I SIMPOSIO NACIONAL
de ONCOLOGÍA de PRECISIÓN

Vigo, del 28 de febrero al 1 de marzo de 2019

Biomarcadores: lo que ya tenemos

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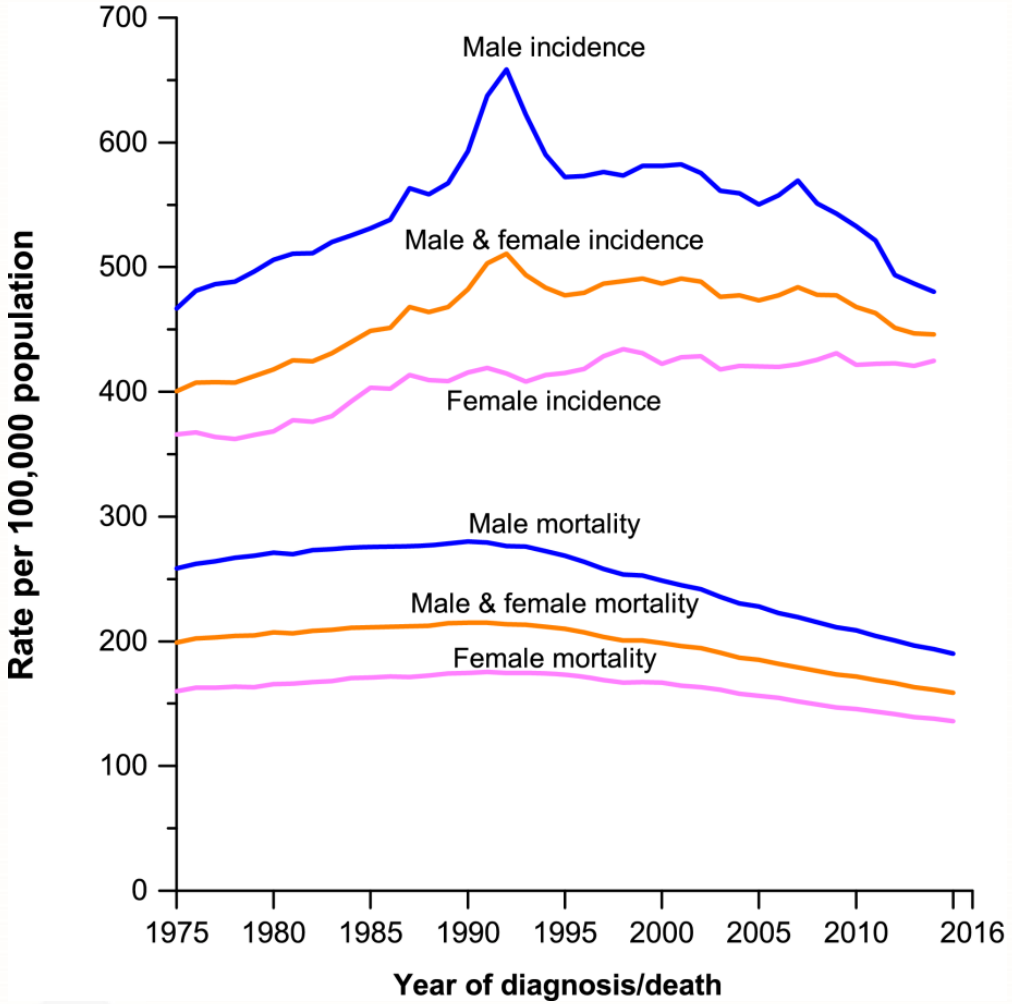
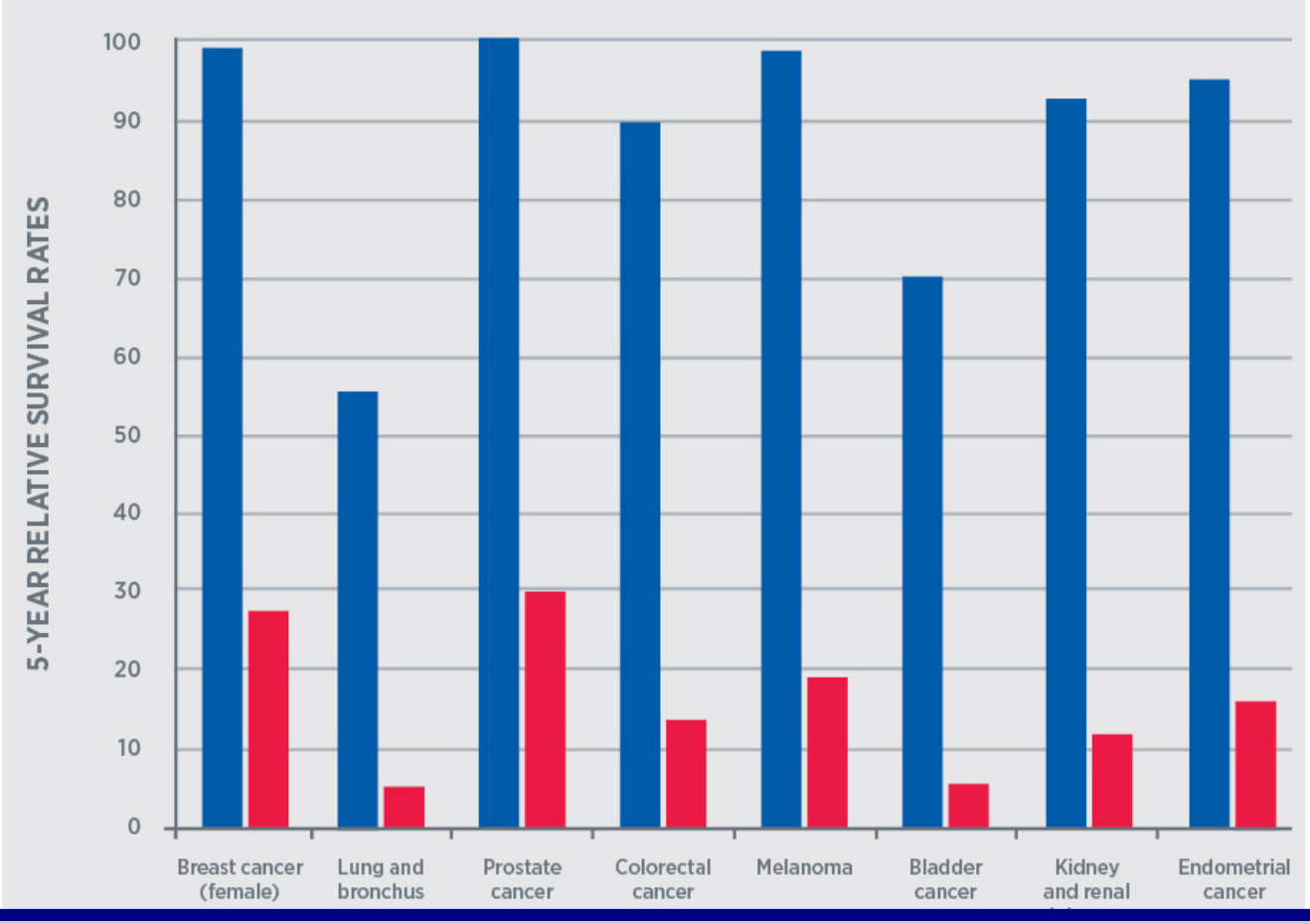
índice

1. Donde estamos: premisas básicas
2. Situación actual Biomarcadores (mama, pulmón, colon, otros)
3. Pitfalls en diagnóstico
4. Donde vamos



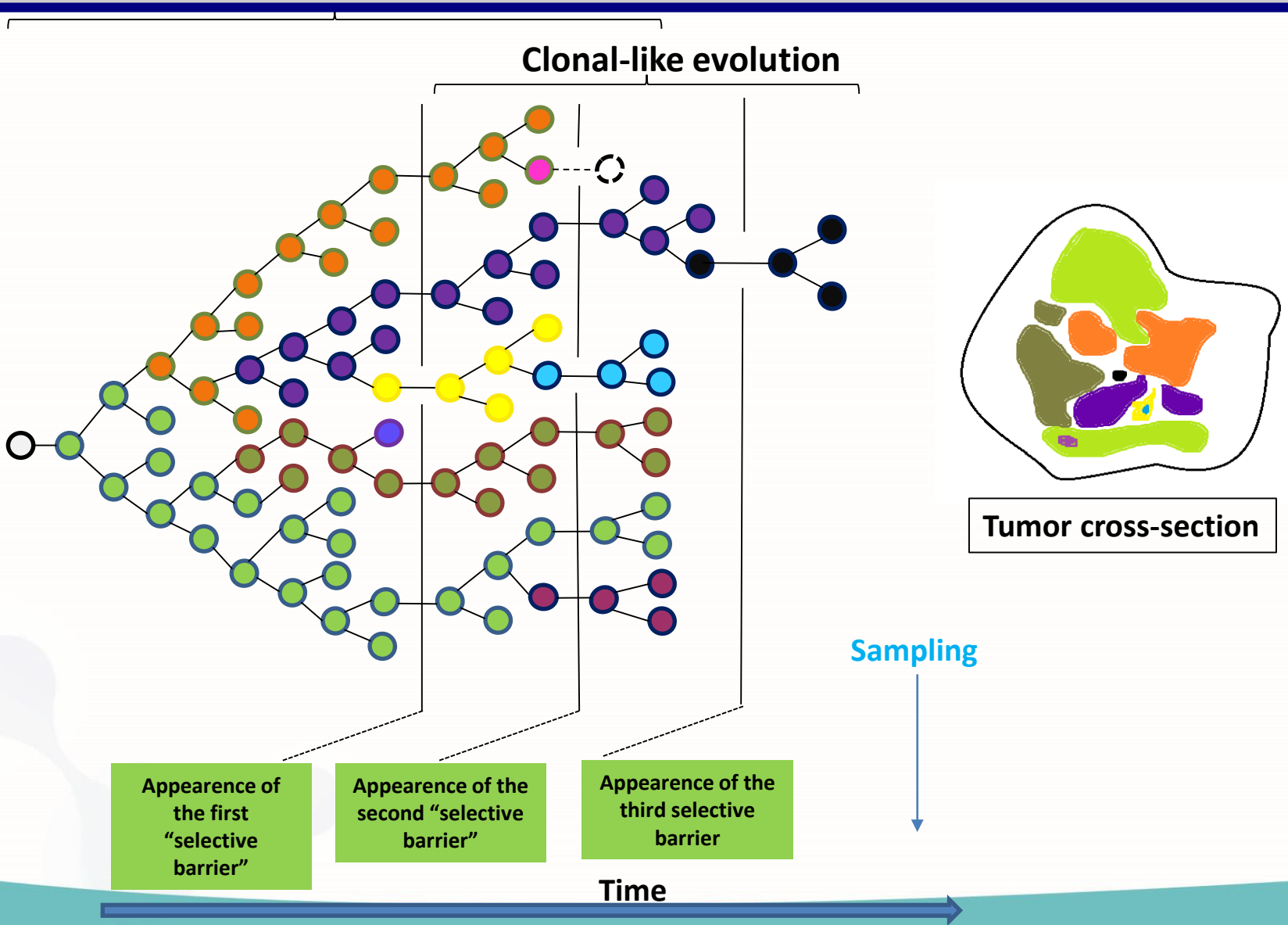
CA: A Cancer Journal for Clinicians, Volume: 69, Issue: 1, Pages: 7-34, First published: 08 January 2019, DOI: (10.3322/caac.21551)

