ESMO ADVANCED COURSE ON BIOMARKERS FOR PRECISION MEDICINE:
Session 2: New Technologies for Precision Medicine
GENE FUSIONS

Caterina Marchiò - University of Turin, Pathology Unit at FPO-IRCCS Candiolo Cancer Institute
Zürich, 28-29 November 2019

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DISCLOSURE OF INTEREST

Consultancy fees from Daiichi Sankyo, MSD, Bayer, Roche, Cor2ED
OUTLINE
Gene Fusions

- Gene fusions: what are they?
- Gene fusions in cancer
- Identification/testing methodologies and challenges
  - Available techniques: rationale, outputs, caveats
  - Strategy for testing: possible algorithms
  - Open questions, challenges
Cancer is a genetic disease at the cellular level, with two dominating types of initiating genetic events identified:

① the inactivation of genes by deletion, mutation or epigenetic mechanisms

① the activation or deregulation of genes as a consequence of point mutation, amplification or balanced cytogenetic abnormalities
GENE FUSION

A hybrid gene formed from two previously separate genes

=> It can occur as a result of:

- Translocation
- Chromosomal inversion
- Interstitial deletion

Images from Mertens F et al, Nature Reviews Cancer 2015
EFFECT OF A GENE FUSION

Gene fusions exert their tumorigenic action by two alternative mechanisms: overexpression of a gene in one of the breakpoints or the creation of a hybrid gene through the fusion of two genes, one in each breakpoint.

Images from Mertens F et al, Nature Reviews Cancer 2015
Constitutive activation and augmented downstream signalling with deregulation of the expression of genes affecting various cellular and physiological processes:
- Proliferation (↑)
- Motility (↑)
- Apoptosis (↓)
- Differentiation (↓)

Fusion genes have oncogenic properties
FUSION GENES IN CANCER AND THERAPEUTIC OPPORTUNITIES

The first specific translocation identified in human neoplasia:
t(9;22)(q34;q11) => resulting in the Philadelphia chromosome,
revealing a fusion of the BCR and ABL1 genes
**FDA drug approvals in 2018**

<table>
<thead>
<tr>
<th>Disease Category</th>
<th>Drugs</th>
</tr>
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<tbody>
<tr>
<td>Cutaneous squamous cell cancer</td>
<td>Cemiplimab-rwlc</td>
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<tr>
<td>Merkel cell cancer</td>
<td>Pembrolizumab</td>
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<td>Melanoma</td>
<td>Encorafenib + binimetinib</td>
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<td>Dabrafenib + trametinib</td>
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<td>Thyroid cancer</td>
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<td>Adrenal gland (PPGL)</td>
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<td>Breast cancer</td>
<td>Talazoparib</td>
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<td>Rubocilib</td>
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<td>Abemaciclib</td>
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<td>Olaparib</td>
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<td>Trastuzumab-pkrb</td>
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<tr>
<td>Colorectal cancer</td>
<td>Ipilimumab + nivolumab</td>
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<td>GEP-NET</td>
<td>Lutetium Lu-177 dotate</td>
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<td>Hepatocellular cancer</td>
<td>Pembrolizumab</td>
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<td>Lenvatinib</td>
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<td>Cervical cancer</td>
<td>Pembrolizumab</td>
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<td>Ovarian cancer</td>
<td>Bevacizumab</td>
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<td></td>
<td>Rucaparib</td>
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<td>Olaparib</td>
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<tr>
<td>Cervical cancer</td>
<td>Pembrolizumab</td>
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<td>EMA approval</td>
<td>Larotrectinib</td>
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<tr>
<td>Renal cancer</td>
<td>Ilulizumab + nipulimab</td>
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<td>Urothelial cancer</td>
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<td>HLH</td>
<td>Emapalumab</td>
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<tr>
<td>Supportive care</td>
<td>Pegfilgrastim-jmdb</td>
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<td></td>
<td>Epoetin alfa-epbx</td>
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</tbody>
</table>

**2019 update:**
- FDA approval: Larotrectinib
- Entrectinib
- EMA approval: Larotrectinib

**TSC-associated seizures**
- Everolimus

**Lung cancer**
- Dacomitinib
- Lorlatinib
- Pembrolizumab
- Nivolumab
- Osimertinib
- Durvalumab
- Aftinib
- Atezolizumab

**NTRK fusion-positive cancers (histology agnostic)**
- Larotrectinib

**Nature Reviews Clinical Oncology 2019**
RET inhibitor selpercatinib (Loxo-292) in patients with advanced solid tumors, including RET fusion-positive solid tumors, MTC, and other tumors with RET activation.

