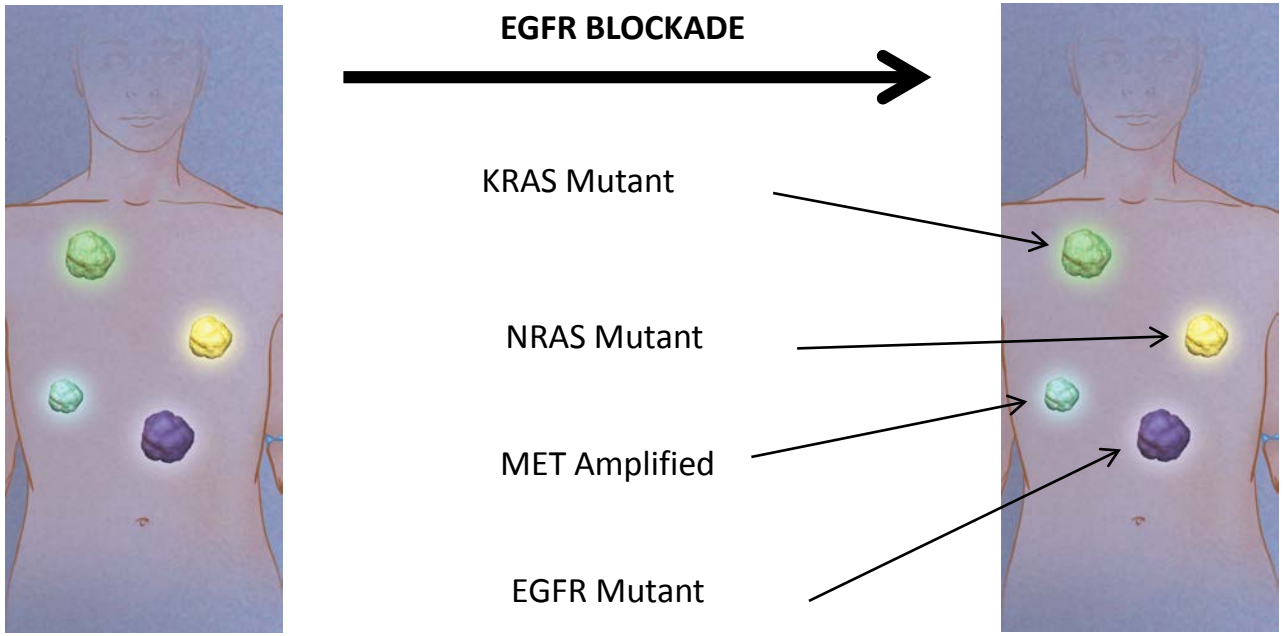
A total of 630 Gb of sequence data were obtained, corresponding to 145x sequence coverage of cell-free plasma genomic DNA.

Identified in plasma sample following clinical resistance:

- Q61H mutation in KRAS
- focal high-level (>9 fold) amplification of *MET*
- focal high-level (>3 fold) amplification of *CDK8*

These were not detected in pre-treatment tumor samples

Tracking Resistance



KRAS WT
NRAS WT
EGFR WT
MET WT

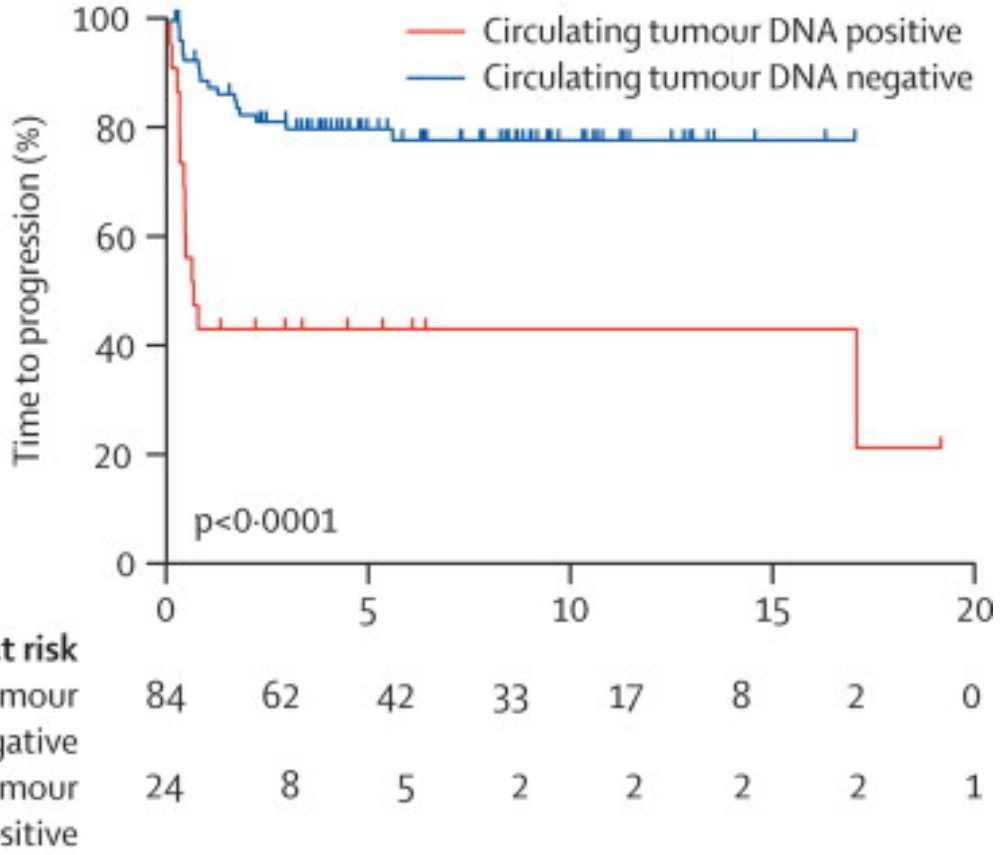
Potential of Liquid Biopsies in Precision Medicine

Molecular Remission

ctDNA monitoring in patients with diffuse large B-cell lymphoma

- **108 patients**
- **VDJ gene segments of the rearranged immunoglobulin receptor genes**
- **ctDNA measured after 2 cycles of therapy**

***National Cancer Institute and Adaptive Biotechnologies**

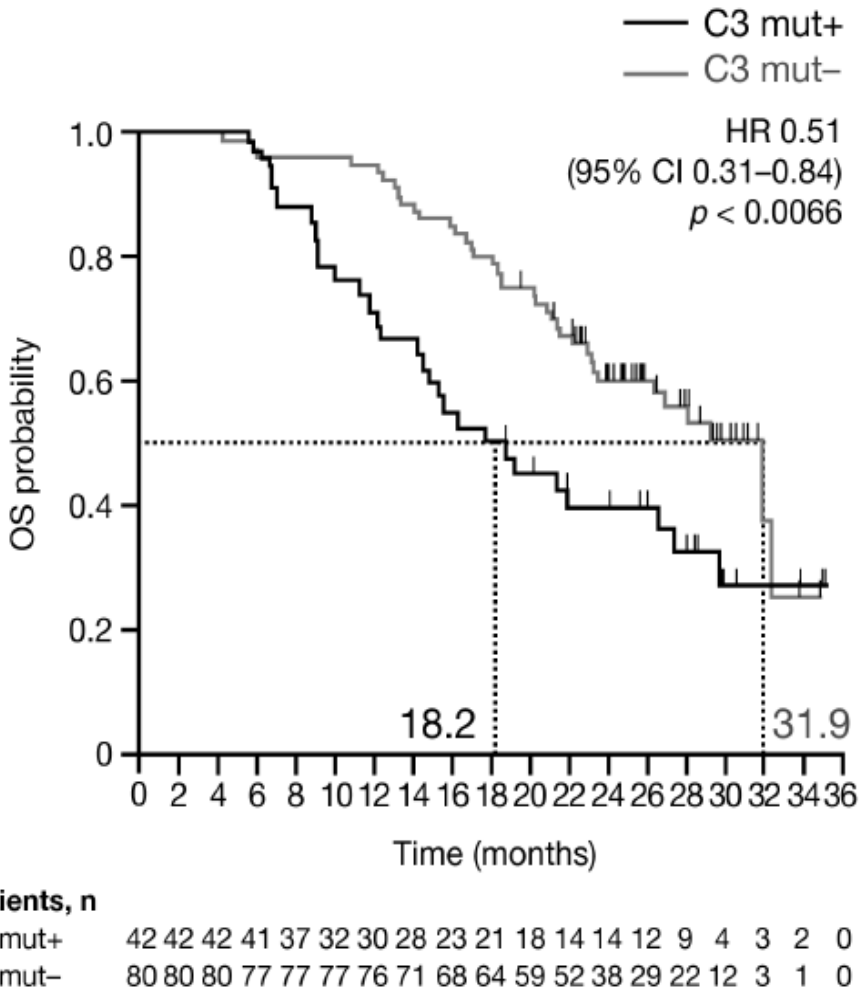


Roschewski M et al. *Lancet Oncol.* 2015.

Clearance of circulating EGFR mutations in metastatic lung cancer

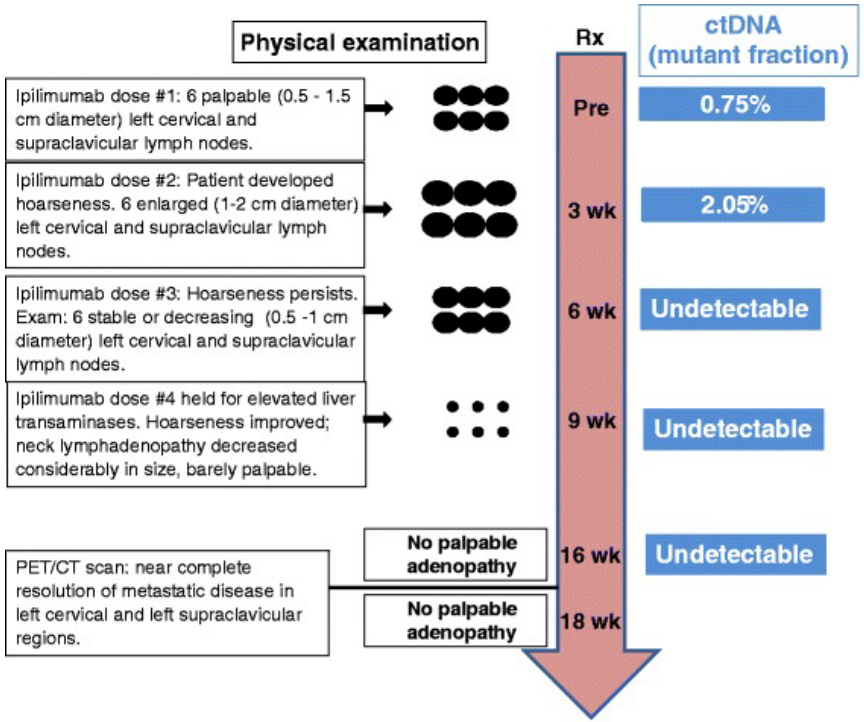
- 122 patients with EGFR mutant NSCLC
- Treated with erlotinib-based regimen
- Determined using allele-specific PCR after 3 cycles of therapy

*Hong Kong Cancer Institute,
Roche, Genentech



Mok T et al. *Clin Cancer Res.* 2015.

