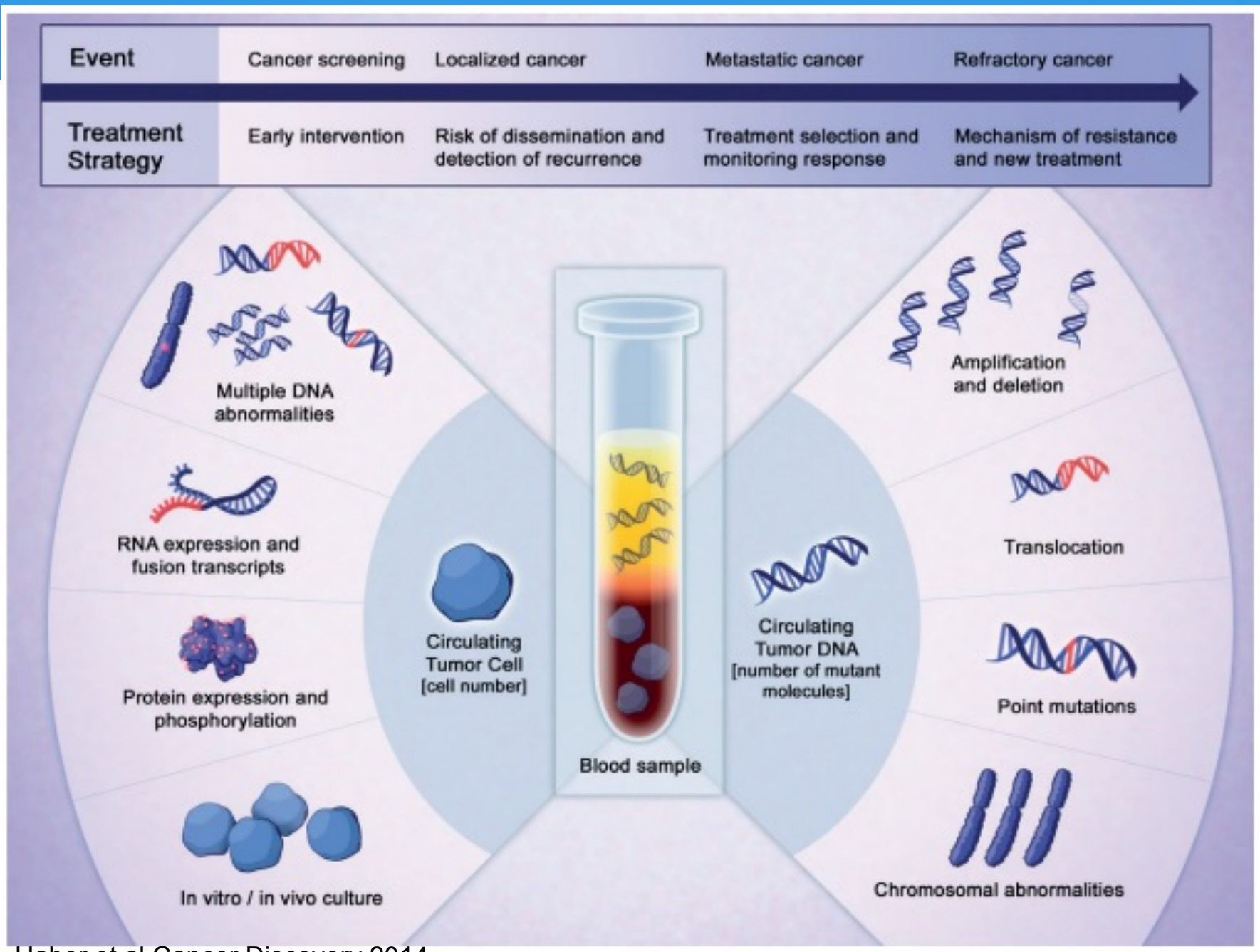
Memorial Sloan Kettering Cancer Center

A Clear and Balanced Look at Liquid Biopsy

Dana Tsui, Ph.D.
Assistant Attending Faculty
Department of Pathology
Center for Molecular Oncology

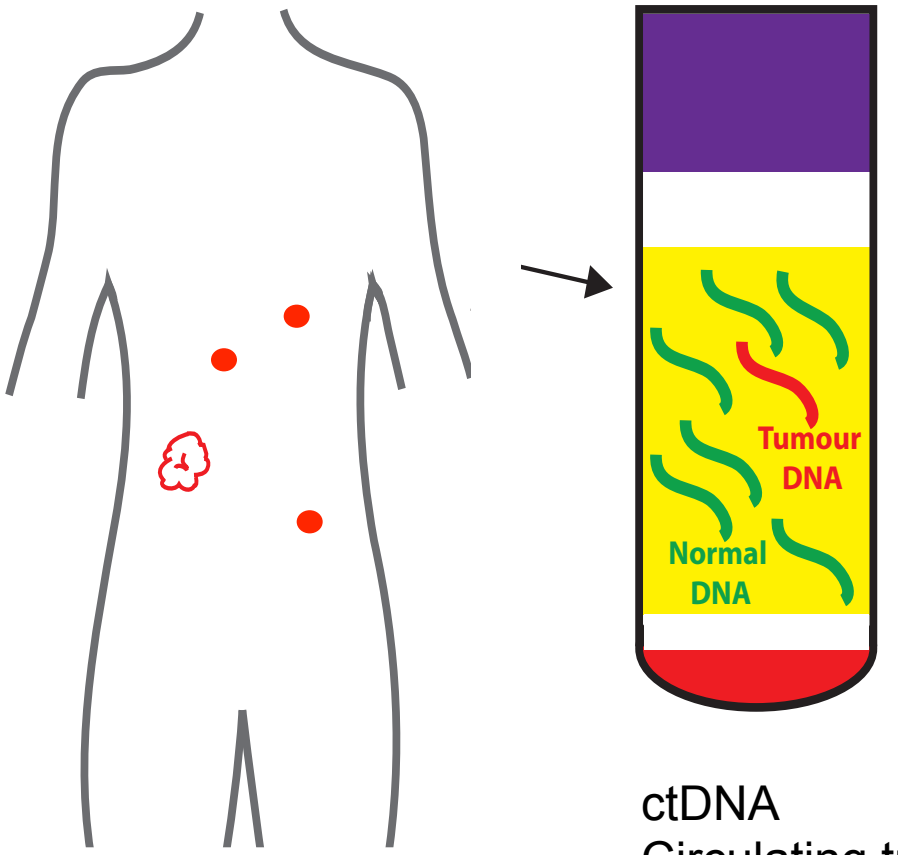
Outline

- Definition of liquid biopsy
- Clinical applications
- Current state of research
- Promises and challenges



Haber et al Cancer Discovery 2014

Cell-free circulating tumor DNA



ctDNA
Circulating tumor DNA

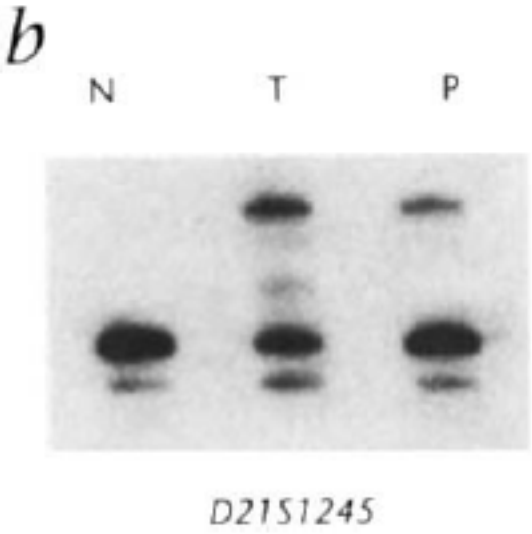
Background about cfDNA

The potential of cfDNA profiling was long recognized

Microsatellite alterations in serum DNA of head and neck cancer patients

HOMAIRA NAWROZ[1], WAYNE KOCH[1], PHILIPPE ANKER[2], MAURICE STROUN[2] & DAVID SIDRANSKY[1]