Early detection vs metastasis. 5 years survival

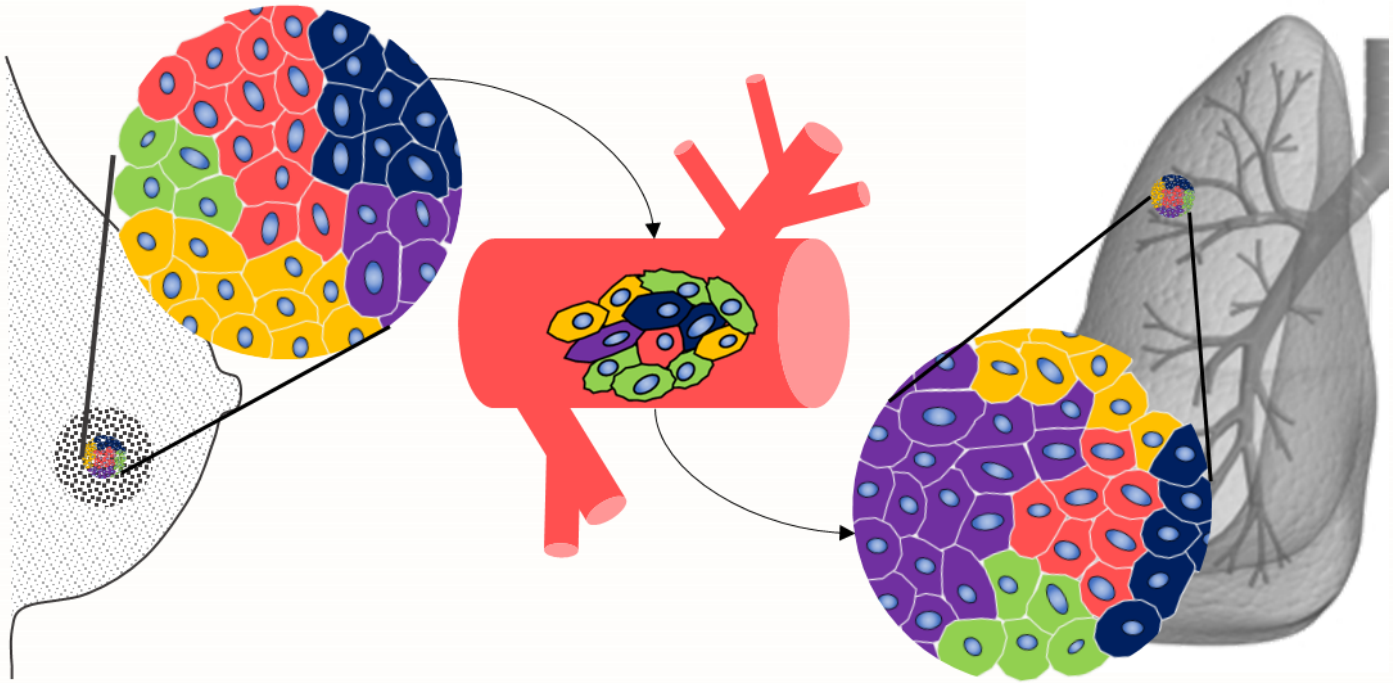


In most carcinomas survival is less than 30% with metastasis

In most tumors, there are areas with different genetic alterations.



primary breast tumor → circulating tumor cell (CTC) clusters → lung metastasis



Features of clonal populations:

- Breast tissue specialized metabolism
- Extracellular Matrix Degradation
- Survial in bloodstream
- Immuno-suppressive Potential
- Lung tissue specialized metabolism

Tumor heterogeneity

There are a huge number of molecular alterations in malignant tumors.

> 2,000 constitutive genetic alterations

- genes drivers, passenger genes

> 1,000 translocations

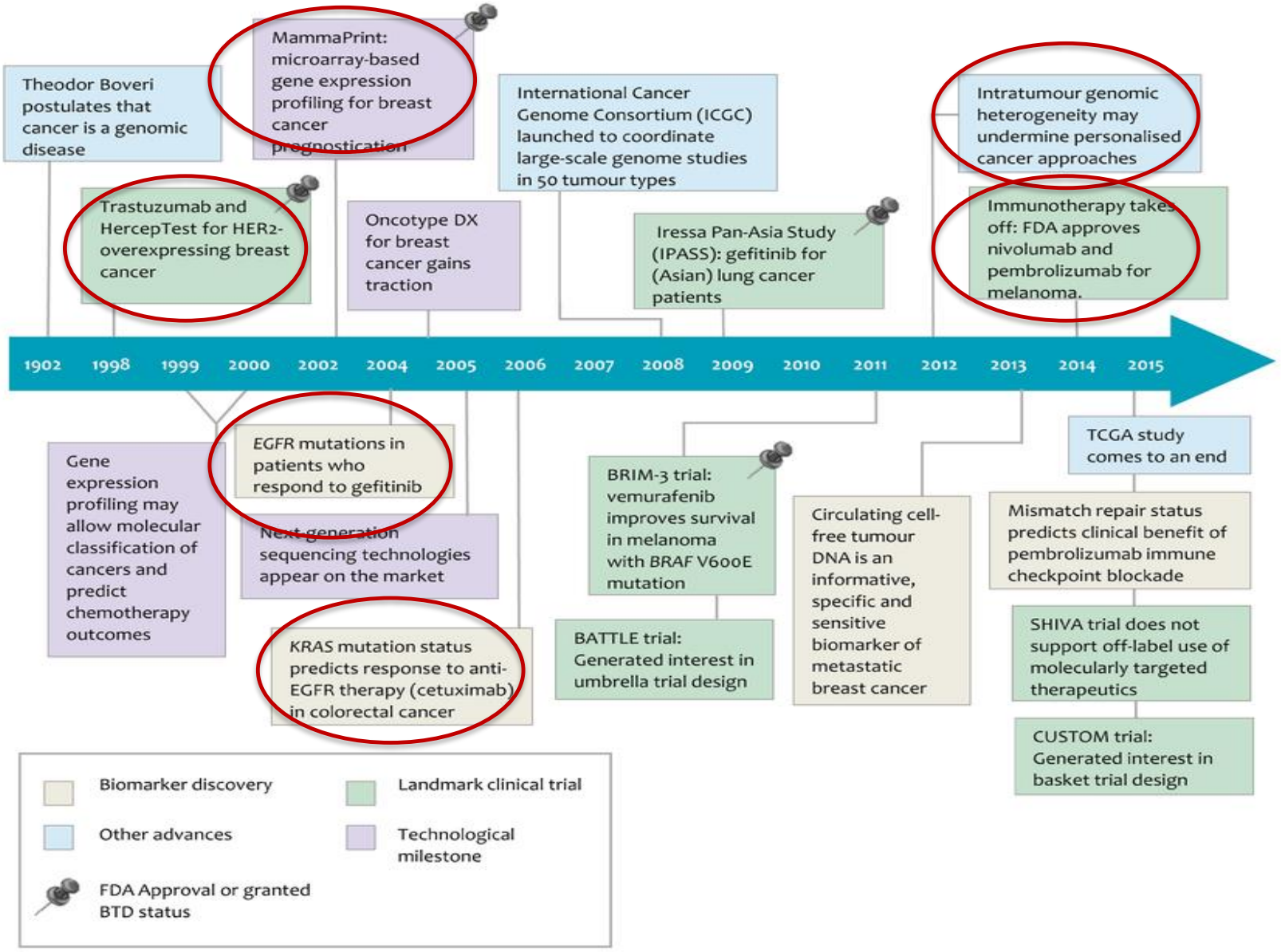
> Thousands of non-coding RNA (lncRNAs, microRNAa

- Hundreds of epigenetic alterations
- Polymorphisms (SNPs)

Dozens / hundreds of genetic alterations accumulate

índice

2. Situación actual Biomarcadores (mama, pulmón, colon, otros)


Theodor Boveri postulates that cancer is a genomic disease
MammaPrint: microarray-based gene expression profiling for breast cancer prognostication
Trastuzumab and HercepTest for HER2-overexpressing breast cancer
Oncotype DX for breast cancer gains traction
International Cancer Genome Consortium (ICGC) launched to coordinate large-scale genome studies in 50 tumour types
Iressa Pan-Asia Study (IPASS): gefitinib for (Asian) lung cancer patients
Intratumour genomic heterogeneity may undermine personalised cancer approaches
Immunotherapy takes off: FDA approves nivolumab and pembrolizumab for melanoma.
1902
1998
1999
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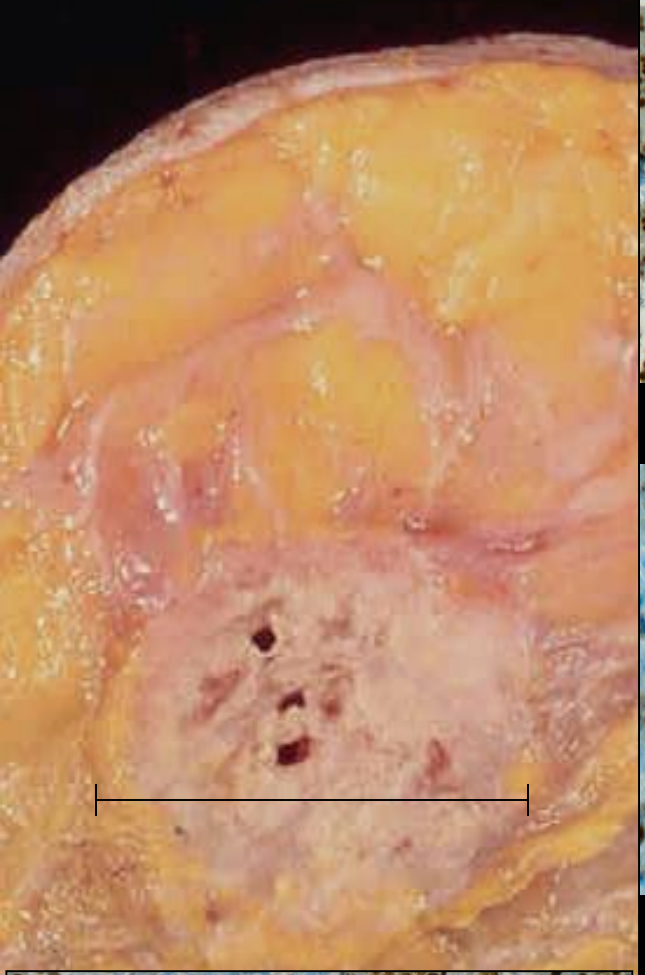
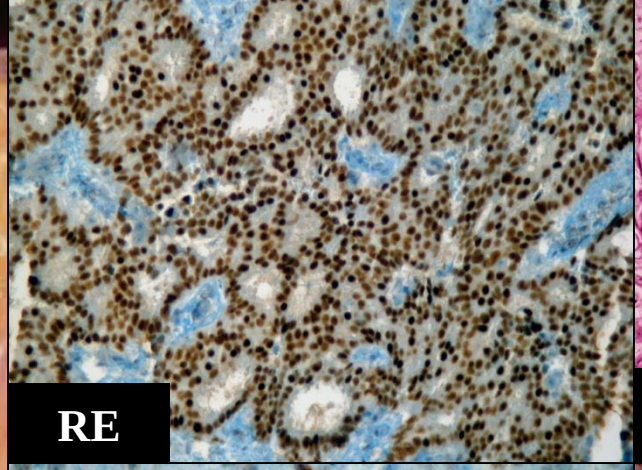
Gene expression profiling may allow molecular classification of cancers and predict chemotherapy outcomes
EGFR mutations in patients who respond to gefitinib
Next-generation sequencing technologies appear on the market
KRAS mutation status predicts response to anti-EGFR therapy (cetuximab) in colorectal cancer
BRIM-3 trial: vemurafenib improves survival in melanoma with BRAF V600E mutation
BATTLE trial: Generated interest in umbrella trial design
Circulating cell-free tumour DNA is an informative, specific and sensitive biomarker of metastatic breast cancer
TCGA study comes to an end
Mismatch repair status predicts clinical benefit of pembrolizumab immune checkpoint blockade
SHIVA trial does not support off-label use of molecularly targeted therapeutics
CUSTOM trial: Generated interest in basket trial design
Biomarker discovery
Landmark clinical trial
Other advances
Technological milestone
FDA Approval or granted BTD status