Monitoring response to checkpoint inhibitors using ctDNA



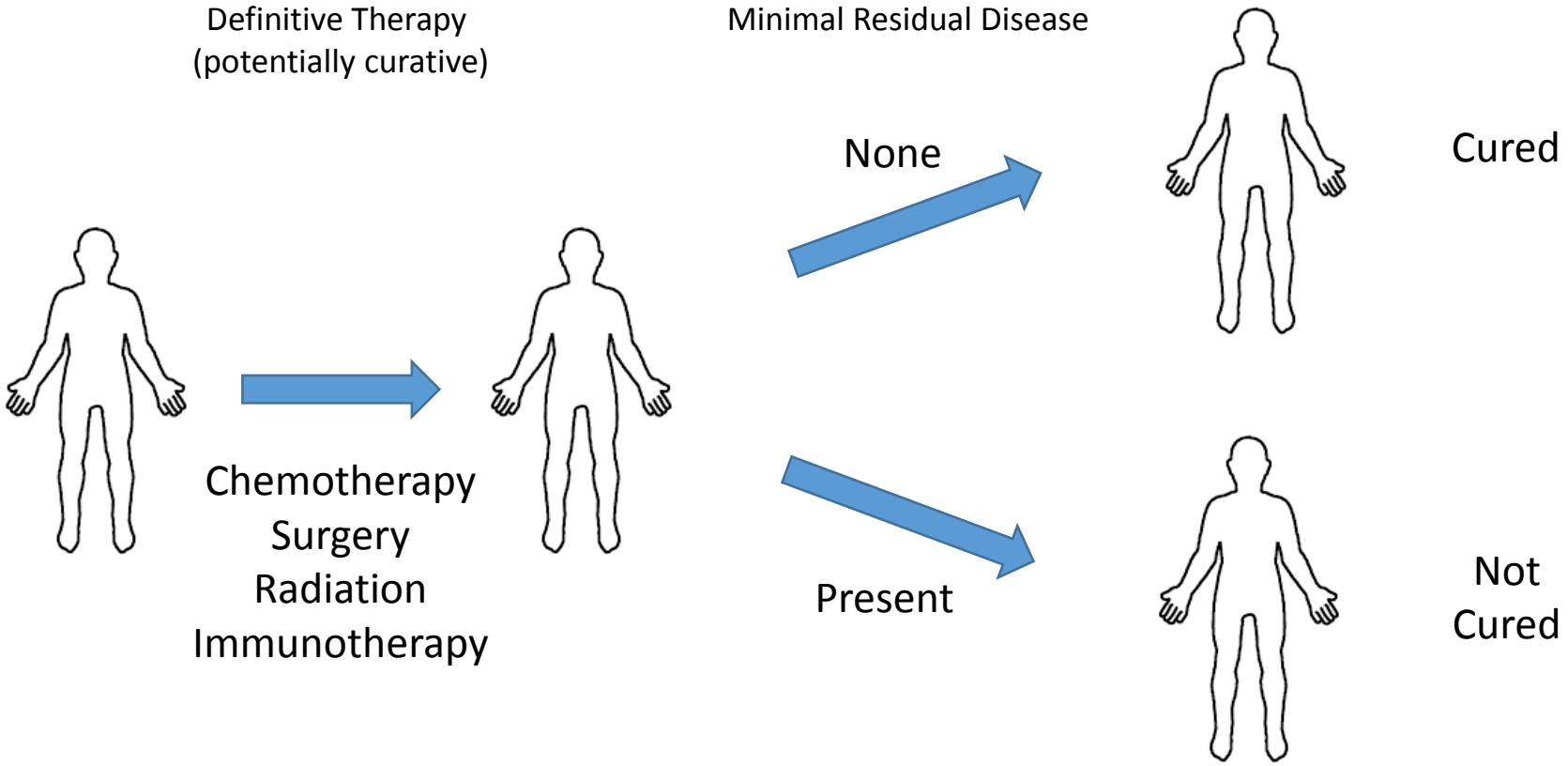
ctDNA levels increased initially as lymphadenopathy progressed by examination, but then became undetectable 3 weeks prior to clinical improvement.

Lipson et al. J. of Immunotherapy 2014.

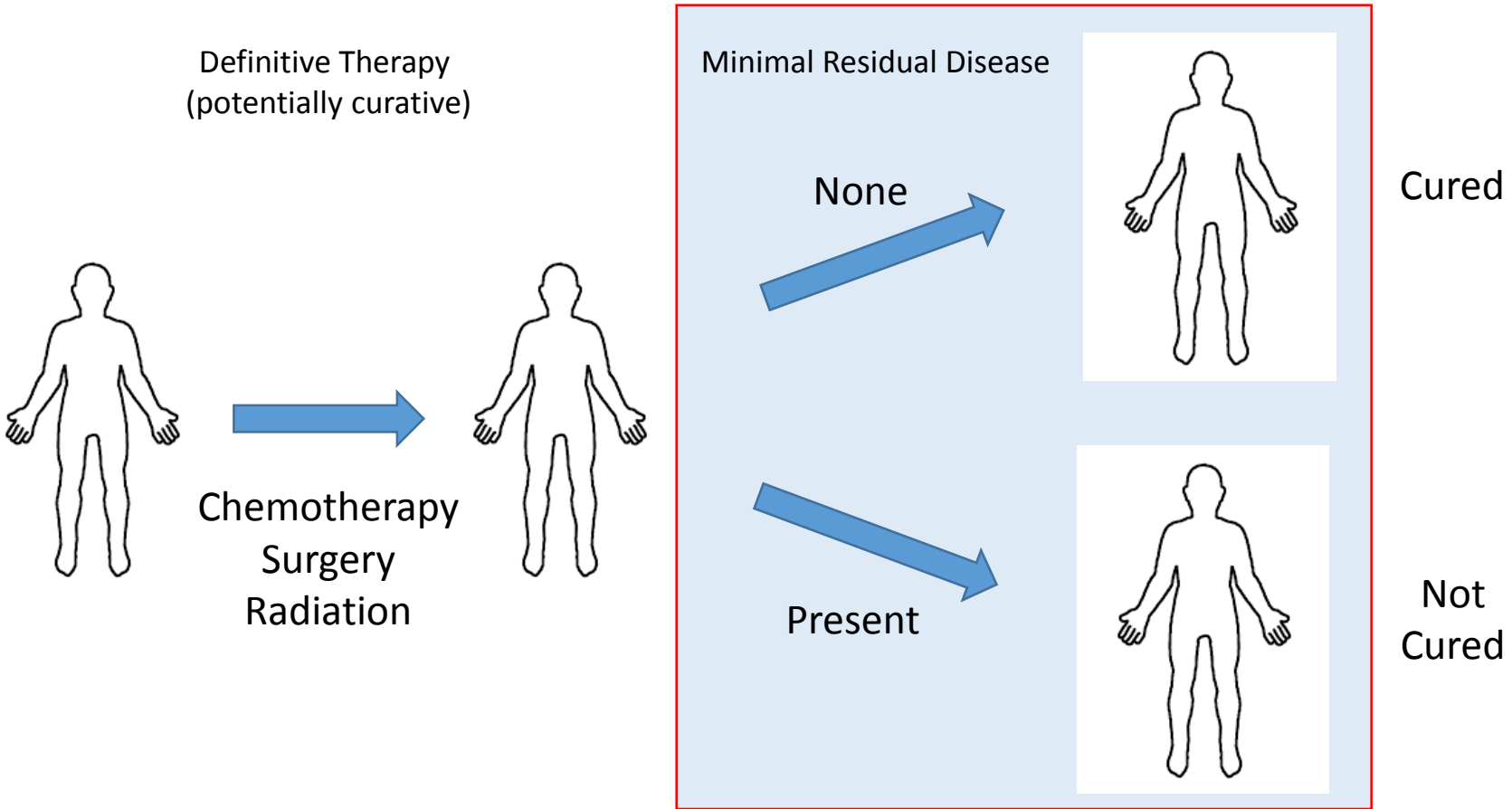
Clinical Application of Cancer Genetics

Minimal Residual Disease

What is Minimal Residual Disease (MRD)?



What is Minimal Residual Disease (MRD)?



Systemic Approaches to Detect MRD

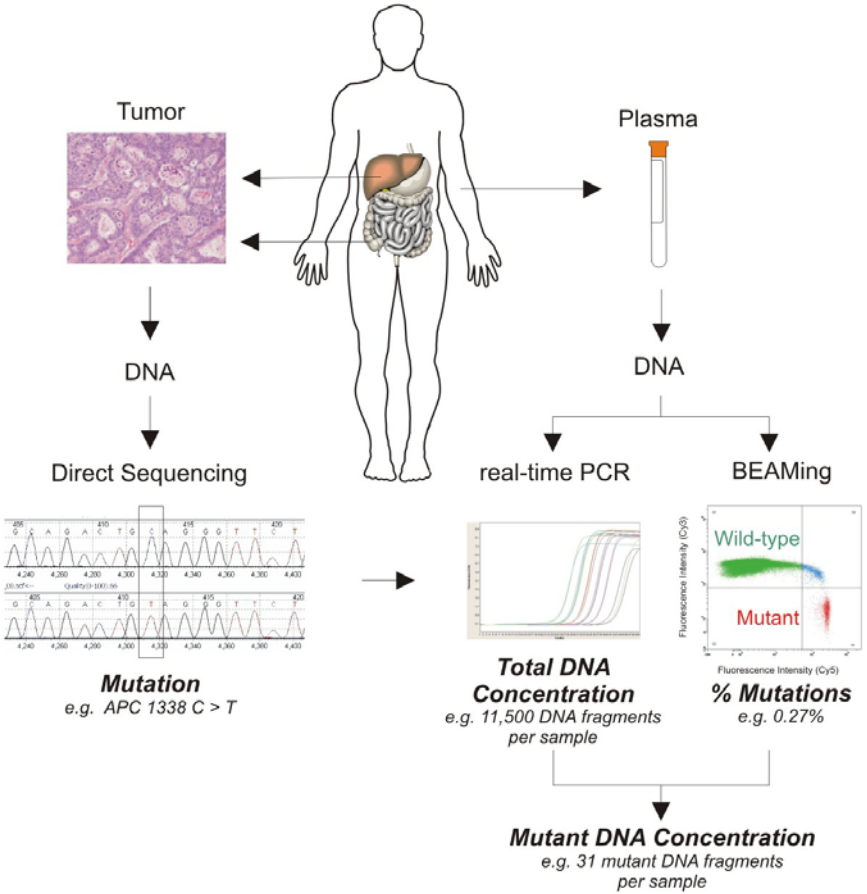
Imaging (FDG-PET or CT Scan)
- Poor sensitivity for microscopic disease
- Variable specificity