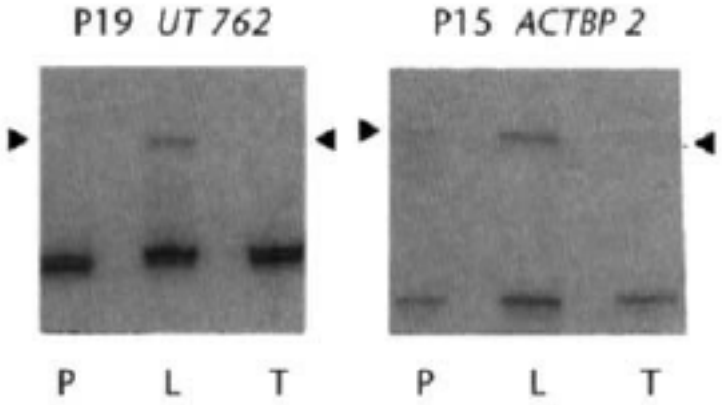
[1]Johns Hopkins, [2]Uni of Geneva



Microsatellite alterations in plasma DNA of small cell lung cancer patients

XU QI CHEN[1], MAURICE STROUN[1], JEAN-LUC MAGNENAT[2], LAURENT P. NICOD[2], ANNE-MARIE KURT[3], JACQUELINE LYAUTEY[1], CHRISTINE LEDERREY[1] & PHILIPPE ANKER[1]

[1]Pavillon des Isotopes, [2,3]Uni of Geneva



Nature Medicine 1996

Memorial Sloan Kettering Cancer Center

BBC Sign in News Sport Weather iPlayer TV Radio

NEWS

Home UK World Business Politics Tech Science Health Education Entertain

Health

Blood test for Down's syndrome 'gives better results'

