OverView Circulating Nucleic Acids (CFNA) in Cancer Patients

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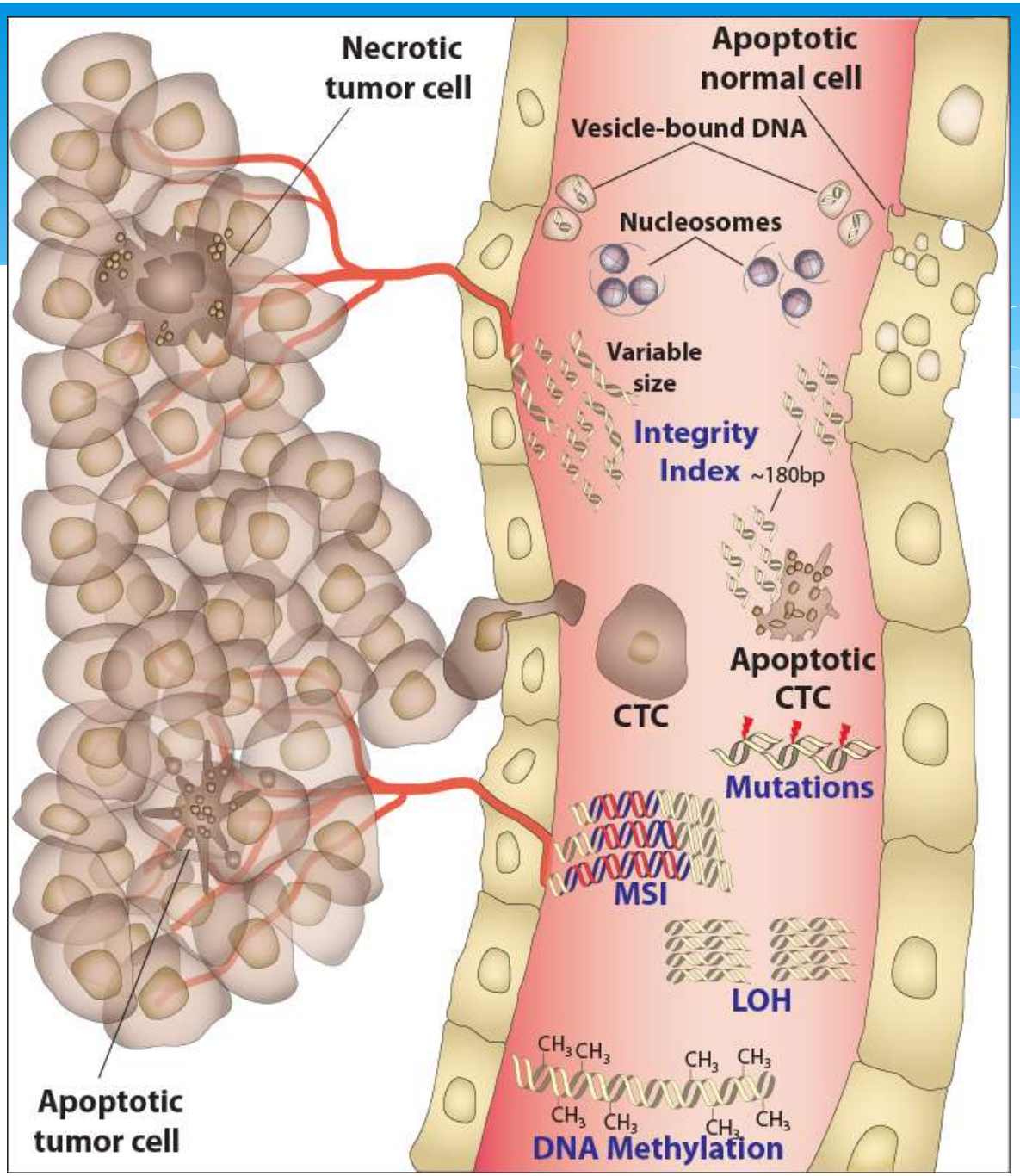
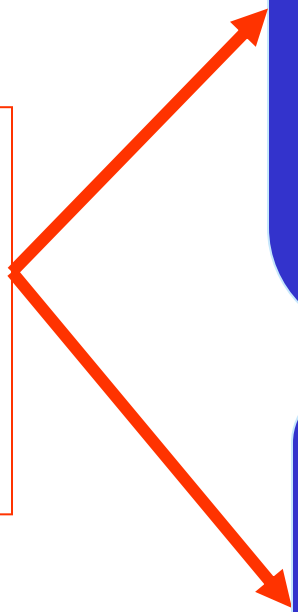

Necrotic tumor cell
Apoptotic normal cell
Vesicle-bound DNA
Nucleosomes
Variable size
Integrity Index
~180bp
CTC
Apoptotic CTC
Mutations
MSI
LOH
DNA Methylation
Apoptotic tumor cell

cfNA Blood Assays



DNA

Microsatellite (LOH)
Mutation
CpG site(s) promotor hypermethylation
DNA Integrity

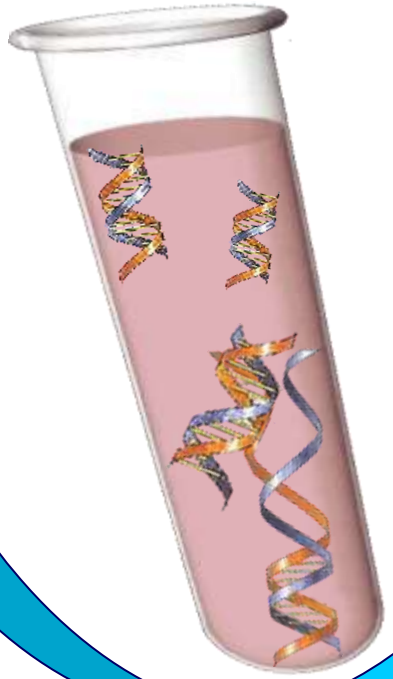
RNA

mRNA
miR

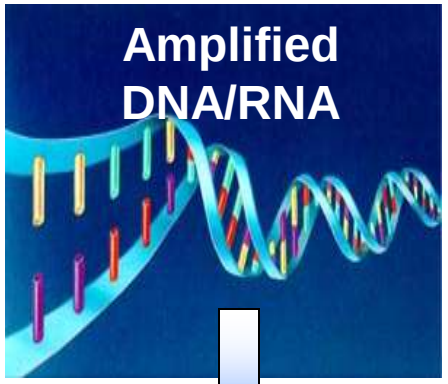
Cell-free nucleic acids as biomarkers in cancer patients.
Schwarzenbach H, Hoon DS, Pantel K.
Nat Rev Cancer. 2011

