**LAUREA MAGISTRALE BIOLOGIA MOLECOLARE E APPLICATA- Curriculum Biosanitario e della Nutrizione**

# Corso di ONCOLOGIA

**9 dicembre 2019**

ONCOLOGIA AA 2019-2020 lezione 15

✓ Cancer is a <u>genetic disease</u> since it is due to alterations in patients' DNA.

✓ Deciphering the genetic changes is necessary to <u>understand</u> the disease.

✓ Unraveling the genetic bases of cancer allows us to design the best <u>treatment</u> protocols for each single patient.

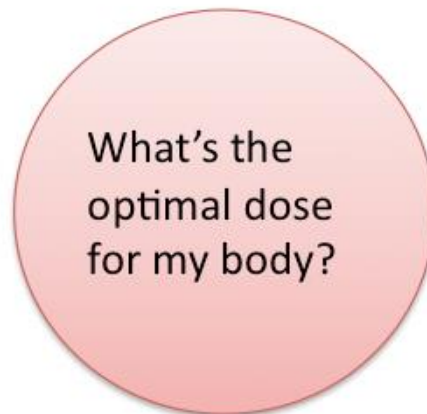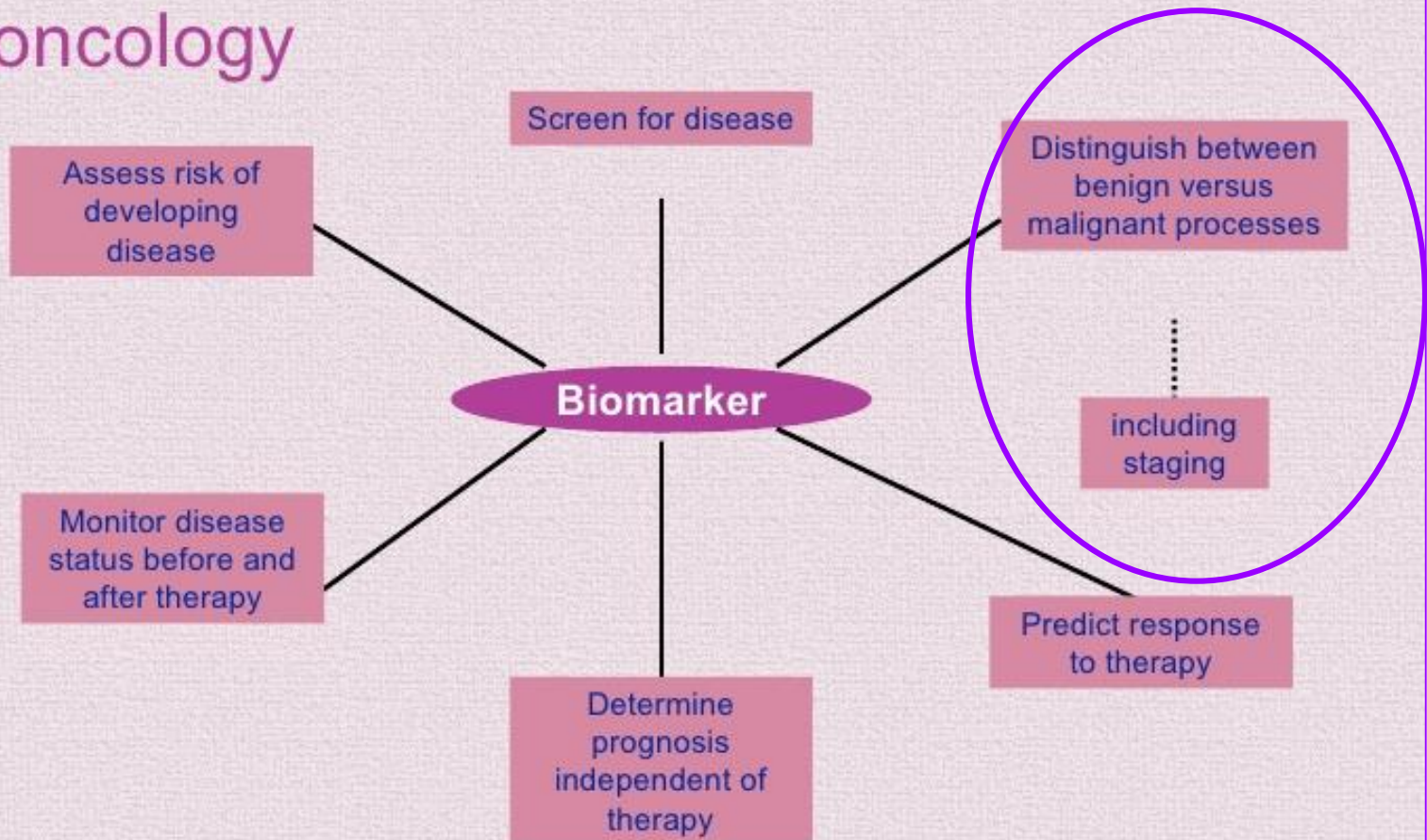Questions that can be answered by cancer biomarkers

**Prognostic** — Is it likely to develop this cancer?

**Diagnostic** — What type of cancer is it?

**Predictive** — Is this the optimal drug for my cancer?

**Pharmacodynamics** — What's the optimal dose for my body?

**Recurrence** — Will the cancer return?

Potential uses for biomarkers in oncology

# DIAGNOSTIC TESTS

➢ Identification of the <u>presence/amount of a specific protein</u>. It can be performed on blood samples (es. CEA, CA-125) and on tumour tissue (es. ER, HER2/Neu).

➢ Evaluation of the <u>expression of a set of genes (microarray technology</u>): diagnostic tests development, better classification, identification of new therapeutic targets, setting up of personalised treatments).
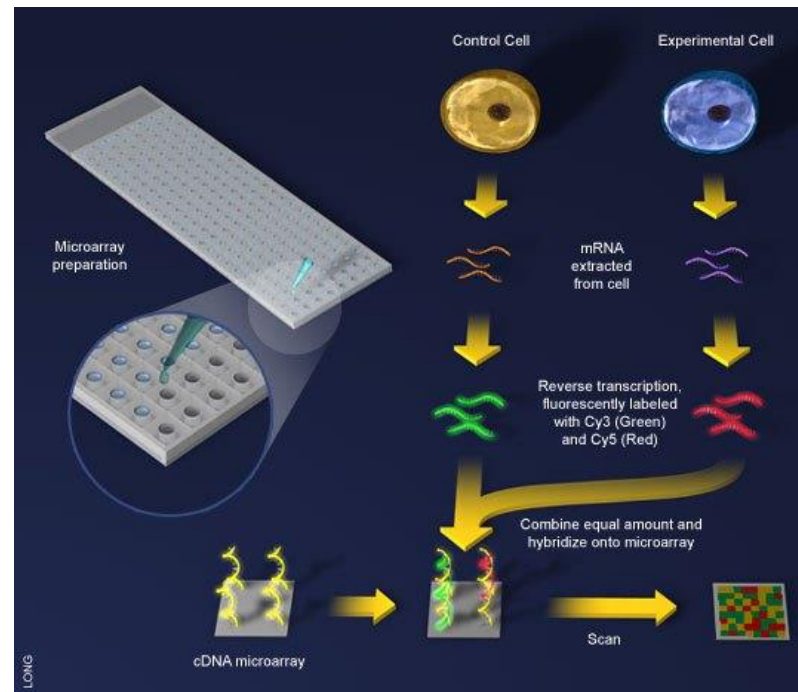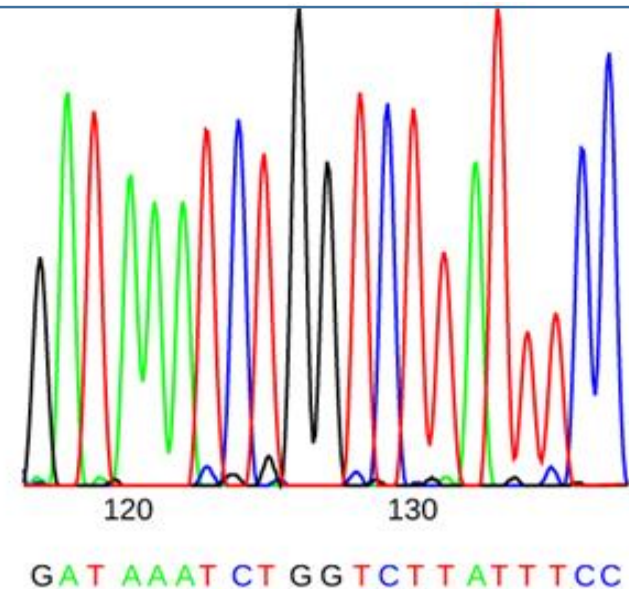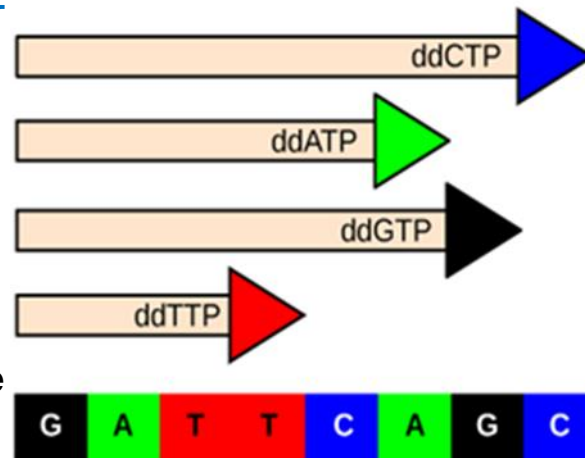
# DIAGNOSTIC TESTS

➢ Evaluation of mutations and epigenetic changes: gene sequencing (Sanger sequencing, Pyrosequencing, Next Generation Sequencing), Beaming.