By James Gallagher
Health editor, BBC News website

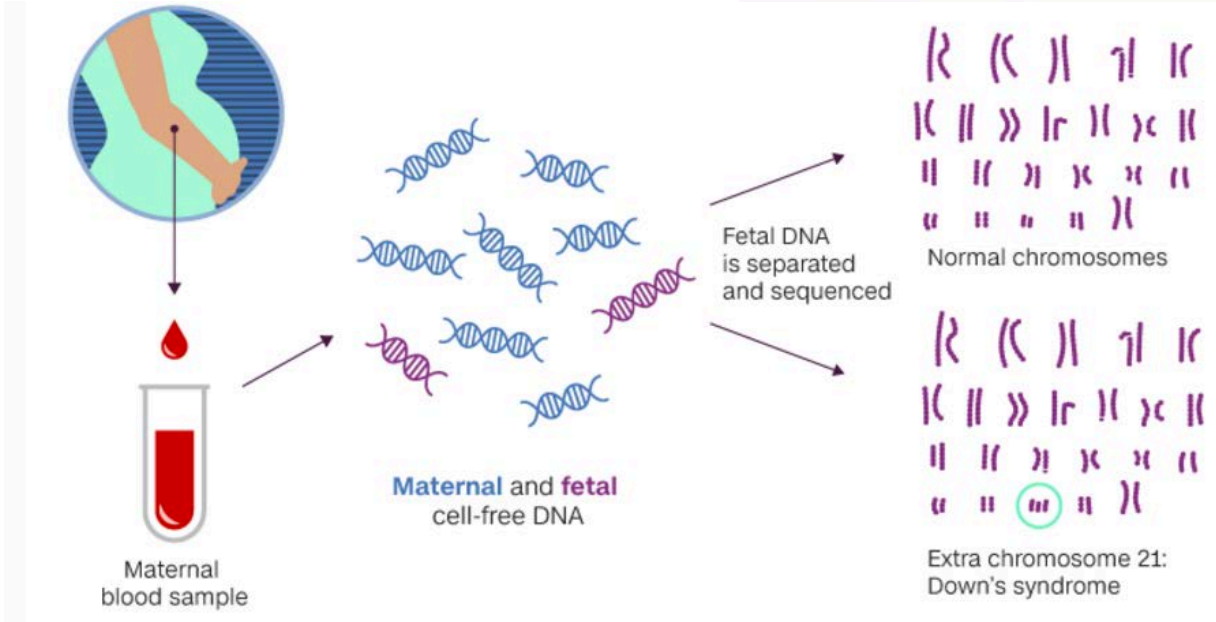
1 April 2015 | Health

CNN Health » A blood test before birth could predict your medical destiny

A blood test before birth could predict your medical destiny

By Jenni Marsh, CNN

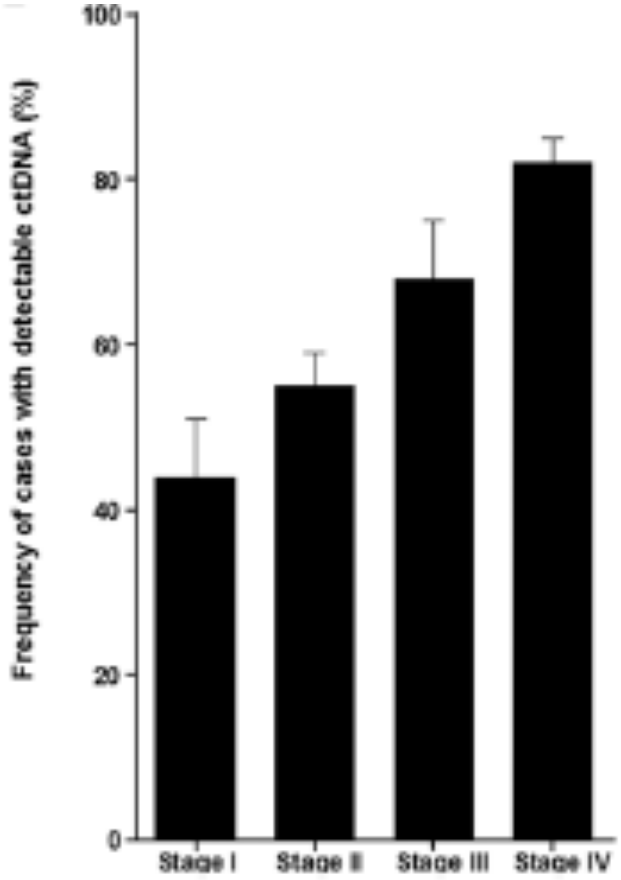
Updated 7:39 AM ET, Tue March 29, 2016



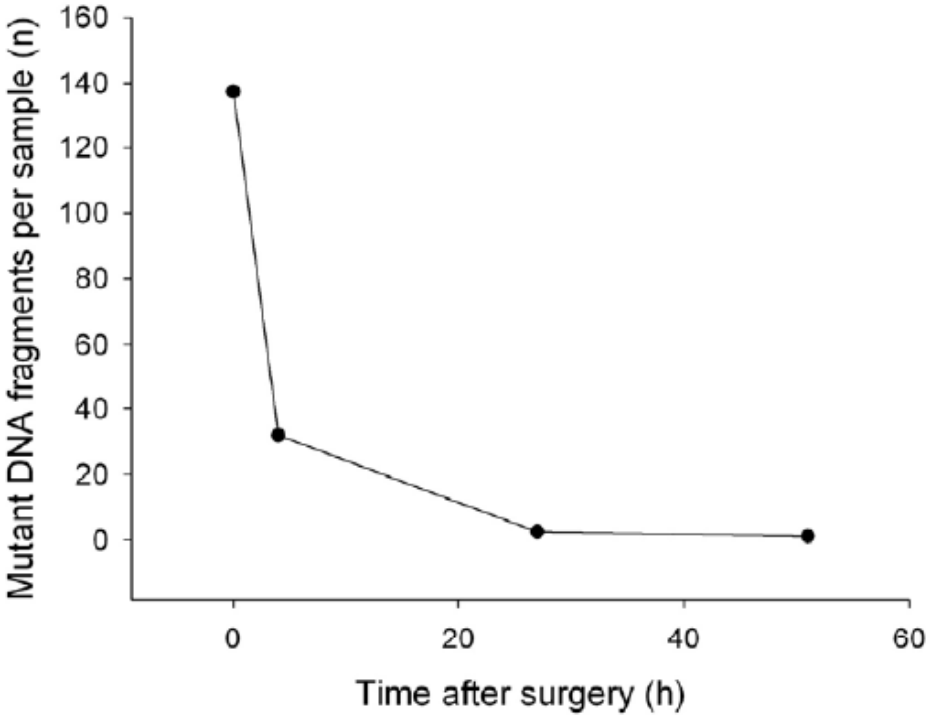
Memorial Sloan Kettering Cancer Center

Picture from http://www.cnn.com/2016/03/24/health/dennis-lo-dna-discovery/

Dynamics of cfDNA in plasma correlate with clinical status



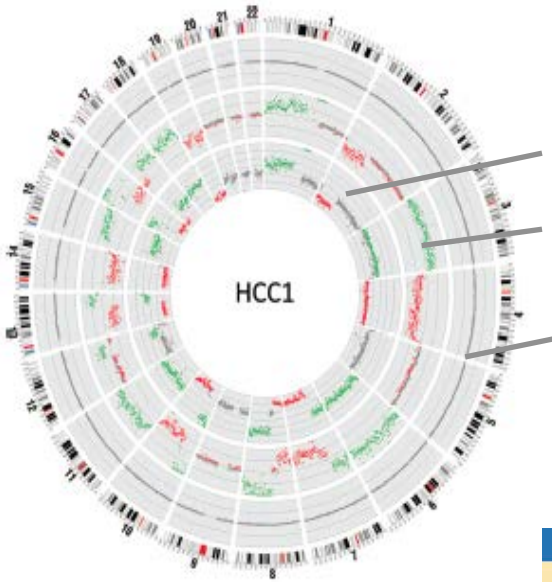
Bettegowda, Vogelstein, Diaz STM 2014



Diehl, Vogelstein, Diaz Nat Med 2008

Memorial Sloan Kettering Cancer Center

Comprehensive representation of cancer genome



Tumor tissue

Plasma before surgery

Plasma after surgery

Chan, Lo et al Clin Chem 2012

Patient A:
Tissue and plasma cfDNA concordance
73yo female metastatic urothelial carcinoma to lung, liver, bone, and lymph nodes

Gemcitabine Carboplatin

Tissue biopsy

Gene		Allele fraction	
ERBB2	S310Y	0.772	(3852/4992)
PIK3CA	E81K	0.268	(268/1000)
ATRX	splice	0.591	(159/269)
BRCA1	Q1396H	0.181	(143/790)
CTLA4	R70Q	0.142	(71/500)
MAX	V9L	0.302	(304/1008)
KMT2A	D2488N	0.330	(243/737)
PDGFRA	E1065K	0.377	(339/899)
TERT	promoter	0.712	(588/826)
TP53	splice	0.460	(291/632)
NUP93	E105D	0.258	(131/508)
FGF19	V133M	0.233	(275/1178)
ARID5B	E765D	0.407	(468/1149)

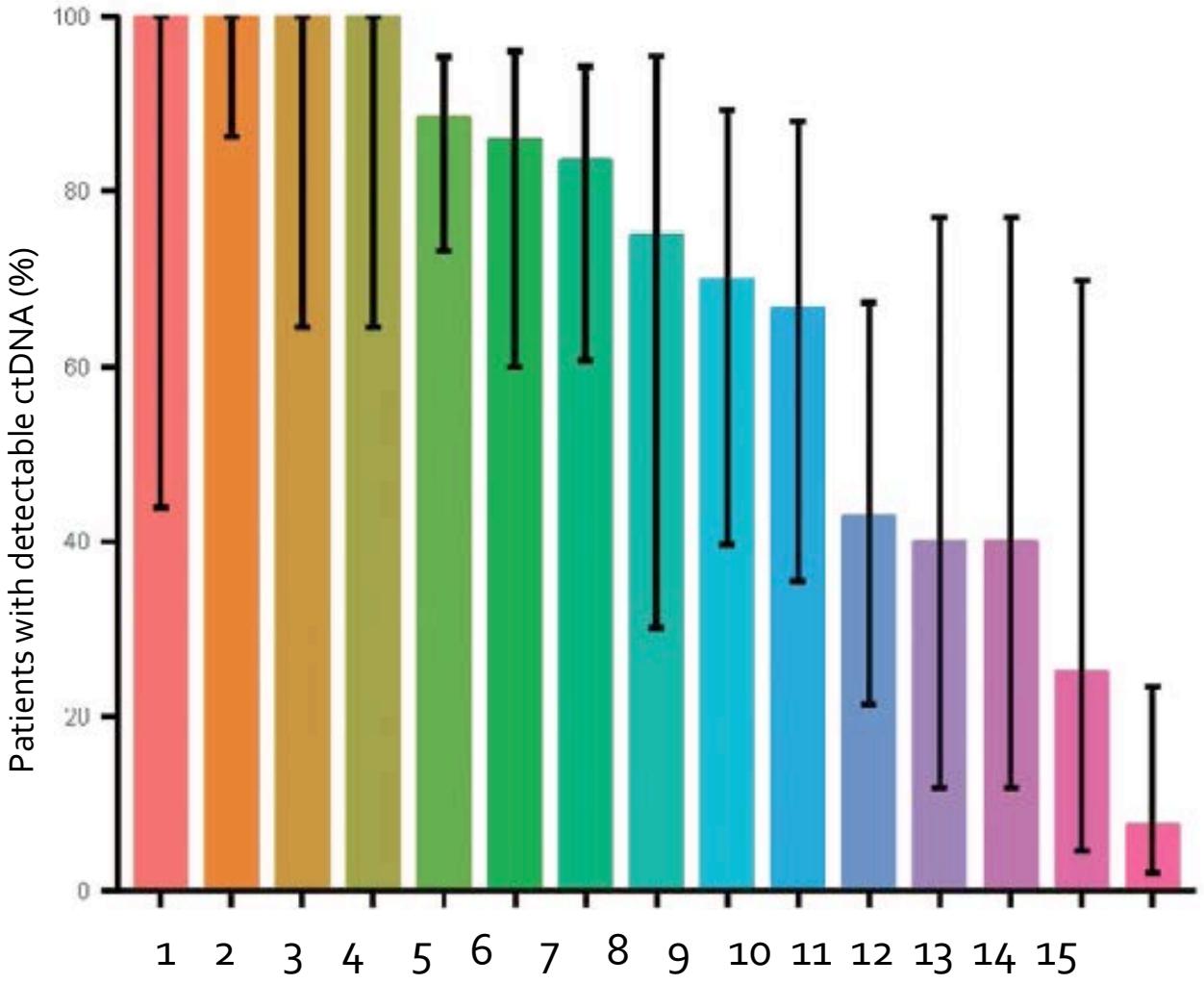
cfDNA draw

Gene		Allele fraction	
ERBB2	S310Y	0.721	(1733/2405)
PIK3CA	E81K	0.254	(543/2138)
ATRX	splice	0.506	(249/492)
BRCA1	Q1396H	0.147	(78/530)
CTLA4	R70Q	0.161	(120/745)
MAX	V9L	0.258	(134/520)
KMT2A	D2488N	0.281	(291/1037)
PDGFRA	E1065K	0.302	(261/863)
TERT	promoter	0.461	(514/1114)
TP53	splice	0.376	(213/566)
NUP93	E105D	0.155	(160/1033)
FGF19	V133M	0.194	(151/779)
ARID5B	E765D	0.324	(112/346)

