Circulating Tumor DNA

Matthias Holdhoff, MD, PhD
Assistant Professor of Oncology
Johns Hopkins University

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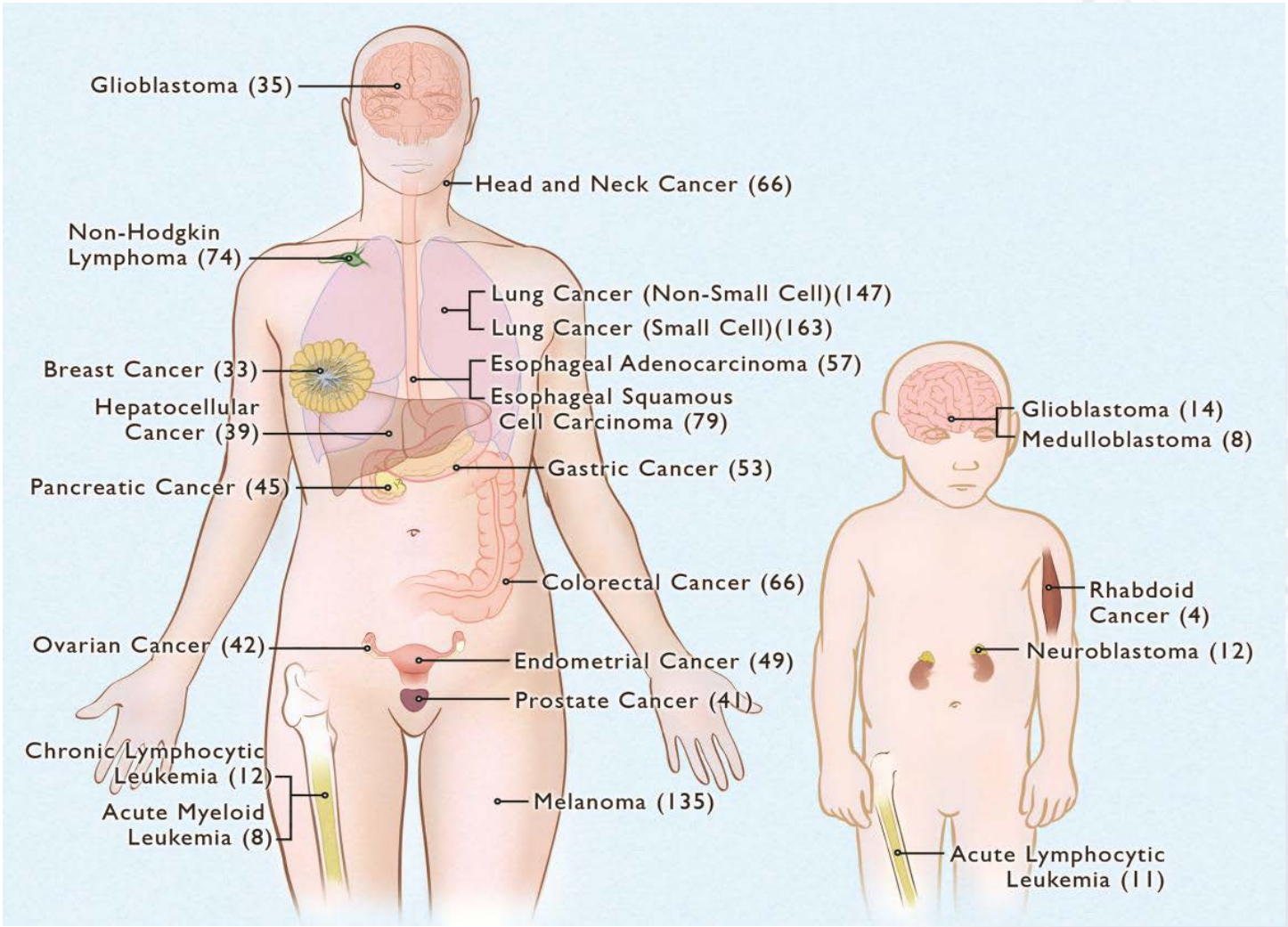
Conflict of Interest

- No financial conflict of interest
- I will not discuss off label use and/or investigational use in my presentation

Objectives

- What is circulating tumor DNA (ctDNA)?
- Methods of detection
- Potential use in clinical oncology
- Challenges in clinical implementation

