Application of laboratory genetic testing in cancer diagnosis, prognosis and disease monitoring & treatment

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Outline

- Cancer genetics
- Cancer genetics laboratory methods
- Pharmacogenomics
- Liquid biopsy

Cancer genetics

Gene and Chromosome Abnormalities Observed in Cancer

- Gene mutations (oncogenes, tumor suppressor genes)
- Chromosome structural abnormalities (translocations, deletions, insertions)
- Chromosome number abnormalities (aneuploidy, polysomy)

Important molecular genetic markers of cancer

- **CHROMOSOMAL INSTABILITY**
- **THE DISTURBANCE IN THE MISMATCH REPAIR SYSTEM**
- **CpG ISLAND METHYLATOR PHENOTYPE**
- **MUTATIONS IN POLYMERASE GENES**
- **SIGNAL TRANSDUCERS (*KRAS, NRAS, BRAF, PIK3CA*)**
- ***TP53* MUTATIONS**
- **GERMINAL VARIATIONS IN GENES encoding DRUG METABOLISING ENZYMES**
- **Liquid Biopsy: CtC, CfDNA &CtDNA**

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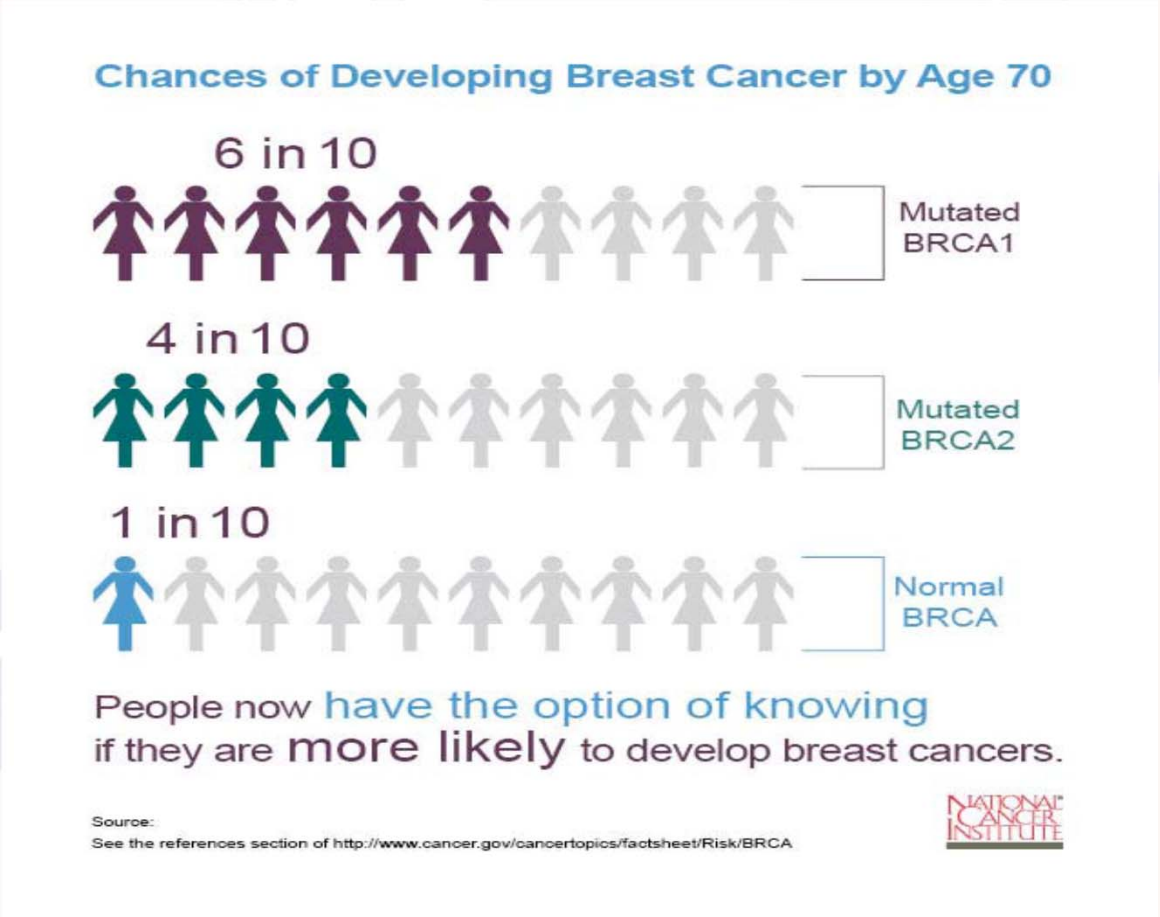
Inherited Cancer Gene Mutations

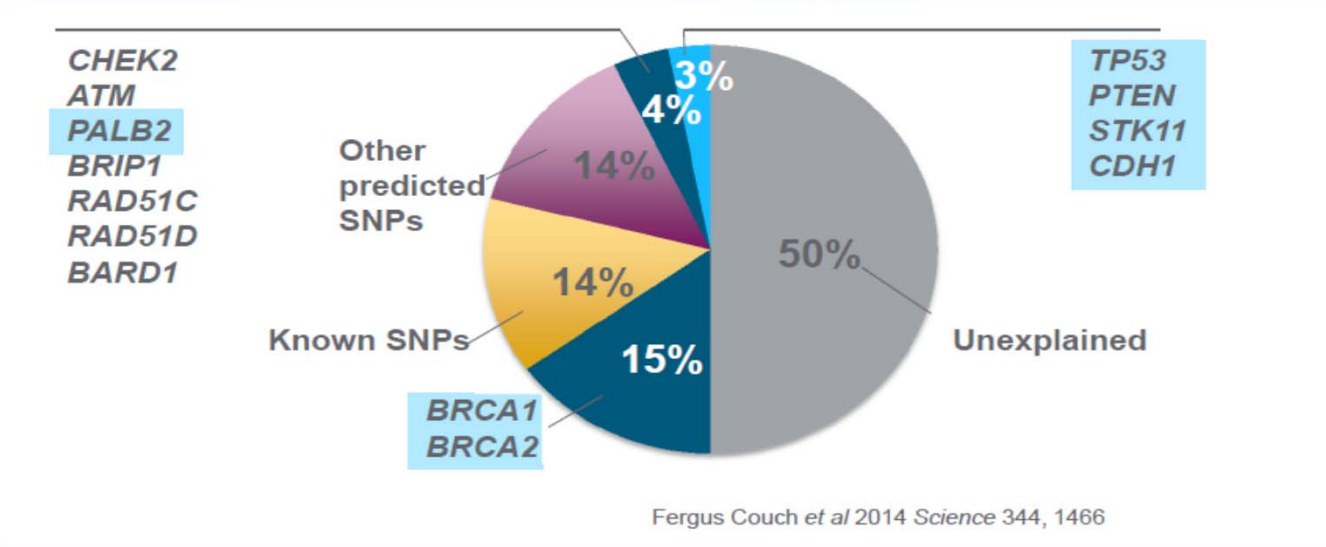
- Inherited tumor suppressor gene mutations are recessive for the malignant phenotype.
- Tumor suppressor gene mutations are dominant with respect to increased risk of malignancy.
- Loss of heterozygosity exposes the recessive mutant allele in a hemizygous state.
- This is explained by the two-hit hypothesis.

Hereditary Cancers
Lifetime Risks: Breast Cancer



Other Hereditary Breast Cancer Genes

- Every year, more than 200,000 women in the U.S. will be diagnosed with breast cancer
- Hereditary breast cancer accounts for about 5% to 10% of female breast cancer and 4% to 40% of male breast cancer
- Genes with highest increased risk for breast cancer (highlighted):



Fergus Couch *et al* 2014 *Science* 344, 1466

“Red Flags” for Inherited Susceptibility to Cancer

- Cancer in 2 or more closely related relatives
- Multiple generations affected
- Early age at diagnosis
- Multiple primary tumors
- Bilateral or rare cancers
- Constellation of tumors consistent with a specific cancer syndrome
- Certain ethnic backgrounds (e.g. Ashkenazi Jewish ancestry)

Molecular Abnormalities in Solid Tumors, HER2/neu

- The *HER2/neu* gene encodes one of a family of human epidermal growth-factor receptors.
- This gene is frequently amplified in breast cancer cells, resulting in increased amounts of HER2 cell surface protein.
- HER2-expressing tumors are sensitive to herceptin, a monoclonal antibody therapy.
- HER2 protein is detected by immunohistochemistry (IHC).
- *HER2/neu* gene amplification is detected by fluorescence in situ hybridization (FISH).

Cancer Genetic laboratory methods

Hereditary Nonpolyposis Colorectal Carcinoma

- Hereditary nonpolyposis colorectal carcinoma (HNPCC) accounts for about 5% of colon cancer.
- HNPCC is the most common form of hereditary colon cancer.
- HNPCC is associated with mutations in genes encoding components of the MMR system, most frequently MLH1 and MSH2.