**SANGER SEQUENCING**

Evaluation of 200-300bp amplicons

Result= presence/absence

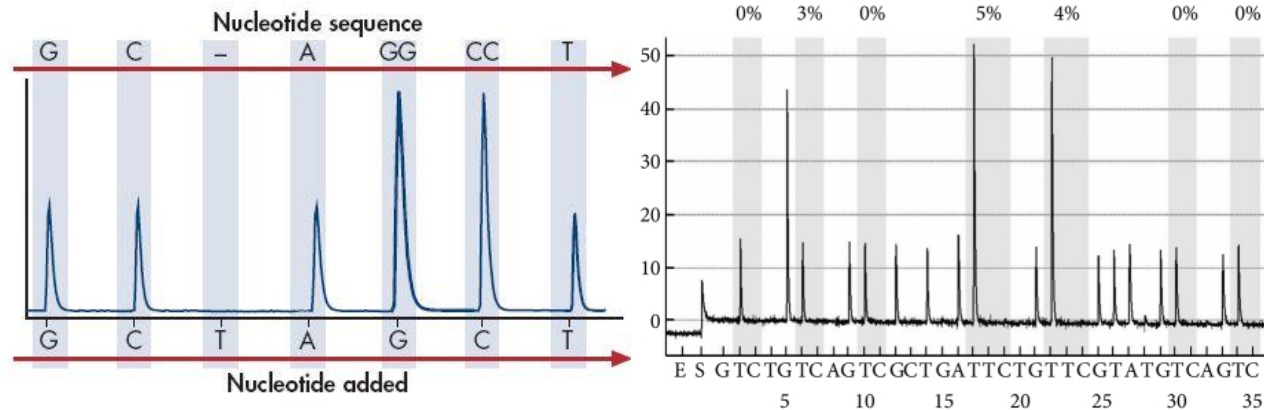Variants determined by the operator

# DIAGNOSTIC TESTS

## PYROSEQUENCING

Evaluation of 100-200bp amplicons

Result= percentage

Variants determined by the operator

# DIAGNOSTIC TESTS

## NGS

Evaluation of the whole genome or exome

Selection of tumour-specific genes

Result= percentage

Variants automatically determined in databases
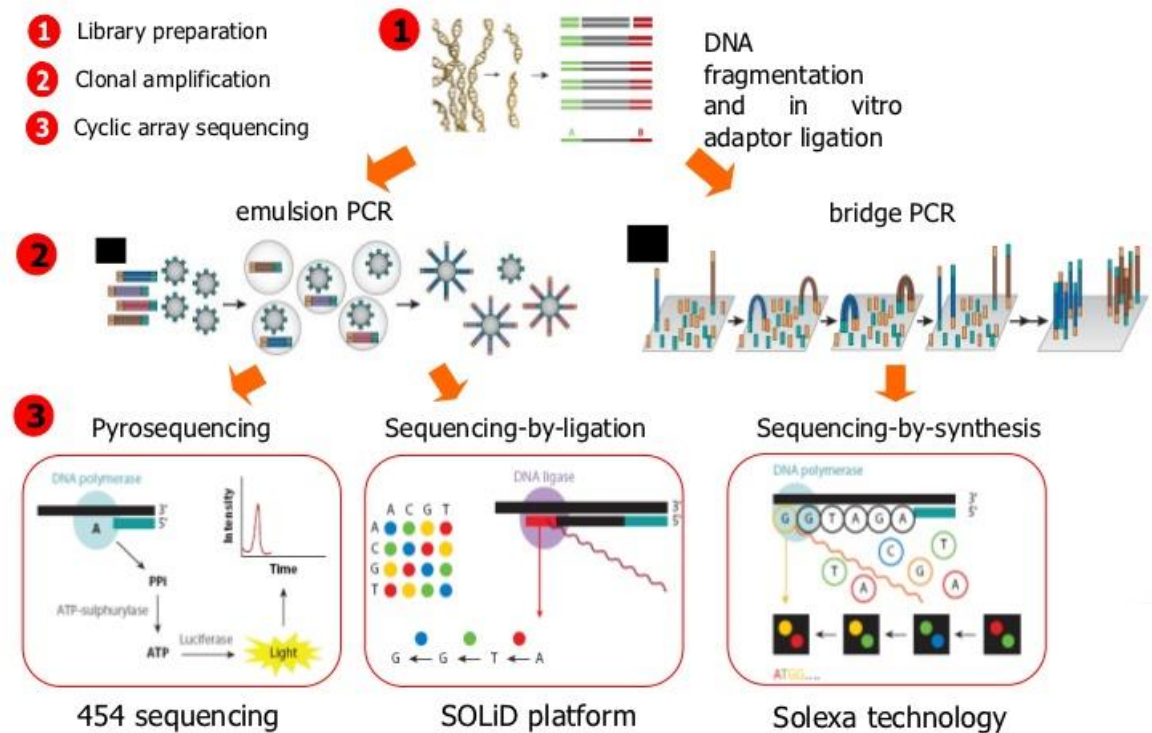
# DIAGNOSTIC TESTS

## BEAMing (performed on cell-free tumour DNA)



Liquid biopsy

- Early detection of cancer (diagnosis)
- Determine risk for metastatic relapse (prognosis)
- Identification of therapeutic targets
- Stratification for treatment decision
- Real-time monitoring of treatment
- Detection of resistance mechanisms
- Early detection of metastatic relapse

CTCs

ctDNA

Gastrointestinal cancer

Precision medicine

**Nature Reviews** | Gastroenterology & Hepatology
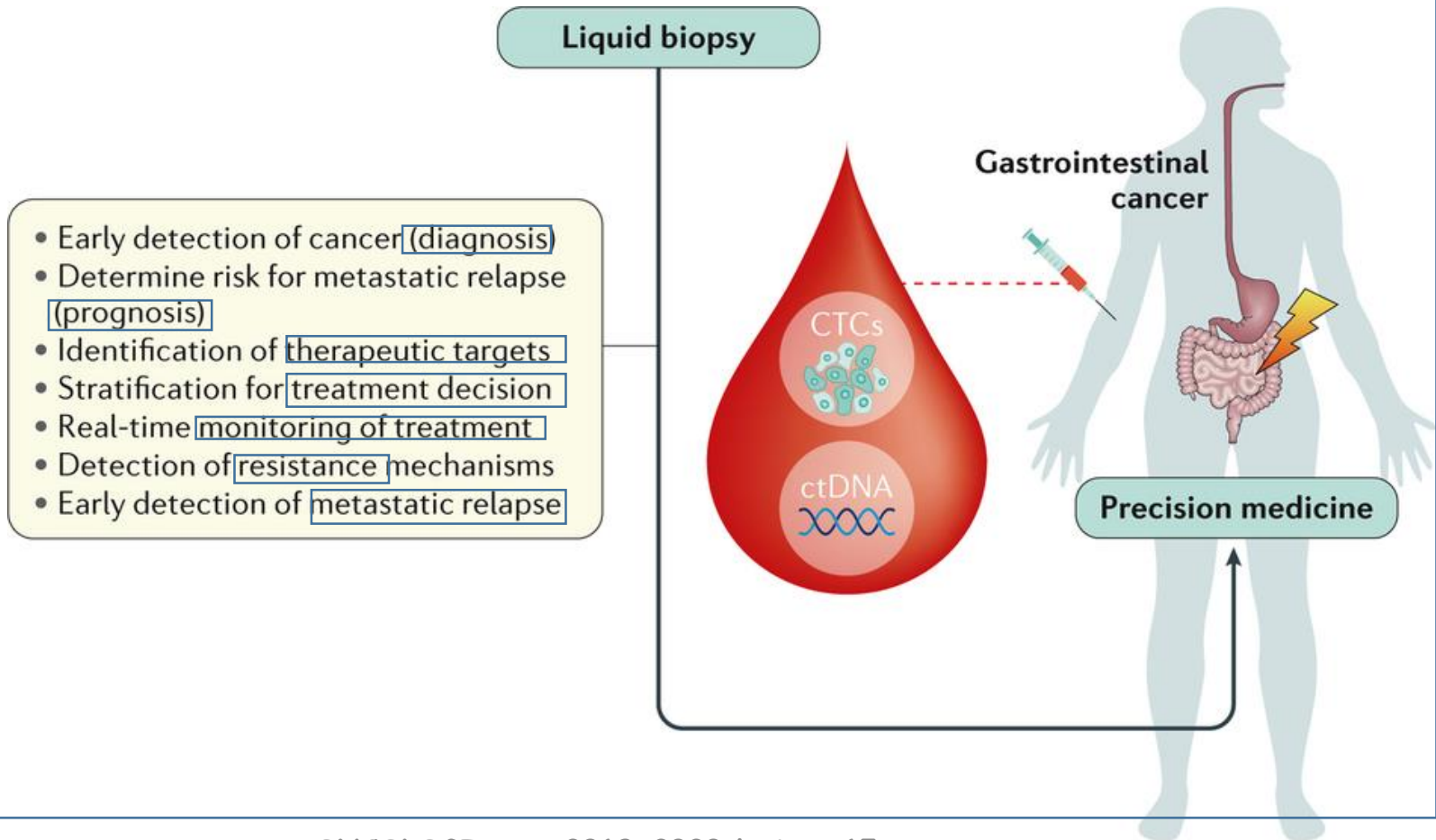
# DIAGNOSTIC TESTS

## BEAMing (performed on cell-free tumour DNA)
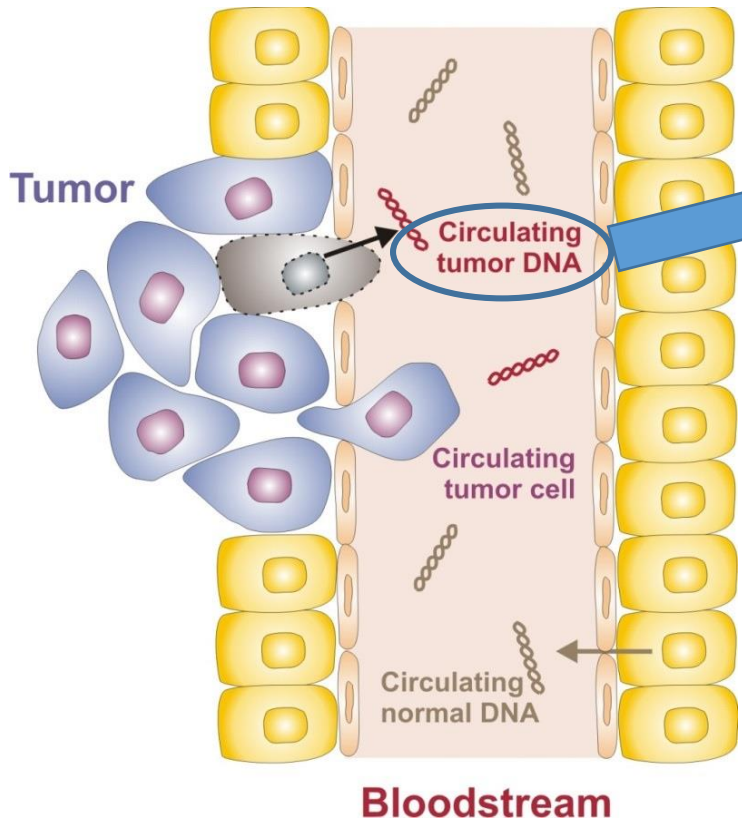


- Released from necrotic and apoptotic tumour cells into the bloodstream
- Short half-life after surgery
- Small DNA fragments (<120 bp)
- Low concentration (0.01% of total circulating DNA)
- More frequent in metastatic patients

