VHIO VALL D'HEBRON Institute of Oncology

The pathologist of the future

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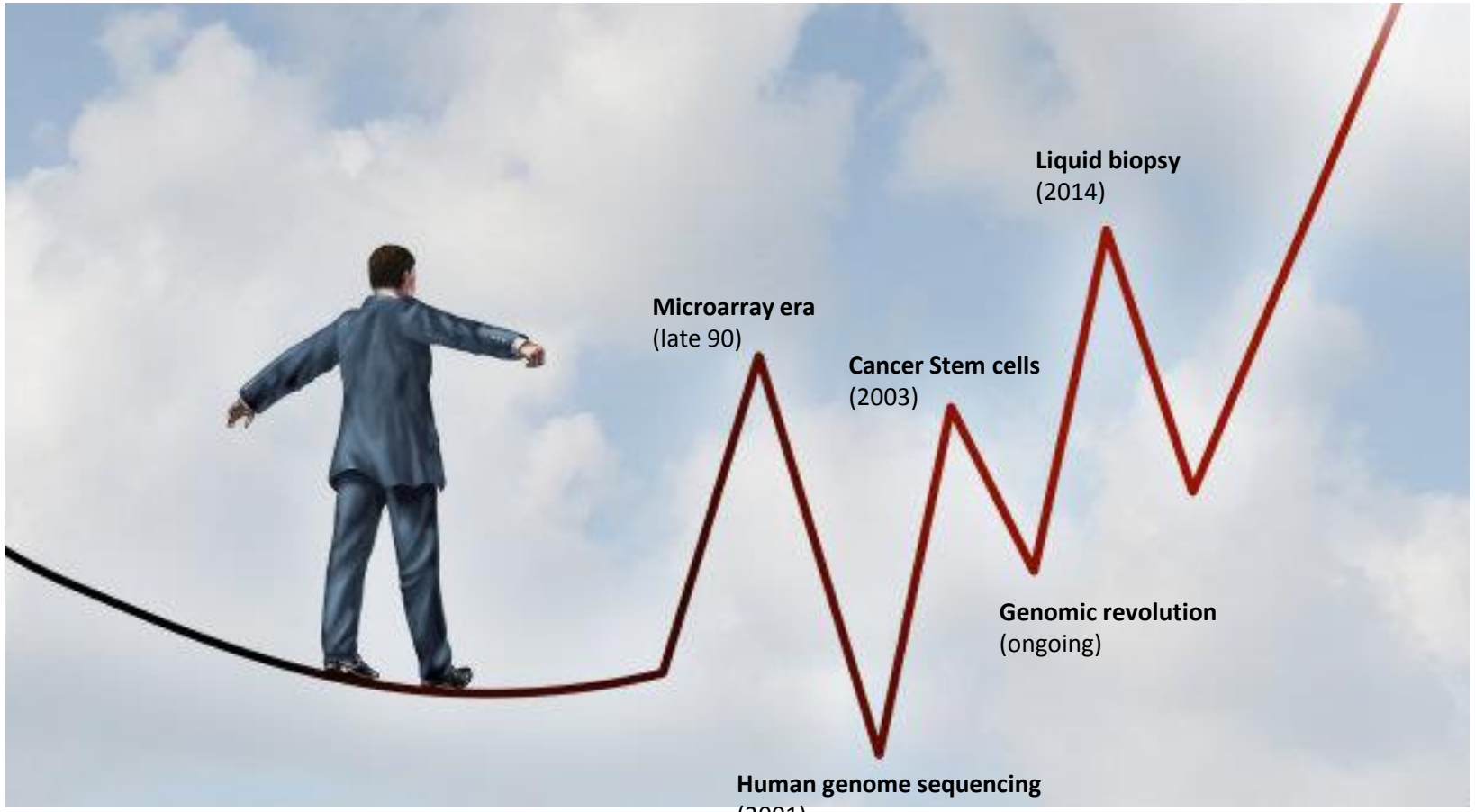
Vall d'Hebron Institute of Oncology

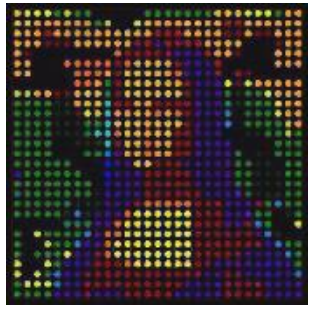
19th World Congress on Gastrointestinal Cancer

28 June -1 July, Barcelona

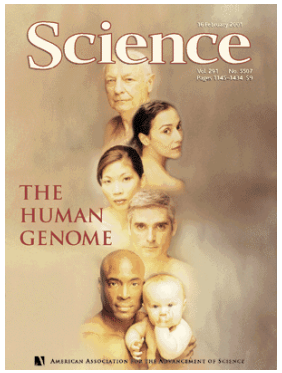
The (hard) life of a Pathologist

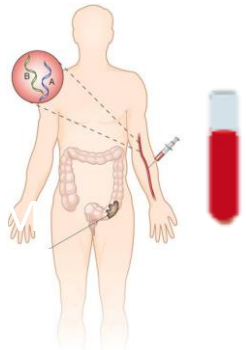


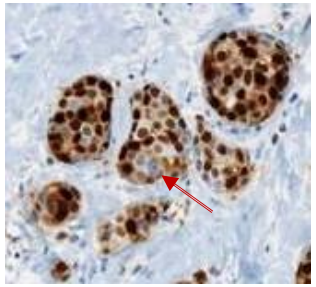
Subjective
Morphology-limited
Tumor complexity-limited

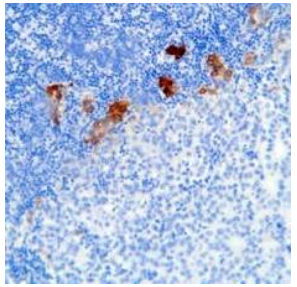


Objective
True biology
Global tumor profile









CSC population in CRC = 1/5.7x10000[2]

[1] Nuciforo, Fraggetta. **Cancer stem cell theory: pathologists' considerations and ruminations about wasting time and wrong evaluations,** JCP 2004; [2] O'Brien et al, 2007

The future of Pathology

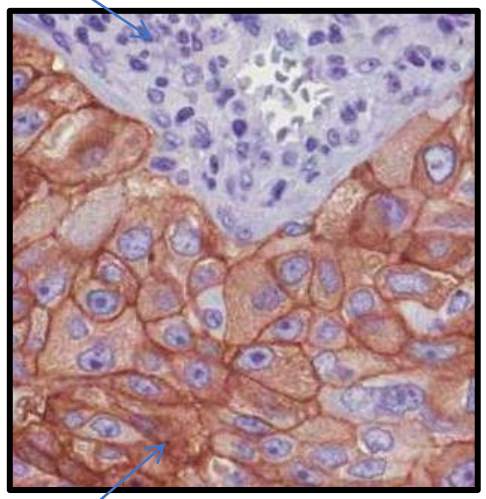
FROM GLASS

TO DIGITAL

From glass to digital

TUMOR INFILTRATING LYMPHOCYTES (TILS)