Michael Cheng, Maha Shady, Preethi Srinivasan,
Dana Rathkopf, Gopakumar Iyer, Mike Berger, David Solit

Memorial Sloan Kettering Cancer Center

Tumor-derived cfDNA in different types of cancers

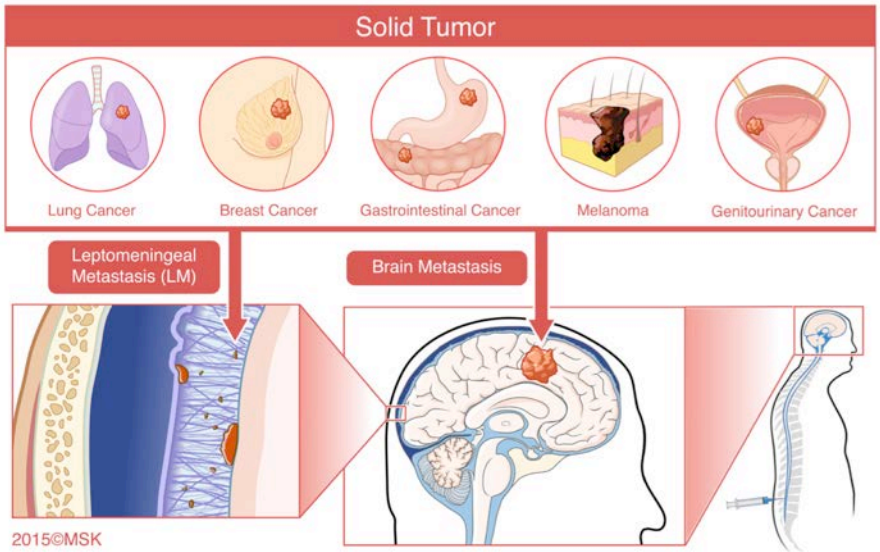


1) Bladder
2) Colorectal
3) Gastroesophageal
4) Ovarian
5) Pancreatic ductal
6) Breast
7) Melanoma
8) Hepatocellular
9) Head and neck
10) Neuroblastoma
11) Medulloblastoma
12) Prostate
13) Renal cell caricinoma
14) Thyroid
15) Glioma

Bettegowda, Vogelstein, Diaz STM 2014

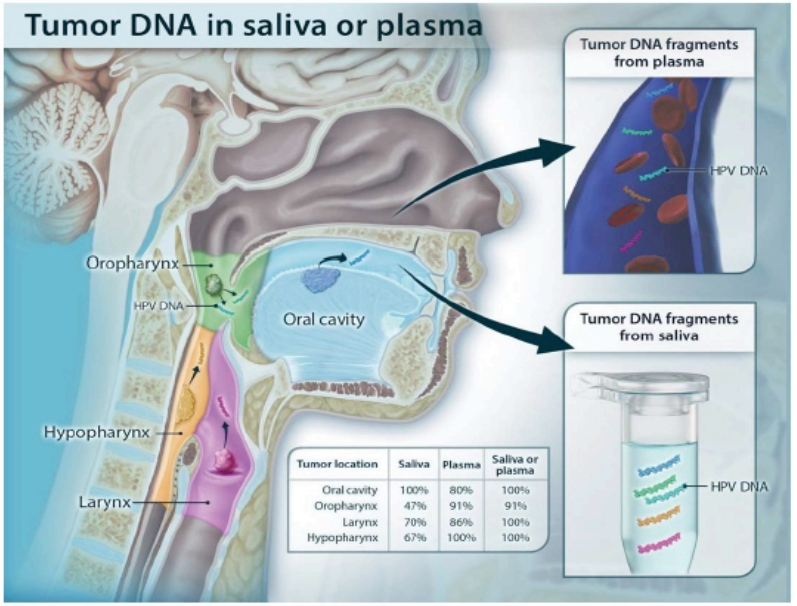
Memorial Sloan Kettering Cancer Center

cfDNA in multiple body fluids



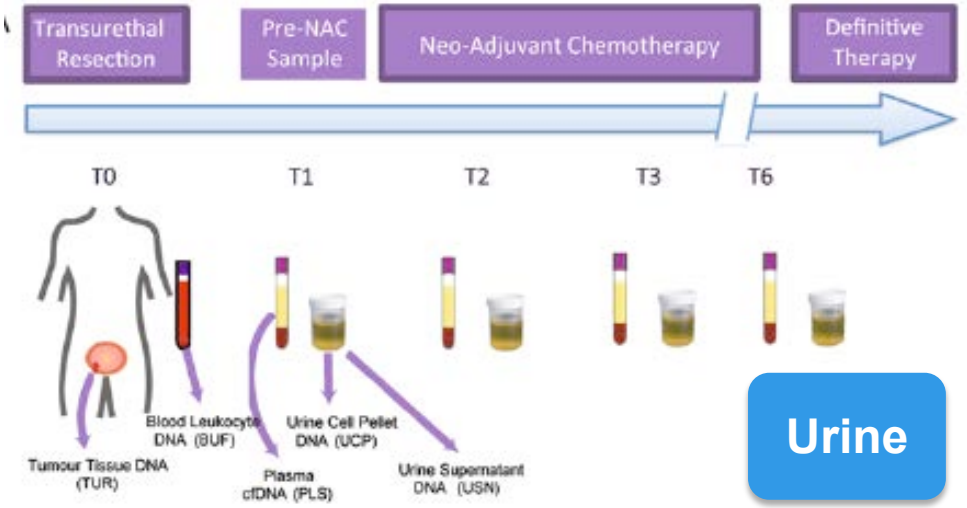
Pentsova et al JCO 2016

Cerebrospinal Fluid (CSF)