Localized (Stages I to III) vs Metastatic (Stage IV) Disease
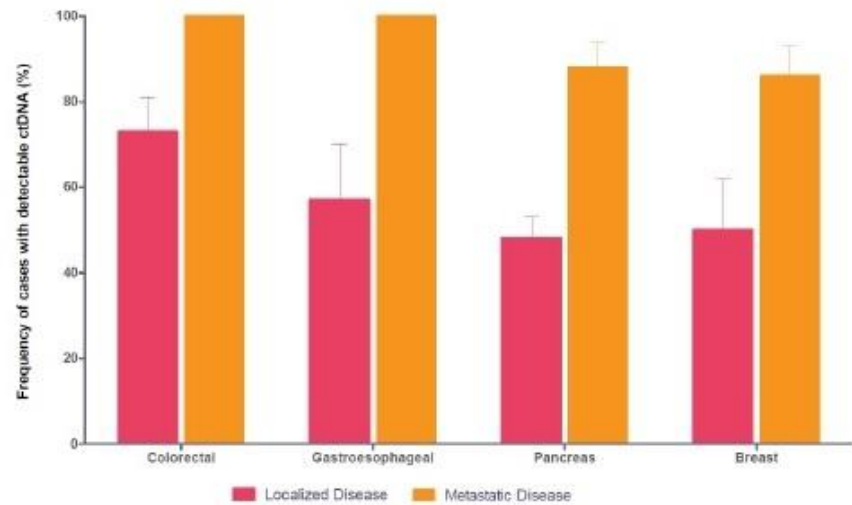


Detectable levels of ctDNA present in 49-78% of patients with localized tumors
Detectable levels of ctDNA present in 86-100% of patients with metastatic tumors

Bettegowda et al, Sci Tran Med Feb 2014

# DIAGNOSTIC TESTS

## BEAMing (performed on cell-free tumour DNA)
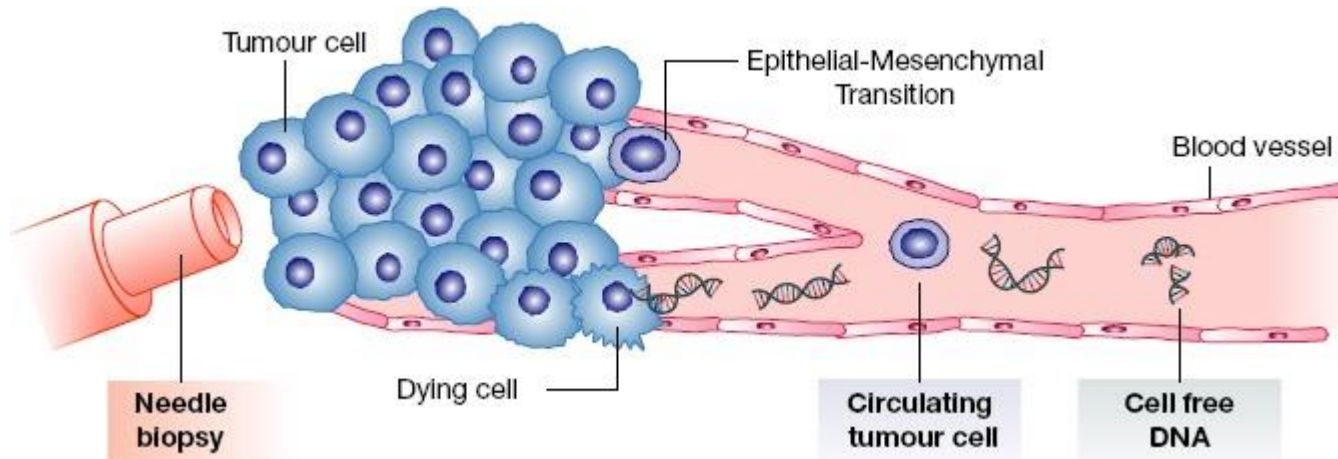


| | Biopsy | CTC | cfDNA |
|---|:---:|:---:|:---:|
| Invasive | + | – | – |
| All patients eligible | – | + | + |
| Instrumentation required | + | + | – |
| WGA required | – | + | +/– |
| RNA profiling | + | + | – |
| Research applicability | +++ | ++ | + |
| Biomarker applicability | – | ++ | +++ |

# DIAGNOSTIC TESTS

## BEAMing (performed on cell-free tumour DNA)



| 1st gene amplification process | Emulsion PCR | Hybridization | Detection |
|---|---|---|---|
| Amplification of the target DNA area | Gene amplification within a droplet emulsion containing one DNA molecule and magnetic bead by PCR. | Forming of a complementary conjugate with fluorescent probe of a gene and a magnetic bead. | Detecting mutations of the cancer gene by FCM. |

DNA extraction

Primer coat
Magnetic bead

Amplified DNA

Tumor DNA
Wild-type DNA

Deoxy nucleotide
tri-phosphate
(dNTP) polymerase
primer

Droplet
emulsion*

sysmex

# DIAGNOSTIC TESTS

BEAMing (performed on cell-free tumour DNA)

DISEASE CLASSIFICATION is central to <u>understand</u> the bases of the diseases, make <u>diagnosis</u> and assign <u>treatment</u>.
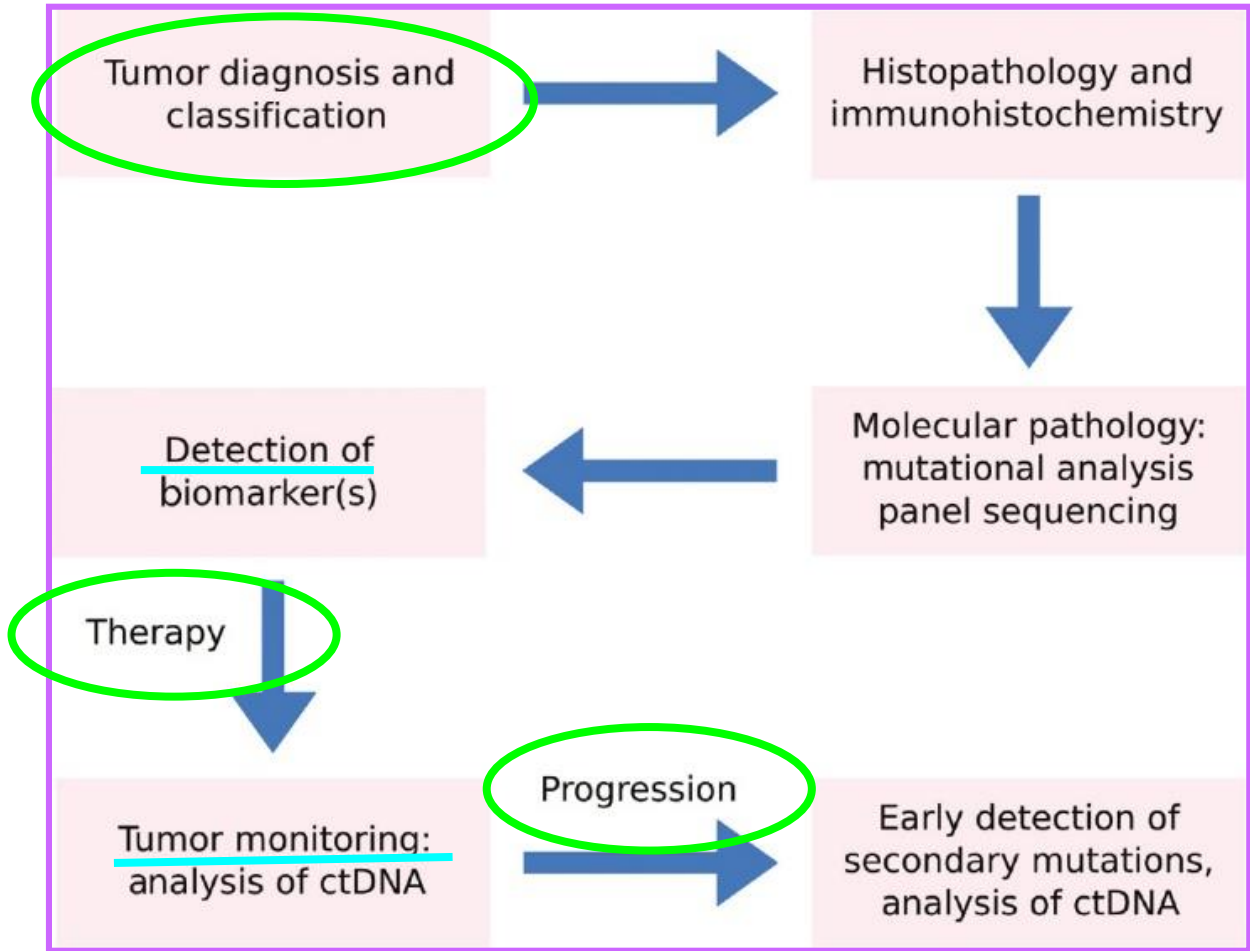
Cancer nomenclature is based on:
1. <u>Localization</u> (breast cancer, lung cancer…..)

