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 DEVELOPMENT AND GENETIC ABNORMALITIES

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*

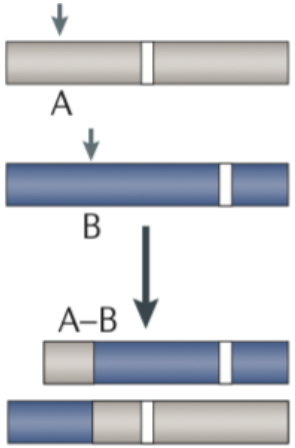
① the **activation or deregulation of genes** as a consequence of *point mutation*, *amplification* or *balanced cytogenetic abnormalities*

GENE FUSION

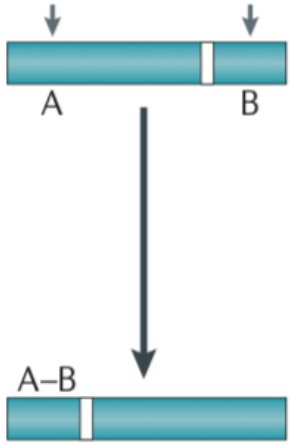
A hybrid gene formed from two previously separate genes

=> It can occur as a result of:

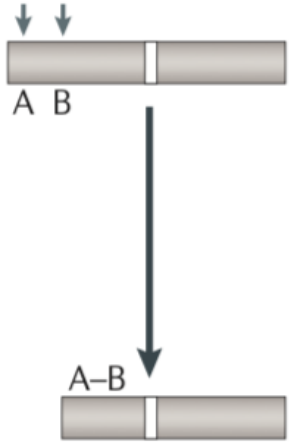
Translocation



Chromosomal inversion



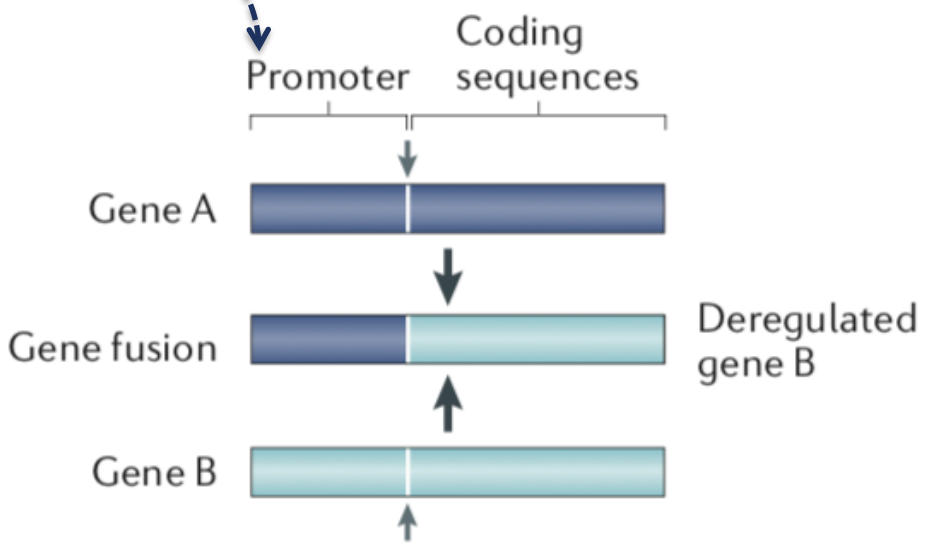
Interstitial deletion

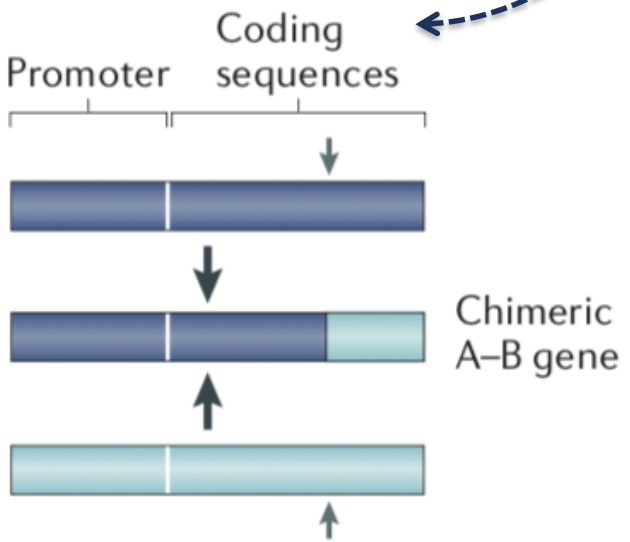


Images from Mertens F et al, Nature Reviews Cancer 2015

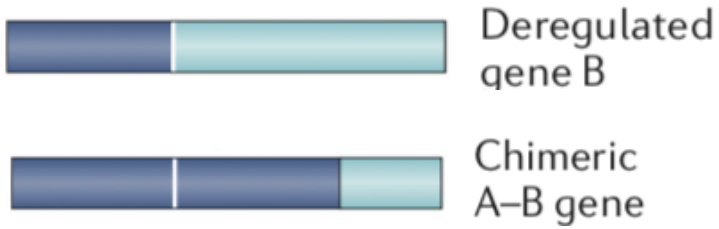
EFFECT OF A GENE FUSION

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.



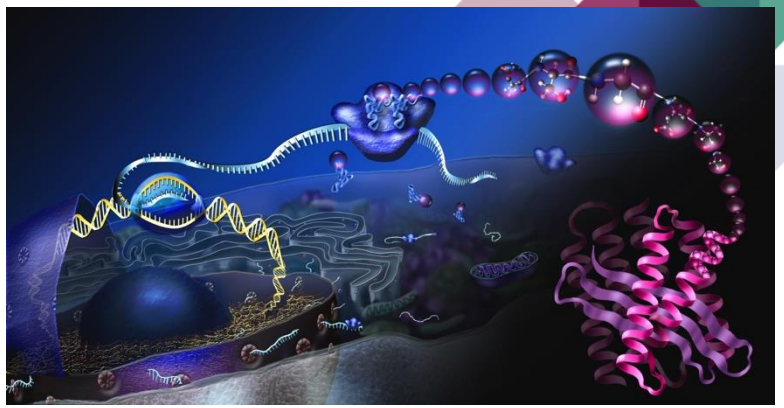


Images from Mertens F et al, Nature Reviews Cancer 2015



One partner in such fusions is often a kinase and the fusion event often maintains kinase activity

In-frame fusion



Transcription and translation

Expression of the protein

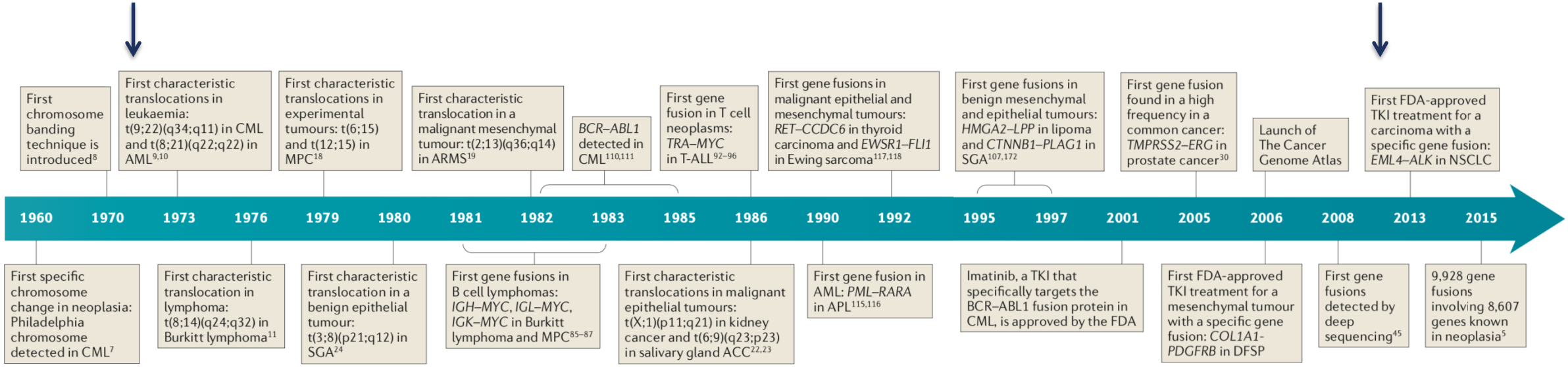
Fusion genes have oncogenic properties

Constitutive activation and augmented downstream si lling with deregulation of t ion of genes affecti ellular and physi esses:

- Pr (↑)
- (↑)
- ptosis (↓)
- Differentiation (↓)

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



Modified from Mertens F et al, Nature Reviews Cancer 2015

Approvals in 2018: a histology-agnostic new molecular entity, novel end points and real-time review