Human Cancer Exomes Sequenced



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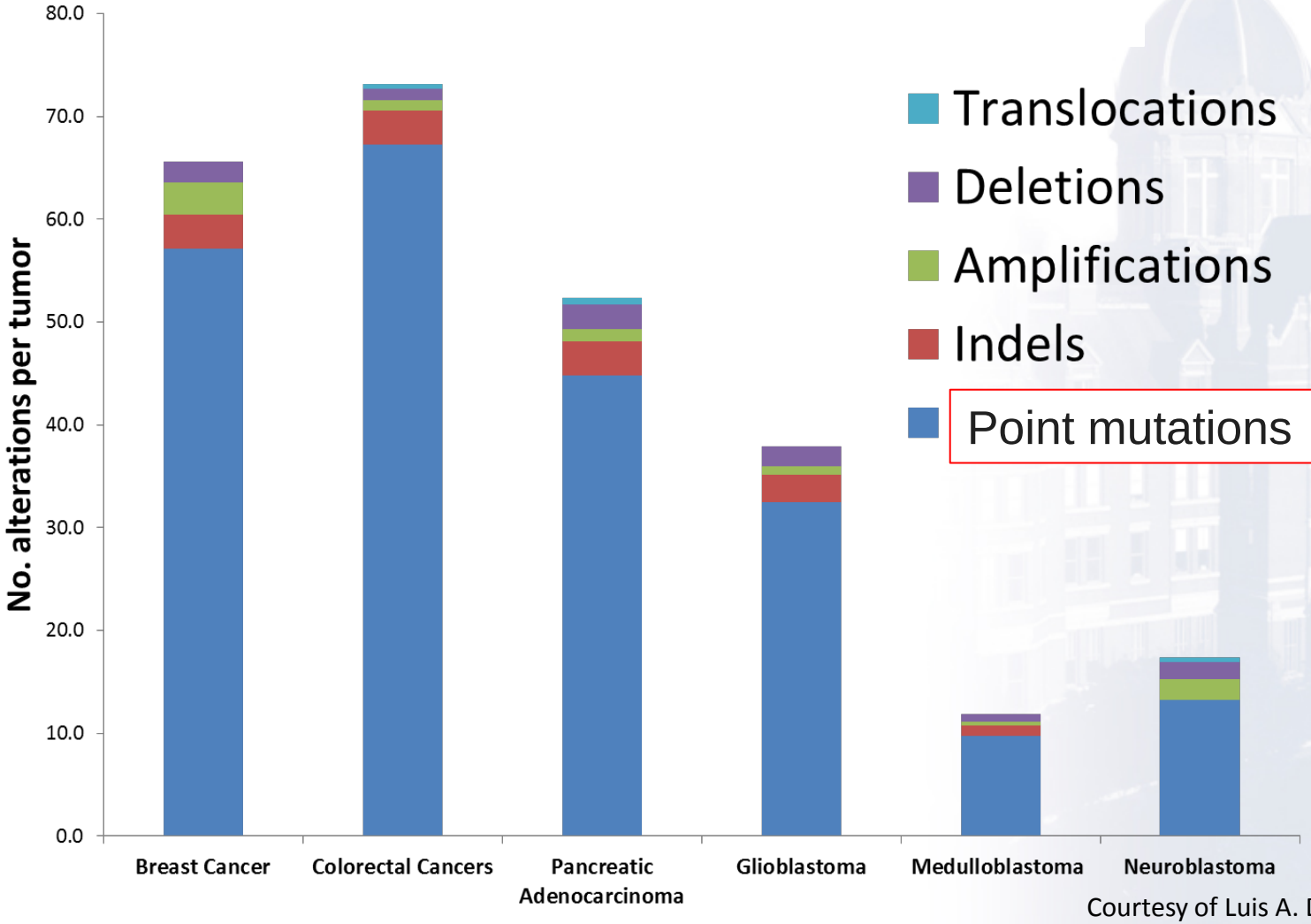
Genetic Alternations from Genome-wide Studies



Courtesy of Luis A. Diaz, Jr.

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Access to Tumor-specific Mutations

Tumor tissue

- FFPE
- Frozen tissue

Blood & other bodily fluids

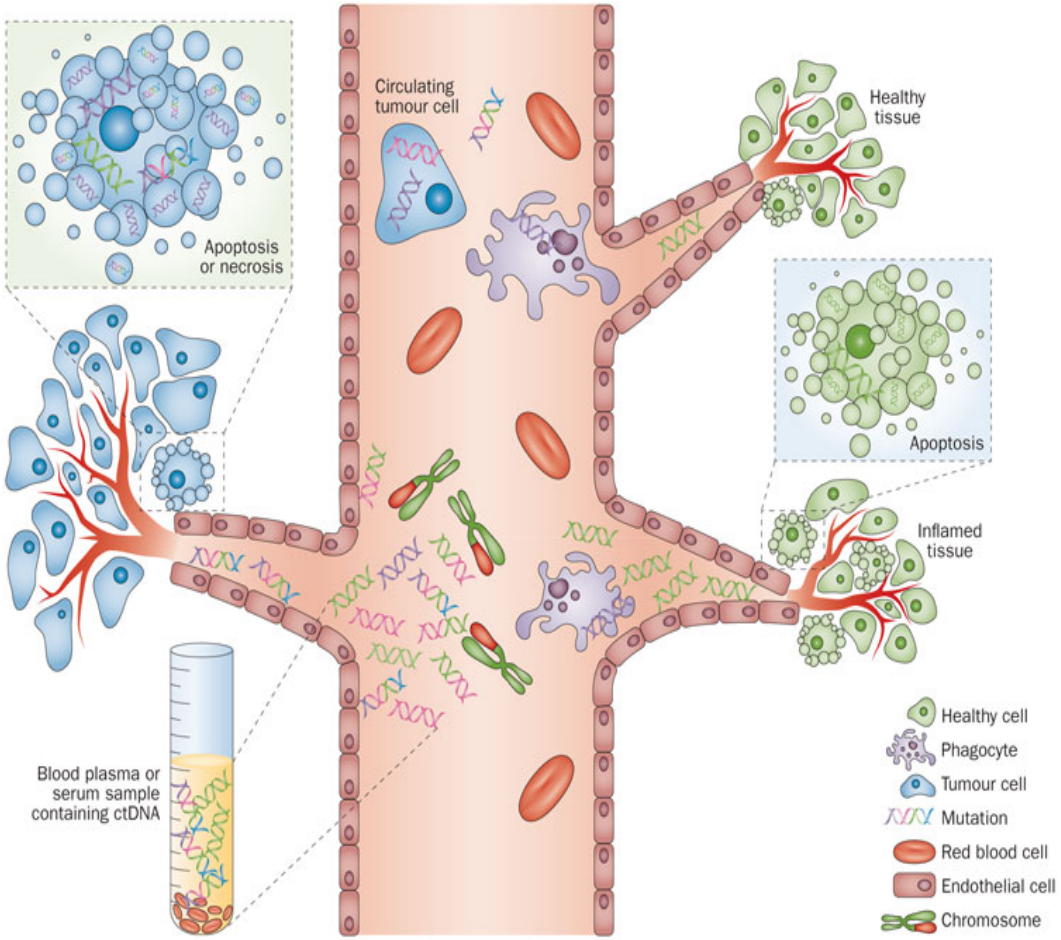
- Cell-free DNA
- Circulating tumor cells (CTCs)

Circulating Tumor DNA (ctDNA)

- ctDNA ≠ not circulating tumor cells
- Cell-free DNA released during cellular turnover
- ctDNA fragments are small 180-200bp
- ctDNA fragments can contain tumor specific somatic mutations