1. Within each organ-specific major type several <u>subgroups</u> are defined, taking into account cell type, histological grades and MOLECULAR MARKERS

## cTNM (CLINICAL)

Essential to select and evaluate therapeutic options
Defined before treatment
Based on evidences aquired by clinical examination, imaging, endoscopy, biopsy....

**+**

## pTNM (PATHOLOGICAL)

Assessed after surgery
Essential to guide adjuvant therapy
Provides data useful for prognosis estimation

**+**

## G (HISTOPATHOLOGICAL GRADING)

**=**

## TNM STAGING

**Tumor grade:** description of a tumor based on how abnormal the tumor cells and the tumor tissue look under a microscope.
It is an indicator of how quickly a tumor is likely to grow and spread.

If a grading system for a tumor type is not specified, the following system is generally used:

GX: Grade cannot be assessed (undetermined grade)
G1: Well differentiated (low grade)
G2: Moderately differentiated (intermediate grade)
G3: Poorly differentiated (high grade)
G4: Undifferentiated (high grade)

# BIOMOLECULAR STAGING

➢ Identification of <u>tumour markers</u> involved in different processes that lead to tumour progression.

➢ Better <u>patients' stratification</u> into TNM staging-defined risk groups.

➢ Potentially applicable to: primary tumour, lymphnodes, bone marrow, serum.

➢ Useful for: <u>early diagnosis, prognosis estimation, occulte metastases identification, predictive markers for chemotherapy resistence or response</u>.

➢ Panels of biomarkers depending on the tumour type.

# HISTOLOGICAL CLASSIFICATION



Histological classification of breast cancers
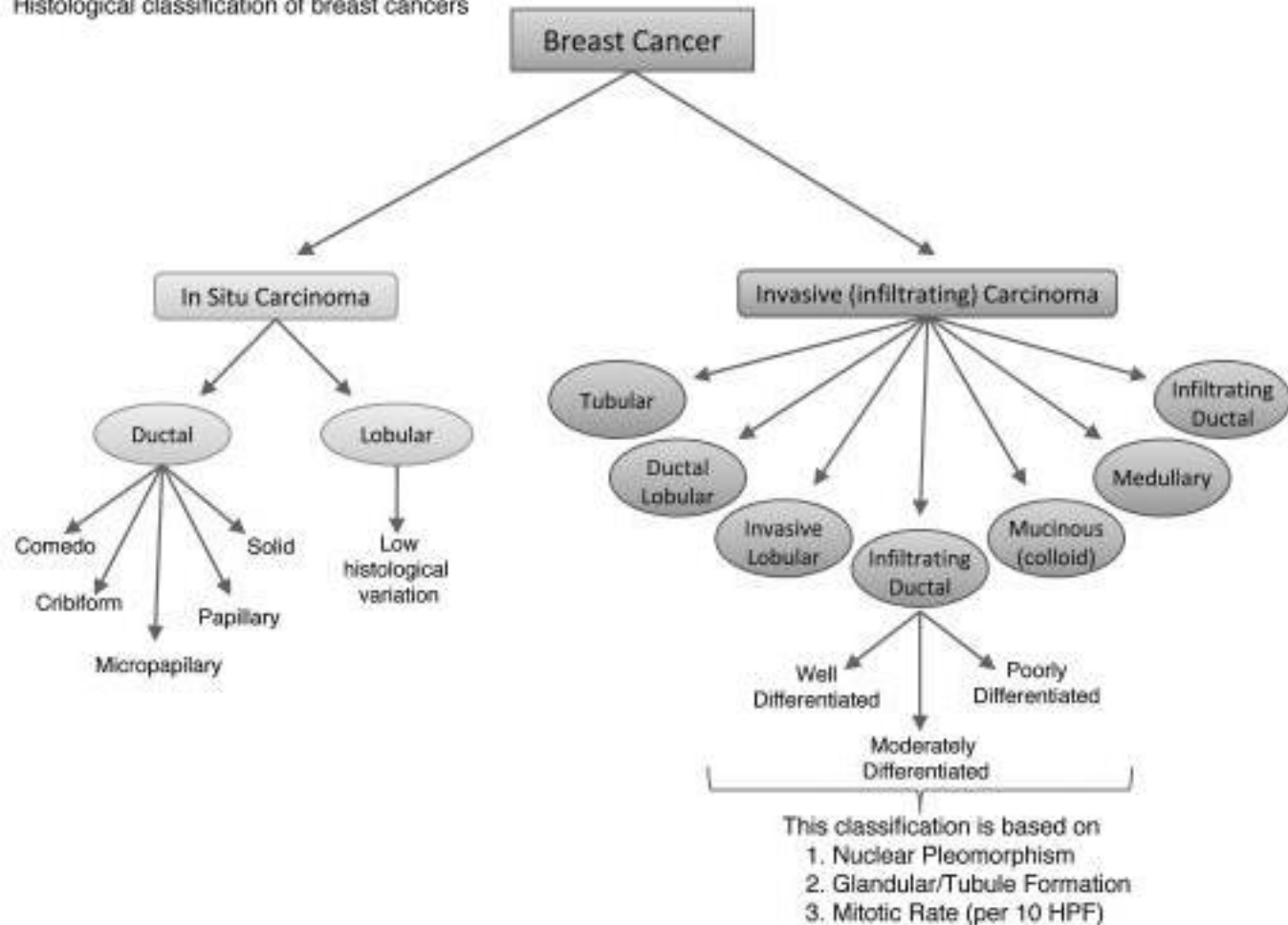
# TNM CLASSIFICATION

| ANATOMIC STAGE/PROGNOSTIC GROUPS | | | |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage IA | T1* | N0 | M0 |
| Stage IB | T0 | N1mi | M0 |
| | T1* | N1mi | M0 |
| Stage IIA | T0 | N1** | M0 |
| | T1* | N1** | M0 |
| | T2 | N0 | M0 |
| Stage IIB | T2 | N1 | M0 |
| | T3 | N0 | M0 |
| Stage IIIA | T0 | N2 | M0 |
| | T1* | N2 | M0 |
| | T2 | N2 | M0 |
| | T3 | N1 | M0 |
| | T3 | N2 | M0 |
| Stage IIIB | T4 | N0 | M0 |
| | T4 | N1 | M0 |
| | T4 | N2 | M0 |
| Stage IIIC | Any T | N3 | M0 |
| Stage IV | Any T | Any N | M1 |

The **One-Step Nucleic acid Amplification (OSNA)** assay is a molecular procedure that can identify deposits of breast cancer cells in the sentinel lymph node.
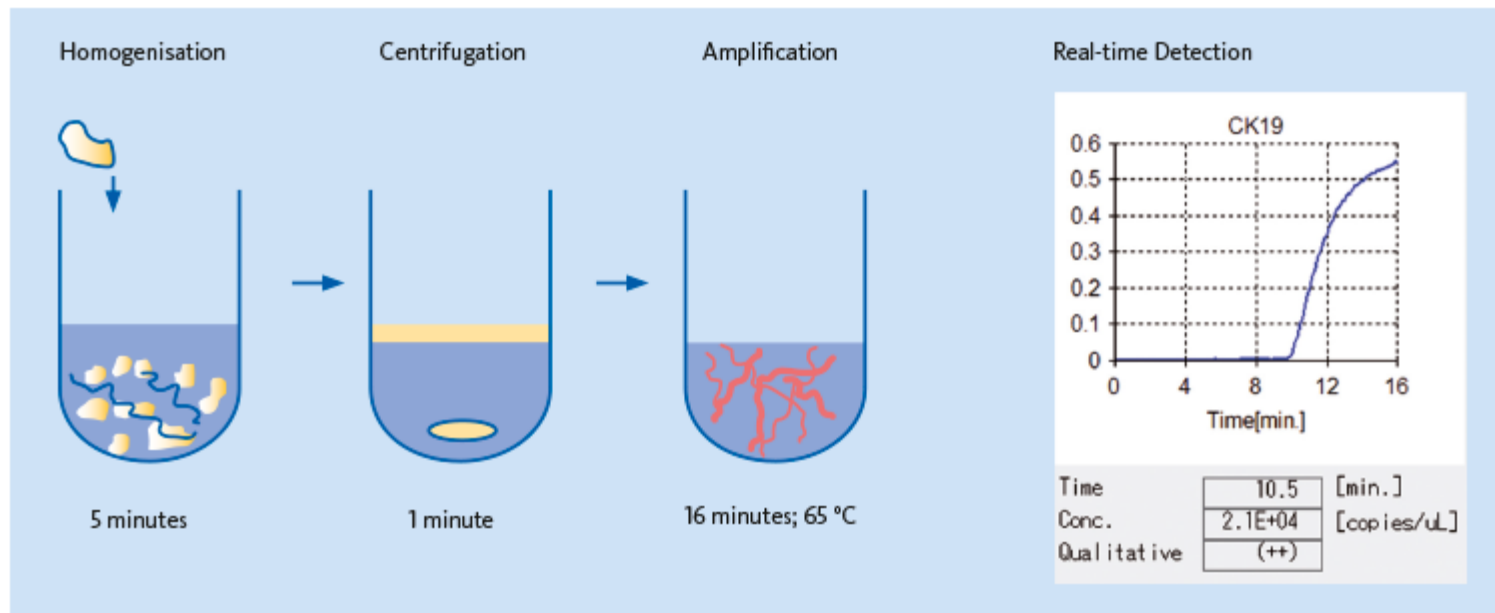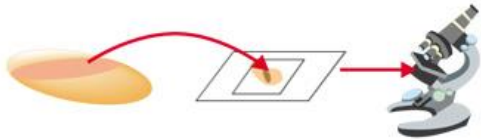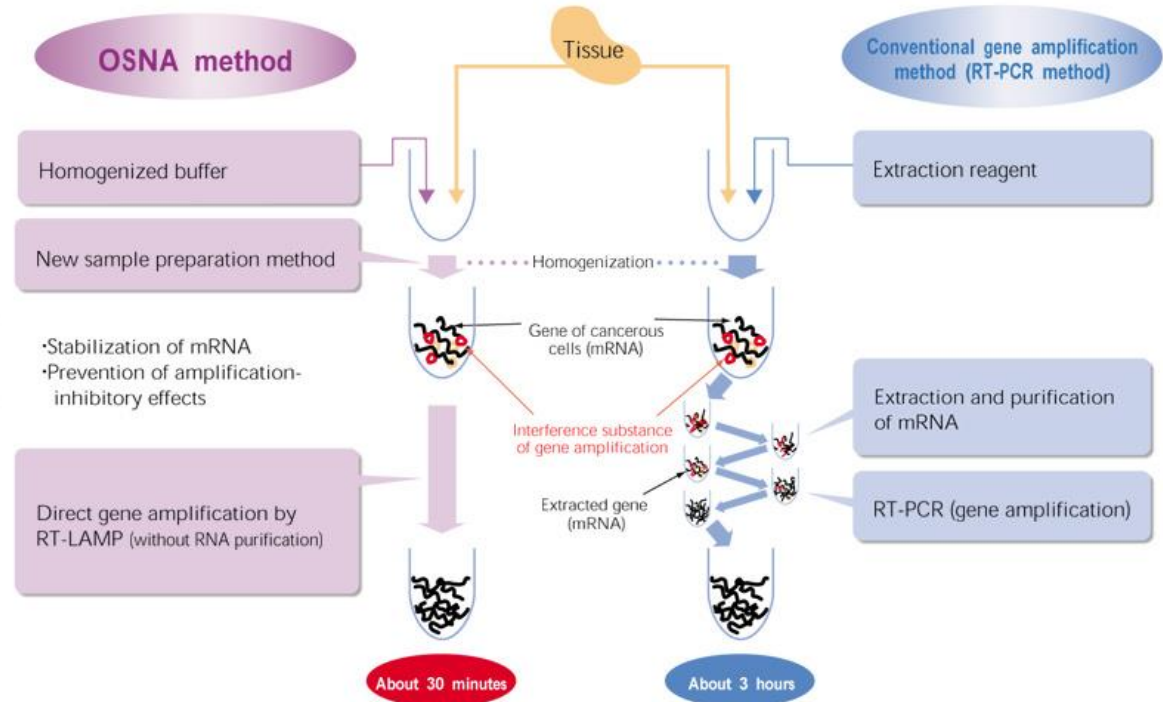
# MOLECULAR CLASSIFICATION