Investigators in the LIBRETTO-001 phase I/II trial presented new data on the experimental RET inhibitor selpercatinib at the 2019 World Conference on Lung Cancer. The agent produced robust responses in patients with RET-altered non–small cell lung cancer who had already received multiple therapies, raising hopes that it will soon receive FDA approval.
Identification of selected oncogenic fusions in various malignancies and the methods used to detect them.
NEXT GENERATION SEQUENCING

Bioinformatics

Mutations
Amplifications
Homozygous deletions
Rearrangements

Mutation signatures

Fusion genes

Carcinogens (smoking, UV)
DNA repair defects (HRD, MSI)
Genotoxic insults

DNA repair defects
MLH1
PMS2/1
MSH2
MSH6

HRD
Microsatellite instability

Pathogens
Viruses (HPV)
Bacteria

Images modified from Zehir A et al, Nature medicine 2018
NEXT GENERATION SEQUENCING

**Bioinformatics**

- Mutations
- Amplifications
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**DNA repair defects**

- MLH1
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- MSH2
- MSH6

- HRD
- Microsatellite instability

**Pathogens**

- Viruses (HPV)
- Bacteria

**Fusion genes**

Images modified from Zehir A et al, Nature medicine 2018
Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients

- Genomic rearrangements, many of which produced putative gene fusions, were reported in 1,597 individuals (15%)

- Of all the gene fusions identified by MSK-IMPACT, 35% (n = 268 fusions) involved kinase genes and encompassed all or part of the kinase domain
DETECTION OF GENE REARRANGEMENTS ACROSS DIFFERENT TUMOR TYPES

Spectrum of kinase fusions identified by MSK-IMPACT in a clinical sequencing of 10,000 malignancies

N=268, ≈2.7%

=> Although certain kinase fusions were enriched in particular lineages, others occurred widely across cancers

Zehir A et al, Nature Medicine 2018
In the MSKCC series, gene fusions involving ALK, RET and ROS1, for which effective targeted therapies exist in lung cancer, were found in 11 additional tumor types.

Zehir A et al, Nature Medicine 2018
**NTRK FUSIONS ACROSS TUMOR TYPES**

Cancers enriched for TRK fusions
- Frequency >90%

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

**Adult cancers**
- High-grade glioma
- Head and neck cancer
- MASC
- Thyroid cancer
- Lung cancer
- Breast cancer
- Secretory breast carcinoma
- Gastrointestinal stromal tumour (pan-negative)
- Cholangiocarcinoma
- Melanoma
- Spitzoid tumours
- Sarcoma
- Acute lymphoblastic leukaemia, acute myeloid leukaemia, histiocytosis, multiple myeloma and dendritic cell neoplasms
- Renal cell carcinoma
- Pancreatic cancer
- Colorectal cancer

**Paediatric cancers**
- High-grade glioma
- Papillary thyroid cancer
- Secretory breast carcinoma
- Infantile fibrosarcoma
- Cellular and mixed congenital mesoblastic nephroma

Cocco 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
NEUROTROPHIC TROPOMYOSIN RECEPTOR KINASE (NTRK)

- **NTRK1**
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- **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**
    *(in adult tissues: smooth muscle, testes and neuronal components)*
NEUROTROPHIC TROPOMYOSIN RECEPTOR KINASE (NTRK)

- **NTRK1**
  - 1q21-q22 – TRKA

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- **NTRK3**
  - 15q25 – TRKC

Congenital fibrosarcoma
Cellular mesoblastic nephroma
Secretory carcinoma
**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

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

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**ETV6-NTRK3 rearrangement**
>95%

**NTRK1, NTRK2, NTRK3 rearrangements**
<1% (≈0.2%)
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, Ann Oncol. 2019 Jul 3. pii: mdz204
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

153 patients
Median DOR: 35.2 months
Median PFS: 26.8 months
Median OS: 44 months

