Stanford
Cancer Institute
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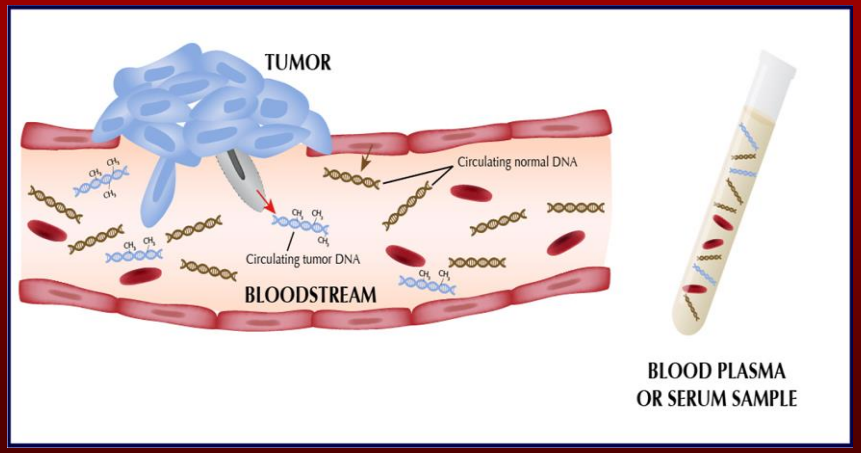
Stanford MEDICINE

Role of Circulating Tumor DNA Profiling in Cancer Management



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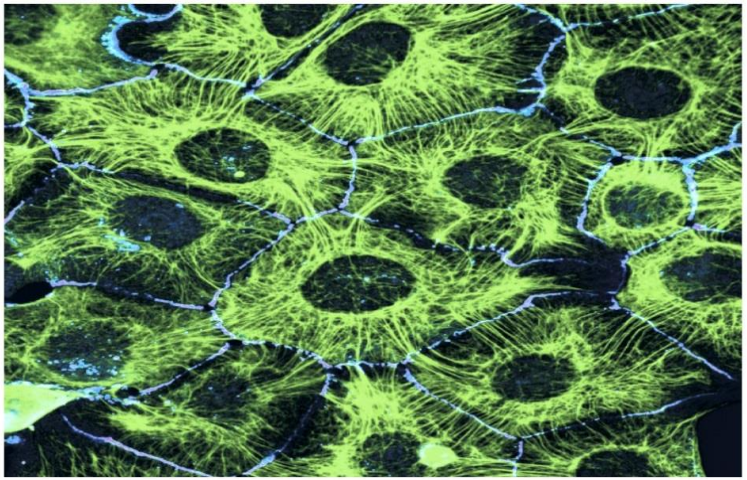
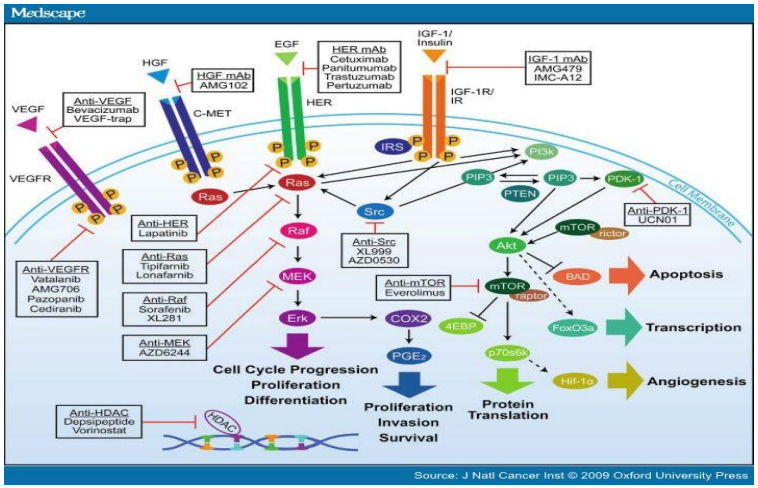
September 30, 2016

Changing Landscape of Drug Development

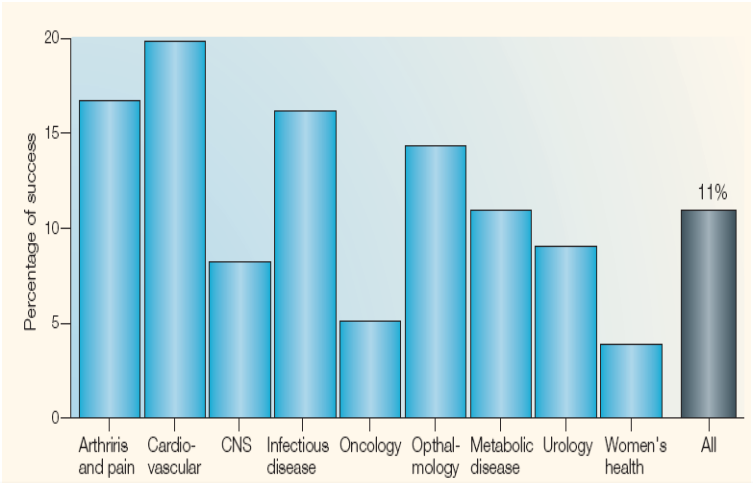
Increased Understanding of Cancer Biology