Tumor DNA → Blood

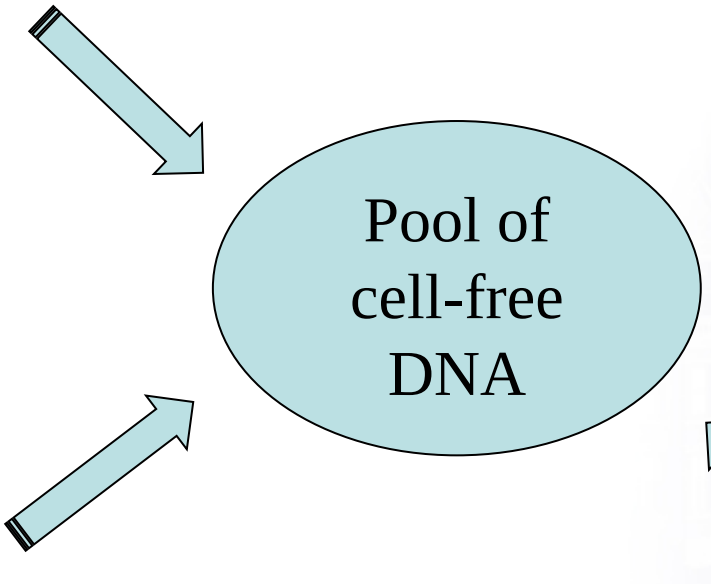
Cancer cells



Normal cells

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

Sources of Circulating Cell-free DNA



- Fluctuations with trauma, surgery, chemotherapy, radiaton
- Variations between individuals

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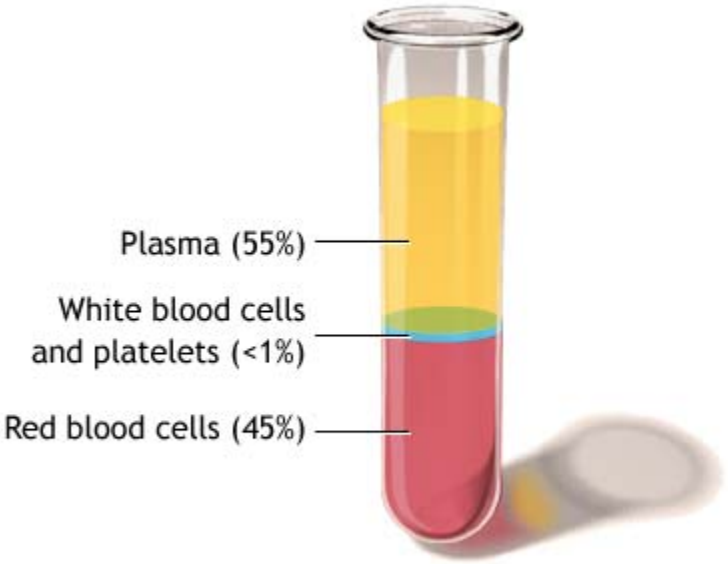
“Liquid Biopsy”



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)

Needle in the Haystack

5 mutant tumor DNA Fragment in a pool of 10,000 DNA fragments

=

0.05%

Technical limit of Sensitivity of Traditional Semi-Quantitative PCR is 1%

Digital Genomics

Assessment of DNA sequence variations by counting individual DNA molecules

Vogelstein & Kinzler, *Proc. Natl. Acad. Sci., USA* 96: 9236-9240, 1999

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Conventional “Analog” Approach

