Liquid biopsies

Sabine Tejpar, MD PhD
University Hospital Leuven, KULeuven
Belgium

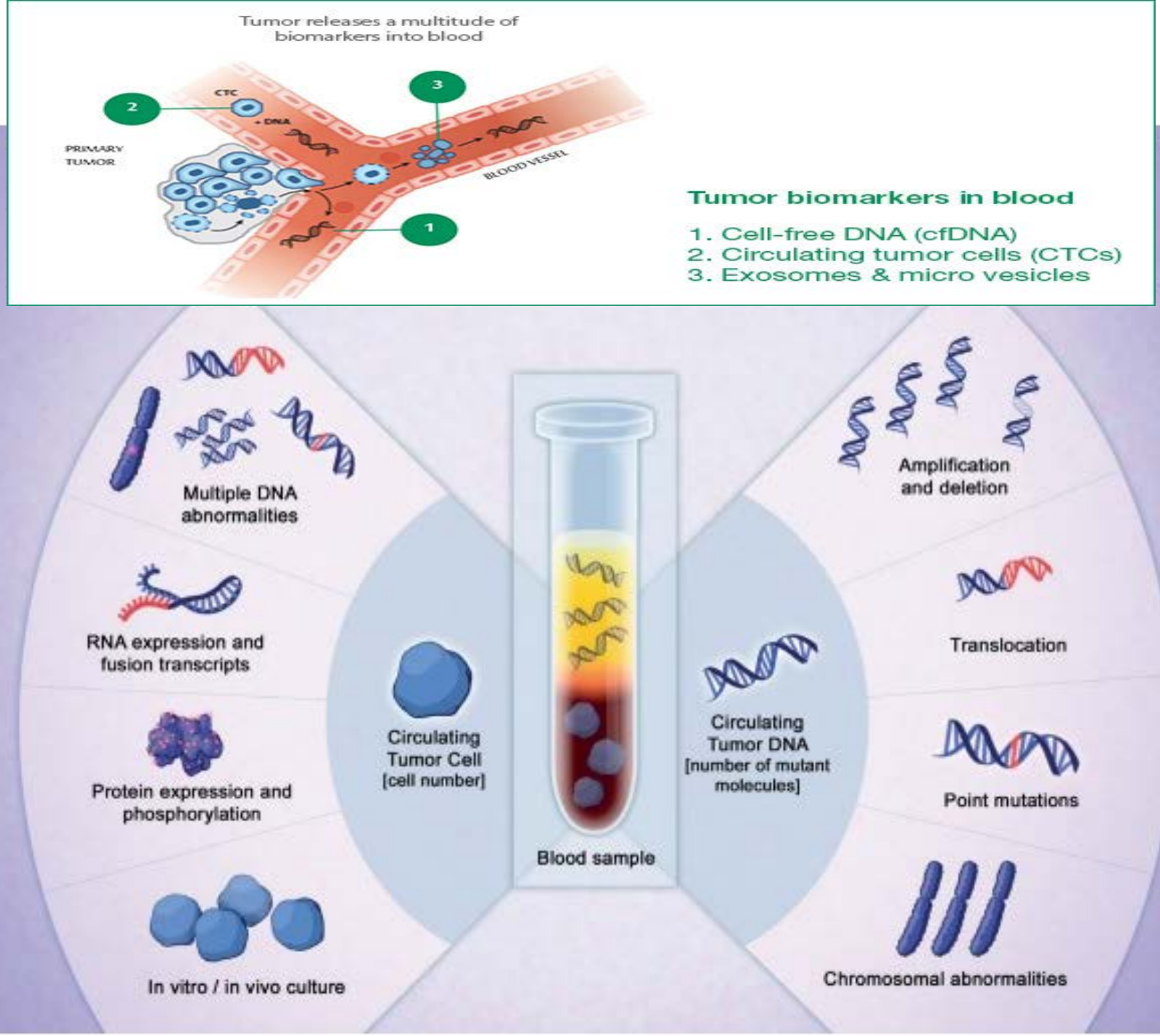
Outline

- Amazing principles/ technologies
- Many relevant applications
- Burden of proof
- How to navigate the (hyped) market?

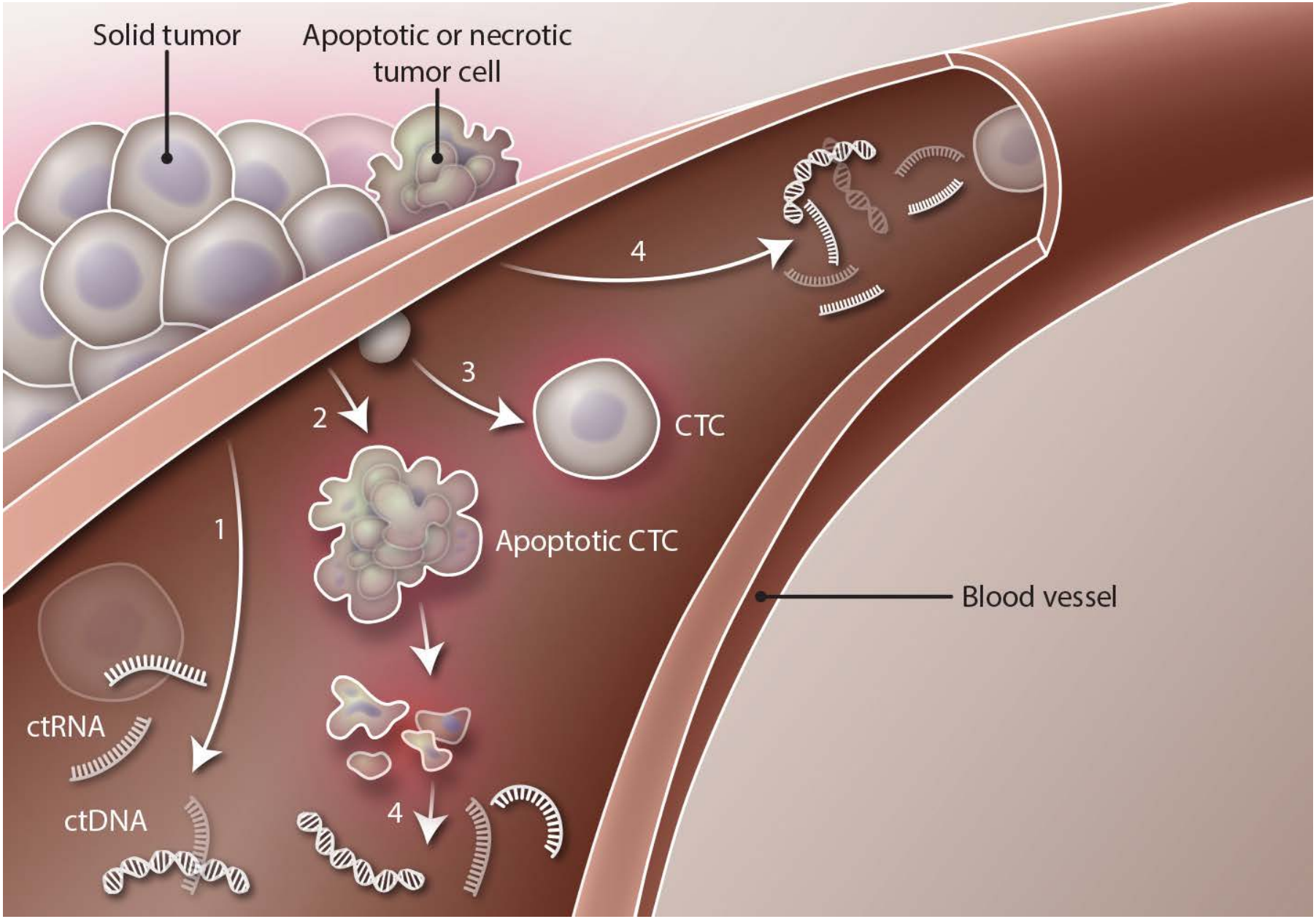
What

- All non tissue based diagnostics

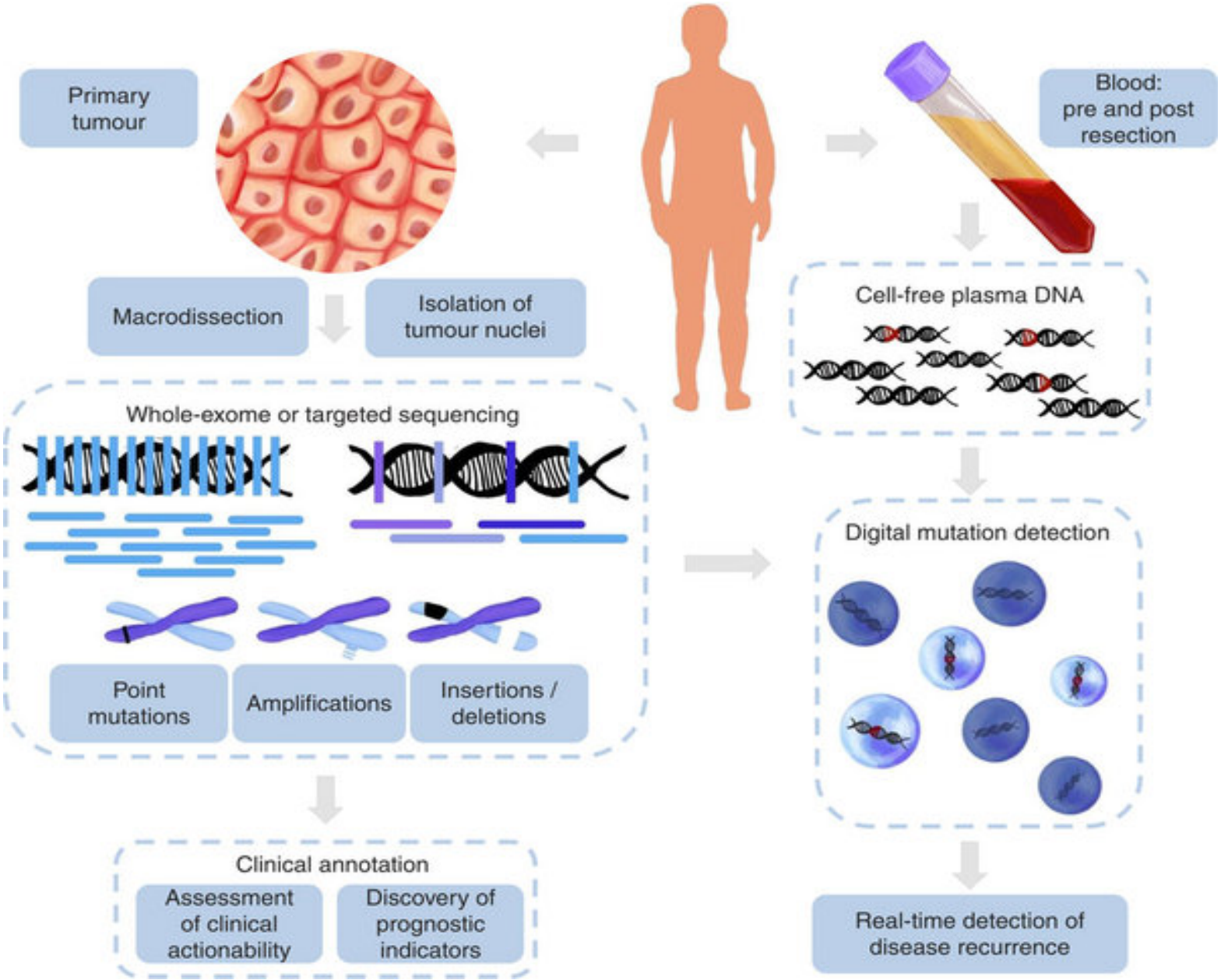
- Tissue is the issue
- Meric-Bernstam JCO 2015 at MDACC found 23% of patients referred for studies were ineligible due to tissue inadequacy for genomic testing
- Biopsy related complications, cost
- Liquid biopsies -> the stethoscope for the next 200 years Wall St Journal, 2015


Tumor releases a multitude of biomarkers into blood
CTC
+ DNA
PRIMARY TUMOR
BLOOD VESSEL
1
2
3
Tumor biomarkers in blood
1. Cell-free DNA (cfDNA)
2. Circulating tumor cells (CTCs)
3. Exosomes & micro vesicles
Multiple DNA abnormalities
RNA expression and fusion transcripts
Protein expression and phosphorylation
In vitro / in vivo culture
Circulating Tumor Cell [cell number]
Blood sample
Circulating Tumor DNA [number of mutant molecules]
Amplification and deletion
Translocation
Point mutations
Chromosomal abnormalities

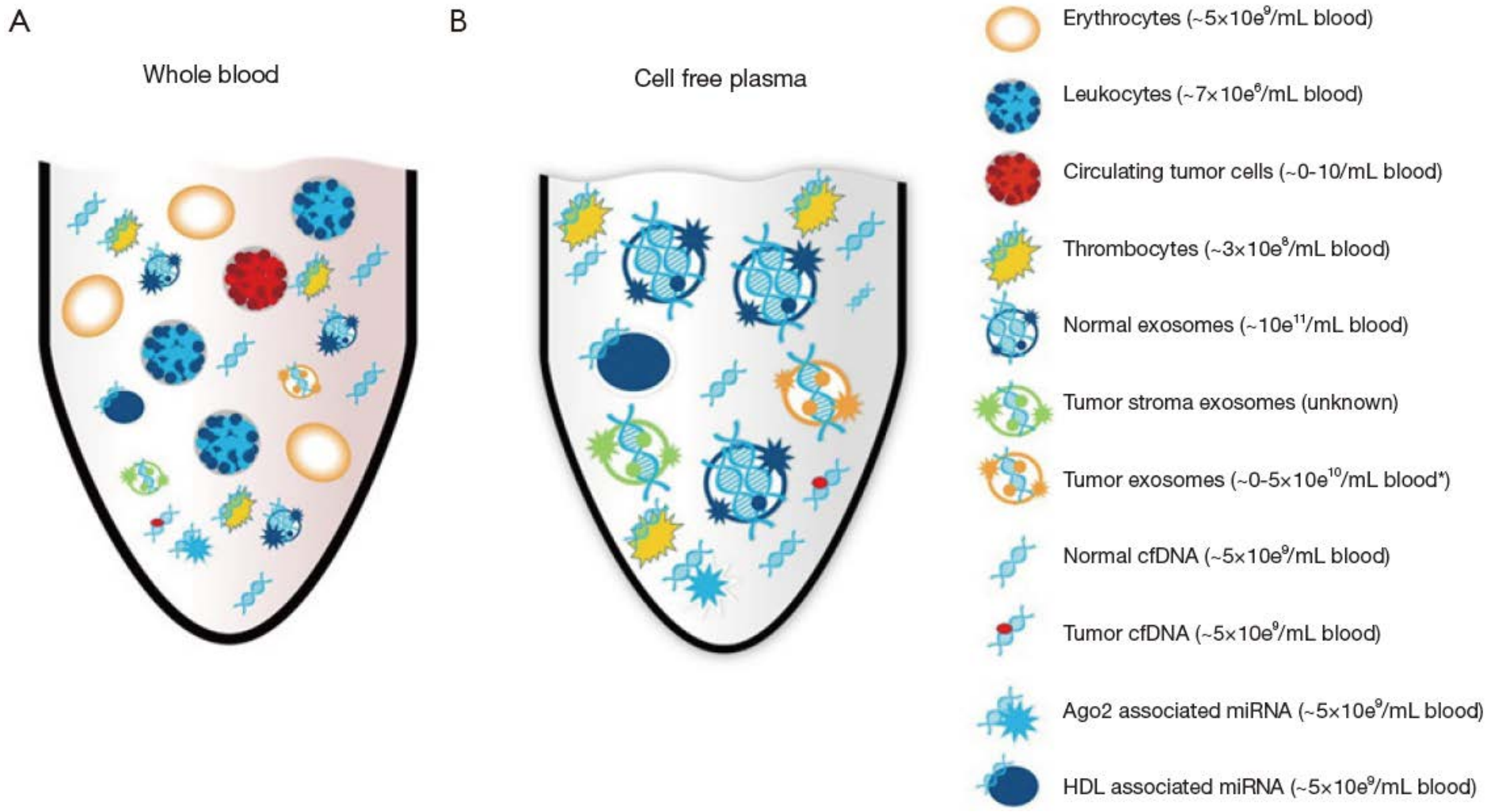
Bias according to source?