# MOLECULAR CLASSIFICATION

| Molecular subtypes | Triple negative ER−, PR−, HER2− | HER2+ | Luminal B | Luminal A |
|---|---|---|---|---|
| % of breast cancers | 15-20% | 10-15% | 20% | 40% |
| Receptor expression | | HER2 | | ER+/PR+ |
| Histologic grade Level of cell differentiation | High (grade III) | | | Low (grade I) |
| Prognosis Correlates to histologic grade | Poor | | | Good |
| Response to medical therapy | Chemotherapy | Trastuzumab | | Endocrine |
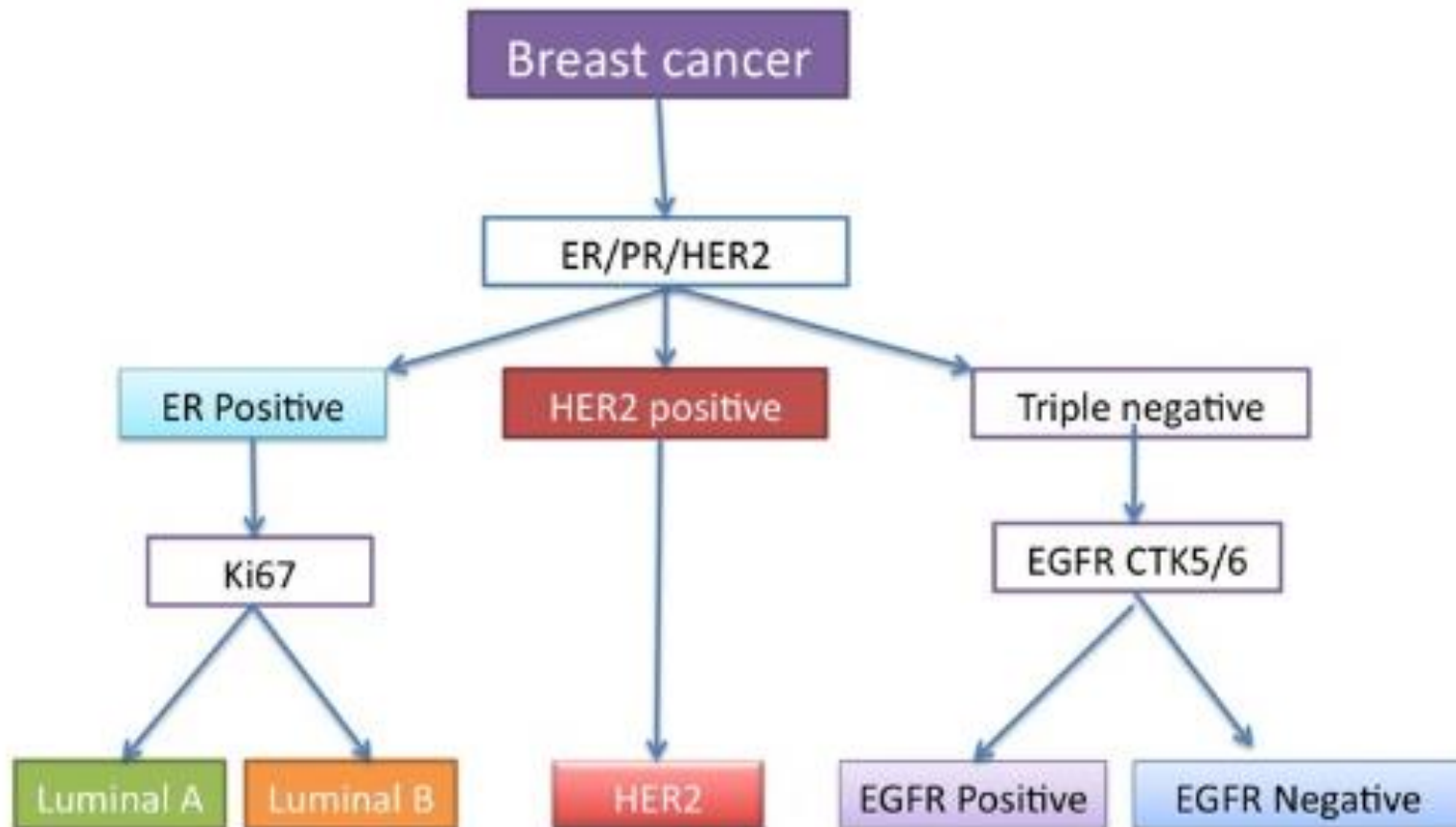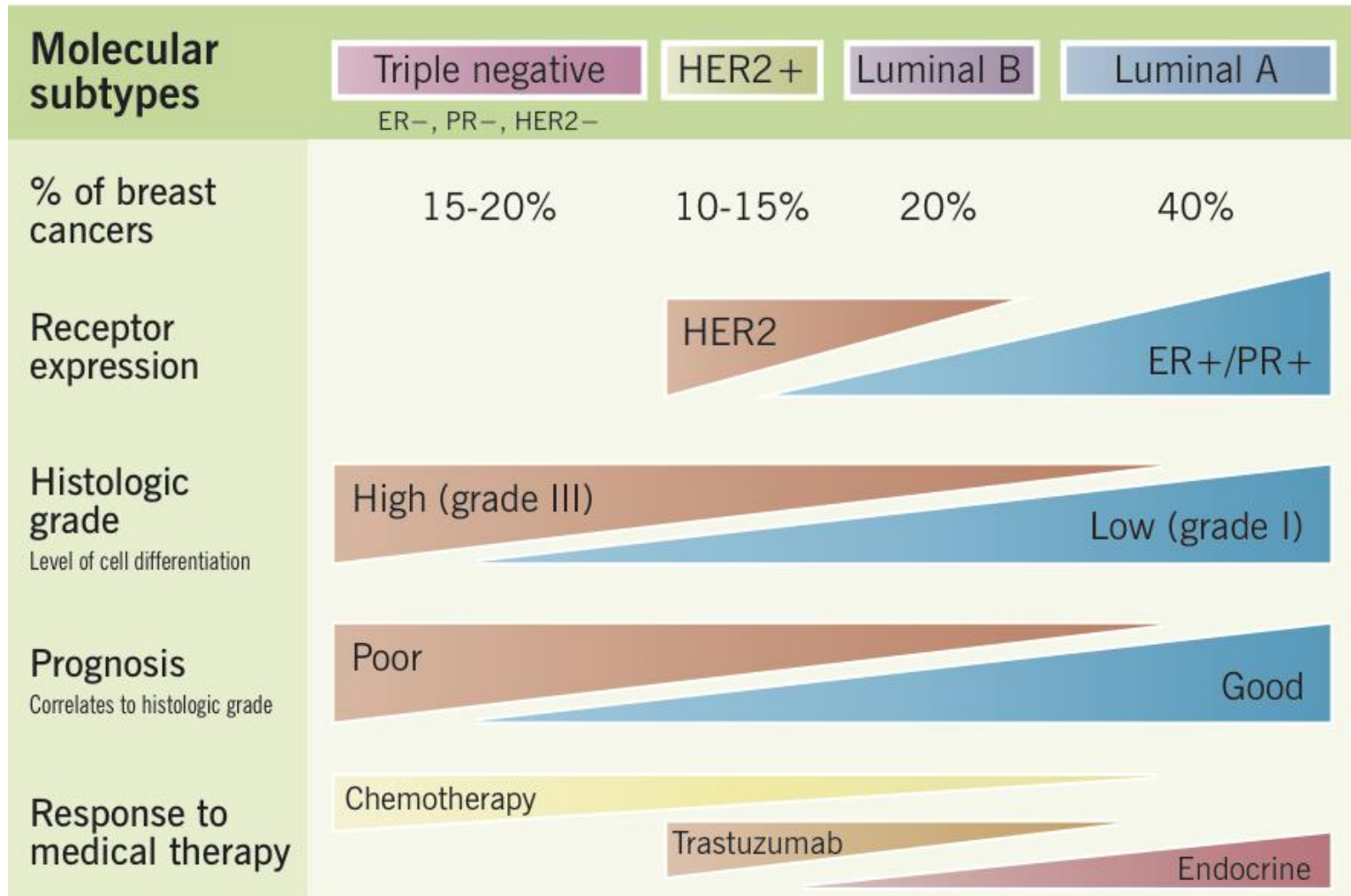
Triple negative tumours respond best to chemotherapy, similar to other aggressive cancers.