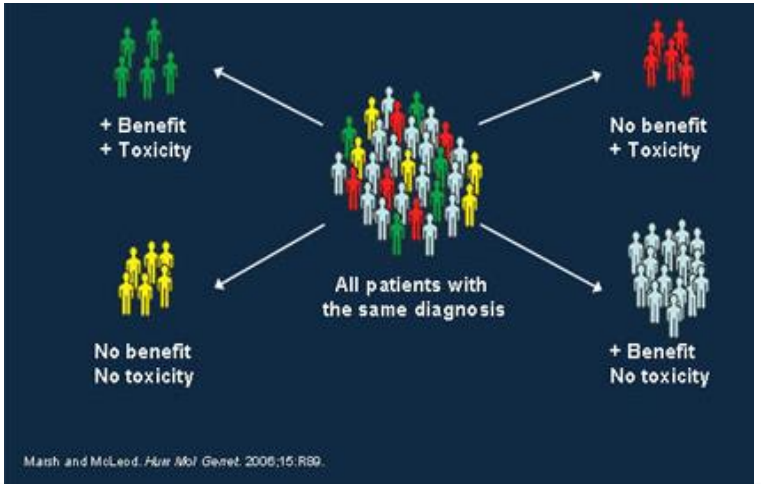
Advent of Targeted Therapies



High Attrition Rates/High Costs

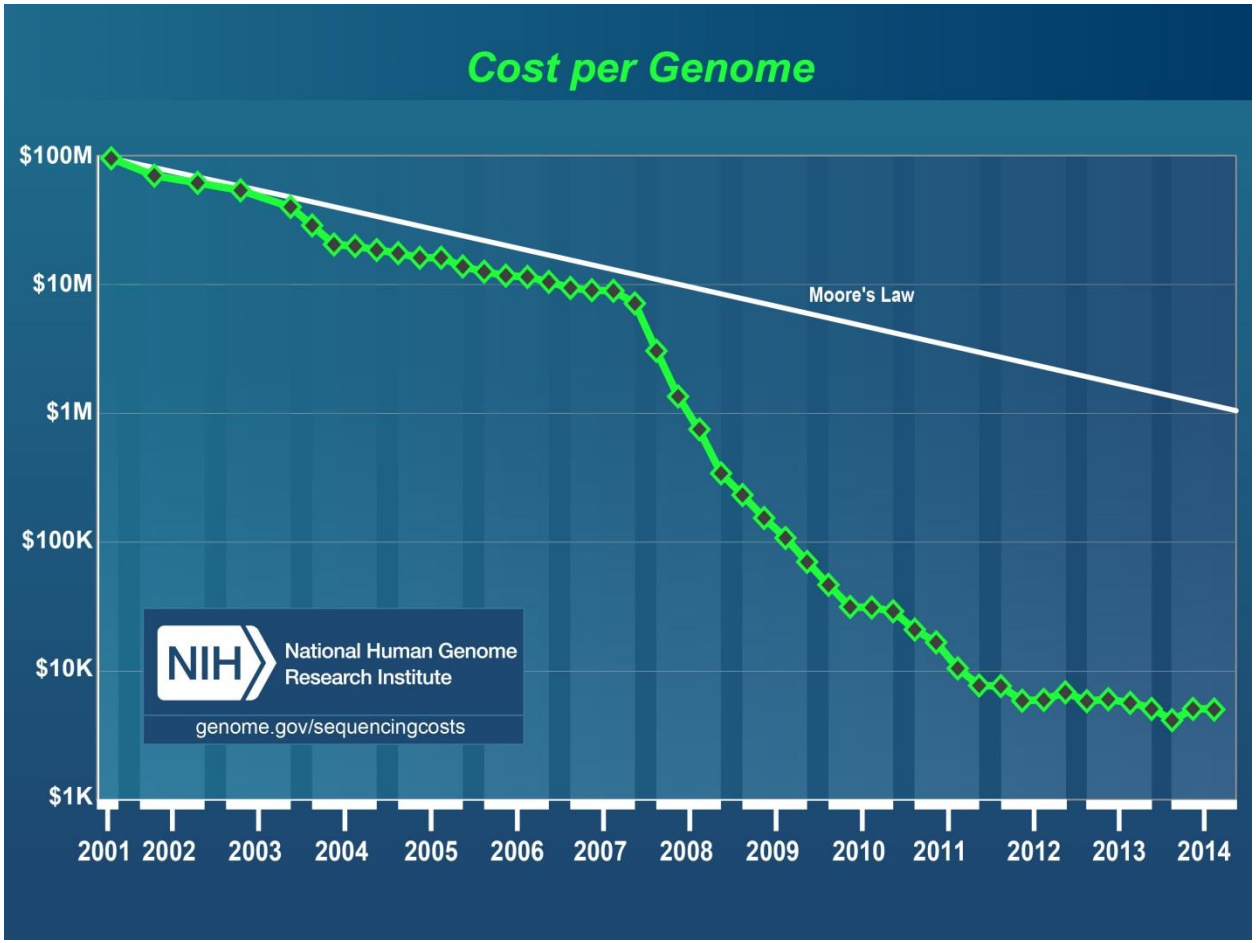


Personalized Medicine



Molecular Characterization for Patient Selection

Declining costs of sequencing: massively parallel next-generation sequencing and subsequent computational analysis



Date	Cost per Mb	Cost per Genome
Sep-01	$5,292.39	$95,263,072
Mar-02	$3,898.64	$70,175,437
Sep-02	$3,413.80	$61,448,422
Mar-03	$2,986.20	$53,751,684
Oct-03	$2,230.98	$40,157,554
Jan-04	$1,598.91	$28,780,376
Apr-04	$1,135.70	$20,442,576
Jul-04	$1,107.46	$19,934,346
Oct-04	$1,028.85	$18,519,312
Jan-05	$974.16	$17,534,970
Apr-05	$897.76	$16,159,699
Jul-05	$898.90	$16,180,224
Oct-05	$766.73	$13,801,124
Jan-06	$699.20	$12,585,659
Apr-06	$651.81	$11,732,535
Jul-06	$636.41	$11,455,315
Oct-06	$581.92	$10,474,556
Jan-07	$522.71	$9,408,739
Oct-07	$397.09	$7,147,571
Jan-08	$102.13	$3,063,820
Oct-08	$3.81	$342,502
Jan-09	$2.59	$232,735
Oct-09	$0.78	$70,333
Jan-10	$0.52	$46,774
Oct-10	$0.32	$29,092
Jan-11	$0.23	$20,963
Apr-11	$0.19	$16,712
Jul-11	$0.12	$10,497
Oct-11	$0.09	$7,743
Jan-12	$0.09	$7,666
Apr-12	$0.07	$5,901
Jul-12	$0.07	$5,985
Oct-12	$0.07	$6,618
Jan-13	$0.06	$5,671
Oct-13	$0.06	$5,096
Jan-14	$0.04	$4,008
Apr-14	$0.05	$4,920
Jul-14	$0.05	$4,905

COSMIC: Catalog of Somatic Mutations in Cancer

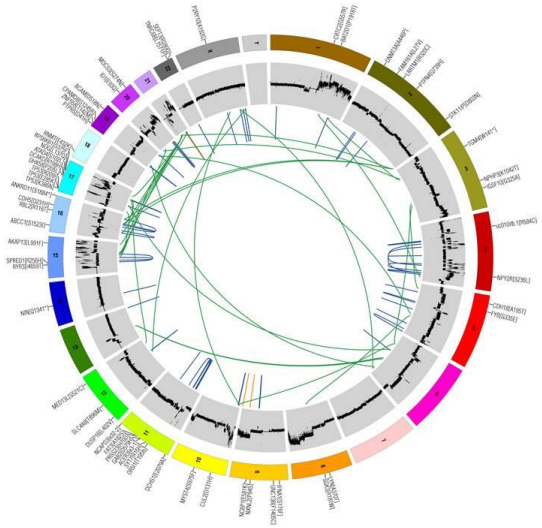
- COSMIC launched in 2004, detailed 4 cancer genes
- 2014: world's largest and most comprehensive resource
  - 2, 002, 811 coding point mutations in over one million tumor samples
  - 6 million noncoding mutations,

    10, 534 gene fusions,

    61 299 genome rearrangements

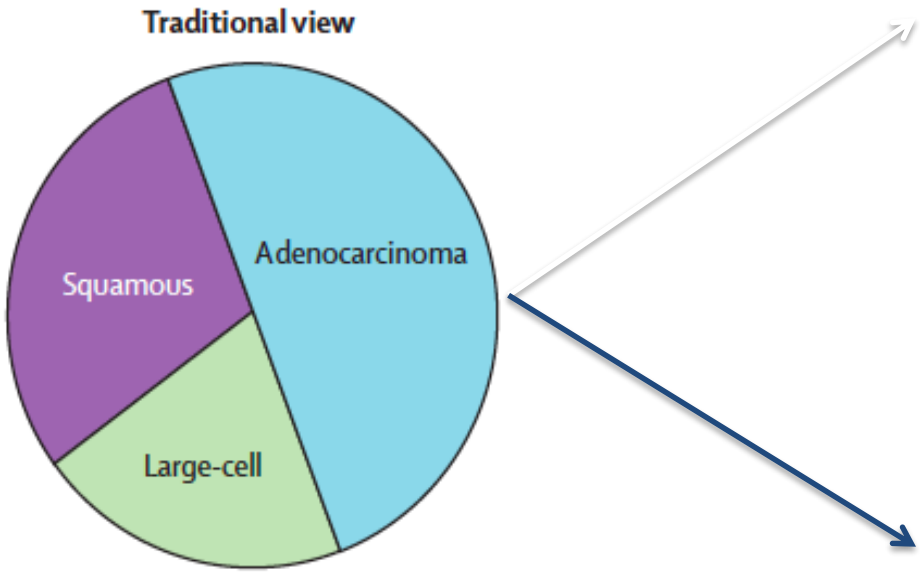
    695, 504 abnormal copy number segments and