Wang, Agrawal STM 2015

Saliva

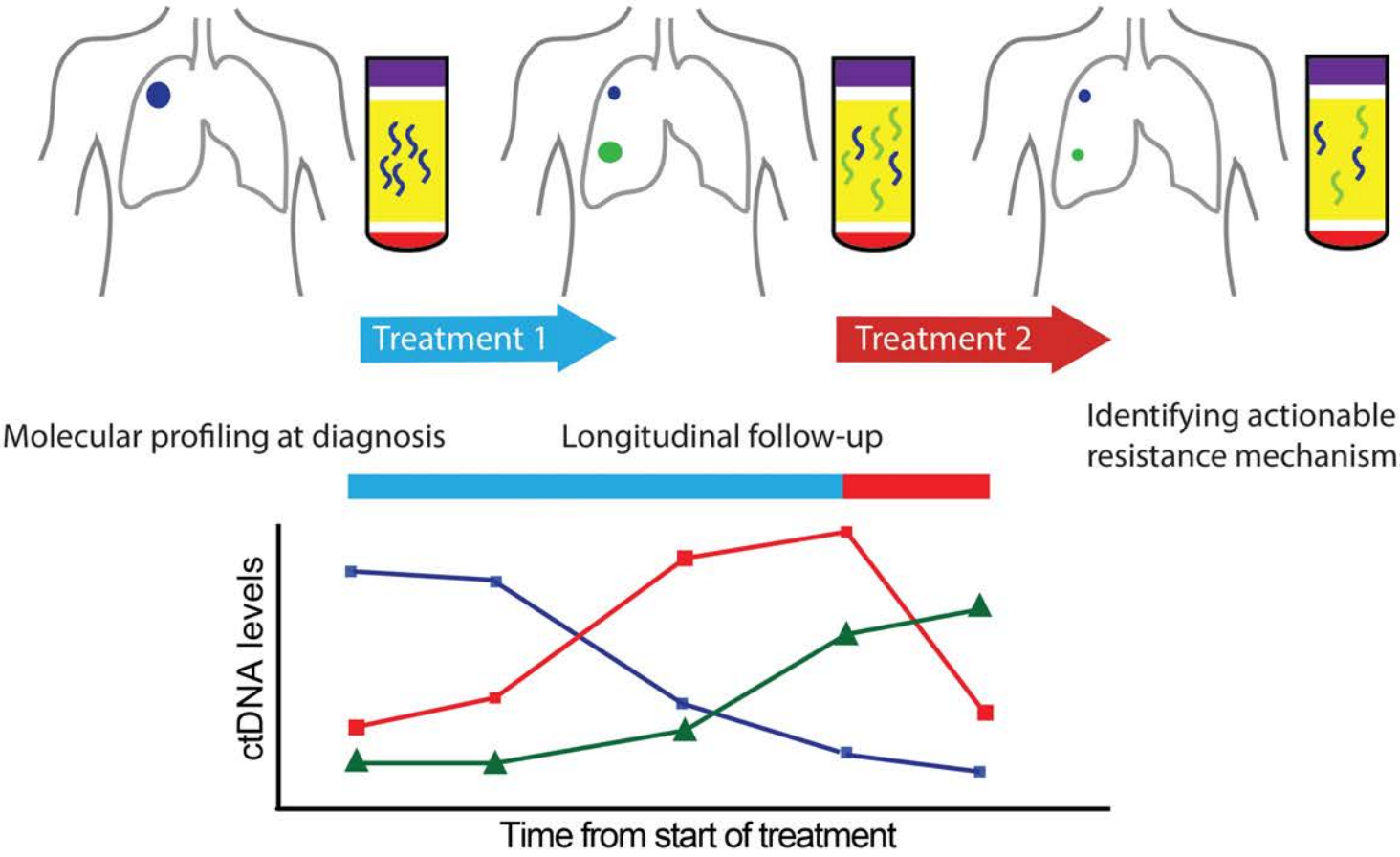


Urine

Petal et al Scientific Reports 2017

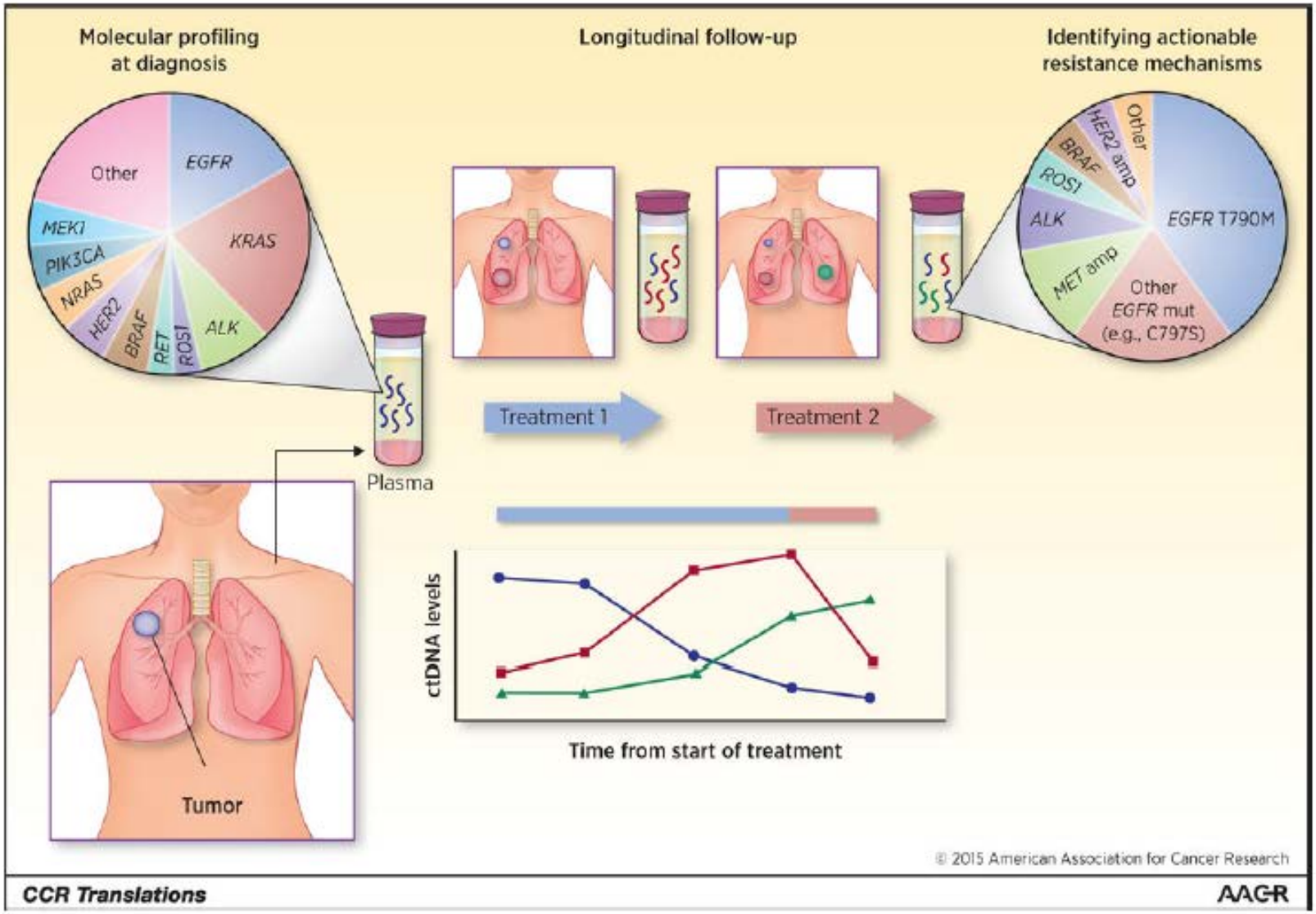
Memorial Sloan Kettering Cancer Center

Clinical applications



Memorial Sloan Kettering Cancer Center

Molecular profiling using plasma

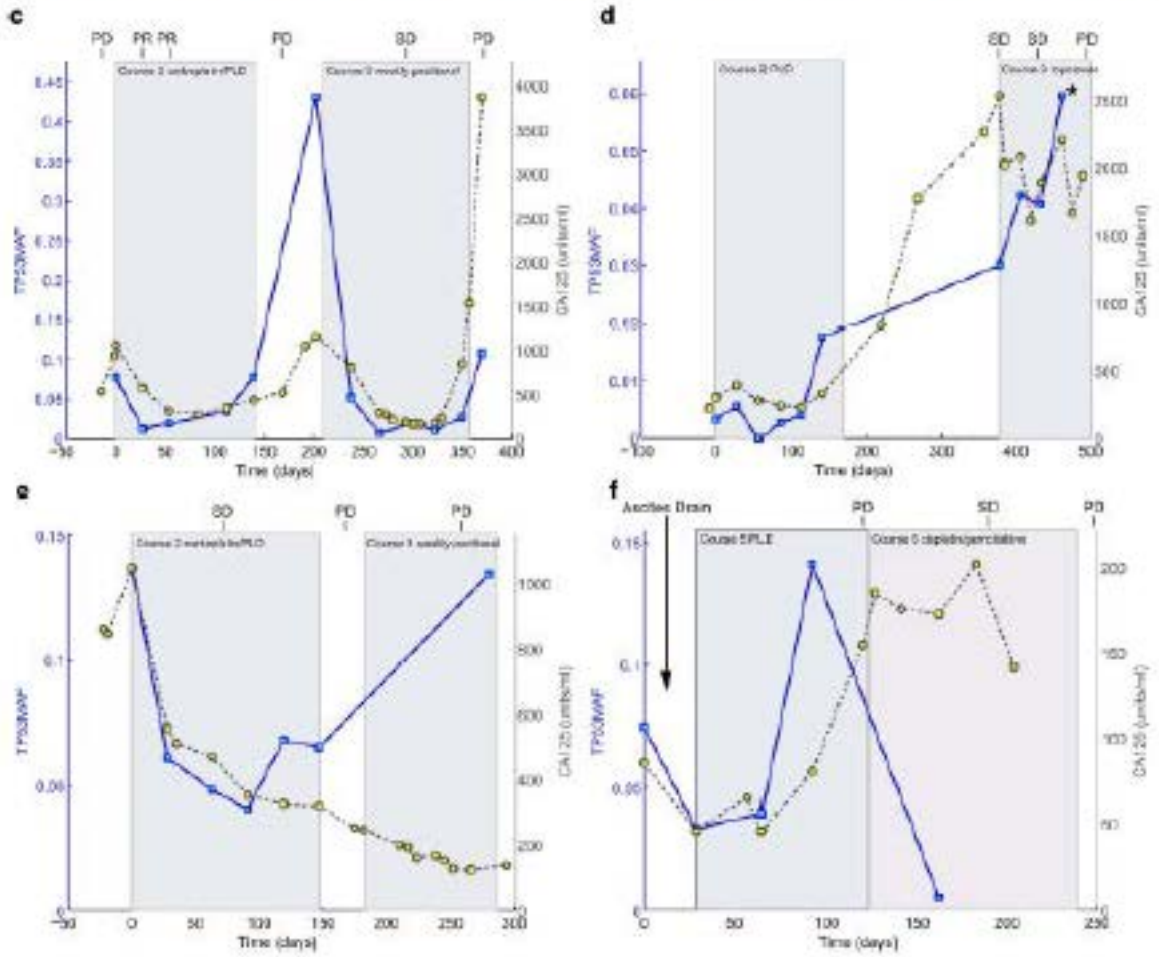


Tsui Berger CCR 2016

Memorial Sloan Kettering Cancer Center

Molecular tumor responses

Dynamics of *TP53* cfDNA in High-grade serous ovarian cancer



Parkinson et al PloS Medicine 2016

Memorial Sloan Kettering Cancer Center

Early detection of cancer

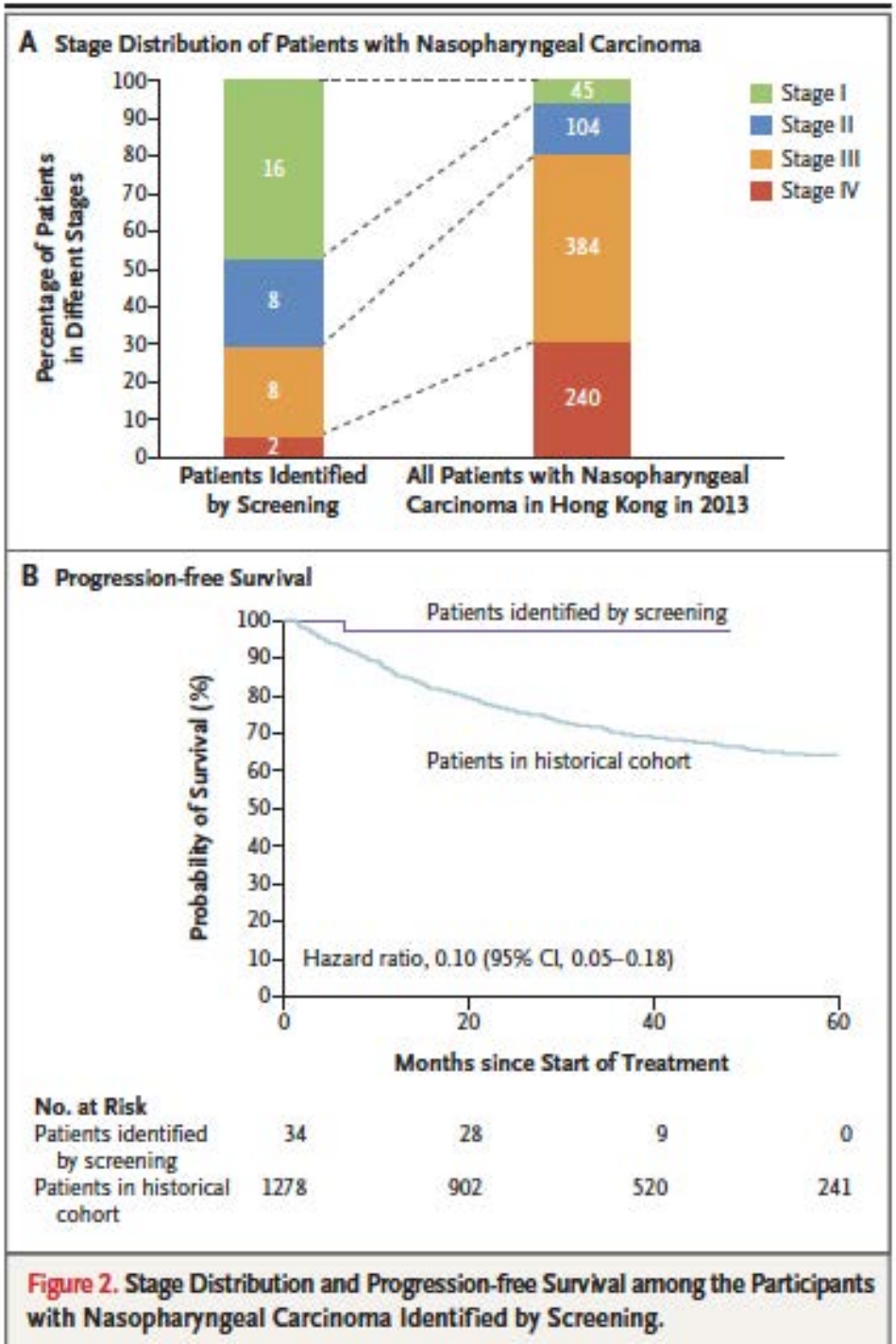


Figure 2. Stage Distribution and Progression-free Survival among the Participants with Nasopharyngeal Carcinoma Identified by Screening.

Targeting cancer-specific markers:

Detecting EBV viral DNA in plasma for detection of nasopharyngeal carcinoma

Chan, Lo et al NEJM 2017

Memorial Sloan Kettering Cancer Center

Analytical challenges: ctDNA present at a low fraction in plasma

Mutant allele fraction:

$$\frac{\text{Tumor-derived DNA}}{\text{[Normal+tumor derived DNA]}}$$

Low total cfDNA yield from plasma

Healthy volunteers
<1 – 12.5 ng/ml of plasma

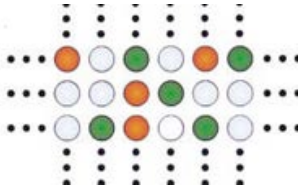
Cancer patients
10 ng DNA = ~3000 genome equivalent