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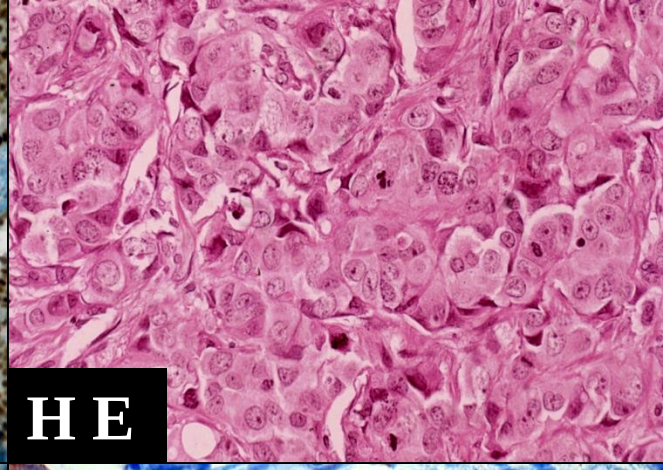
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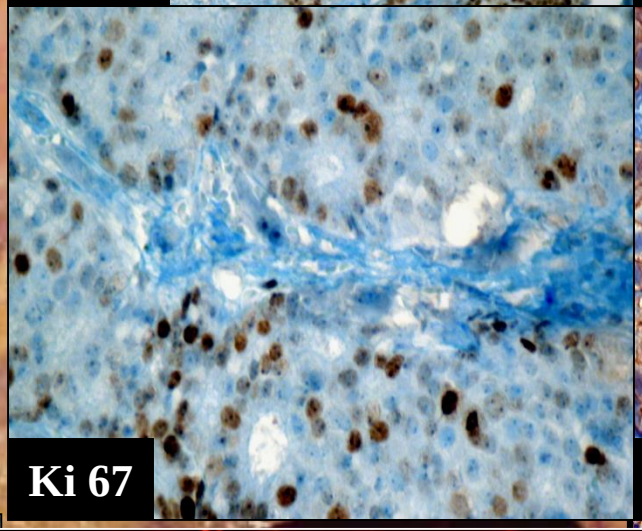
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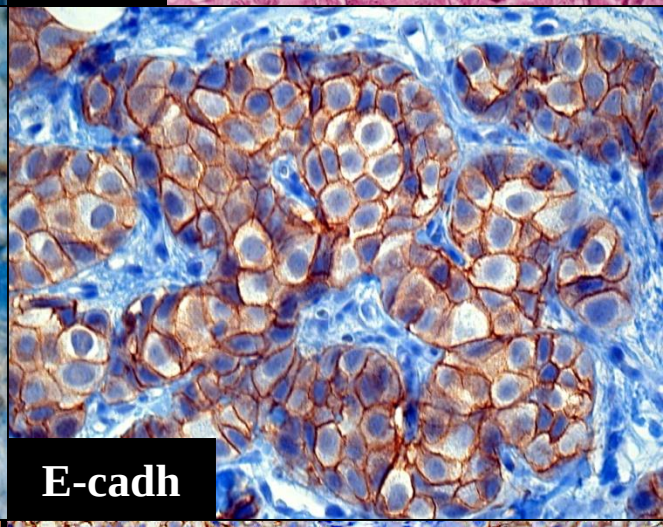
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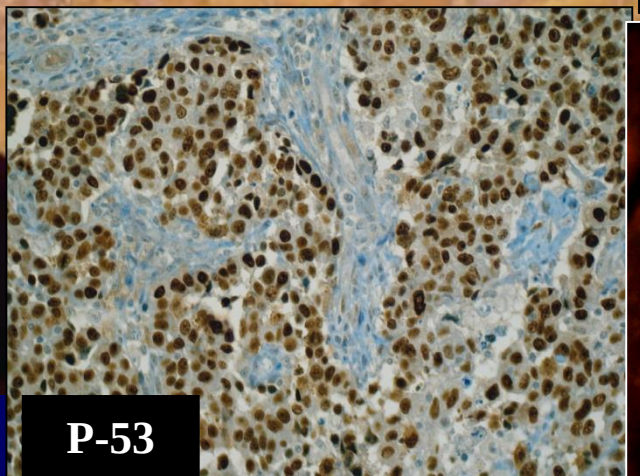
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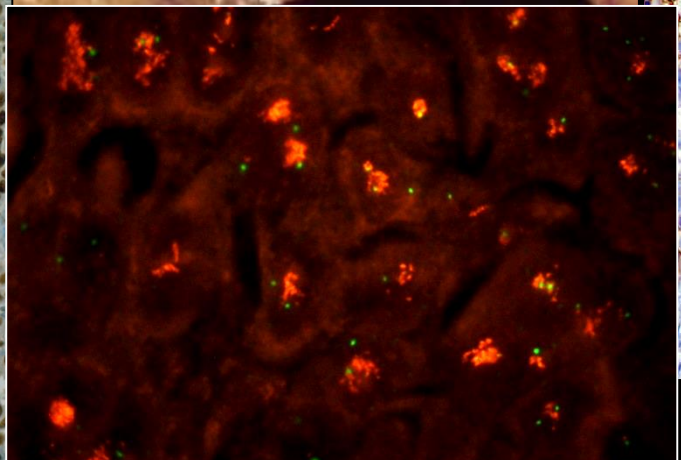
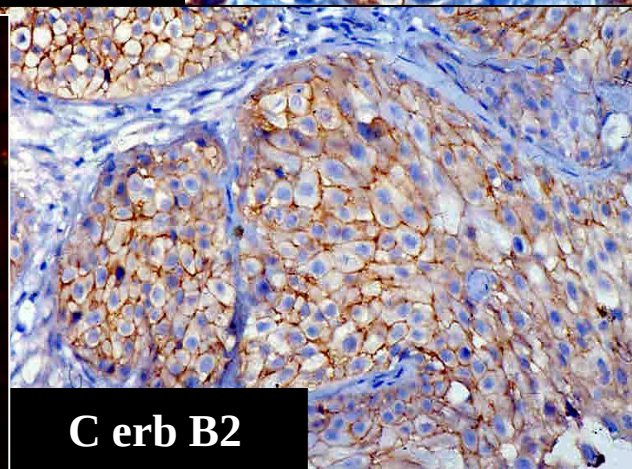



C erb B2

Biomarkers and Tumor Heterogeneity

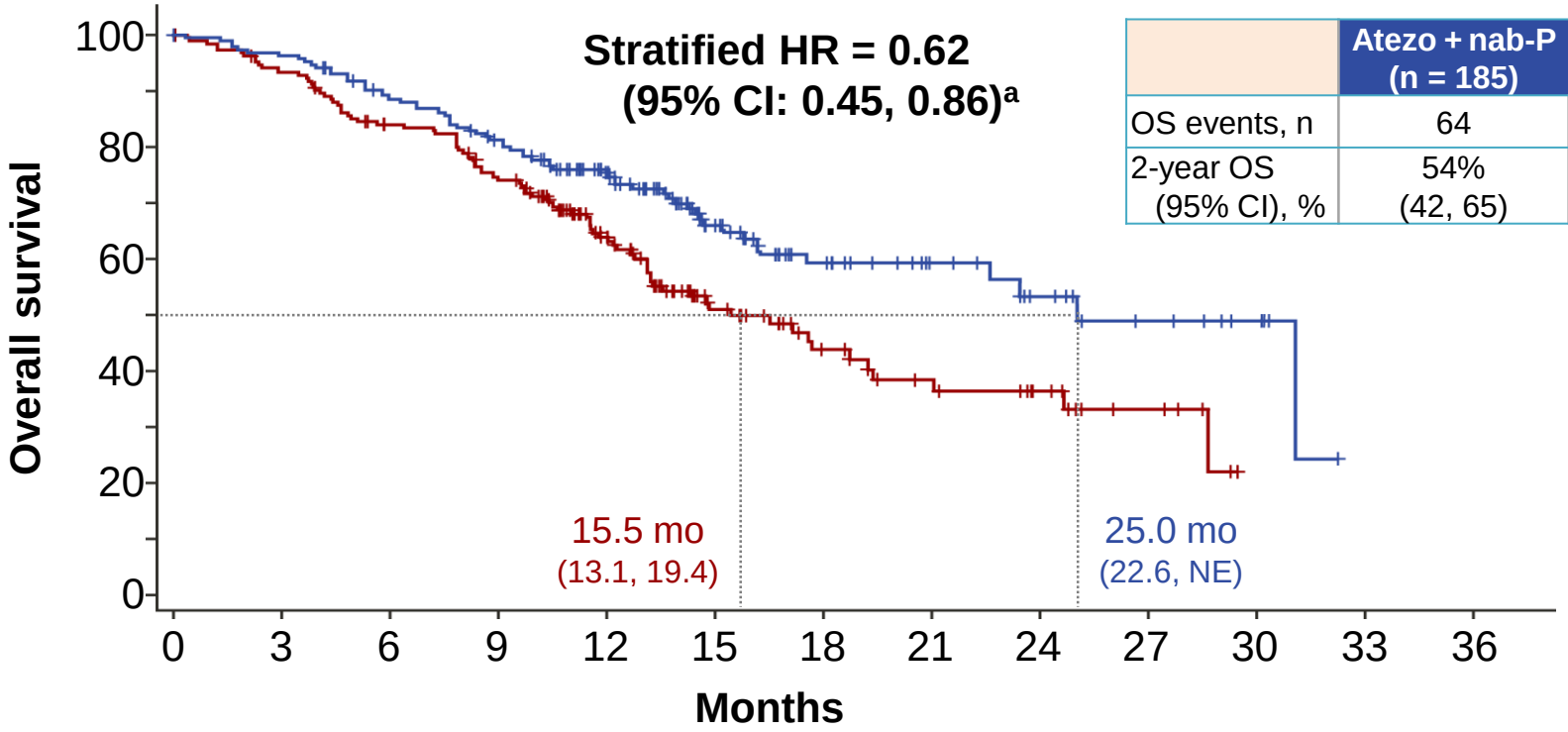
RE: >1%

HER2: >10% IHQ

PDL1: > 1%

PD-L1
Triple negative breast carcinoma

Interim OS analysis: PD-L1+ population



	Atezo + nab-P (n = 185)	Plac + nab-P (n = 184)
OS events, n	64	88
2-year OS (95% CI), %	54% (42, 65)	37% (26, 47)

No. at risk:

Atezo + nab-P	185	177	160	142	113	61	36	22	15	9	5	NE	NE
Plac + nab-P	184	170	147	129	89	44	27	19	13	6	NE	NE	NE

Schmid P, et al. IMpassion130
ESMO 2018 (LBA1_PR) http://bit.ly/2DMhayg

Prospective Clinical Trials to Assess Role of Multigene Prognostic Tests

TAILORx (Onco*type*DX)

–*Trial Assigning Individualized Options for Treatment*

–RxPONDER (Onco*type*DX)

–*Rx for Positive Node Endocrine Responsive Breast Cancer*

MINDACT (MammaPrint)

–*Microarray in Node Negative Disease May Avoid Chemotherapy*

- *PAM 50*

The TAILORx trial demonstrated that women with low recurrence scores had a 98% survival suporting its ability to lower cost of care

B Doble, 2016

PAM50, Risk of Recurrence (ROR) Score provided more prognostic information in endocrine-treated, ER+, node- patients than Onco*type*Dx recurrence score, especially in HER2-negative group. More patients scored as high risk and fewer as intermediate risk ***JCO, 2013***