Combine all the pictures into a single mixture


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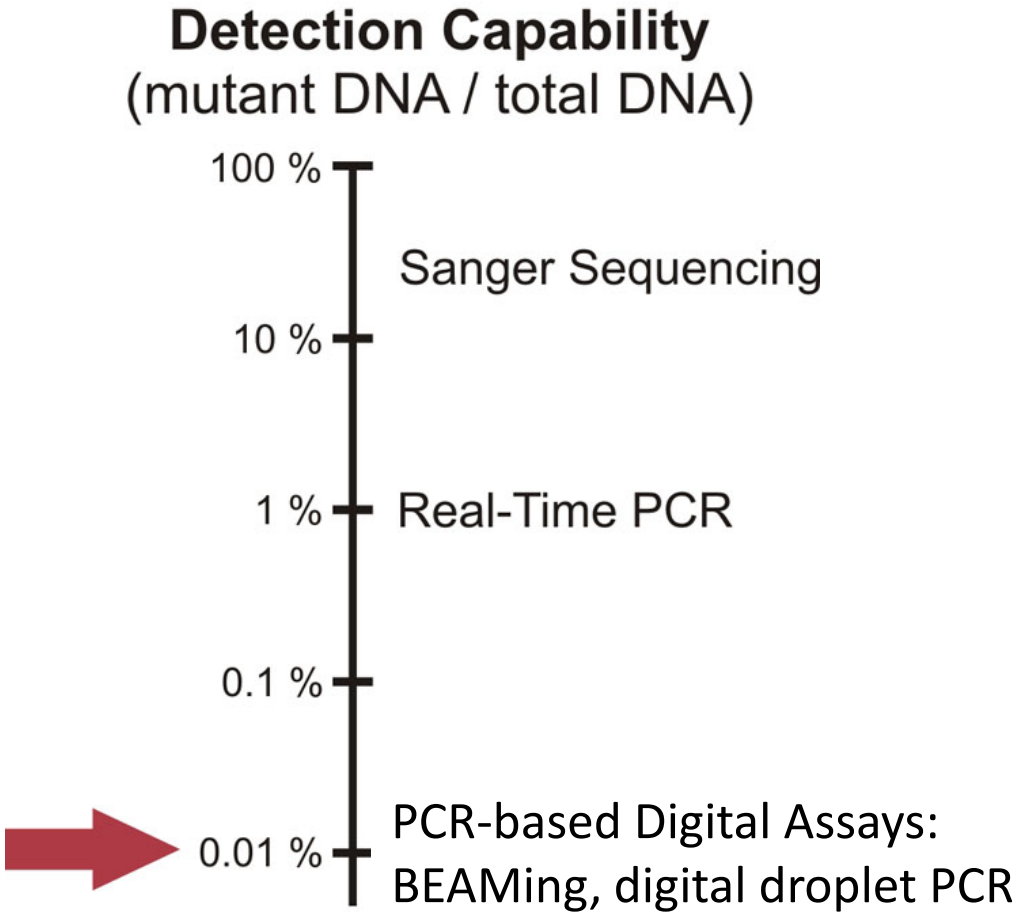
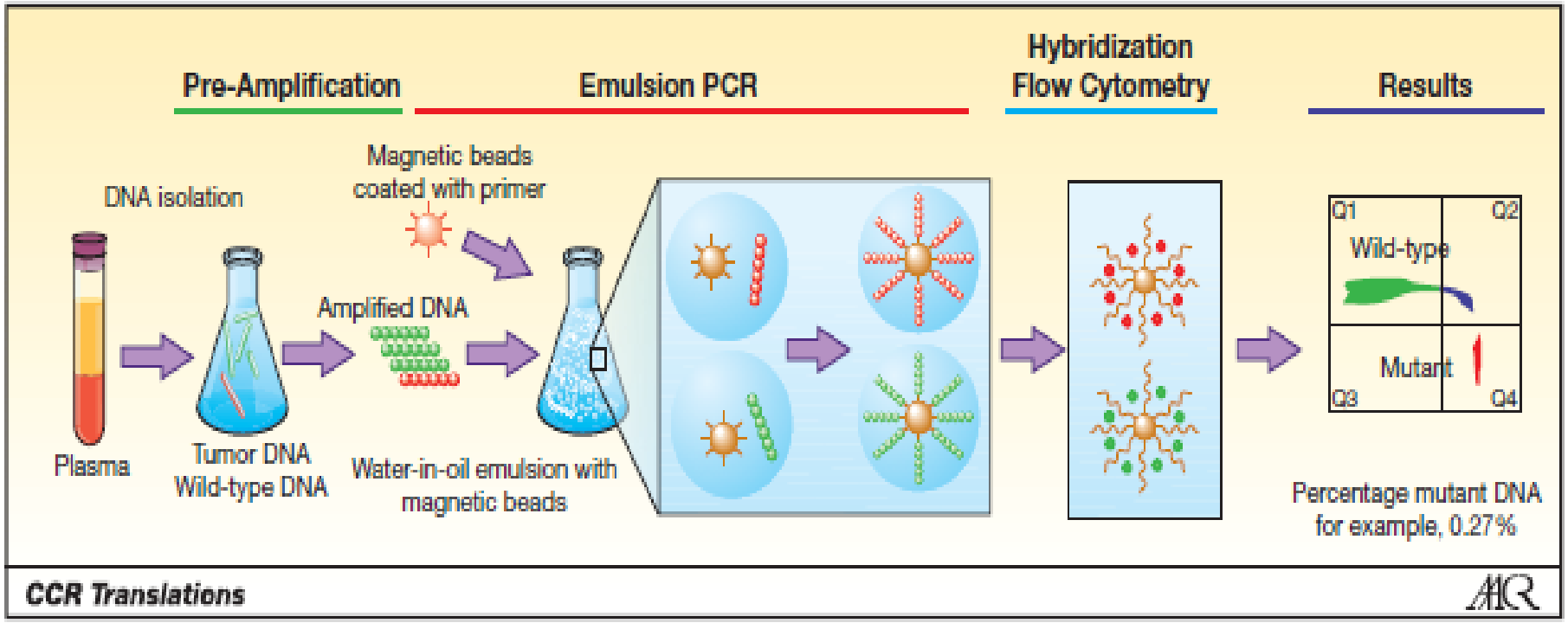
Technologies to assess ctDNA