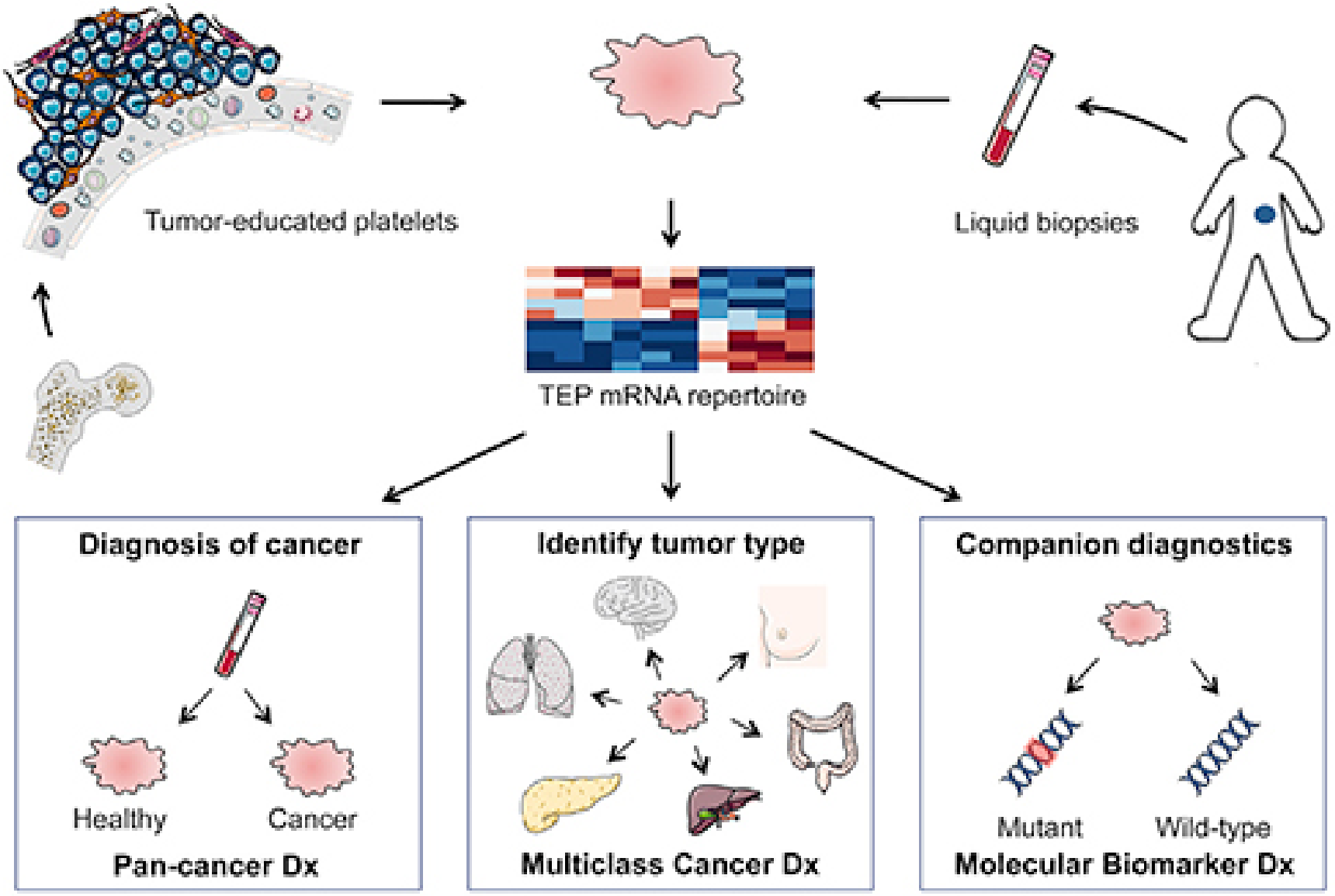


Equivalence for clinical relevance?



Many sources of data, appropriate extraction



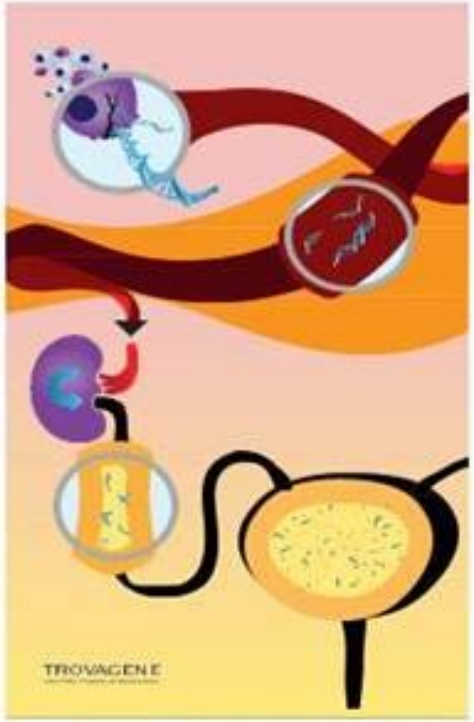

Tumor-educated platelets
Liquid biopsies
TEP mRNA repertoire
Diagnosis of cancer
Healthy
Cancer
Pan-cancer Dx
Identify tumor type
Multiclass Cancer Dx
Companion diagnostics
Mutant
Wild-type
Molecular Biomarker Dx

TROVAGENE
CELL-FREE MOLECULAR DIAGNOSTICS

Cell-free DNA Filters Into The Urine

Tracking-cell free DNA

- Cells in the body die continuously; cancer cells die at an accelerated rate
- DNA is released into the bloodstream, which is then broken down into smaller segments and filtered by the kidney
- Small, stable DNA fragments collect in urine where Trovagene technology can identify and quantify mutations of interest



Urine sample

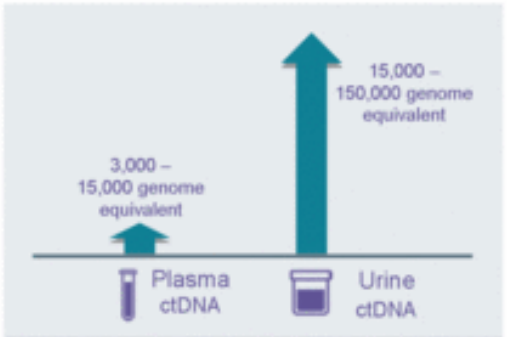
Enabling Benefits

- ✓ Large sample volume
- ✓ Hours of continuous cfDNA collection
- ✓ Cell-free DNA stable in urine
- ✓ High frequency of collection possible

Logistics/Convenience

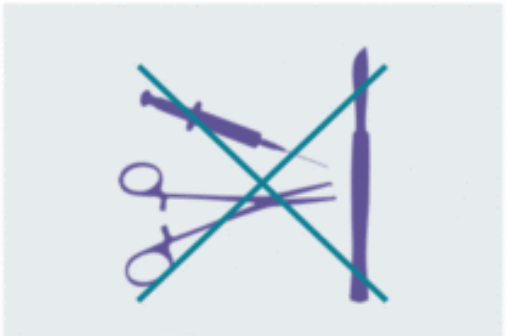
- ✓ Truly non-invasive
  - Patient can self-sample at home or clinic
  - No medical professional required for specimen
- ✓ No refrigeration required
- ✓ No infection risk
- ✓ Lower cost

Why Urine?



Unlimited Volume

Frequency of Testing



Completely Non-invasive

Convenient

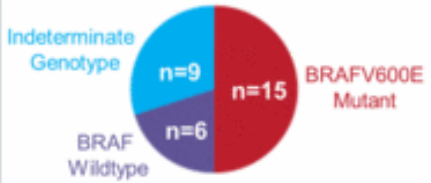
Urinary ctDNA Outperforms Tissue Biopsies[1]

Tissue Biopsies

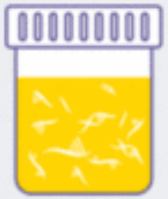
70%

BRAF mutational status obtained

Tissue BRAFV600E genotype



Urine



100%

BRAF mutational status obtained

Urinary ctDNA BRAFV600E genotype

100% concordance between tissue, urine and plasma in treatment naive patients

[1]*Hyman et al., Cancer Discovery 2015 Jan;5(1):64-71*

Why

New Standard of Care Enabled by Trovagene Technology

Minimal Residual Disease (MRD)	Detection	Quantitate Drug Response	Monitoring

Current Cancer Standard of Care

Surgery — Adjuvant Therapy — Tissue Biopsy — Therapy — Imaging (every 6-8 weeks)

Trovagene PCM adds information that imaging does not currently provide at much earlier time points.

	Minimal Residual Disease (MRD)	Detection	Quantitate Drug Response	Monitoring
TROVAGENE PCM	• Monitoring for Minimal Residual Disease • Tumor Recurrence	• Molecular Detection of Clinically Actionable Mutations	• Week 1: Assess Tumor Cell Kill by Therapy • Beyond Week 1: Monitor Tumor Mutation Burden	• Monitoring for Progression and Emergence of Resistance
TROVAGENE CLINICAL UTILTY	• Earlier Detection of Metastatic Disease	• Alternative to Tissue Biopsy • Right Patients Treated with Right Therapy • Eliminates Patient Morbidity due to Tissue Biopsy Procedure	• Immediate Assessment of Drug Effect on Tumor • Predict Best Response Weeks in Advance of Imaging • Enables Early Switch from Ineffective Therapy	• Anticipates and Enables Next Therapy to Target Tumor Resistance

Trovagene Urine-based High Risk HPV Assay

Cervical Cancer

265K
Deaths in 2012

HPV Testing Market Globally

$6B
Market in 2013

$9B
Market in 2020

- Screening is a viable solution:
  75% reduction in incidence in US from 1940 to 1980 w/ National Screening Program
- Screening not available globally:
  - Cost, Technical expertise, Healthcare infrastructure, Quality Control, Cultural
- High-risk HPVs cause virtually all cervical cancers

Urine-based High Risk HPV Assay established in Trovagene's CLIA lab since 2013

New Clinical Evidence

NCI:
Comparative Urine Study
J Clin Virol. 2014 Aug;60(4):414-7

UNC:
Urine collection methodology for detecting HPV associated with CIN2+/CIN3

Queen Mary, UL:
High sensitivity of test for detecting HPV associated with CIN2+, CIN3 (malignancy)