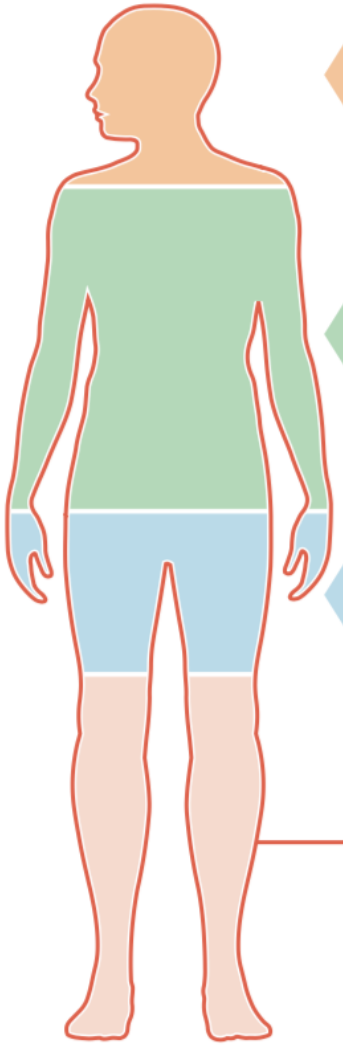
Gideon M. Blumenthal and Richard Pazdur*

FDA drug approvals in 2018

2019 update:

FDA approval
- Larotrectinib
- Entrectinib

EMA approval
Larotrectinib



Cutaneous squamous cell cancer
- Cemiplimab-rwlc

Merkel cell cancer
- Pembrolizumab

Melanoma
- Encorafenib + binimetinib
- Dabrafenib + trametinib

Thyroid cancer
- Dabrafenib + trametinib

TSC-associated seizures
- Everolimus

Adrenal gland (PPGL)
- Iobenguane I-131

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

Colorectal cancer
- Ipilimumab + nivolumab

GEP-NET
- Lutetium Lu-177 dotatate

Hepatocellular cancer
- Pembrolizumab
- Lenvatinib

Lung cancer
- Dacomitinib
- Lorlatinib
- Pembrolizumab
- Nivolumab
- Osimertinib
- Durvalumab
- Afatinib
- Atezolizumab

Renal cancer
- Ipilimumab + nivolumab

NTRK fusion-positive cancers (histology agnostic)
- Larotrectinib

Cervical cancer
- Pembrolizumab

Ovarian cancer
- Bevacizumab
- Rucaparib
- Olaparib

Prostate cancer
- Enzalutamide
- Apalutamide
- Abiraterone acetate

Urothelial cancer
- Pembrolizumab
- Atezolizumab

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

Lymphoma
- Tisagenlecleucel
- Brentuximab vedotin
- Duvelisib
- Rituximab-abbs
- Pembrolizumab

MF/SS
- Mogamulizumab-kpkc

BPDCN
- Tagraxofusp-erzs

HLH
- Emapalumab

Supportive care
- Pegfilgrastim-jmdb
- Epoetin alfa-epbx

Nature Reviews Clinical Oncology 2019

Sept 2019

CANCER DISCOVERY

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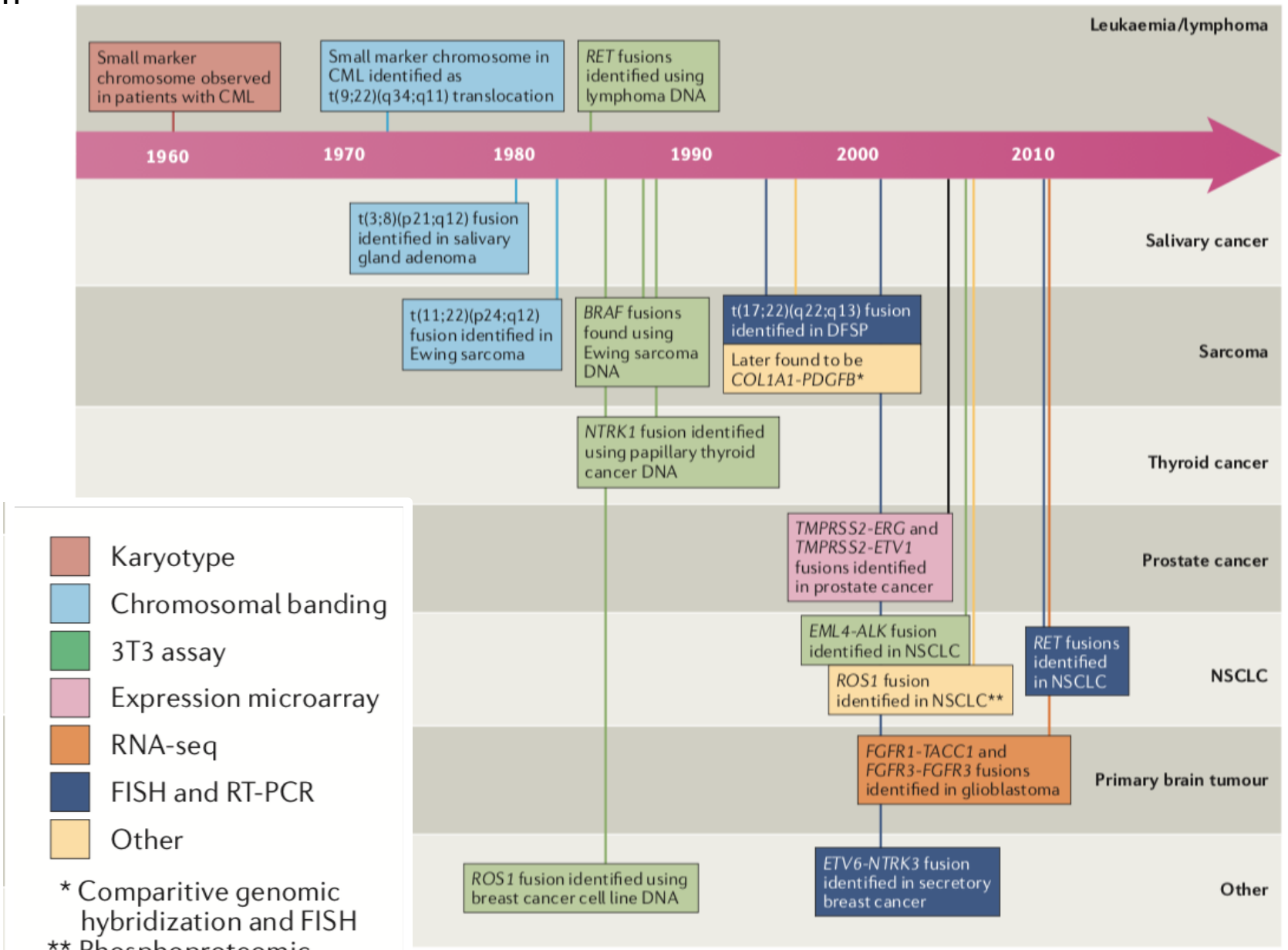
First RET Inhibitor on Path to FDA Approval

LIBRETTO-001 phase I/II trial

RET inhibitor selpercatinib (Loxo-292) in patients with advanced solid tumors, including *RET* fusion-positive solid tumors, MTC, and other tumors with *RET* activation

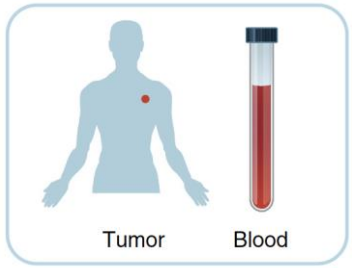
Investigators in the LIBRETTO-001 phase I/II trial presented new data on the experimental RET inhibitor selpercatinib at the 2019 World Conference on Lung Cancer. The agent produced robust responses in patients with *RET*-altered non–small cell lung cancer who had already received multiple therapies, raising hopes that it will soon receive FDA approval.

Identification of selected oncogenic fusions in various malignancies and the methods used to detect them



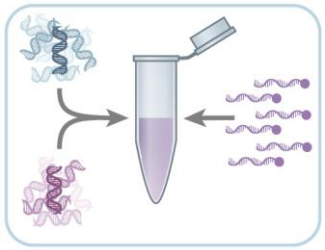
Schram AM et al, Nature Reviews Clinical Oncology 2017

NEXT GENERATION SEQUENCING



2. Sample accessioning

Sequencing



3. Sample preparation

Bioinformatics

Mutations

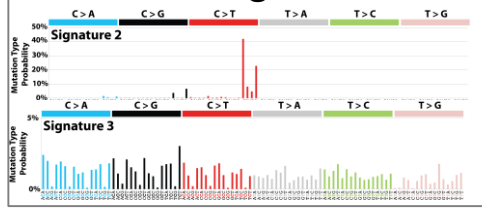
Amplifications

Homozygous deletions

Rearrangements

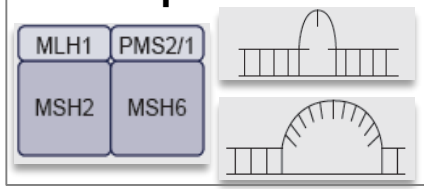
Fusion genes

Mutation signatures



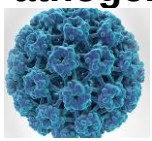
Carcinogens (smoking, UV)
DNA repair defects (HRD, MSI)
Genotoxic insults