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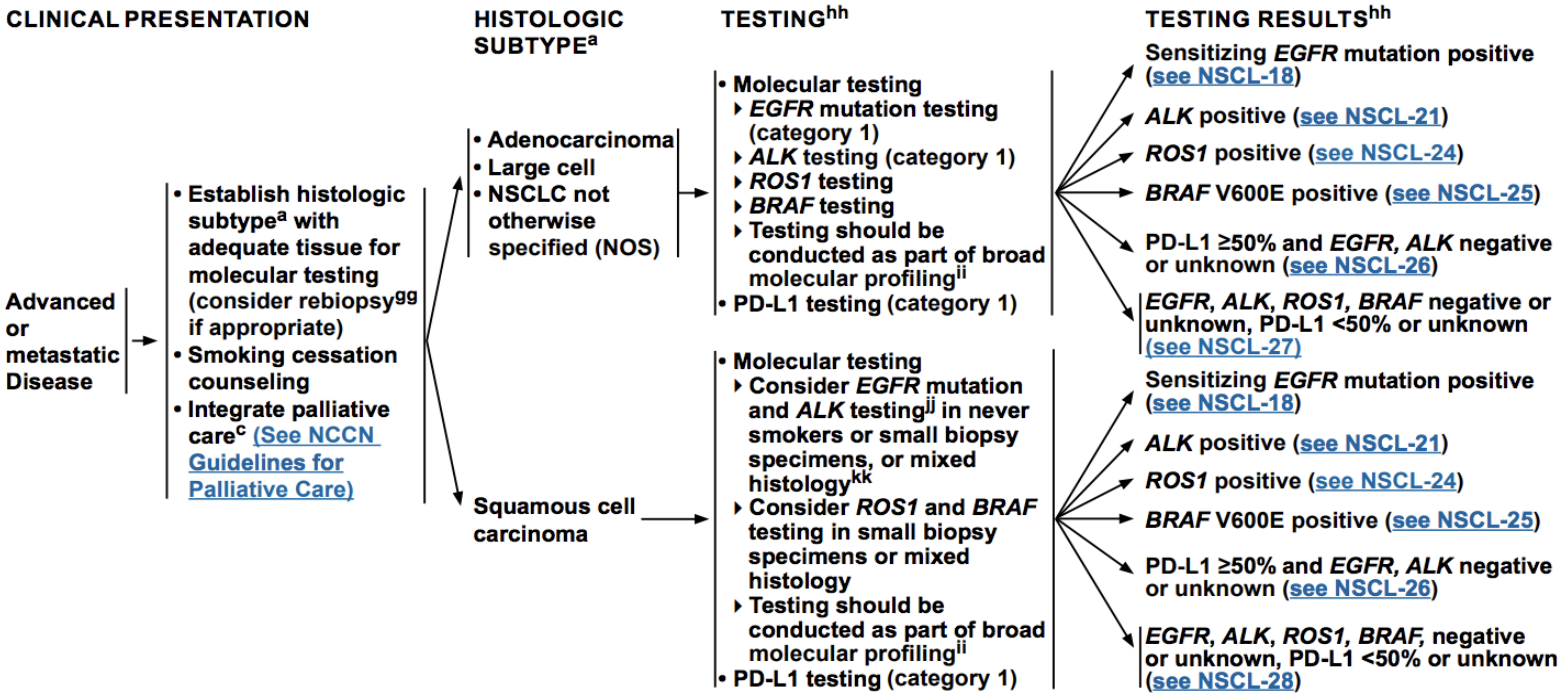
National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2019
Non-Small Cell Lung Cancer

NCCN Guidelines Index
Table of Contents
Discussion



[a] See Principles of Pathologic Review (NSCL-A).
[c] Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med 2010;363:733-742.
[gg] If repeat biopsy is not feasible, plasma testing should be considered.
[hh] See Principles of Molecular and Biomarker Analysis (NSCL-G).
[ii] The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. See Emerging Biomarkers to Identify Patients for Therapies (NSCL-H).
[jj] In patients with squamous cell carcinoma, the observed incidence of *EGFR* mutations is 2.7% with a confidence that the true incidence of mutations is less than 3.6%. This frequency of *EGFR* mutations does not justify routine testing of all tumor specimens. Forbes SA, Bharma G, Bamford S, et al. The catalogue of somatic mutations in cancer (COSMIC). Curr Protoc Hum Genet 2008;chapter 10:unit 10.11.
[kk] Paik PK, Varghese AM, Sima CS, et al. Response to erlotinib in patients with *EGFR* mutant advanced non-small cell lung cancers with a squamous or squamous-like component. Mol Cancer Ther 2012;11:2535-2540.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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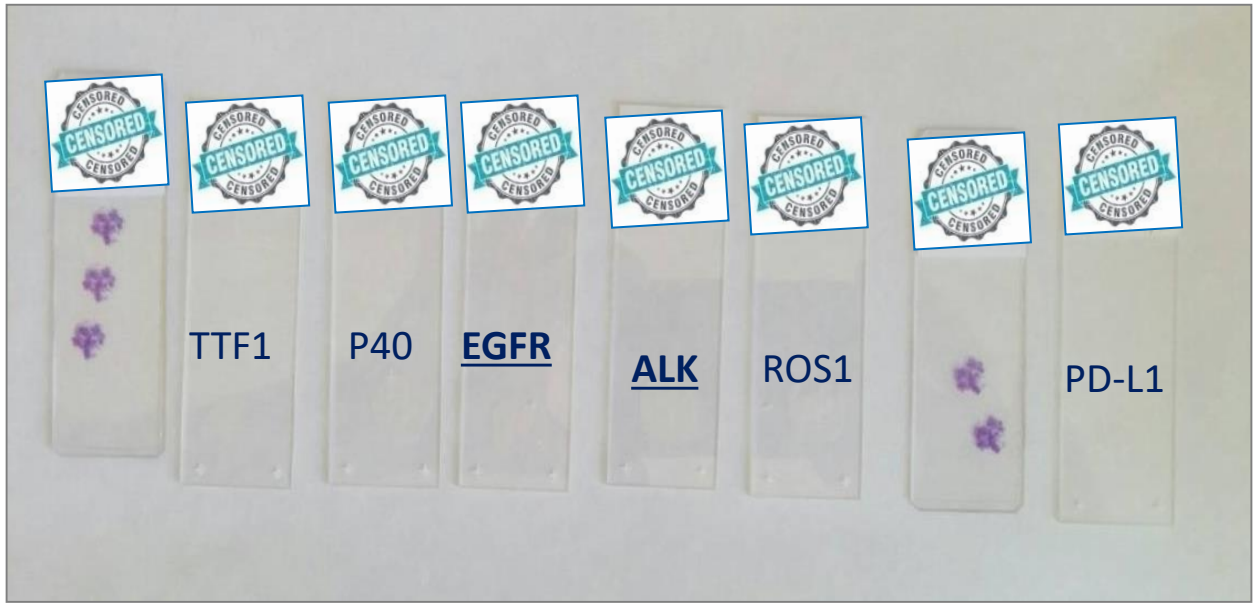
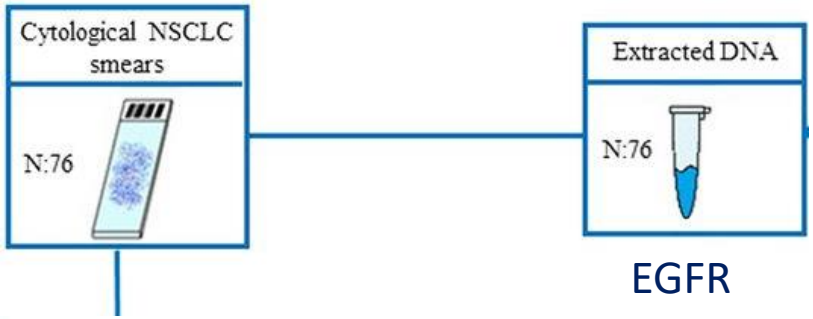

TTF1
P40
EGFR
ALK
ROS1
PD-L1


Cytological NSCLC smears
N:76
Extracted DNA
N:76
EGFR

PRIORIZACIÓN DE BIOMARCADORES

- En el momento del diagnóstico, determinamos EGFR, ALK, ROS1 y PD-L1 a la vez en todos los CCNP no escamosos en estadio metastásico

- En el momento del diagnóstico, determinamos PD-L1 en todos los carcinomas escamosos en estadio metastásico

- En el momento de la recidiva a petición de Oncología

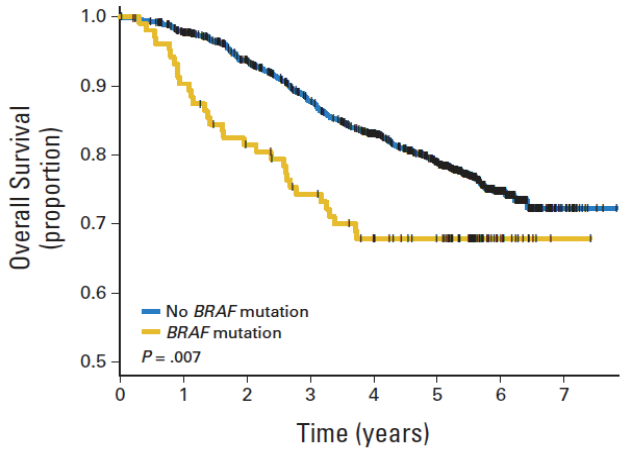
- Otros genes (cmet, ret, BRAF, RAS, HER2,..): NGS

NCCN Guidelines Version 2.2018
Colon Cancer

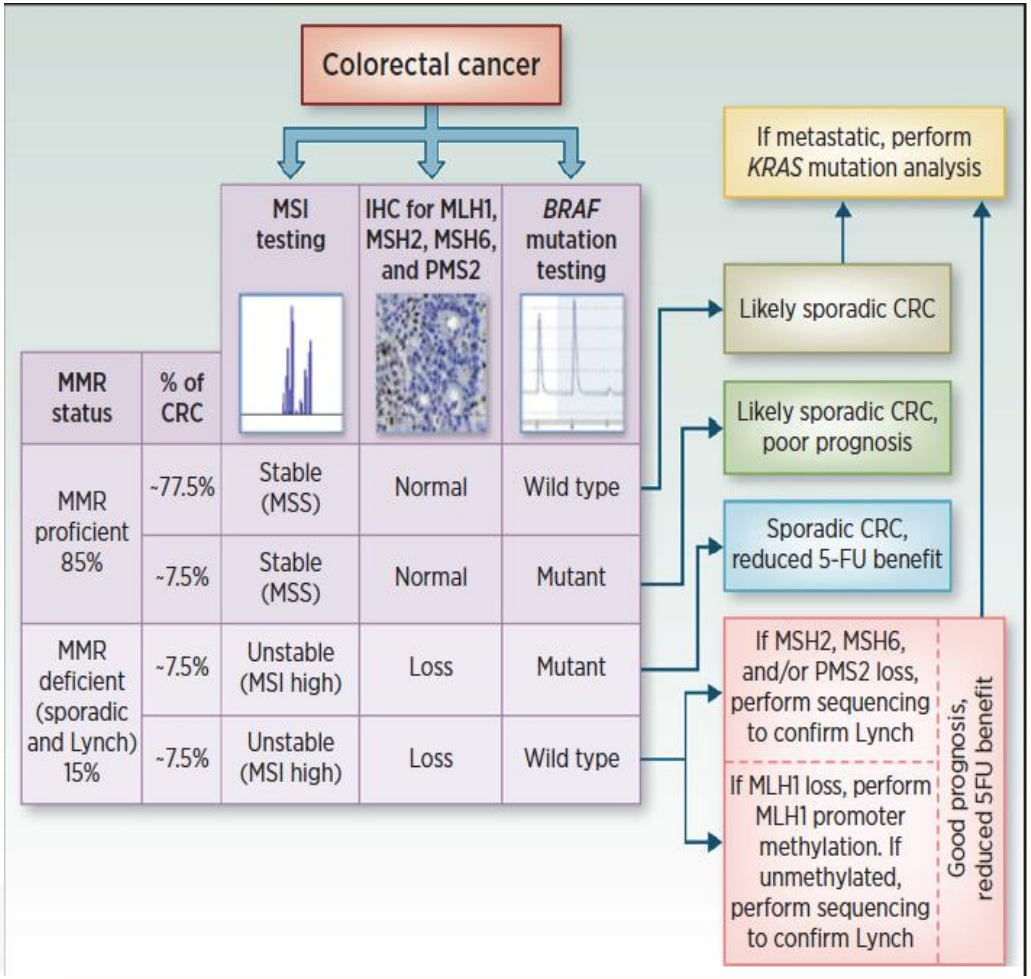
03-2018

KRAS, NRAS, and BRAF Mutation Testing

- All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations. Patients with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.[43,44,45] *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor.[46-48]