Source: Transparency Market Research

Early resected disease, relapse monitoring

Colorectal Cancer Standard of Care

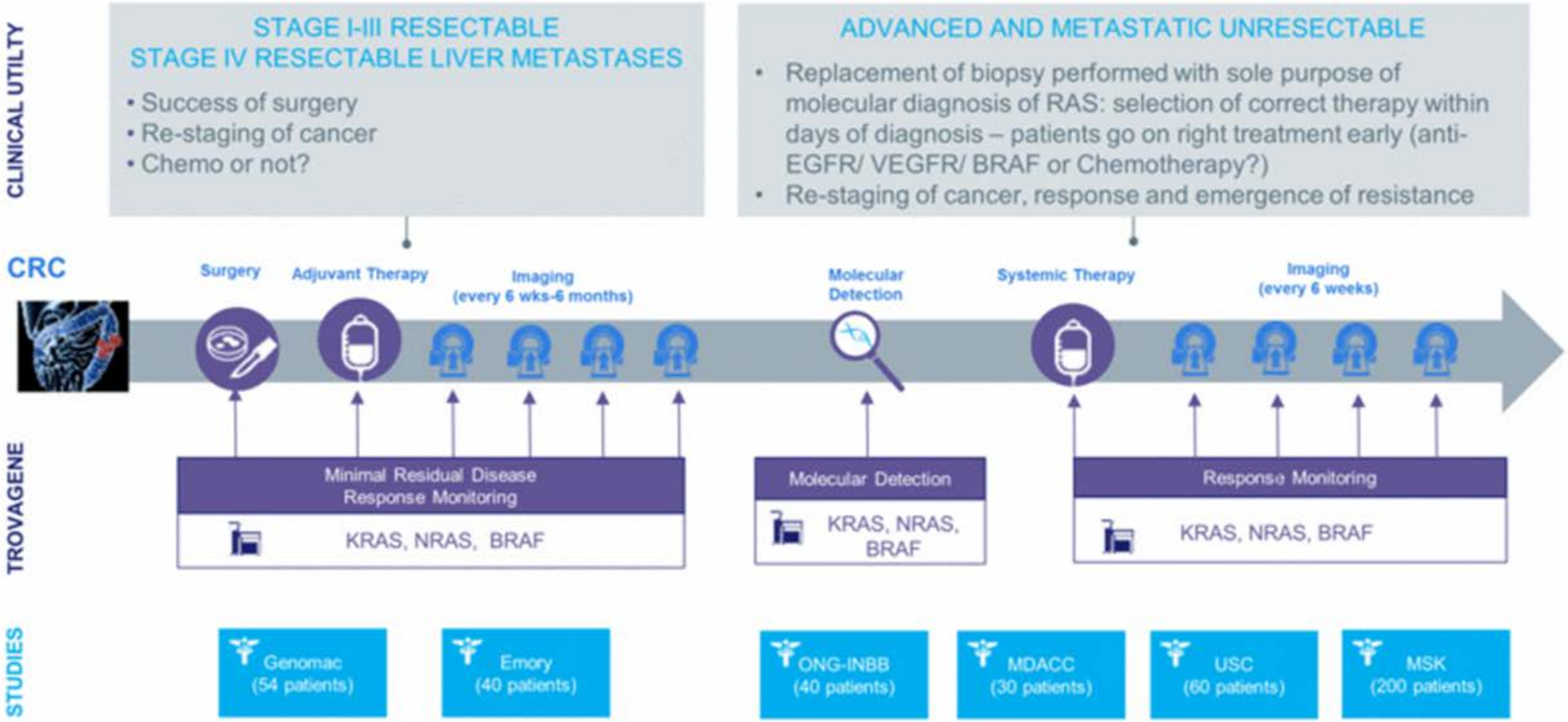
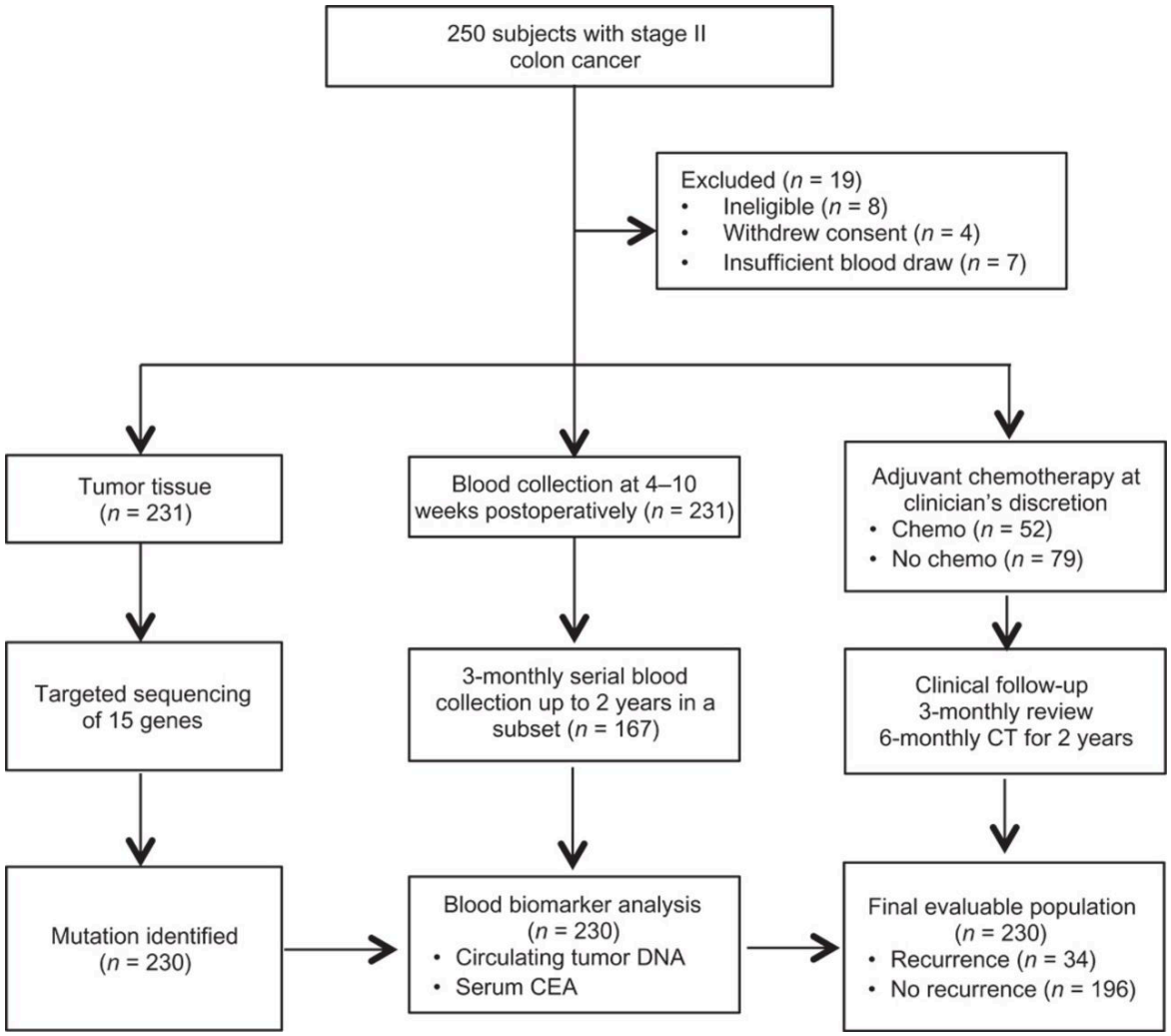
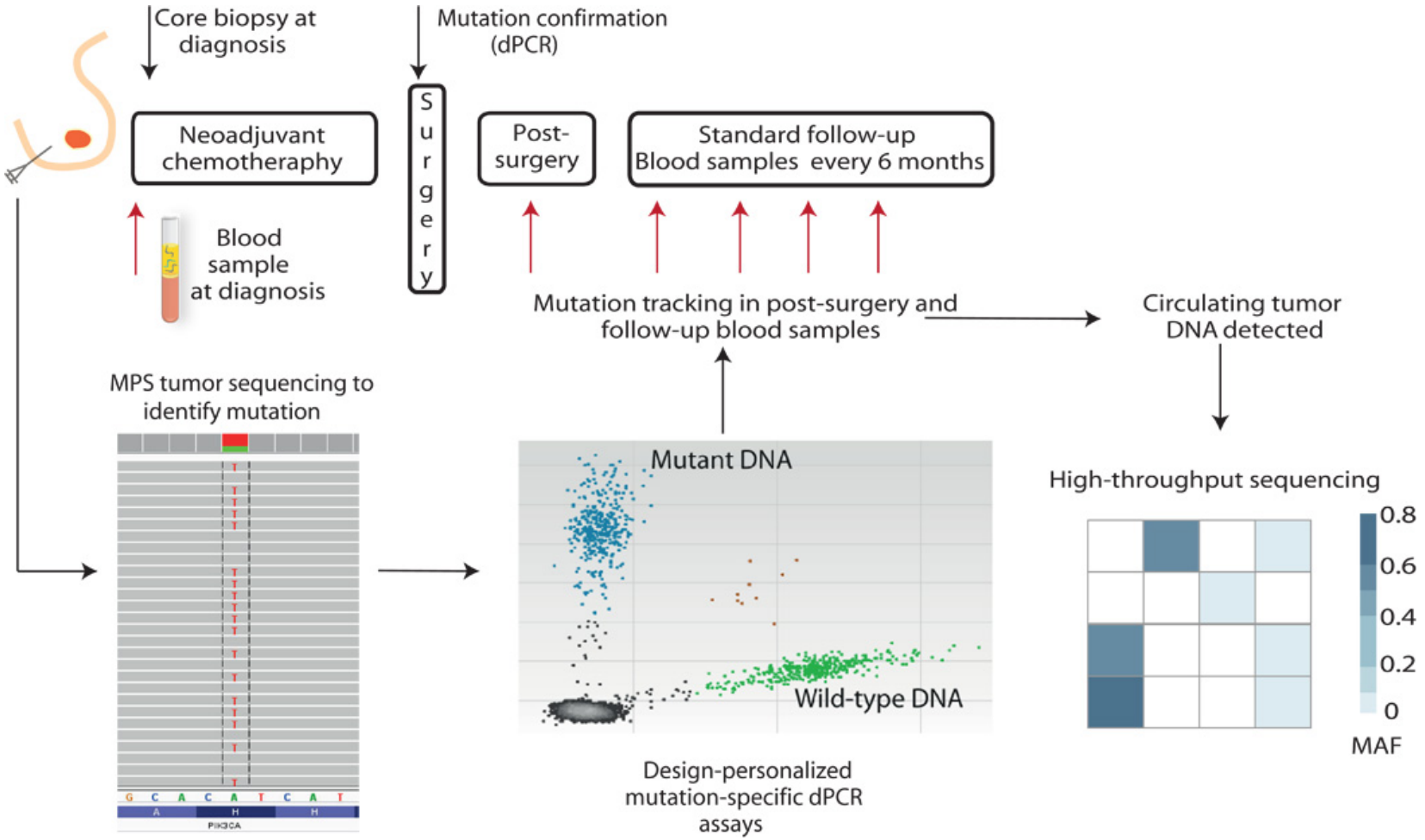


Fig. 1. Patient enrolment and sample collection.



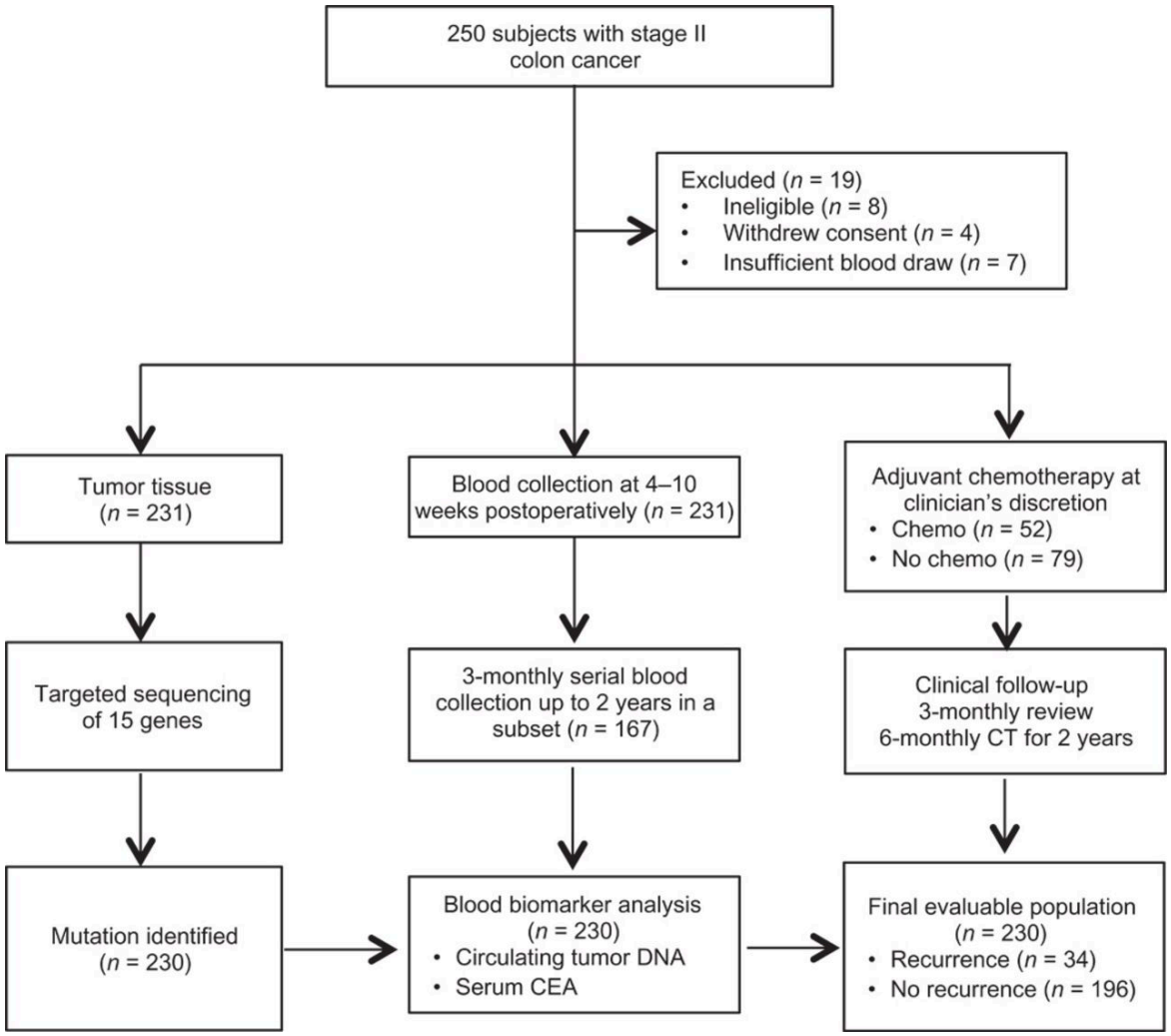
Jeanne Tie et al., Sci Transl Med 2016;8:346ra92

Fig. 1. Personalized dPCR assays for mutation tracking of ctDNA in plasma of patients with early breast cancer.



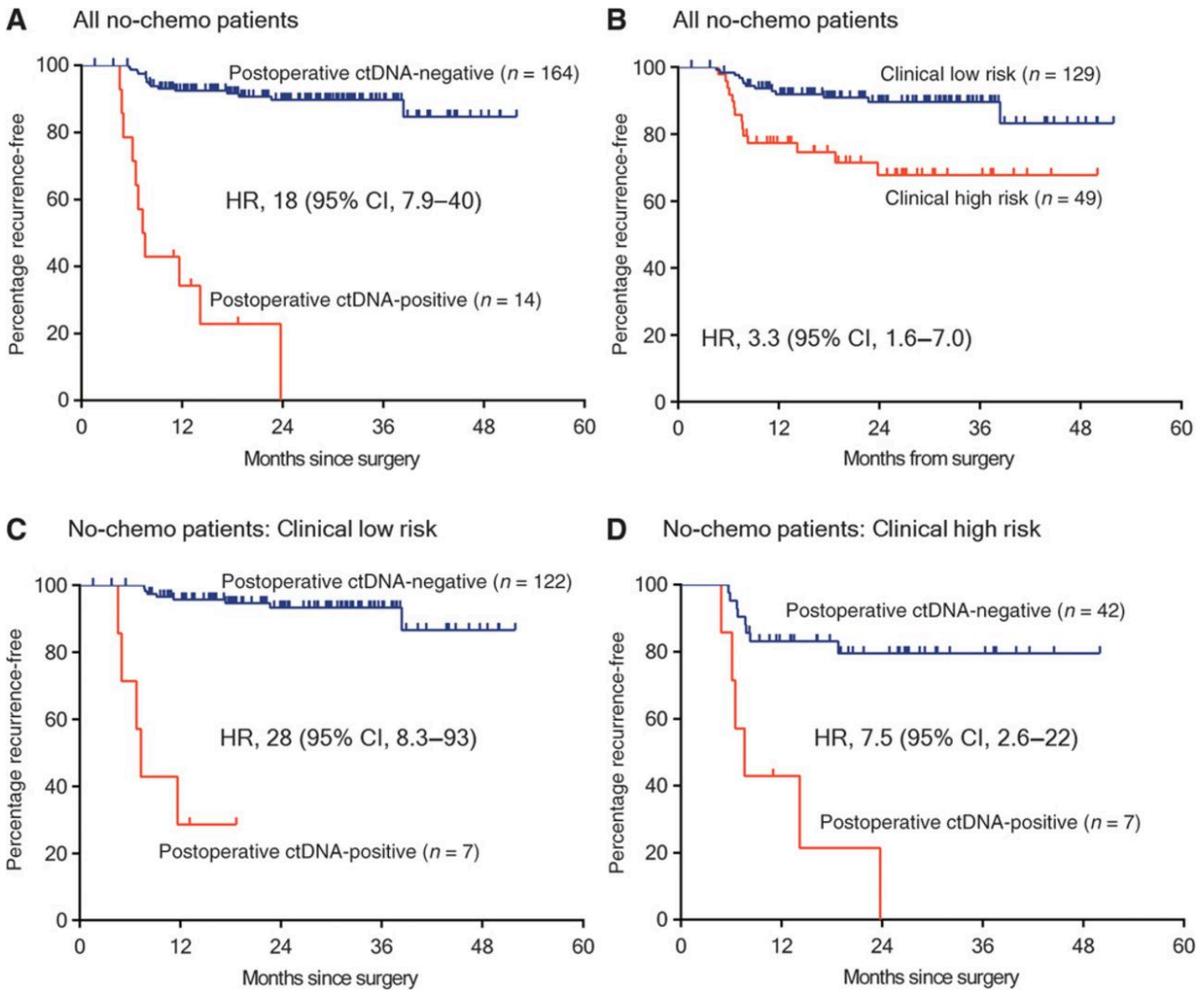
Isaac Garcia-Murillas et al., Sci Transl Med 2015;7:302ra133

Fig. 1. Patient enrolment and sample collection.