DNA repair defects



HRD
Microsatellite instability

Pathogens



Viruses (HPV)
Bacteria

Images modified from Zehir A et al, Nature medicine 2018

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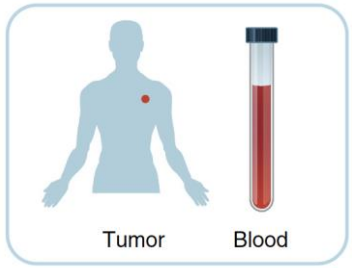

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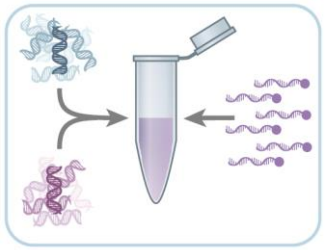

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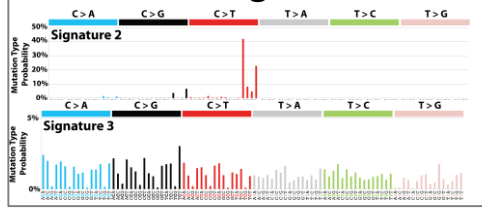

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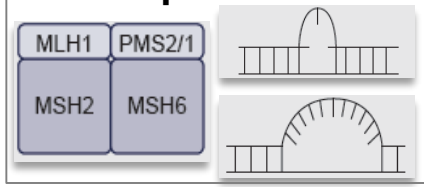

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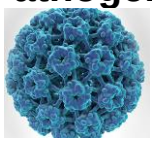


Viruses (HPV)
Bacteria

Images modified from Zehir A et al, Nature medicine 2018

DETECTION OF GENE REARRANGEMENTS ACROSS DIFFERENT TUMOR TYPES

Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients

Memorial Sloan Kettering Cancer Center

- 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

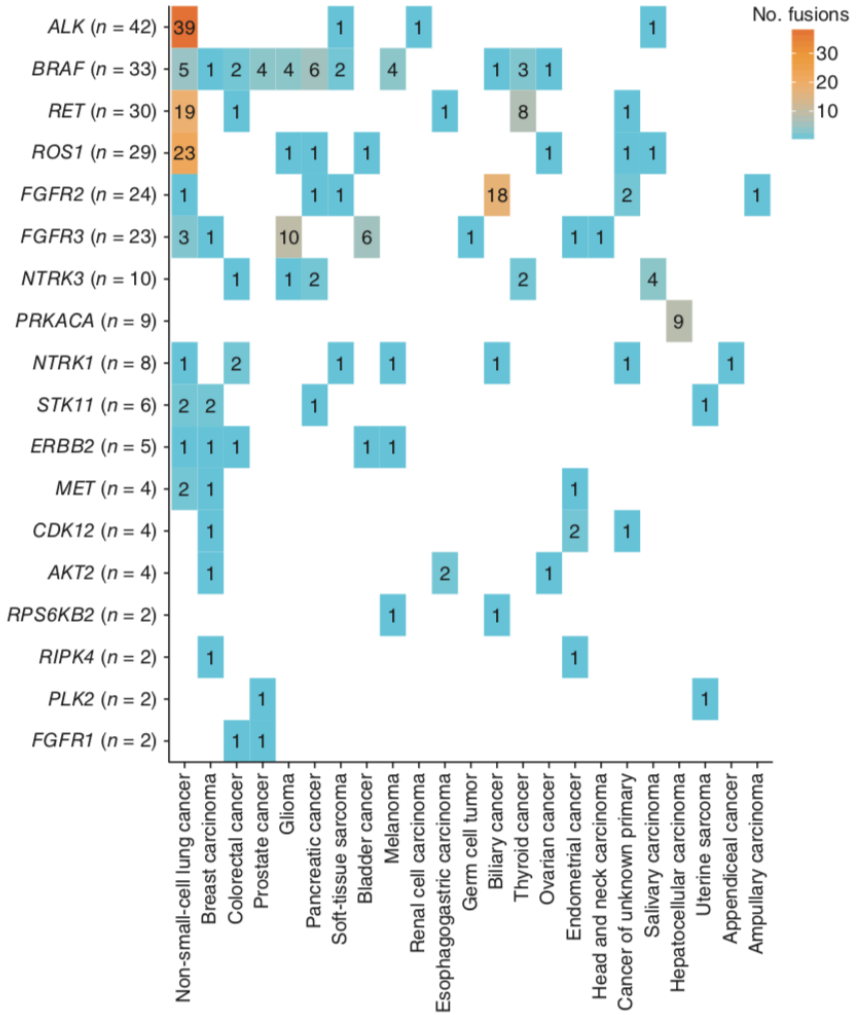
ESMO

Zehir A et al, Nature Medicine 2018

DETECTION OF GENE REARRANGEMENTS ACROSS DIFFERENT TUMOR TYPES

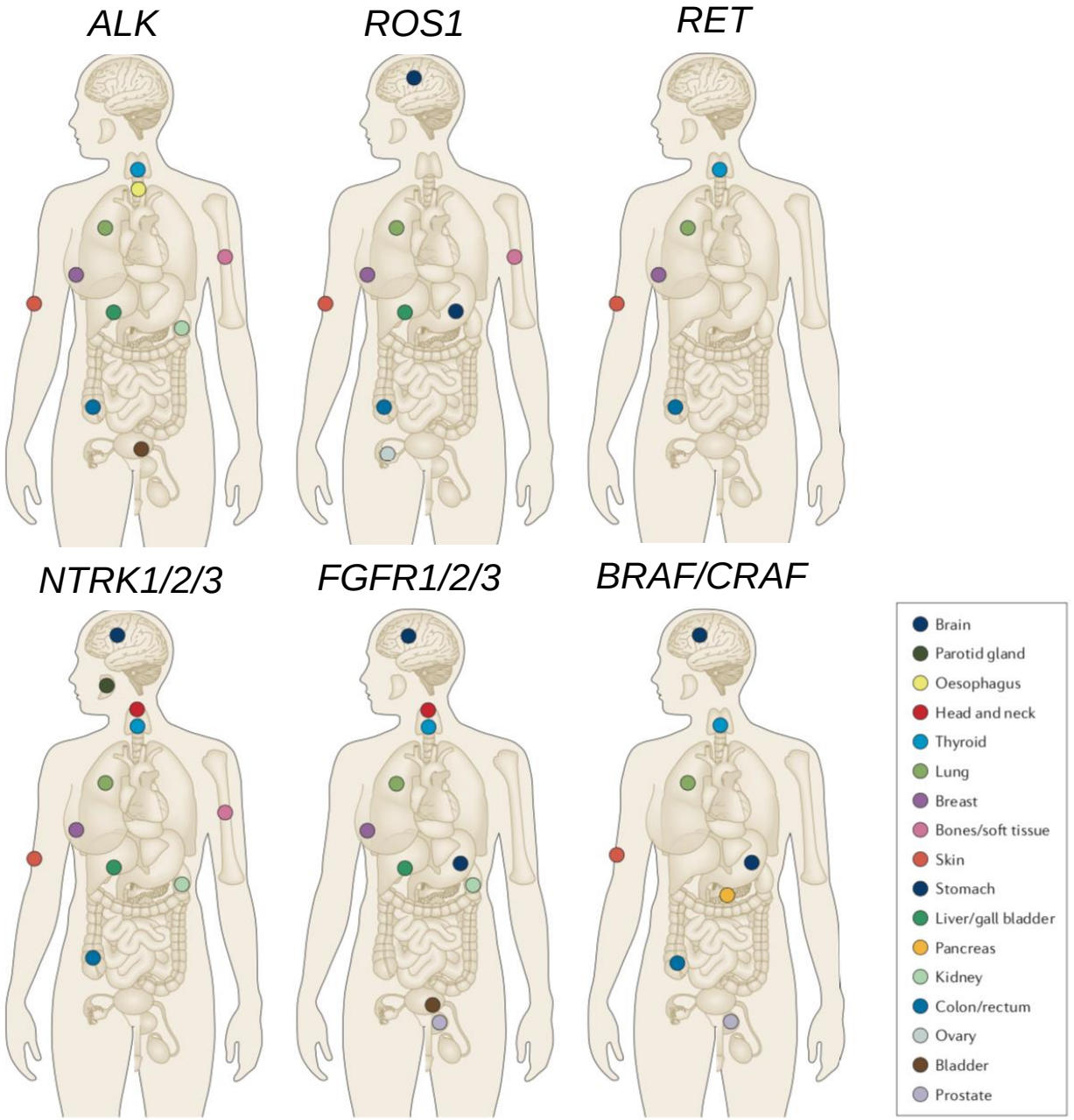
Spectrum of kinase fusions identified by **MSK-IMPACT** in a **clinical sequencing of 10,000 malignancies**