    60,119,787 abnormal expression variants

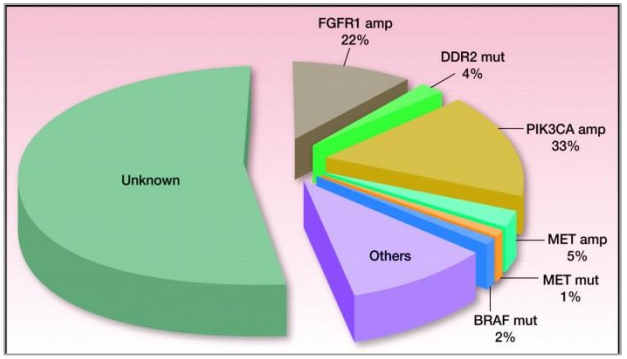


Forbes SA, et al. *Nucl. Acids Res. 2015; 43 (D1): D805*

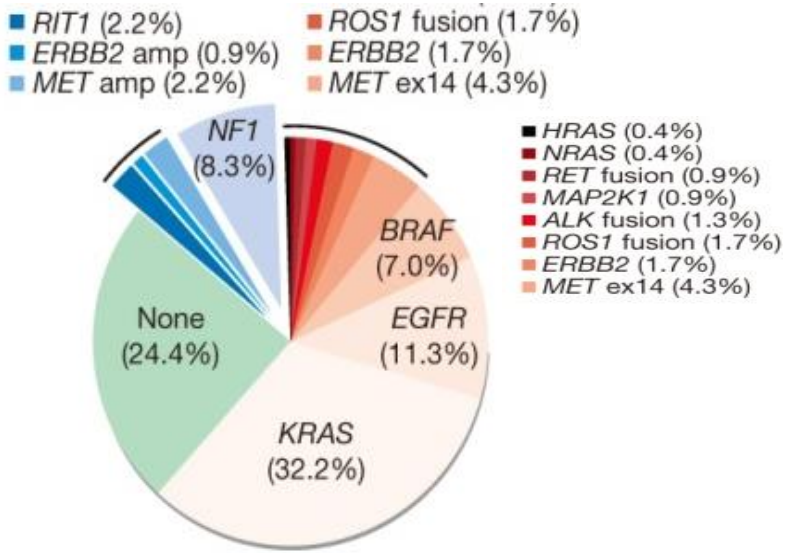
Transition From Histology → Genomic Driver Mutations



SQUAMOUS

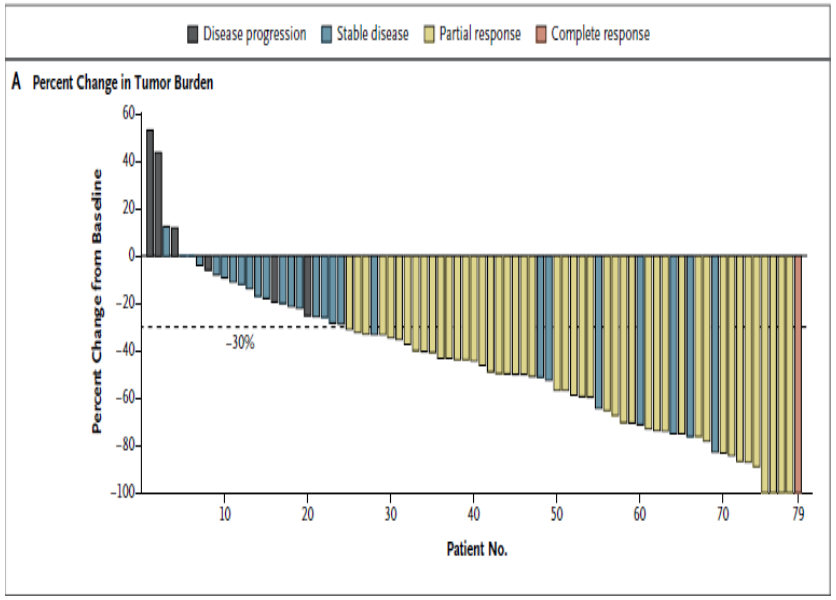


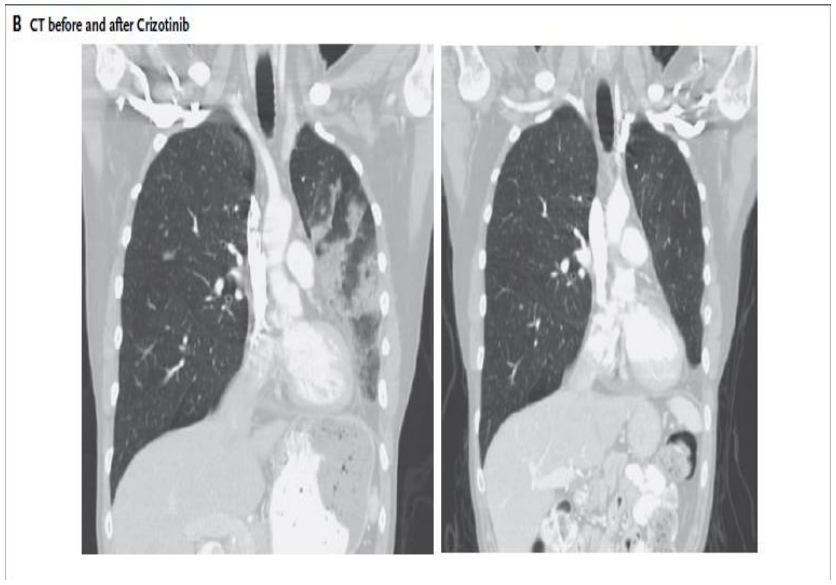
ADENOCARCINOMA



Pao W, Girard N. *Lancet Oncol*. 2011;12:175-180;
Perez-Moreno P, et al. *Clin Cancer Res*. 2012;18:2443-2451;
Cancer Genome Atlas Research Network. *Nature*. 2012;489:519-525;
Cancer Genome Atlas Research Network. *Nature*. 2014;511:543-550.

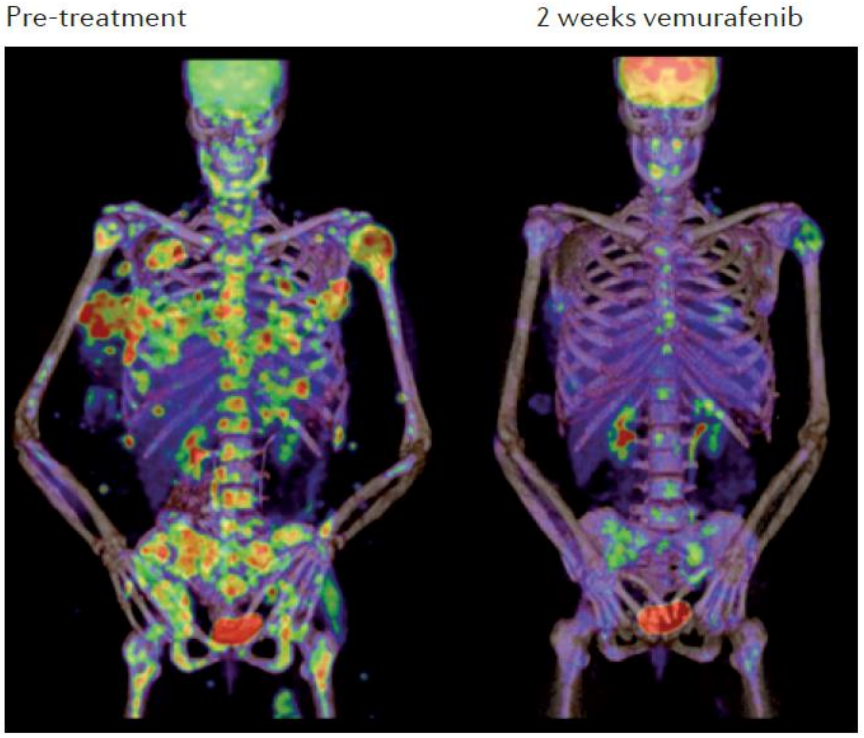
ALK inhibition in EML4-ALK + NSCLC





Vemurafenib in BRAF V600E mutant melanoma

- High response rate in early phase trials (>50%)
- Around 5 years from filing of Investigational New Drug (IND) application and approval by the FDA in August 2011



Tumor Profiling

- Exciting, dramatic responses, accelerated drug approvals

  Phase I for Crizotinib –standard dose escalation in solid tumors, 2 pts responded→ profiling showed ALK rearrangement→protocol amended to include an expansion cohort→1500 patients screened from 2008-2010 to enroll 82 patients with FISH+ ALK rearrangement→57% objective confirmed partial/complete response. 2011-Crizotinib approved by the FDA