Inflammatory T cells



Tumor cell

- MSI-H
- Better overall survival
- Lesser venous/lymphatic invasión
- Lower pTNM stage
- Expansive growth
- Proximal location
- Younger patients
- EBV infection
- *Response to immunotherapy*

Gullo I, Carneiro F, 2016

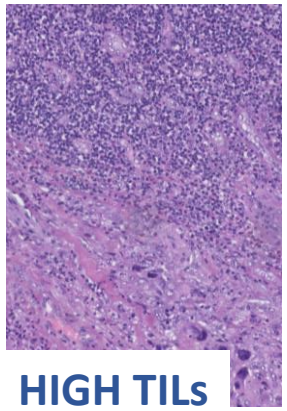
From glass to digital

TUMOR INFILTRATING LYMPHOCYTES (TILS)

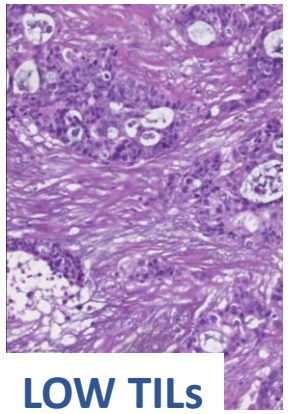
1. How to quantify TILs ?

No guidelines, No consensus, but worth doing it…

Glass-based semiquantitative assessment



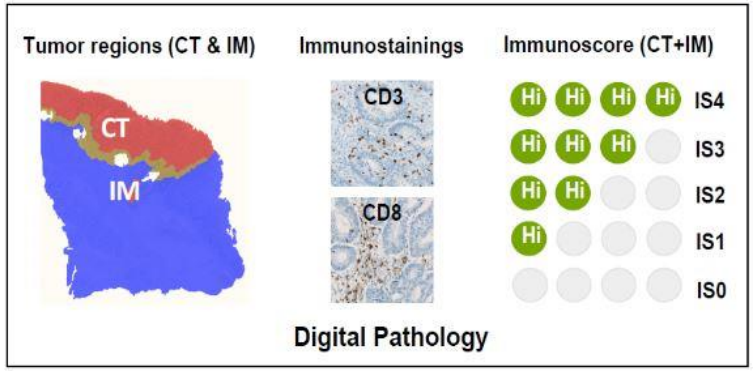
HIGH TILs



LOW TILs

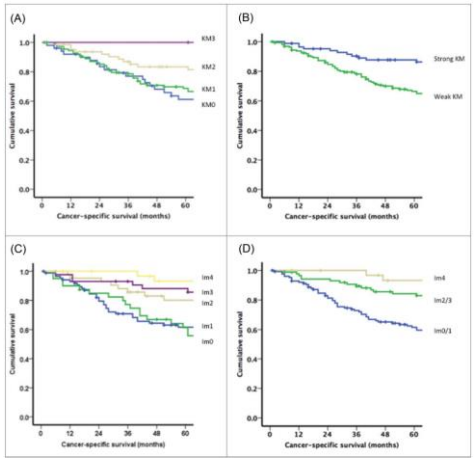
- Jass JR, et al. J Clin Pathol 1986; 39: 585-589.
- Klintrup K, et al. Eur J Cancer 2005; 41: 2645-2654.
- Richards CH, et al. Eur J Cancer 2014; 50: 309-319

IHC-based (digital) quantitation



- Galon J, Science 2006; 313: 1960-1964.
- Pages, J Clin Oncol 2009; 27: 5944-5951.
- Galon J, Journal of Translational Medicine 2012; **10**: 205

Brindging studies



- Vayrynen JP, *et al. Virchows Arch* 2012; **460**: 455-465.
- Richards CH, et al. Eur J Cancer 2014; 50: 309-319.
- Park JH, et al. OncoImmunology 2016; 5: e1098801.

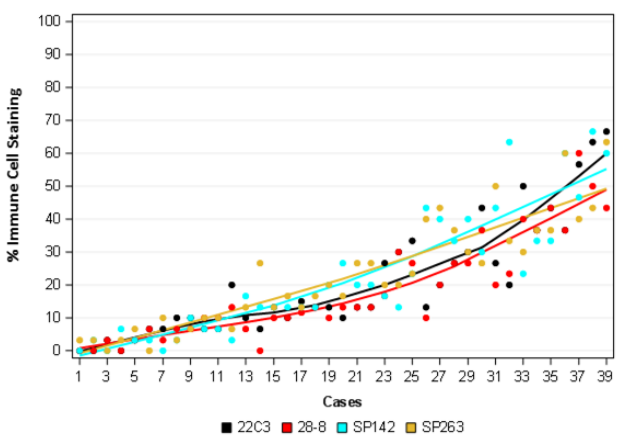
From glass to digital

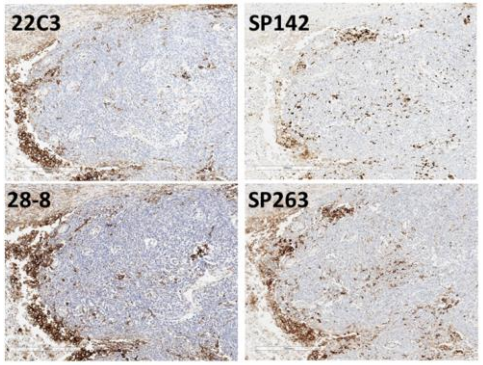
TUMOR INFILTRATING LYMPHOCYTES (TILS)

2. **How to quantify a biomarker expressed in TILs ?**

Better digital!

Mean PDL1 <u>immune cells</u> proportion score per case (3 readers)





BluePrint Study, Adapted from Hirsch, IASCL, AACR 2016

PD-L1 IHC Intra-class Correlation Coefficient (ICC)

ICC for Pathologists by Each Antibody in Tumor					
	22C3	28-8	SP142	E1L3N	SUMMARY
All, N=90	0.882	0.832	0.869	0.859	0.86 (0.02)

ICC for Pathologists by Each Antibody in Immune Cells					
	22C3	28-8	SP142	E1L3N	SUMMARY
All, N=90	0.207	0.172	0.185	0.229	0.19 (0.03)

ICC or kappa agreement measure assessment:
<40: poor
0.40–0.59: fair
0.60–0.74: good
0.75–1.00: excellent

Rimm et al. JAMA Oncol 2017

The future of Pathology

FROM GLASS → TO DIGITAL

FROM QUASI-QUANTITATIVE → TO DYNAMIC RANGE

From quasi-quantitative to dynamic range

HER2 in GEC

OS by HER2 status **(ToGa)** [1]

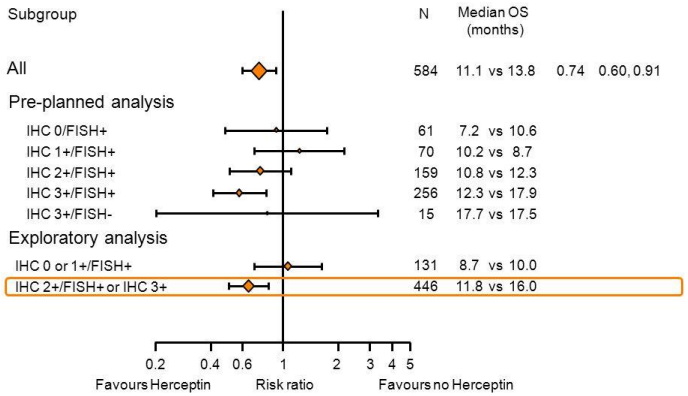


Table 1 Major clinical trials in gastric adenocarcinoma (GAC) with *HER2/neu* targeted agents [2]