Change in tumor diameter
<table>
<thead>
<tr>
<th>Gene fusion</th>
<th>Drug</th>
<th>Disease</th>
<th>Year of approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK fusions</td>
<td>Crizotinib</td>
<td>NSCLC</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td>Ceritinib</td>
<td>NSCLC</td>
<td>2014</td>
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<tr>
<td>BCR–ABL1</td>
<td>Imatinib</td>
<td>CML and ALL</td>
<td>2001</td>
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<tr>
<td></td>
<td>Dasatinib</td>
<td>CML and ALL</td>
<td>2006</td>
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<td></td>
<td>Nilotinib</td>
<td>CML</td>
<td>2007</td>
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<td></td>
<td>Bosutinib</td>
<td>CML</td>
<td>2012</td>
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<td>COL1A1–PDGFRB</td>
<td>Imatinib</td>
<td>DFSP</td>
<td>2006</td>
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<tr>
<td>FIP1L1–PDGFRB</td>
<td>Imatinib</td>
<td>HES/CEL</td>
<td>2006</td>
</tr>
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<td>PDGFR fusions</td>
<td>Imatinib</td>
<td>MDS/MPN</td>
<td>2006</td>
</tr>
<tr>
<td>NTRK1/2/3 fusions</td>
<td>Larotectinib</td>
<td>NA (histology agnostic)</td>
<td>2018</td>
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<td>ROS1 fusions</td>
<td>Entrectinib</td>
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<td>FGFR2/3 fusions</td>
<td>Erdafinib</td>
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Modified from Mertens F et al, Nature Reviews Cancer 2015
HOW CAN WE DETECT FUSION GENES?
GENE FUSION DETECTION: POSSIBLE TOOLS

- **IHC**
  - Ab against a specific protein
  - Pan-proteins Ab
  - Cocktail of Abs

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

- **RT-PCR**
  - Specific probes designed for the gene fusion of interest

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

- **NGS**
  - Global RNAseq
  - Targeted panels
    - DNA-based
    - RNA-based
    - DNA/RNA panels
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
- It is (relatively) inexpensive: LDT versus Kit preparation
EXAMPLE: IHC FOR NTRK

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

**Pos controls**

- KM12 (*TPM3-NTRK1*), MO-91 (*ETV6-NTRK3*) and CUTO-3.29 (*MPRIP-NTRK1*) cells
- Peripheral nerves

**Neg controls**

Non-neoplastic tissues

*Tissue in which the protein are expressed*

Neuronal components => NOT good for CNS tumors!

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Ann Oncol. 2019 Jul 3. pii: mdz204
USE OF IHC

“Two-step approach”

IHC as a screening method

FISH or NGS to confirm the presence of rearrangement

Sensitivity is crucial
FISH

- Genes on a glass slide

Hicks and Tubbs, Human Pathology 2005
FISH

• It is a commonly used method for detecting chromosomal rearrangement fusions in solid tumours (see ALK, ROS1 and RET…)

• **Split-apart** rearrangement **probes** are invariably easier in FFPE samples
FISH

⇒ FISH cannot ascertain the 5’ partner or whether the rearrangement results in a productive in-frame chimaeric transcript

Secretory carcinoma of the breast
ETV6/NTRK3 split apart probe
FISH

The utility of FISH for screening cancer when more than one gene has to be assayed (e.g. NTRK1/2/3 fusions) is limited, given the multitude of partners involved, the expertise required and its labour-intensive nature

⇒ Ideal technique when we have to confirm the presence of a fusion
⇒ For NTRK rearrangements: useful in lesions where it is predicted to be found at high frequency => ETV6-NTRK3
Gene Fusion Detection: Possible Tools

Technical options

In Vitro Nucleic Acid-Based Assays

- **IHC**
  - Ab against a specific protein
  - Pan-proteins Ab
  - Cocktail of Abs

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    - DNA-based
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    - DNA/RNA panels
IN VITRO NUCLEIC ACID-BASED ASSAYS OTHER THAN NGS

RT-PCR

- Typically used as an orthogonal validation method in studies exploring the genetic landscape of subgroups of neoplasms by high-throughput techniques
- The partner has to be known
- Specific primers to be designed
- Used in the context of confirmation of ETV6-NTRK3 in several studies
### IN VITRO NUCLEIC ACID-BASED ASSAYS OTHER THAN NGS