- Challenges:

  Responses may be short-lived (e.g.vemurafenib)-development of resistance

  Tumor heterogeneity

  Defining a genetic aberration as ‘actionable’

  Finding enough patients

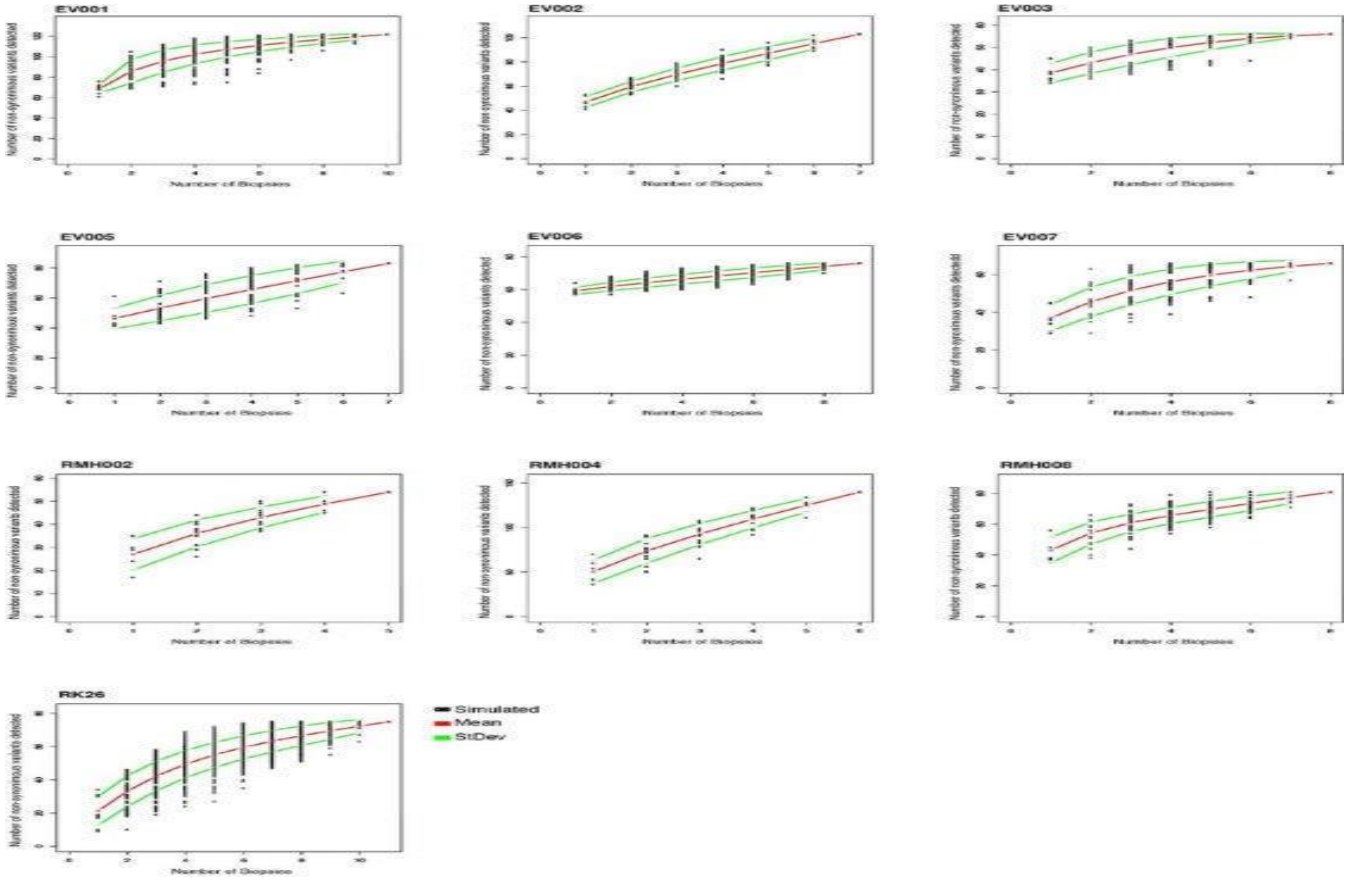
Tumor heterogeneity

- Difference within regions of a given tumor and between primary and metastases
- Only 34% of all mutations detected by multiregion sequencing in the nephrectomy specimen were present in all regions
- So far, clinical decisions are based on data generated from one or 2 core biopsy specimens from any one lesion
- 'Ongoing regional clonal evolution'
- Prognostic gene expression signatures shown to classify into good or bad prognostic categories for RCC varied by region

Gerlinger M, et al. N Eng J Med 2012; 366(10): 883

Is the sample representative of the disease being treated?
Archival vs fresh tissue?

How many biopsies need to be analyzed



The number of mutations that would have been detected from each tumor by sampling one to *n* biopsies (where *n* was the number of biopsies sampled from that tumor)

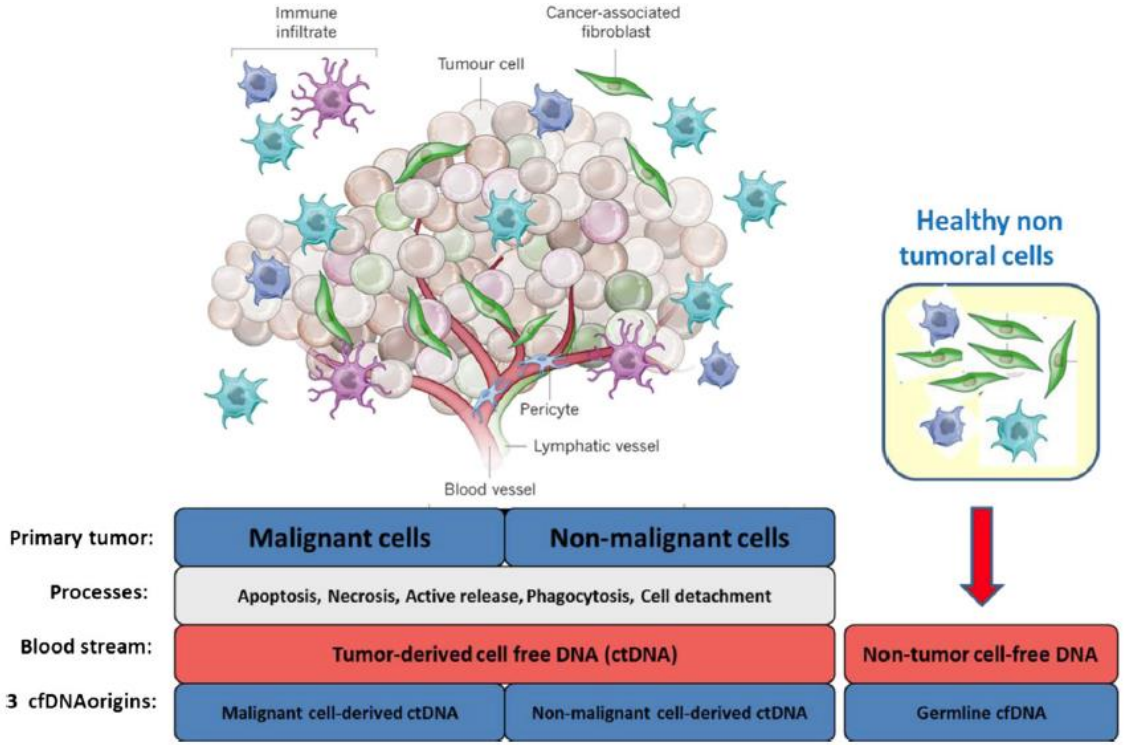
Gerlinger M, et al. *Nat Gen* 2014

Cell-free circulating tumor DNA (ctDNA)?