CFNA Direct Quantitative PCR Assays

Clinic

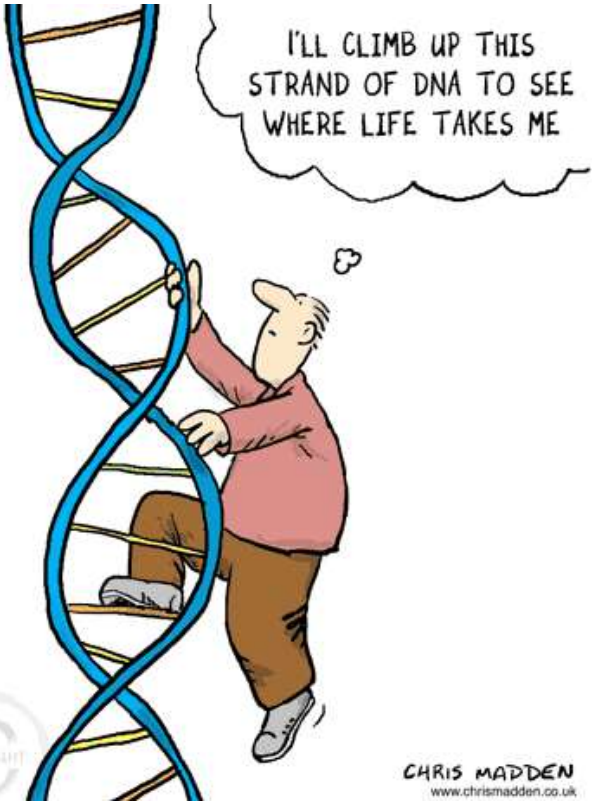


Laboratory

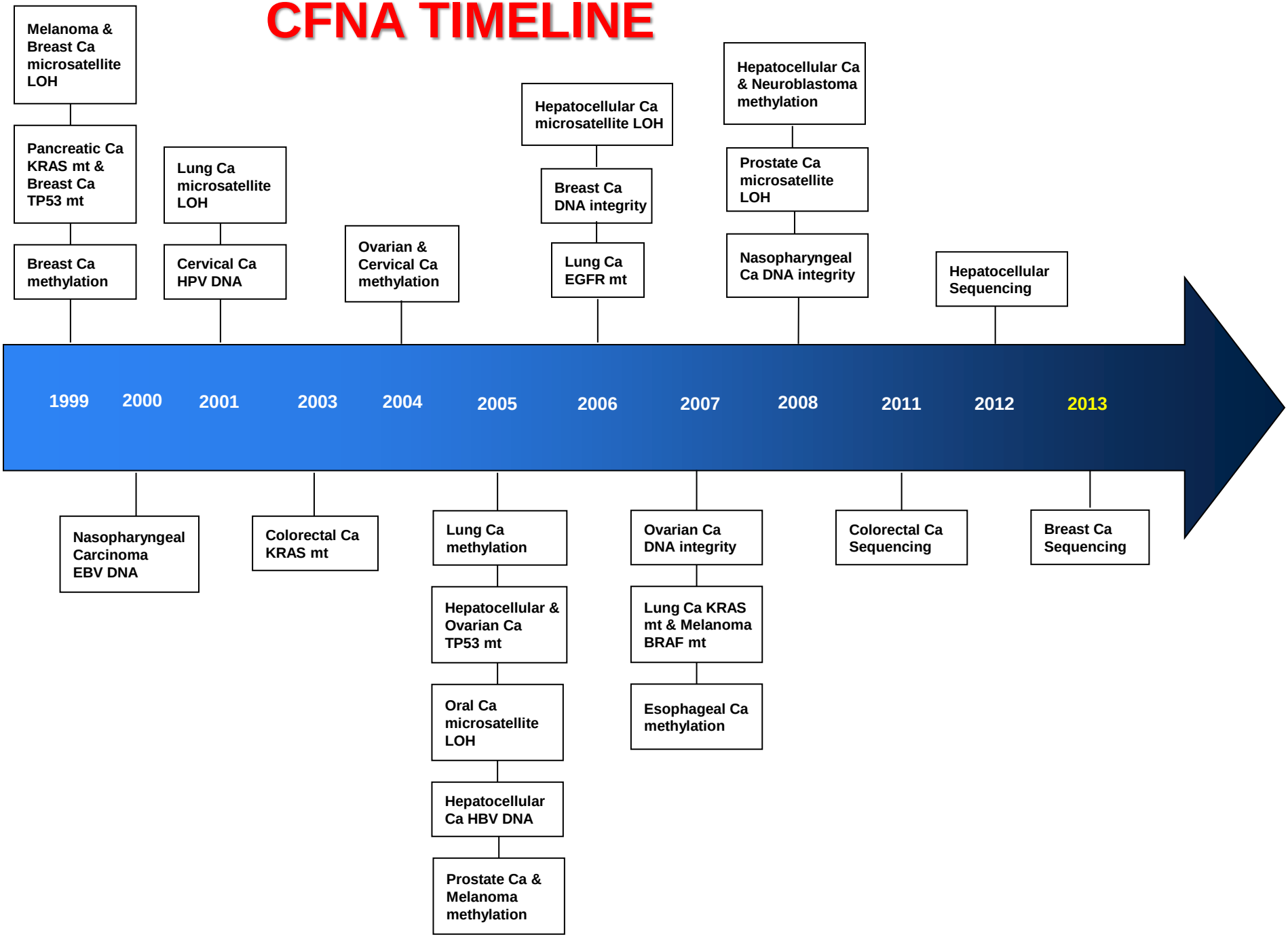


Thermocycler

CFNA Genomic Sequencing

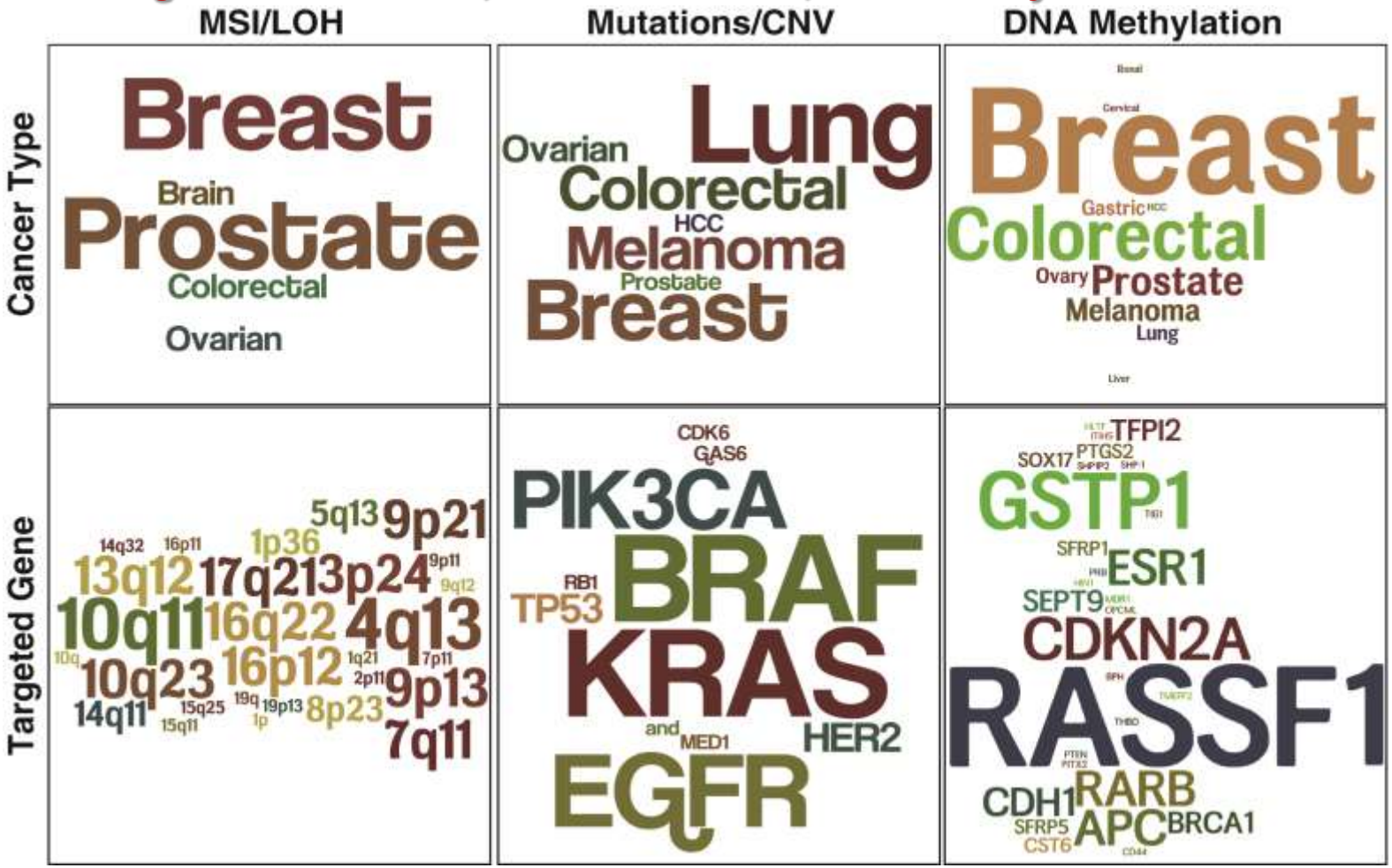


CFNA TIMELINE

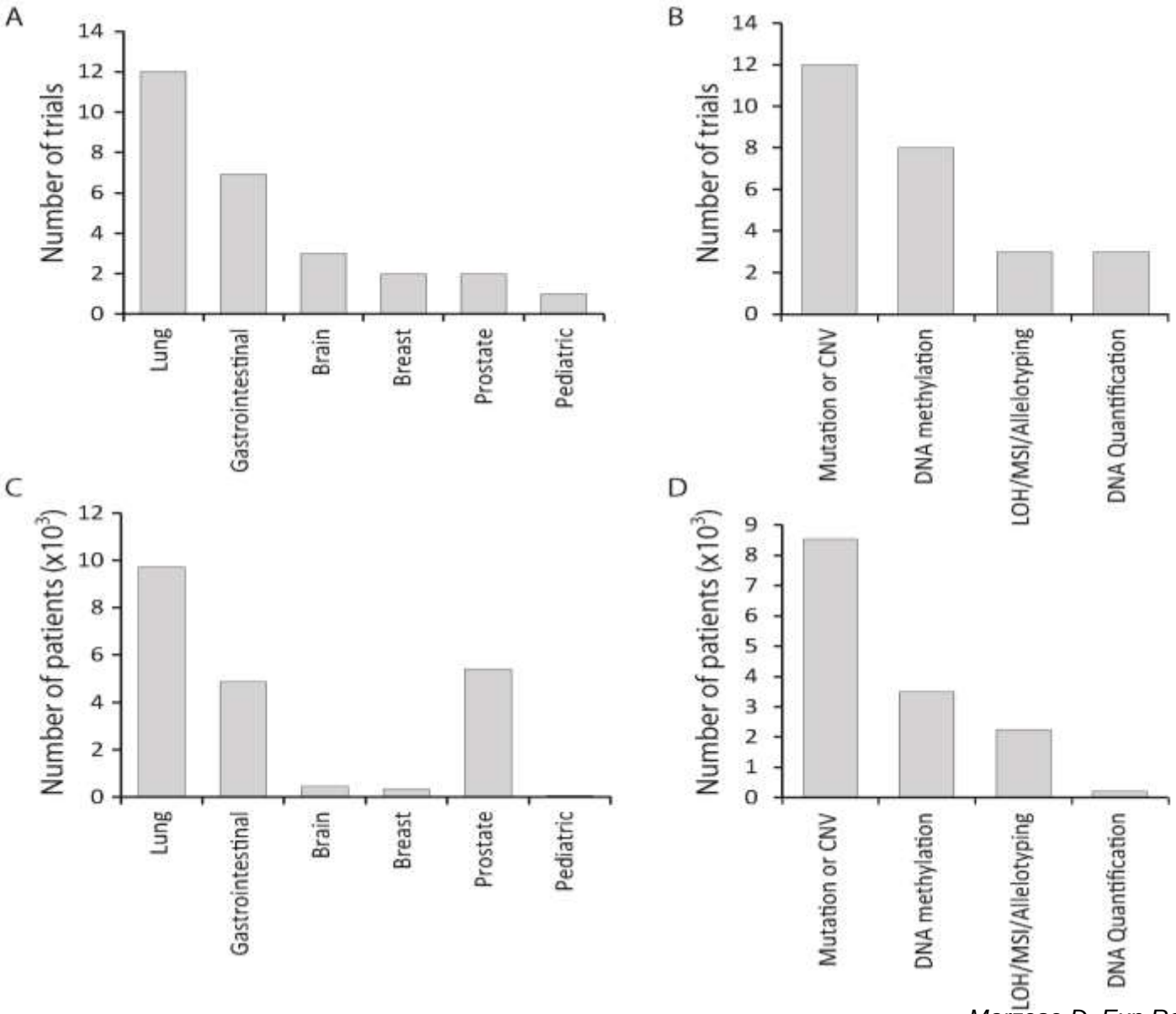


Word Analysis of Recent Papers

More than 50 patients, newer than >2008, Focused on three categories: MSI/LOH, Mutations/CNV, DNA methylation



Trends in Cancer Types and Technological Approaches of Ongoing and Complete cfDNA Clinical Trials