Luminal A tumours respond best to endocrine therapy, e.g. antiestrogen or aromatase inhibitor.

# MOLECULAR DIAGNOSTICS TESTS

## MAMMAPRINT®

Stage 1 and 2 Breast Cancer, node negative

10% chance of recurrence within 10 years with no treatment
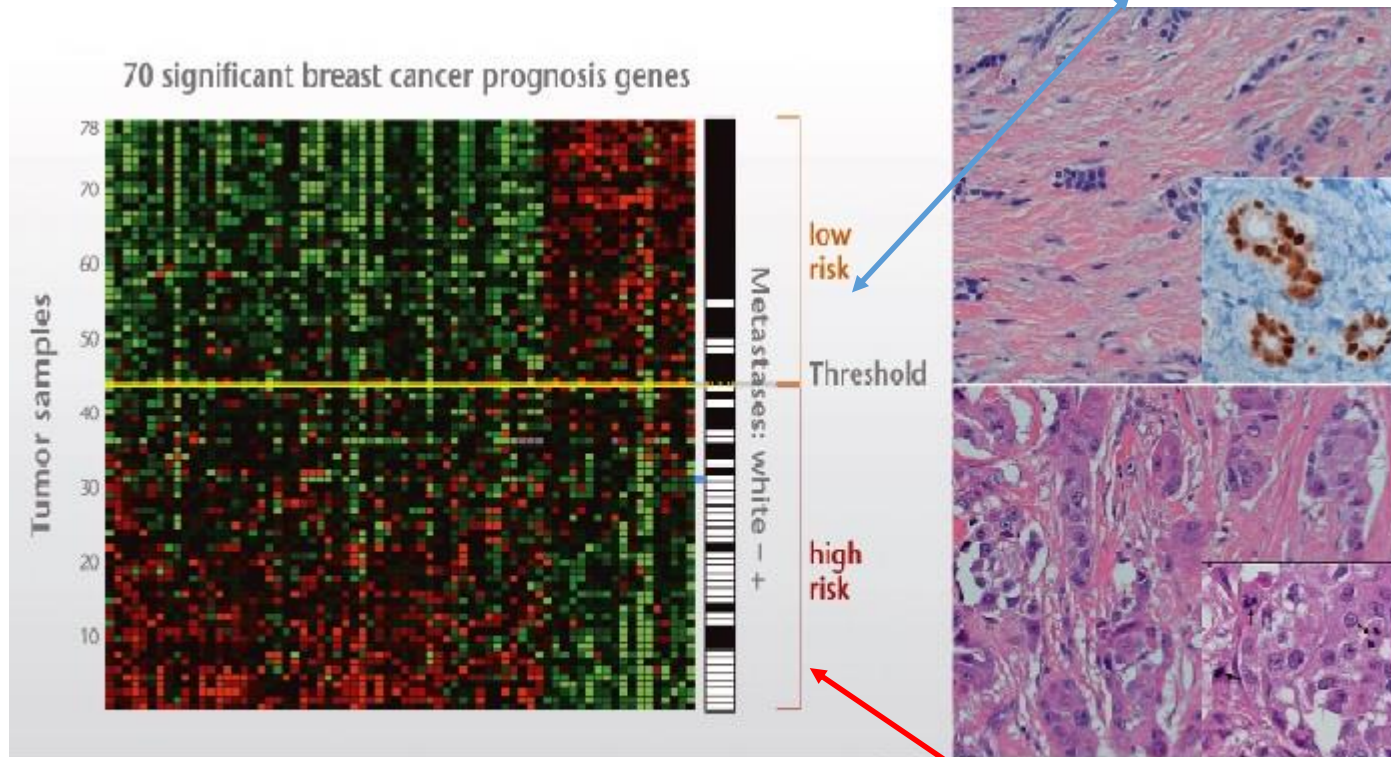


29% chance of recurrence within 10 years with no treatment

# MOLECULAR DIAGNOSTICS TESTS

## ONCOTYPE DX®

Stage 1 and 2 Breast Cancer, node negative, ER+; expression of 21 genes (16 genes known to be related with breast cancer and 5 reference genes)

| Proliferation | HER2 | Estrogen |
|---|---|---|
| Ki67 | GRB7 | ER |
| STK15 | HER2 | PGR |
| Survivin | | BCL2 |
| CCNB1 (cyclin B1) | | SCUBE2 |
| MYBL2 | GSTM1 | |

| Invasion | CD68 | Reference |
|---|---|---|
| MMP11 (stromolysin 3) | | ACTB (β-actin) |
| CTSL2 (cathepsin L2) | | GAPDH |
| | BAG1 | RPLPO |
| | | GUS |
| | | TFRC |
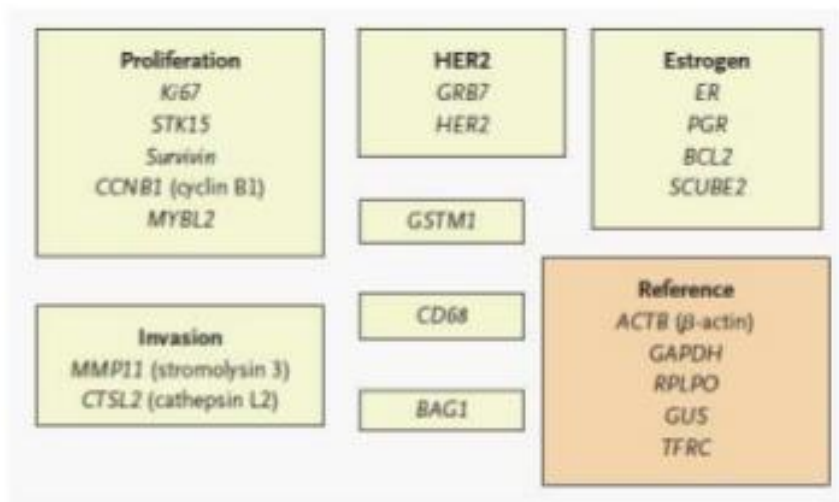
- Quantifies the standard pathologic characterization
- Complex algorithm that adds the HER2, proliferation, and invasion scores, and subtracts the estrogen score in a weighted fashion

- Reported as a Recurrence Score (RS)
- RS < 18 = low risk
- 18 ≤ RS < 31 = intermediate risk
- RS ≥ 31 = high risk

Paik et al. *NEJM*, 2004

# MOLECULAR DIAGNOSTICS TESTS

## PROSIGNA®



Compare patient profile to intrinsic subtypes[5,6]
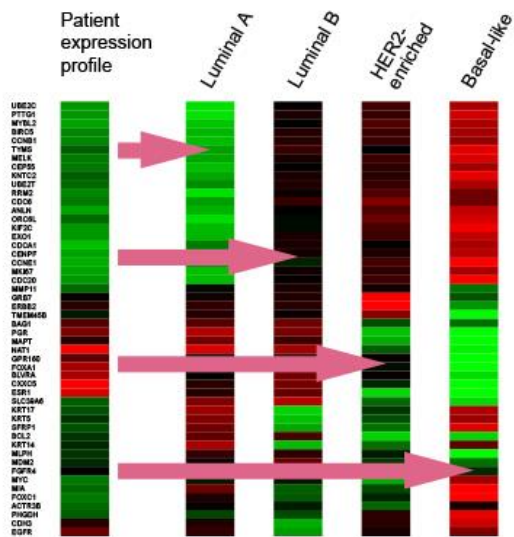
Adapted from Nielsen T et al, 2013, and Gnant M et al, 2012.

The end result is the Risk of Recurrence (ROR, 0-100) estimating the risk of relapse within 10 years.

ROR is calculated taking into account the PAM50 gene signature, intrinsic subtype, tumour size, nodal status, and proliferation score.