MSI testing algorithm proposed in 2012 by the Association for Molecular Pathology

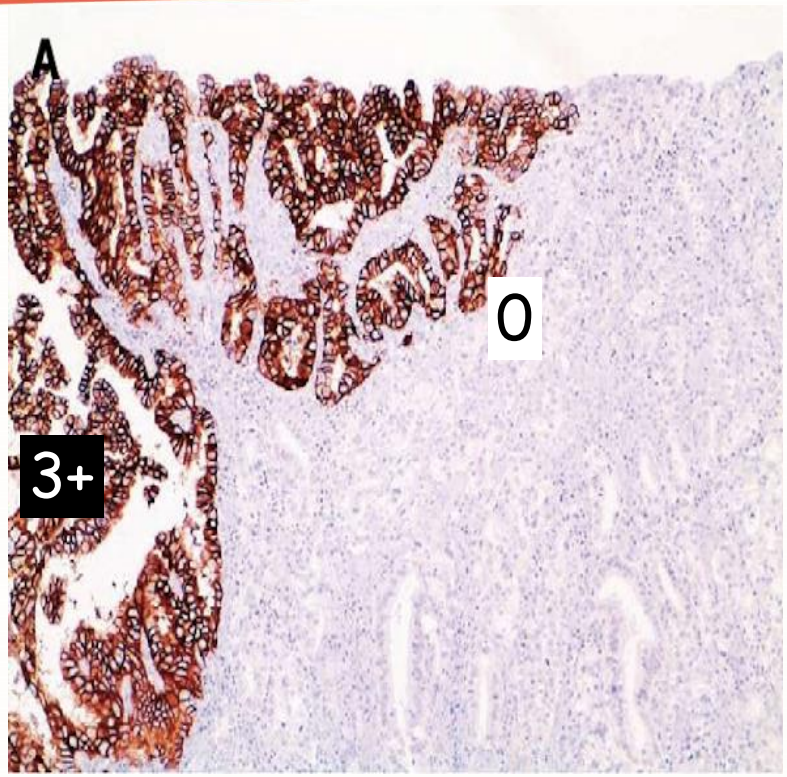


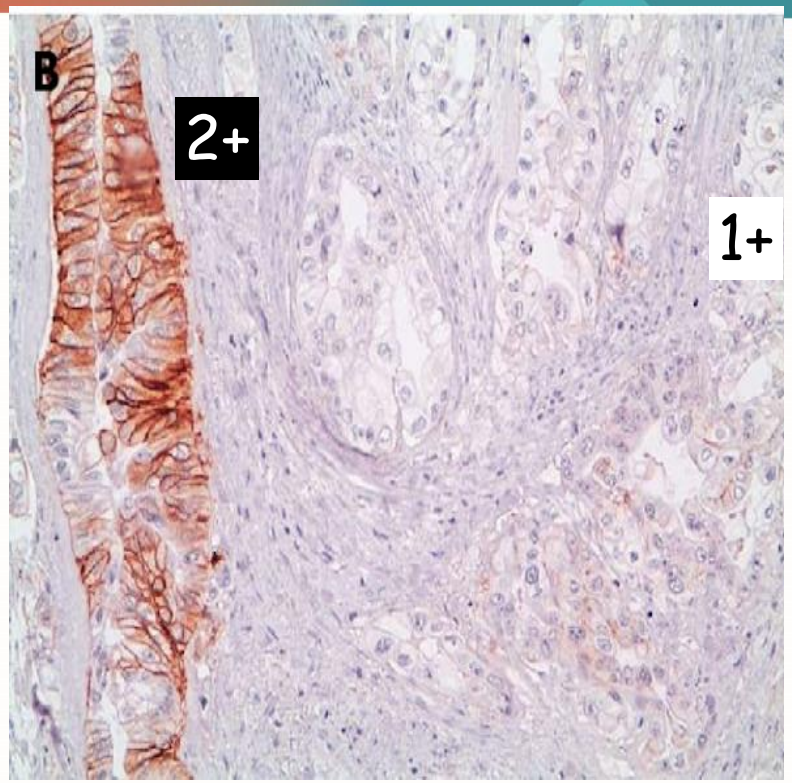
MMR status	% of CRC	MSI testing	IHC for MLH1, MSH2, MSH6, and PMS2	*BRAF* mutation testing
MMR proficient 85%	~77.5%	Stable (MSS)	Normal	Wild type
	~7.5%	Stable (MSS)	Normal	Mutant
MMR deficient (sporadic and Lynch) 15%	~7.5%	Unstable (MSI high)	Loss	Mutant
	~7.5%	Unstable (MSI high)	Loss	Wild type

Jonathan C. Dudley et al. Clin Cancer Res 2016;22:813-820

In gastric carcinomas…

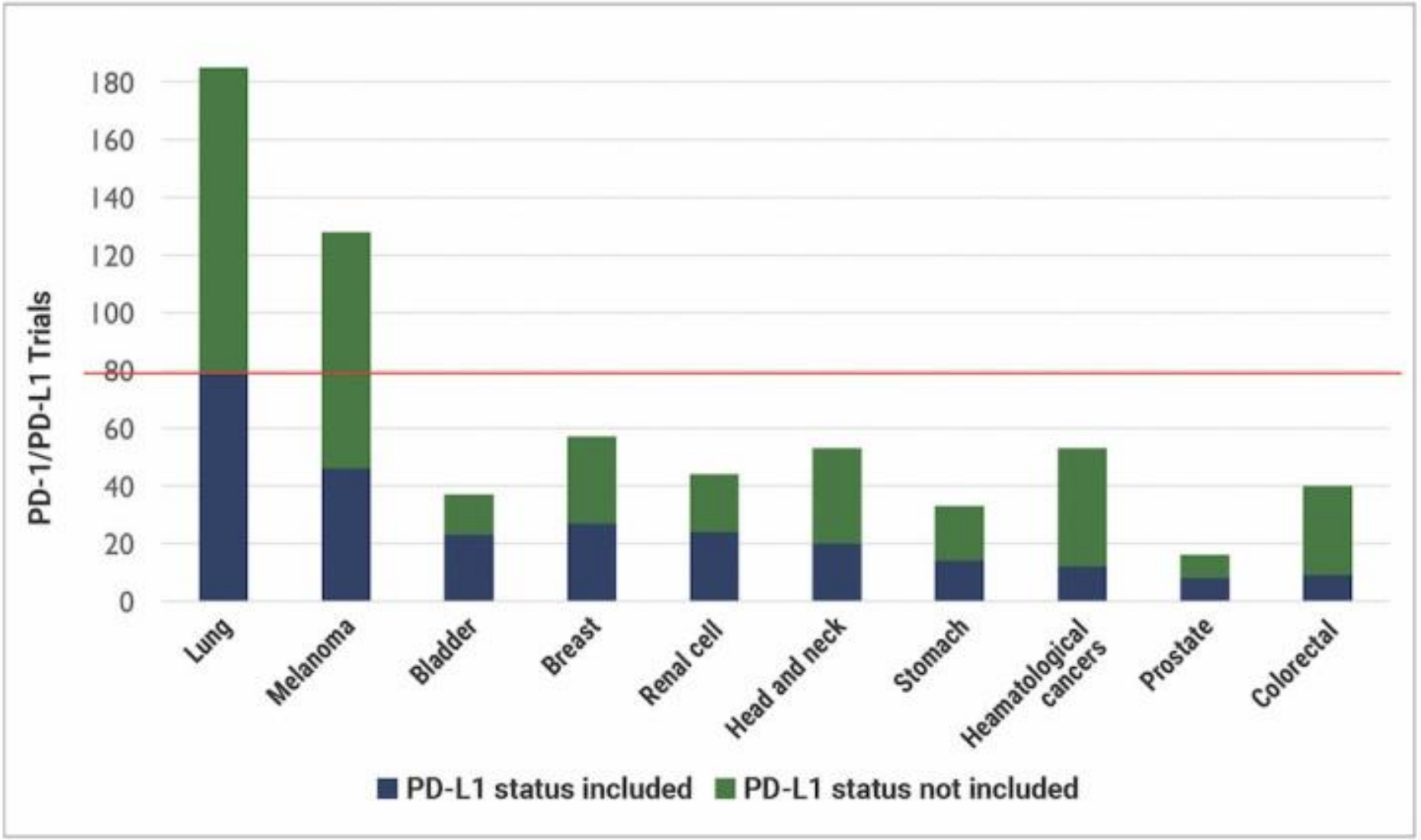
	Biomarker type	Indication for testing	GC patients who should be tasted
MSI/MMR proteins	Diagnostic	Lynch Syndrome Screening	All patients
	Predictive	Immunotherapy	
	Pronostic	Choose therapy in early stages GCs	
HER2	Predictive	Trastuzumab in advance HER2+ GC	All patients
EBV	Predictive	Immunotherapy	Metastatic GCs
PD-L1	Predictive	Immunotherapy	Metastatic GCs





- **Heterogeneous staining** is more common in gastric and GE junction cancer than in breast cancer
- In gastric cancer, tumour heterogeneity occurs in **5–30% of cases**

Hofmann M, et al. Histopathology 2008; 52:797–805
Rüschoff J, et al. Der Pathologe 2010


PD-1/PD-L1 Trials
180
160
140
120
100
80
60
40
20
0
Lung
Melanoma
Bladder
Breast
Renal cell
Head and neck
Stomach
Heamatological cancers
Prostate
Colorectal
PD-L1 status included
PD-L1 status not included

índice

3. Pitfalls en diagnóstico

a) HG intratumoral

b) sesgos diagnóstico molecular

IHQ vs NGS vs arrays

especificidad tisular

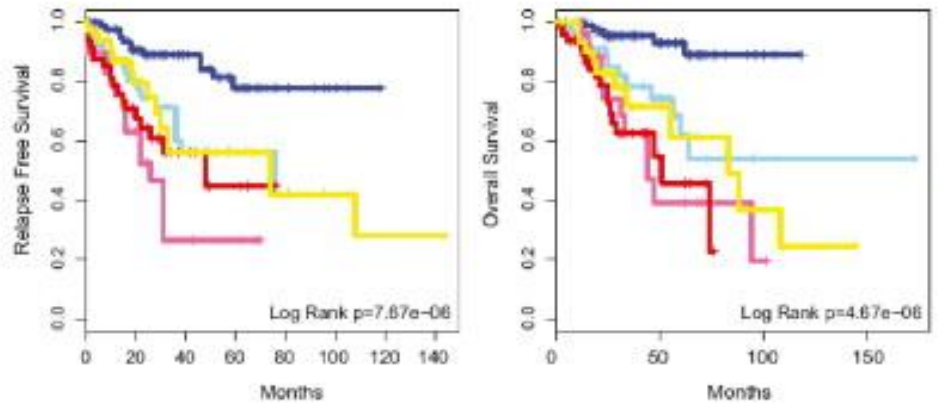
c) control calidad

Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways

A

Molecular Subtype	Frequency	ER/PRg/HER2	CK5/6 EGFR	Genes of Proliferation	Characteristics genes	Histologic grade	TP53 mutations	Prognostic
Basal-like	10-20%	ER- PRg- HER2-	+	High	KRT5, CDH3, ID4, FABP7, KRT17, TRIM29, LAMC2	High	High	Bad
HER2-enriched	10-15%	ER- PRG- HER2+	+/-	High	ERBB2, GRB7	High	High	Bad
Normal breast-like	5-10%	ER-/+ HER2-	+	Low	PTN, CD36, FABP4, AQP7, ITGA7	Low	Low	Intermediate
Luminal A	50-60%	ER+ PRg+ HER2-	-	Low	ESR1, GATA3, KRT8, KRT18, XBP1, FOXA1, TFF3, CCND1, LIV1	Low	Low	Excelent
Luminal B	10-20%	ER+/- PRg+/- HER2-/+	-	High	ESR1, GATA3, KRT8, KRT18, XBP1, FOXA1, TFF3, SQLE, LAPTM4B	Intermediate /High	Intermediate	Intermediate /Bad
Claudin-low	12-14%	ER- PRg- HER2-	+/-	High	CD44, SNAI3	High	High	Bad