Target	Trial	Type of study/line	Patients selection method	Regimen	Results (primary endpoint)	Reference
HER2	ToGa	Phase III/first	HER2 IHC	5-FU/capecitabine cisplatin ± trastuzumab	Positive (OS)	Bang *et al*. 2010
HER2	LOGIC	Phase III/first	HER2 amplification	Lapatinib *vs*. XELOX	Negative (OS)	Hecht *et al*. 2016
HER2	TYTAN	Phase III/second	HER2 amplification	Paclitaxel + lapatinib *vs*. paclitaxel	Negative (OS)	Satoh *et al*. 2014
HER2	GATSBY	Phase II/III/second	HER2 IHC	TDM1 *vs*. paclitaxel or docetaxel	Negative (OS)	Knag *et al*. 2016

IHC, immunohistochemical; OS, overall survival.

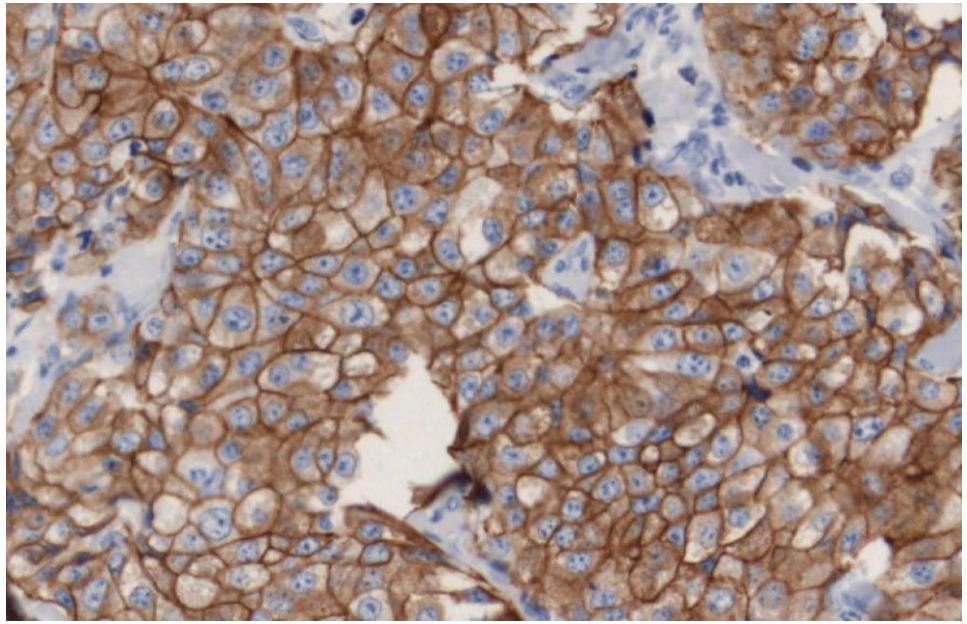
TYTAN, HER2 FISH+/IHC 3+ had better OS when treated with lapatinib (HR, 0.59; P=.0176) [3]

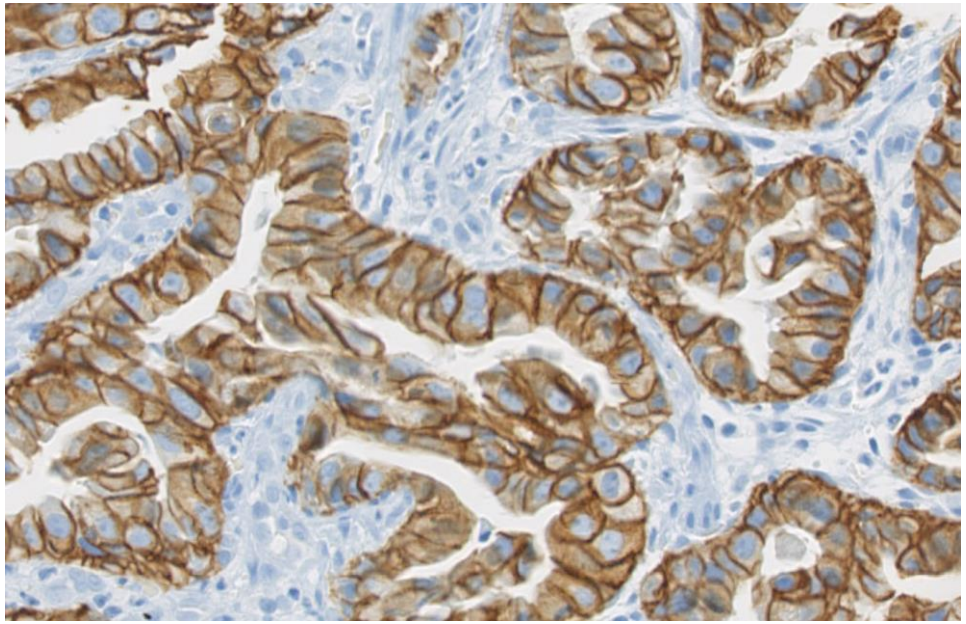
LOGIC, HER2 ratio >10 (n=176, 33%) had better PFS when treated with lapatinib (HR, 0.62 P=.0033) [4]

Quantifying HER2 may better predict response to HER2 inhibition

[1]Van Cutsern, J Clin Oncol 2009
[2]Cetin B, Ozet A, Transl Gastroenterol Hepatol 2016
[3]Satoh et al, 2014
[4]Press et al, Mol Cancer Ther 2017

HER2 in Breast versus Gastric cancer





“Ad hoc” interpretation criteria exclusive of GEJ cancers:

- Membrane staining pattern
- Heterogeneity
- Biopsy versus surgical specimen

Bartley, Arch Pathol Lab Med 2016

HER2 positivity by Immunohistochemistry

10%

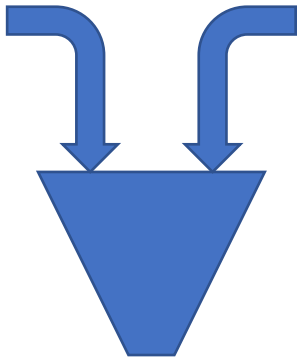
IHC 3+

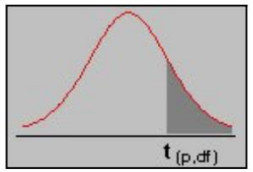
Equal or greater than 10% strong membrane staining or Cancer cell cluster (5 cells in GC biopsies)

Breast Cancer

87%-96% homogeneous[1-3]

[1]Brunelli M, et al. Am J Clin Pathol 2009; 131: 678–82.
[2]Seol H, et al. Mod Pathol 2012; 25: 938–48.
[3]Chang MC, et al. Mod Pathol 2012; 25: 683–8.