Development of Prosigna™ is Based on PAM50 Gene Signature

| 2000 | 2009 | 2010 | 2012/13 |
|------|------|------|---------|
| Researchers first describe breast cancer intrinsic subtypes based on microarray experiments | Researchers first describe "PAM50" gene expression signature | NanoString exclusively licenses PAM50 gene expression signature | Prosigna launches after receiving CE Mark for Europe & Israel; FDA 510k clearance in US |
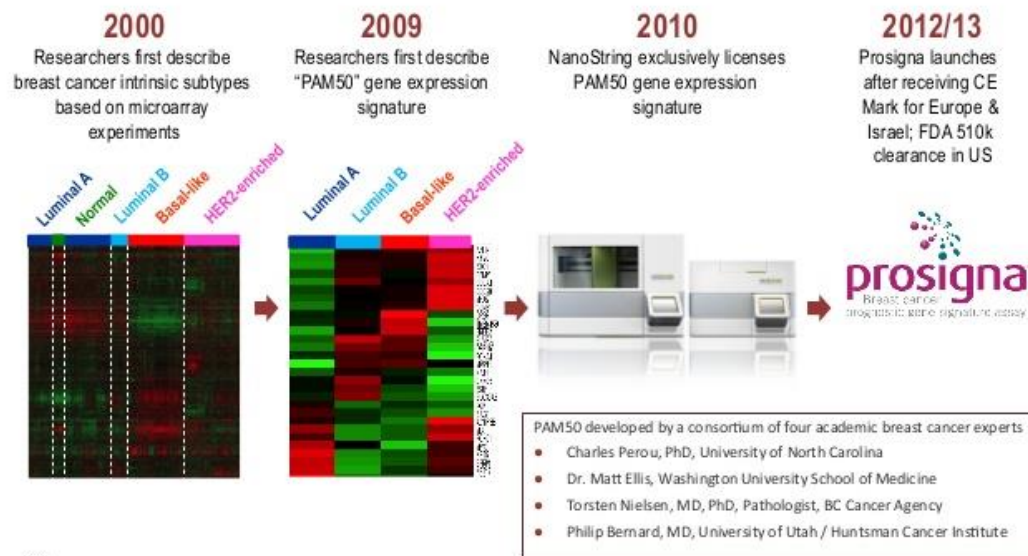
PAM50 developed by a consortium of four academic breast cancer experts
- Charles Perou, PhD, University of North Carolina
- Dr. Matt Ellis, Washington University School of Medicine
- Torsten Nielsen, MD, PhD, Pathologist, BC Cancer Agency
- Philip Bernard, MD, University of Utah / Huntsman Cancer Institute

12 *Source: Molecular portraits of breast cancer. Nature. 2000 May 25;.*
*Source: Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes, JCO.2009*

# HISTOLOGICAL CLASSIFICATION

- **Adenocarcinoma (85%)**
- **Mucinous adenocarcinoma (10%)**
- **Signet-ring cell carcinoma**
- **Medullary carcinoma**
- **Undifferentiated carcinoma**
- **Small cell carcinoma**
- **Adenosquamous carcinoma**
- **Squamous carcinoma**

# CLASSIFICATION
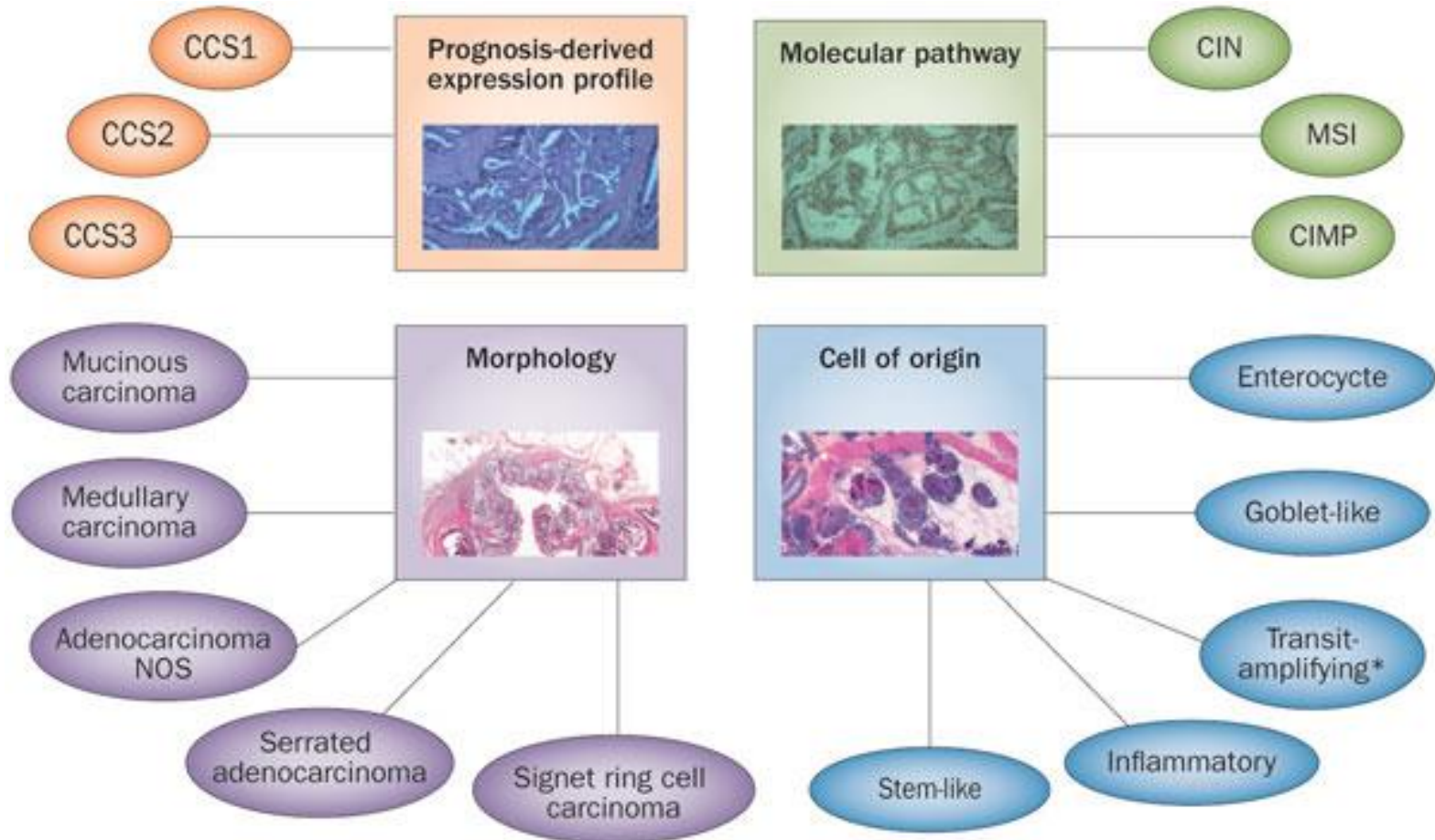
# TNM CLASSIFICATION   Colorectal Cancer

| ANATOMIC STAGE/PROGNOSTIC GROUPS | | | | | |
|---|---|---|---|---|---|
| Stage | T | N | M | Dukes* | MAC* |
| 0 | Tis | N0 | M0 | – | – |
| I | T1 | N0 | M0 | A | A |
| | T2 | N0 | M0 | A | B1 |
| IIA | T3 | N0 | M0 | B | B2 |
| IIB | T4a | N0 | M0 | B | B2 |
| IIC | T4b | N0 | M0 | B | B3 |
| IIIA | T1–T2 | N1/N1c | M0 | C | C1 |
| | T1 | N2a | M0 | C | C1 |
| IIIB | T3–T4a | N1/N1c | M0 | C | C2 |
| | T2–T3 | N2a | M0 | C | C1/C2 |
| | T1–T2 | N2b | M0 | C | C1 |
| IIIC | T4a | N2a | M0 | C | C2 |
| | T3–T4a | N2b | M0 | C | C2 |
| | T4b | N1–N2 | M0 | C | C3 |
| IVA | Any T | Any N | M1a | – | – |
| IVB | Any T | Any N | M1b | – | – |

NOTE: cTNM is the clinical classification, pTNM is the pathologic classification. The y prefix is used for those cancers that are classified after neoadjuvant pretreatment (for example, ypTNM). Patients who have a complete pathologic response are ypT0N0cM0 that may be similar to Stage Group 0 or I. The r prefix is to be used for those cancers that have recurred after a disease-free interval (rTNM).
* Dukes B is a composite of better (T3 N0 M0) and worse (T4 N0 M0) prognostic groups, as is Dukes C (any TN1 M0 and Any T N2 M0). MAC is the modified Astler-Coller classification.

Up to 30% of all patients classified in stage II suffer from local recurrence or distant metastases within 5 years of undergoing surgery, leading to significantly poorer survival rates.
These patients are classified in a lower lymph node status (false-negative rates up to 24%), which impacts on the decisions made concerning their further therapy options.

OSNA® allows the investigation of the entire lymph node and its results are comparable with ultra-staging (IHC). Studies have shown that a lymph node analysis of pN0 patients with OSNA® yielded an upstaging rate of approximately 26%, compared with the standard histological test method. These patients' therapies could thus be adjusted accordingly.

Parts of lymph nodes are surgically sampled.

Lymph node

OSNA stands for One-step Nucleic Acid Amplification. With the OSNA method, sample lymph nodes are dissolved, and cancer genes contained in the samples are amplified and detected to determine cancer lymph node metastasis. This is a one-step testing method.

[Features]
- Results are rapidly obtained.
- Automated diagnosis eliminates accuracy disparities among medical institutions.

**Testing with the OSNA method**

Measurement by a proprietary instrument and reagent are used to amplify the cancer cell genes for measurement.