B



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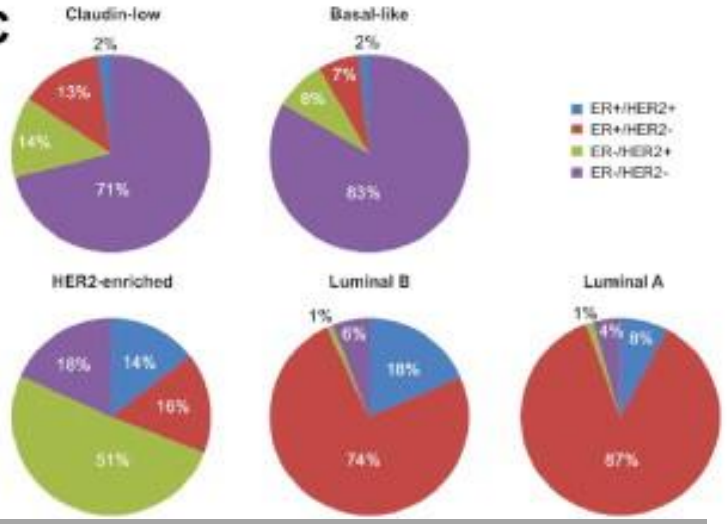


Poca concordancia con los estudios de IHQ

Entorno al 50% de los HER2+ y > 20% de los tumores Luminal B y claudin low

Fig. 1. (A) Features of molecular subtypes of breast cancer. (B) Kaplan–Meier curves of disease-free survival and overall survival based on UNC337 database. Dark blue, luminal A; light blue, luminal B; red, basal-like; pink, HER2-enriched; yellow, Claudin-low....

Pilar Eroles, Ana Bosch, J. Alejandro Pérez-Fidalgo, Ana Lluch

Cancer Treatment Reviews, Volume 38, Issue 6, 2012, 698–707

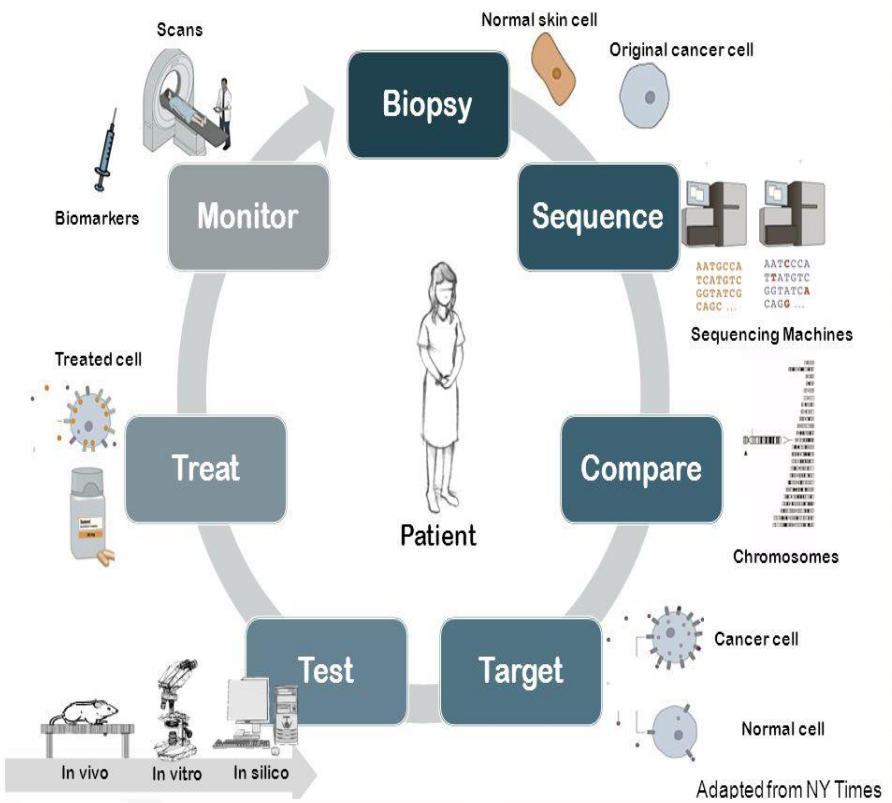
Alteraciones genómicas y especificidad tisular

A) **BRAF**: mutations and translocations in melanocytic nevi, malignant melanomas, colon adenocarcinomas, glioblastomas or pilocytic astrocytomas,..

B) **EGFR**: mutations in lung adca, amplificado en gliomas, sobreexpresado en adca.colon,….

C) ***ALK*** gene fusions: IMT, ALCL anaplastic lymphomas, pulmonary/breast/ovary/colon adenoCA, renal medullary CA, Spitz nevi and atypical Spitz neoplasms

Precision Oncology 2.0 (Today)

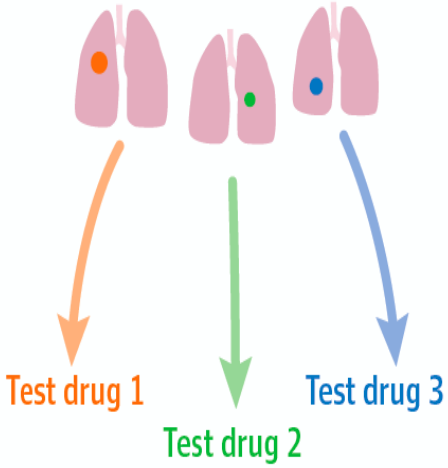


Novel precision medicine trial designs

Umbrella trial

1 type of cancer

Different genetic mutations (●●●)



Basket trial

Multiple types of cancer

1 common genetic mutation (●)

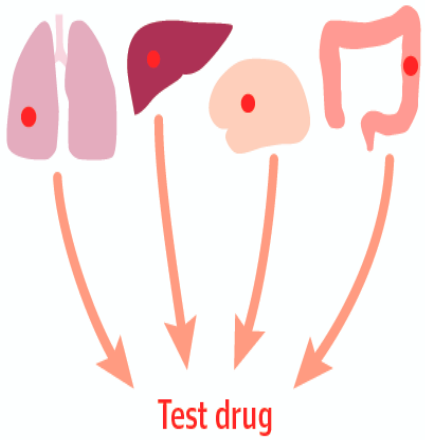
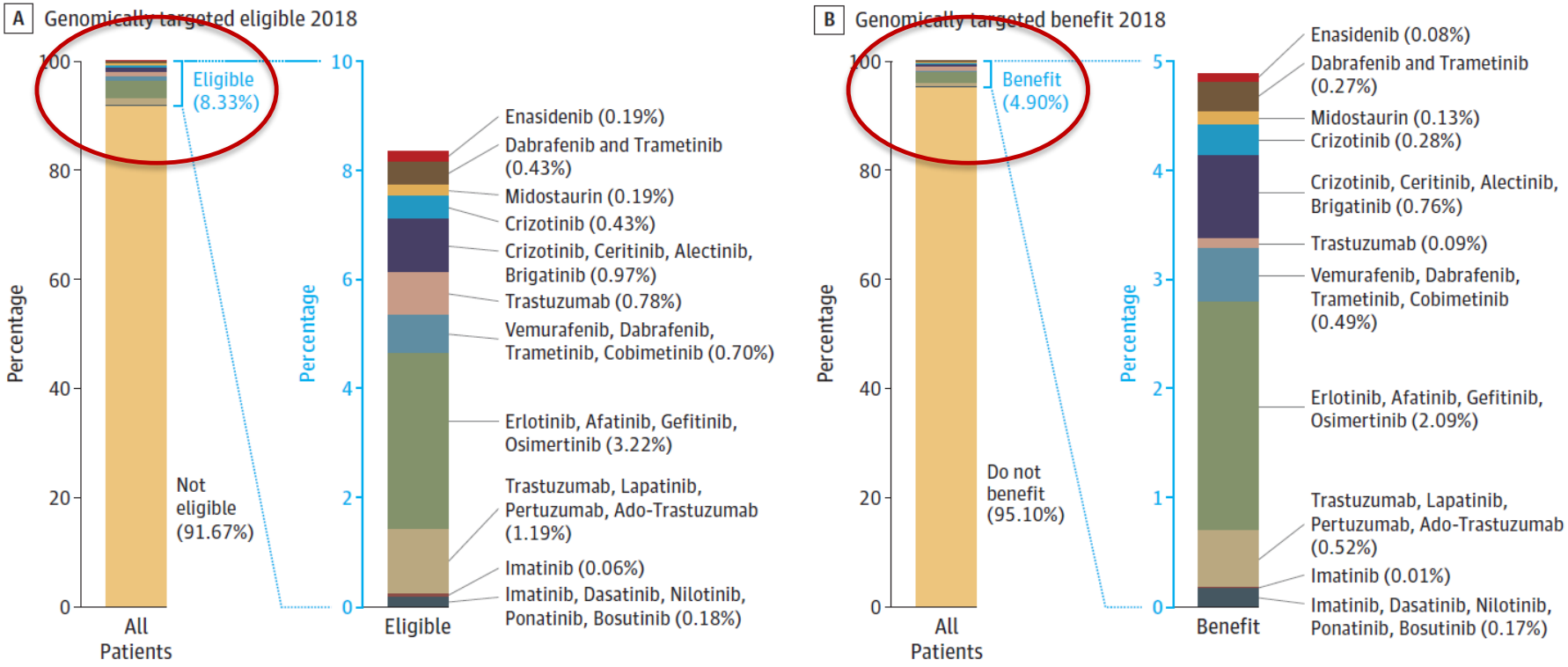


Figure 1. Estimated US Patient Eligibility and Benefit From Genomically Targeted Benefit, 2018



Alteraciones moleculares "No" especificas tipo tumor": **clasificación molecular**

- Drivers compartidos por varios tipos de tumores: BRAF, ALK, EGFR,..

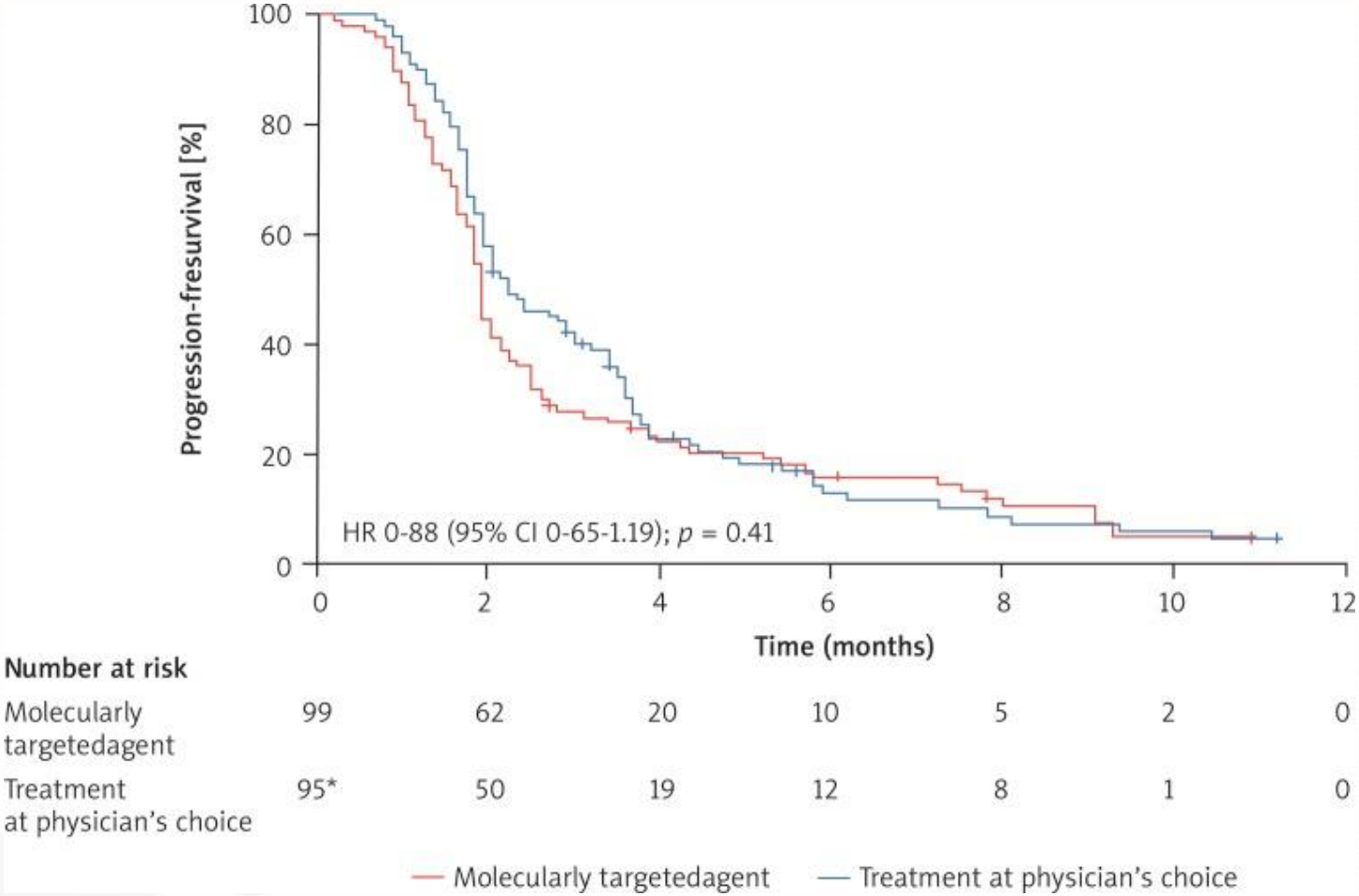
  (Umbrella and basket trials: NCI-MATCH,..)