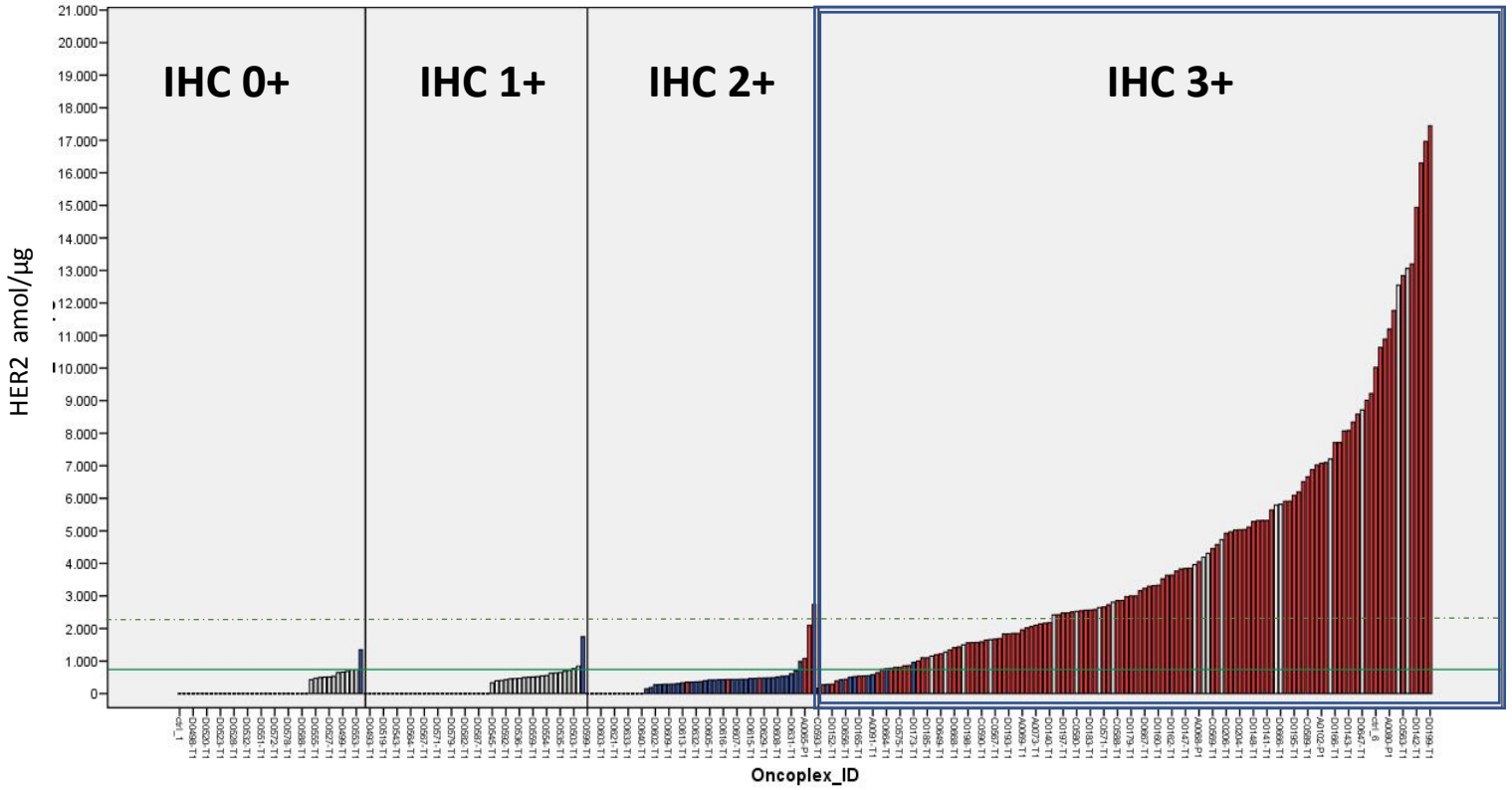


Gastric Cancer

31%-95% homogeneous[4-6]

[4]Van Cutsem E, et al. *Gastric Cancer* 2015; 18: 476-484.
[5]Hofmann M, et al. *Histopathology* 2008; 52: 797-805.
[6]Ahn S, et al. *Oncotarget* 2015; 6: 38372-38380.

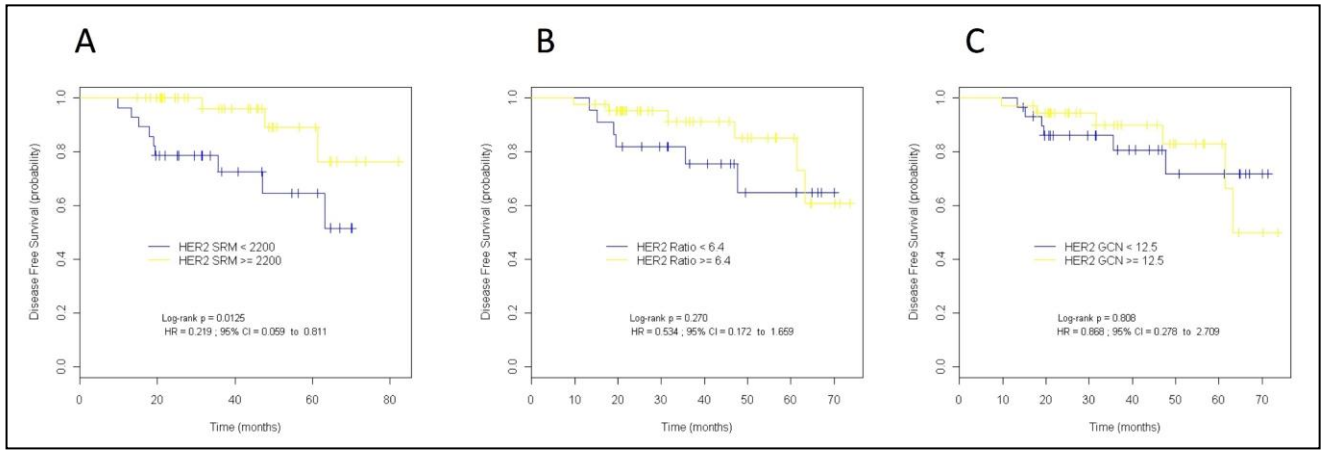
HER2 quantification by Mass Spectrometry - Breast Cancer (n=277)



HER2 3+ tumors dynamic range:
BC= <164-17.447 amol/ug **(105 fold range)**[1]