<table>
<thead>
<tr>
<th>RT-PCR</th>
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<th>Real Time PCR</th>
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<td></td>
<td>Custom-made panels</td>
<td>Simple workflow and short TAT</td>
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<tr>
<td></td>
<td>Technology used also for other types of diagnostic testings</td>
<td>Low costs</td>
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<tr>
<td></td>
<td>Not many studies so far</td>
<td>Detection of specific alterations</td>
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| - Specific primers to be designed | | |
| - Used in the context of confirmation of *ETV6-NTRK3* in several studies | | |

**Confirmatory technique**  
**Can be used for screening**  
**Can be used for screening**
RNA next generation targeted sequencing assays

They enable *de novo* detection of fusion genes that are transcribed

- the Oncomine assays (ThermoFisher Scientific) cover fusion variants (including *NTRK1*, *NTRK2* and *NTRK3*)

- GeneTrails Solid Tumor Fusion Gene Panel (Knight Diagnostic Laboratories), designed to detect fusions involving 20 target genes (including *NTRK1*, *NTRK2*, *NTRK3*)

- the Universal Fusion/Expression Profile (Neogenomics), an assay capable of detecting different classes of genomic abnormalities such as fusion transcripts and transcriptomic gene expression levels in 1,385 genes (*NTRK1*, *NTRK2*, *NTRK3* included)
NGS

RNA next generation targeted sequencing assays

Anchored Multiplex PCR (AMP) has become a widely adopted methodology for fusion gene detection

=> commercial ready to use kits and customisable assays

=> high technical sensitivity and specificity even in FFPE-derived RNA samples

The sequencing library targets fusion exons in multiple oncogenes (including the three members of the NTRK family)
AMP

A target enriched chemistry that creates target enriched libraries for NGS

Able to detect and identify gene fusions without prior knowledge of fusion partners

Works on both Illumina and Ion Torrent platforms

Images from https://archerdx.com
Targeted next generation DNA sequencing assays

Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™) assay, a deep-coverage assay encompassing the entire coding regions and selected intronic and regulatory regions of >400 key cancer genes.

This tumour-profiling multiplex panel can detect missense mutations, indels, copy number alterations, and selected gene fusions.

Zehir A et al, Nature medicine 2018
Other DNA targeted sequencing assays

- FoundationOneCDx test (Foundation Medicine)
- UW Oncoplex and the UCSF500 Cancer Gene Panel
- SmartGenomics Complete –(PathGroup) Expanded Solid Tumor
- Solid Tumor Focus Oncomine NGS Panel (Cancer Genetics)
Targeted next generation DNA sequencing assays – key concepts

1) DNA-based NGS has proven to be effective to detect gene rearrangements and predicted fusions

2) Detected rearrangements by DNA-based assays may not result in fusions

3) NOT ALL of the rearrangements can be practically detected using targeted assays: for instance, those fusions involving \(\textit{NTRK2}\) and \(\textit{NTRK3}\) where large intronic regions can render DNA-based detection challenging
OPEN QUESTIONS/CHALLENGES

Is there a strategy when we have to screen for fusion in an agnostic way?

(e.g. NTRK fusion genes)
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?

NO*  YES

Is there a sequencing platform available?

NO  YES

Use IHC as a screening tool

IHC to confirm protein expression in positive cases

NO TRK expression

Detection of TRK expression

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

ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019
Is the histologic tumor type known to harbor highly recurrent NTRK fusions?

YES

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

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

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Detection of TRK expression

NO TRK expression

Use IHC as a screening tool

Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019
OPEN QUESTIONS/CHALLENGES

Any other relevant issues for gene fusion testing?
RESISTANCE TO THERAPY

Despite durable responses to drugs targeting kinase fusions, it is expected that acquired resistance to therapy may ultimately emerge in most patients.