Solubilization

One-step amplification detection of the target gene and judgment of the results

Diagnosis → Approx. 1 day

Preparation of samples

Confirmation using a microscope

Diagnosis → Approx. 1 week

Confirmation of the presence of cancer cells by a pathologist using a microscope

**Conventional pathological diagnosis**

sysmex
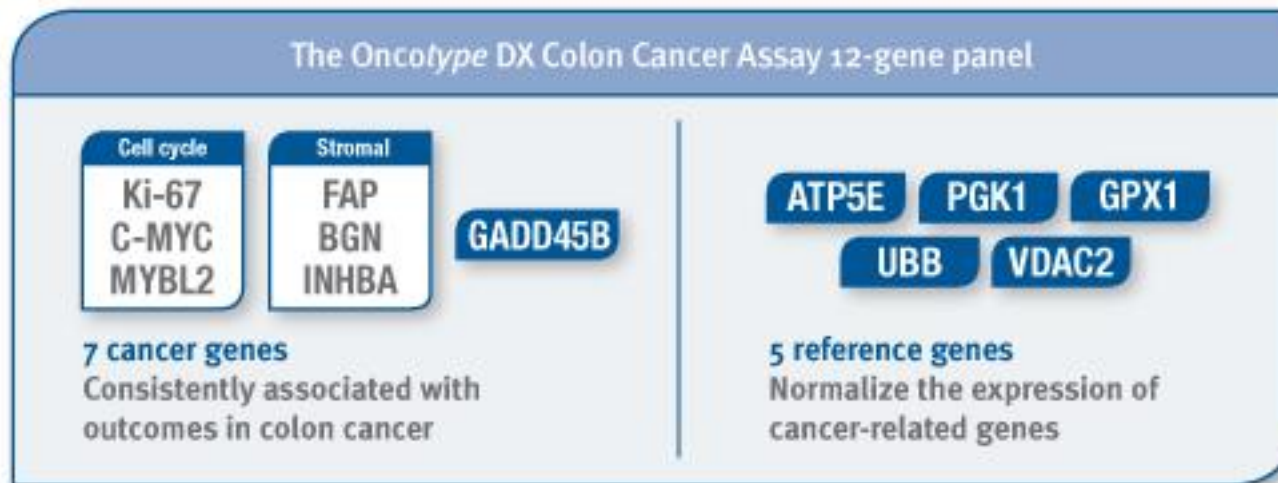
Colon Cancer

# MOLECULAR CLASSIFICATION

- *k-ras* mutations
- *P53* mutations
- LOH 17p (p53)
- LOH 18q (dcc)
- Microsatellite instability (MMR)
- DNA methylation
- Altered expression of TGFb
- *Apc* mutation/loss

# MOLECULAR DIAGNOSTICS TESTS

**ONCOTYPE DX COLON®**

Stage 2 Colon Cancer; expression of 12 genes
(7 genes known to be related with colon cancer and 5 reference genes)



The Oncotype DX Colon Cancer Assay 12-gene panel

**Cell cycle**
Ki-67
C-MYC
MYBL2

**Stromal**
FAP
BGN
INHBA

GADD45B

**7 cancer genes**
Consistently associated with outcomes in colon cancer

ATP5E    PGK1    GPX1
UBB    VDAC2

**5 reference genes**
Normalize the expression of cancer-related genes

The end result of the testing is a Recurrence Score (0-100) indicating the risk of recurrence in the three years after surgery.

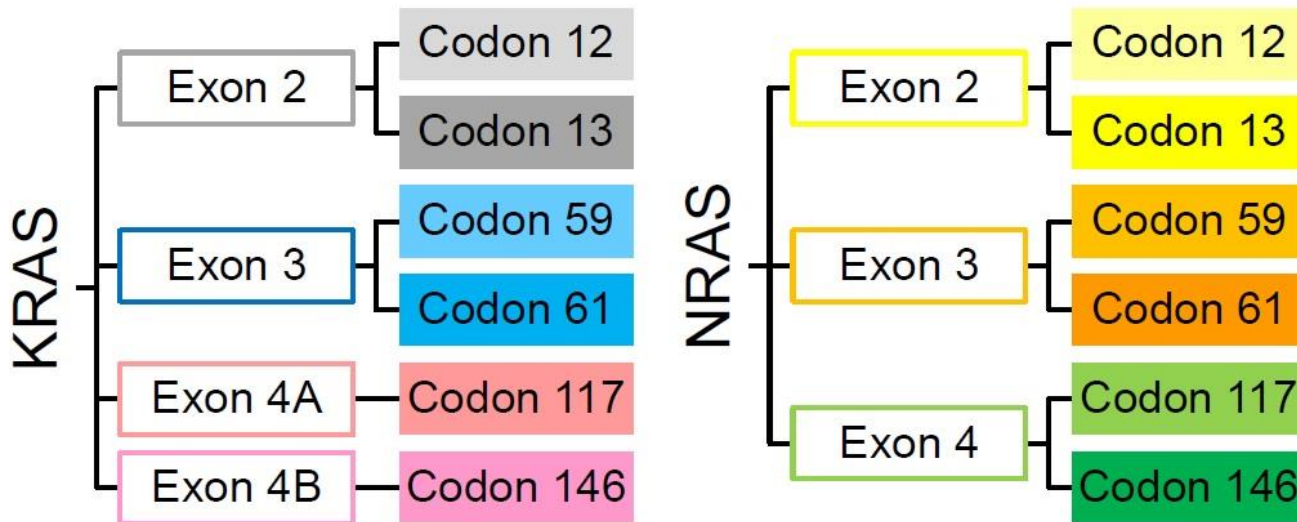The test has been validated but it's not currently included in standard clinical practice.

Colon Cancer

# MOLECULAR DIAGNOSTICS TESTS

## ONCOBEAM™ RAS CRC ASSAY

Stage 4 Colon Cancer; evaluation of *K-* and *N-RAS* mutations in specific codons

**KRAS**

Exon 2 → Codon 12, Codon 13

Exon 3 → Codon 59, Codon 61

Exon 4A → Codon 117

Exon 4B → Codon 146

**NRAS**

Exon 2 → Codon 12, Codon 13

Exon 3 → Codon 59, Codon 61

Exon 4 → Codon 117, Codon 146

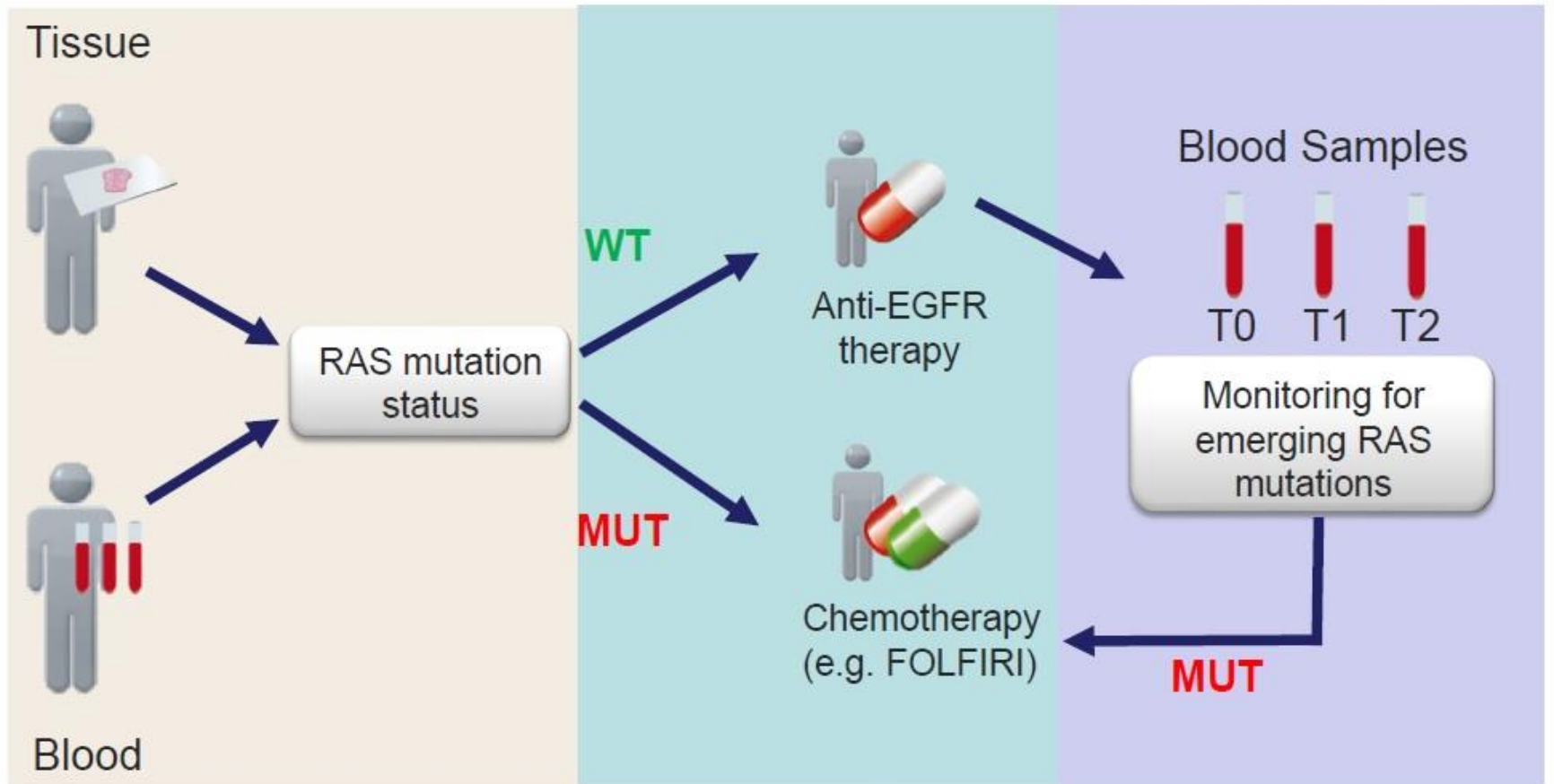The end result of the testing is the mutational status of *K-* and *N-RAS* in plasma (ctDNA).

The test has been validated but it's not included in standard clinical practice yet.

# MOLECULAR DIAGNOSTICS TESTS

**ONCOBEAM™ RAS CRC ASSAY**



Example: Blood-based RAS testing for colorectal cancer

HISTOLOGICAL CLASSIFICATION

Clinico-Pathological Staging (TNM, Dukes....)

**+**

BIOMOLECULAR CLASSIFICATION

Tumor with Favorable Prognosis

RNA Extraction and Labeling

Tumor with Unfavorable Prognosis

Hybridization to High-Density Arrays

Multiple Genes

Tumors with Favorable Prognosis

Tumors with Unfavorable Prognosis

Clustering Analysis

CLUSTER OF MOLECULAR MARKERS PREDICTIVE OF CLINICAL OUTCOME

a Whole-genome analysis

b Generate signature of genes that correlate with clinical parameter

c Apply to independent validation cohorts

d Evaluate clinical performance

Survival probability (%)

ColoPrint
— 1
---- 0

Time

e Regulatory approval

U.S. Food and Drug Administration
CENTER FOR DEVICES AND RADIOLOGICAL HEALTH

f Clinical implementation

ONCOLOGIA AA 2019-2020 lezione 15