Protein Biomarkers(e.g. CA19-9, CEA, CA-125)
- Long half-life
- Often Non-specific

CTCs
- Poor sensitivity for microscopic disease
- Does not localize disease

Circulating Nucleic Acids
- Does not localize disease
- Highly specific

Molecular Analysis



Wild-type fragments

Before Surgery
Day 0

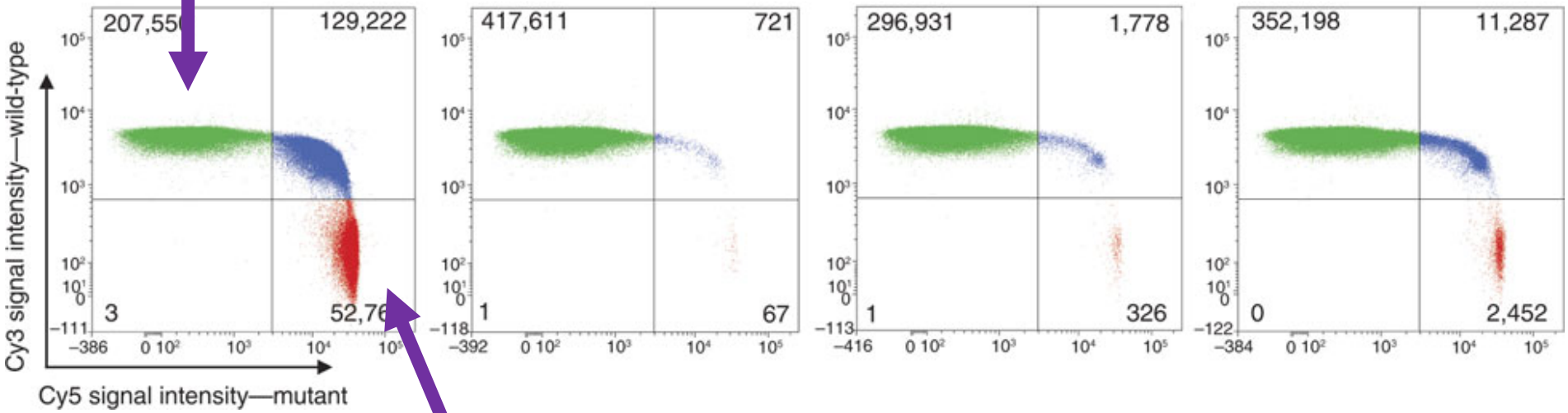
After Surgery
Day 1

CT scan negative

After Surgery
Day 42

CT scan positive

After Surgery
Day 244



13.4 %

0.015 %

0.11 %

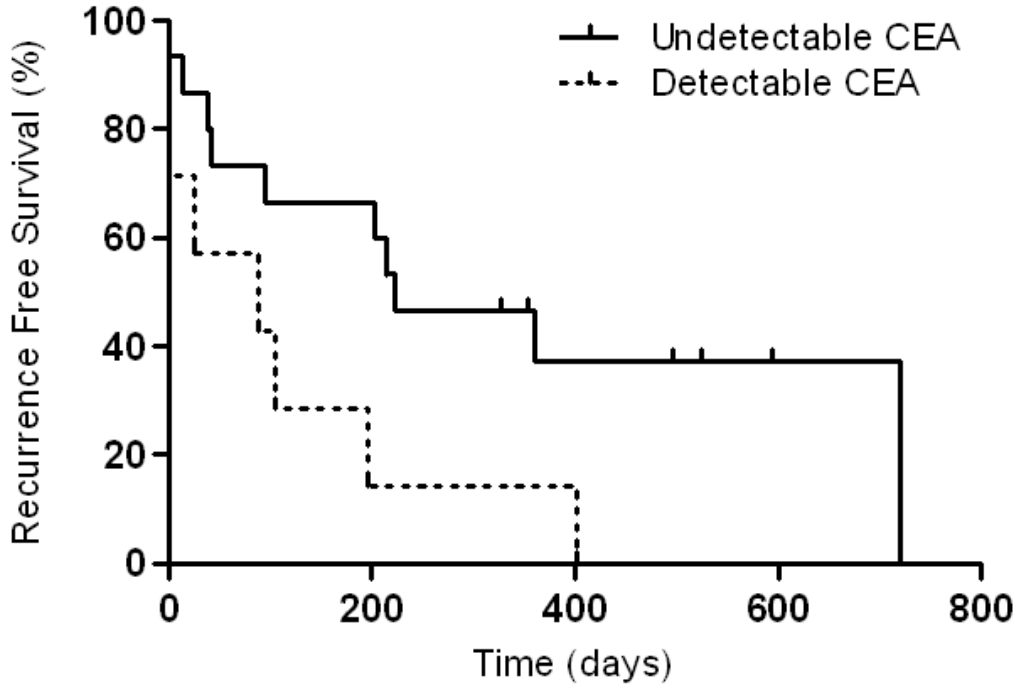
0.66 %

Mutant fragments

Percent Mutant APC

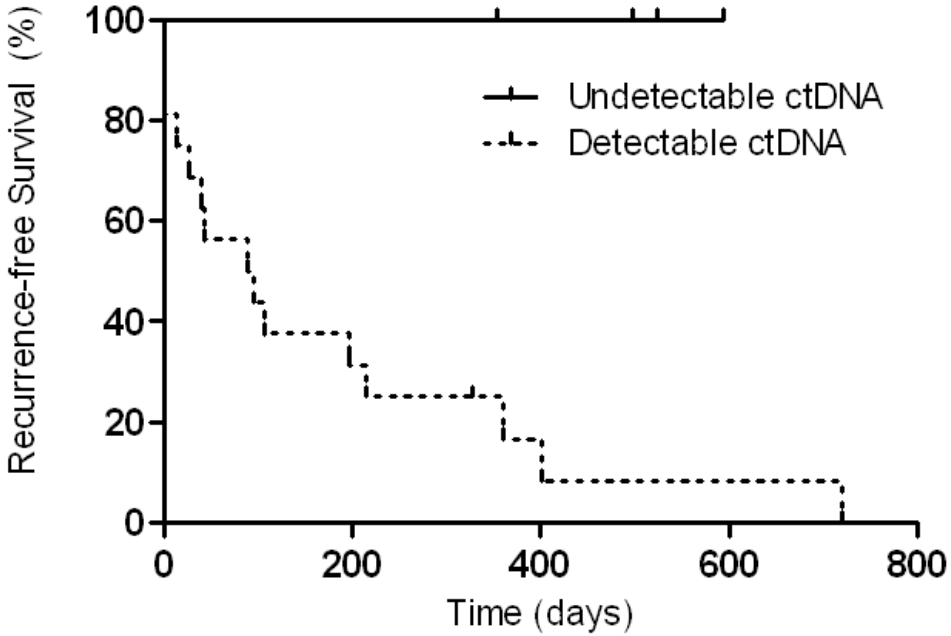
Diehl et al Nature Medicine, 2008

CEA measured 6-8 weeks following curative resection of mCRC

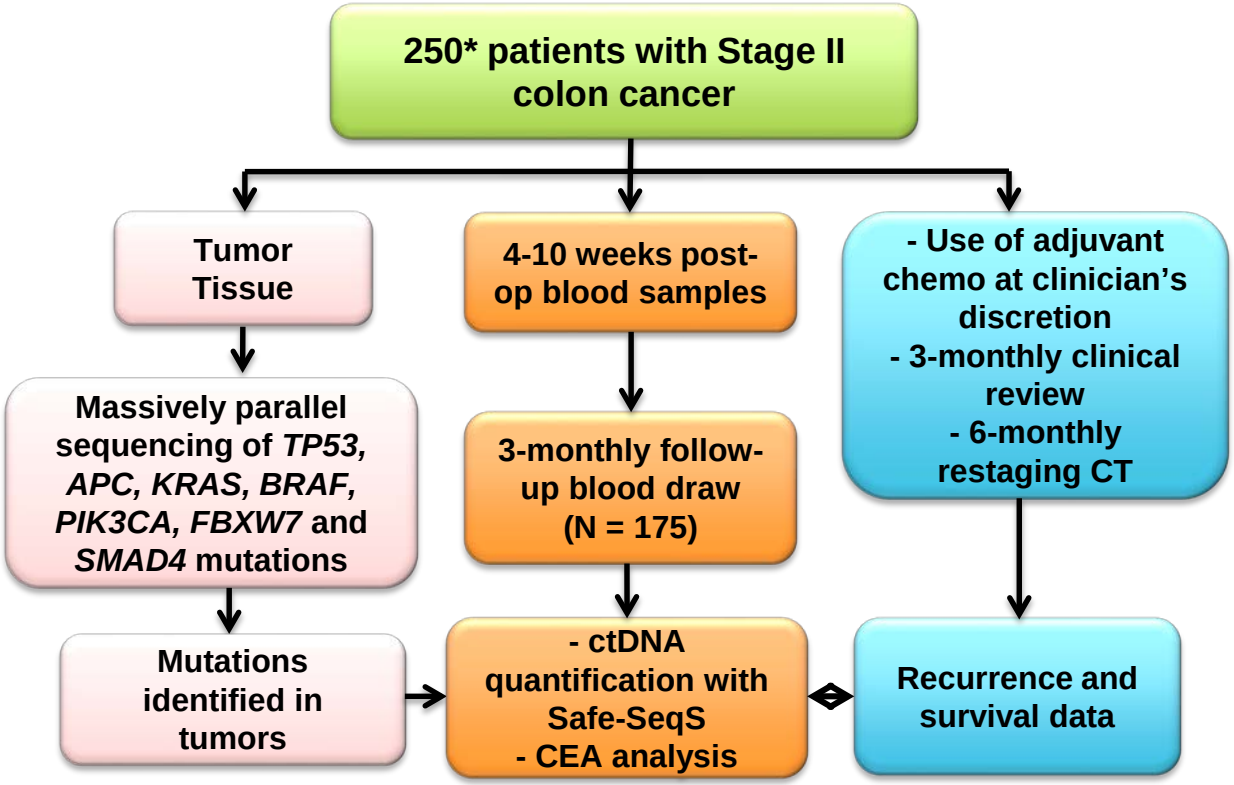


Diehl et al Nature Medicine, 2008