Circulating DNA

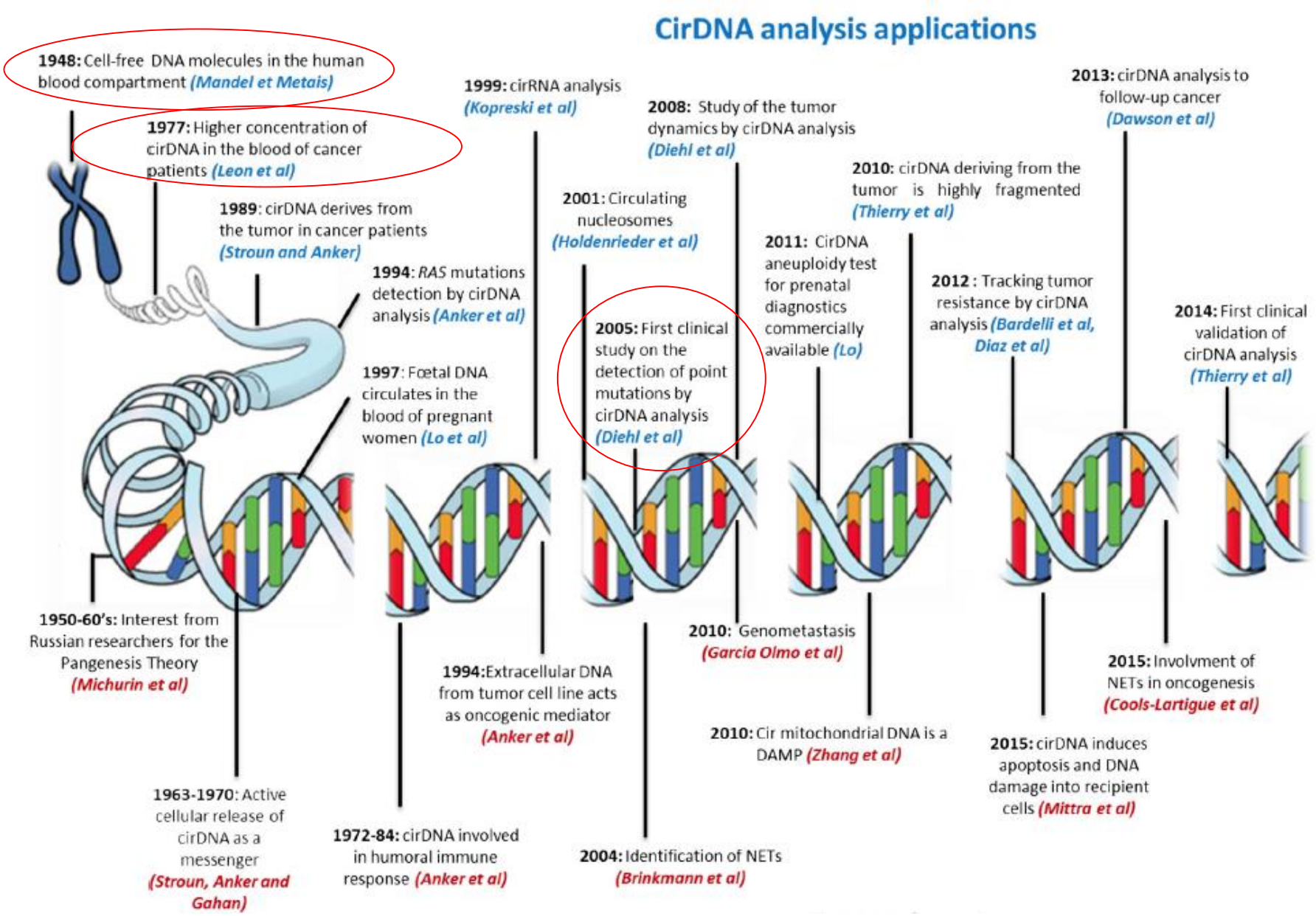
Can arise from various cell populations, could be nuclear or mitochondrial

Exist in number of structural forms: particulate structures (exosomes, microparticles, apoptotic bodies) or macromolecular structures (nucleosomes, virtosomes/proteolipidnucleic acid complexes, DNA traps, links with serum proteins or to cell-free membrane parts



Thierry AR, et al. Cancer Metastasis Rev 2016

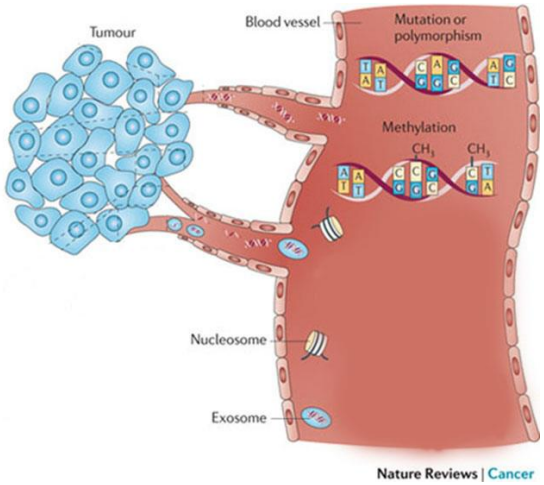
Timeline for important discoveries about circulating DNA



Thierry AR, et al. Cancer Metastasis Rev 2016

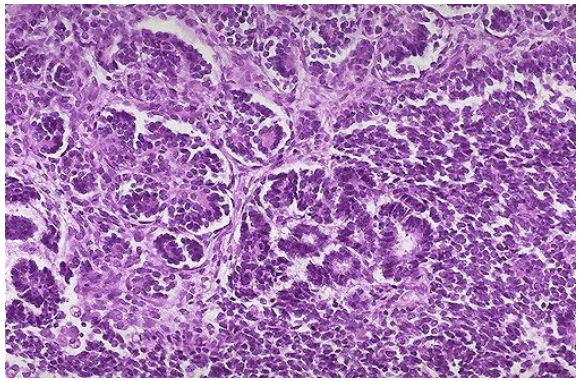
ctDNA

- Minimally invasive
- Longitudinal sampling
- Low cost
- Potentially represents multiple disease sites



Tumor biopsy

- Invasive, associated risks
- Limited sampling
- Expensive, resource intense
- Limited sample from one site