Low fraction of tumor-derived DNA

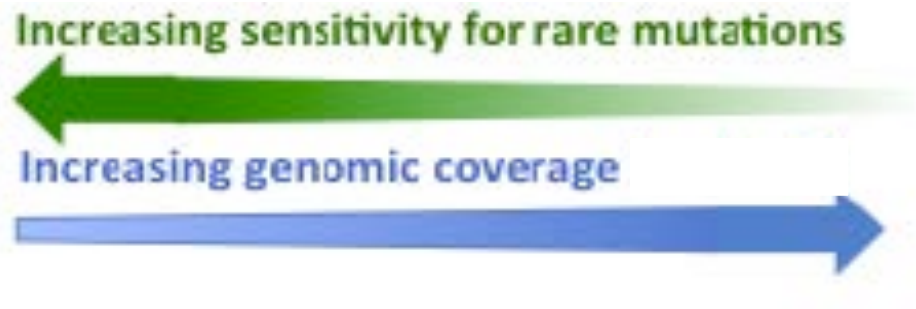
Metastatic
~5%, average 8-10%

Early stage
<1%

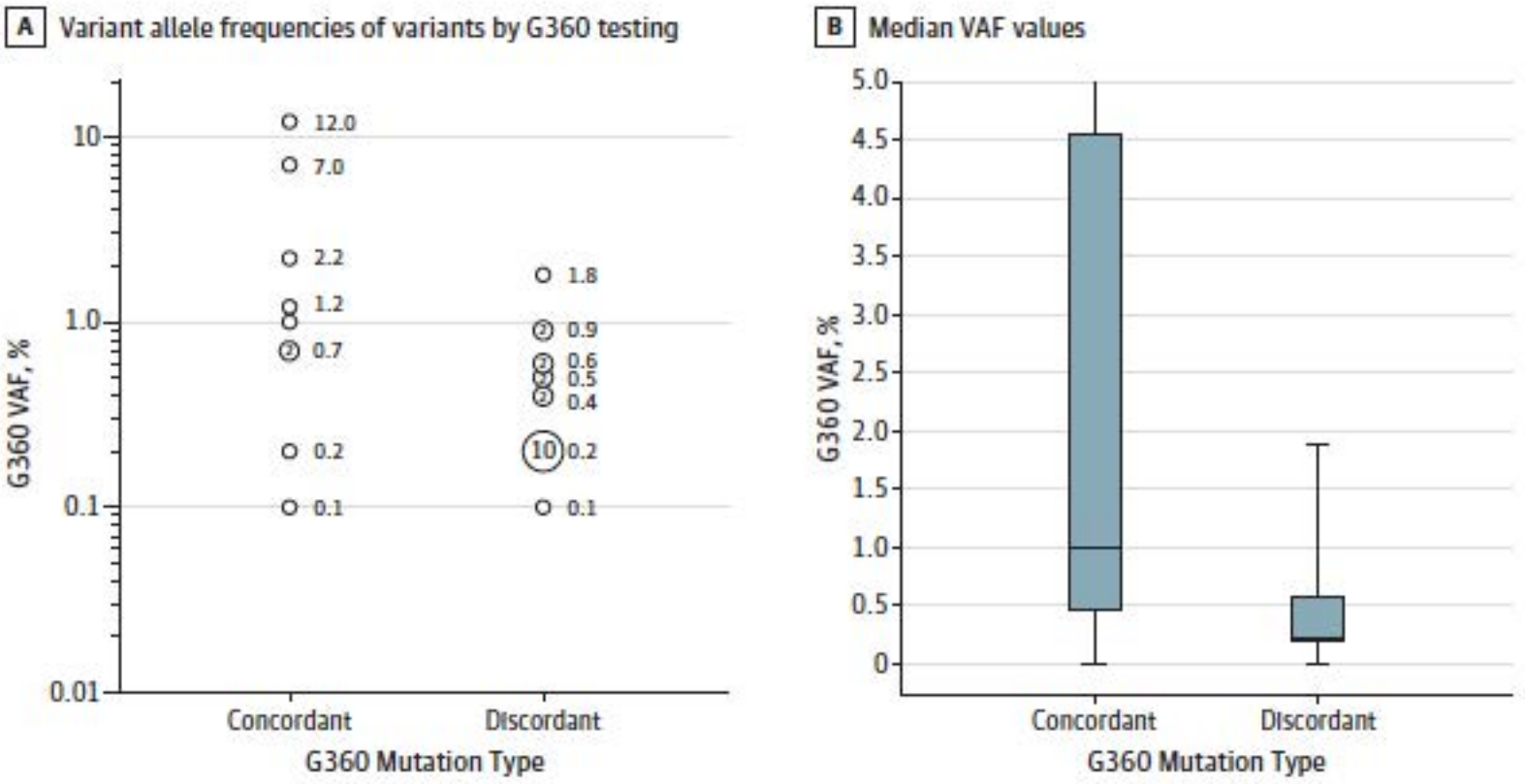
Analytical strategies



	Single-locus assay	Targeted sequencing	Whole genome analysis
Genomic bases screened	1~10	10^4	10^8
Mutation detection	<0.1%	1%	10%