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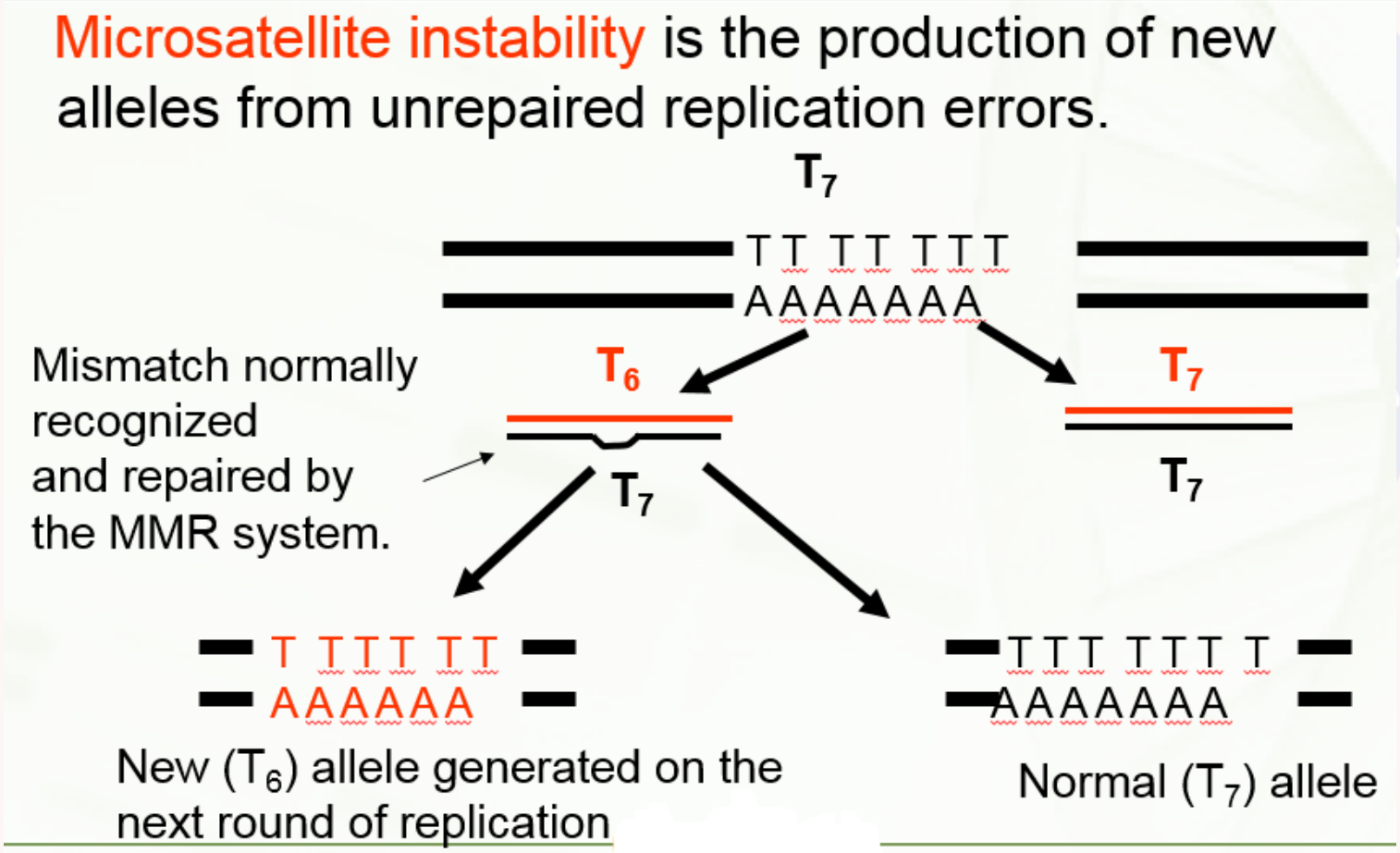
Microsatellite Instability (MSI)



HNPCC and MSI

- 85–90% HNPCC tumors have MSI.
- Mutations in genes of the MMR system (loss of function) are inferred by testing for MSI.
- MSI analysis determines gene function. Direct sequencing is used to detect the actual gene mutation.
- MSI is analyzed by assessing stability of at least five microsatellite loci as recommended by the National Cancer Institute.

Molecular Detection of Leukemia and Lymphoma

- Targets:
  - Antibodies, gene rearrangements, translocations, point mutations, polymorphisms, viruses
- Methods:
  - Hybridization, blotting
  - Standard PCR, RT-PCR, electrophoresis
  - Real-time PCR with gene or patient-specific probes

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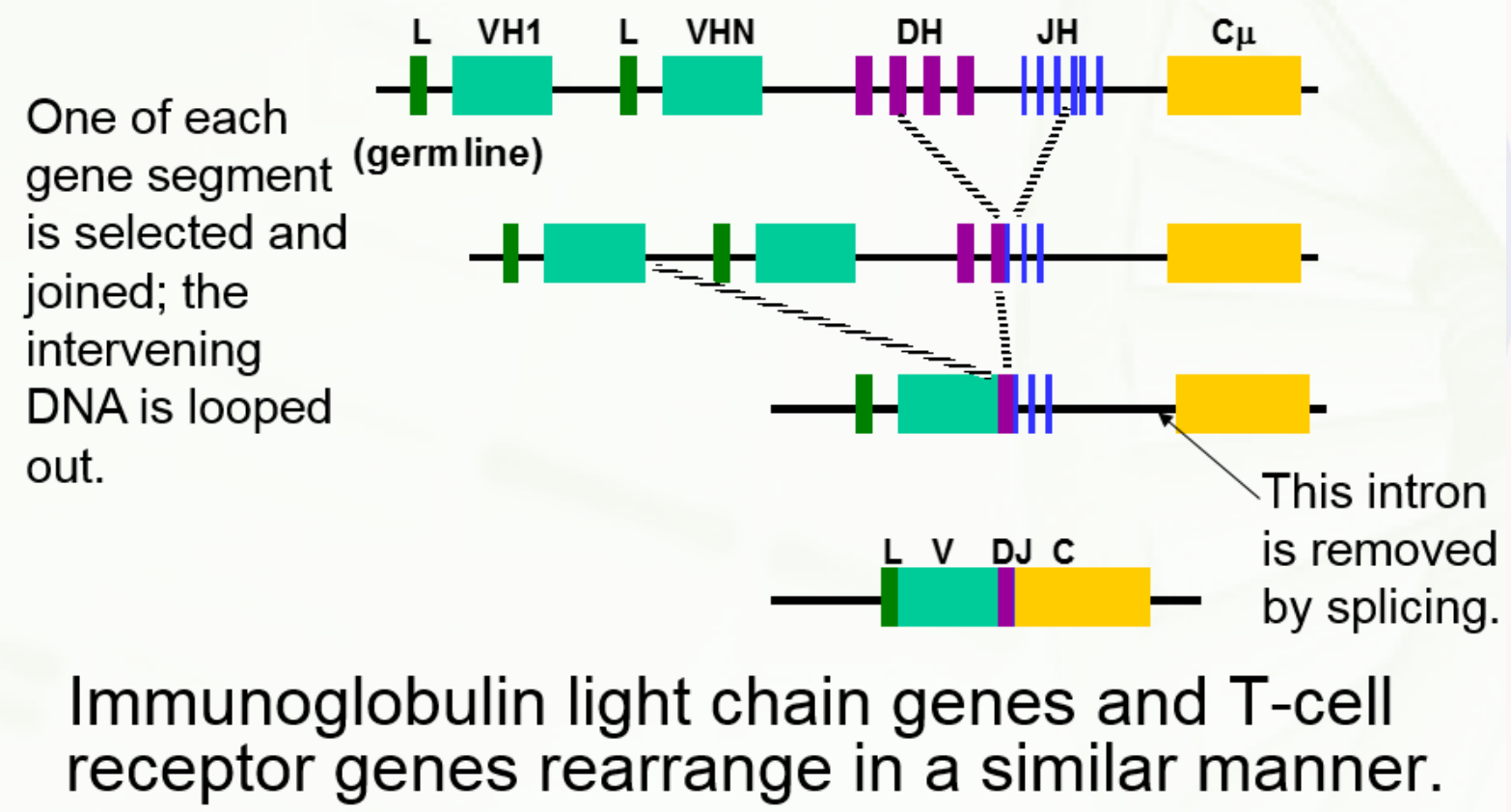
Immunoglobulin Heavy Chain (IgH) Gene Rearrangement



Immunoglobulin light chain genes and T-cell receptor genes rearrange in a similar manner.

