The pathologist of the future

Paolo G Nuciforo, MD PhD
Vall d’Hebron Institute of Oncology

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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 = 1/5.7x10000²
The future of Pathology

FROM GLASS  TO DIGITAL
From glass to digital

TUMOR INFILTRATING LYMPHOCYTES (TILS)

- MSI-H
- Better overall survival
- Lesser venous/lymphatic invasion
- 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

IHC-based (digital) quantitation

Brindging studies

2. How to quantify a biomarker expressed in TILs?

Better digital!

Mean PDL1 immune cells proportion score per case (3 readers)

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

<table>
<thead>
<tr>
<th>Antibody</th>
<th>22C3</th>
<th>28-8</th>
<th>SP142</th>
<th>E1L3N</th>
<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>All, N=90</td>
<td>0.882</td>
<td>0.832</td>
<td>0.869</td>
<td>0.859</td>
<td>0.86 (0.02)</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Antibody</th>
<th>22C3</th>
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<th>SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>All, N=90</td>
<td>0.207</td>
<td>0.172</td>
<td>0.185</td>
<td>0.229</td>
<td>0.19 (0.03)</td>
</tr>
</tbody>
</table>

ICC or kappa agreement measure assessment:
- <0.40: 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>
<thead>
<tr>
<th>Subgroup</th>
<th>N</th>
<th>Median OS (months)</th>
<th>HR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-planned analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 0/FISH+</td>
<td>61</td>
<td>7.2 ± 10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 1/FISH+</td>
<td>70</td>
<td>10.2 ± 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 2/FISH+</td>
<td>156</td>
<td>10.8 ± 12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 3/FISH+</td>
<td>256</td>
<td>12.3 ± 17.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 4/FISH+</td>
<td>10</td>
<td>17.7 ± 17.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploratory analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 0.1/FISH+</td>
<td>131</td>
<td>8.7 ± 10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2 0.2/FISH+ or 0.2C 3+</td>
<td>446</td>
<td>11.8 ± 18.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Van Cutsem, 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

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

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

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
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

IHC 3+
Equal or greater than 10% strong membrane staining or Cancer cell cluster (5 cells in GC biopsies)

Breast Cancer
87%-96% homogeneous\textsuperscript{1-3}


Gastric Cancer
31%-95% homogeneous\textsuperscript{4-6}

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

HER2 3+ tumors dynamic range:
BC = <164-17.447 amol/µg (105 fold range)\(^1\)

CB: 2200 amol/µg\(^1\)
ST: 740 amol/µg (AUC=0.96)\(^2\)

\(^1\) 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/µg (105 fold change)\(^1\)
GC = <200-23.055 amol/µg (115 fold range)\(^2\)

CB: 2200 amol/µg\(^1\)
ST: 740 amol/µg (AUC=0.96)\(^2\)
GC: <200-23.055 amol/µg (115 fold range)\(^2\)

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 exhausted

**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**

**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, ...).

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

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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 mutations²
- Require a priori knowledge of the mutation status of the tumor determined in tissue

¹Tie et al. Sci Transl Med 2016
## Tissue vs liquid biopsy

<table>
<thead>
<tr>
<th></th>
<th>Tissue biopsy</th>
<th>CTC</th>
<th>ctDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomics</td>
<td>HIGH</td>
<td>LOW</td>
<td>MODERATE</td>
</tr>
<tr>
<td>Gene expression</td>
<td>HIGH</td>
<td>MODERATE</td>
<td>LOW</td>
</tr>
<tr>
<td>Protein</td>
<td>HIGH</td>
<td>MODERATE</td>
<td>N/A</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>HIGH</td>
<td>MODERATE</td>
<td>MODERATE</td>
</tr>
<tr>
<td>Spatial context</td>
<td>HIGH</td>
<td>LOW</td>
<td>LOW</td>
</tr>
<tr>
<td>Quality</td>
<td>HIGH (FF)/MODERATE (FFPE)</td>
<td>LOW</td>
<td>LOW</td>
</tr>
<tr>
<td>Quantity</td>
<td>HIGH/MODERATE</td>
<td>VERY LOW</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Tumor content</td>
<td>HIGH</td>
<td>LOW</td>
<td>LOW</td>
</tr>
<tr>
<td>False negative</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td>False positive</td>
<td>LOW</td>
<td>MODERATE</td>
<td>MODERATE</td>
</tr>
</tbody>
</table>

**Note:**
- **HIGH**: High level of feature
- **LOW**: Low level of feature
- **MODERATE**: Moderate level of feature
- **N/A**: Not applicable
- **VERY LOW**: Very low level of feature
- **FF**: Fresh frozen
- **FFPE**: Formalin-fixed paraffin-embedded
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
The future of Pathology

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FROM QUASI-QUANTITATIVE TO DYNAMIC RANGE
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 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”

Thanks