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

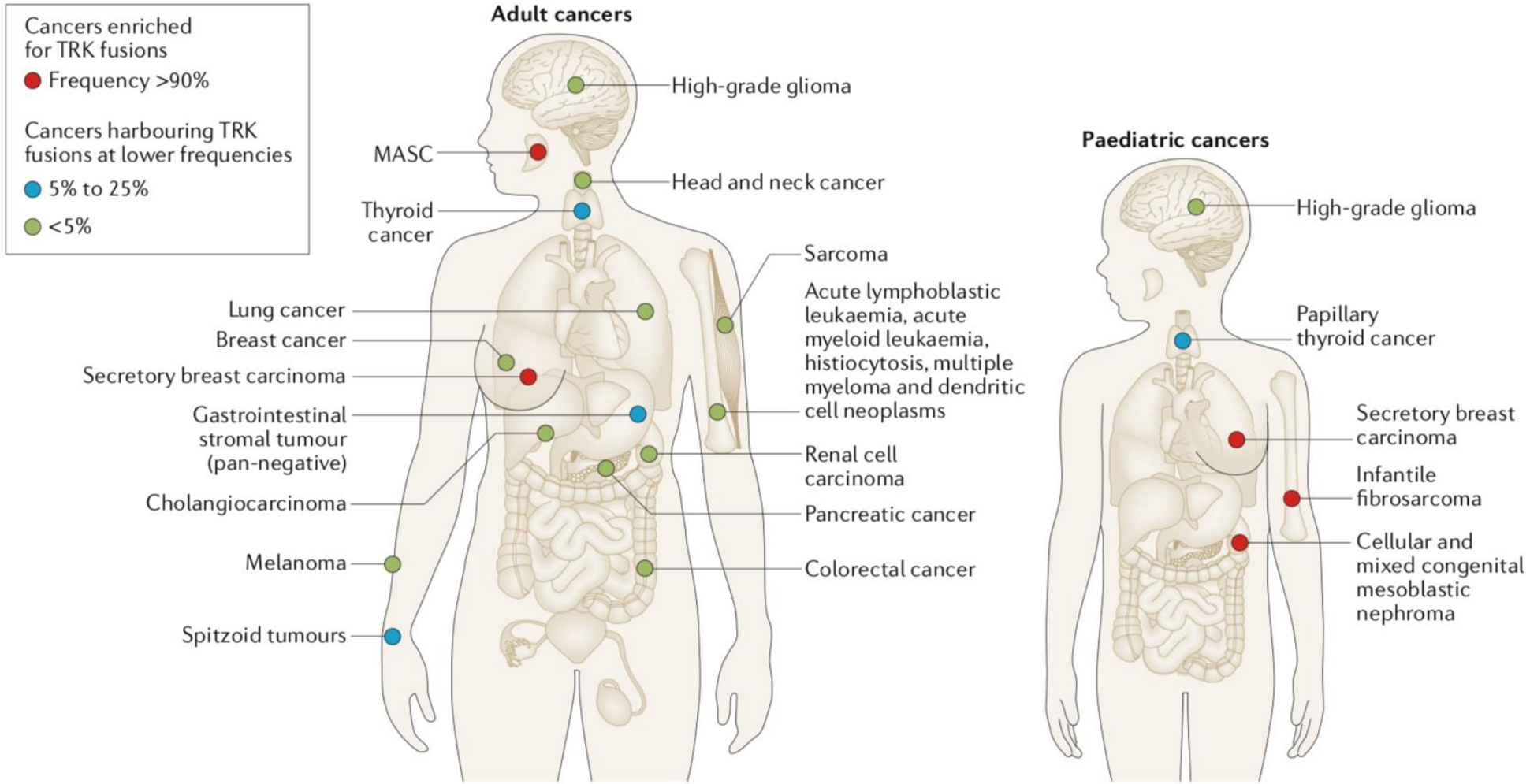


Schram AM et al, Nature Reviews Clinical Oncology 2017

=> 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



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

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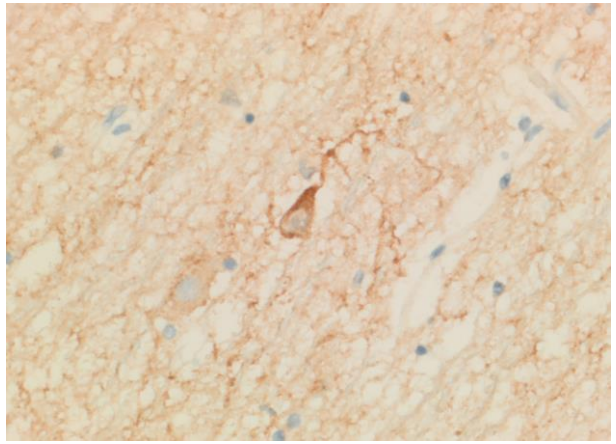
- Tyrosine kinases that play roles in
  - Neuronal differentiation and development, including the growth and function of neuronal synapses and memory development
  - **Expression restricted to specific tissues** *(in adult tissues: smooth muscle, testes and neuronal components)*



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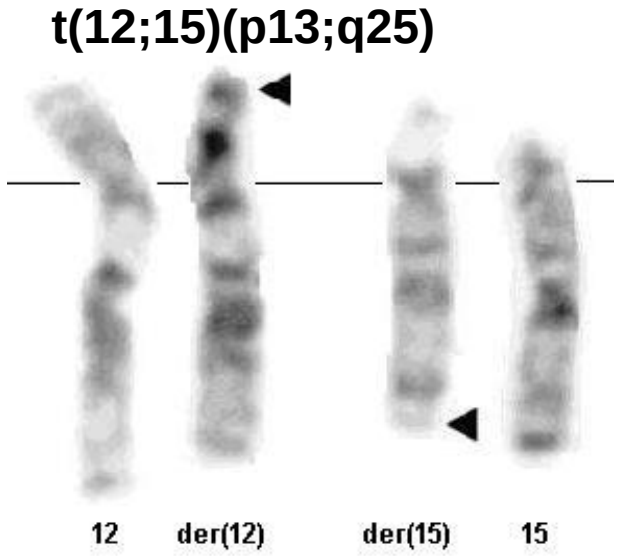
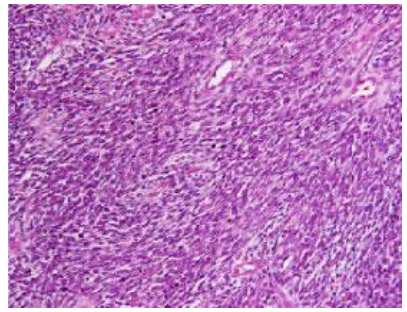
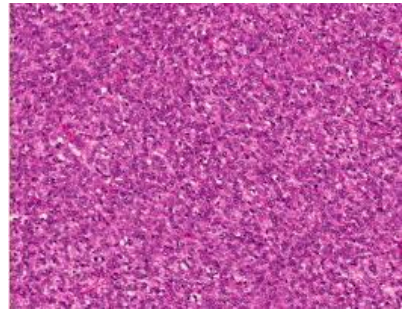
- ***NTRK3***
  - **15q25 – TRKC**