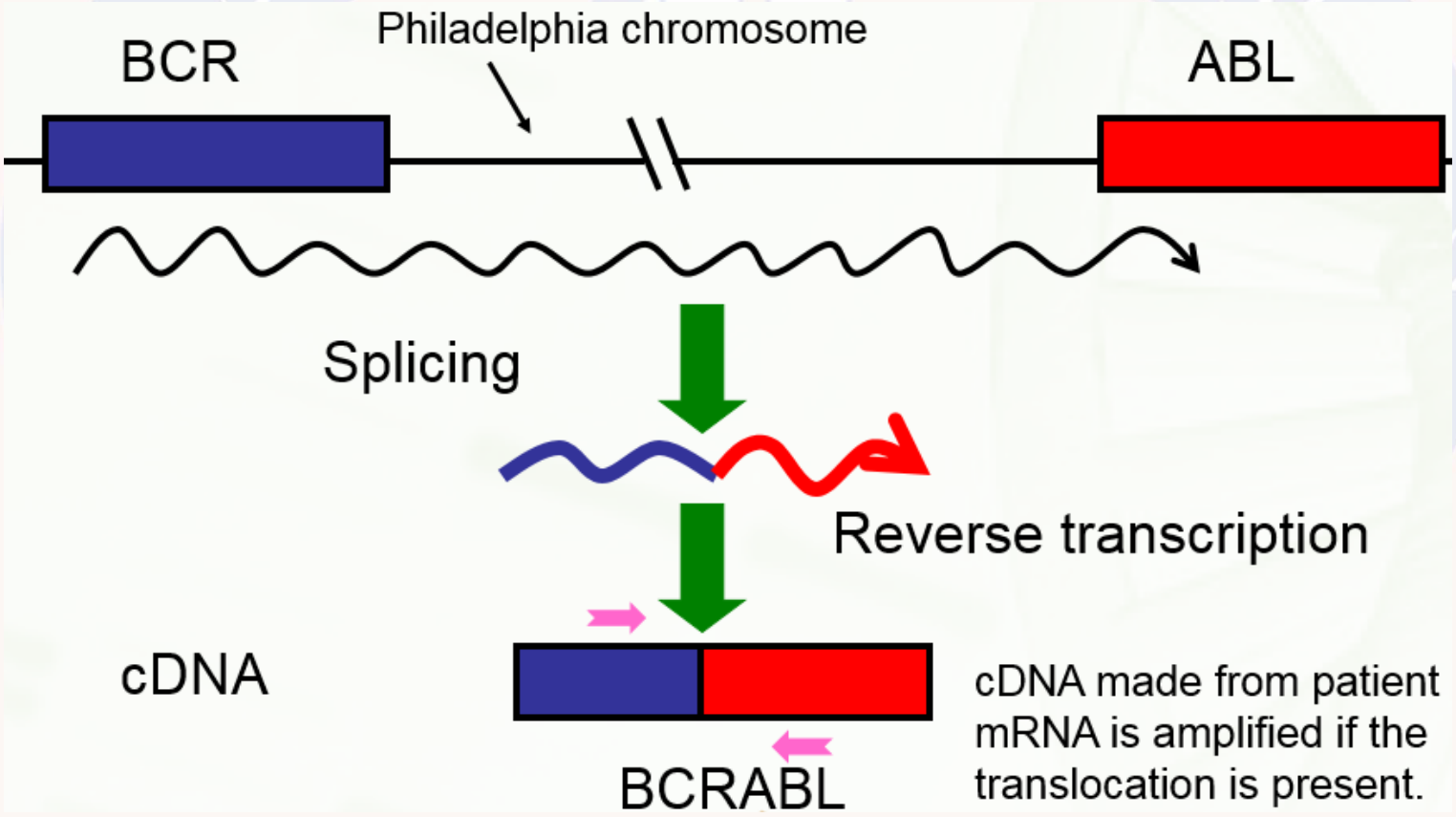
Translocations Used in Diagnosis and Monitoring of Hematological Tumors

PreB ALL	t(1;19)
B-cell leukemia	t(2;8), t(8;14), t(8;22), t(11;14)
Acute TCLL	t(11;14)
AML/MDS	t(11q23)
AML (M2)	t(8;21), t(6;9)
APL (M3)	t(15;17)
AMML (M4)	t(11;21)
AMoL (M5)	t(9;11)

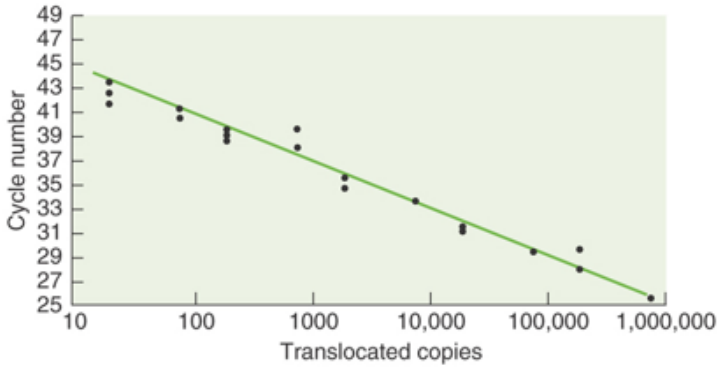
Cont

CML	t(9;22), t(11;22)
ALL	t(9;22), t(12;21), t(8;14), t(2;8), t(8;22), t(11q)
Burkitt	t(8;14), t(2;8), t(8;22)
DLBCL	t(3q27), t(14;18); t(8;14)
TCL	t(8;14)
Follicular	t(14;18), t(8;14)
MCL	t(11;14)
MM	t(14q32)

Translocations Used in Diagnosis and Monitoring of Hematological Tumors: t(9; 22)



Quantification by qPCR (TaqMan®)

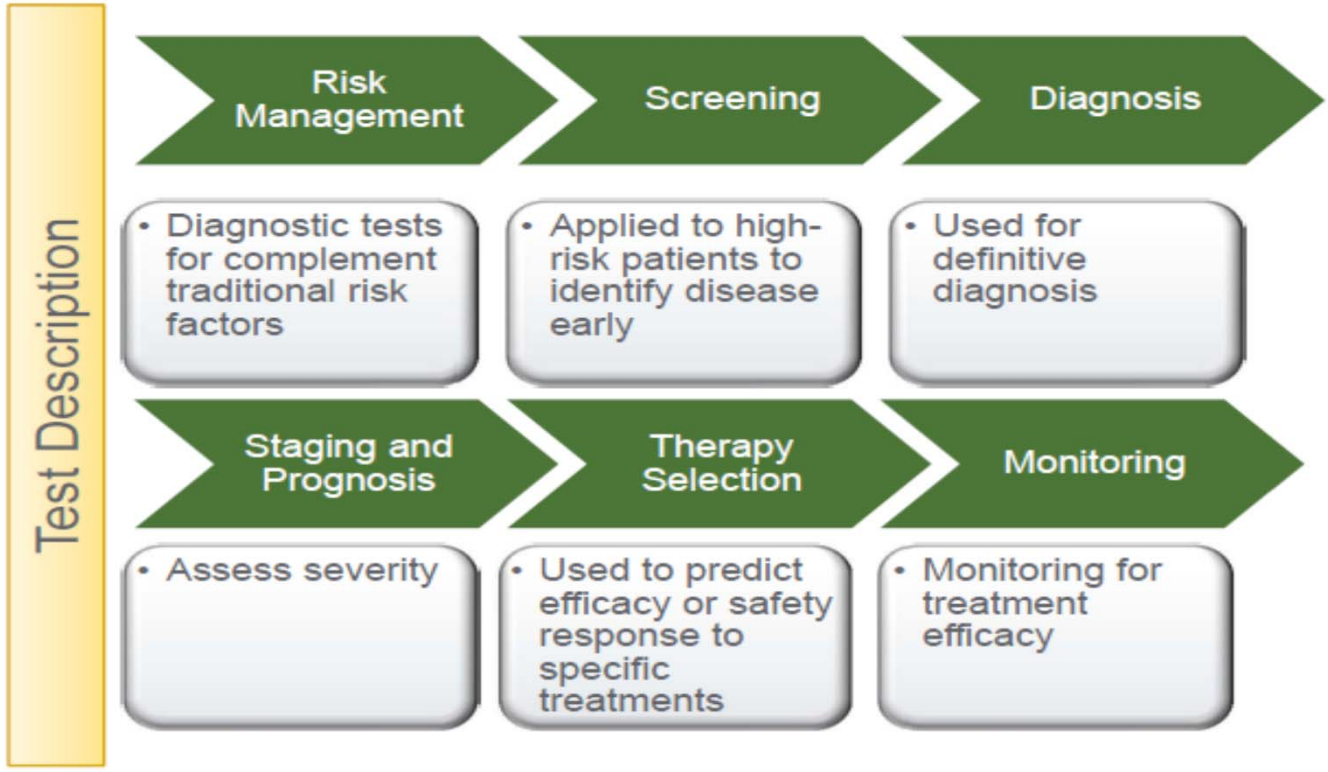


- For qPCR, use a standard curve of tumor cells diluted into normal cells.
- For RT-qPCR, use a standard curve of transcripts of known copy numbers diluted into normal RNA.