Marzese D, Exp Rev Mol Diagn, 2013

Utility of CFNA as Biomarkers

Detection

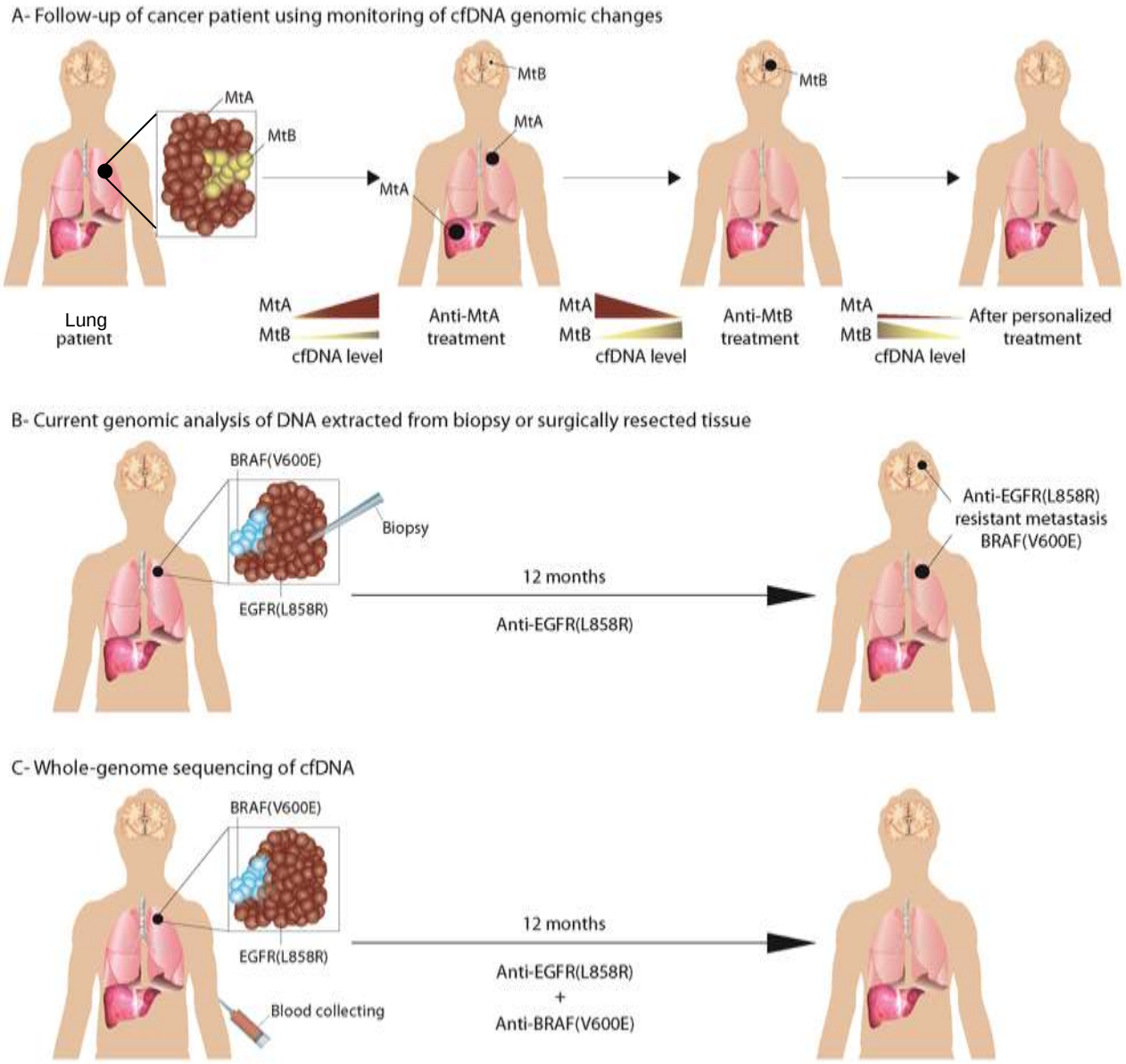
Prognostic

Predictive

CFNA Utility

- ✓ **Different forms of genetic/epigenetic biomarkers can be detected in serum/plasma**
- ✓ **Have clinic utility as single or multiple biomarkers involving different forms**
- ✓ **Identify early disease recurrence**
- ✓ **Used to monitor cancer patients during treatment**

Potential Advantages and Applications of cfDNA Analysis



Marzese D, 2013

Sources of Circulating Tumor-Related DNA?