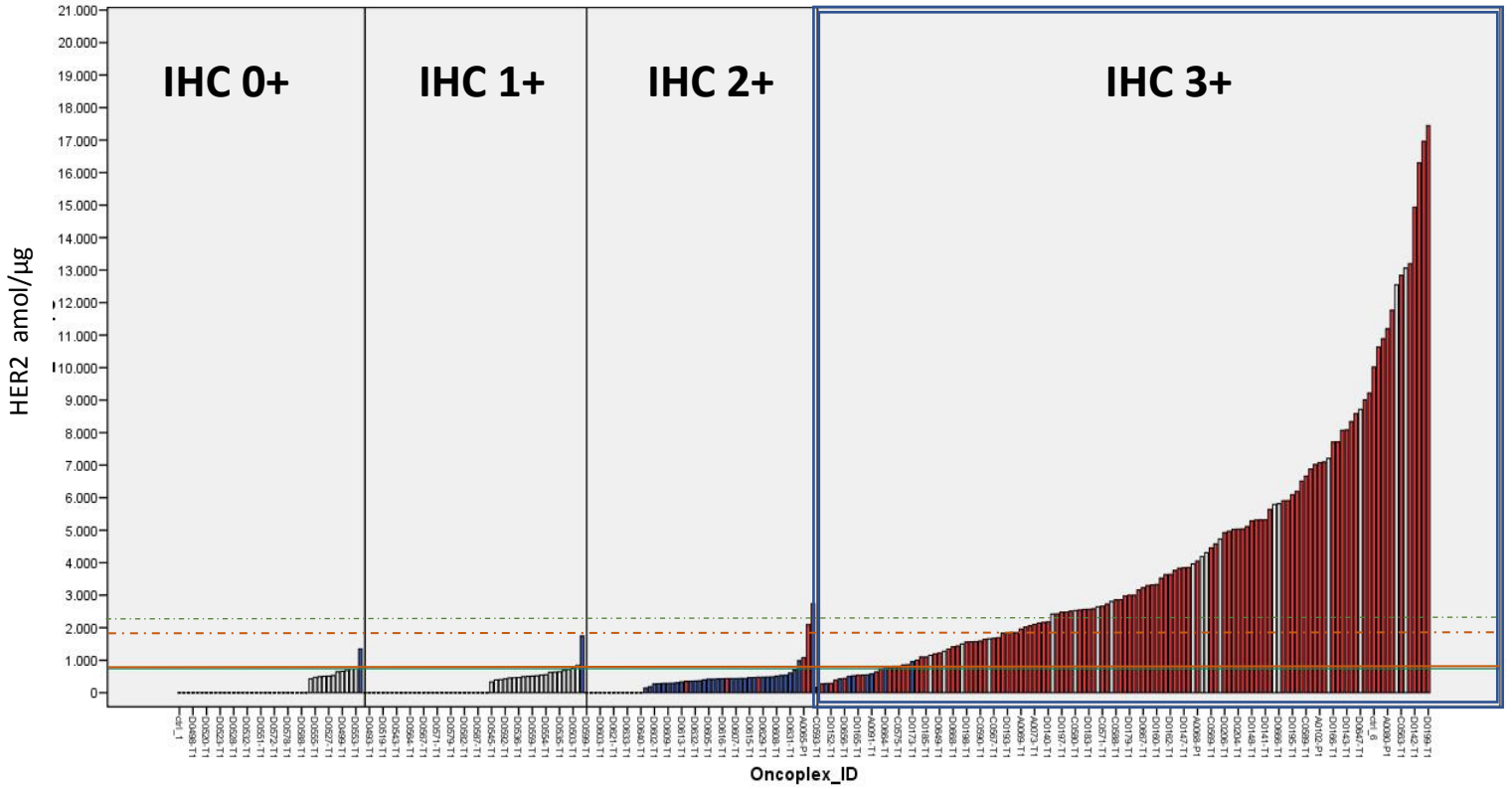
CB: 2200 amol/µg[1]

ST: 740 amol/µg (AUC=0.96)[1]



[1] Nuciforo et al. Mol Oncol 2016

HER2 quantification by Mass Spectrometry - Gastric Cancer (n=237)

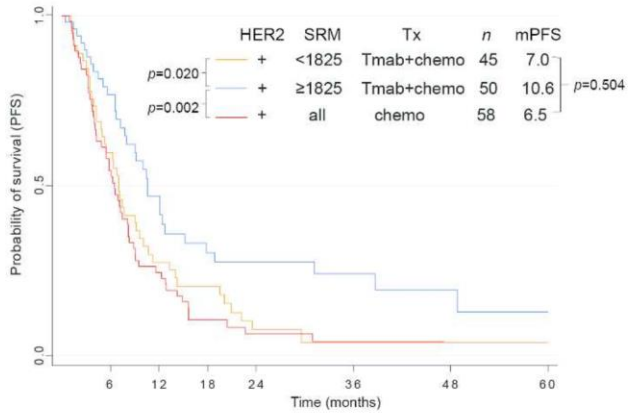


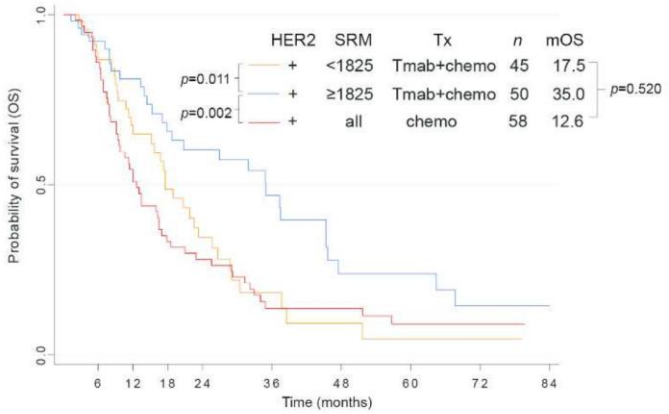
HER2 3+ tumors dynamic range:

BC= <164-17.447 amol/ug **(105 fold change)**[1]

GC=<200-23.055 amol/ug **(115 fold range)**[2]

CB: 2200 amol/µg[1]
CB: 1825 amol/µg[2]
ST: 750 amol/µg (AUC=0.85)[2]
ST: 740 amol/µg (AUC=0.96)[1]





FISH+/No protein expression

9% (BC) vs **31% (GC)**

[1] Nuciforo et al. Mol Oncol 2016

[2] An et al. Ann Oncol 2017

The future of Pathology

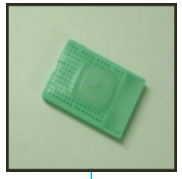
FROM GLASS → TO DIGITAL

FROM QUASI-QUANTITATIVE → TO DYNAMIC RANGE

FROM SINGLE → TO MULTIPLEXING

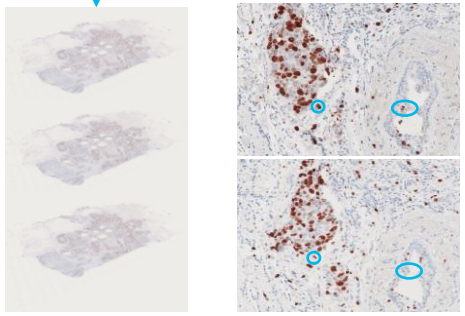
From single biomarker to multiplexing

Traditional approach



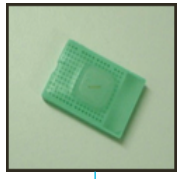
***N* Serial sections**
N markers

Stain 1
Stain 2
Stain n

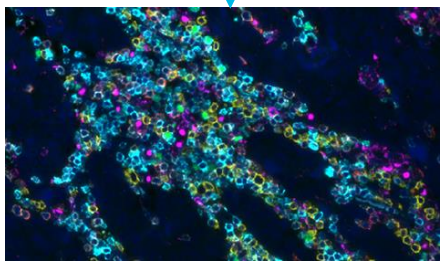


- **PRO:**
  - Easy to evaluate (one BM at a time, bright field)
- **CONS:**
  - Multiplexing at a single cell resolution not possible
  - Sample quickly exahusted