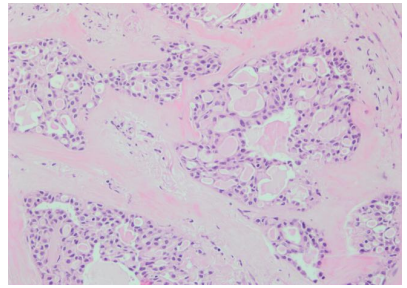




Congenital fibrosarcoma



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

ETV6-NTRK3 rearrangement

>95%

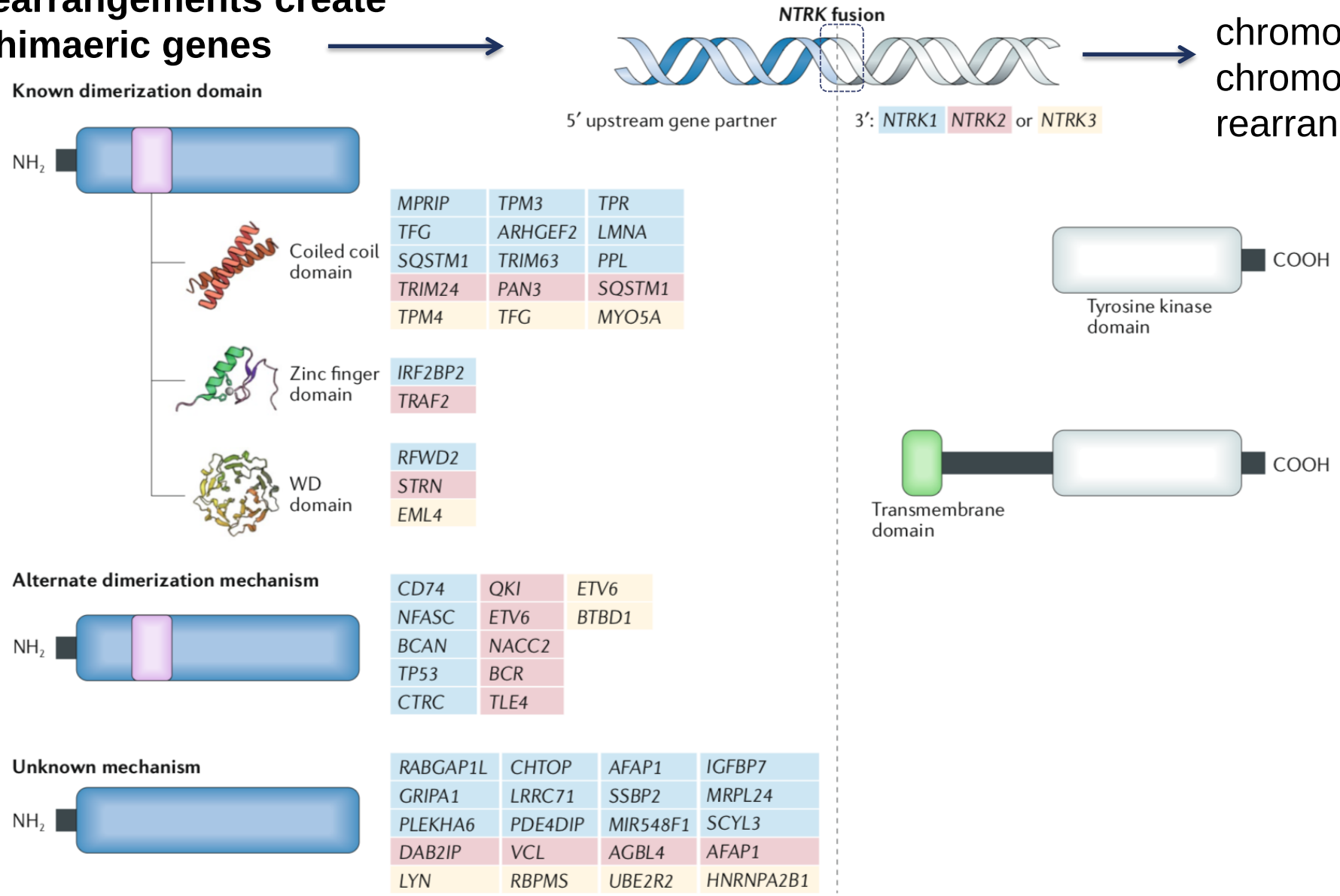
Low frequency in common forms of different types of cancers

- 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

<1% (≅0.2%)

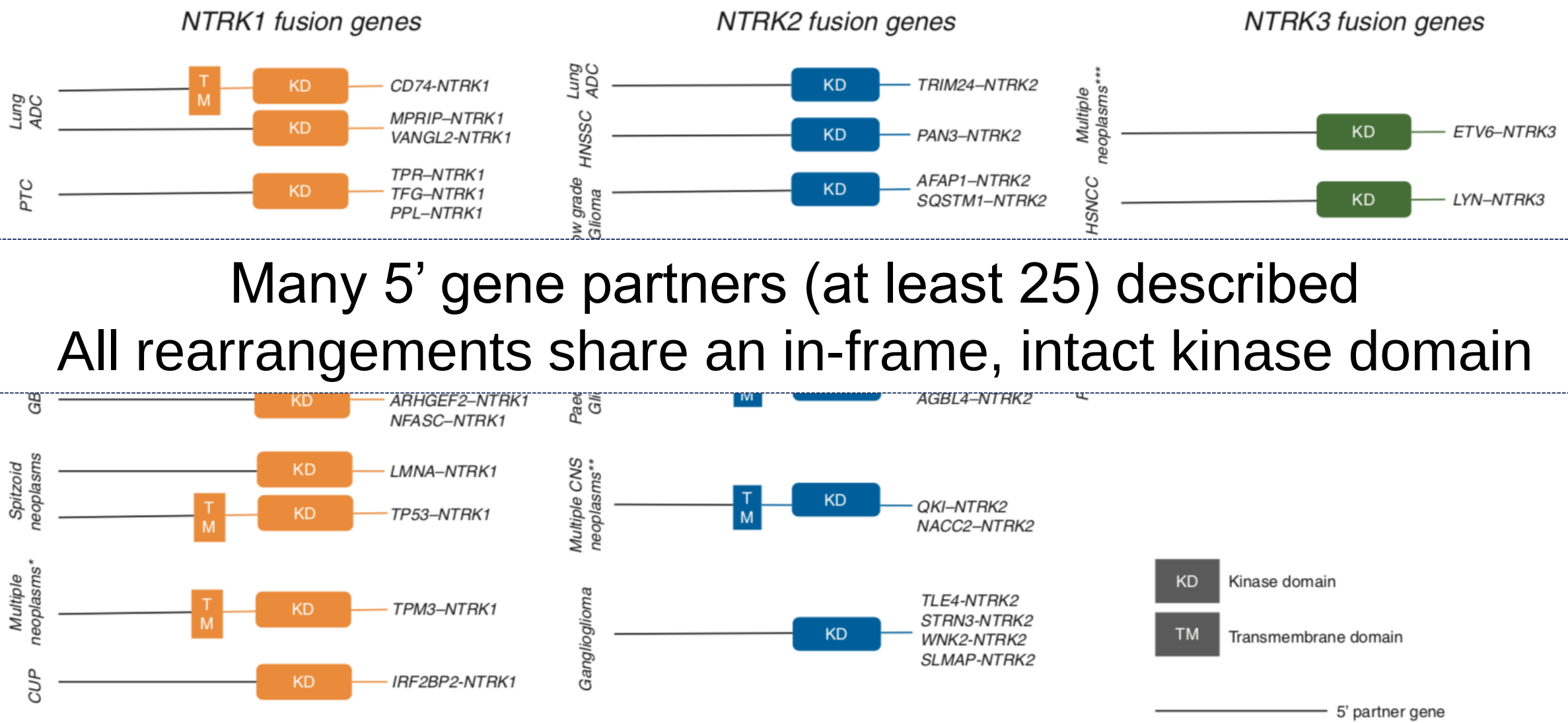
NTRK rearrangements create chimaeric genes



This may stem from intra-chromosomal or inter-chromosomal rearrangements

Cocco E, Scaltriti M & Drilon A, Nature Reviews Clinical Oncol 2018

NTRKs are promiscuous: multitude of 5’ partners



Many 5’ gene partners (at least 25) described

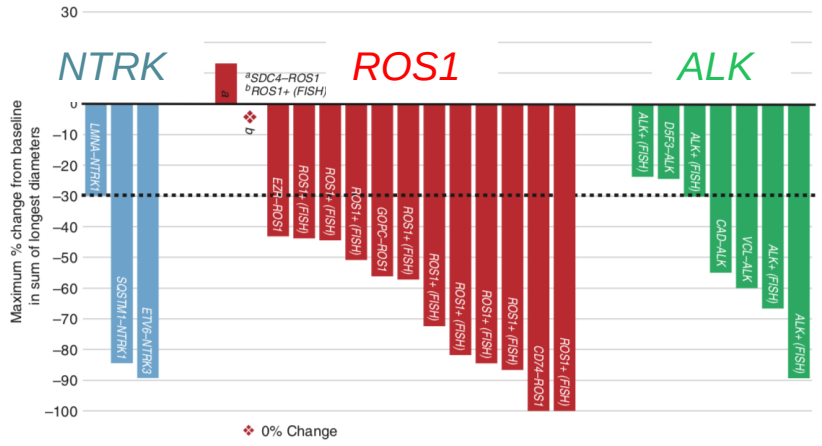
All rearrangements share an in-frame, intact kinase domain

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)