① ‘on-target’ alterations: mutation or amplification of the fusion itself

② ‘off-target’ alterations: when there is activation of parallel bypass pathways
Acquired resistance mutations that cluster around the ATP-binding site of the kinase domain and solvent front

The existence of convergent evolution has been demonstrated across kinase fusions, with paralogous resistance mutations reported in several fusion transcripts

*In silico* structural modelling of the KD of ALK, ROS1, RET, TRKA, TRKB, and TRKC by Schram AM et al, Nature Reviews Clinical Oncology 2017
“Off target” resistance

**NTRK RECEPTOR SIGNALING**

*NTRK* fusions lead to:

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

Amatu A et al, ESMO open 2016
RESISTANCE TO THERAPY

“Off target” resistance

=> Convergent MAPK pathway activation:

*KRAS/BRAF* mut

*MET* amplification

Cocco E et al (Scaltriti’s lab at MSKCC), Nature Medicine Aug 25 2019
### DNA/RNA panels

<table>
<thead>
<tr>
<th>Assay</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>TruSight Tumor 170 (Illumina)</strong></td>
<td>Comprehensive NGS assay targeting DNA and RNA variants from the same FFPE sample, designed to cover 170 genes associated with common solid tumors, is an enrichment-based targeted panel that simultaneously analyzes DNA and RNA, covering a wide range of genes and variant types. <strong>NTRK1</strong>, <strong>NTRK2</strong>, <strong>NTRK3</strong> genes are included in the panel for the fusions.</td>
</tr>
<tr>
<td><strong>TruSight Oncology 500</strong> DNA + RNA* assay targeting 523 genes for assessment of small variants, TMB, MSI, splice variants, and fusions</td>
<td></td>
</tr>
<tr>
<td><strong>Oncomine Assays (ThermoFisher Scientific)</strong></td>
<td>Targeted, multi-biomarker assay that enables to target hotspots, SNVs, indels, CNVs, and gene fusions from DNA and RNA in a single workflow.</td>
</tr>
<tr>
<td><strong>FoundationOne®Heme (Foundation Medicine)</strong></td>
<td>A validated to detect all classes of genomic alterations in 405 cancer-related genes. In addition to DNA sequencing, FoundationOneHeme employs RNA sequencing across 265 genes to capture a broad range of gene fusions, common drivers of hematologic malignancies, and sarcomas.</td>
</tr>
<tr>
<td><strong>Caris Molecular intelligence</strong></td>
<td>Panel of 592 genes including fusion genes (<strong>ALK</strong>, <strong>BRAF</strong>, <strong>NTRK1</strong>, <strong>NTRK2</strong>, <strong>NTRK3</strong>, <strong>RET</strong>, <strong>ROS1</strong>, <strong>RSP03</strong>)</td>
</tr>
<tr>
<td><strong>Omniseq Comprehensive</strong></td>
<td>It identifies somatic variants across 144 genes, including all of the genes that point to either an approved drug or clinical trial. The panel for RNA-seq (23 genes) includes <strong>NTRK1</strong>, <strong>NTRK2</strong>, <strong>NTRK3</strong>.</td>
</tr>
<tr>
<td><strong>Paradigm Cancer Diagnostic (PCDx)</strong></td>
<td>Comprehensive profiling test that has been designed to analyze solid tumor alterations to match the best therapies and clinical trials based on the latest clinical evidence. It measures DNA mutations, copy number alterations, gene fusions, mRNA expression and splice variants (isoforms). In addition, proteins are tested by IHC.</td>
</tr>
<tr>
<td><strong>HANDLE-LCP30 panel (AmoyDx)</strong></td>
<td>Multiplex and targeted deep sequencing of variants in 30 driver genes, including <strong>NTRK1-3</strong> fusions. The assay allows detection of SNVs, InDels, Fusions and CNVs.</td>
</tr>
</tbody>
</table>

Modified from Supplementary material by Marchiò C et al, on behalf of the ESMO TR and PM Working Group, Annals of Oncology 2019
TAKE HOME MESSAGES

• Fusion genes are strong oncogenic drivers
  ➢ Gene fusions frequently involve tyrosine kinases and can cause constitutive kinase activation, augmentation of downstream signalling, and tumour proliferation

• Oncogenic gene fusions are common in patients with solid tumours and occur across a wide spectrum of tumour types:
  ➢ The prevalence of gene fusions varies considerably, from 0–100%, among different tumour types

• Targeted therapies are remarkably effective and are approved for patients with fusions
  ➢ Substantial and durable responses in particular with NTRK inhibitors
TAKE HOME MESSAGES

➢ In the detection of gene fusions there are techniques strategically better in some scenarios than others (histology-driven *versus* histology-agnostic)

➢ There is not a single technique that outperforms the others
  •  *RNA panels (and IHC) may be preferred but be aware of the limitations*

➢ Gene panels enable a more comprehensive profiling
THANK YOU FOR YOUR ATTENTION