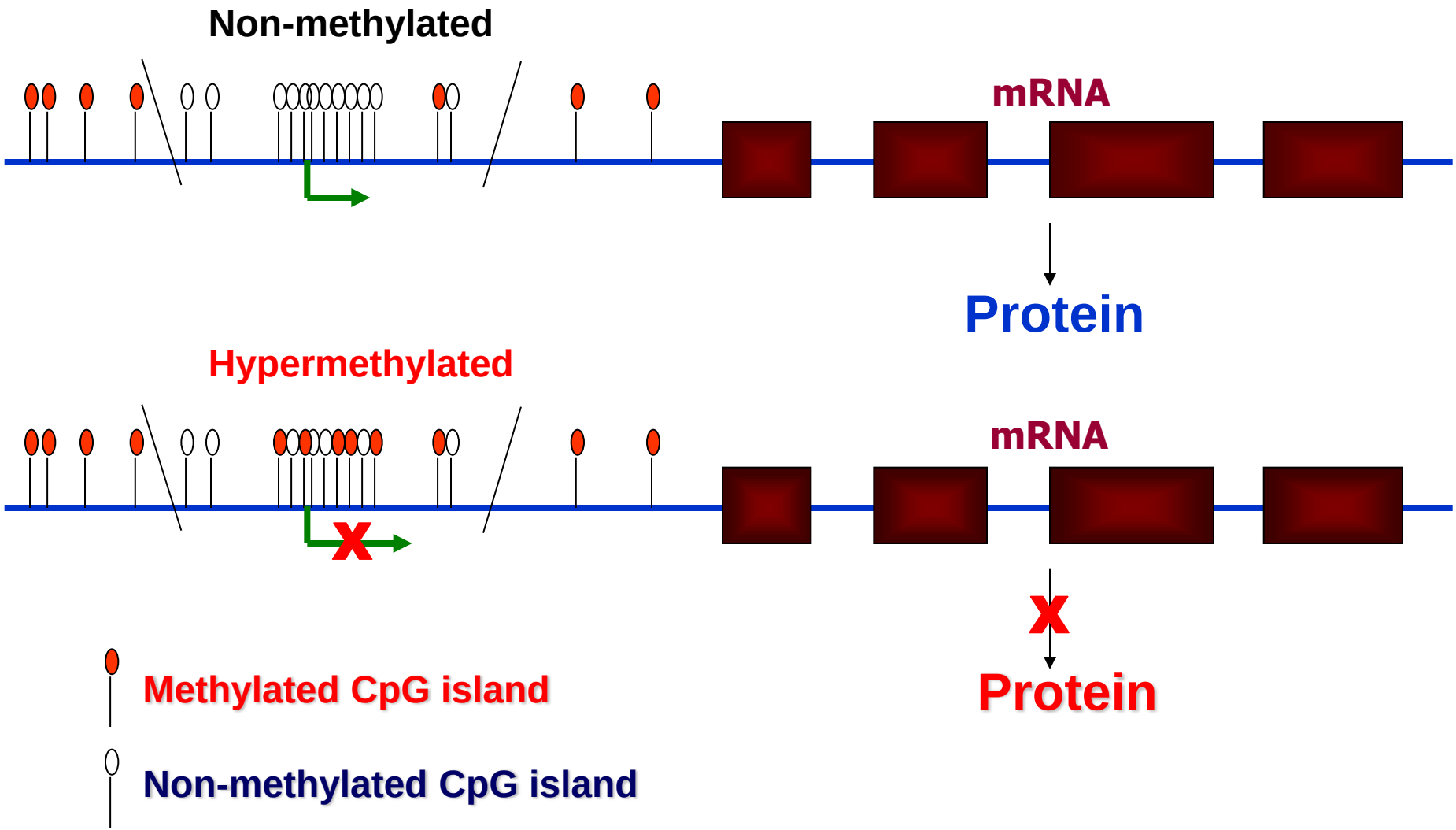
Tumors: Primary/Metastasis

Cancer Cells Programmed and Non Programmed Death

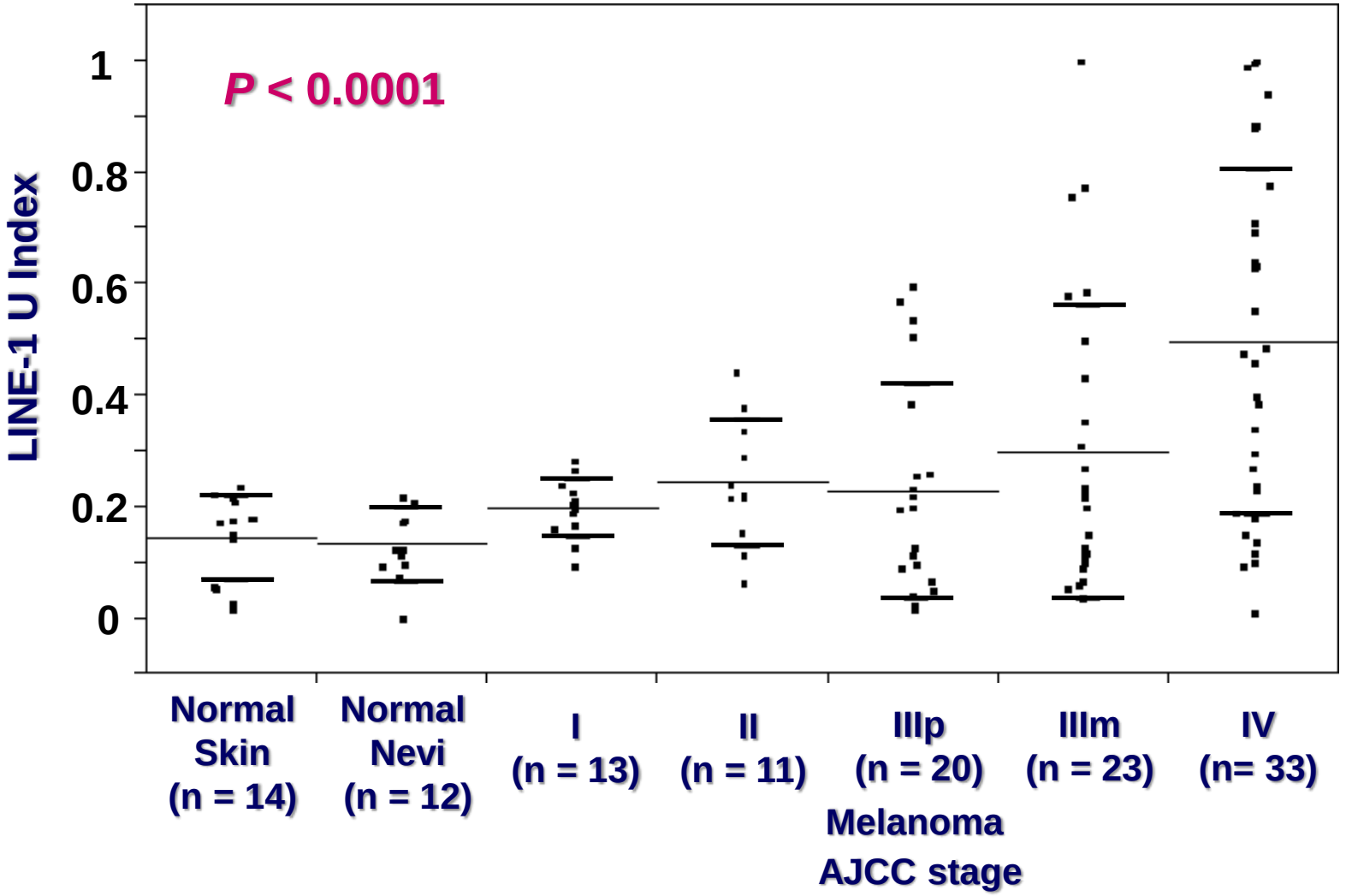
Cancer Cells Secreting

CTC in blood

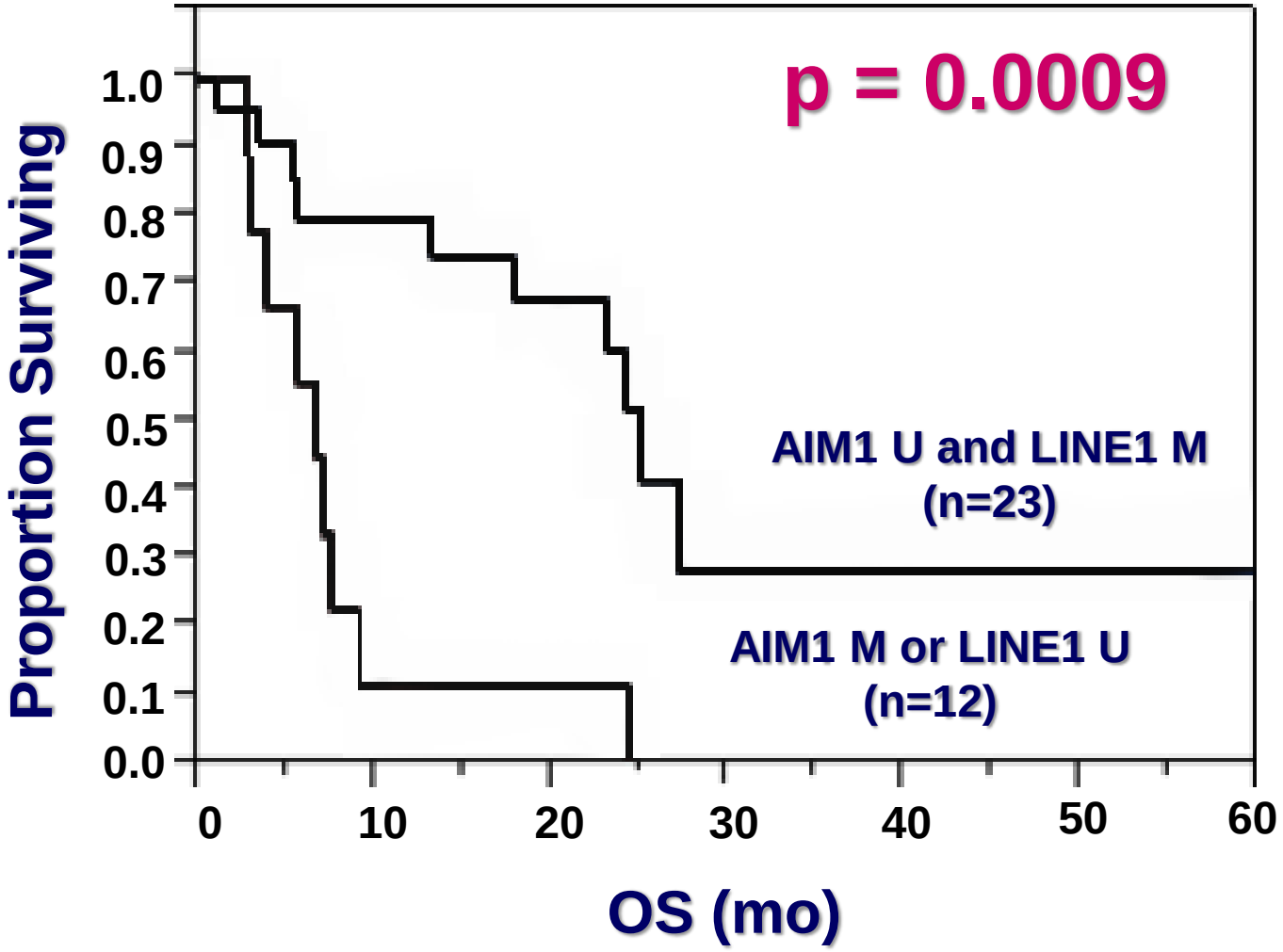
Silencing: Hypermethylated CpG Islands of Promoter Region of Coding and Non-Coding Sequencing



