Immunohistochemistry in clinical research

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Outline

• 3 examples
  – a new biomarker
  – an old biomarker across distinct entities
  – an old biomarker in a new clinical scenario
Outline

• 3 examples
  – a new biomarker
  – an old biomarker across distinct entities
  – an old biomarker in a new clinical scenario
**NTRK fusions across tumor types**

Cancers enriched for TRK fusions
- Frequency >90%

Cancers harbouring TRK fusions at lower frequencies
- 5% to 25%
- <5%

*Image showing various types of cancers and their locations.*

Coco E et al, Nature Clin Review 2018
Neurotrophic tropomyosin receptor kinase (NTRK)

- **NTRK1**
  - 1q21-q22 – TRKA

- **NTRK2**
  - 9q22.1 – TRKB

- **NTRK3**
  - 15q25 – TRKC

- Tyrosine kinases that play roles in
  - Neuronal differentiation and development, including the growth and function of neuronal synapses and memory development
  - Expression restricted to specific tissues
Neurotrophic tropomyosin receptor kinase (NTRK)

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- Tyrosine kinases that play roles in
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  - **Expression restricted to specific tissues**
Binding of neurotrophins to the extracellular region of TRK proteins

Receptor homodimerization

Transactivation of the intracellular tyrosine kinase domains

Recruitment of various cytoplasmic adaptors

Activation downstream signalling pathways, including the MAPK, PI3K and PKC pathways

Differentiation and survival in neuronal cells

Cocco E, Scaltriti M & Drilon A, Nature Reviews Clinical Oncol 2018
**NTRK fusions across tumor types**

**High frequency in special histologic types**
- Secretory breast carcinoma
- Mammary analogue secretory carcinoma of the salivary glands (MASC)
- Congenital infantile fibrosarcoma

**ETV6-NTRK3 rearrangement**

**Low frequency in common forms of different types of cancers**
- Thyroid PTC
- GIST (lacking canonical KIT/PDGFRA/RAS alterations)
- Lung cancer
- Carcinomas of the GI tract
- Melanoma
- Sarcomas
- Gliomas
- Acute myeloid and acute lymphoblastic leukemias

**NTRK1, NTRK2, NTRK3 rearrangements**
DETECTION OF GENE FUSIONS ACROSS DIFFERENT TUMOUR TYPES

Spectrum of kinase fusions identified by MSK-IMPACT

Zehir A et al, Nature Medicine 2018
NTRK rearrangements create chimaeric genes

This may stem from intra-chromosomal or inter-chromosomal rearrangements
NTRKs are promiscuous: multitude of 5’ partners

Many 5’ gene partners (at least 25) described
All rearrangements share an in-frame, intact kinase domain

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
NTRK receptor signaling

NTRK fusions have oncogenic properties:

- induction of cancer cell proliferation
- activation of critical cancer-related downstream signaling pathways (e.g. MAPK and PI3K/AKT)

Amatu A et al, ESMO open 2016
EFFICACY OF NTRK INHIBITORS

Drilon A et al, Cancer Discovery 2017

Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1)

Drilon A et al, NEJM 2018

Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children

Change in tumor diameter
## FDA drug approvals in 2018

### Cutaneous squamous cell cancer
- Cemiplimab-rwlc
- Pembrolizumab

### Merkel cell cancer
- Pembrolizumab

### Adrenal gland (PPGL)
- Iobenguane I-131

### Breast cancer
- Talazoparib
- Ribociclib
- Abemaciclib
- Olaparib
- Trastuzumab-pkrb

### Cervical cancer
- Pembrolizumab

### Colorectal cancer
- Ipilimumab + nivolumab

### GEP-NET
- Lutetium Lu-177 dotatate

### Hepatocellular cancer
- Pembrolizumab
- Lenvatinib

### Ovarian cancer
- Bevacizumab
- Rucaparib
- Olaparib

### Prostate cancer
- Enzalutamide
- Apalutamide
- Abiraterone acetate

### Leukaemia
- Gilteritinib
- Duvelisib
- Moxetumomab pasudotox-tdfk
- Iosidenib
- Venetoclax
- Blinatumomab
- Nilotinib
- Calaspargase pegol-mknl
- Glasdegib

### Lymphoma
- Tisagenlecleucel
- Brentuximab vedotin
- Duvelisib
- Rituximab-abb
- Pembrolizumab

### BPDCN
- Tagraxofusp-erz

### HLH
- Emapalumab

### Supportive care
- Pegfilgrastim-jmdlb
- Epoetin alfa-epbx

### TSC-associated seizures
- Everolimus

### Renal cancer
- Ipilimumab + nivolumab

### Lung cancer
- Daclomitinib
- Lorlatinib
- Pembrolizumab
- Nivolumab
- Osimertinib
- Durvalumab
- Afinitin
- Atezolizumab