ctDNA measured 6-8 weeks following curative resection of mCRC

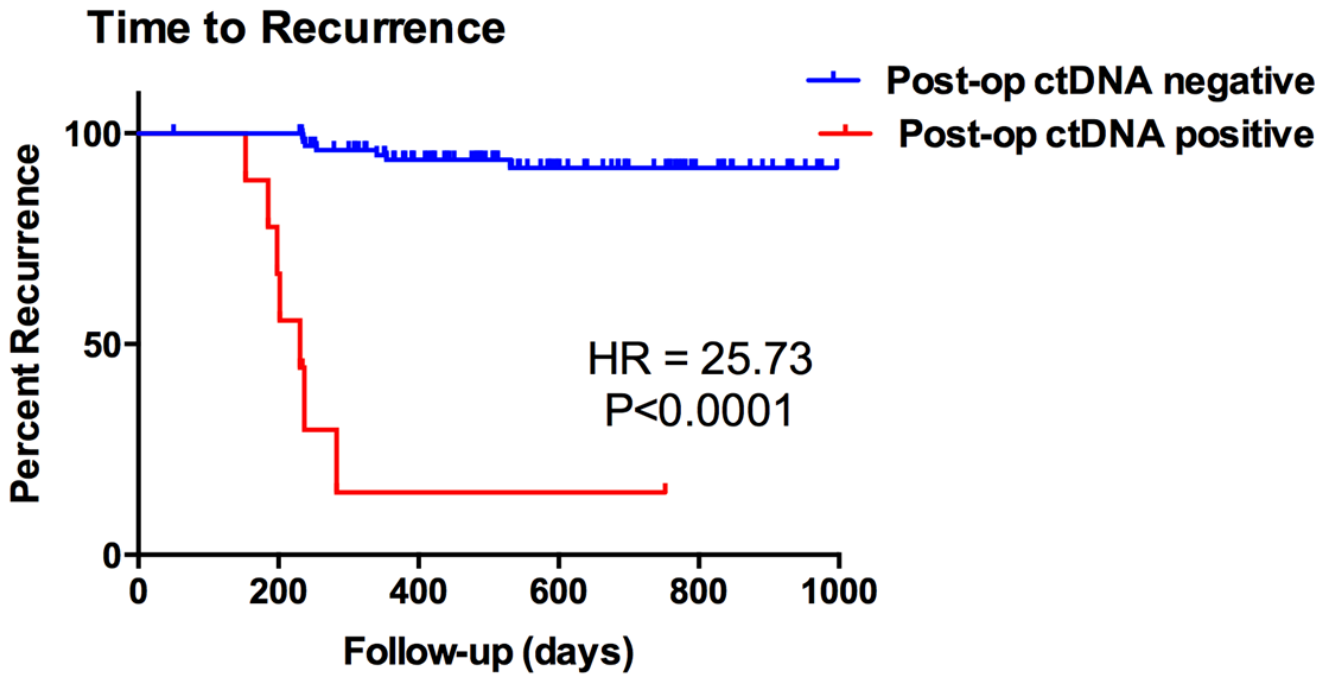


Diehl et al Nature Medicine, 2008



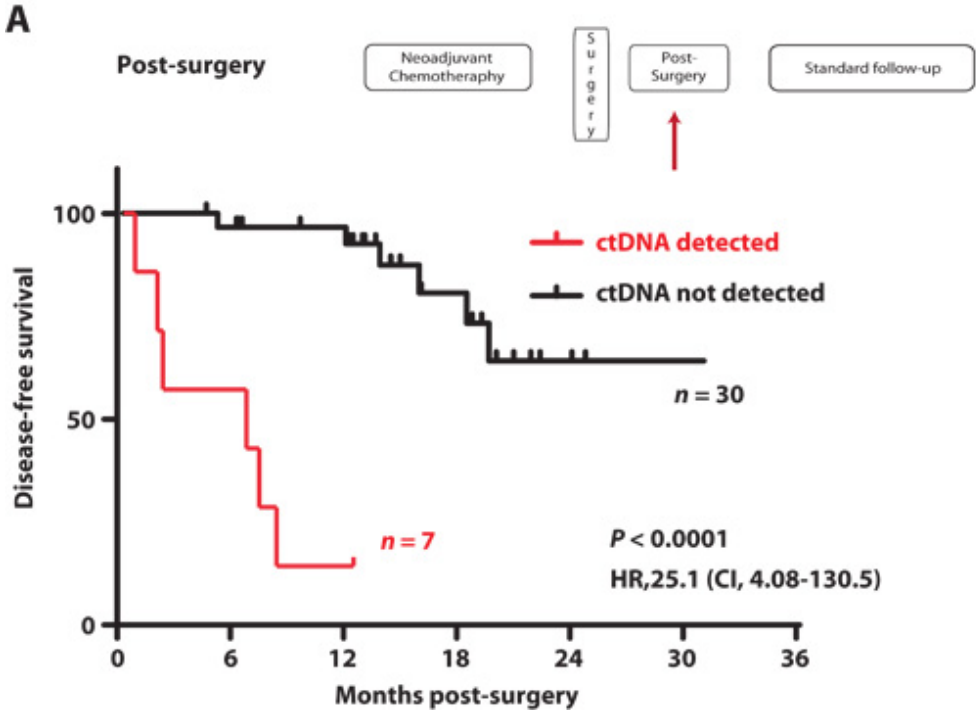
J. Tie and Peter Gibbs

ctDNA measured 6-8 weeks following curative resection of stage II CRC



J. Tie and Peter Gibbs, ASCO 2015

MRD detection with ctDNA in breast cancer.

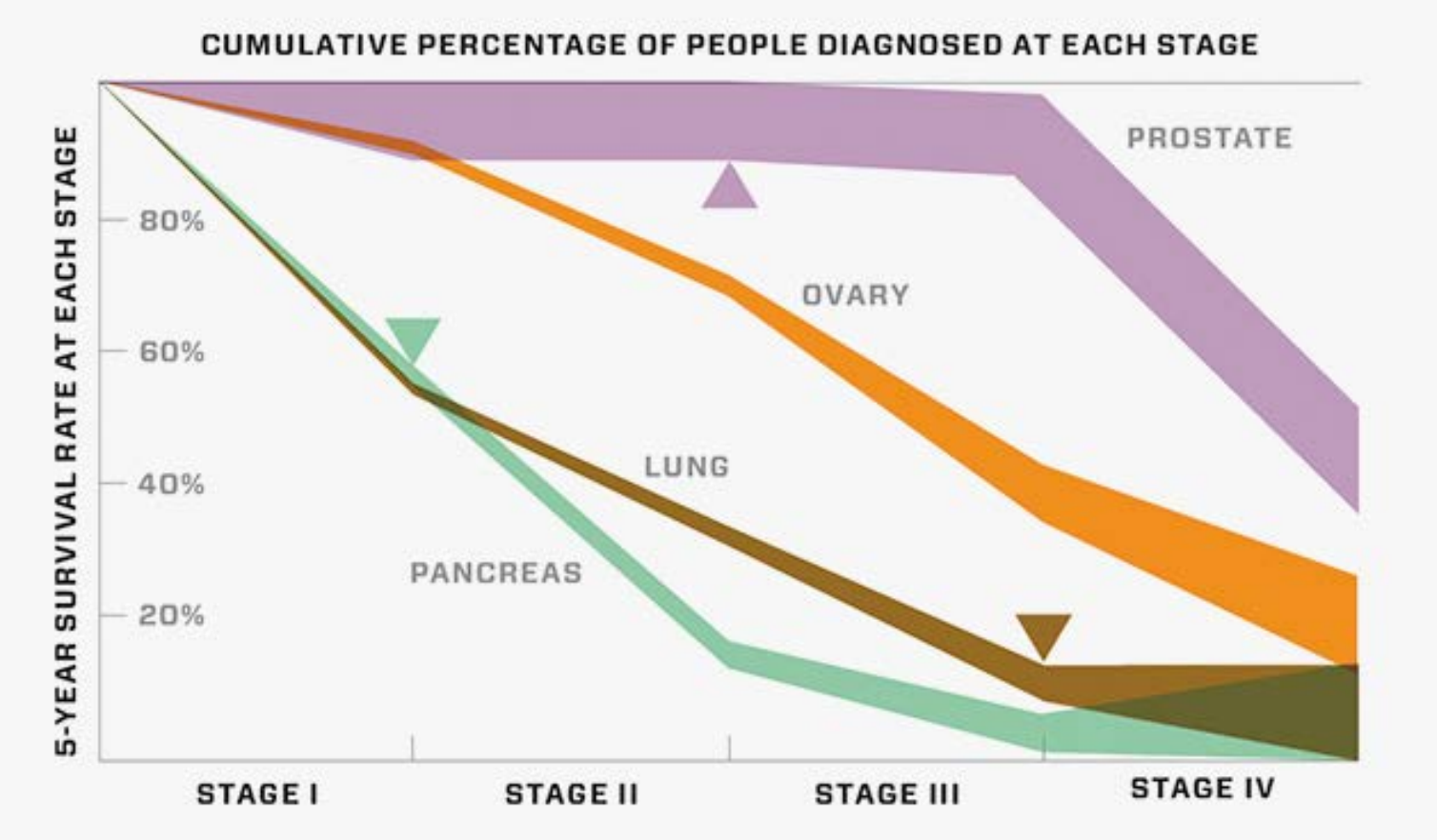


Isaac Garcia-Murillas et al., Sci Transl Med 2015;7:302ra133

Science Translational Medicine
AAAS

Published by AAAS

Philosophy of Early Detection



Philosophy of Early Detection

Relative 5 Year Cancer Survival Rates

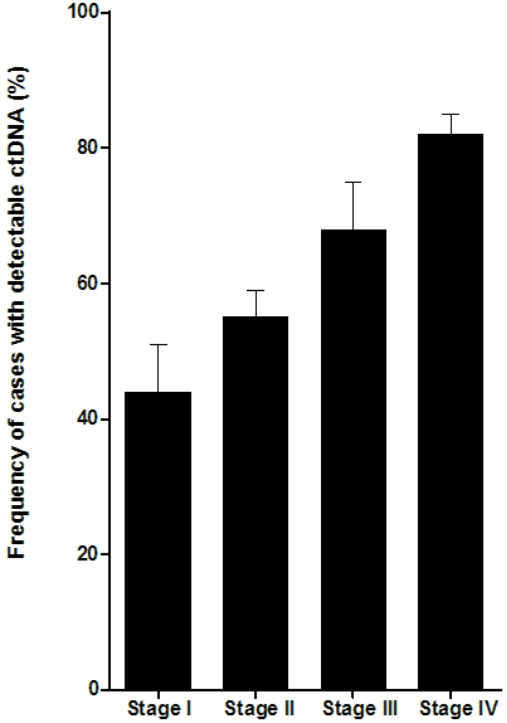
Cancer type	Early detection	Late detection
Colorectal	90%	8%
Breast	97%	21%
Prostate	96%	34%
Melanoma	96%	12%
Cervix	92%	15%