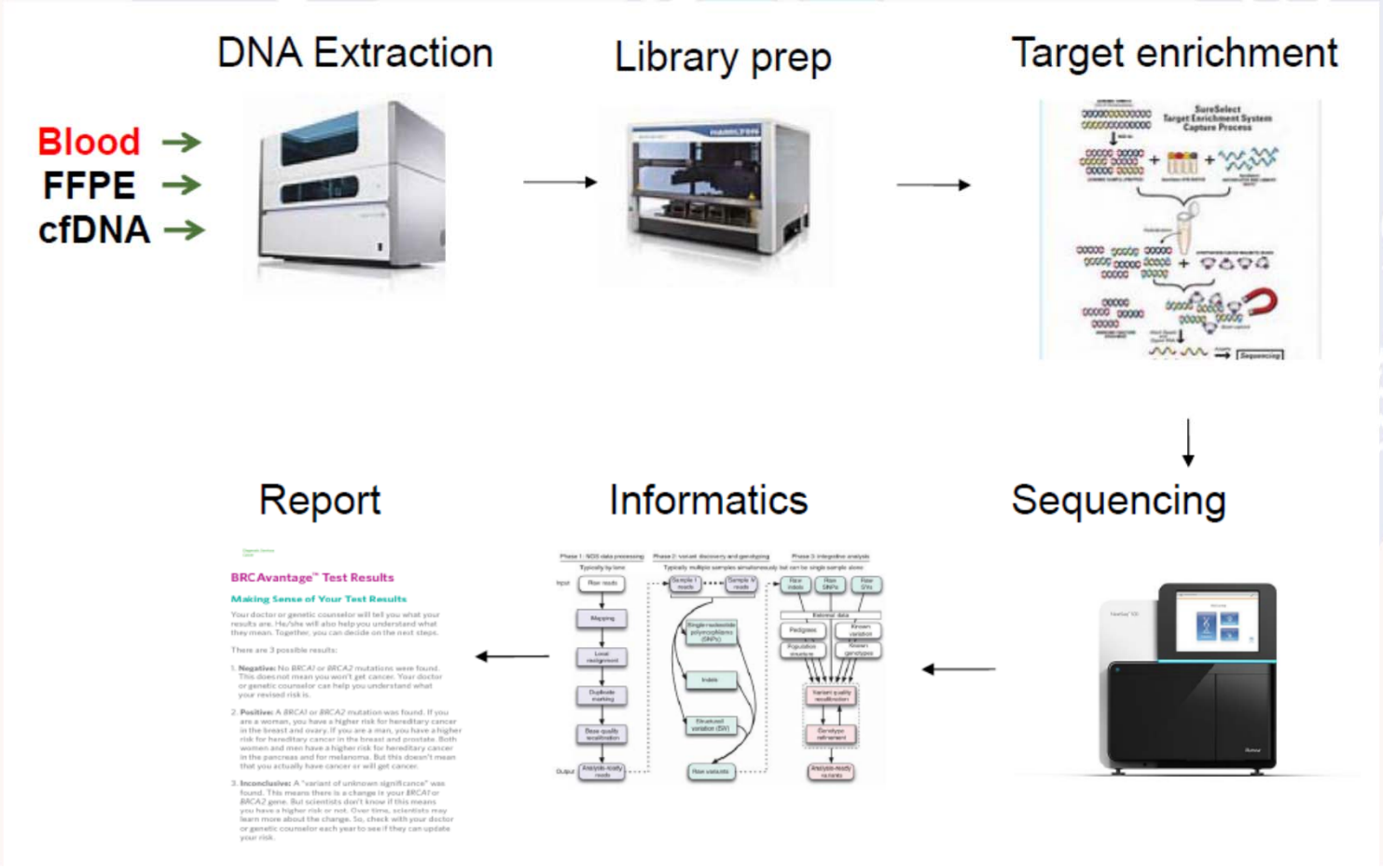
Inherited Breast Cancer Risk

- BRCA1 and BRCA2 are tumor suppressor genes encoding proteins that participate in DNA repair.
- Inherited mutations in BRCA1 or BRCA2 significantly increase risk of breast cancer at an early age.
- Frequently occurring mutations, including 187delAG and 5382insC in BRCA1 and 6174delT in BRCA2 (founder mutations of Ashkenazi), are detected by Sanger sequencing and other methods.
- Most mutations are detected by direct sequencing of both genes.

Diagnostic Applications of Sequencing



Testing Workflow for Cancer Gene Panel



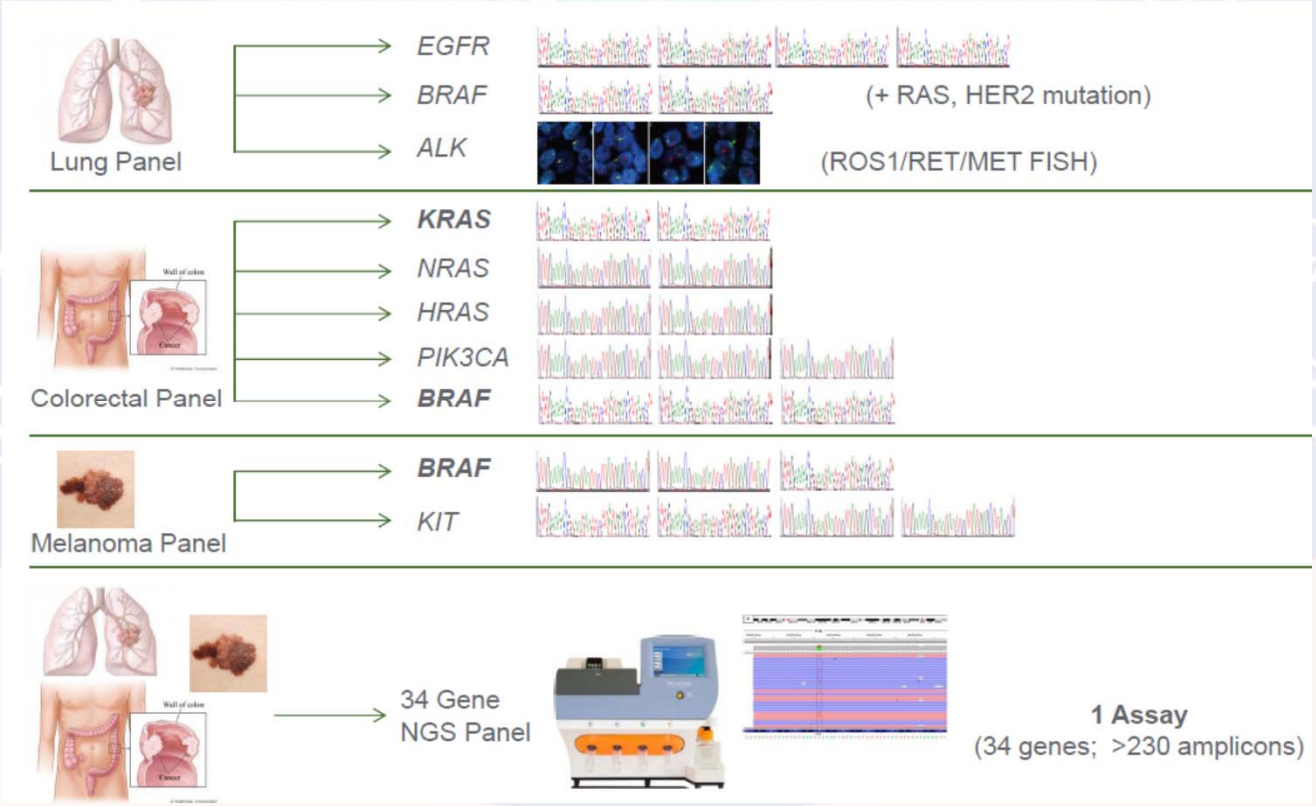
Hereditary Breast Cancer Panel Test Options

Comprehensive	Includes sequencing and large rearrangement analysis of all coding exons in *BRCA1* and *BRCA2*
Ashkenazi Jewish Screen	Includes detection of the 3 HBOC founder mutations (187delAG, 5385insC, 6174delT)
Ashkenazi Jewish Screen with Reflex Comprehensive	Ashkenazi Jewish screen test that reflexes to the comprehensive test when Ashkenazi test is negative
Single Site	Specific mutation testing for a known familial mutation
Rearrangements	*BRCA1* and *BRCA2* complete rearrangement testing not performed