- **Problema**: La inhibición de drivers no tiene el mismo efecto clínico según la localización y tipo de tumor; ej, BRAF, ALK, EGFR,..

- Diferentes feedbacks +/- según tejido, microambiente,..

(Schneider et al Nat.Rev. Cancer 2017)

SHIVA trial results: primary endopoint PFS



Problemas diagnóstico biomarcadores

a) IHC intratumoral

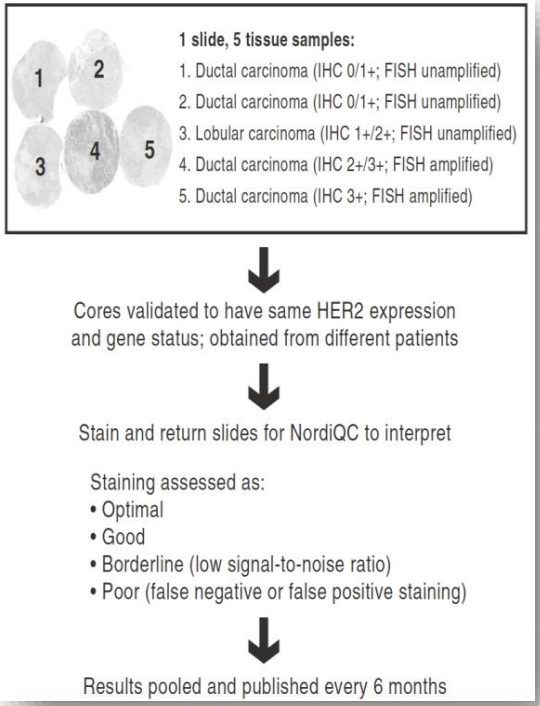
b) sesgos diagnóstico molecular

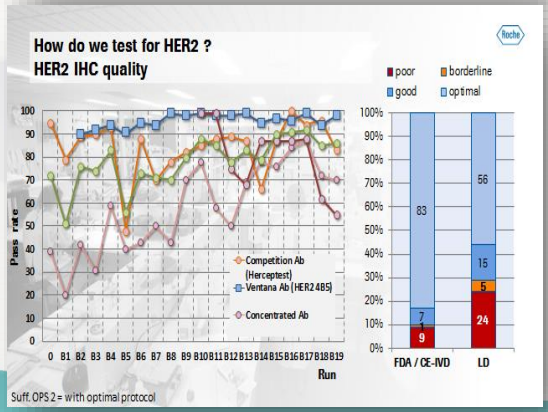
IHC vs NGS vs arrays

especificidad tisular

c) control calidad

False Negative



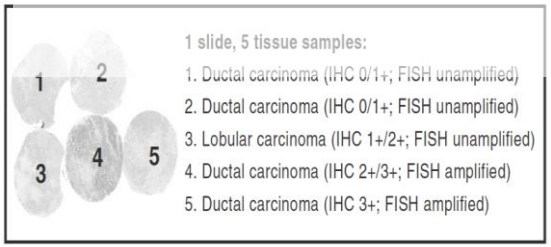


11% For approved IVD

25% For Lab Dev IVD

VyBerg et al 2015

False Positive

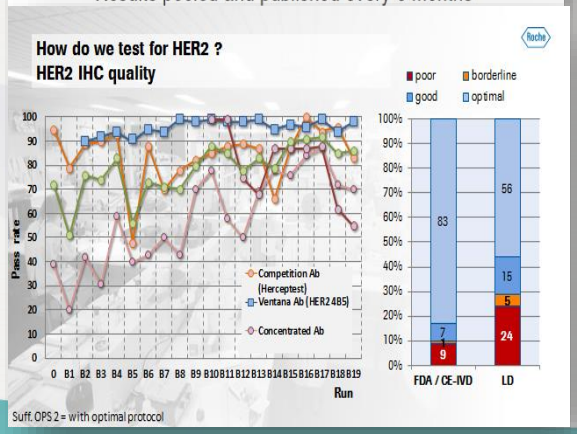


Cores validated to have same HER2 expression and gene status; obtained from different patients

Stain and return slides for NordiQC to interpret

Staining assessed as:
- Optimal
- Good
- Borderline (low signal-to-noise ratio)
- Poor (false negative or false positive staining)

Results pooled and published every 6 months



0% For approved IVD

5% For Lab Dev IVD

VyBerg et al 2015

Controles de calidad SEAP

HER IHQ (>100 labs): puntuacion regular un **18%** y pobre un 1%.

HER 2 FISH (50): valoración regular o pobre en el 6%

IHQ ALK: 57, FISH ALK:40 (27)

Ras: 33 labs, EGFR: 40

EMQN The European Molecular Genetics Quality Network

IQNPath International Quality Network for Pathology

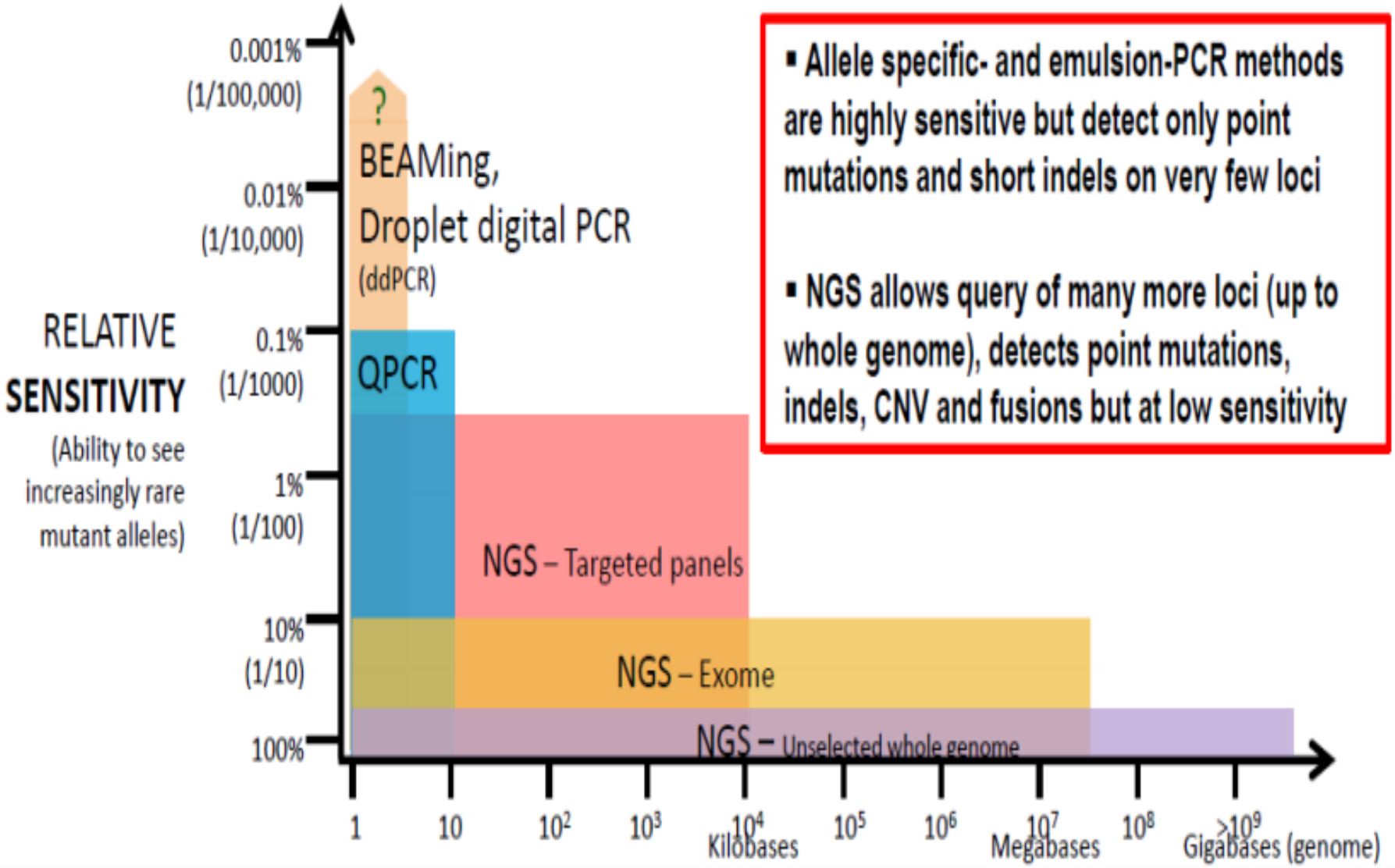
EQA European Society of Pathology

ISO 9001:2015

ISO 15189

Donde vamos

1. Implementación generalizada plataformas NGS y multiplexing
2. La biopsia líquida


RELATIVE
SENSITIVITY
(Ability to see increasingly rare mutant alleles)
0.001% (1/100,000)
0.01% (1/10,000)
0.1% (1/1000)
1% (1/100)
10% (1/10)
100%
?
BEAMing, Droplet digital PCR (ddPCR)
QPCR
NGS – Targeted panels
NGS – Exome
NGS – Unselected whole genome
1
10
10²
10³
10⁴
10⁵
10⁶
10⁷
10⁸
>10⁹
Kilobases
Megabases
Gigabases (genome)
▪ Allele specific- and emulsion-PCR methods are highly sensitive but detect only point mutations and short indels on very few loci
▪ NGS allows query of many more loci (up to whole genome), detects point mutations, indels, CNV and fusions but at low sensitivity