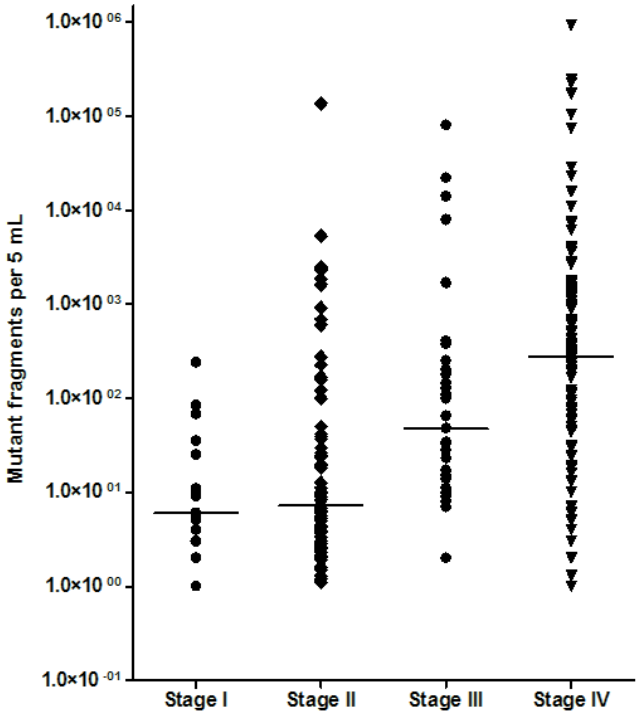
Clinical Application of Cancer Genetics

Early Detection – Blood

Early Detection using ctDNA Analyses

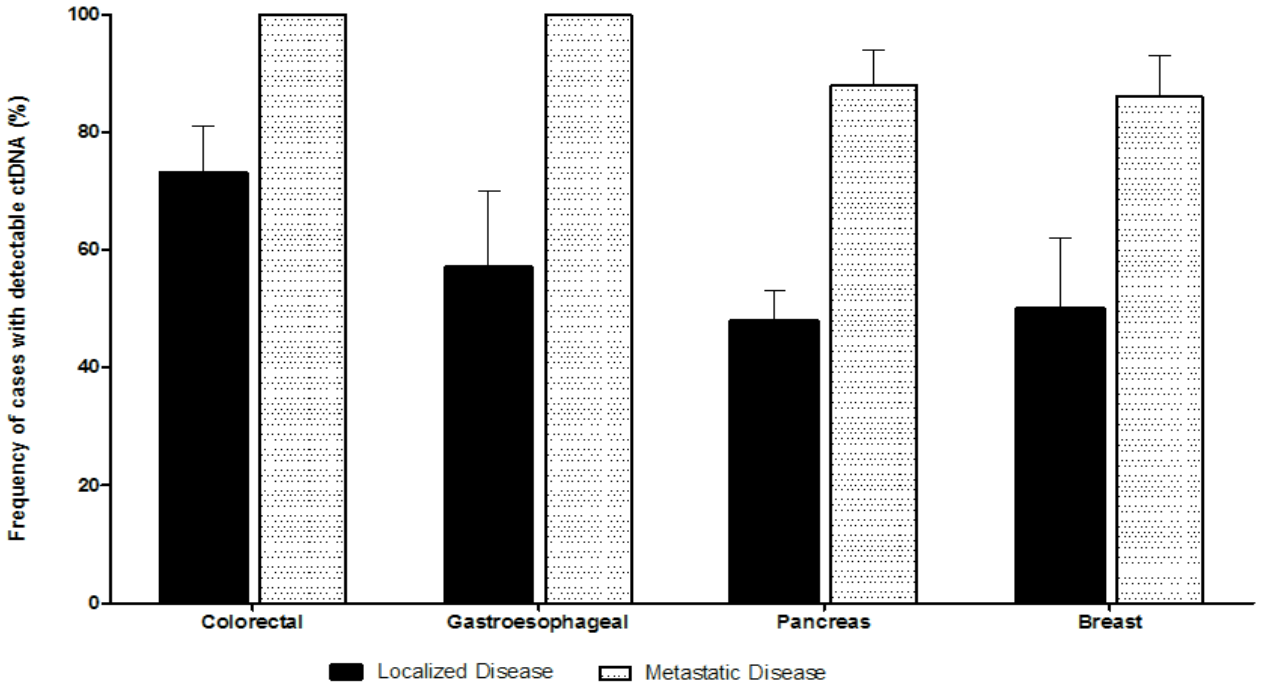
14 Tumor types (n = 684)





Bettegowda et al, Sci Tran Med Feb 2014

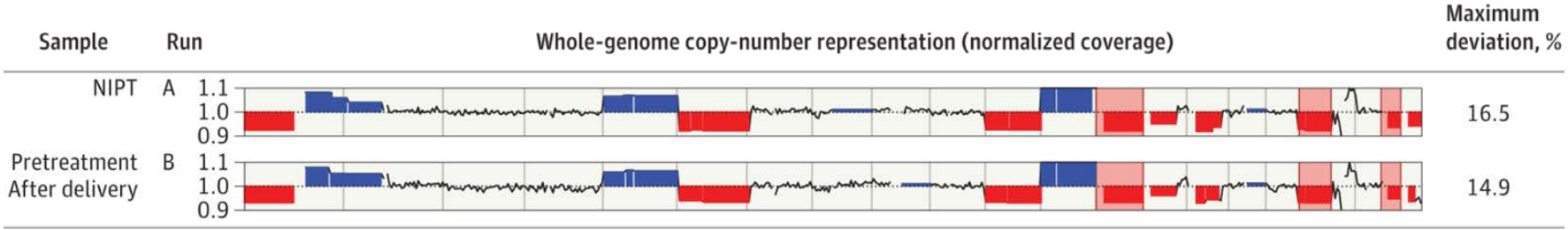
Early Detection using ctDNA Analyses



Bettegowda et al, Sci Tran Med Feb 2014

Detection of Occult Malignancy from analyses of cell free fetal DNA

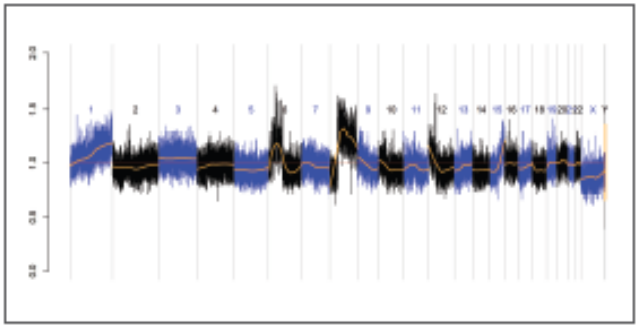
125,426 NIPT tests
3757 (3%) positive for 1 or more aneuploidies
10 cases of maternal cancer were identified



36 year old female at 20 weeks gestation
Monosomy in chromosomes 21, 18 and 13 persisted post-delivery
Diagnosed with stage IIA Hogdkin disease

JAMA. **2015;314(2):162-169.**

Detection of Occult Malignancy from analyses of cell free fetal DNA



400,000 NIPT Tests
38 confirmed aneuploidies with neoplasm
17 Mutant, 15 benign, 6 Unclassified

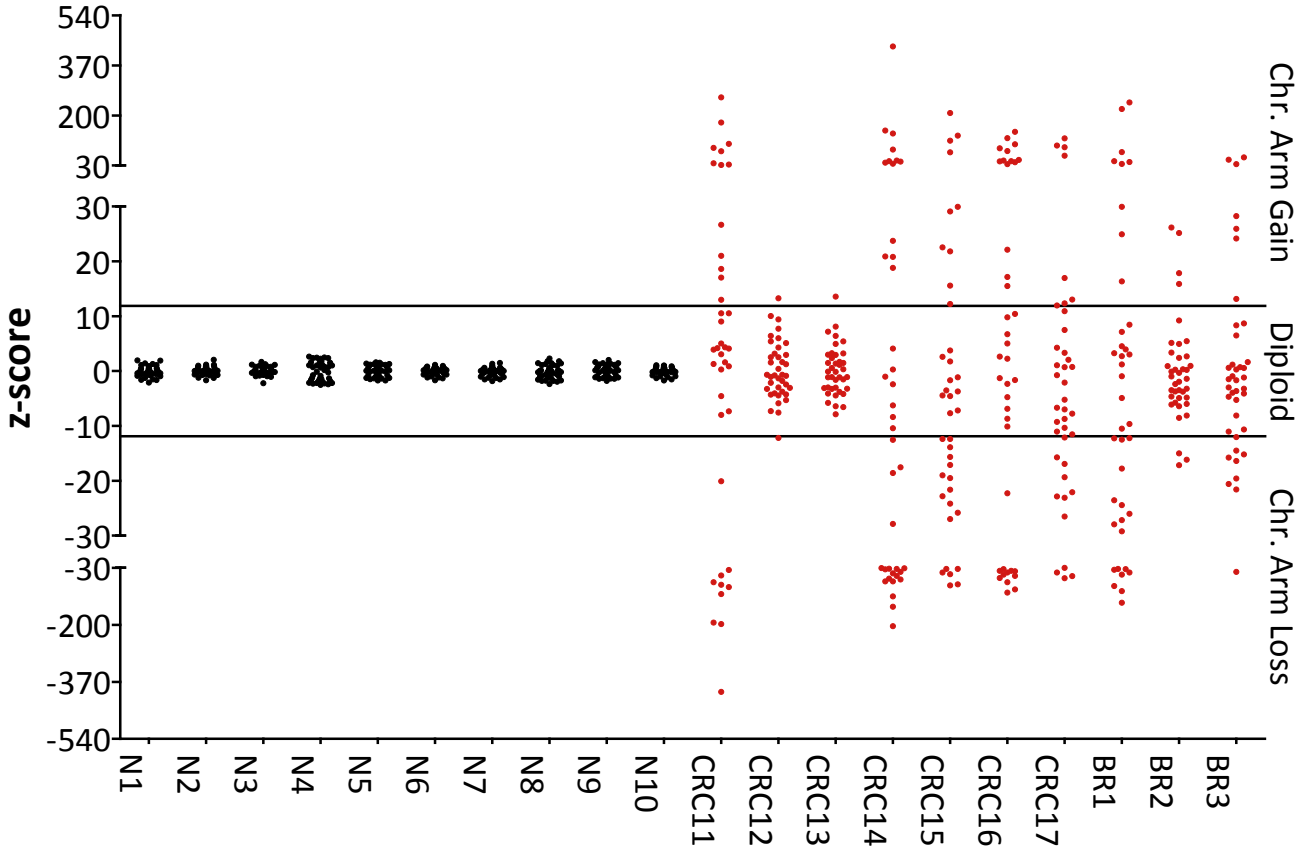
Type and frequency of maternal malignancies identified adventitiously by NIPT.