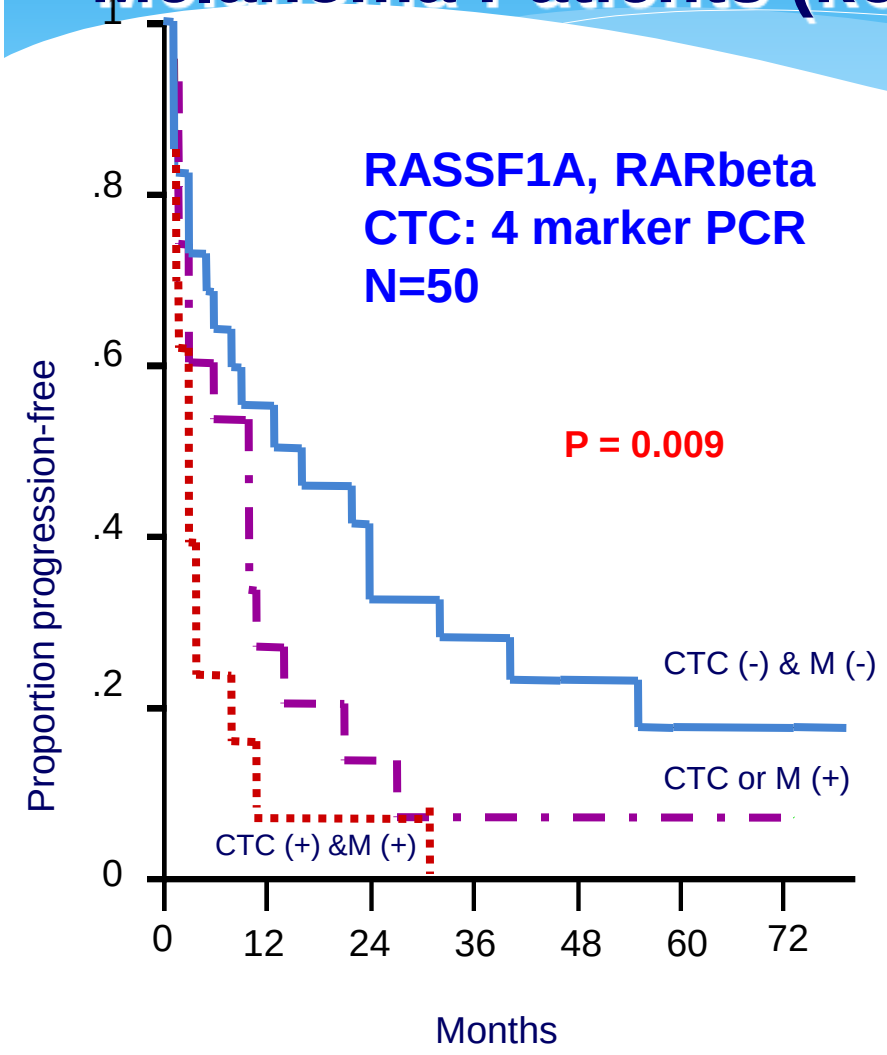
LINE-1 Unmethylated-Index for Melanoma According to AJCC stage

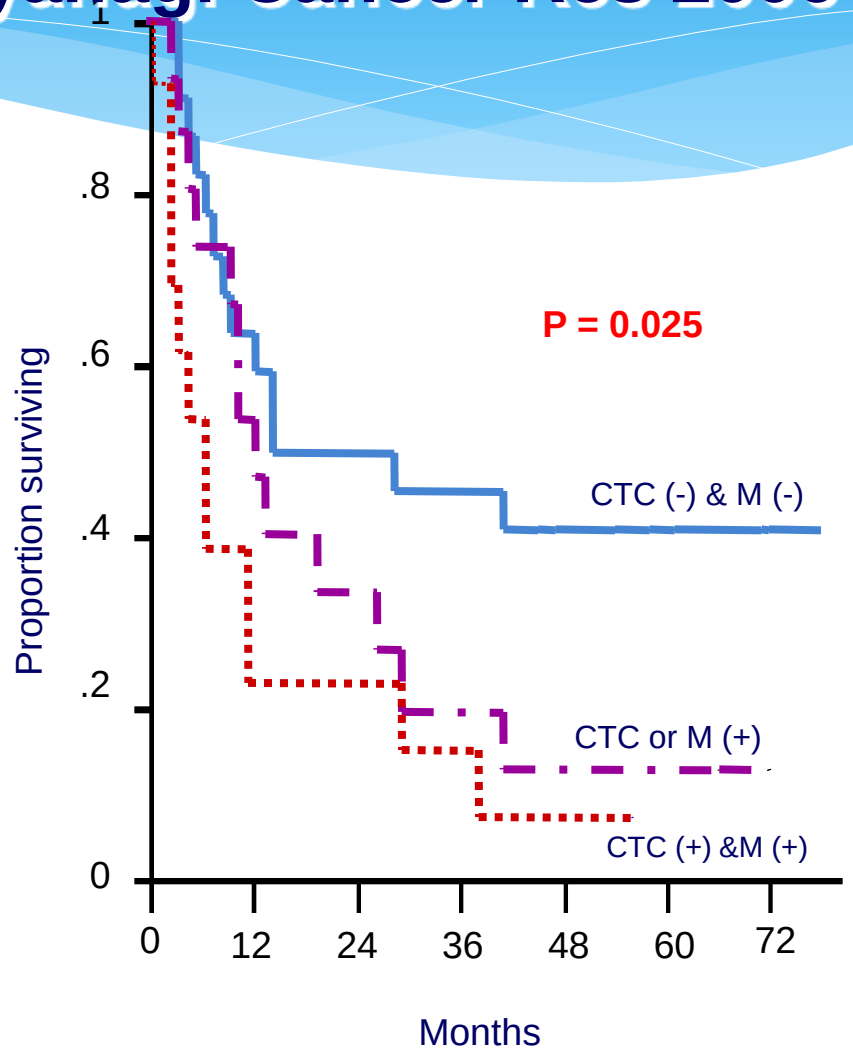


CFNA Analysis of Overall Survival of Stage IV Melanoma Patients: LINE1 Unmethylated and/or *AIM1* Methylated CFDNA vs. Methylated LINE-1 and Unmethylated *AIM1* CFDNA in Serum