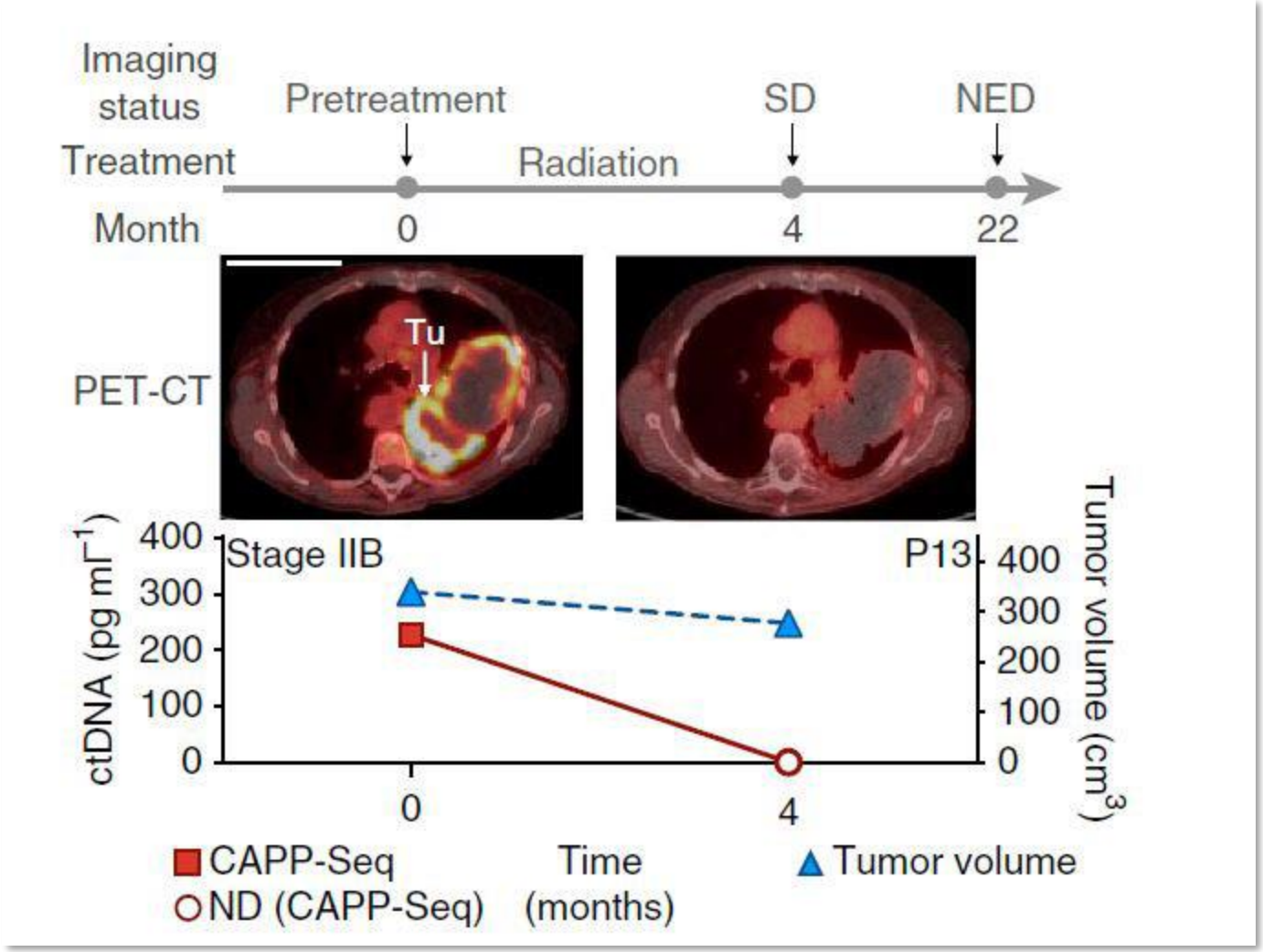
Role of ctDNA in Cancer Management

- Known driver mutations; known aberrations in that disease
  - Disease burden
  - As an early response marker
  - Monitor for recurrence
  - Tumor heterogeneity-does it provide a more complete picture of the presence of various clones?
  - Emergence of resistant clones
  - Diagnostic
    - Presence of actionable mutations
    - Differentiate between benign and malignant disease
- Broad profiling to look for genetic aberrations

Assessing tumor burden, marker of response

Percentage of ctDNA to total cirDNA is a measure for tumor burden

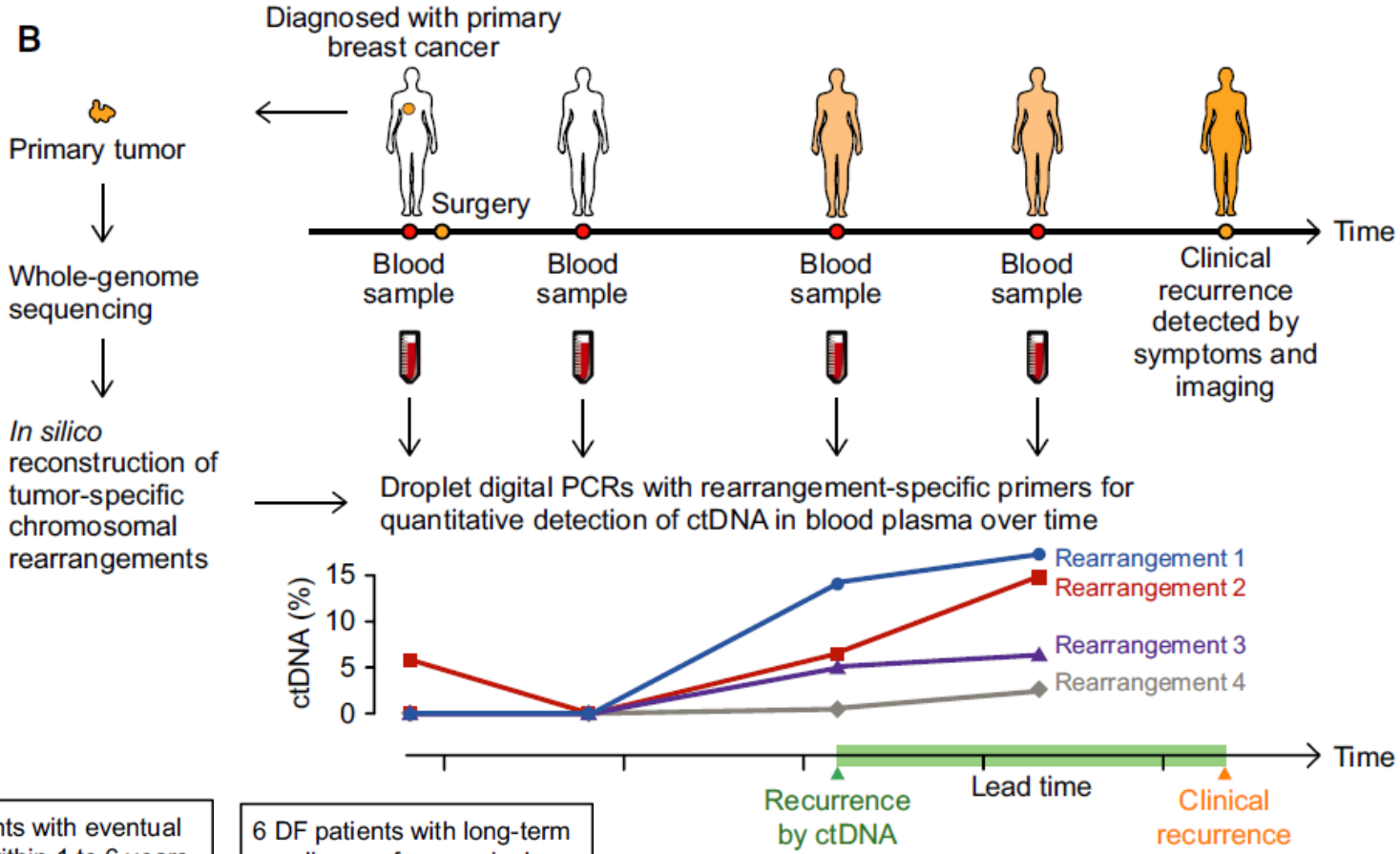
Cancer Personalized Profiling by deep Sequencing [CAPP-Seq] in lung cancer