Diagnosis	No. of Cases
Hodgkins Lymphoma	2
Non-Hodgkin's Lymphoma	2
Follicular Lymphoma	1
Multiple Myeloma	1
Breast Carcinoma	3
Angiosarcoma	1
Colon Carcinoma	2
Uterine Leiomyoma	11
Uterine Leiomyosarcoma	1
Teratoma (Dermoid Cyst) of Right Ovary	1
Mass on Right Fallopian Tube	1
Non-Reportable, Clinical Feedback Pending	12
Total	38

Nilesh Dharajiya et al. AMP Abstract 2015

sequenom Laboratories

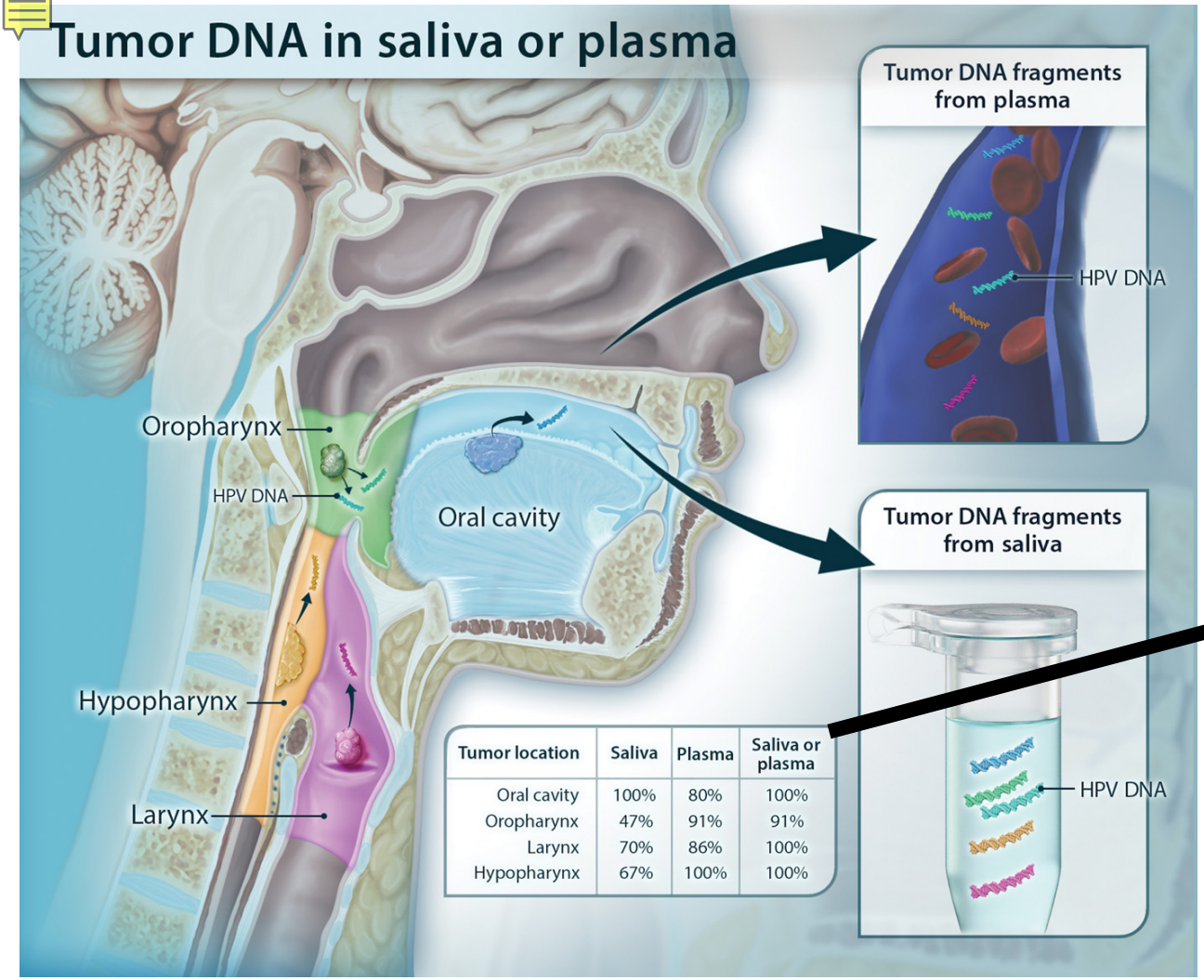
Aneuploidy in normal in cancer patients ctDNA



Leary, Sausen, and Kinde et al. Sci Transl Med. 2012 Nov 28;4(162):162ra154.