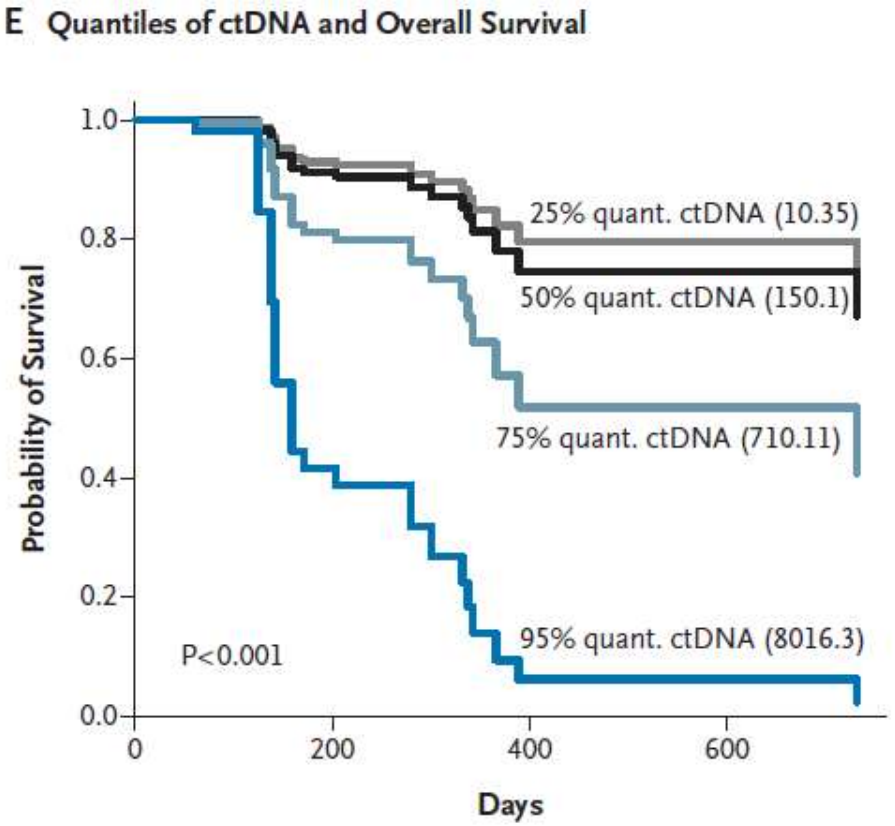


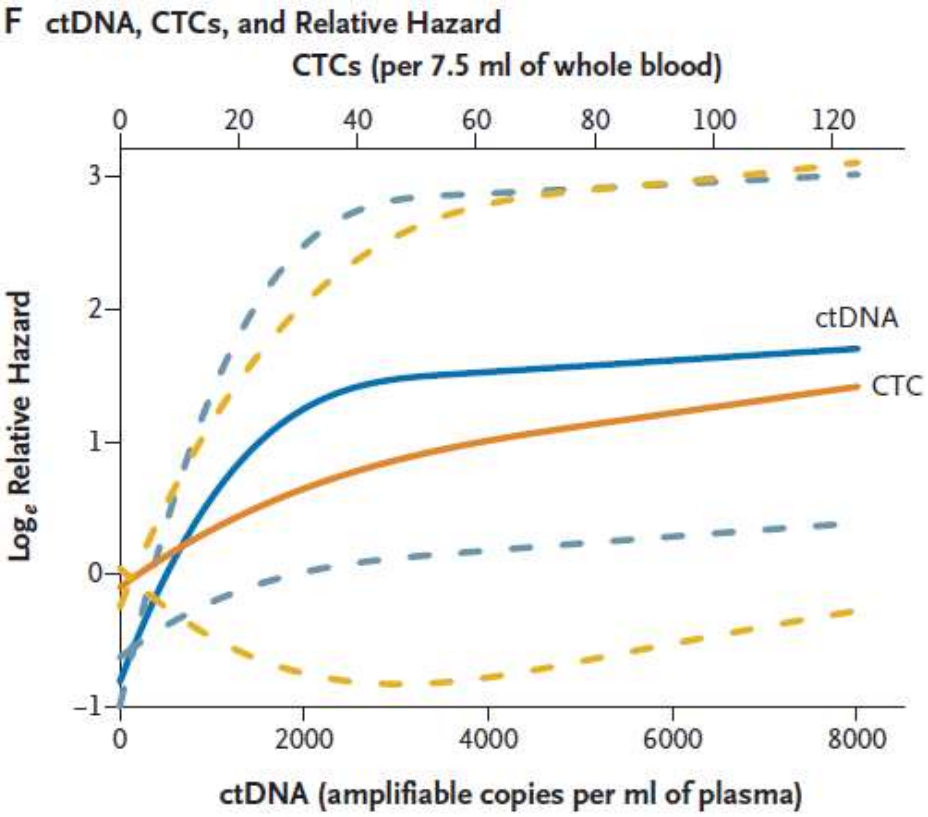
Analysis of Time to Progression and Overall Survival: CTC and Serum DNA Methylation Detection in Stage IV Biochemotherapy Melanoma Patients (koyanagi Cancer Res 2006





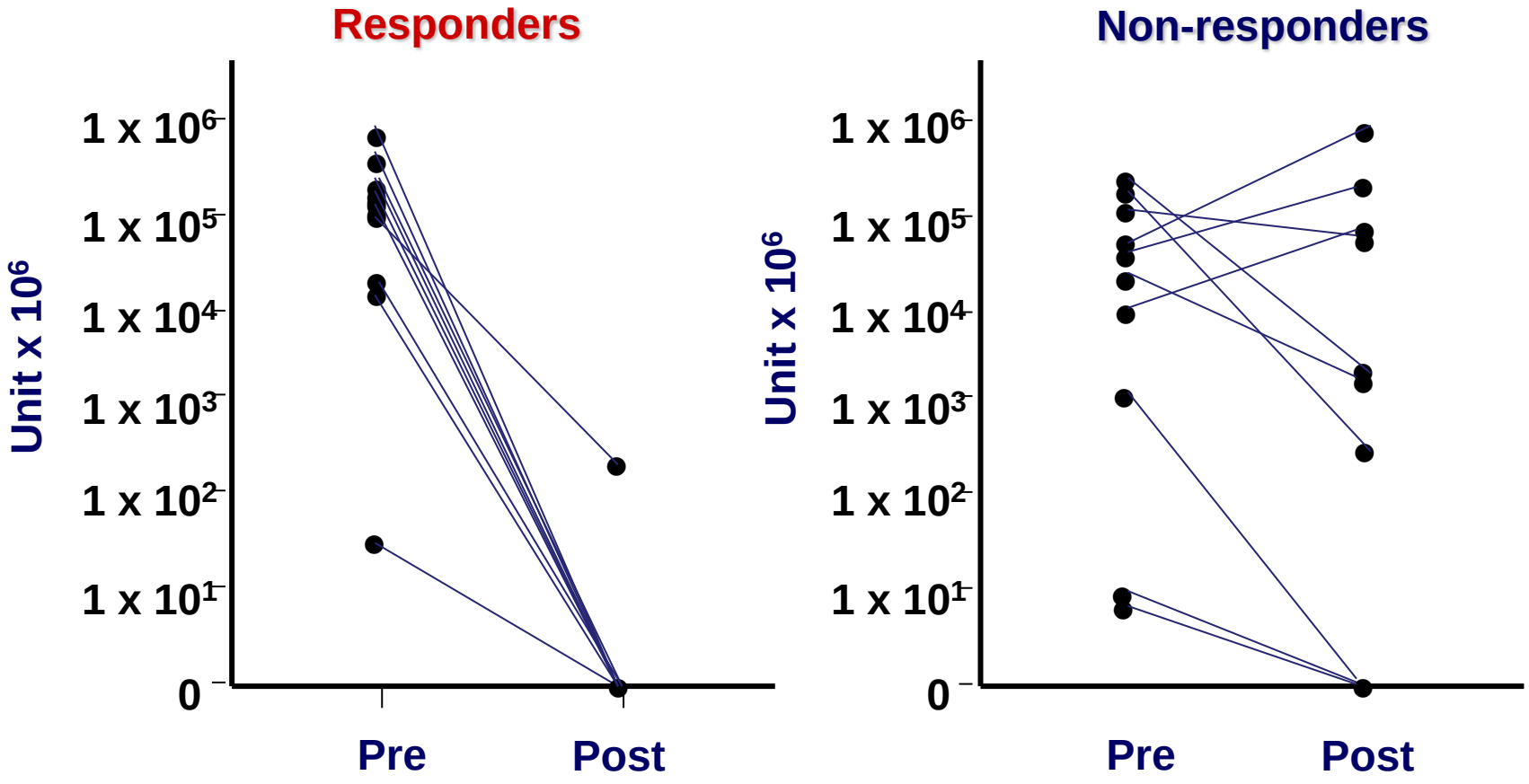
Monitoring Multiple Point Mutations and Structural Variants in cfDNA