### Urothelial cancer
- Pembrolizumab
- Atezolizumab

### NTRK fusion-positive cancers (histology agnostic)
- Larotrectinib
**NTRK FUSION DETECTION: POSSIBLE TOOLS**

- Technical options

- **IHC**
  - Ab anti TRKA
  - Ab anti-TRKB
  - panTRK Ab
  - Cocktail of Abs

- **FISH**
  - Commercially available probes
  - In-house constructed probes

- **RT-PCR**
  - Specific probes designed for NTRK1/NTRK2, NTRK3

- **NanoString**
  - nCounter Lung Fusion Panel
  - Custom panel

- **NGS**
  - Global RNAseq
  - Targeted panels
    - DNA-based
    - RNA-based
    - DNA/RNA panels

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
### ADVANTAGES AND DRAWBACKS OF THE DIFFERENT METHODS AT A GLANCE

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Detection of all fusion genes</th>
<th>Detection of partner</th>
<th>Detection of expression</th>
<th>Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>High</td>
<td>Moderate/High</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>FISH</td>
<td>High</td>
<td>High</td>
<td>One per probe</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>RNA seq NGS</td>
<td>High</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA seq</td>
<td>Moderate</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
Given the restricted expression of TRKA, TRKB and TRKC in adult tissues (i.e. smooth muscle, testes and neuronal components) => IHC is highly sensitive (from 95% to 100%) and specific (from 93% to 100%) for the detection of NTRK rearrangements

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
IHC

Further advantages:

• it is a rapid method that can be easily employed in different laboratory environments => quick turnaround time
• it is able theoretically to detect only transcribed and translated fusion proteins

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
IHC

The pattern of TRK expression detected by IHC can be variable in intensity and subcellular localisation

⇒ **Nuclear** pan-TRK IHC can be considered a diagnostic surrogate of *NTRK3* fusions

⇒ Moderate to strong diffuse **cytoplasmic** pan-TRK IHC staining can be considered diagnostic of *NTRK1/NTRK2* fusions

*ETV6-NTRK3* fusion positive case from *Am J Surg Pathol* 2017;41:1547–1551

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, *Annals of Oncology* 2019; doi:10.1093/annonc/mdz204
The pattern of TRK expression detected by IHC can be variable in intensity and subcellular localisation

=> For tumours with only weak cytoplasmic expression of pan-TRK, an \textit{NTRK} fusion should be confirmed by other molecular/cytogenetic methods to ensure that a fusion is present in patients being considered for targeted therapeutic agents

\textit{“Two-step approach”}

IHC as a screening method \hspace{1cm} \rightarrow \hspace{1cm} Sensitivity is crucial!

NGS to confirm the presence of rearrangement

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019; doi:10.1093/annonc/mdz204
IHC - pitfalls

• Most of the available antibodies are RUO
Pan-Trk immunohistochemical reactions with antibody clone EPR17341 (Abcam, Cambridge, MA)

**Table 3** Sensitivity and specificity of pan-Trk immunohistochemistry for detecting NTRK fusions

<table>
<thead>
<tr>
<th>NTRK</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTRK1</td>
<td>96.2% (26/27)</td>
</tr>
<tr>
<td>NTRK2</td>
<td>100% (5/5)</td>
</tr>
<tr>
<td>NTRK3</td>
<td>79.4% (27/34)</td>
</tr>
</tbody>
</table>

Immunohistochemistry showed overall sensitivity of 87.9% and specificity of 81.1%, with high sensitivity for NTRK1 (96%) and NTRK2 (100%) fusions and lower sensitivity for NTRK3 fusions (79%).
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>87.9% (58/66)</td>
<td>81.1% (257/317)</td>
</tr>
<tr>
<td>Colon</td>
<td>87.5% (7/8)</td>
<td>100% (25/25)</td>
</tr>
<tr>
<td>Lung</td>
<td>87.5% (7/8)</td>
<td>100% (24/24)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>81.8% (9/11)</td>
<td>100% (27/27)</td>
</tr>
<tr>
<td>Salivary</td>
<td>88.9% (8/9)</td>
<td>52% (13/25)</td>
</tr>
<tr>
<td>Breast</td>
<td>80% (4/5)</td>
<td>82.1% (23/28)</td>
</tr>
<tr>
<td>Inflammatory myofibroblastic tumor</td>
<td>100% (3/3)</td>
<td>100% (5/5)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>80% (8/10)</td>
<td>74.4% (29/39)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>(0/0) (^a)</td>
<td>100% (20/20)</td>
</tr>
<tr>
<td>Appendix</td>
<td>100% (1/1)</td>
<td>100% (1/1)</td>
</tr>
<tr>
<td>Cholangio</td>
<td>100% (2/2)</td>
<td>100% (19/19)</td>
</tr>
<tr>
<td>Glioma</td>
<td>100% (6/6)</td>
<td>20.8% (5/24)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>100% (3/3)</td>
<td>100% (17/17)</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>(0/0) (^a)</td>
<td>88.9% (8/9)</td>
</tr>
<tr>
<td>Small round cell tumor(^b)</td>
<td>(0/0)</td>
<td>45.8% (11/24)</td>
</tr>
<tr>
<td>Other(^c)</td>
<td>(0/0)</td>
<td>100% (30/30)</td>
</tr>
</tbody>
</table>