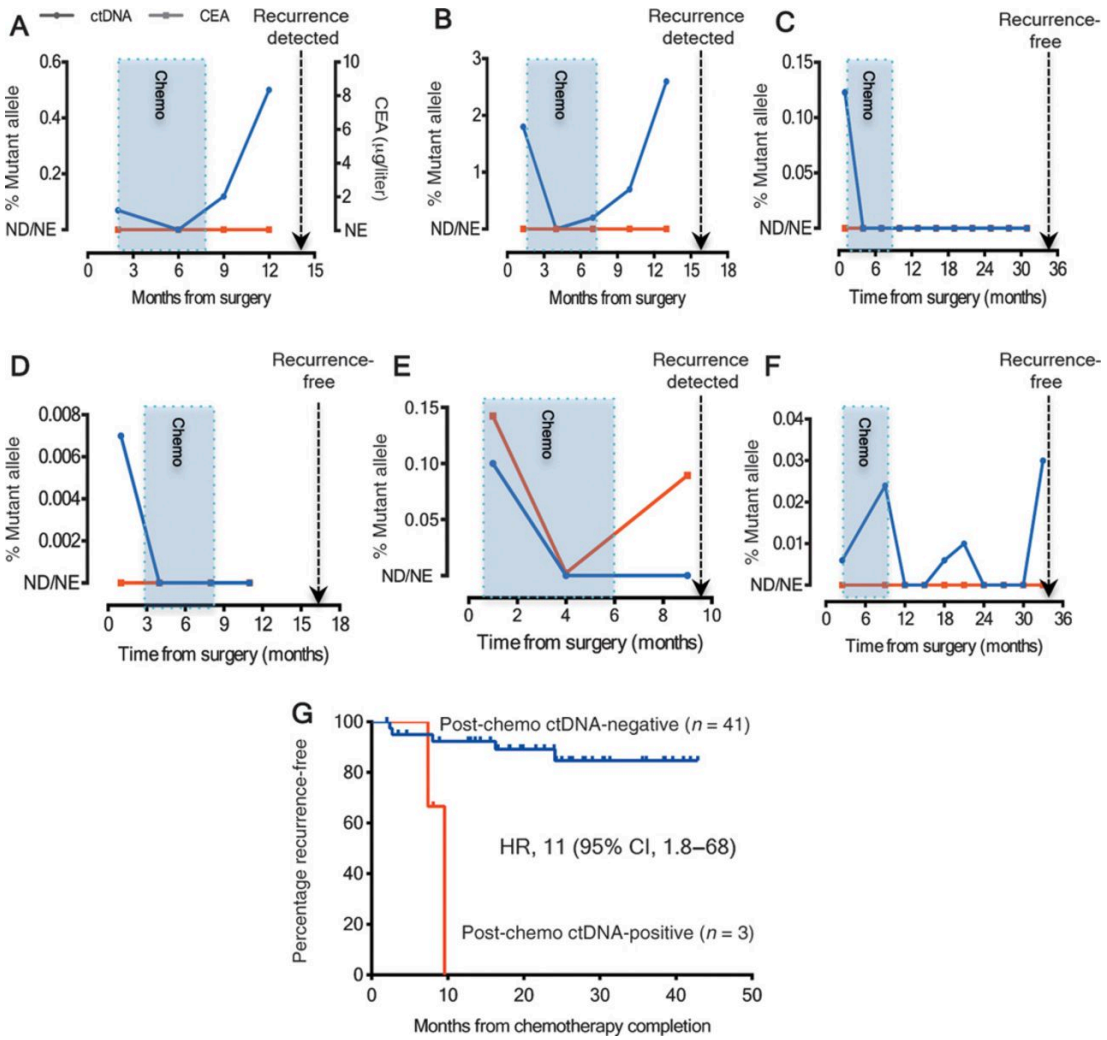
Jeanne Tie et al., Sci Transl Med 2016;8:346ra92

Fig. 2. RFS in patients not treated with adjuvant chemotherapy.

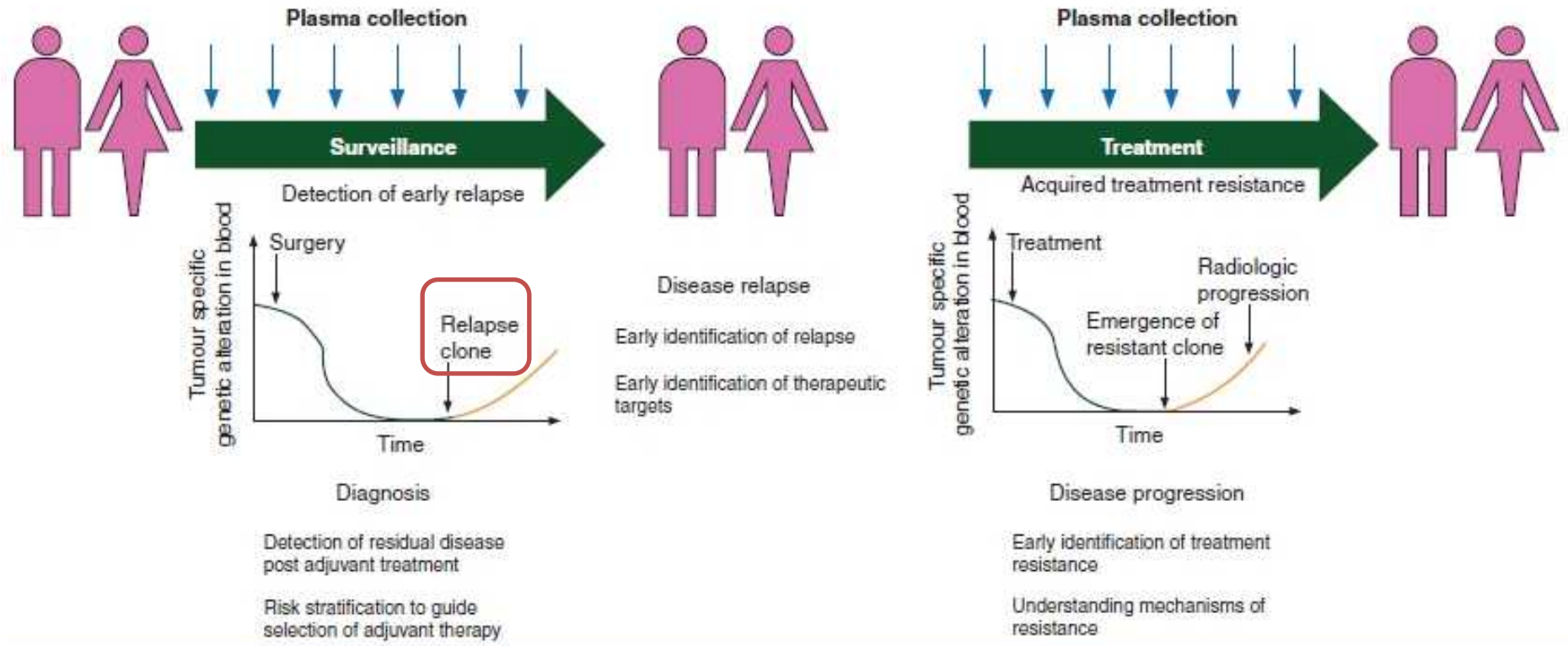


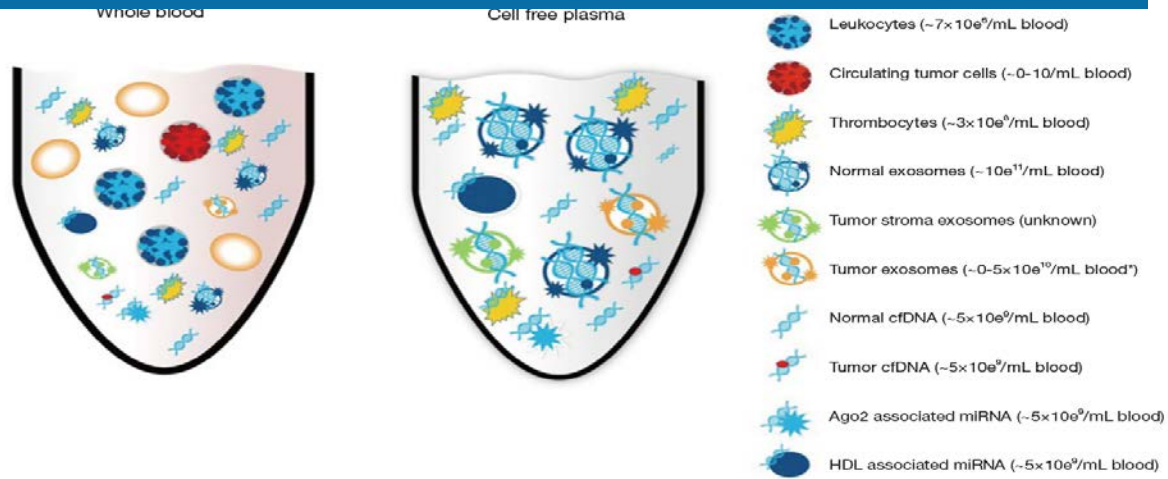
Jeanne Tie et al., Sci Transl Med 2016;8:346ra92

Fig. 3. ctDNA status before, during, and after adjuvant chemotherapy.



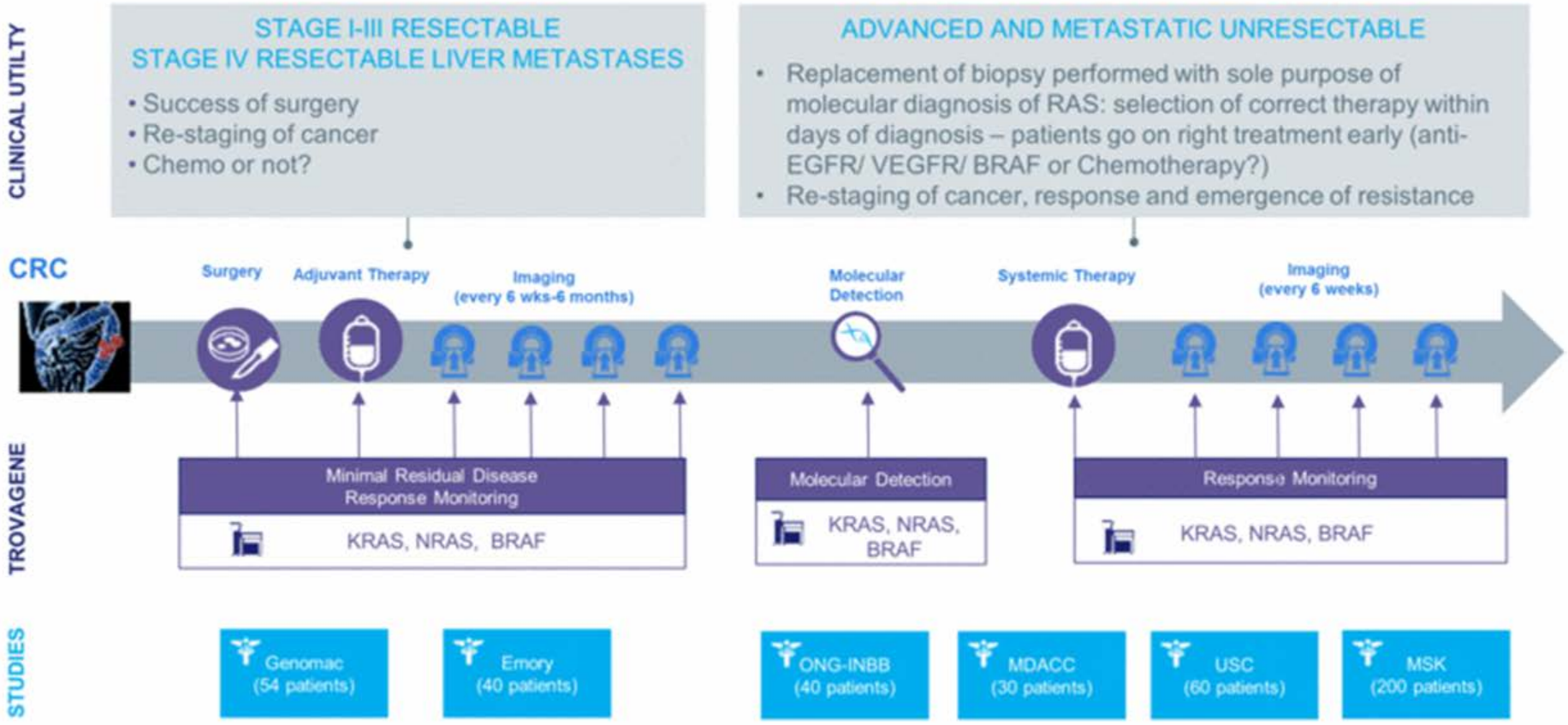
Jeanne Tie et al., Sci Transl Med 2016;8:346ra92