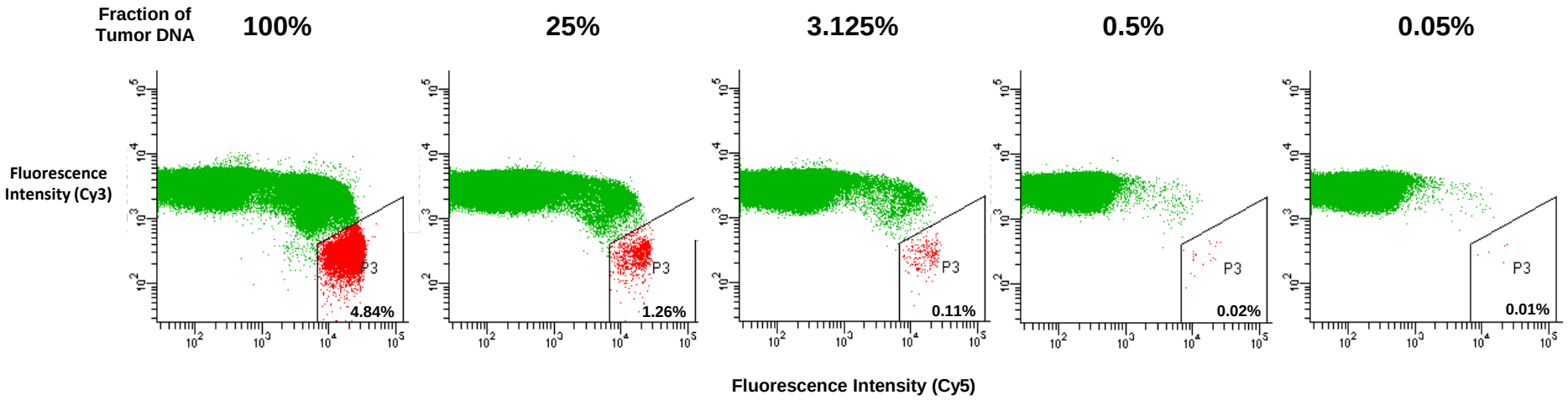


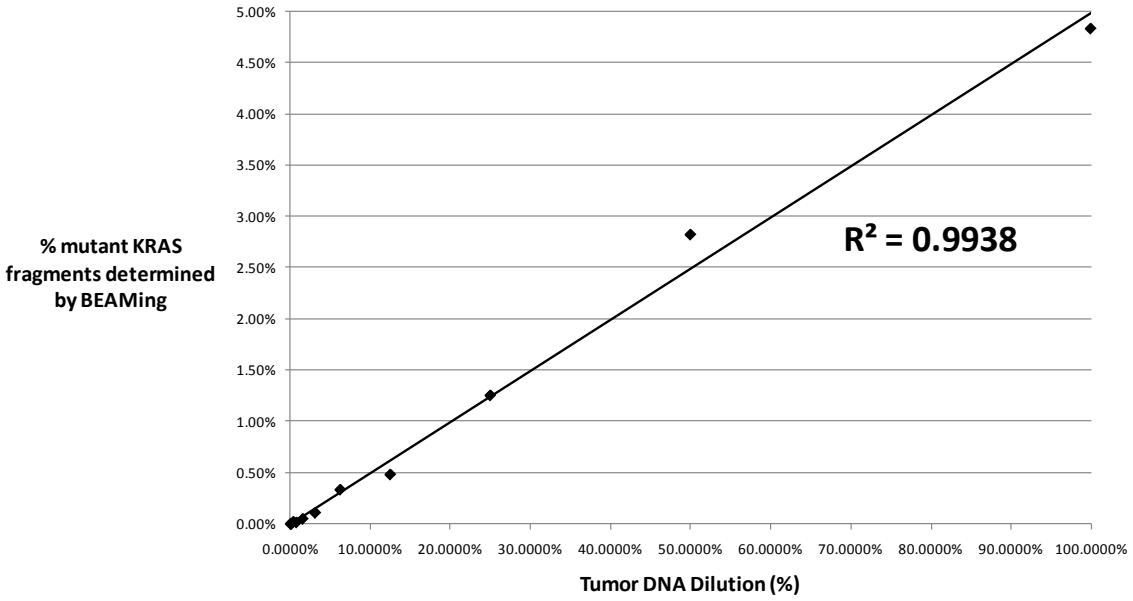
Gene sequencing-based assays:
Safe Seqs, Tam Seq, Capp Seq, PARE

BEAMing (Beads, Emulsion PCR, Amplification, Magnetics)



Lauring and Park, Clin Cancer Res, 2011





Detection rate:

~ 1:10,000

Holdhoff et al, JNCI 2009

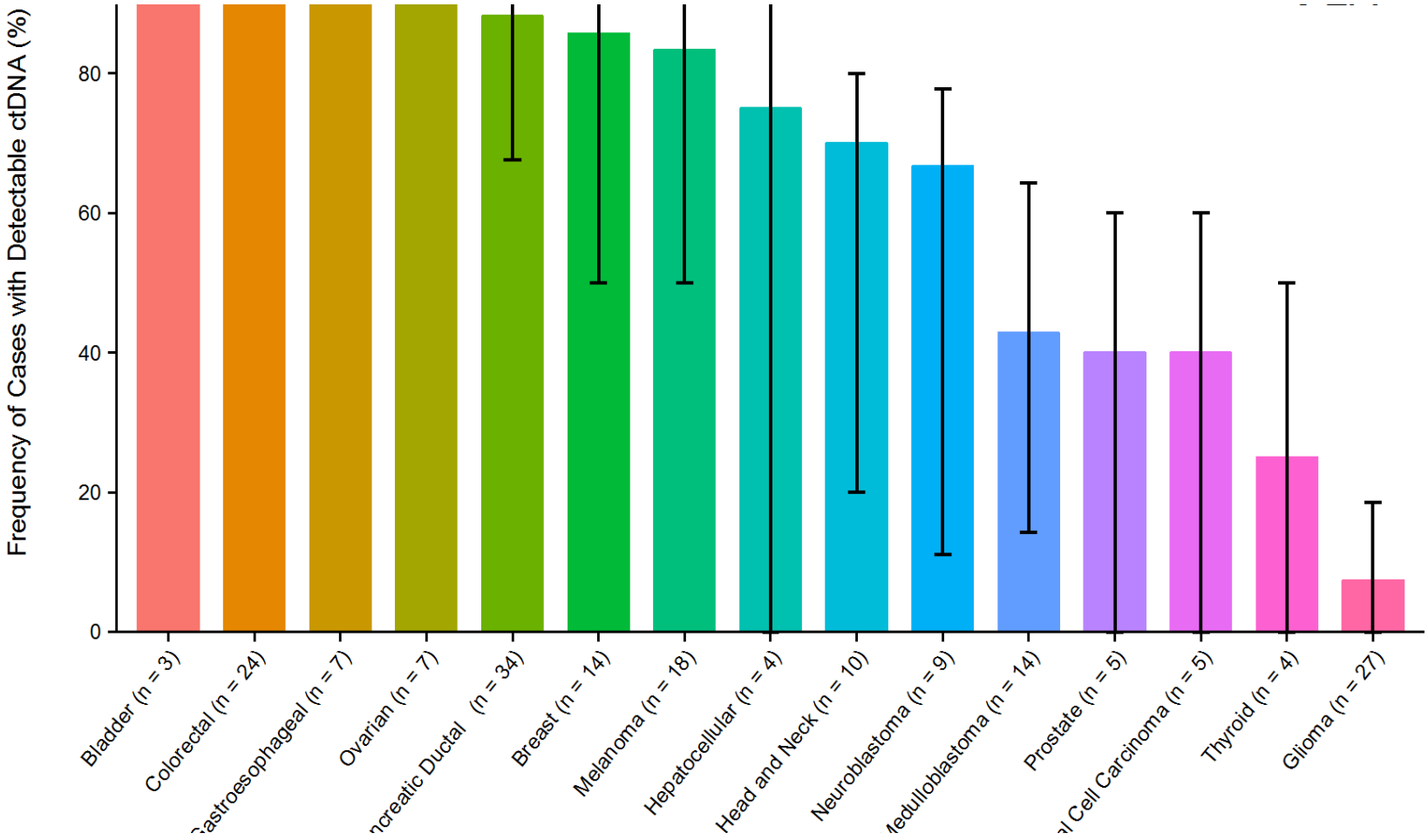
Liquid Biopsy: Appealing Features

- Non-invasive
- Multiple specimens
- Highly specific, highly sensitive assays available
- Fresh source of DNA
- No sampling bias