Donde vamos

3. Biomarcadores y Oncoguías
4. Controles de calidad.
5. Centros de referencia

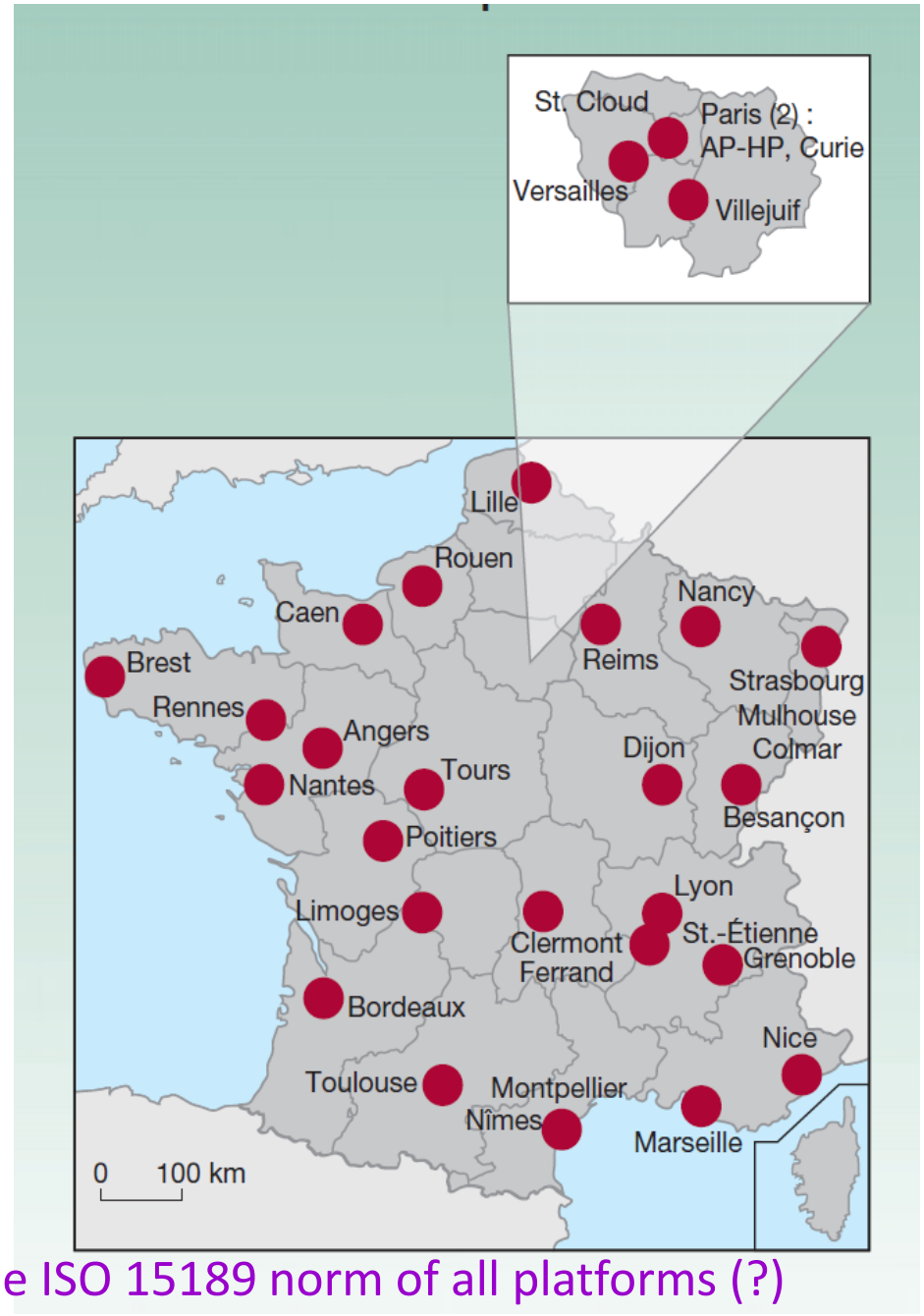
INSTITUT NATIONAL DU CANCER

1. Developement of 28 public molecular laboratories in France for molecular testing

2. Funding for these laboratories came from the French Ministry of Health

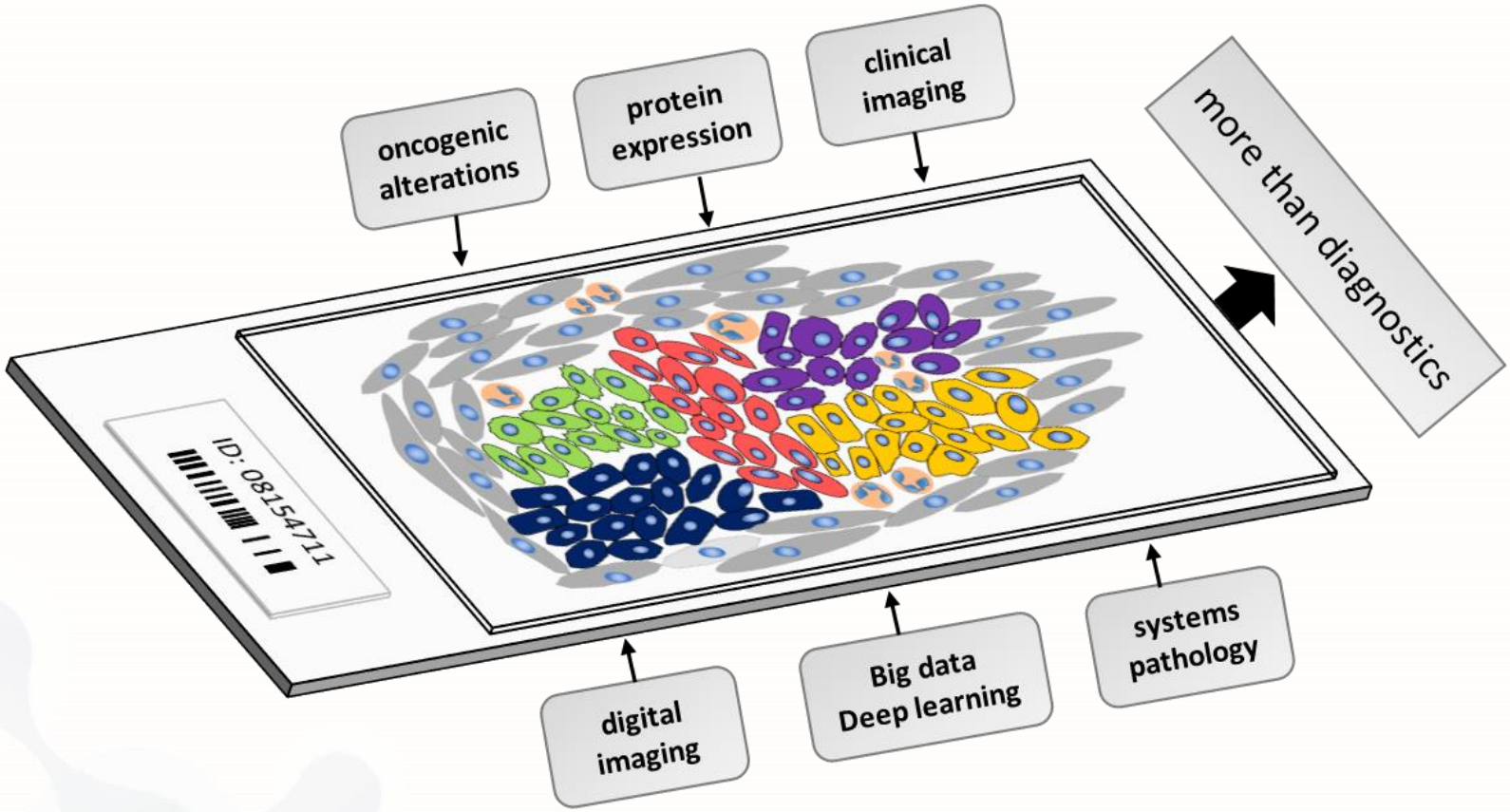
3. Biomarker analyses do not need (currently) to be submitted for reimbursement

Ensuring equal access to personalized cancer treatment is a public health requirement in France



Toward an accreditation according the ISO 15189 norm of all platforms (?)

Integrating clinical, molecular, proteomic and histopathological data within the tissue context: *Tissunomics*

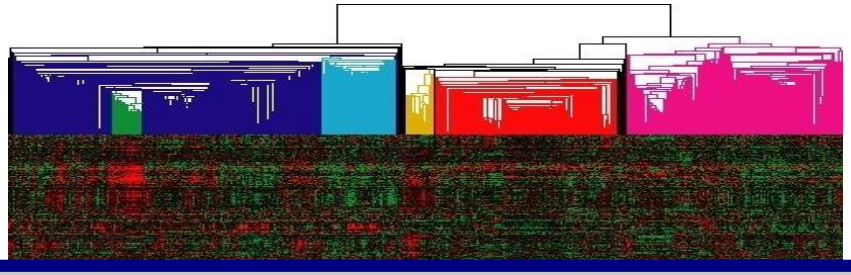


Ramon y Cajal S et al 2019

Muchas gracias!!!

Santiago Ramón y Cajal
Vall d'Hebron University Hospital
Barcelona, Spain

Normal Breast
Claudin-low
HER2-enriched
Luminal A
Luminal B
Basal-like



Intrinsic Subtypes

Perou et al., Nature 2000
Sorlie et al., PNAS 2001
Sorlie et al., PNAS 2003
Hu et al., BMC Genomics 2006
Herschkowitz et al., GB 2007
Cheang et al. JNCI 2008
Parker et al., JCO, Feb 2009
Prat et al., BCR 2010
Nielsen et al., CCR 2010
Cheang et al., CCR 2012

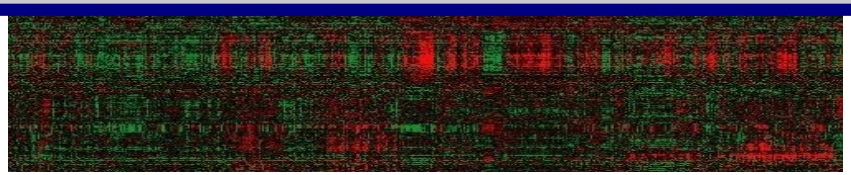
There is heterogeneity within the molecular subtypes:

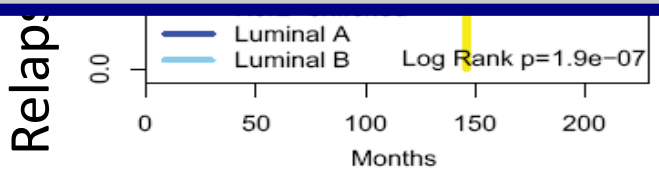
EVEN THE SUBTYPES HAVE SUBTYPES

Prat et al. Breast 2015
Prat et al. BMC Med 2015

High discordance of mRNA levels.

Difficult to apply a single cases

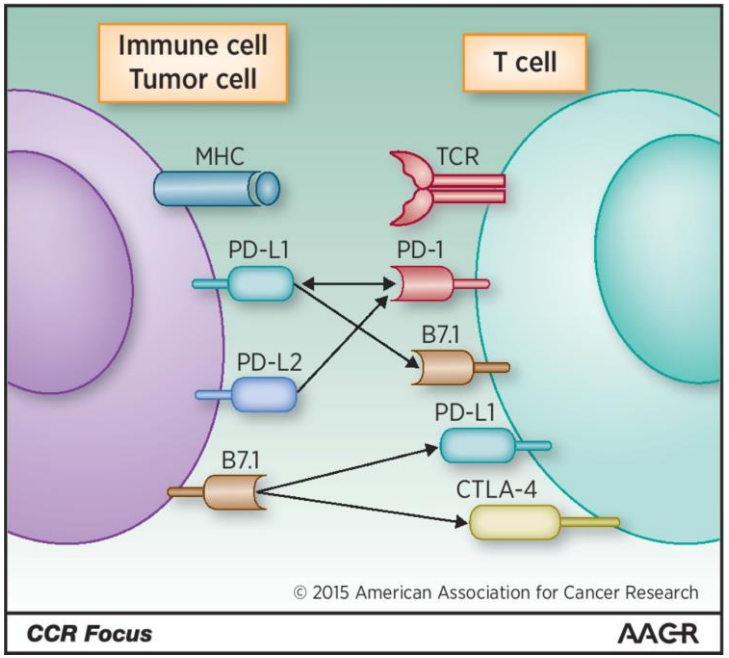




PD-L1

- PD1 se expresa en las células T, NK y algunas B

- Tiene dos ligandos PD-L1 y PD-L2 que se expresan en muchas células del SI y en células tumorales



- La unión de PD1 y sus ligandos inactiva y disminuye la proliferación de los LT

Arch Pathol Lab Med (2016) 140: 249-254