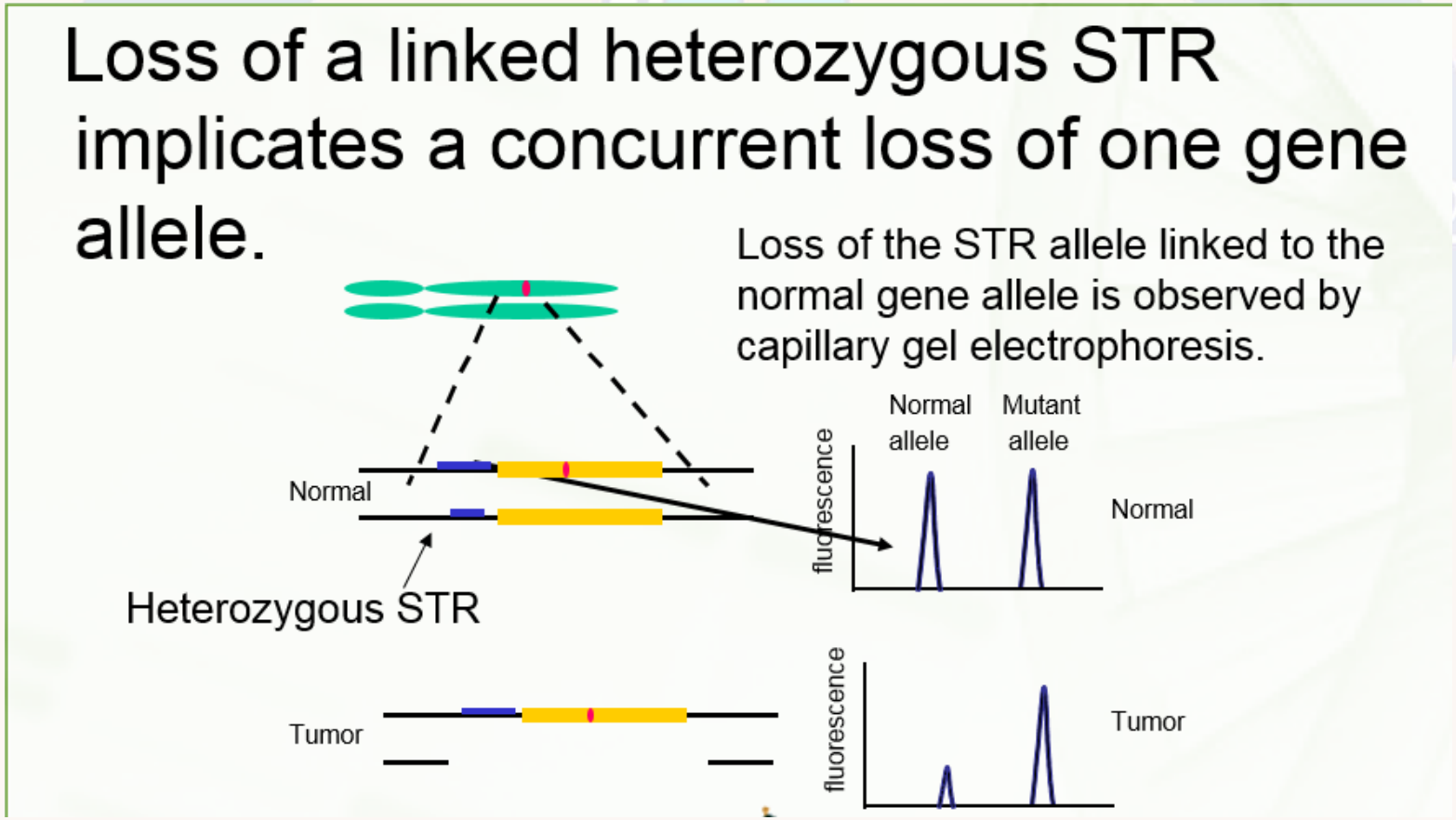
Cancer Predisposition Panel: 34 Genes

	34 genes	Breast	Ovarian	GYN	Colon	Pancreatic	Renal
7G	**BRCA1**	BRCA1	BRCA1	BRCA1		BRCA1	
	BRCA2	BRCA2	BRCA2	BRCA2		BRCA2	
	STK11	STK11	STK11		STK11	STK11	
	CDH1	CDH1	CDH1		CDH1		
	PTEN	PTEN	PTEN	PTEN	PTEN		PTEN
	TP53	TP53	TP53	TP53	TP53	TP53	TP53
	PALB2	PALB2	PALB2			PALB2	
27G	BARD1	BARD1	BARD1				
	BRIP1	BRIP1	BRIP1				
	NBN	NBN	NBN				
	NF1	NF1	NF1				
	RAD51C	RAD51C	RAD51C				
	RAD51D	RAD51D	RAD51D				
	ATM	ATM	ATM			ATM	
	MUTYH	MUTYH	MUTYH		MUTYH		
	CHEK2	CHEK2	CHEK2		CHEK2		
	RET	RET			RET		
	MEN1	MEN1					
	MLH1		MLH1	MLH1	MLH1	MLH1	MLH1
	MSH2		MSH2	MSH2	MSH2	MSH2	MSH2
	MSH6		MSH6	MSH6	MSH6	MSH6	MSH6
	PMS2		PMS2	PMS2	PMS2	PMS2	PMS2
	EPCAM		EPCAM	EPCAM	EPCAM	EPCAM	EPCAM
	APC				APC	APC	
	BMPR1A				BMPR1A		
	SMAD4				SMAD4		
	POLD1				POLD1		
	POLE				POLE		
	CDKN2A					CDKN2A	
	CDK4						
	SDHB						SDHB
	SDHC						SDHC
	SDHD						SDHD
	VHL						VHL