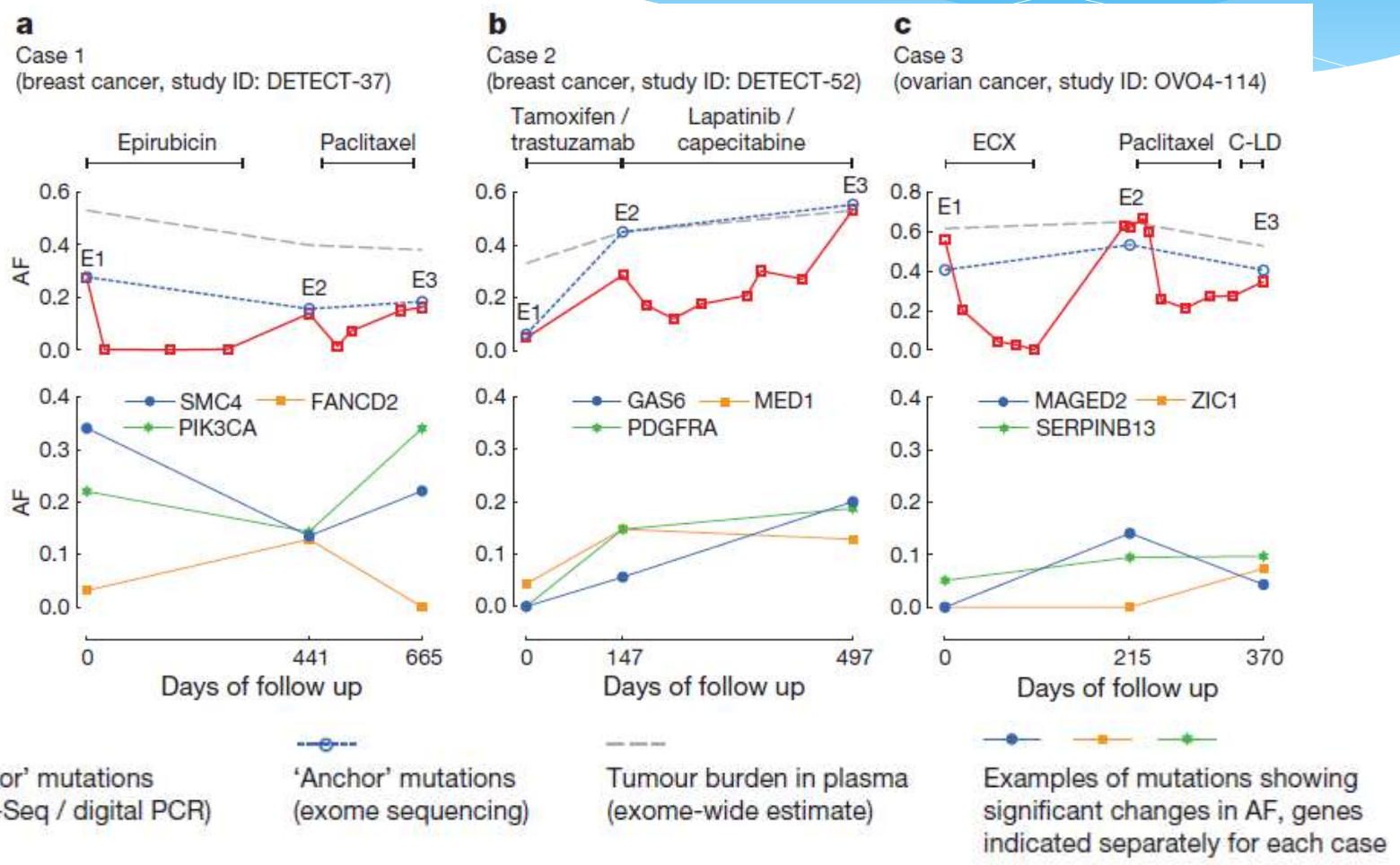
Dawson, SJ. NEJM 2013

Circulating B-RAF V600E in Stage IV Melanoma Patients' Sera: Biochemotherapy Patients Responses



Shinozaki M et al Clin Cancer Res 2007

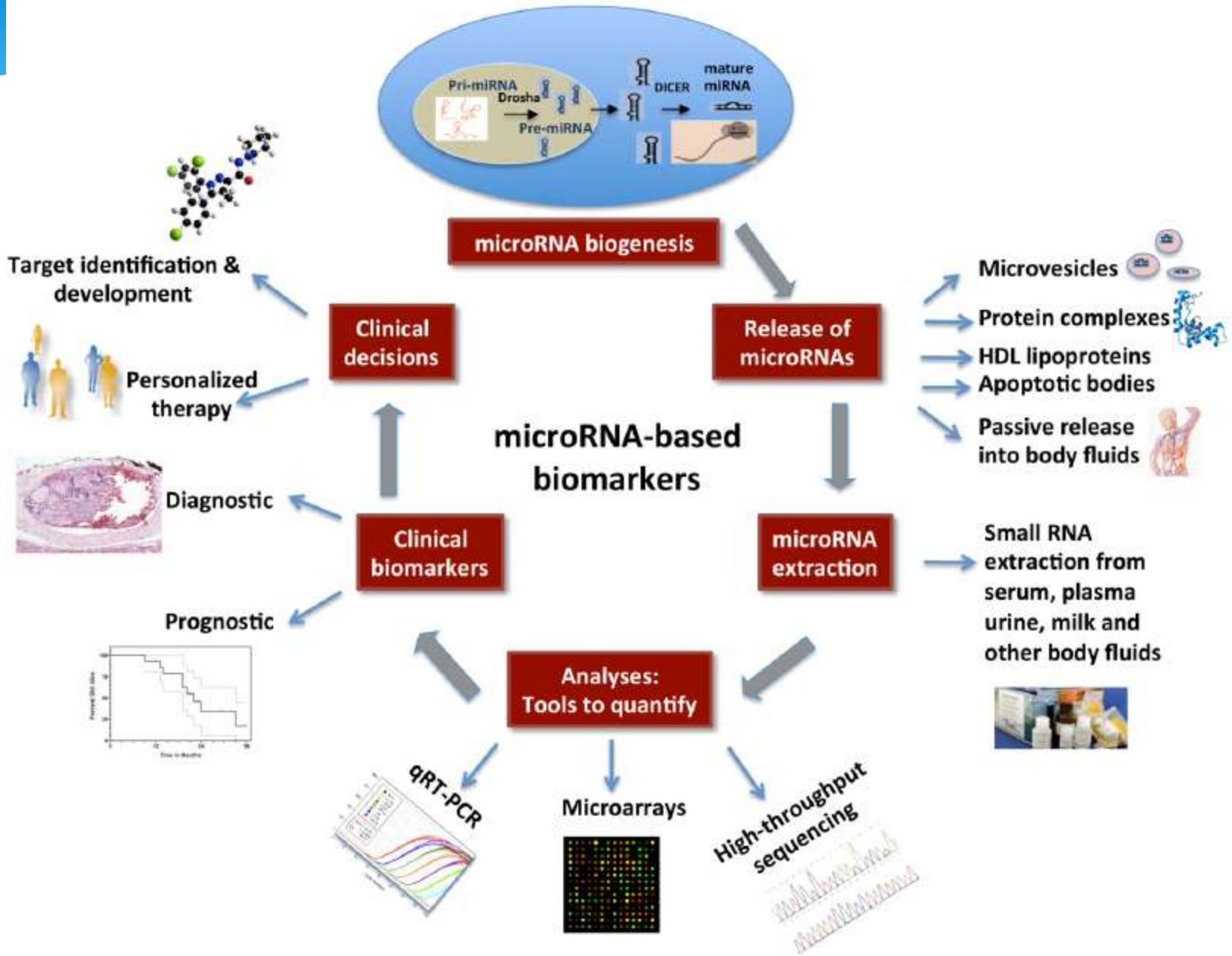
Analysis of Acquired Resistance to Cancer Therapy by cfDNA Sequencing



Mutations showing evidence of genomic tumor evolution

Initial allele fractions (Anchor mutations) used for initial cfDNA screening decreassing and tumor burden increasing during therapy.

Murtaza, M. Nature 2013



Sundarbose et al, Diagnostics, 2013

Advantages of miRs for Blood Assays

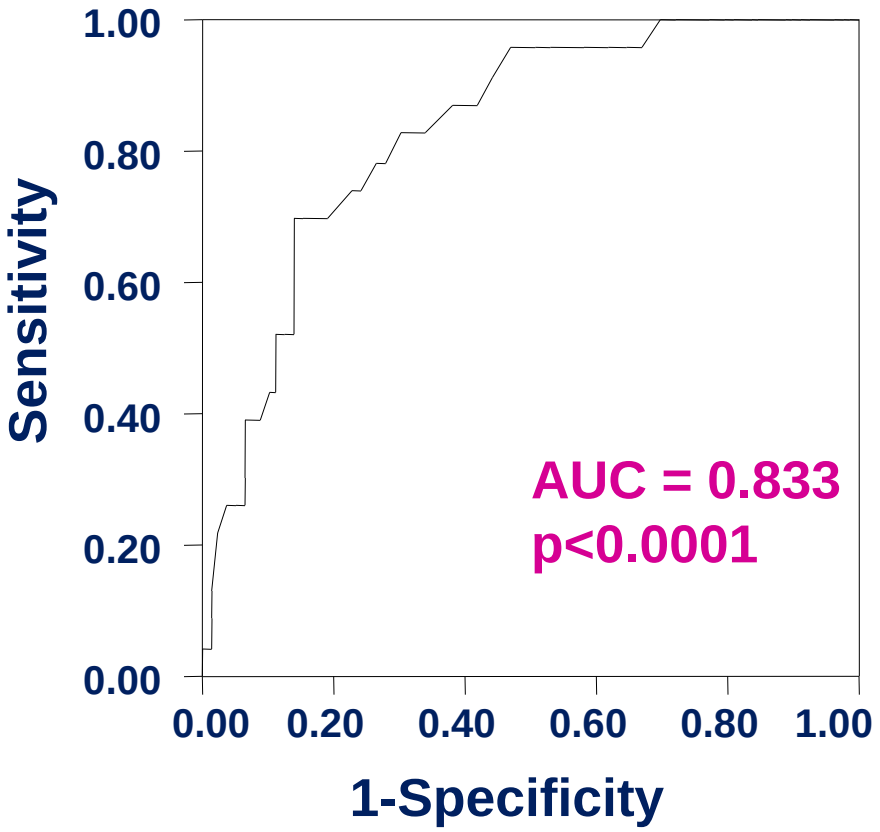
- Low degradation rate (mRNA degrades rapidly)
- Stable at room temperature
- Does not require special blood handling logistics: limited volume
- Functional targets of tumor-related genes