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Modern Pathology 2019; doi.org/10.1038/s41379-019-0324-7

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ETV6-NTRK3
IHC - pitfalls

- Most of the available antibodies are RUO
- Ventana pan-TRK is CE-IVD:
  kit preparation
NTRK FUSION DETECTION: THE ESMO PROPOSAL

Sample to be investigated for the presence of NTRK fusions

As a confirmatory technique use FISH, RT-PCR or targeted RNA NGS assays with specific probes for the fusion involving the known NTRK gene

Is the histologic tumour type known to harbour highly recurrent NTRK fusions?

YES

NO*

Is there a sequencing platform available?

NO

YES

Use IHC as a screening tool

NO TRK expression

Detection of TRK expression

IHC to confirm protein expression in positive cases

Use front line NGS reliably detecting NTRK fusions, preferably including RNA testing when possible
Outline

• 3 examples
  – a new biomarker
  – an old biomarker across distinct entities
  – an old biomarker in a new clinical scenario
In HER2+ patients

NEJM 2001
ISH

**HER2 negative**

- **SCORE 0**
- **SCORE 1+**
- **SCORE 2+**
- **SCORE 3+**

**HER2 POSITIVE**

- **ISH**
  - **NOT Amp**
  - **EQUIV**
  - **AMP**
Unusual Score 2+ staining patterns

Baso-lateral membrane staining
Unusual Score 2+ staining patterns

Complete intense membrane staining in ≤ 10% of tumor cells
Impact of intra-tumor heterogeneity: the neoadjuvant setting
Impact of intra-tumor heterogeneity: the neoadjuvant setting

pCR rates
Impact of intra-tumor heterogeneity: the neoadjuvant setting
HER2 matters beyond breast cancer

- Gastric cancer
- Colorectal Cancer
- Lung Cancer
**Table 1.** Diagnostic and staging investigations in gastric cancer

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full blood count</td>
<td>Assess for iron deficiency anaemia</td>
</tr>
<tr>
<td>Renal and liver function</td>
<td>Assess renal and liver function to determine appropriate therapeutic options</td>
</tr>
<tr>
<td>Endoscopy and biopsy</td>
<td>Obtain tissue for diagnosis, histological classification and molecular biomarkers, e.g. HER2 status</td>
</tr>
<tr>
<td>CT thorax + abdomen ± pelvis</td>
<td>Staging of tumour—to detect local/distant lymphadenopathy and metastatic disease or ascites</td>
</tr>
<tr>
<td>EUS</td>
<td>Accurate assessment of T and N stage in potentially operable tumours Determine the proximal and distal extent of tumour</td>
</tr>
<tr>
<td>Laparoscopy ± washings</td>
<td>Exclude occult metastatic disease involving peritoneum/diaphragm</td>
</tr>
<tr>
<td>PET, if available</td>
<td>May improve detection of occult metastatic disease in some cases</td>
</tr>
</tbody>
</table>

**Table 4.** Personalised medicine synopsis table for lower oesophageal and gastric cancer

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Method</th>
<th>Use</th>
<th>LOE, GOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>Immunohistochemistry for HER2 protein expression or ISH for HER2 gene amplification</td>
<td>Used to select patients with metastatic disease for treatment with a trastuzumab-containing regimen</td>
<td>I, A</td>
</tr>
</tbody>
</table>

**HER2 as a target of treatment**

**2010, ToGA Phase III trial**

=> Clinically and statistically significant improvements in response rate, PFS and OS with the addition of trastuzumab to a cisplatin/fluoropyrimidine doublet  
(median OS 13.8 versus 11.1 months, HR 0.74, 95% CI 0.60–0.91; P = 0.0048).