Current Tumor Panels

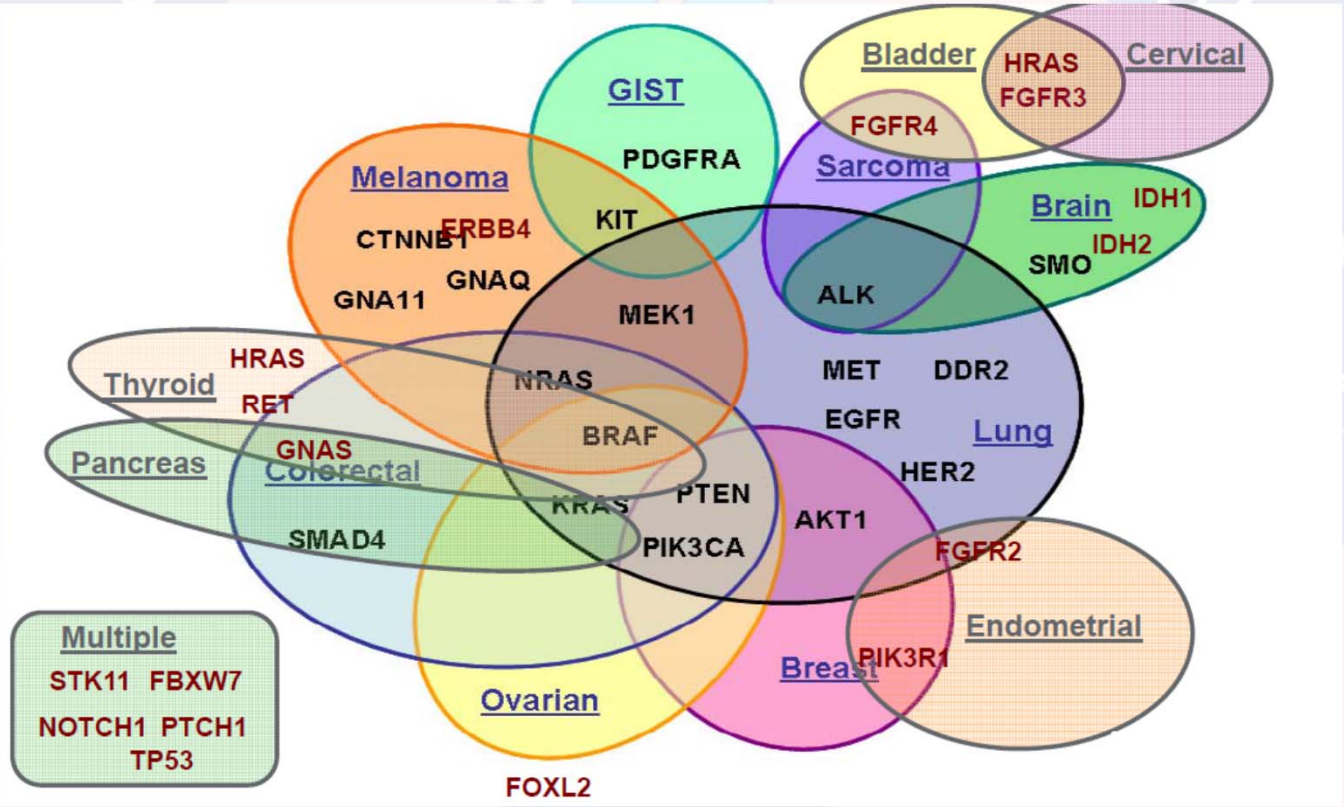


Loss of Heterozygosity Can Be Detected by STR Analysis



Pharmacogenomics

Solid Tumor Gene Mutations Related to Therapy



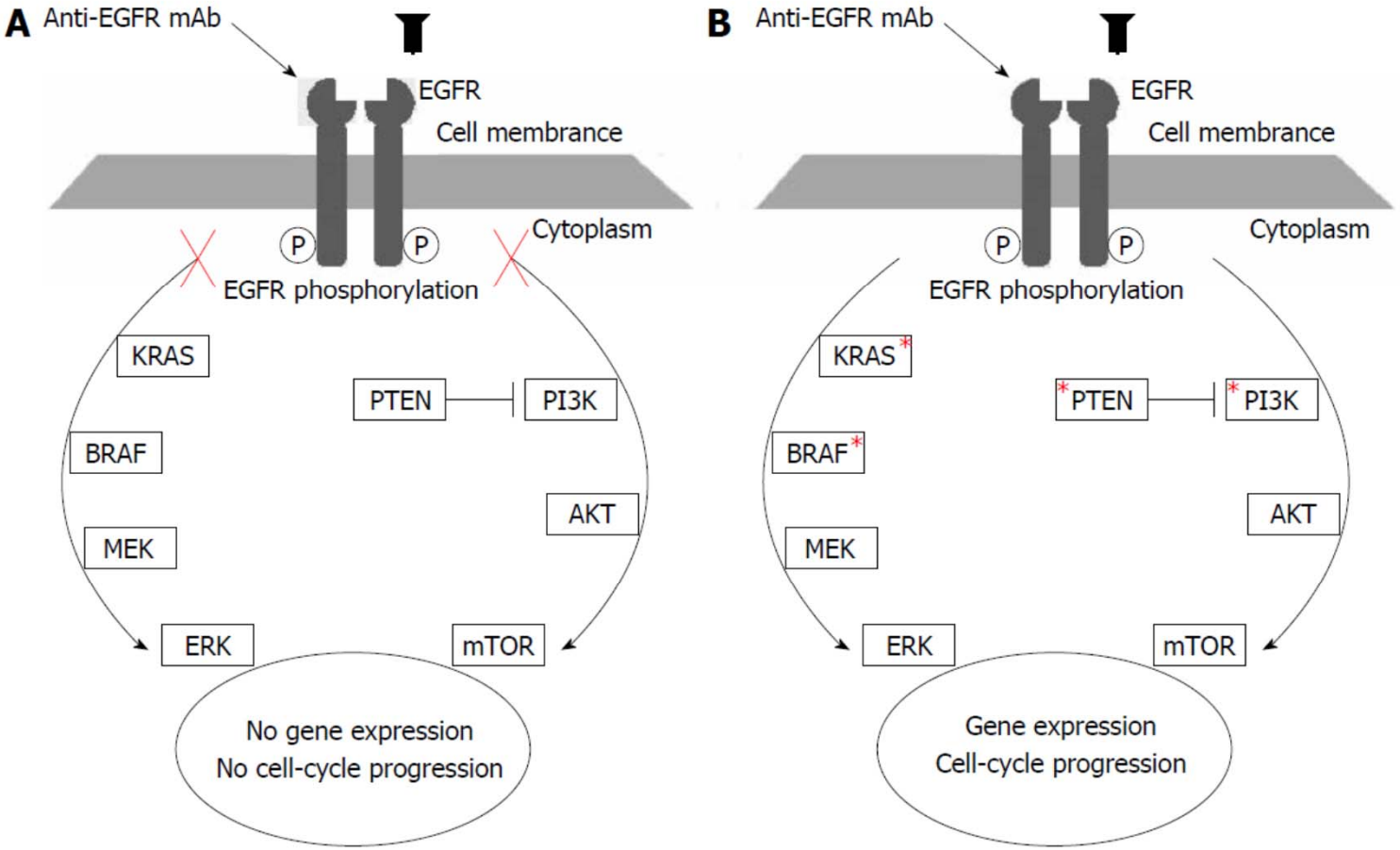
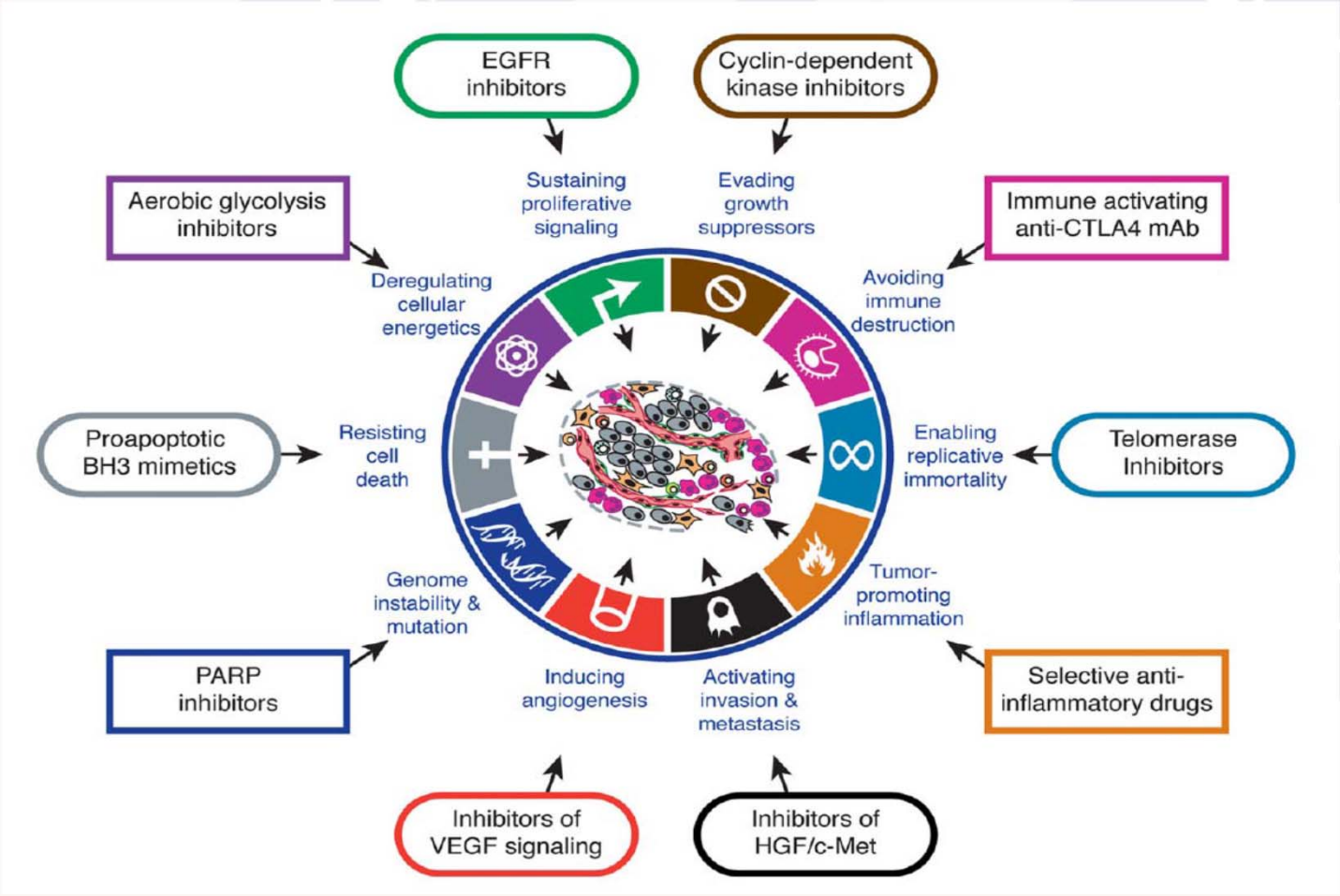
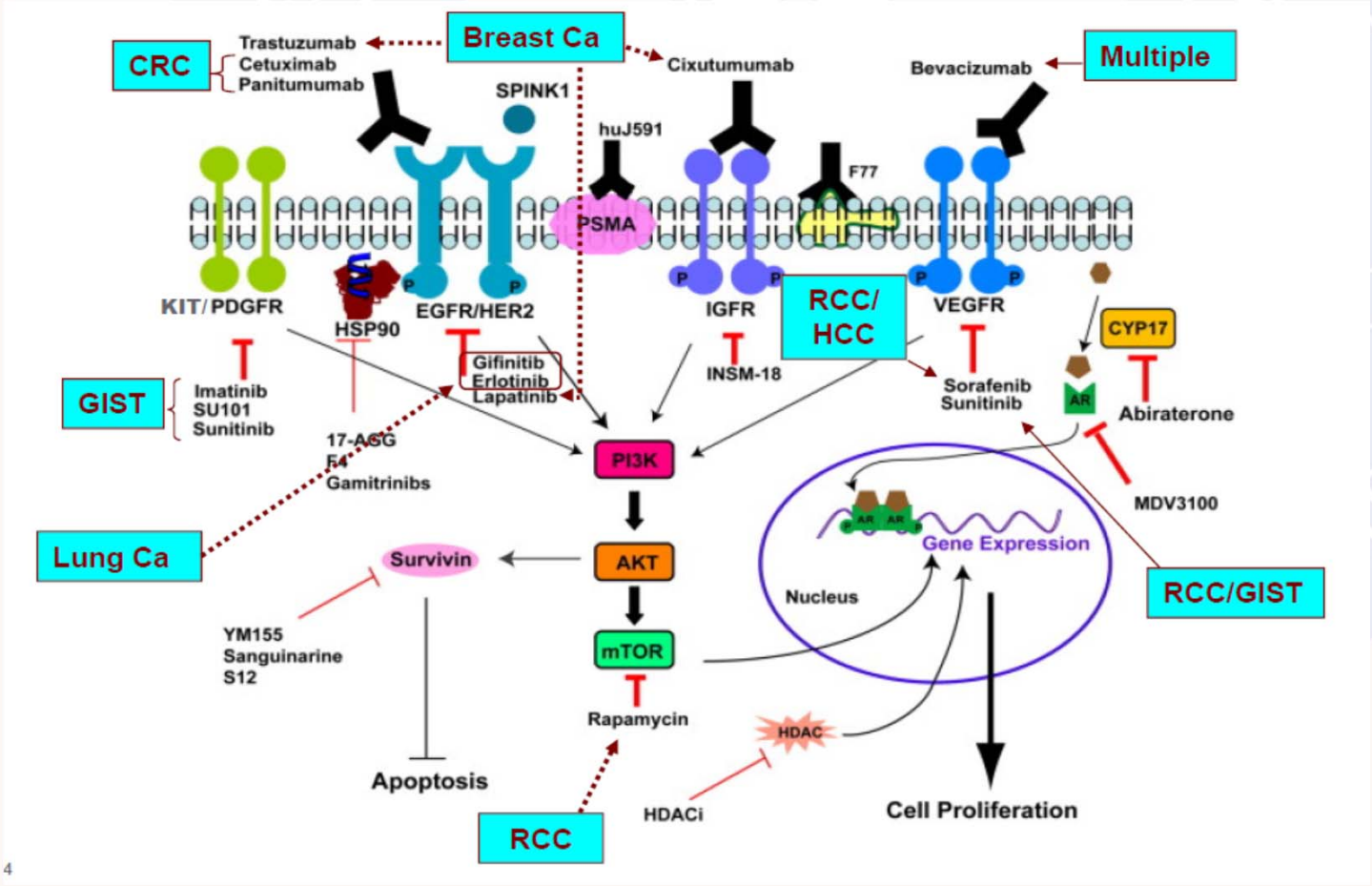


Figure 1 Representation of epidermal growth factor receptor pathway in response to therapy with anti-epidermal growth factor receptor inhibitors in wild-type and mutant patients. A: The binding of monoclonal antibodies (mAb) to EGFR normally causes the blockage (indicated with a red cross) of downstream RAS/RAF/MAPK and PI3K/AKT/mTOR signalling pathways and so the blockage of gene expression and cell cycle progression; B: Mutations in any gene of this pathway (indicated with a red star) cause a constitutive activation of the pathway leading to gene expression, upregulated proliferation, impaired differentiation and no response to monoclonal inhibitors. EGFR: Epidermal growth factor receptor; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog gene; BRAF: V-raf murine sarcoma viral oncogenes homolog B1; MEK: MAPK/ERK kinase; ERK: Extracellular signal-regulated kinases; PTEN: Phosphatase and tensin homolog; PI3K: Phosphatidylinositide-3-kinases; AKT: Protein Kinase B (PKB); mTOR: Mammalian target of rapamycin.