Clinical Application of Cancer Genetics

Early Detection – Saliva



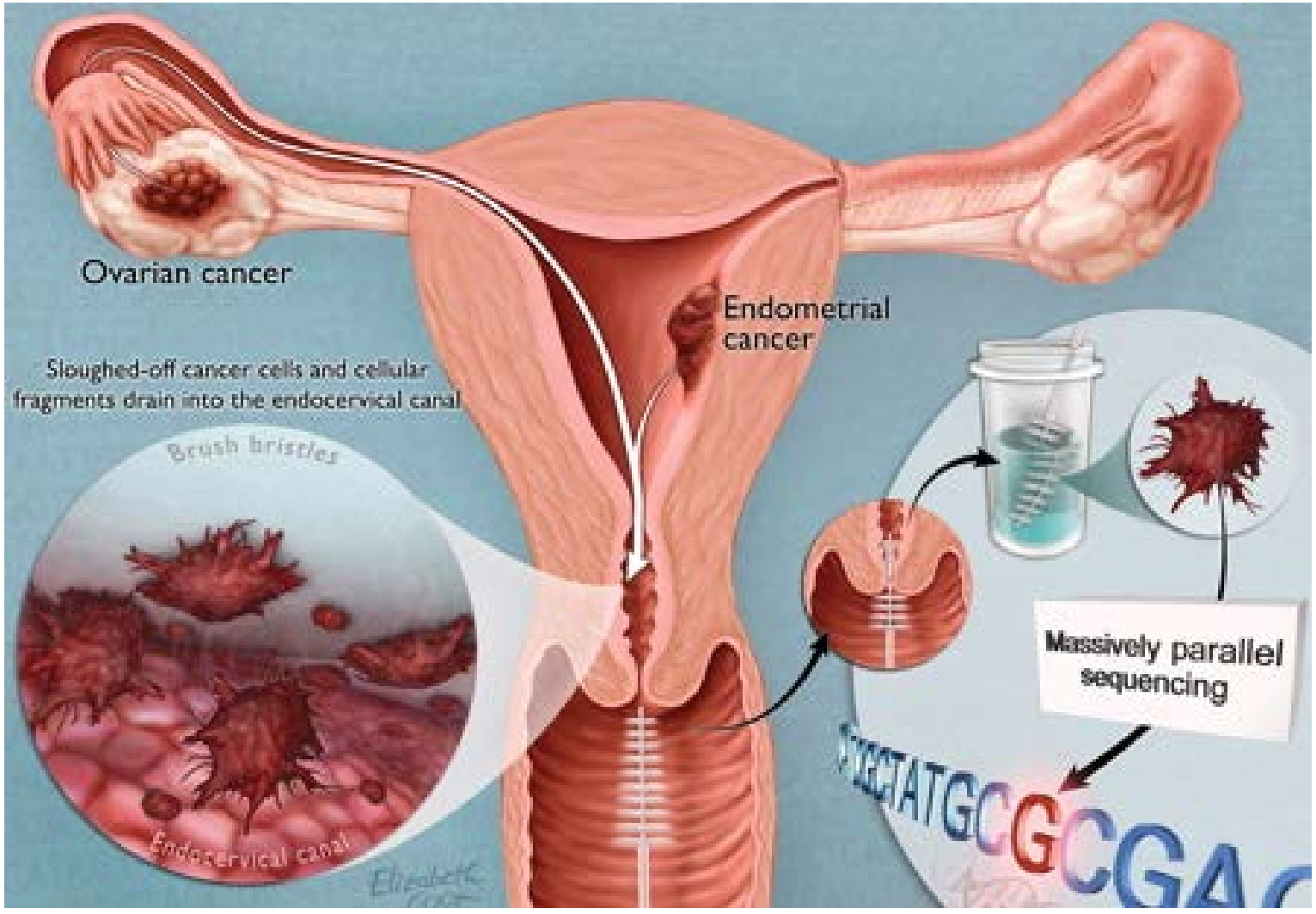
Sensitivity

Tumor location	Saliva
Oral cavity	100%
Oropharynx	47%
Larynx	70%
Hypopharynx	67%

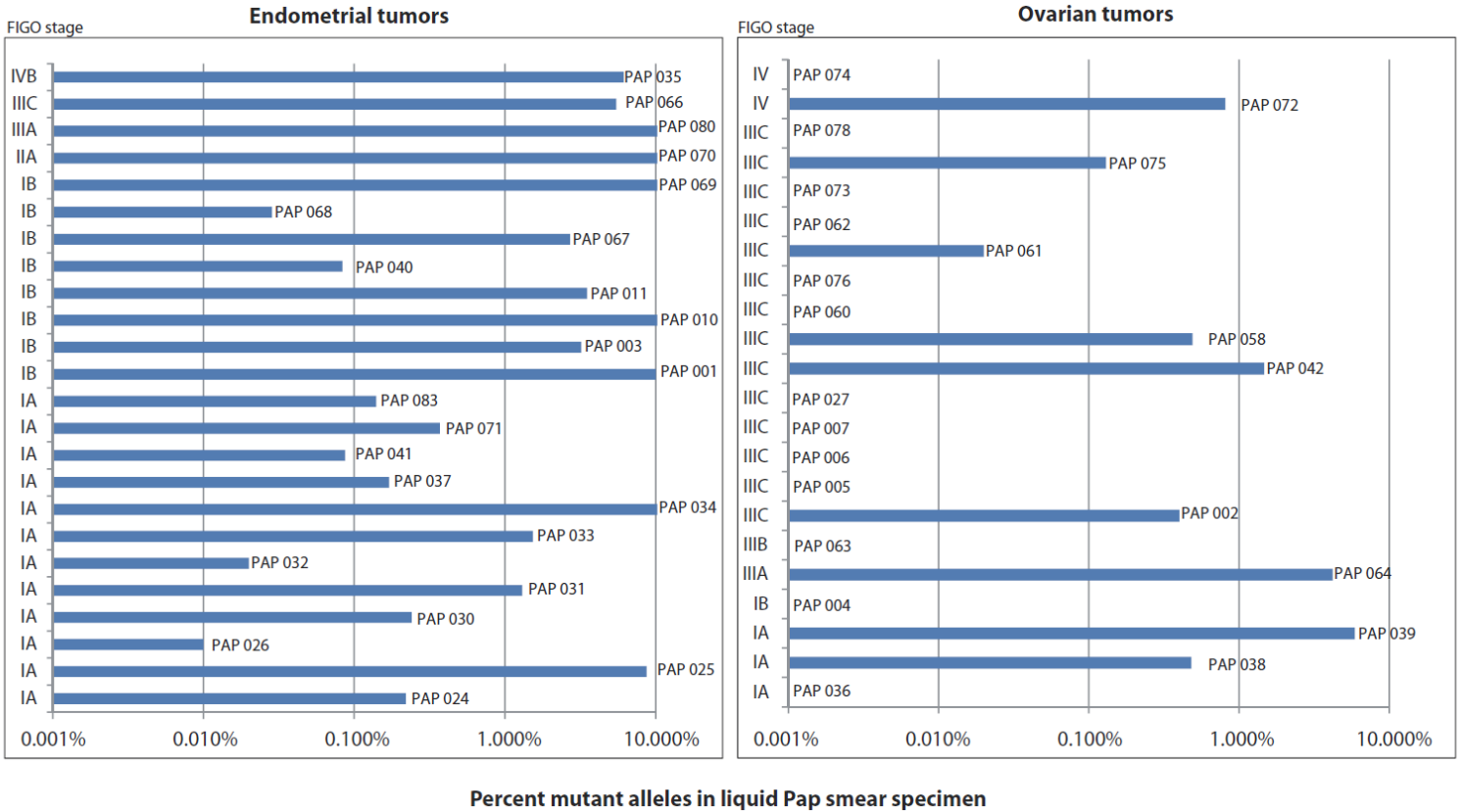
Yuxuan Wang et al., Sci Transl Med 2015;7:293ra104

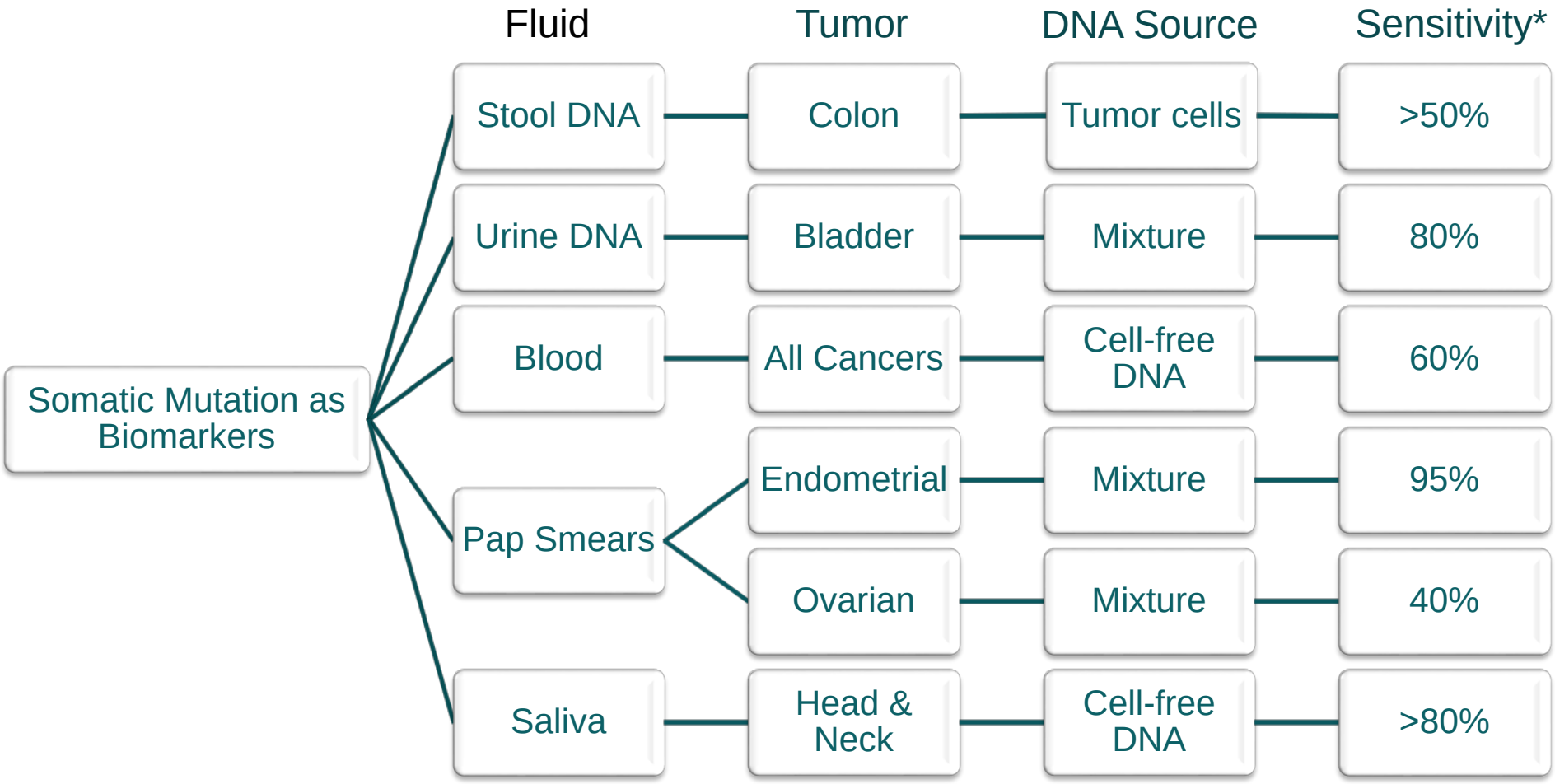
Clinical Application of Cancer Genetics

Early Detection – Pap Smears


Ovarian cancer
Endometrial cancer
Sloughed-off cancer cells and cellular fragments drain into the endocervical canal
Brush bristles
Endocervical canal
Massively parallel sequencing

Early Detection - GenePap





*Stage I and II Disease

Clinical Application of Cancer Genetics

Challenges

Not all clonal events are cancer

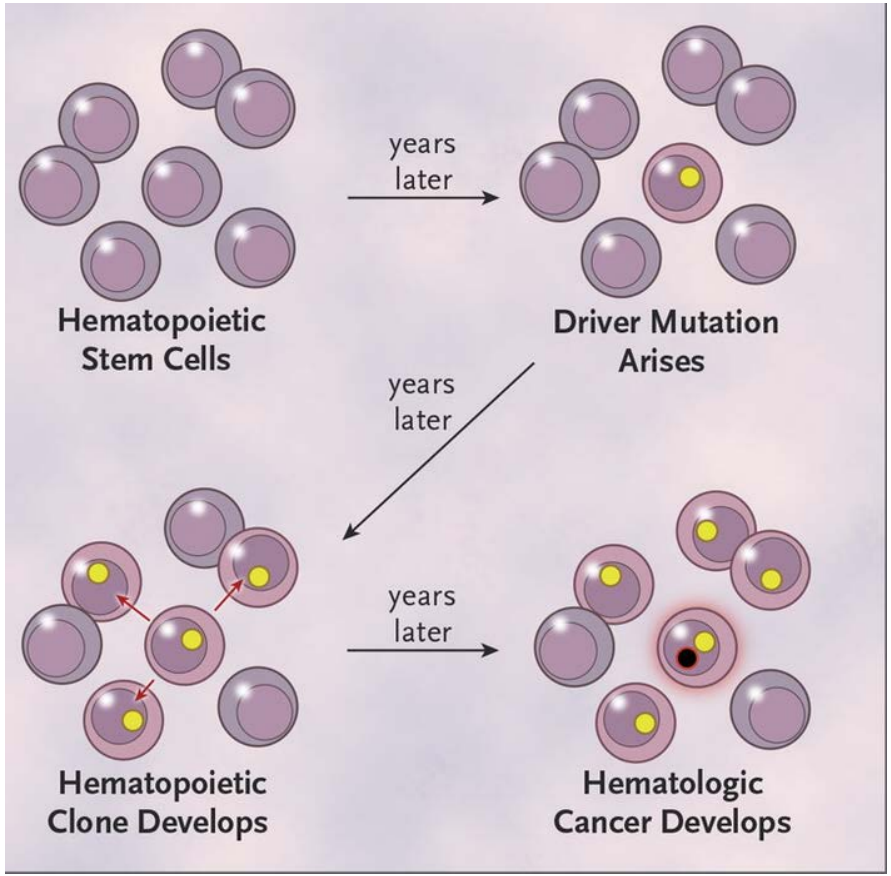
The NEW ENGLAND JOURNAL of MEDICINE

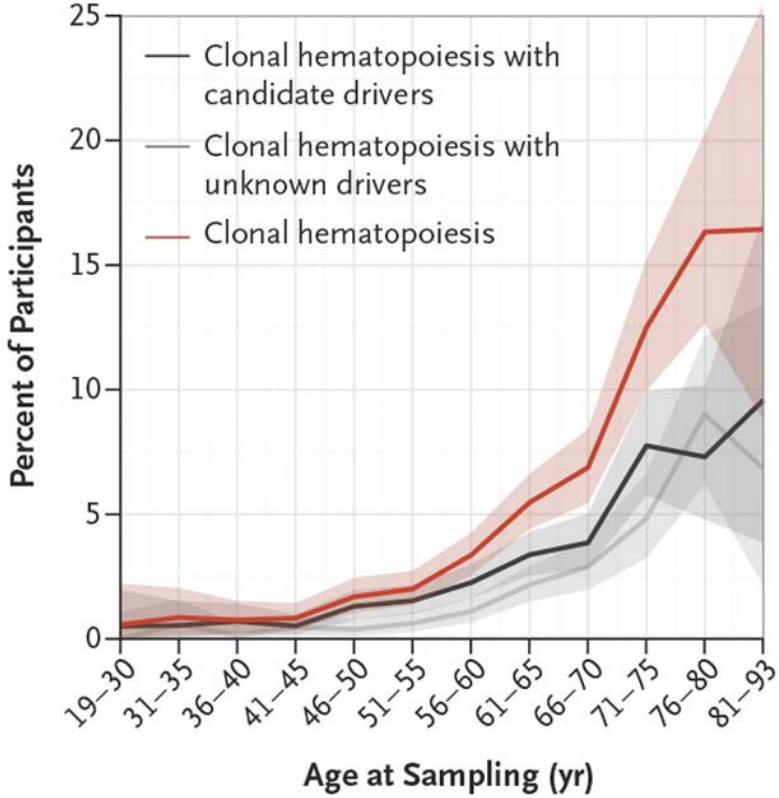
ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Whole-exome sequencing of DNA in peripheral-blood cells from 12,380 persons → somatic mutations characteristic of hematologic malignancies were observed in 10% of persons older than 65 years of age