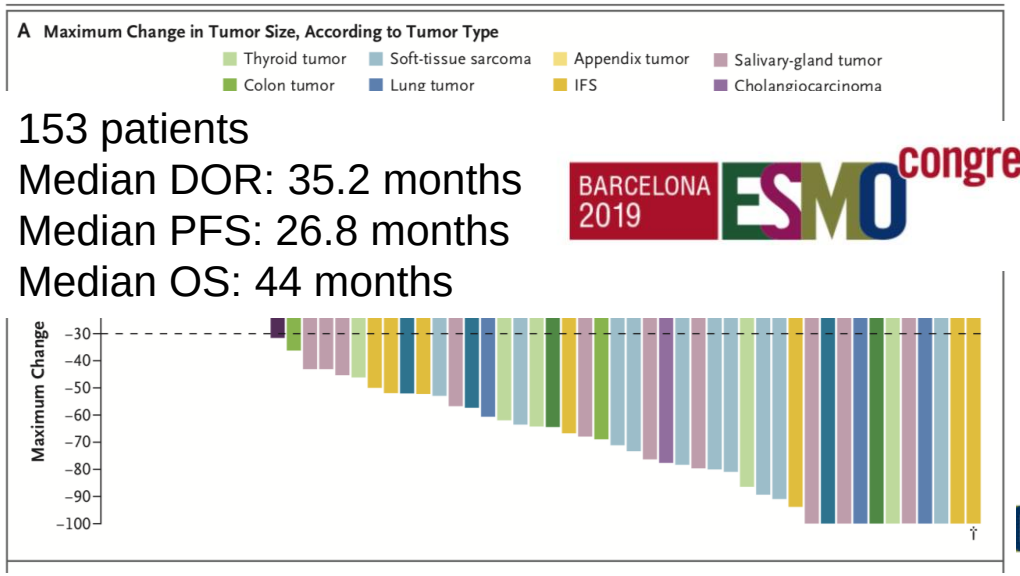
Change in tumor diameter

Drilon A et al, NEJM 2018

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

BARCELONA 2019 ESMO congress

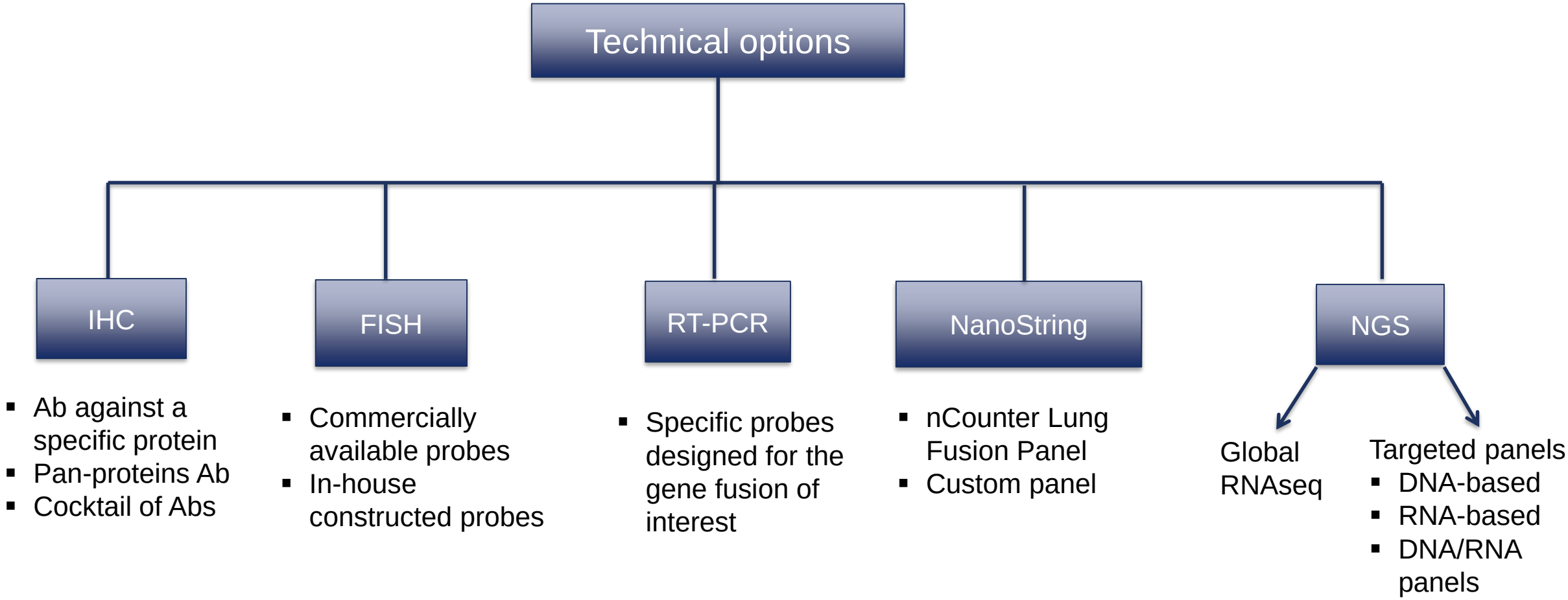
Table 2 | **FDA-approved drugs targeting gene fusions in malignant disorders***

Gene fusion	Drug	Disease	Year of approval
ALK fusions	Crizotinib	NSCLC	2011
	Ceritinib	NSCLC	2014‡
BCR–ABL1	Imatinib	CML and ALL	2001
	Dasatinib	CML and ALL	2006
	Nilotinib	CML	2007
	Bosutinib	CML	2012
	Ponatinib	CML and ALL	2012
COL1A1–PDGFRB	Imatinib	DFSP	2006
FIP1L1–PDGFRA	Imatinib	HES/CEL	2006
PDGFR fusions	Imatinib	MDS/MPN	2006
NTRK1/2/3 fusions	Larotectinib	NA *(histology agnostic)*	2018
NTRK1/2/3 fusions	Entrectinib	NA *(histology agnostic)*	2019
ROS1 fusions	Entrectinib	NSCLC	2019
FGFR2/3 fusions	Erdafinib	Urothelial carcinoma	2019

Modified from Mertens F et al, Nature Reviews Cancer 2015

HOW CAN WE DETECT FUSION GENES?

GENE FUSION DETECTION: POSSIBLE TOOLS



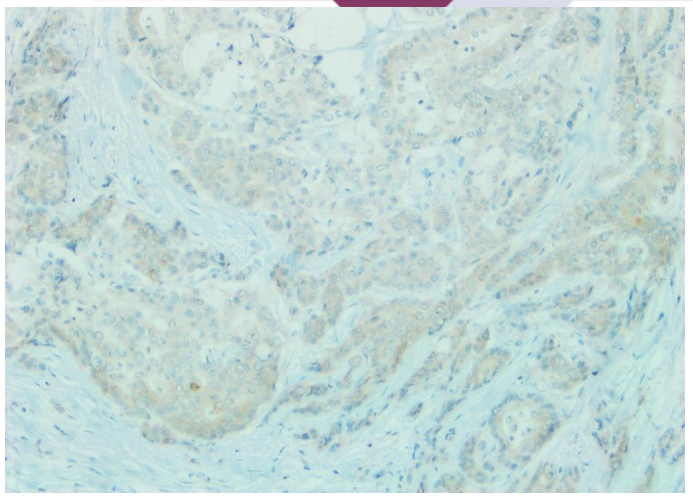
IMMUNOHISTOCHEMISTRY (IHC)

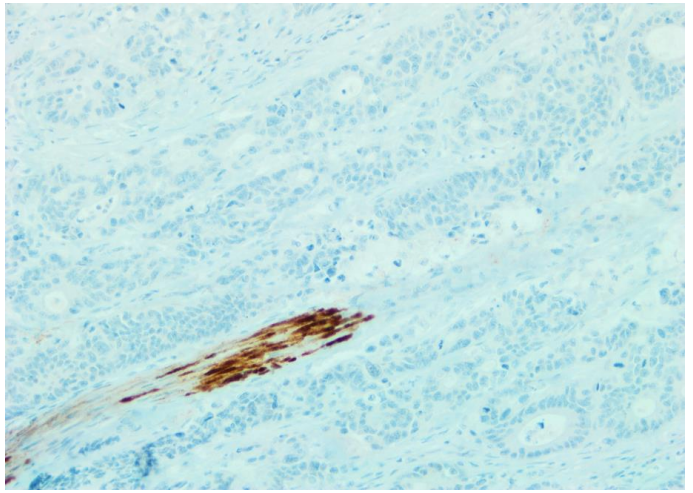
Advantages:

- 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





Colorectal adenocarcinoma

Pos controls

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

Neg controls

Non-neoplastic tissues

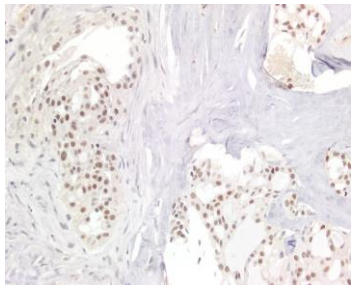
Tissue in which the protein are expressed

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

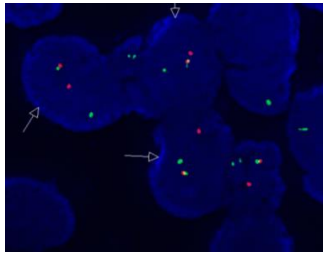
USE OF IHC

“Two-step approach”



IHC as a screening method

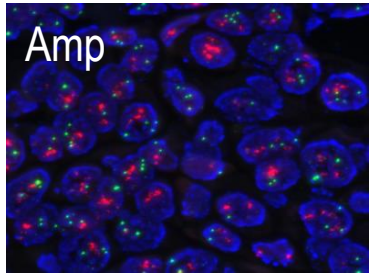
Sensitivity is crucial

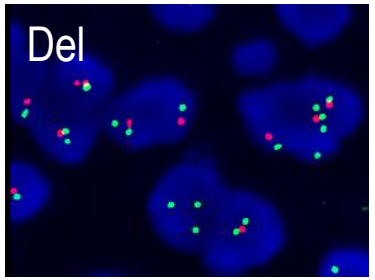


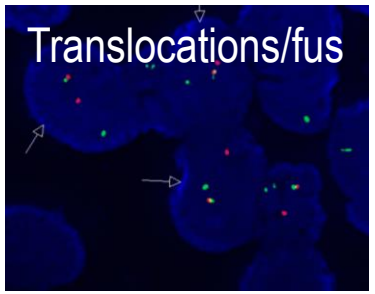
FISH or NGS
to confirm the presence of rearrangement