Clinical View of Cancer



Cancer Pathways and Targeted Treatments



Lung Cancer

NCCN Guidelines Version 3.2014
Non-Small Cell Lung Cancer

TARGETED AGENTS FOR PATIENTS WITH GENETIC ALTERATIONS

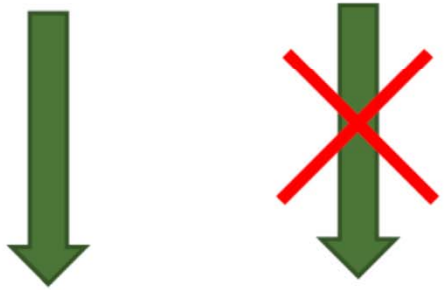
Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
EGFR mutations	erlotinib,[1] gefitinib,[2] afatinib[3]
ALK rearrangements	crizotinib[4]
HER2 mutations	trastuzumab,[5] afatinib[6]
BRAF mutations	vemurafenib,[7] dabrafenib[8]
MET amplification	crizotinib[9]
ROS1 rearrangements	crizotinib[10]
RET rearrangements	cabozantinib[11]

Treatment Options Based on Molecular Profile

EGFR exon 18, 19 or 21 mutation

EGFR T790M

KRAS codon 12, 13, 61, etc.



Tarceva® (erlotinib)

Summary

- During recent years, access to genetic testing for cancer predisposition and solid tumor genetic profile has broadened.
- Important technical advances have made it possible to perform multi-gene sequencing assays
- Existing mutation databases and a newly created public/private organization are expected to enhance the clinical utility of genetic results
- Overall, the field of genetic testing for predisposition to cancer is becoming fundamentally important and proving clinical validity and utility
- Solid tumor genetic profile influence drug selection, enabling targeted therapy

Liquid biopsy

Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology

- Apoptotic or necrotic cancer cells release circulating cell-free DNA fragments, designated as circulating tumor DNA (ctDNA), as well as exosomes (EXOs), namely membrane-encapsulated subcellular structures containing proteins and nucleic acids released by the tumor cells.

Integrating liquid biopsies into the management of cancer

- At present, the molecular landscapes of solid tumours are established using surgical or biopsy tissue samples.
- Tissue-based tumour profiles are, however, subject to sampling bias, provide only a snapshot of tumour heterogeneity, and cannot be obtained repeatedly.

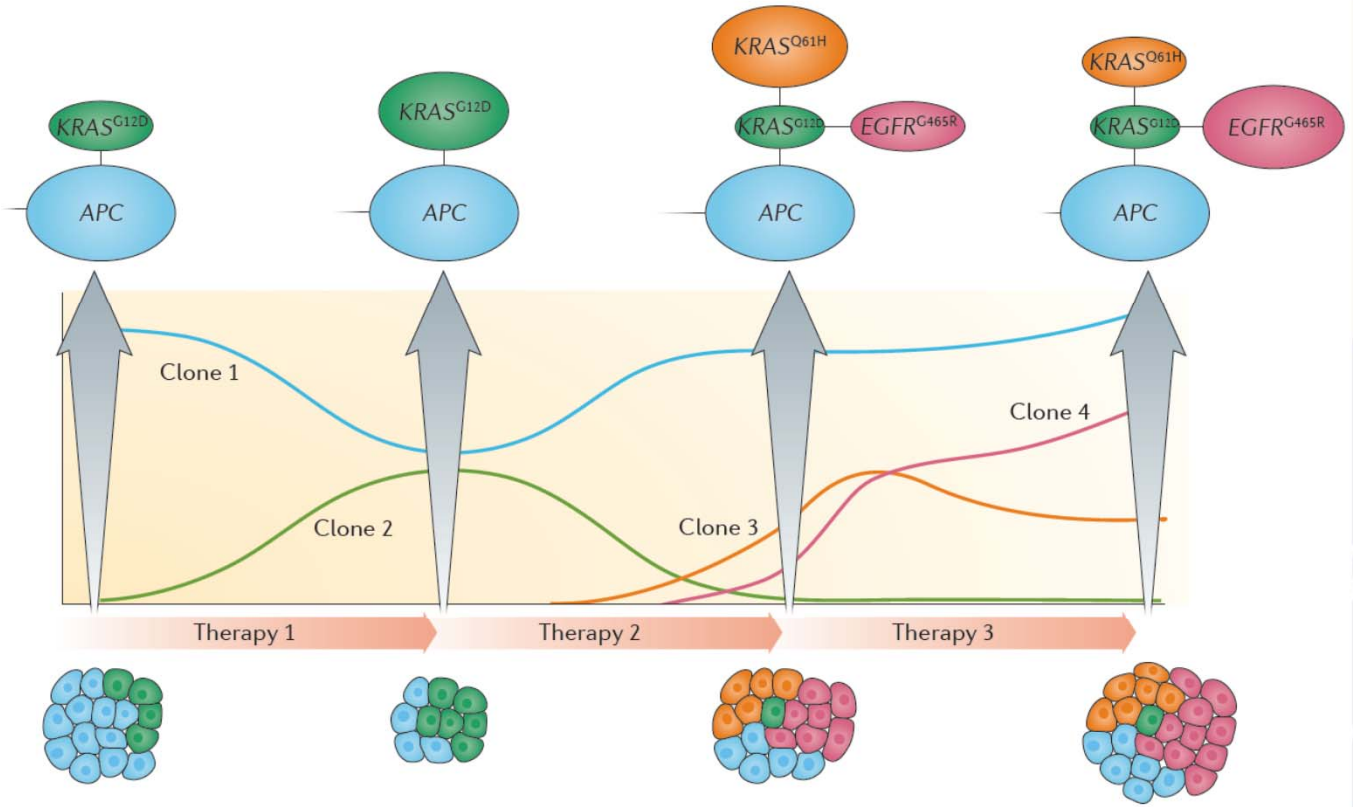


Figure 3 | **Monitoring clonal evolution using liquid biopsies.** Treatment with targeted therapies places tumour cells under selective pressure, thereby triggering clonal evolution that can be captured using liquid biopsy approaches. The data obtained can provide important insights into mechanisms of resistance, and can guide therapeutic decision-making. This schematic depicts the dynamic changes in the abundance of tumour-cell subclones harbouring different mutations in a patient with metastatic colorectal cancer treated with anti-EGFR antibodies. Monitoring of a clonal mutation in *APC* that is present in essentially all tumour cells (clone 1, blue line) tracks tumour burden, while subclonal mutations (*KRAS*^{G12D}, *KRAS*^{Q61H}, and *EGFR*^{G465R} in clones 2, 3 and 4, respectively) provide a measure of clonal evolution during therapy. Subclonal mutations can be lesion-specific, leading to variations in responses at different disease sites. In this hypothetical patient, primary treatment with the anti-EGFR antibody leads to a substantial decline in tumour burden through targeting of the majority of tumour cells, but outgrowth of the resistant *KRAS*^{G12D}-mutant subclone eventually causes tumour regrowth, necessitating a therapeutic switch. The change in therapy decreases the size of the *KRAS*^{G12D}-cell population, but resistant subclones (clones 3 and 4) with other mutations expand and drive tumour growth. A third-line of treatment restricts the growth of clone 3, but clone 4 continues to proliferate.

Cont’d

- ‘liquid biopsies’, can be used to monitor response to treatment, assess the emergence of drug resistance, and quantify minimal residual disease.
- In addition to blood, several other body fluids, such as urine, saliva, pleural effusions, and cerebrospinal fluid, can contain tumour-derived genetic information.