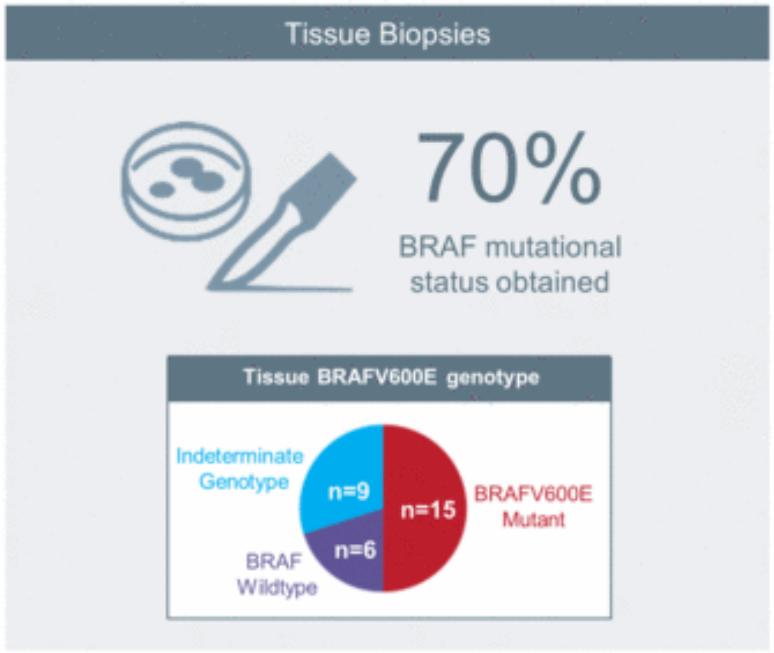


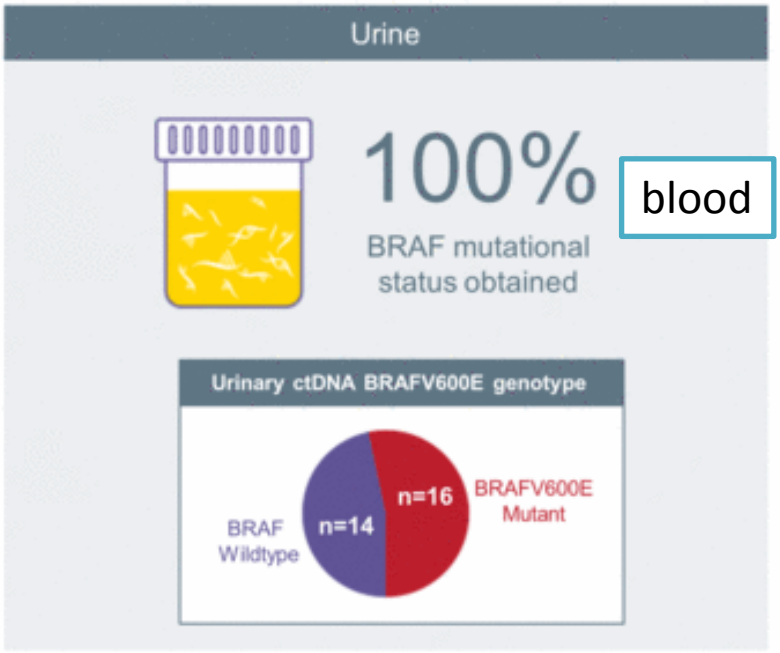
Predictive biomarkers, replace tissue baseline

Colorectal Cancer Standard of Care



Equivalence studies ongoing (Sysmex, Biocartis, ..)
At baseline/diagnosis for colon and lung

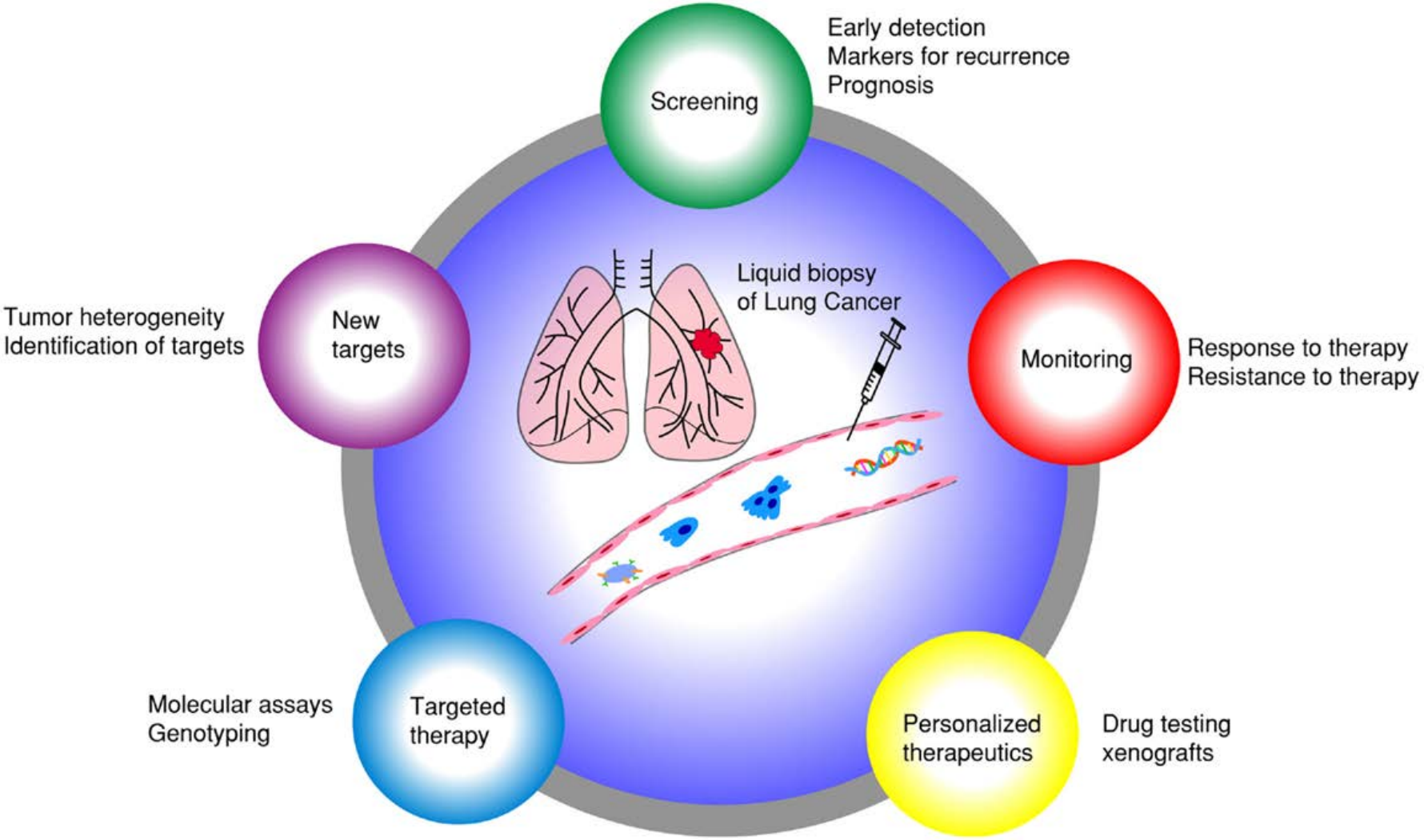




100% concordance between tissue, urine and plasma in treatment naive patients

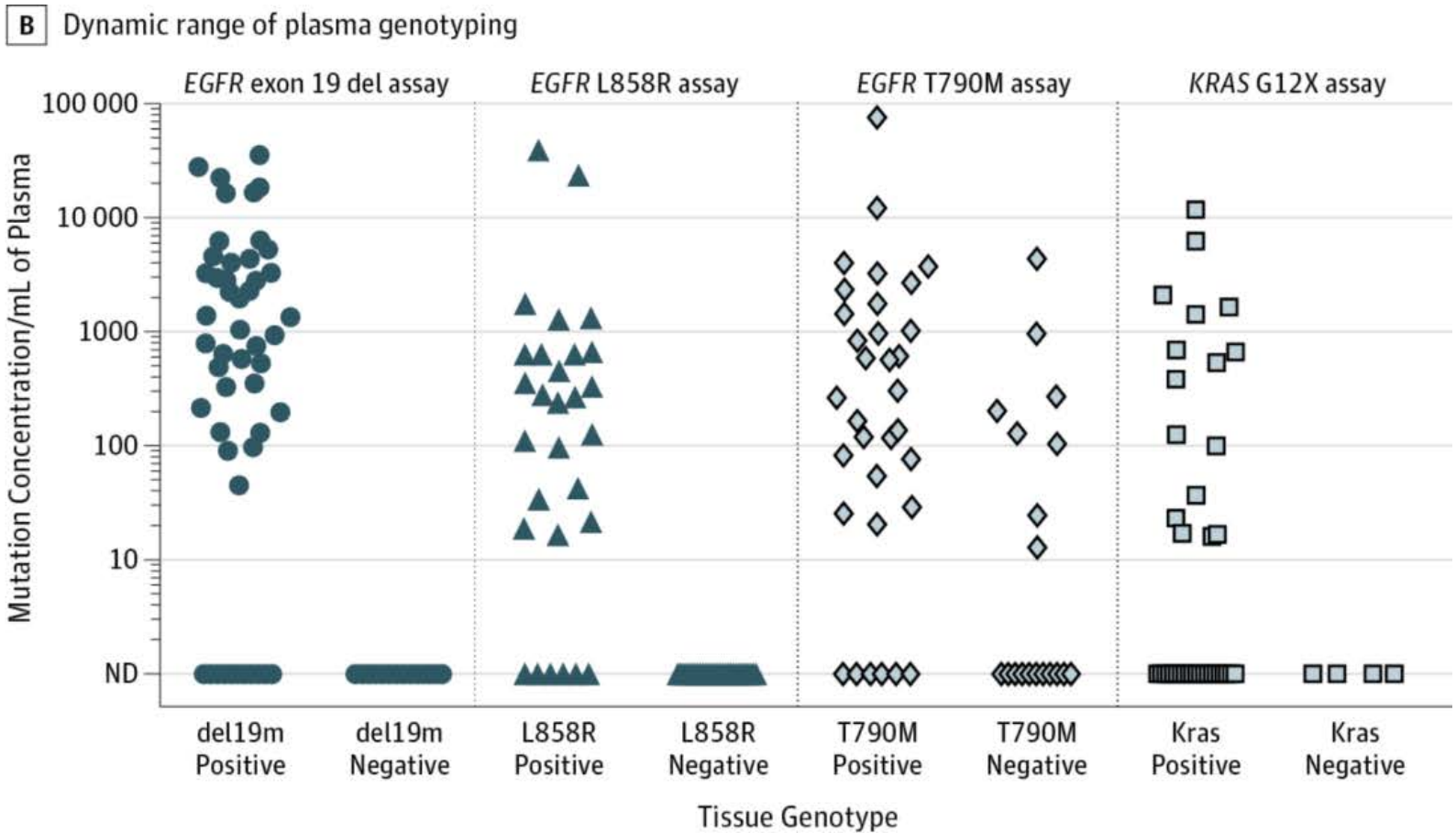
[1]*Hyman et al., Cancer Discovery 2015 Jan;5(1):64-71*

Some tissues can be more difficult to get



Prospective validation of EGFR mt testing. Sacher and Oxnard JAMA Oncol 2016

- •180 pts •ctDNA tested via ddPCR for EGFR exon 19 del, L858R, T790M and KRAS mutations •Turnaround times for plasma ctDNA vs tissue is 3 (1-7) vs 12/27 (1-146) days
- •High specificity – 100% for all mtns except T790M 79%
- •Sensitivity 72-84% for EGFR mts and 64% for KRAS mtns



Drugs

Approved Drugs

Hematology/Oncology (Cancer) Approvals & Safety Notifications

Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book)

FDA

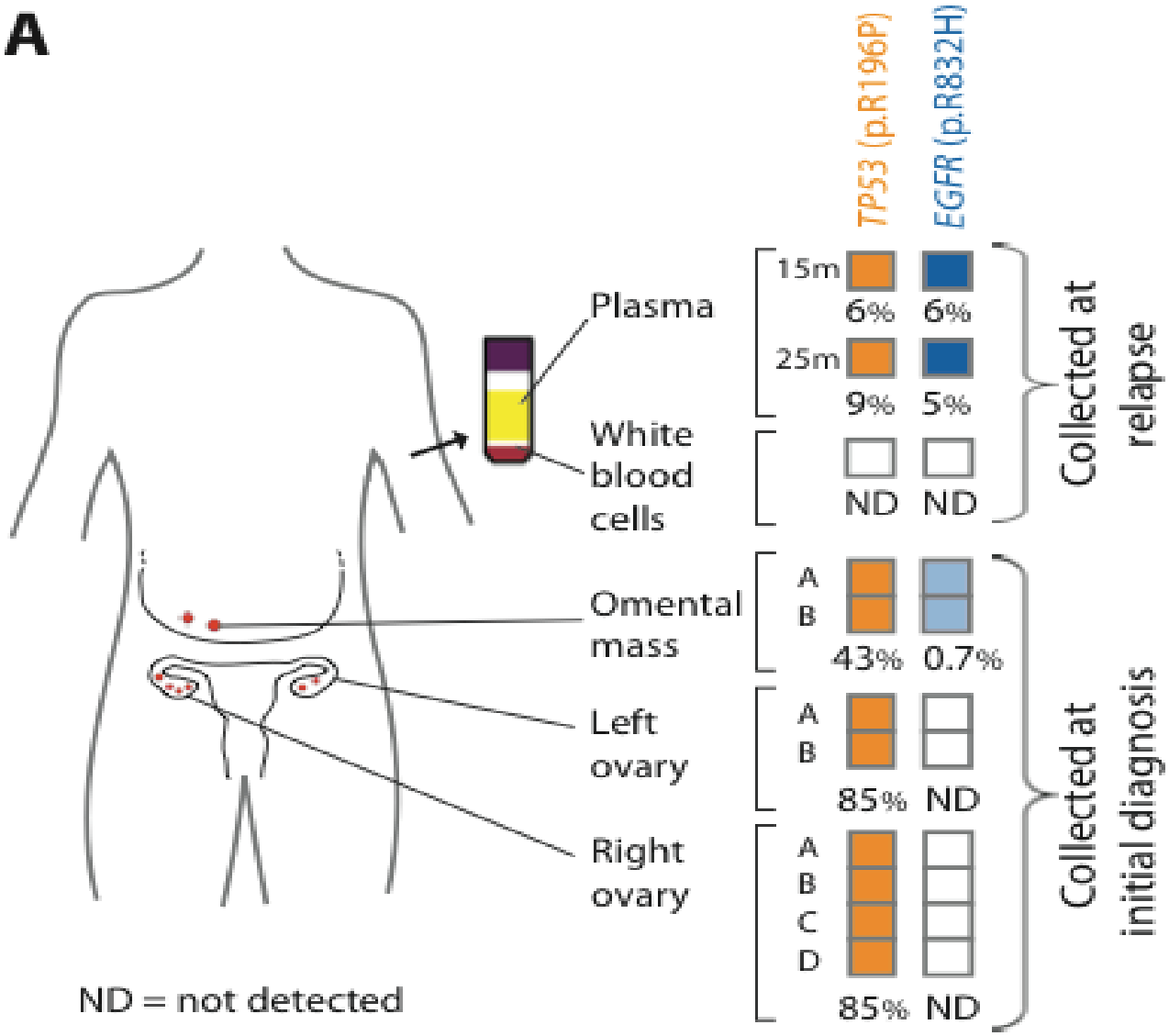
cobas EGFR Mutation Test v2

On June 1, 2016, the U. S. Food and Drug Administration approved **cobas** EGFR Mutation Test v2 (Roche Molecular Systems, Inc.) using plasma specimens as a companion diagnostic test for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations in the epidermal growth factor recptor (EGFR) gene to identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with Tarceva® (erlotinib). The **cobas** EGFR Mutation Test v2 is already approved for this indication using formalin-fixed paraffin-embedded (FFPE) tissue specimens. The new use is for detection of these specific mutations in circulating-free tumor DNA (cfDNA) isolated from plasma specimens, also called liquid biopsy specimens, to aid physicians in identifying patients who may be treated first with TARCEVA (erlotinib). This is the first "liquid biopsy test" approved for use by FDA. This new test may benefit patients who may be too ill or are otherwise unable to provide a tumor specimen for EGFR testing. Patients positive by **cobas** EGFR Mutation Test v2 using plasma specimens for the presence of EGFR exon 19 deletions or L858R mutations are candidates for treatment with Tarceva (erlotinib). Patients who are negative by this test should undergo routine biopsy and testing for EGFR mutations with the FFPE tissue sample type.