Lancet 2010; 376: 687–97
HER2 heterogeneity in gastric cancer

HER2 expression heterogeneity found in about 50% of cases, being highest in the IHC1+ and 2+ categories
HER2 in CRC

- HER2 expression can be heterogeneous
- Assessment is a 2-tier process:
  - 0/1+ and 2+ <50% and score 3+ <10% => no further action, not eligible
  - Score 3+ >50% => no further action, eligible
  - Score 2+>50%, score 3+ >10%<50% => ISH

HER2 in CRC

- HER2 expression can be heterogeneous
- Assessment is a 2-tier process:
  - 0/1+ and 2+ <50% and score 3+ <10%=> no further action, not eligible
  - Score 3+ >50% => no further action, eligible
  - Score 2+>50%, score 3+ >10%<50%=> ISH

Anti-HER2 treatment in CRC

Patients with KRAS exon 2 (codons 12 and 13) WT and HER2-positive metastatic colorectal cancer refractory to standard of care (including cetuximab or panitumumab).

HER2 positivity: tumours with 3+ HER2 score in more than 50% of cells by IHC, or tumors with 3+ HER2 score in >10% but <50% or with 2+ HER2 score in more than 50% of cells and a HER2:CEP17 ratio higher than two in more than 50% of cells by ISH.

HER2 in gastric and colorectal cancers

• Highly heterogeneous expression

• Scoring criteria mimic those reported in recommendations for breast cancer, however patterns of expression/cut-offs may differ (pathologists ought be aware of that!)
Outline

• 3 examples
  – a new biomarker
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  – an old biomarker in a new clinical scenario
In HER2+ patients
Population of “HER2-low” breast cancer patients
Score 1+/2+ not amp (HER2/CEP17 <2 and HER2 copy number < 4)

**B-47: Invasive Disease-Free Survival**

**HR 0.98 (95% CI 0.77-1.26) P=0.90**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Events</th>
<th>5 year EFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChemoRx</td>
<td>1603</td>
<td>134</td>
<td>89.2%</td>
</tr>
<tr>
<td>ChemoRx+Trast</td>
<td>1599</td>
<td>130</td>
<td>89.6%</td>
</tr>
</tbody>
</table>

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Treatment options for HER2+ patients
# HER2-directed ADC in clinical development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Anti-HER2 MAb/payload (target)</th>
<th>Drug to antibody ratio</th>
<th>Linker drug</th>
<th>Phase of development</th>
<th>ORR in HER2-positive</th>
<th>ORR in HER2 low (IHC1+/2+ /ISH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab-DM1 (T-DM1)9</td>
<td>Trastuzumab/DM1 (anti-tubulin)</td>
<td>3.5</td>
<td>Noncleavable</td>
<td>US FDA Approved</td>
<td>43.6%</td>
<td>———</td>
</tr>
<tr>
<td>Trastuzumab duruxtecan (DS-8201a)39</td>
<td>Trastuzumab/ exatecan derivative (topoisomerase I inhibitor)</td>
<td>8</td>
<td>Cleavable</td>
<td>II/III</td>
<td>54.5%</td>
<td>50%</td>
</tr>
<tr>
<td>SYD98540</td>
<td>Duocarmycin derivative (alkylating agent)</td>
<td>2.8</td>
<td>Cleavable</td>
<td>III</td>
<td>33%</td>
<td>HR + 27% HR - 40%</td>
</tr>
<tr>
<td>XMT-152241</td>
<td>XMT-1519/ monomethyl auristatin (anti-tubulin)</td>
<td>12</td>
<td>Cleavable</td>
<td>I</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>ARX788</td>
<td>Anti-HER2 MAb/ auristatin analog 269 (AS269) (anti-tubulin)</td>
<td>1.9</td>
<td>Non-cleavable</td>
<td>I</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>DHES0815A</td>
<td>Trastuzumab/ alkylator</td>
<td>2</td>
<td>Cleavable</td>
<td>I</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Pernas S & Tolaney S, *Therapeutic Advances in Medical Oncology* 2019
What’s next?

An old biomarker in a new clinical scenario
ASCO/CAP Recommendations in 2018

SCORE 0
SCORE 1+
SCORE 2+
SCORE 3+

ISH

NOT Amp  EQUIV  AMP

HER2 negative
HER2 POSITIVE
ASCO/CAP Recommendations in ...?

SCORE 0

SCORE 1+

SCORE 2+

SCORE 3+

ISH

NOT Amp

EQUIV

AMP

HER2 negative

“HER2 low”

HER2 POSITIVE
The Dilemma of HER2 Double-equivocal Breast Carcinomas
Genomic Profiling and Implications for Treatment

Molecular subtyping by PAM50

Luminal B in the large majority (76%) HER2 mRNA levels much closer to HER2-neg HER2-enriched in 5% of cases

Possible benefit from anti-HER2 therapies to be investigated in RCT?

Marchiò C et al, Am J Surg Pathol 2018
N=45
Summary

• IHC can be a powerful tool in clinical research
• When used as a screening assay, sensitivity is crucial
• One size may not fit all => follow specific recommendations
• With drug development biomarker assessment may evolve and recommendations may change
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