Genovese et al., N Engl J Med 2014; 371:2477-2487





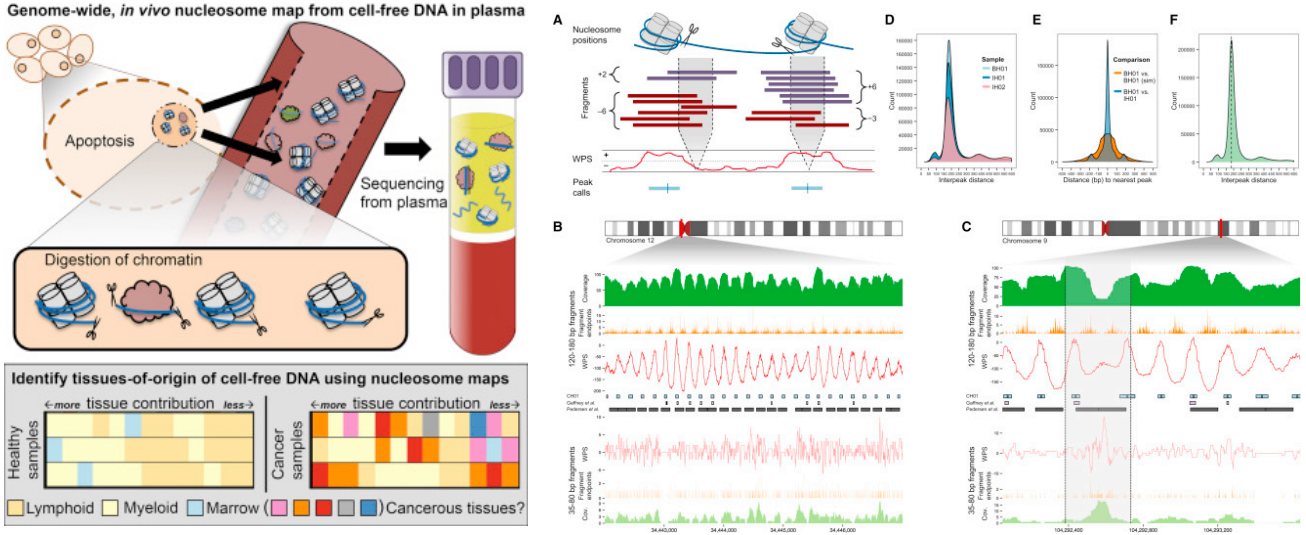
Genovese G et al. N Engl J Med 2014;371:2477-2487.

Localization

CASE: A 55 year old male was found to have a persistent KRAS mutation (G12D) in ctDNA at >0.8%

CT Scan, PET Scan, Colonoscopy and PSA are normal.

What is this? Lung, Colon Pancreas?

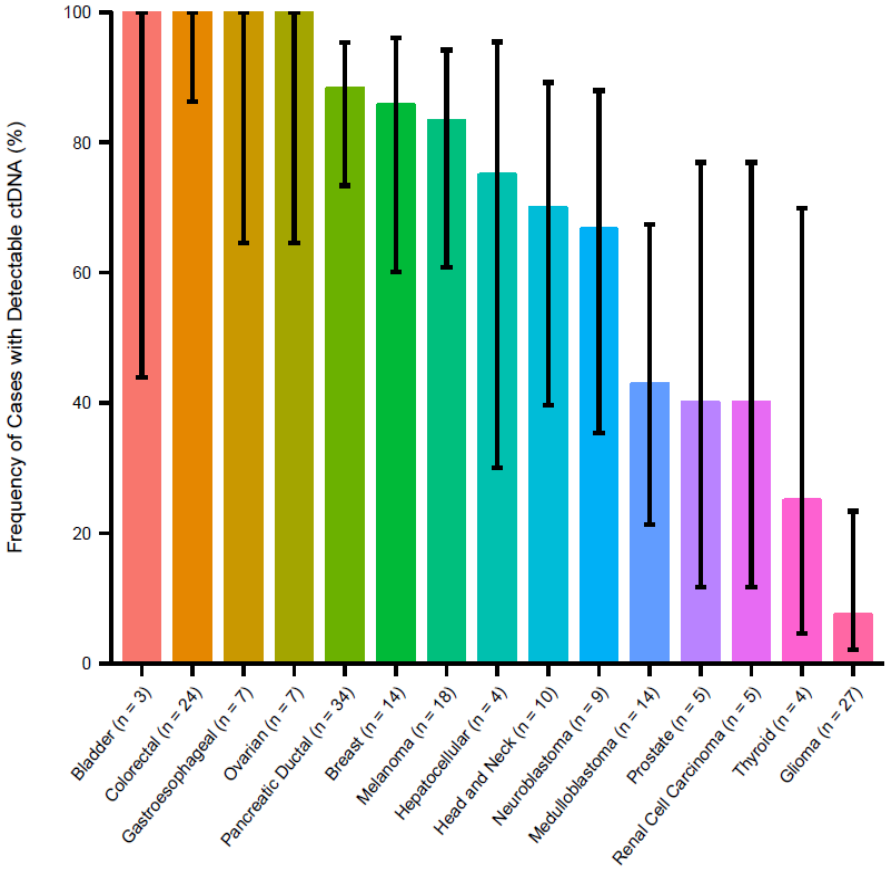


Matthew W. Snyder, Martin Kircher, Andrew J. Hill, Riza M. Daza, Jay Shendure

Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin

Cell, Volume 164, Issues 1–2, 2016, 57–68

Heterogeneity



- ~80% late stage tumors shed ctDNA
- Anatomic barriers to tumor DNA release into circulation
- Heterogeneity in shedding

Future for ctDNA

Incremental improvements in technology

- Increase in comprehensive panels
- Limited by biology more that technology
- Need a biologic based discovery to drive dramatic improvement

Future for ctDNA

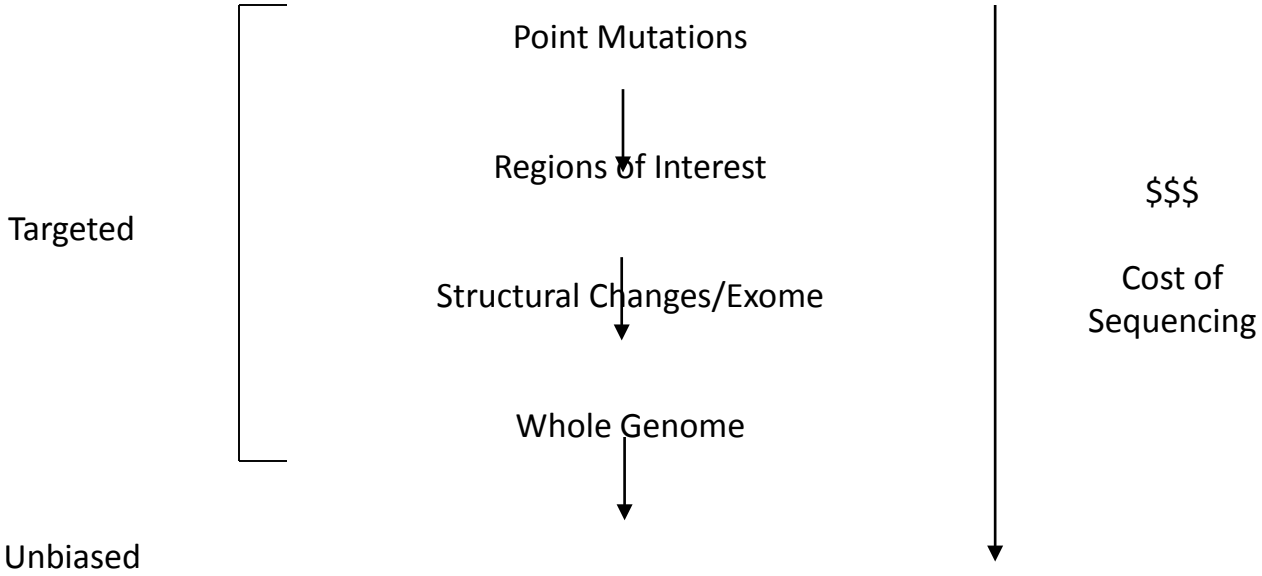
Incremental improvements in technology

- Increase in comprehensive panels
- Limited by biology more that technology
- Need a biologic based discovery to drive dramatic improvement

Clinical Application

- Tumor genotyping in plasma will be integrate into routine practice – based on concordance studies
- High impact applications that drive improvements in OS will require prospective clinical trials and partnership with FDA.

Summary


Point Mutations
Regions of Interest
Structural Changes/Exome
Whole Genome
Targeted
Unbiased
$$$
Cost of Sequencing

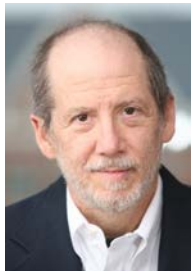
Summary

- Somatic mutations can be effective biomarkers largely because of specificity
- Digital Genomics has improved sensitivity and throughput sufficient for real clinical application
- Applications for detecting occult disease for minimal residual disease detection and screening for cancer
- Broad commercialization will require overcoming cost, regulatory, payor and definitive clinical studies demonstrating clinical benefit.

Acknowledgments

Acknowledgments

Thank you



B. Vogelstein

Ken Kinzler

N. Papadopoulos

V. Velculescu

Shibin Zhou

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