Liquid Biopsy: Potential Applications in Oncology

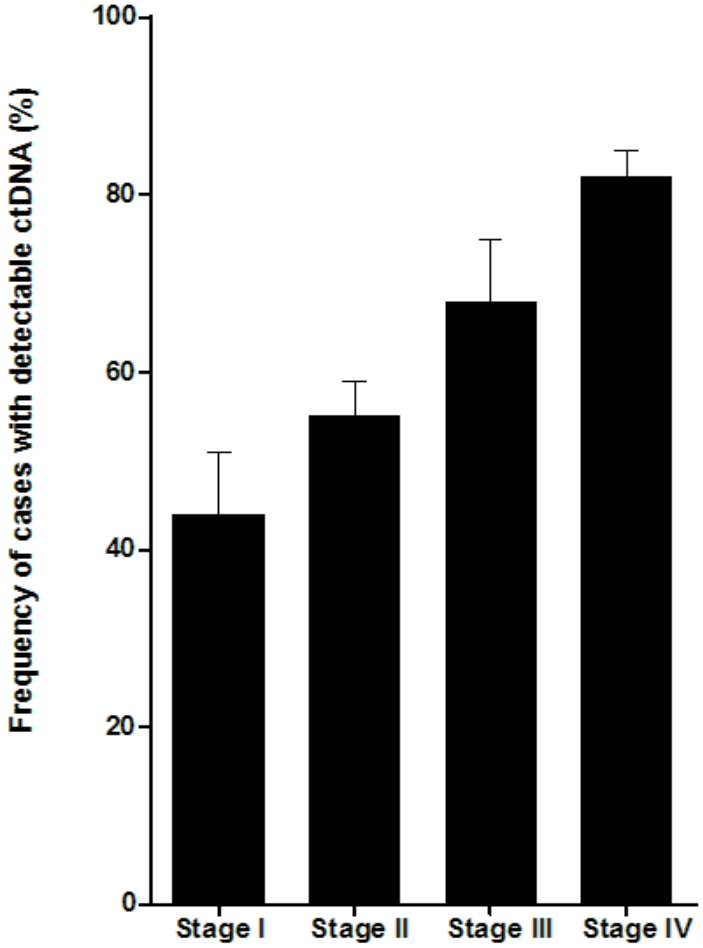
- Non-invasive determination of mutational status
- Monitoring of disease burden
- Tracking resistance
- Assessment of minimally residual disease
- Early detection