IF approach



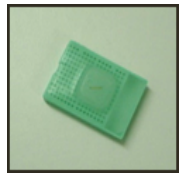
1 section
5 markers



Ventana 5-plex

- **PRO:**
  - Multiplexing at a single cell resolution
  - Preserve sample
- **CONS:**
  - Limited by the number of fluorochromes
  - Difficult to evaluate

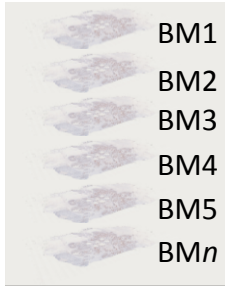
Next Gen IHC



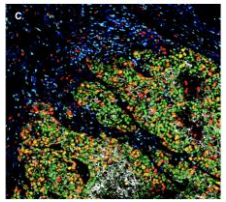
1 section
n markers

Sequencial cycles of

1. Stain
2. Scan
3. Strip



Integrated virtual image

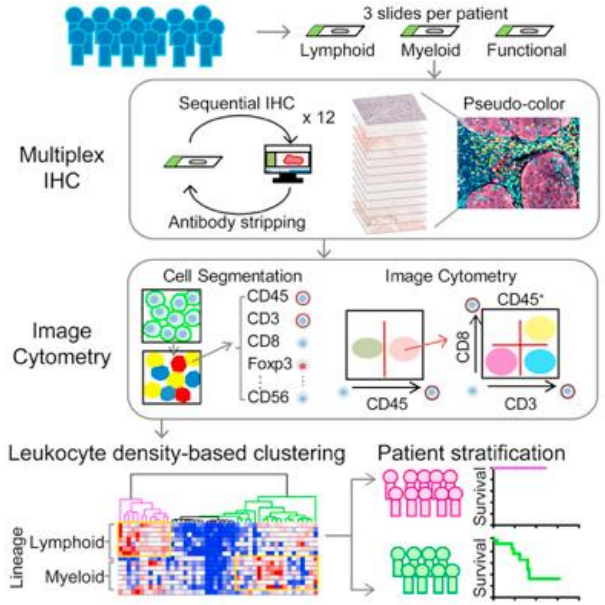


- **PRO:**
  - All PROs of Traditional and IF approaches
- **CONS:**
  - Find one!

From single biomarker to multiplexing

NEXT GENERATION IHC

- Characterization of the expression of ***multiple biomarkers in the same cell*** using a single FFPE tissue section.
- ***Not*** limited by the available ***fluorochromes*** as for IF.
- ***Easy to manually score*** as a single biomarker, powerful when automatized for multiplexing.
- Studying ***intratumor heterogeneity***.
- Exploring ***spatial interaction*** between tumor and its microenvironment.
- ***Maximizing sample use*** for diagnostic and research analyses.
- ***Overcoming limitations of small biopsy*** samples to provide sufficient material for diagnostic IHC/FISH and Molecular analyses (Sequencing, Transcriptomic, …).



Tsujikawa et al. Quantitative multiplex immunohistochemistry reveals myeloid.-inflamed tumor-immune complexity associated with poor prognosis. Cell Reports 2017 19, 203-217.

The future of Pathology

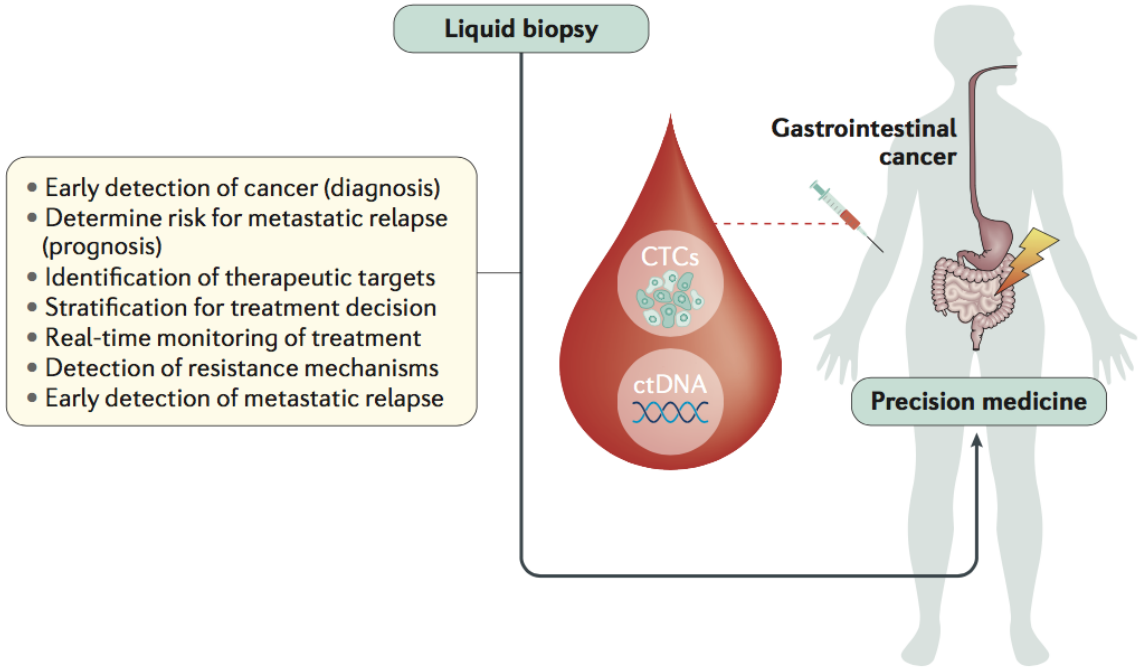
FROM GLASS → TO DIGITAL

FROM QUASI-QUANTITATIVE → TO DYNAMIC RANGE

FROM SINGLE → TO MULTIPLEXING

FROM TISSUE → TO LIQUID

From tissue to liquid



• Genomic heterogeneity in colorectal cancer is associated with acquired resistance to targeted agents[1]

• Circulating DNA persistence after colon cancer surgery is associated with an increased risk of relapse in Stage II CRC[2]

- No adjuvant chemo, 79% vs 9.8% recurrence in ctDNA positive vs negative patients
- ctDNA positivity after adjuvant chemo associated with poored RFS (R11; 95% CI, 1.8 to 68, P=0.001)

• Dynamic monitoring of circulating tumour cells (CTCs) evaluates therapeutic efficacy in advanced gastric cancer[3]

- >3 CTCs per 7.5ml blood correlated with poor therapeutic outcome
- Treatment induced conversion to a favorable CTCs levels improved prognosis

Klaus Pantel and Catherine Alix-Panabières, Jan 2017 NATURE REVIEWS | **GASTROENTEROLOGY & HEPATOLOGY**

1.Russo, M. et al. Cancer Discov. 6, 147–153 (2016).
2.Tie, J. et al. Sci. Transl. Med. 8, 346ra92 (2016).
3.Li, Y. et alBr. J. Cancer 114, 138–145 (2016).