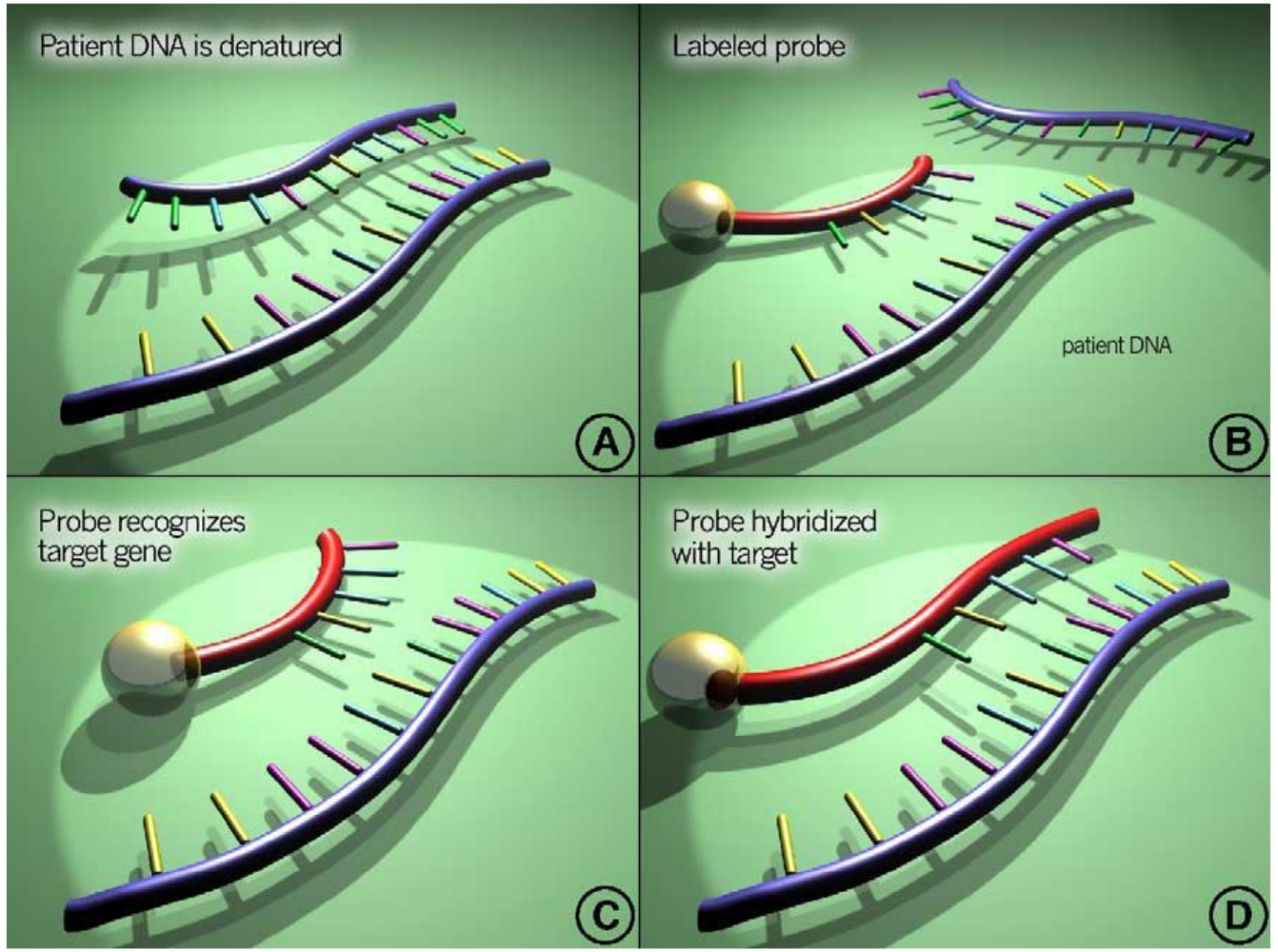
FISH

- Genes on a glass slide







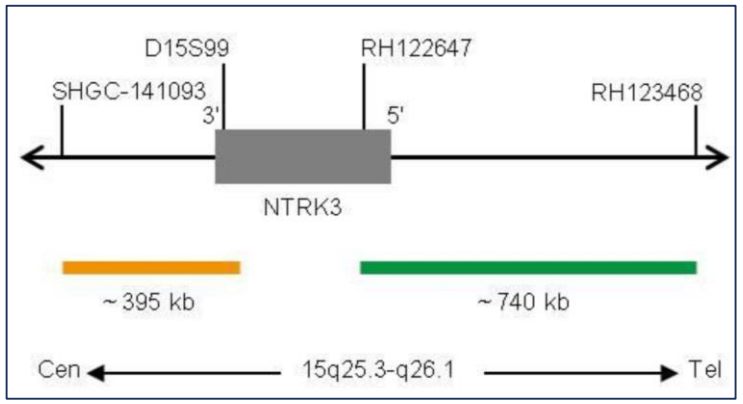


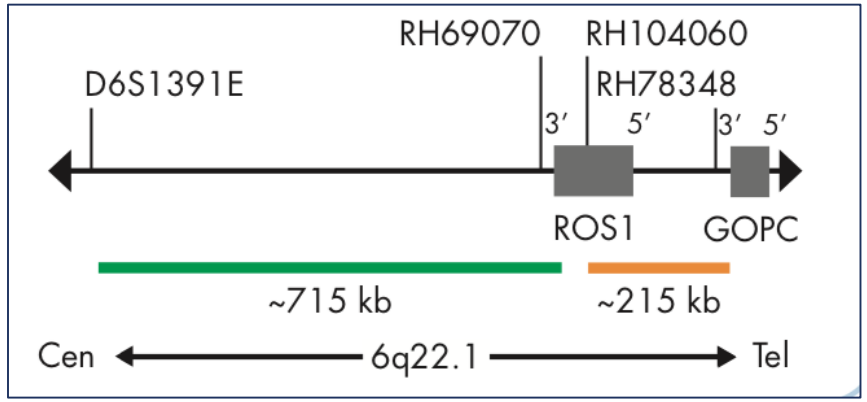
Hicks and Tubbs, Human Pathology 2005

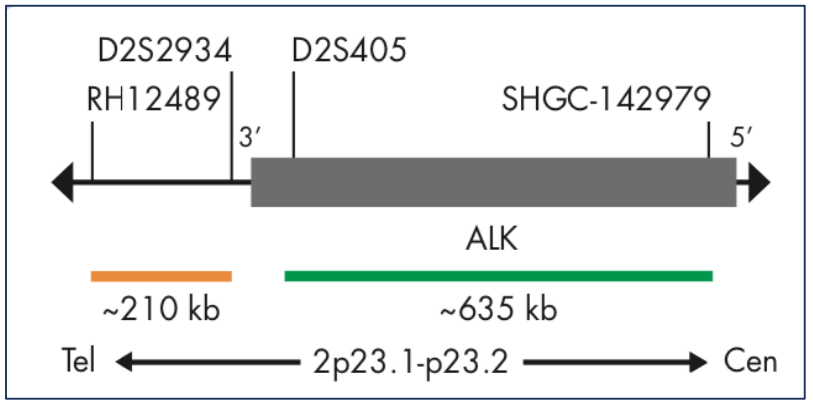
FISH

- 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

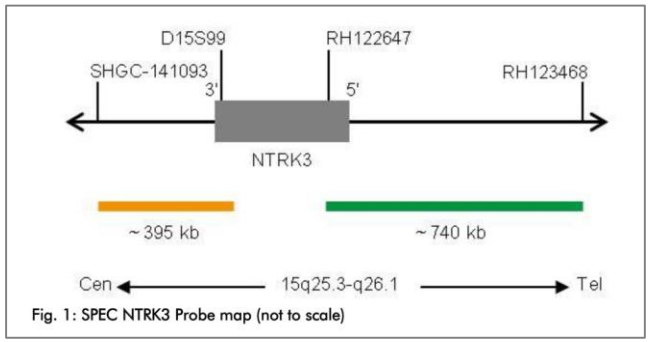
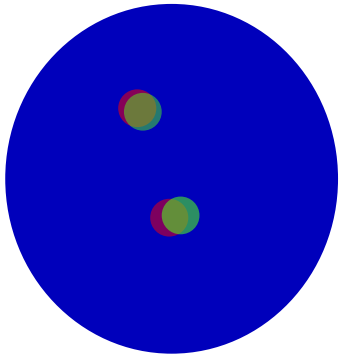
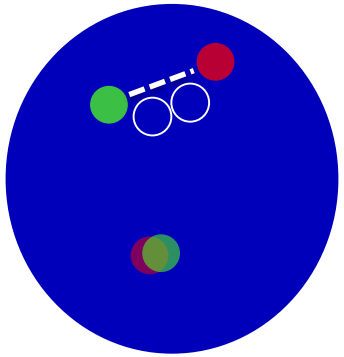


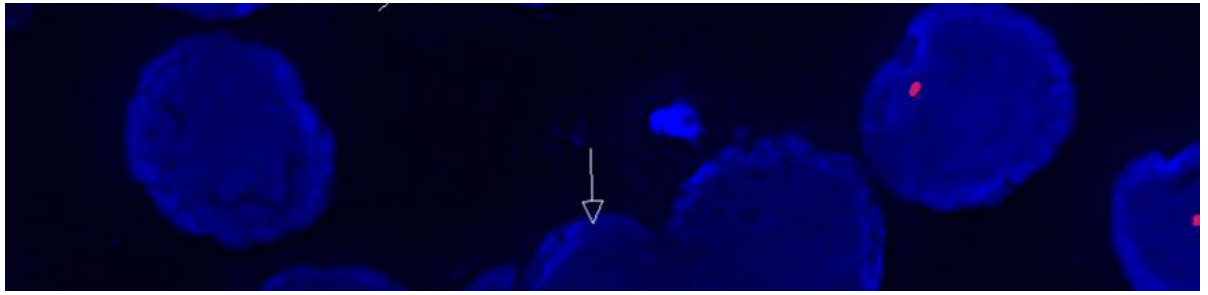
Fig. 1: SPEC NTRK3 Probe map (not to scale)