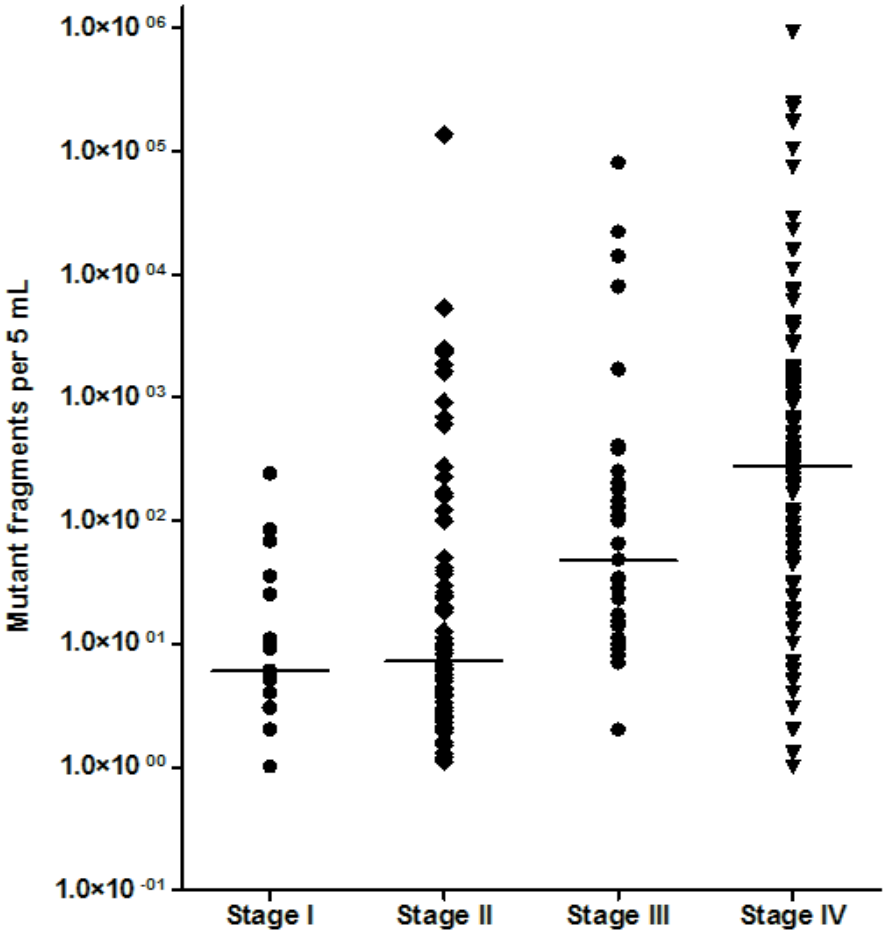
ctDNA and Different Cancer Types



Bettegowda et al, Science Transl Med 2014

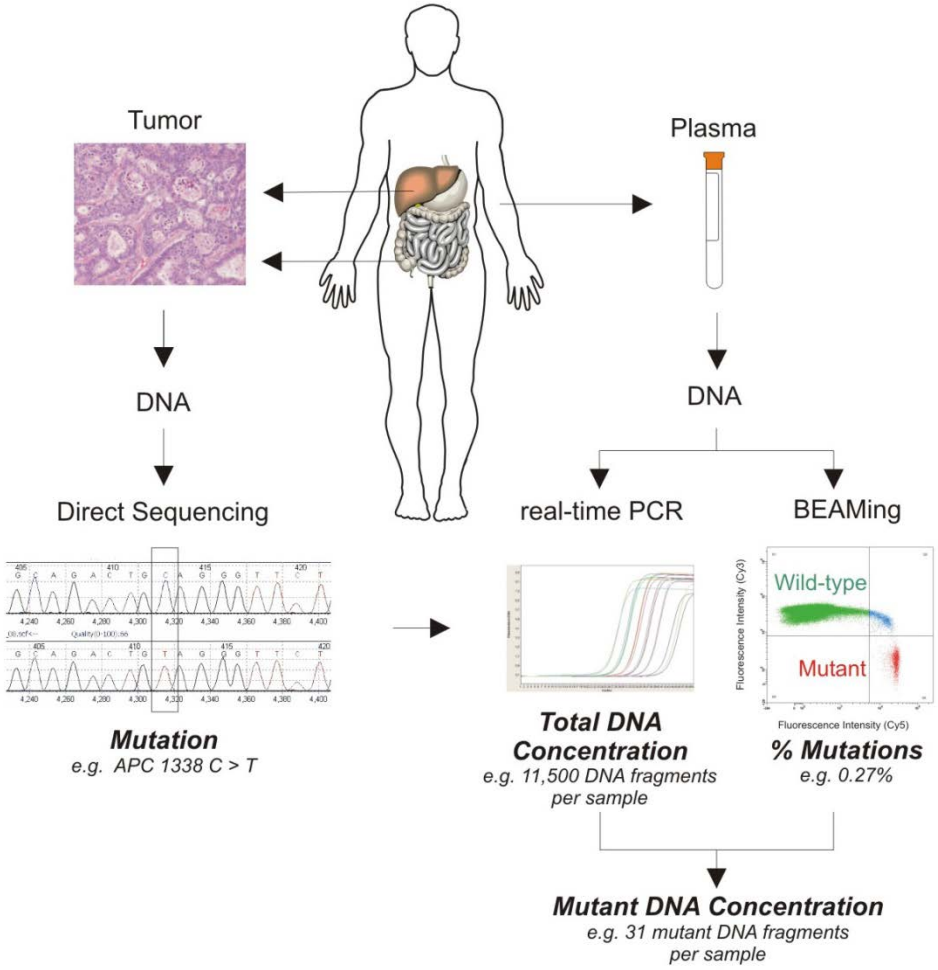
ctDNA and Tumor Stage





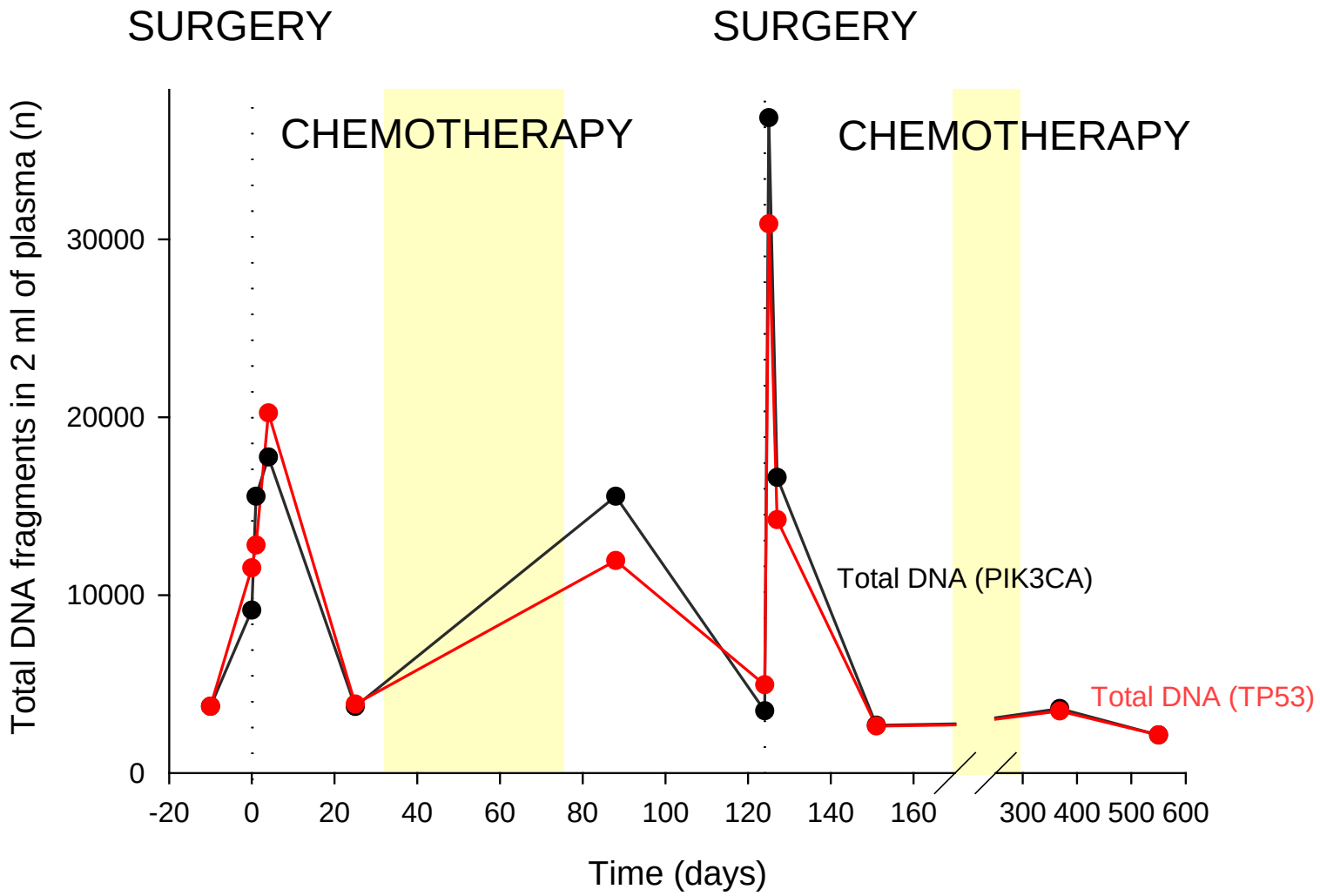
Bettegowda et al, Science Transl Med, 2014