The approval was based on a multicenter, open-label, randomized, Phase III study, to evaluate the efficacy and safety of Tarceva versus gemcitabine plus cisplatin as first-line treatment for stage IIIB/IV NSCLC patients (ENSURE study). Patients entering the ENSURE study had tumor tissue specimens that tested positive for the EGFR exon 19 deletion or L858R mutations as determined by the **cobas** EGFR Mutation Test v1. Five hundred seventeen of the 601 (86.0%) patients screened for the ENSURE study with valid **cobas** EGFR Mutation Test v1 test results had available plasma samples available. Of the patients enrolled, 98.6% (214/217) had a plasma sample available for testing. The agreement between the **cobas** EGFR Mutation Test v2 in plasma and the **cobas** EGFR Mutation Test v1 in tissue was evaluated for detection of EGFR mutations (Ex. 19del and L858R mutations) in NSCLC patients screened for participation in ENSURE. 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. The drug efficacy of TARCEVA, based on the **cobas** EGFR Mutation Test v2 in plasma, was evaluated by bridging to the drug efficacy based on the **cobas** EGFR Mutation Test v1 in tissue in the ENSURE study.

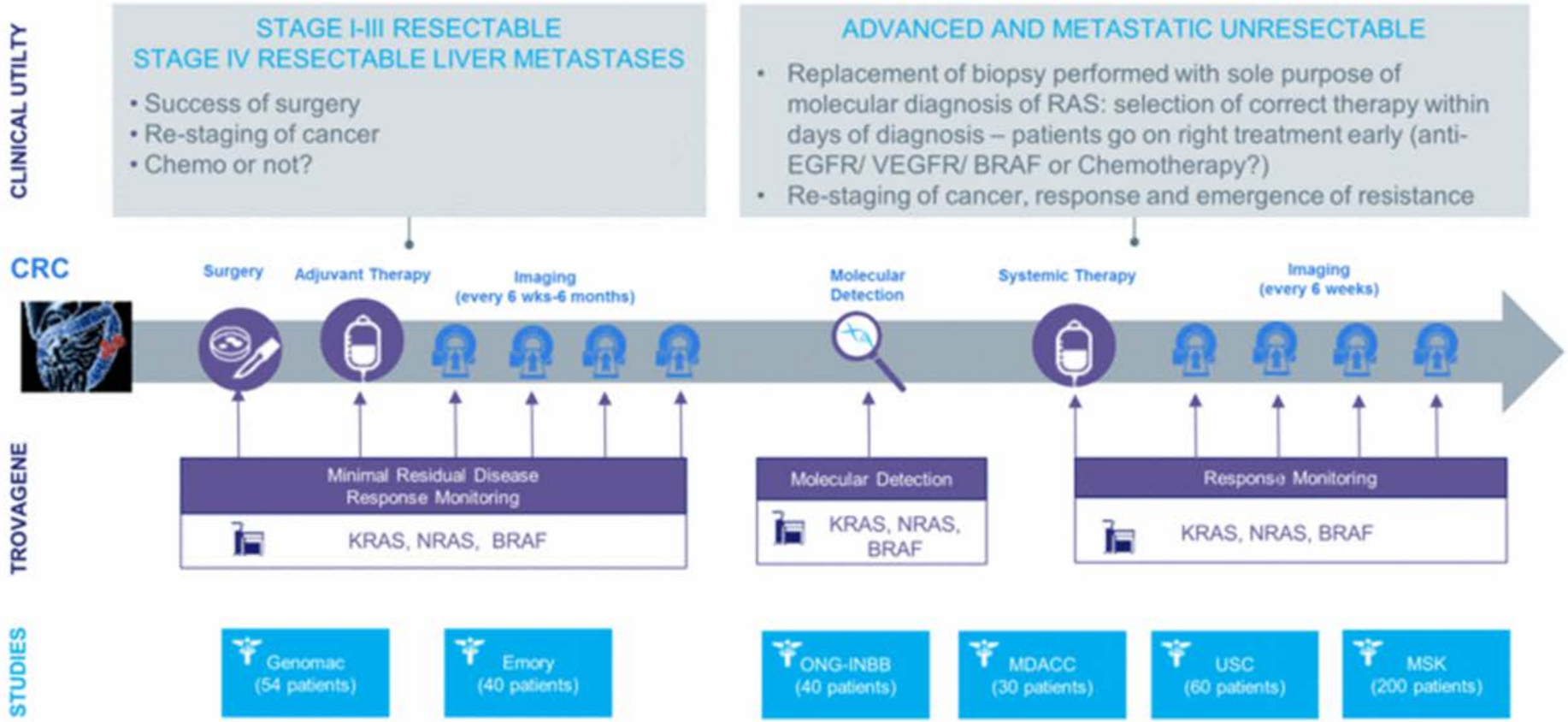
The patients whose plasma results were positive for exon 19 deletion and/or an L858R mutations treated with

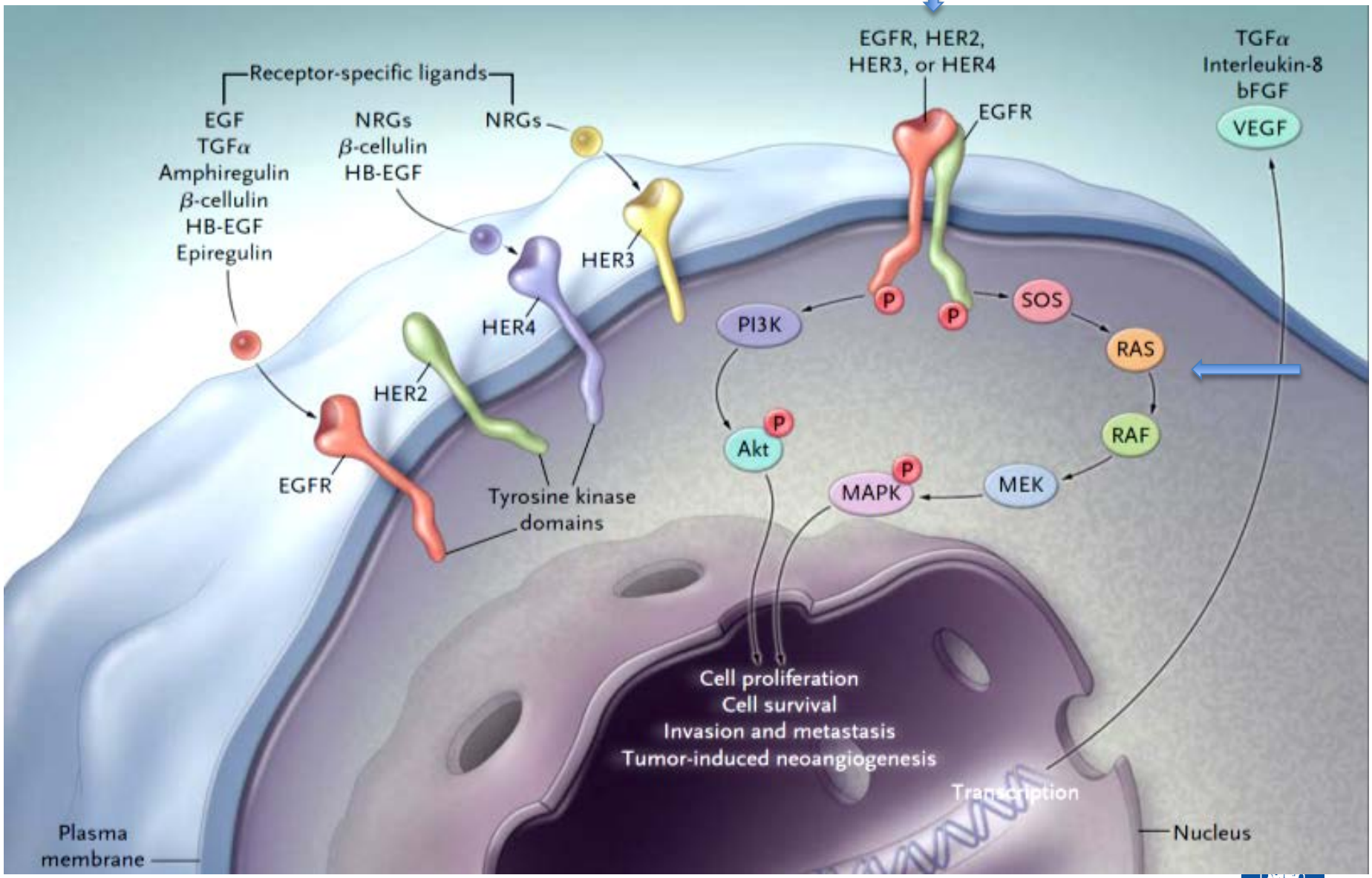
Is primary tumor representative of metastatic disease?
Tumor heterogeneity at baseline, does plasma tell you more