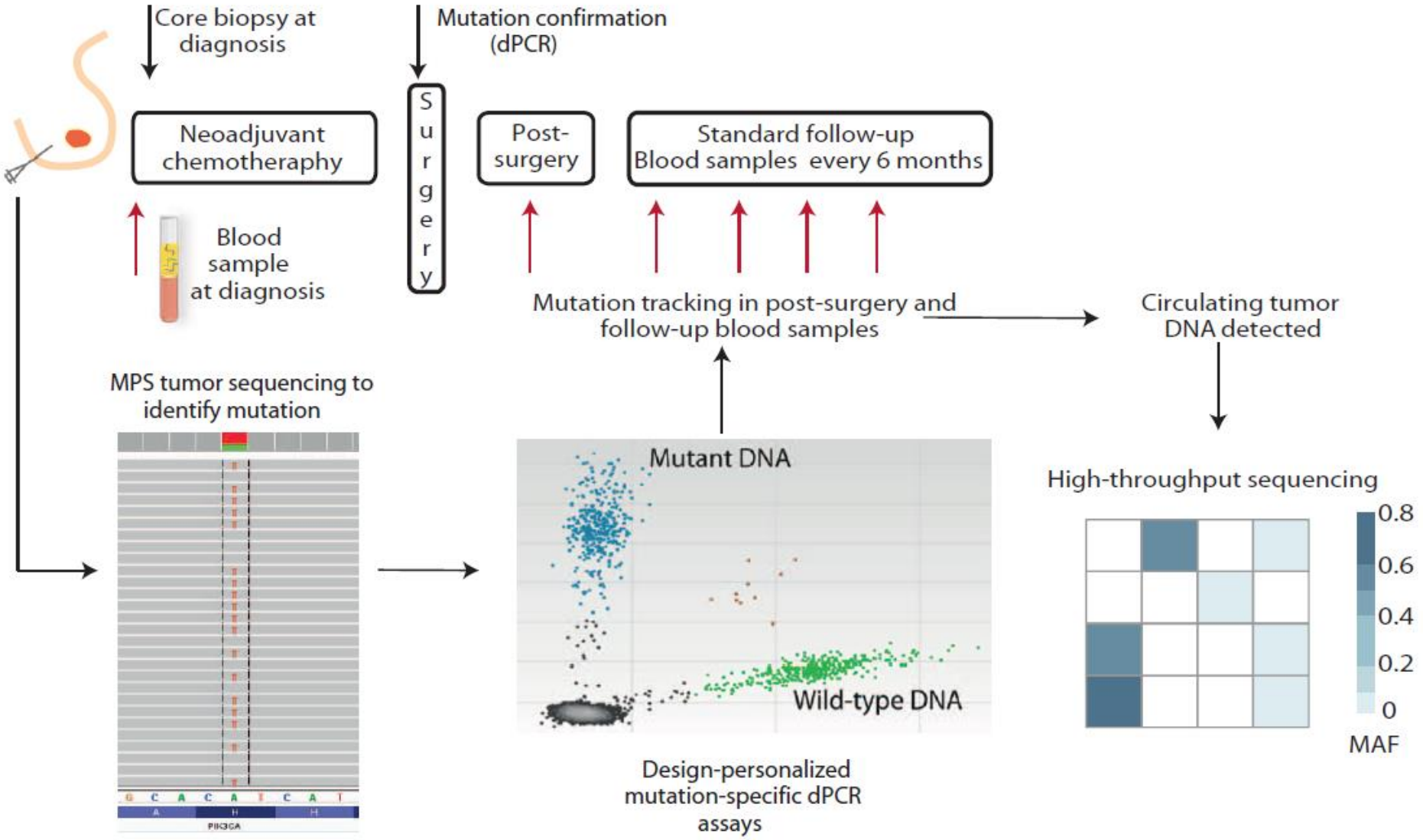
Newman AM, et al. Nat Med 2014;20(5):548

Monitoring ctDNA for risk of recurrence

Hypothesis: Monitoring of tumor-specific chromosomal rearrangements in ctDNA can detect occult metastatic disease and serve as a sensitive, specific, and thus potentially clinically useful noninvasive biomarker in the early stage disease setting



Olsson E, et al. EMBO Mol Med 2015; 7(8)



- 55 women with early stage breast cancer who received neoadjuvant chemo → definitive t/t
- Mutation tracking with serial ctDNA samples was predictive of relapse (median of 7.9 mos lead time over clinical relapse)
- In the 3 pts with CNS only relapse, no ctDNA was detected before or at relapse

Garcia-Murillas I, et al. Sci Trans Med 2015, 7(302)

ctDNA: Is it better able to define tumor heterogeneity?

- Analysis of tumor biopsies from patients with NSCLC progressing on EGFR TKIs demonstrated presence of potential additional resistance mechanisms in ~15% of cases.
- In 41 pts with T790M mutation+ NSCLC progressing on EGFR TKIs, additional putative resistance mutations were detected in 46% (19 pts) in pre-treatment plasma.

  14 pts had increased copy number in MET or ERBB2

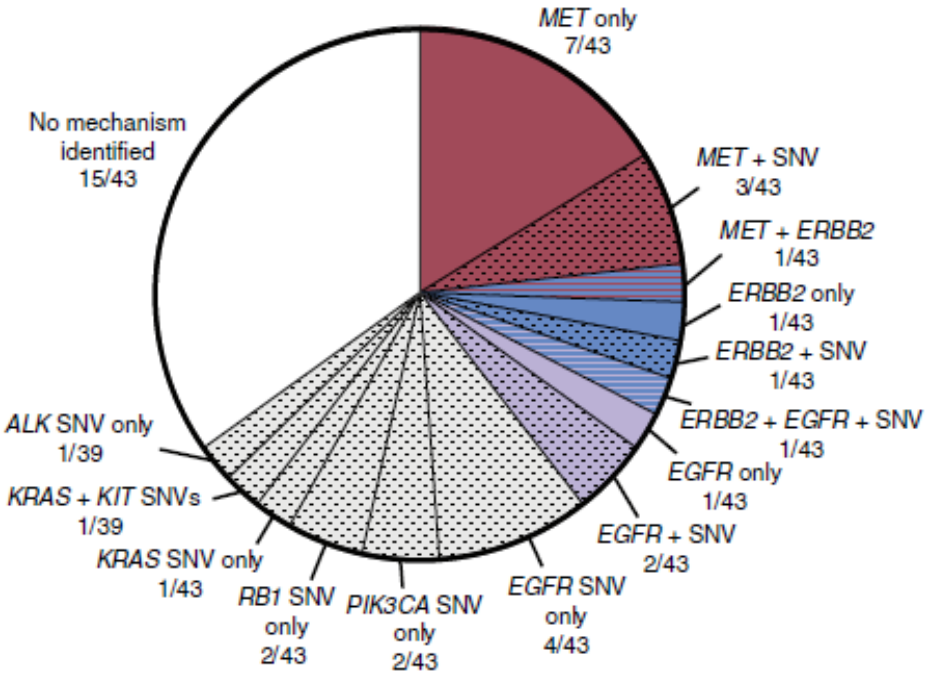
  3 pts had SNVs in EGFR, PIK3CA or RB1

  2 had both an increased copy number in MET and SNVs in PIK3CA or RB1
- The ability to detect additional abnormalities at baseline may impact choice of subsequent therapy and better define innate resistance.
- Not a direct comparison of tumor biopsy vs ctDNA in the same patient

Chabon JJ, et al. Nat Commun 2016;7:11815

Defining resistance in pts with EGFR+ NSCLC

Rociletinib selectively targets T790M containing subclones
43 pts with T790M EGFR + NSCLC-samples baseline and at progression
At progression, 28/43 pts (65%) had one or more putative resistance mechanism



Red, blue, purple: SCNA affecting MET, ERBB2, EGFR resp.

SNV: single nucleotide variants
SCNA: somatic copy-number alterations

Gene	Number of patients	Baseline alterations	Emergent alterations
EGFR	5	A750P (1)	C797S (1) L798I (1) L692V (1) E709K (1)
PIK3CA	5	E545K (1)	E542K (3) E545K (3) E81K (1)
KRAS	3		G12A (1) Q61H (1) A146T (1)
CDKN2A	3	D74A (1)	D74A (2)
RB1	2	G587* (1)	R787Q (1)
ALK	1		R1061Q(1)
KIT	1		L576P (1)
MET	1	D1304H (1)	