ctDNA: Initial Studies



Diehl et al, Nature Medicine, 2008

ctDNA: Monitoring of Disease Burden



ctDNA and Tissue: Liquid Biopsy

Stage IV Breast Cancer (Higgins et al. Cancer Res. 2012)

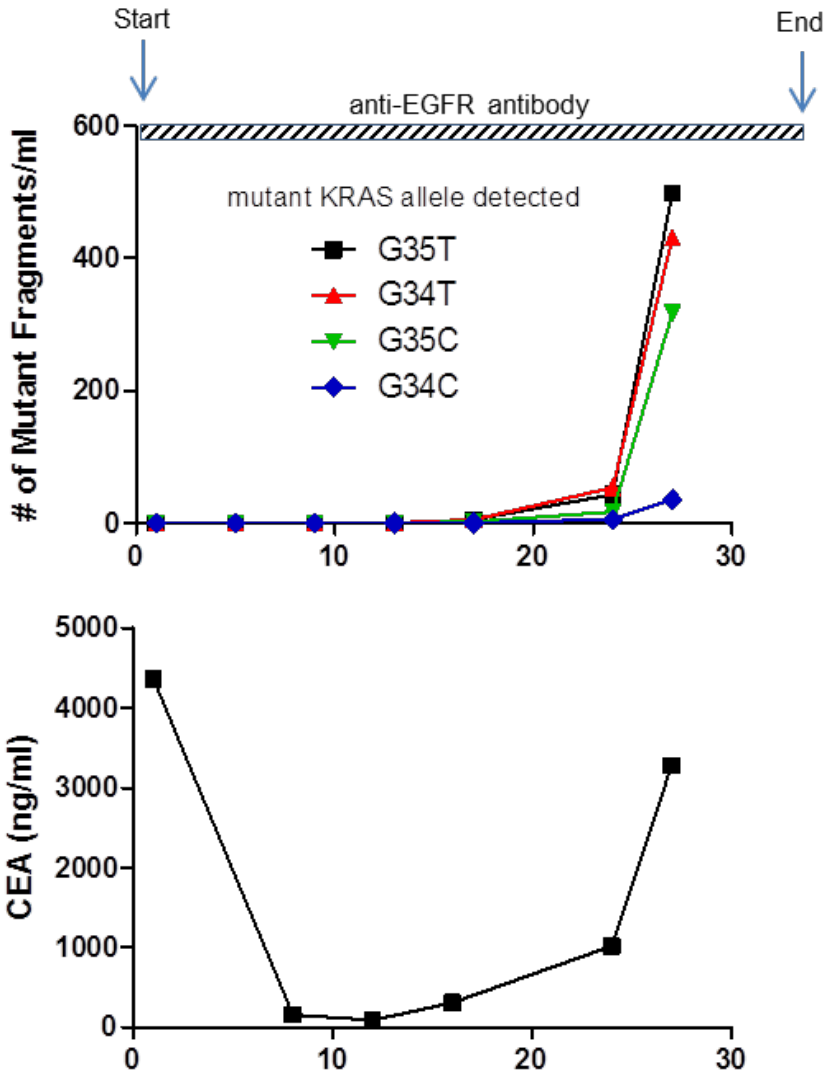
- Gene – PIK3CA
- n = 49
- Specificity – N/A
- Sensitivity - 100%
- Concordance - 100% (FFPE Tissue vs. Plasma DNA)

Stage IV Colon Cancer (Bettegowda et al. STM 2014)

- Gene – KRAS Codons 12 and 13
- n = 206
- Specificity- 99.2%
- Sensitivity- 87.2%
- Concordance - 95% (FFPE Tissue vs. Plasma DNA)

ctDNA: Tracking Resistance

Example:
Monitoring the emergence of resistant mutations in KRAS WT patients treated with EGFR blockade



Diaz et al, Nature, 2012

Clinical Implementation

- To date no clinical application of ctDNA been prospectively validated
- Hurdles
  - Challenges of appropriate sample collection in prospective clinical trials
  - High cost
  - Administrative barriers
  - Investigator time commitment
  - Minimal residual disease and screening studies: large patient numbers are needed

Where to Start?

- Dedicated funding and personal resources for large scale companion studies in conjunction with prospective clinical trials
- Standardization of specimen sampling, processing and storage
- Start with straight forward, simple question studies to optimize methodology
- Involve ctDNA experts early in clinical trial design

ctDNA: Summary

- Highly sensitive, specific and dynamic tumor marker
- Qualitative and quantitative: Provides a simultaneous snapshot of tumor genotype and tumor burden
- Many potential clinical applications
- No ‘one size fits all’: Different detection rates in different cancers, tumor stages, etc.

ctDNA: Summary

- **What we have: Good proof of principle data**
- **What we need: Prospective validation, large scale studies to determine the value of ctDNA for clinical practice**

Thank You

- Institute of Medicine
- Our patients and their families
- Bert Vogelstein, Ken Kinzler, Luis Diaz, Chetan Bettegowda, Ben Park, Nickolas Papadopoulos, Victor Velculescu and many others

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