Detection of ctDNA depends of

- Abundance of ctDNA in the blood (0,01% to 60% of total DNA, early vs late stage disease);
- Sensitivity of the method used and sequencing depth;
- Number of features interrogated.

- Sensitivity of 50%[1]
- Risk of detecting age-related somatic mutations[2]
- Require a priori knowledge of the mutation status of the tumor determined in tissue

[1]Tie et al. Sci Transl Med 2016
[2]Jaiswal et al, N Engl J Med 2014

Tissue vs liquid biopsy

	Tissue biopsy	CTC	ctDNA
Genomics	HIGH	LOW	MODERATE
Gene expression	HIGH	MODERATE	LOW
Protein	HIGH	MODERATE	N/A
Heterogeneity	HIGH	MODERATE	MODERATE
Spatial context	HIGH	LOW	LOW
Quality	HIGH (FF)/MODERATE (FFPE)	LOW	LOW
Quantity	HIGH/MODERATE	VERY LOW	VERY LOW
Tumor content	HIGH	LOW	LOW
False negative	LOW	HIGH	HIGH
False positive	LOW	MODERATE	MODERATE

Tissue vs liquid biopsy: Platform comparison

- Foundation one (F1, tissue) vs Guardant 360 (G360, cfDNA)

- **Concordance between platforms**:
  - 10/45 (22%) alterations detectable by both platforms
  - 9/36 (25%) drugs recommended for the same patient by both platforms
  - Higher mutation frequency in G360 as compared to F1 (MAF <1%)

- **Possible reasons of discordance**:
  - Timing between the 2 tests (7 of 8 patients, <2.5 months)
  - Tumor heterogeneity
  - Variant calling process

Kuderer et al, Jama Oncol 2017

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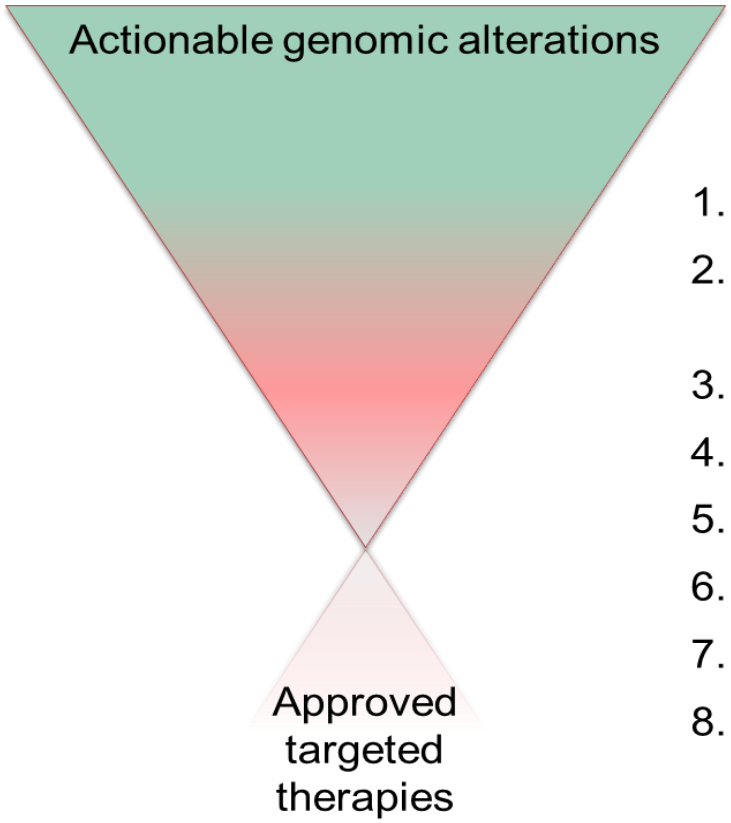
FROM SINGLE → TO MULTIPLEXING

FROM TISSUE → TO LIQUID

FROM ACTIONABLE → TO APPROVED TX

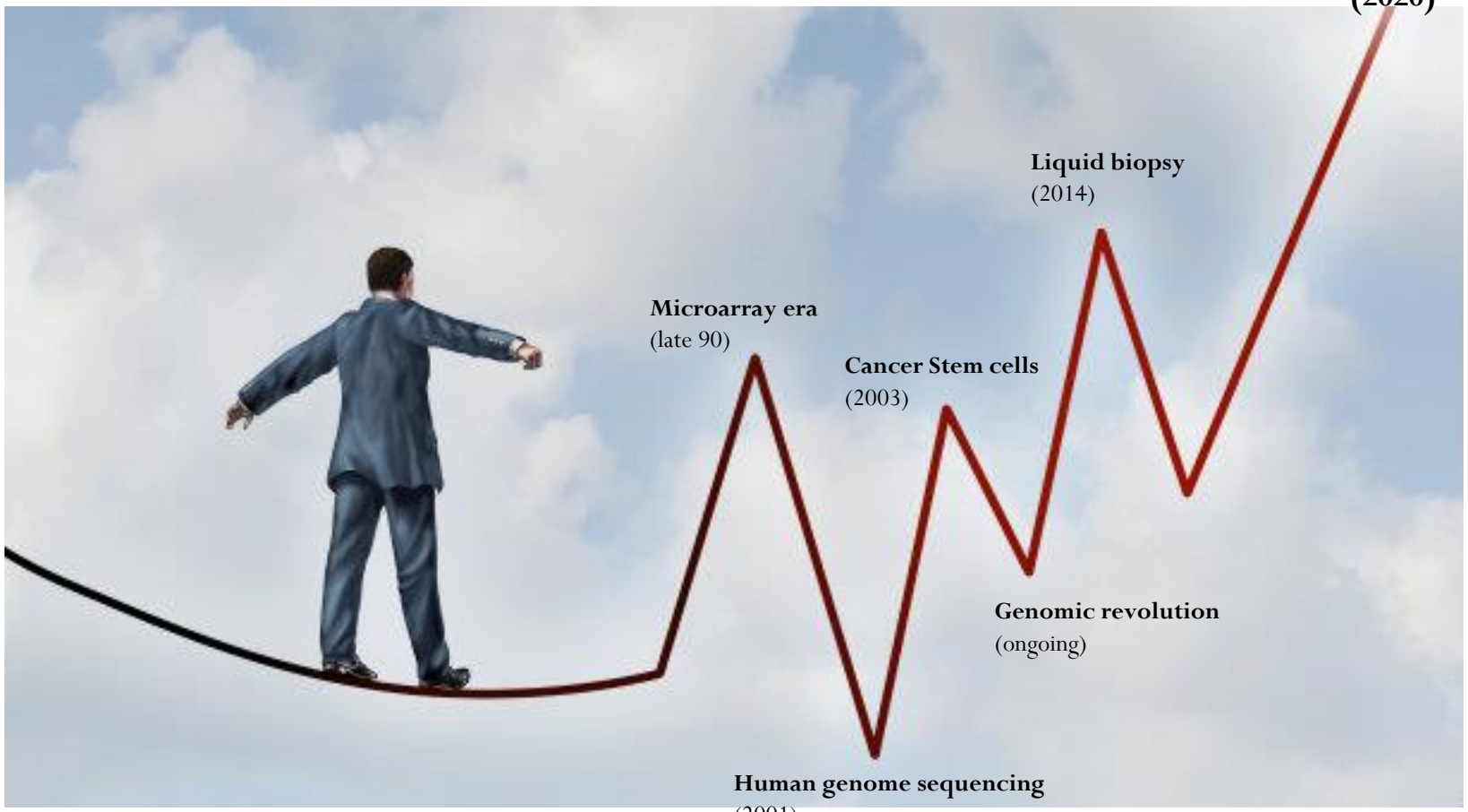
From actionable genomic alterations to approved targeted therapies