Disadvantages of miRs for Blood Assays

- Specificity and robustness of assays
- Normal healthy donors or other disease effects
- Cut-off quantification values; standardization
- Isolation/detection processes; robustness

Identification of Circulating miR-21 in Breast Cancer by RT-qPCR-DS(Direct Serum Assay) of Circulating miR-21

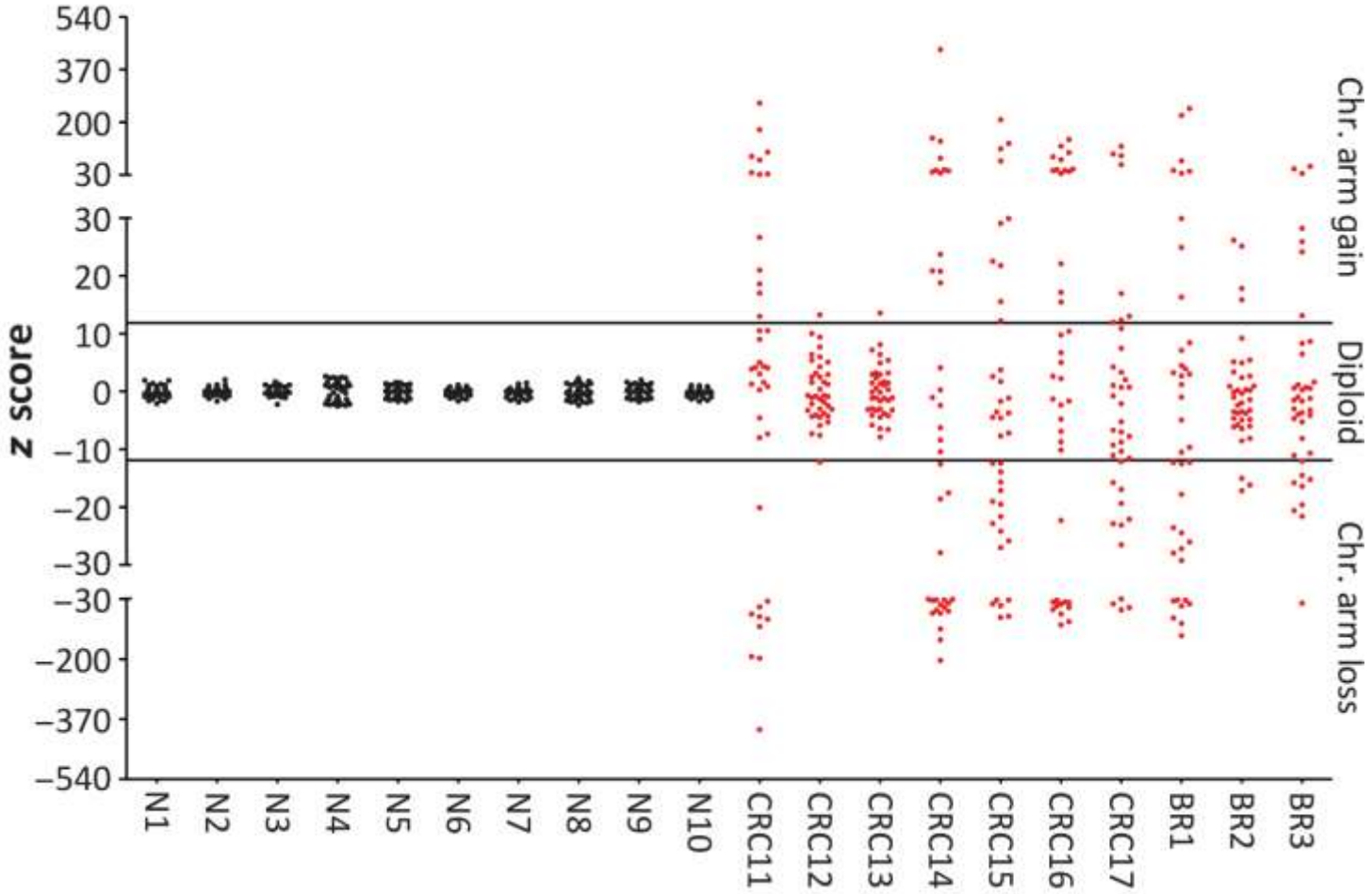
AJCC Stage IV vs. Stage I, II, or III Breast Cancer Patients



Patients' status and -dC_q
AJCC Stage IV vs. Stage I-III breast cancer
N=102 patients

Asaga S et al, Clin Chem, 2010

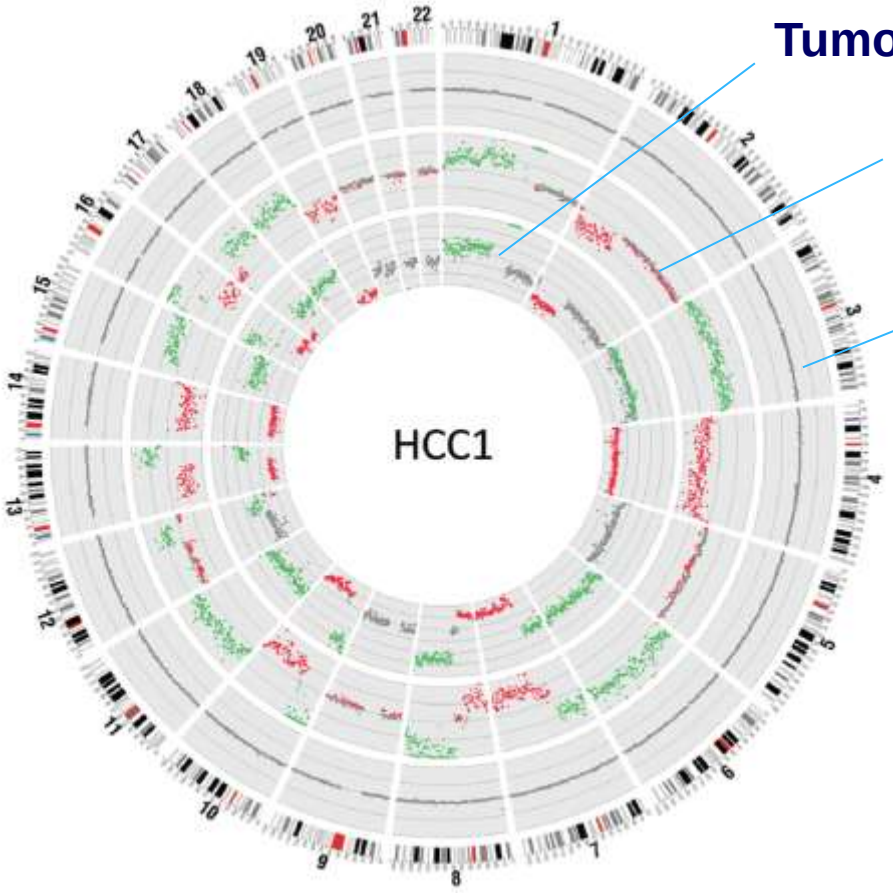
Detection of Chromosomal Alterations in cfDNA of Cancer Patients by Whole-Genome Sequencing



Detection of Chromosomal aberrations in all the cancer patients

Leary, R. Sci Transl Med 2012

cfDNA Clearance After HepatoCellular Carcinoma Surgery



Tumor Tissue

Pre Surgery cfDNA

Post Surgery cfDNA

Copy number aberrations detected in the tumor tissue sample (inner ring), presurgery plasma sample (middle ring), and postsurgery plasma sample (outer ring) for a HCC case

Chan, A. Clin Chem 2013

Issues of CFNA That Need to be Addressed

- **Degradation** and **Half-life** of CFNA in blood
- **Isolation** of CFNA: tedious process and losses
- **Quantification** of CFNA after extraction; how much is put into each assay, **robustness**, **reproducibility, standardization**
- **Sensitivity** and **specificity** of assays: certain CFNA types better than others
- **Regardless of how interesting CFNA are they must follow standard cancer biomarker validation regulatory requirements for clinical approval. Competition with other biomarkers; analytes, proteins, etc.**

CFNA vs CTC Utility

- ✓ CTC represents a detection of a realtime "metastasis" event occurring. CFNA does not
- ✓ CFNA detection occurs at any stage; CTC very limited in earlier tumor stages.
- ✓ Tumor volume often relates to CFNA levels whereas CTC does not.
- ✓ Both differ in utility relative to cancer type and natural disease history
- ✓ CFNA analysis requires far less amount of blood and less logistic problems in multicenter trials.