Predictive biomarkers during therapy, replace very difficult tissue

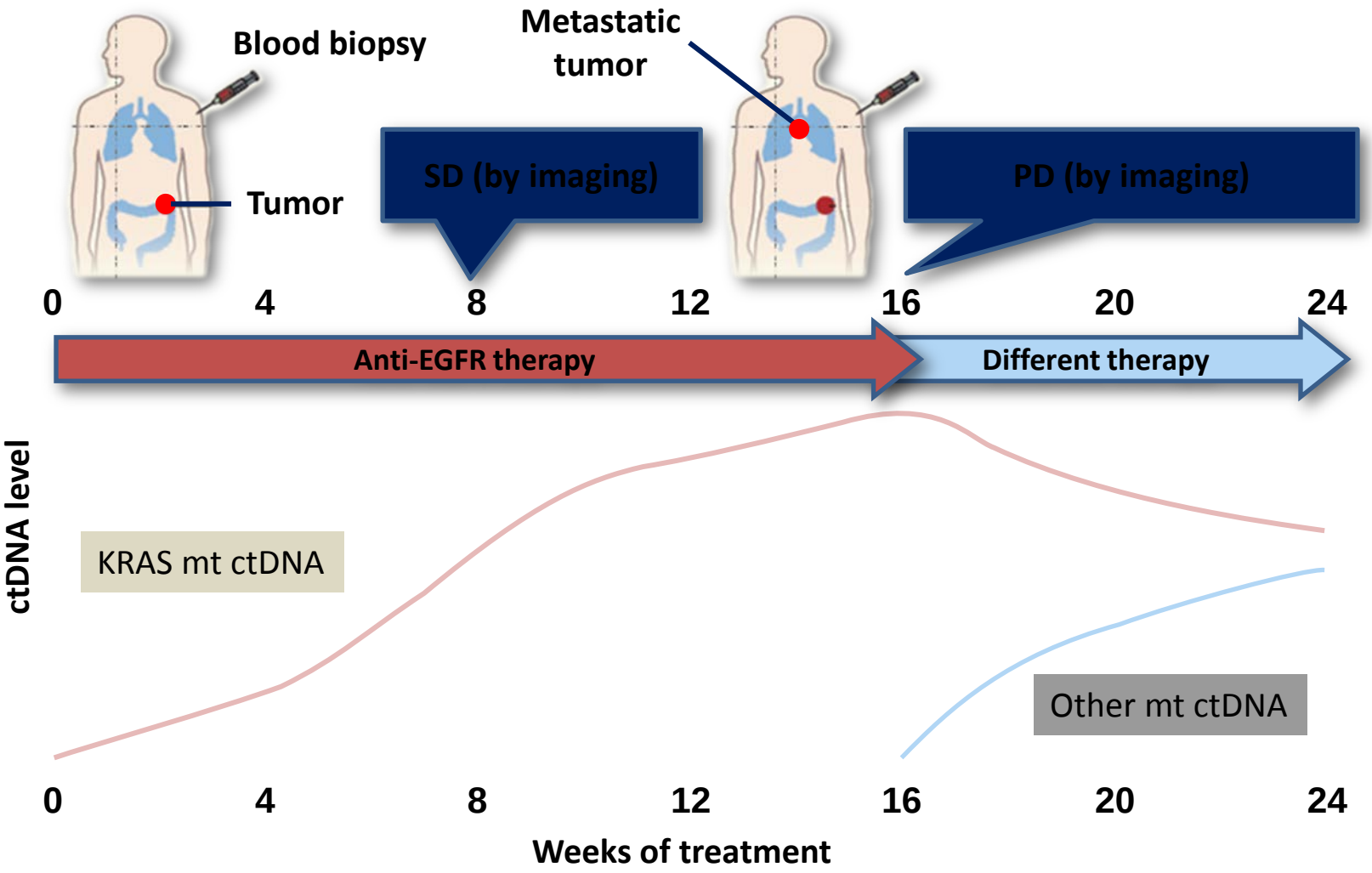
Colorectal Cancer Standard of Care



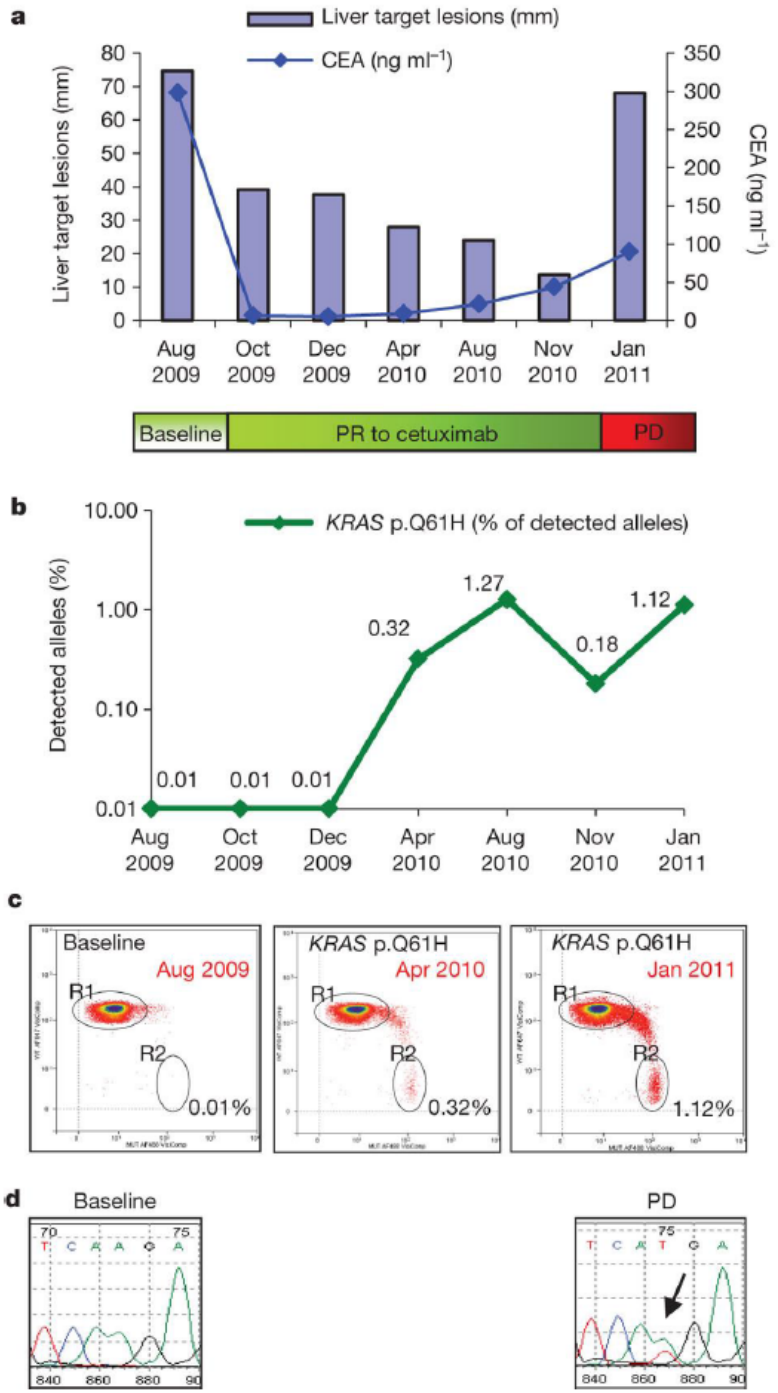


Gastro-intestinal Oncology

IF not present at Baseline, RAS mutations are frequently Gained during therapy!



Detection of circulating *KRAS* mutant DNA in a patient with acquired resistance to cetuximab therapy.



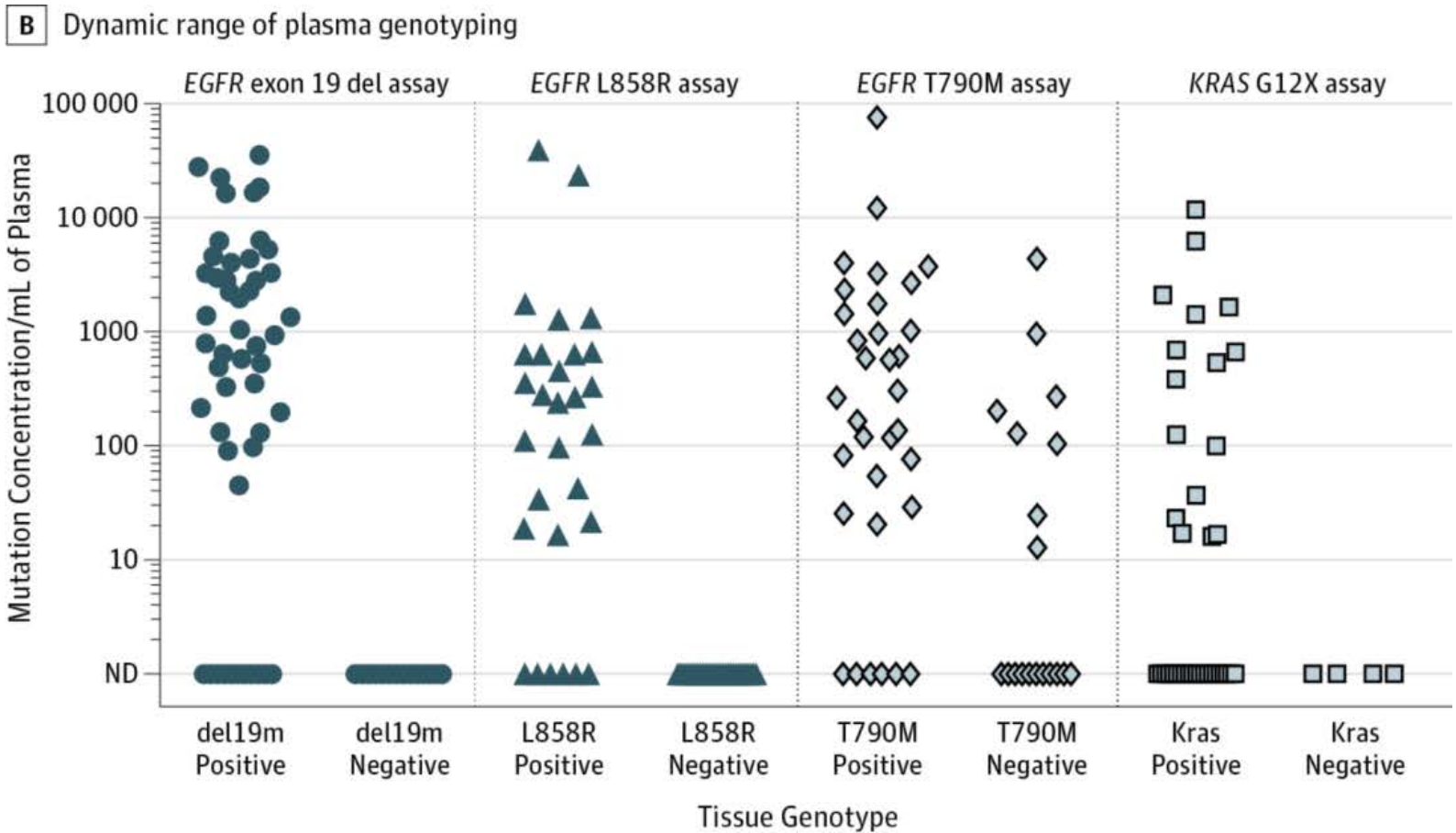
a, Size of liver metastasis (blue bars) and carcinoembryonic antigen (CEA) levels in blood (blue line) at the indicated time points, showing an initial response to cetuximab followed by progression (patient 8). PR, partial response; PD, progressive disease. **b**, Quantitative analysis of KRAS(Q61H) mutant DNA in plasma, as assessed by BEAMing. **c**, Two-dimensional dot plot showing quantitative analysis of the KRAS(Q61H) mutation in plasma using BEAMing at individual time points. **d**, Mutational analysis of *KRAS* on tumour samples collected before cetuximab treatment and at the time of disease progression.

S Misale *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature11156

nature

Prospective validation of EGFR mt testing. Sacher and Oxnard JAMA Oncol 2016

- •180 pts •ctDNA tested via ddPCR for EGFR exon 19 del, L858R, T790M and KRAS mutations •Turnaround times for plasma ctDNA vs tissue is 3 (1-7) vs 12/27 (1-146) days
- •High specificity – 100% for all mtns except T790M 79%
- •Sensitivity 72-84% for EGFR mts and 64% for KRAS mtns



Other cancers, predictive?
Ongoing data generation

Murtaza et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013; 497: 108.

- ctDNA used to track markers of resistance in breast, ovarian and lung cancers
- 6 pts followed over 1-2 yrs & sampled at intervals >3wks.
- Mutant alleles detected in therapy resistance

–PIK3CA mtn on paclitaxel

–T790M mtn on gefitinib

–RB1 mtn on cisplatin

–MED1mtn on tamoxifen and trastuzumab

Technology

ct-dna

- Quantitative PCR amplification methods

–Requires primers specific for the detection of certain mutations
–Lowest cost and ease of use
–limited sensitivity

- Digital PCR

–Absolute quantification of allele of interest
–highest accuracy and sensitivity
–Limited genomic loci

- Targeted deep sequencing and NGS

–high-sensitivity
–Broad range of genomic coverage
-CAPP seq, Safe SEqS ,..

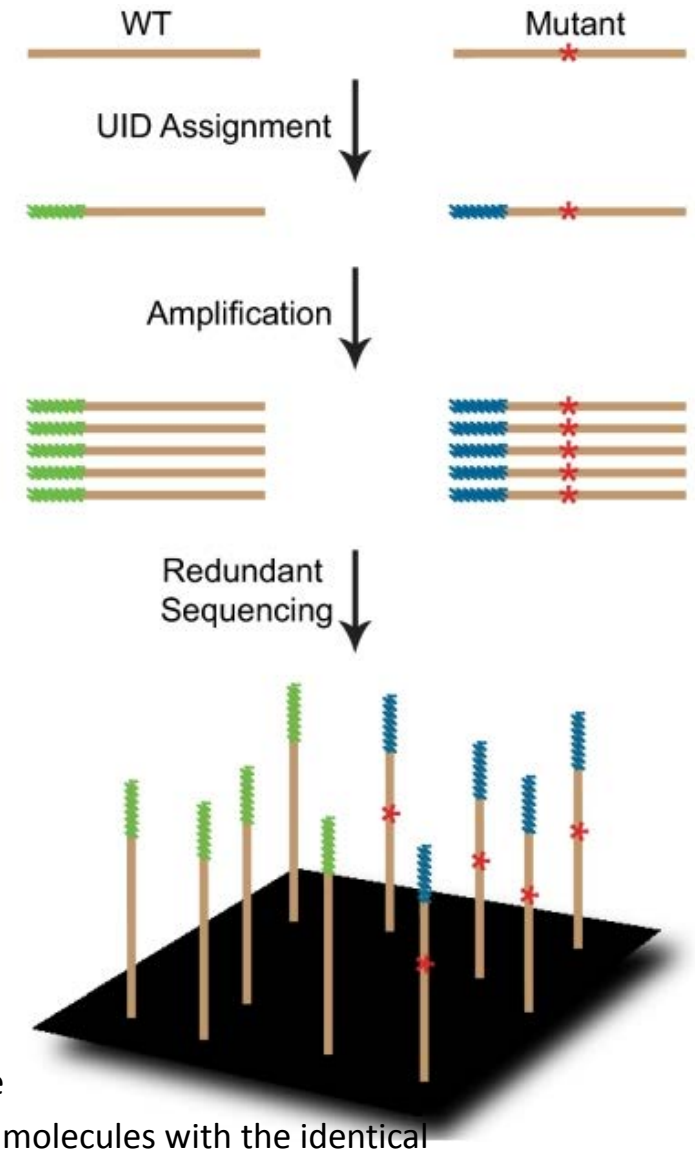
What is droplet digital PCR?

- A real-time PCR reaction that is partitioned into many droplets – a PCR reaction happens in each droplet.
- A portion of these reactions contain the target molecule while others do not.
- Positive reactions are counted.
- Fraction of negative reactions is used to deduce the absolute number of target molecules in the sample.
- Currently in use to test for JAK2 V617F mutation.

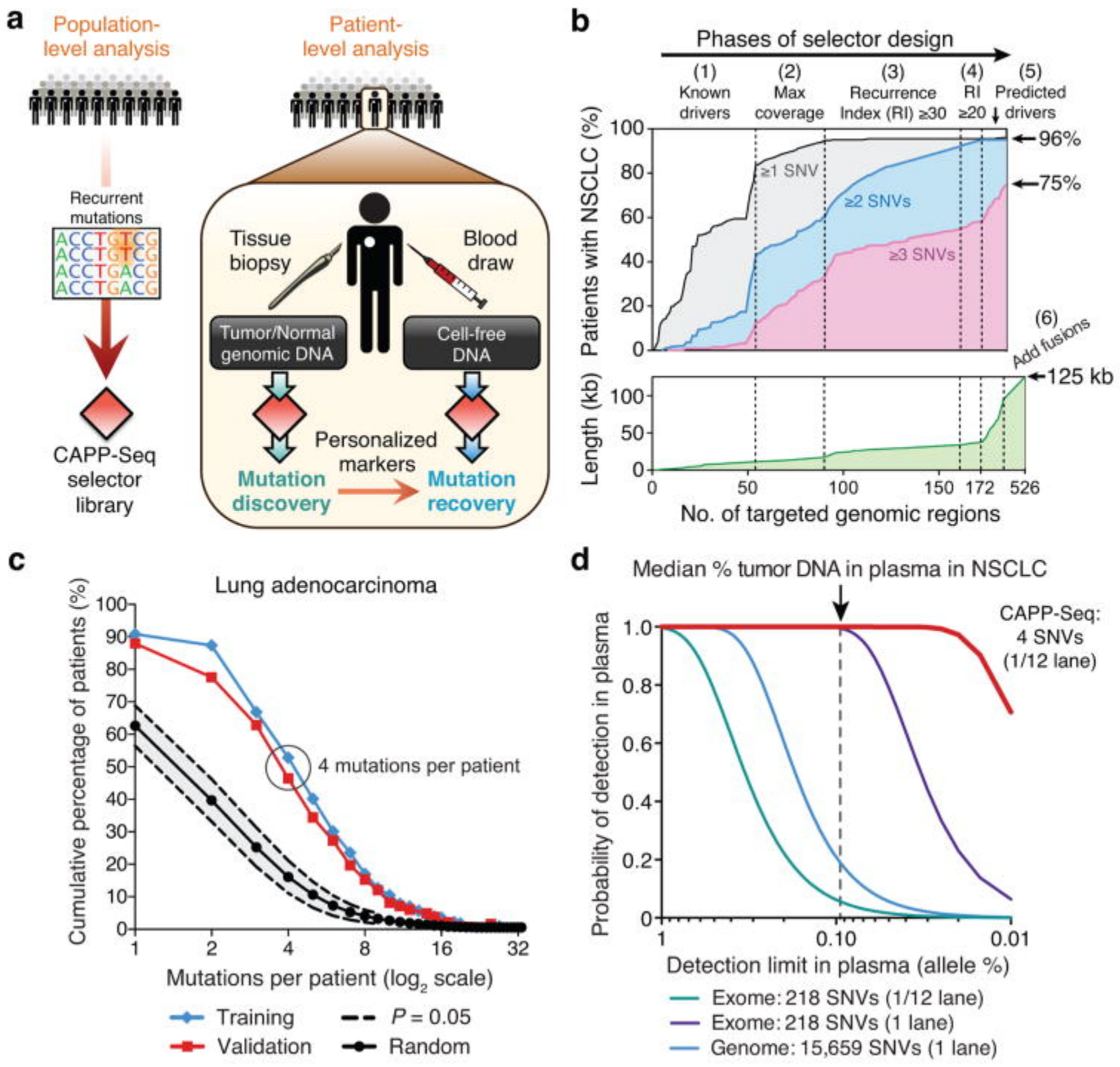
One measurement

Nanodroplet PCR reactions are independent, single amplification events

Many thousands of discrete measurements



- Safe-Sequencing System (“Safe-SeqS
- assignment of a unique identifier (UID) to each DNA template molecule
- amplification of each uniquely tagged template, so that many daughter molecules with the identical sequence are generated (defined as a UID family). If a mutation preexisted in the template molecule used for amplification, that mutation should be present in every daughter molecule containing that UID (barring any subsequent replication or sequencing errors).
- A UID family in which at least 95% of family members have the identical mutation is called a “supermutant”.