1. Technical reproducibility of “omics” platforms
2. Quality and size of available library used to identify molecules
3. False-positive results in global “omics”
4. Statistical reproducibility
5. Lack of prospective validation
6. Intra- and inter-sample heterogeneity
7. Disconnection between genotype and phenotype
8. Tissue levels BM are invasive

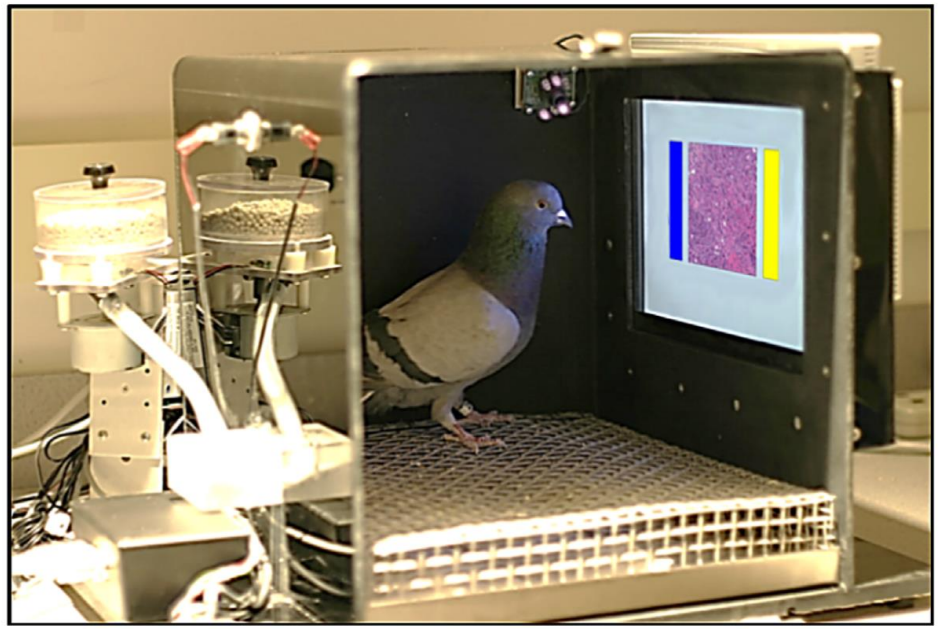
THE PATHOLOGIST'S RESURRECTION

The (hard) life of a Pathologist: the next challenge ???

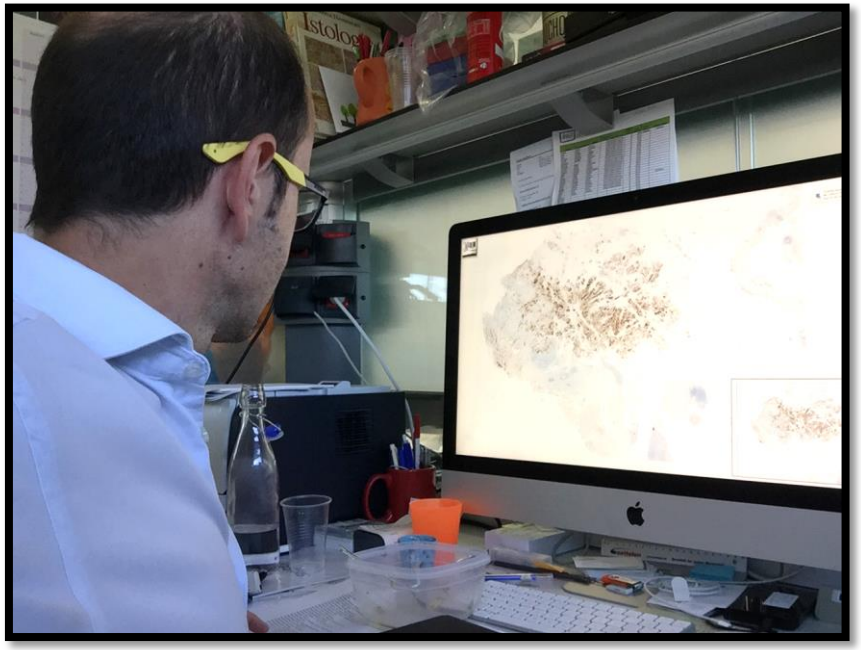


Pigeons (Columba livia) as Trainable observers of Pathology and Radiology Breast Cancer Images

Levenson et al, Plos one 2015



The pigeons' training environment



The pathologist's training environment

THE FUTURE OF PATHOLOGY

To the Editor:—Pathologists will doubtless agree in general with the Dorland dictionary definition of pathology as "that branch of medicine which treats of the essential nature of disease." Many, therefore, were doubtless disturbed, as I was, to read in the editorial comment in THE JOURNAL, January 1, page 50, under the heading given above, most of a column devoted to the activities of the hospital and private laboratory (i. e., largely diagnostic procedures). Granting the correctness of the statements about pathology as applied to hospital and private laboratories, though much of this work may be technically biochemical, bacteriologic or serologic, should it not have indicated that only one phase of pathology was being considered? The future of pathology as a whole will be chiefly affected by its efficiency in maintaining and improving pathologic teaching and investigation and by the ability of all kinds of pathologists to adapt their specialty to the ever changing aspects of medical progress. Such factors as the pathologist's adequate control of hospital laboratory work, the part he takes in organized medicine and even his interest in clinical medicine, desirable and important though these features are, would seem to be of less importance to the future of the discipline.

When Dr. Kracke made his presidential address to the American Society of Clinical Pathologists, it is obvious that his hearers correctly understood his use of the term "pathology" as referring to the type of work that his society was concerned with. As a topic in the editorial comment, however, the "future of pathology," interpreted in this way, presents Dr. Kracke's special and narrower meaning to such a large number of the medical profession that serious misconception of the proper scope of pathology is unavoidable. I ask, then, that you publish this reminder that pathology is a basic branch of medical science which has been defined as dealing with "the causation, development, nature of and disturbances—structural and functional—produced by disease."

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"The future of pathology as a whole will be chiefly affected by … the ability of all kinds of pathologists to adapt their specialty to the ever changing aspects of medical progress"

E.B. KRUMBHAAR, JAMA, 1938; 110 (6):457.

Thanks