Chabon JJ, et al. Nat Commun 2016;7:11815

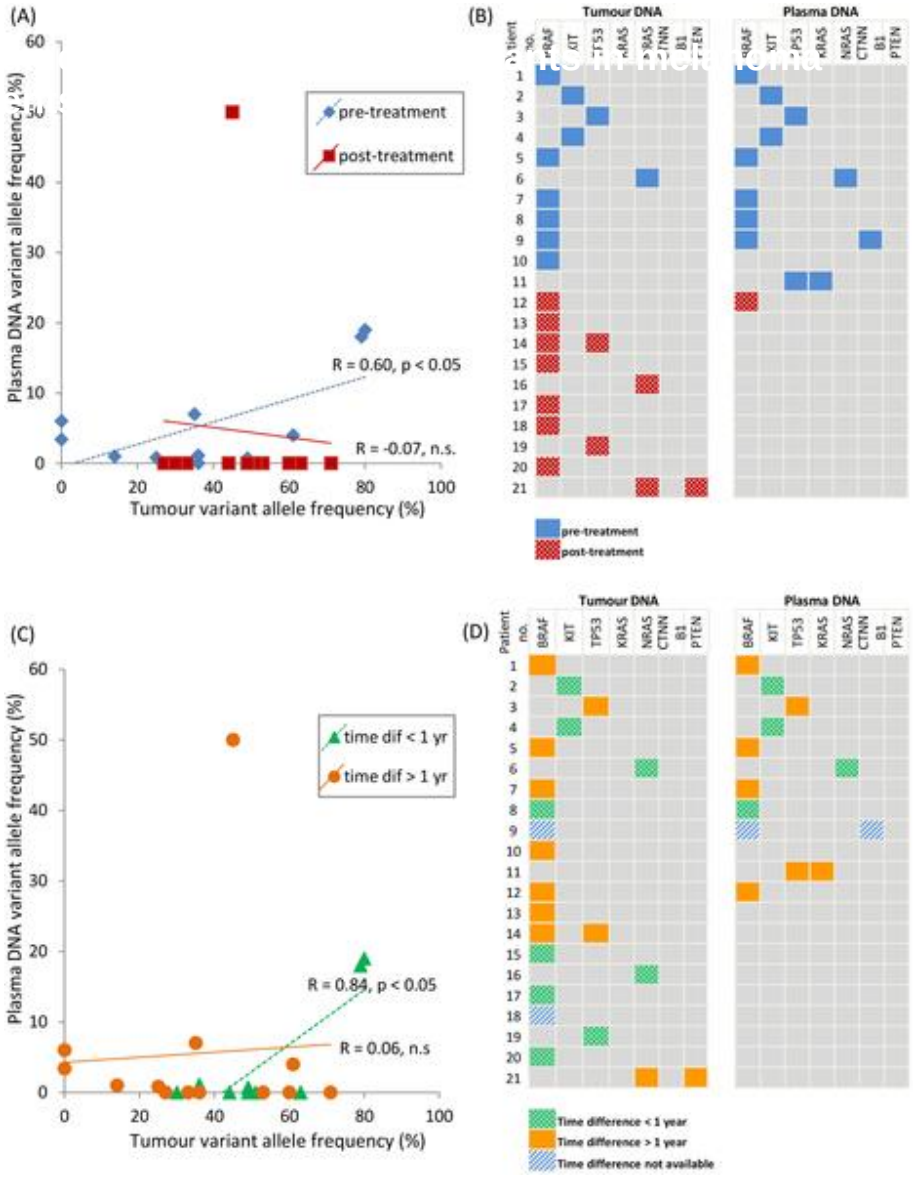
ctDNA Analysis as a Companion Diagnostic

- June 2016: FDA approved **cobas**® EGFR Mutation Test v2 using plasma specimens as a companion diagnostic test for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations in EGFR gene to identify patients with metastatic NSCLC eligible for treatment with Tarceva® (erlotinib).
- Ph III ENSURE trial: Efficacy and safety of Tarceva versus gemcitabine plus cisplatin as first-line treatment for stage IIIB/IV NSCLC pts. T/t was assigned based on tumor tissue results, 98.6% of pts also had plasma samples available.

  In 76.7% (70.5%, 81.9%) of tissue-positive specimens, plasma was also positive for an EGFR mutation.

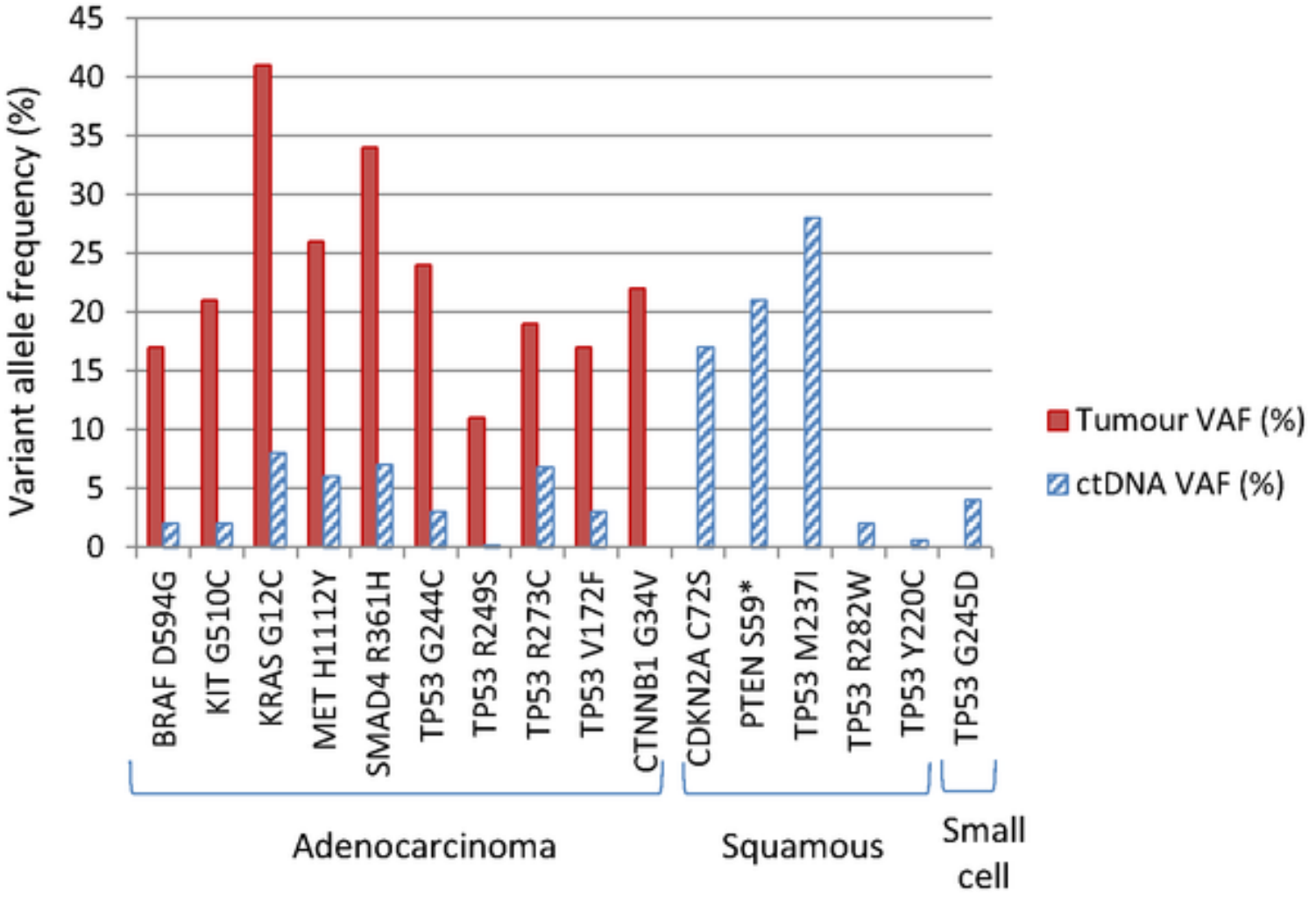
  Plasma was negative for EGFR mutation in 98.2% (95.4%, 99.3%) of tissue-negative cases.
- Test approved for pts too ill or otherwise difficult to get tumor tissue
- If ctDNA result is positive then proceed with EGFR inhibitor, if negative then obtain tumor tissue.

Good concordance between melanoma ctDNA and primary tumors when samples were collected before treatment or less than one year apart



Kaisaki PJ, Cutts A, Popitsch N, Camps C, Pentony MM, et al. (2016) Targeted Next-Generation Sequencing of Plasma DNA from Cancer Patients: Factors Influencing Consistency with Tumour DNA and Prospective Investigation of Its Utility for Diagnosis. PLoS ONE 11(9): e0162809. doi:10.1371/journal.pone.0162809
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0162809

Somatic variants in lung cancer tumor (diagnostic biopsy) and plasma DNA



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http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0162809

Definitions

- ***Analytical performance (analytical validity):*** how accurately the test detects the analyte(s) of interest

- ***Clinical Validity***: How well does the assay result correlate with outcome?

- ***Clinical Utility***: How does use of the assay improve outcome?