a
Population-level analysis
Patient-level analysis
Recurrent mutations
ACCTGTCG
ACCTGTCG
ACCTGACG
ACCTGACG
CAPP-Seq selector library
Tissue biopsy
Blood draw
Tumor/Normal genomic DNA
Cell-free DNA
Personalized markers
Mutation discovery
Mutation recovery
b
Phases of selector design
(1) Known drivers
(2) Max coverage
(3) Recurrence Index (RI) ≥30
(4) RI ≥20
(5) Predicted drivers
Patients with NSCLC (%)
≥1 SNV
≥2 SNVs
≥3 SNVs
96%
75%
(6) Add fusions
125 kb
Length (kb)
No. of targeted genomic regions
c
Lung adenocarcinoma
Cumulative percentage of patients (%)
4 mutations per patient
Mutations per patient (log2 scale)
Training
Validation
P = 0.05
Random
d
Median % tumor DNA in plasma in NSCLC
CAPP-Seq: 4 SNVs (1/12 lane)
Probability of detection in plasma
Detection limit in plasma (allele %)
Exome: 218 SNVs (1/12 lane)
Exome: 218 SNVs (1 lane)
Genome: 15,659 SNVs (1 lane)

ct-dna

- Quantitative PCR amplification methods

–Requires primers specific for the detection of certain mutations
–Lowest cost and ease of use
–limited sensitivity

- Digital PCR

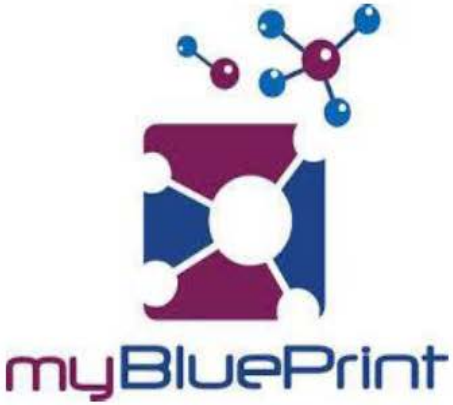
–Absolute quantification of allele of interest
–highest accuracy and sensitivity
–Limited genomic loci

- Targeted deep sequencing and NGS

–high-sensitivity
–Broad range of genomic coverage
-CAPP seq, Safe SEqS ,..

ctDNA Platforms

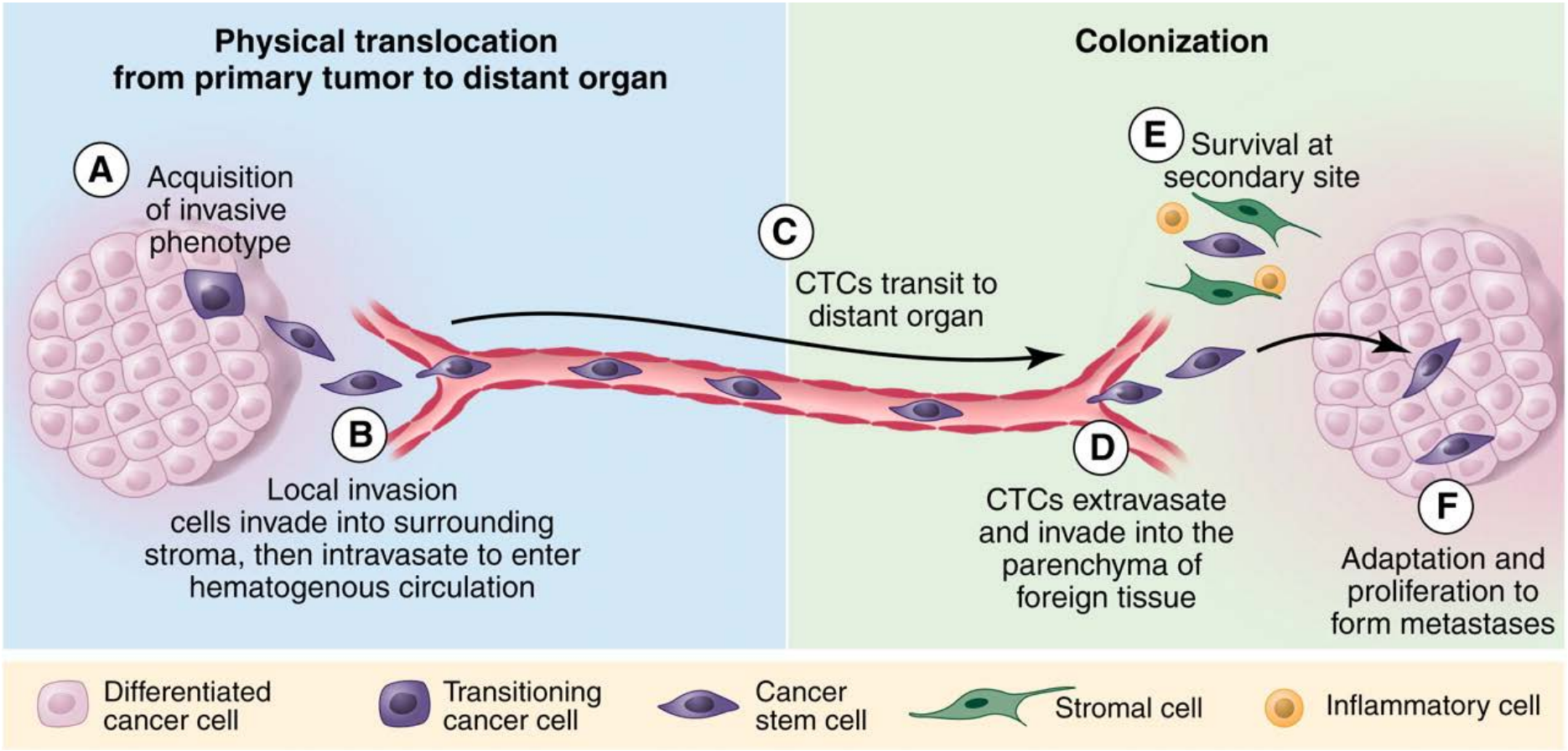
Commercial entities –over 60 in USA!



Detection method	Commercial test	Analytical sensitivity	Analytical specificity	Cost ($USD)	TOT (days)
Next-generation sequencing	Guardant 360	>85%	99.99%	$5600	14
	Foundation ACT	>95%	99%	$5800	14
Digital PCR	Biodesix	>85%	100%	$1800	3
Quantitative PCR & sanger	Biocept	97%	99.5%	$1900	7
Quantitative PCR & NGS	Trovagene	93%	99%	$1500	14

From Johnson ML, ASCO 2016 education session: Biomarkers, Blood-based testing and the heterogenous tumour.

Nucleic acids from Circulating tumor cells



Technology	Approach	Flow rate (ml/h)	Recovery cell lines	Purity WBCs (ml)	Patient samples	Whole blood	Genomic analysis	Live cells	Culture	Drug testing
CellSearch	EpCAM-coated magnetic beads	NA	>80%	Low	<50% in breast (43) 32% in lung cancer, 5CTCs/7.5ml (19)	N	N	N	N	N
Epic Sciences	No enrichment, RBCs lysed blood deposited on slides	NA	NA	None	73% in lung cancer (38), 55% in melanoma (112)	N	Y, single cell for copy number	N	N	N
Mag Sweeper	Flow through immunomagnetic capture		62 ± 7%		100% in metastatic breast cancer, 12CEpCs/9 ml (113)	Y, need dilution	Y	Y	N	NA
ISET	Size-based filtration	NA	One CTC per 1 ml of blood	NA	80% in lung cancer (36, 40, 68)	N	Y, FISH	N	N	N
CTC iChip	Size-based separation +ve or −ve selection with mag beads	9.6	>95% for −ve 78–98% for +ve	>10,000 for −ve <10,000 for +ve	90% from multiple types of metastatic cancers, including lung cancer (64)	Y, not a single step	Y, single-cell RNA expression	Y	Y	Y
FACS Sorting	Surface marker-based selection	Very low	NA	Very Low	<10% (99)	Y	Y	Y	Y	N
RosetteSep kit	Depletion of WBCs	NA	NA	NA	NA (42, 101, 109)	Multiple steps	Y	Y	Y	NA
CTC chip	Positive selection	1	>95%	NA	72% in lung cancer (27)	Y	Y	Y	Y	N
GO Chip	Nanopillars with graphene oxide	1–3	>95% 2–5 CTCs	<1,000	>95% sensitivity, 10 CTCs/ml (46)	Y	Y	Y	Y	N

Detection method	Commercial test	Analytical sensitivity	Analytical specificity	Cost ($USD)	TOT (days)
Next-generation sequencing	Guardant 360	>85%	99.99%	$5600	14
	Foundation ACT	>95%	99%	$5800	14
Digital PCR	Biodesix	>85%	100%	$1800	3
Quantitative PCR & sanger	Biocept	97%	99.5%	$1900	7
Quantitative PCR & NGS	Trovagene	93%	99%	$1500	14

From Johnson ML, ASCO 2016 education session: Biomarkers, Blood-based testing and the heterogenous tumour.

Until now, what's been missing from liquid biopsy research is evidence from large controlled studies that the information the test provides is both accurate and useful.

MIT Technology Review

Drugs

Approved Drugs

Hematology/Oncology (Cancer) Approvals & Safety Notifications

Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book)

FDA

cobas EGFR Mutation Test v2

On June 1, 2016, the U. S. Food and Drug Administration approved **cobas** EGFR Mutation Test v2 (Roche Molecular Systems, Inc.) using plasma specimens as a companion diagnostic test for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations in the epidermal growth factor recptor (EGFR) gene to identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with Tarceva® (erlotinib). The **cobas** EGFR Mutation Test v2 is already approved for this indication using formalin-fixed paraffin-embedded (FFPE) tissue specimens. The new use is for detection of these specific mutations in circulating-free tumor DNA (cfDNA) isolated from plasma specimens, also called liquid biopsy specimens, to aid physicians in identifying patients who may be treated first with TARCEVA (erlotinib). This is the first "liquid biopsy test" approved for use by FDA. This new test may benefit patients who may be too ill or are otherwise unable to provide a tumor specimen for EGFR testing. Patients positive by **cobas** EGFR Mutation Test v2 using plasma specimens for the presence of EGFR exon 19 deletions or L858R mutations are candidates for treatment with Tarceva (erlotinib). Patients who are negative by this test should undergo routine biopsy and testing for EGFR mutations with the FFPE tissue sample type.

The approval was based on a multicenter, open-label, randomized, Phase III study, to evaluate the efficacy and safety of Tarceva versus gemcitabine plus cisplatin as first-line treatment for stage IIIB/IV NSCLC patients (ENSURE study). Patients entering the ENSURE study had tumor tissue specimens that tested positive for the EGFR exon 19 deletion or L858R mutations as determined by the **cobas** EGFR Mutation Test v1. Five hundred seventeen of the 601 (86.0%) patients screened for the ENSURE study with valid **cobas** EGFR Mutation Test v1 test results had available plasma samples available. Of the patients enrolled, 98.6% (214/217) had a plasma sample available for testing. The agreement between the **cobas** EGFR Mutation Test v2 in plasma and the **cobas** EGFR Mutation Test v1 in tissue was evaluated for detection of EGFR mutations (Ex. 19del and L858R mutations) in NSCLC patients screened for participation in ENSURE. 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. The drug efficacy of TARCEVA, based on the **cobas** EGFR Mutation Test v2 in plasma, was evaluated by bridging to the drug efficacy based on the **cobas** EGFR Mutation Test v1 in tissue in the ENSURE study.

The patients whose plasma results were positive for exon 19 deletion and/or an L858R mutations treated with

COST

Cases

New cases of cancer, 2012, UK

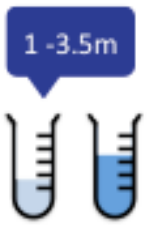
Common cancers

More than half of new cases of cancer are breast, lung, prostate or bowel cancer, 2012, UK

Survival

Survive cancer for 10 or more years, 2010-11, England and Wales

Tests?



Number of ctDNA "liquid biopsy" tests 2020, UK

Detection method	Commercial test	Analytical sensitivity	Analytical specificity	Cost ($USD)	TOT (days)
Next-generation sequencing	Guardant 360	>85%	99.99%	$5600	14
	Foundation ACT	>95%	99%	$5800	14
Digital PCR	Biodesix	>85%	100%	$1800	3
Quantitative PCR & sanger	Biocept	97%	99.5%	$1900	7
Quantitative PCR & NGS	Trovagene	93%	99%	$1500	14

Commercial versus Home Brew

Focus group test development
Test comparison/equivalence