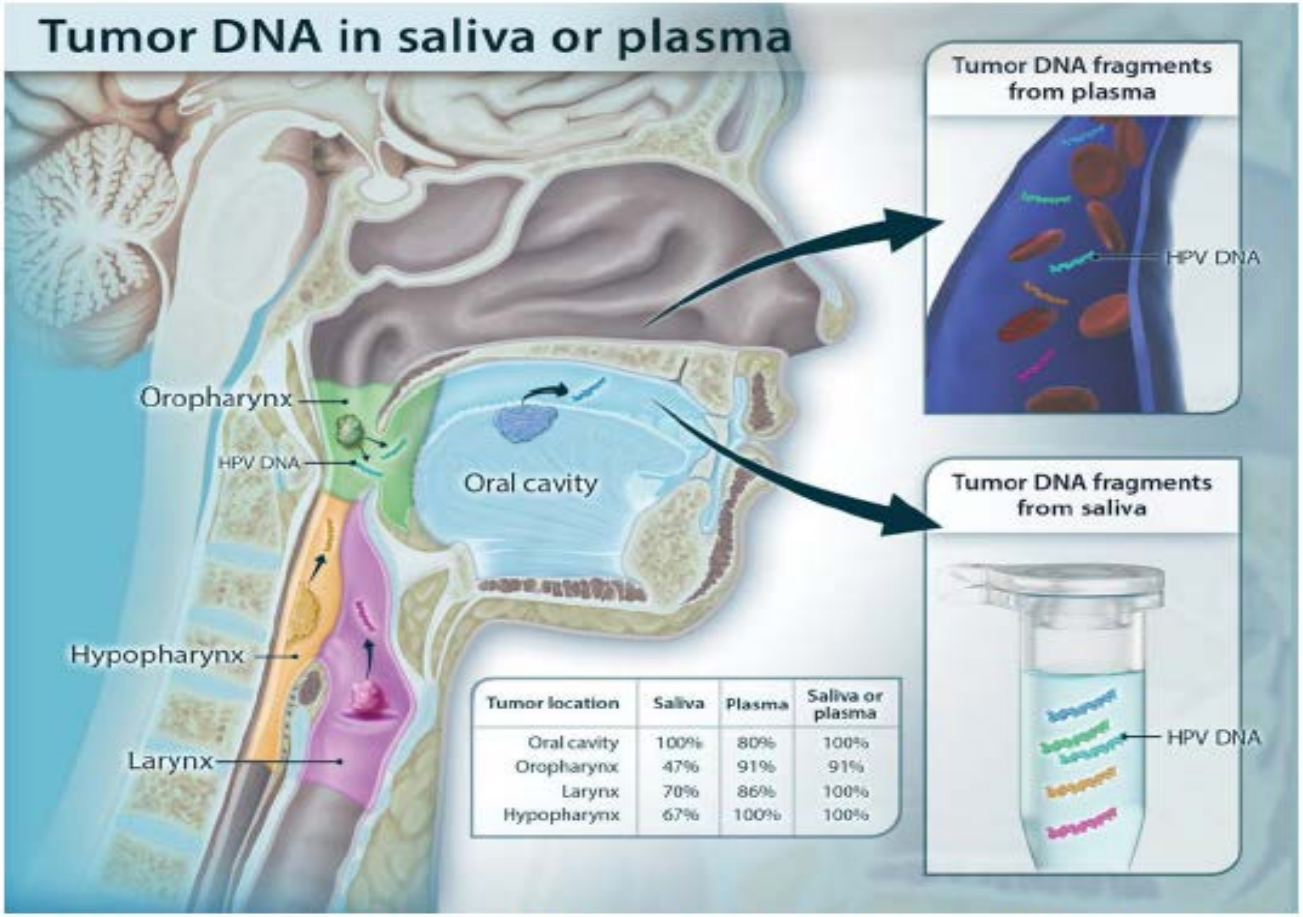


False positives and negatives



Kuderer et al JAMA Oncology 2016

Leverage the power of multiple body fluids



HPV – Human papillomaviruses

Wang, Agrawal STM 2015

Quality control: Assay validation

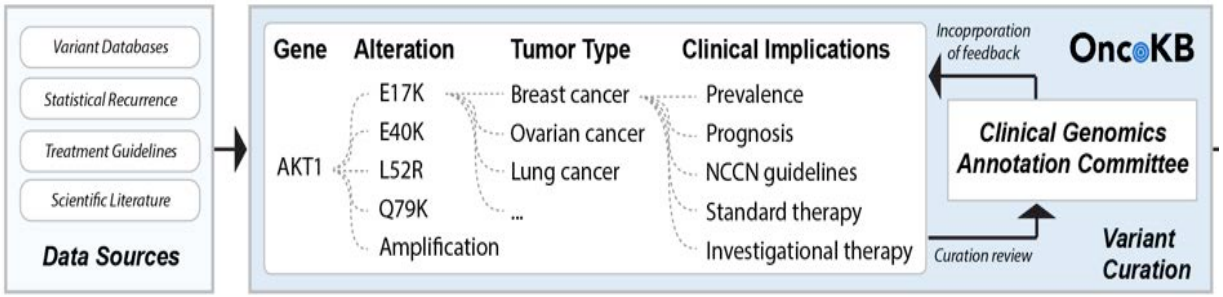
New York State Department of Health NGS assay validation guideline

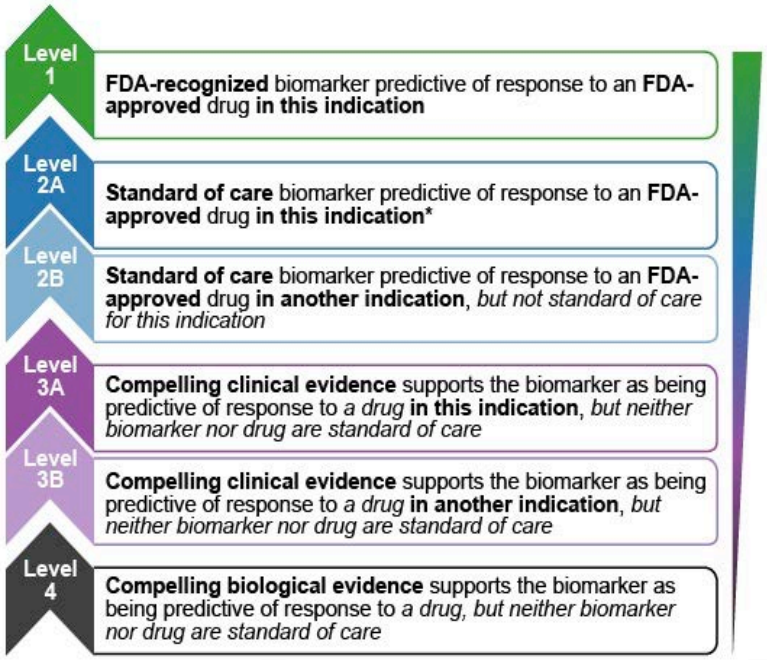
NYSDOH Guidelines for NGS-based somatic genetic variant detection	
SOP	Laboratory protocols
QC	Bioinformatic criteria for read quality, depth, coverage, etc.
Controls	No template control, negative control and positive control
Reports	Criteria for reporting
Validaiton	
Accuracy	A minimum of 3 well-characterized reference samples
Initial validation	A minimum of 50 patient samples of intended context
Full validation	10 positive samples for each type of intended variant
Precision (within run)	A minimum of 3 positive patient samples containing variants in the same run using different barcodes
Reproducibility (Between runs)	A minimum of 3 positive patient samples containing variants in three separate runs on 2 different days by 2 different technologists.
Contamination assessment	Verify no cross talk between samples and barcodes
Analytical sensitivity	Establish the analytical sensitivity for each type of variant using control materials

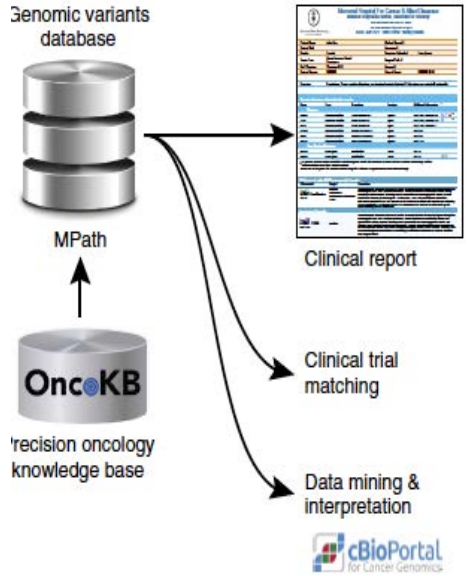
https://www.wadsworth.org/

What does it mean? Clinical annotations

OncoKB: MSK precision oncology knowledgebase of clinically relevant alterations



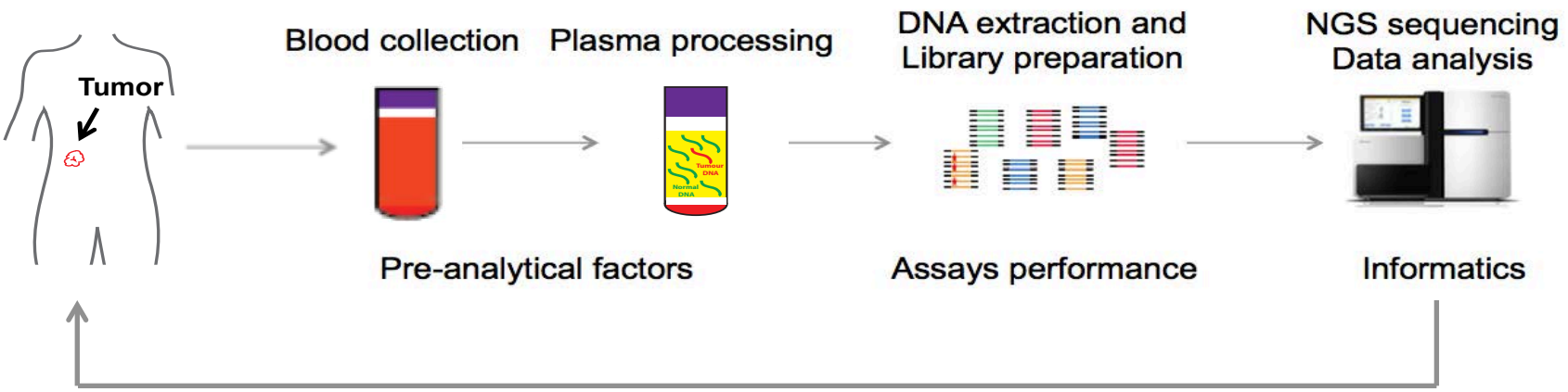




Chakravarty *et al.*, *JCO Precision Oncology*, 2017

Memorial Sloan Kettering Cancer Center

Liquid biopsy: promises and challenges



Promises

- Screen for actionable targets
- Monitor response and resistance
- Early detection

Challenges

- False positives and negatives
- Clinical interpretation
- Cost and reimbursement

Memorial Sloan Kettering Cancer Center