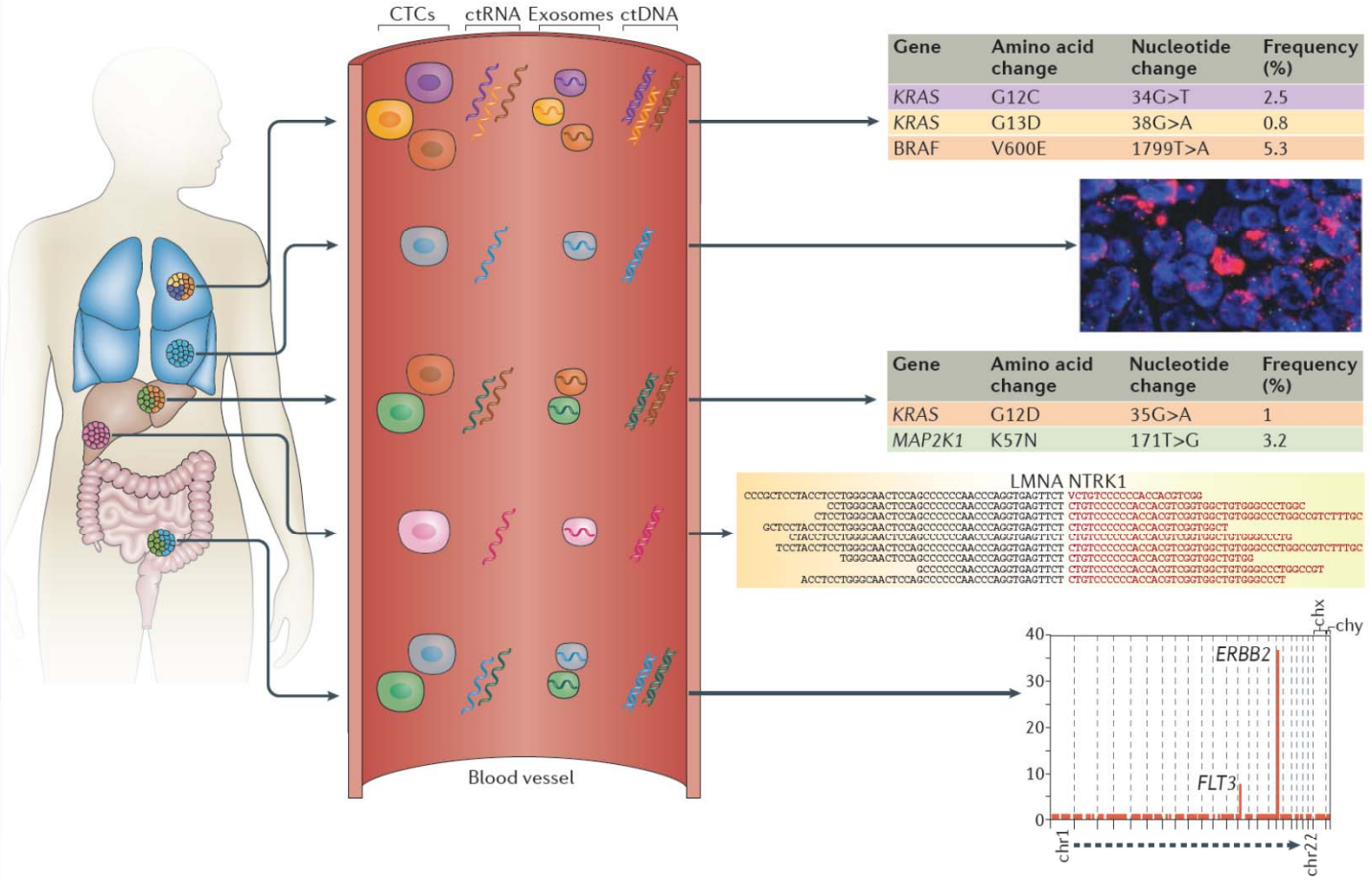


Figure 1 | **Liquid biopsies capture the molecular heterogeneity of metastatic cancers.** Liquid biopsies of tumour components in the blood, including circulating cell-free tumour DNA (ctDNA) and RNAs (ctRNA), exosomes, and circulating tumour cells (CTCs), can be leveraged to capture the molecular heterogeneity of distinct tumour lesions harbouring different genetic alterations. The tables illustrate the detection of point mutations in oncogenes (G12D, G12C, and G13D mutations in *KRAS*; V600E mutation of *BRAF*; and K57N mutation of *MAP2K1* (MEK1)) through candidate-gene or next-generation sequencing analysis of ctDNA in plasma. Identification of a *LMNA–NTRK* gene fusion is depicted in the sequence alignment. Gene copy-number variations can also be detected using fluorescence *in situ* hybridization analysis of CTCs isolated from the blood, as shown in the fluorescence micrograph, and via whole-exome analysis of blood-derived tumour DNA by next-generation sequencing (as demonstrated for *FLT3* and *ERBB2 (HER2)* amplification).

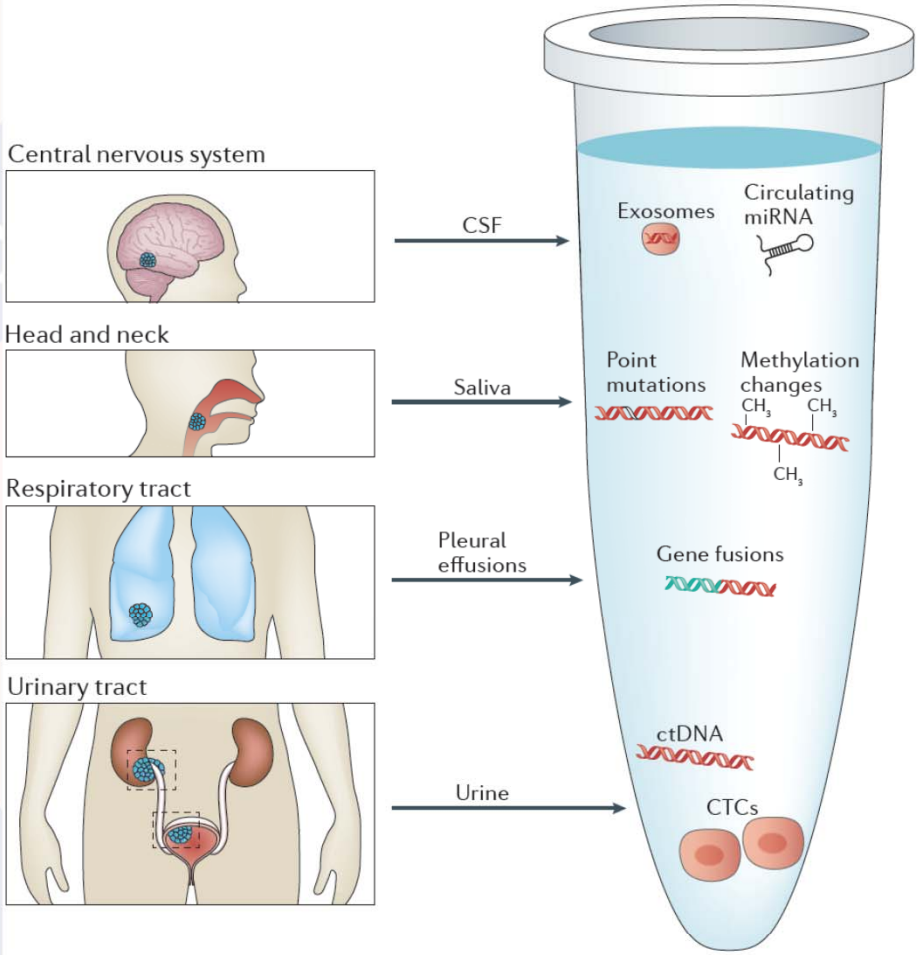


Figure 2 | **Body fluids as a source of tumour-derived molecular information.** Schematic representation of different body fluids (other than blood) that can contain tumour-derived molecular information, specifically, urine, cerebrospinal fluid (CSF), saliva, and pleural effusions. The localization of the primary tumour and of any metastatic lesions influences the presence of circulating tumour-derived nucleic acids, cells, and microvesicles in individual body fluids. As indicated, point mutations, gene fusions, and methylation changes associated with the cancer can be detected through analysis of DNA or mRNA derived from these blood-borne tumour materials. CTCs, circulating tumour cells; ctDNA, circulating cell-free tumour DNA; miRNA, microRNA.

Clinical applications of liquid biopsies
CTDNA as biomarkers and their clinical utility

- ***ctDNA in cancer diagnosis.***
- ***Methylation profiles in ctDNA — predicting response to chemotherapy.***
- ***MRD monitoring and early diagnosis of relapse — ctDNA as prognostic biomarker***
- ***Evaluating response and predicting resistance to therapy.***

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Liquid biopsy in the clinical setting

- The first attempt at tissue–plasma pairwise comparison of *EGFR* mutations was reported a decade ago.
- In two meta-analyses, have confirmed that *EGFR*-sensitizing mutations are detectable in ctDNA with high specificity (>93%), but improvable sensitivity (<70%), making plasma genotyping of NSCLCs a viable alternative to tissue-based genotyping when the latter is not feasible.

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Key points

- Longitudinal surveillance of clonal evolution is essential for precision medicine, but cannot be effectively achieved using tissue biopsy specimens, owing to sampling issues.
- The blood of patients with cancer contains diverse tumour-derived materials, including circulating cell-free tumour DNA (ctDNA), circulating tumour cells, and exosomes.
- The sampling and analysis of ctDNA or other circulating tumour components present in biological fluids, termed ‘liquid biopsy’, enables minimally invasive monitoring of tumour evolution over time in the clinic.
- Two different liquid biopsy companion diagnostic tests for *EGFR* mutations in plasma ctDNA have been approved by the regulatory agencies in Europe and the USA for the selection of patients with non-small-cell lung cancer for anti-EGFR treatment in clinical practice

Thanks for your attention