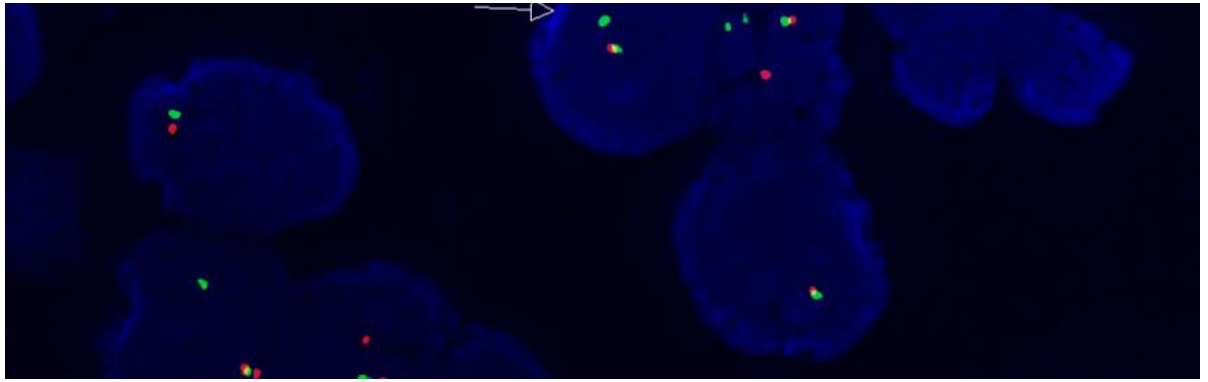
Normal



Aberrant



⇒ 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

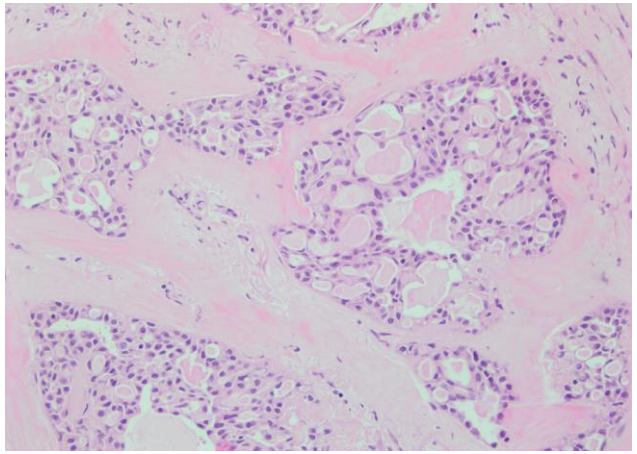
FISH

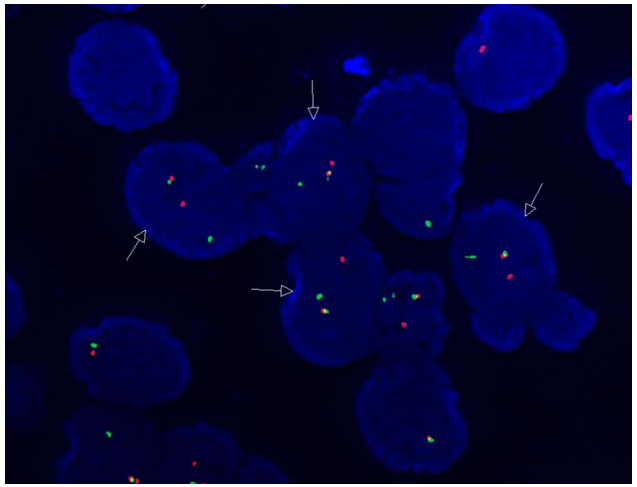
The utility of FISH for screening cancer when more than one gene has to be assayed (e.g. *NTRK1/2/3* fusions) is limited, given the multitude of partners involved, the expertise required and its labour-intensive nature

⇒ 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***

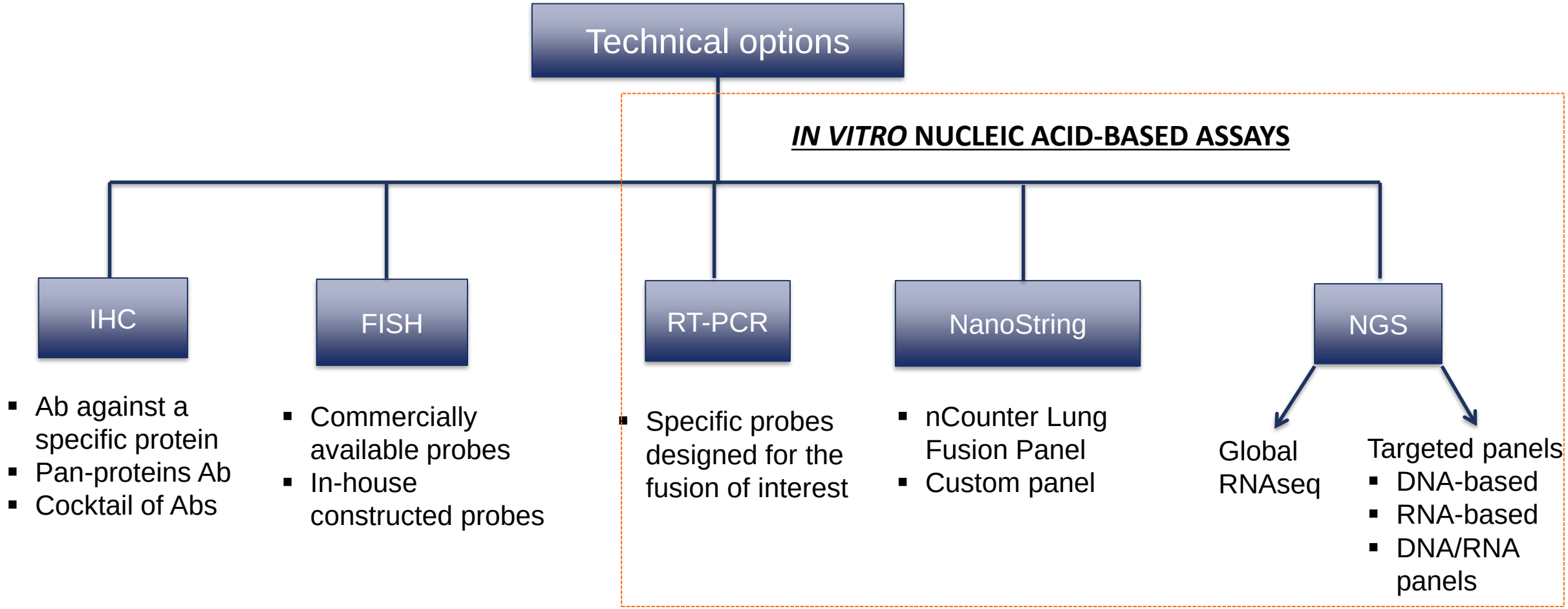
Secretory carcinoma of the breast





ETV6/NTRK3 split apart probe

GENE FUSION DETECTION: POSSIBLE TOOLS



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

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Nanostring

- nCounter Lung Fusion Panel (only selected fusions)
- Custom-made panels
- Technology used also for other types of diagnostic testings
- Not many studies so far

Real Time PCR

- Commercially kits available (even suggested as companion diagnostic)
- Simple workflow and short TAT
- Low costs
- Detection of specific alterations

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Confirmatory technique

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Can be used for screening

Real Time PCR

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- Simple workflow and short TAT
- Low costs
- Detection of specific alterations

Can be used for screening

NGS

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

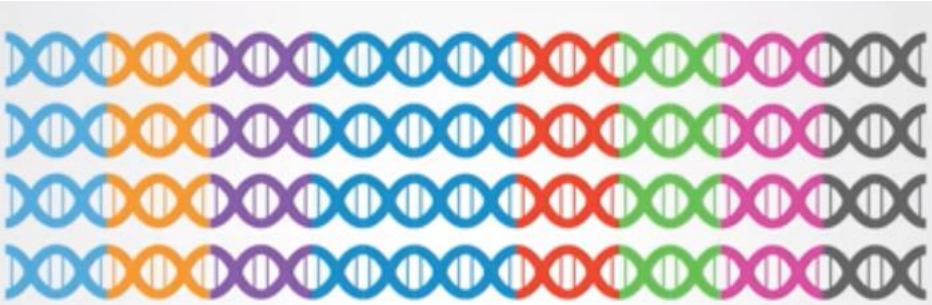
=> commercial ready to use kits and customisable assays

=> 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

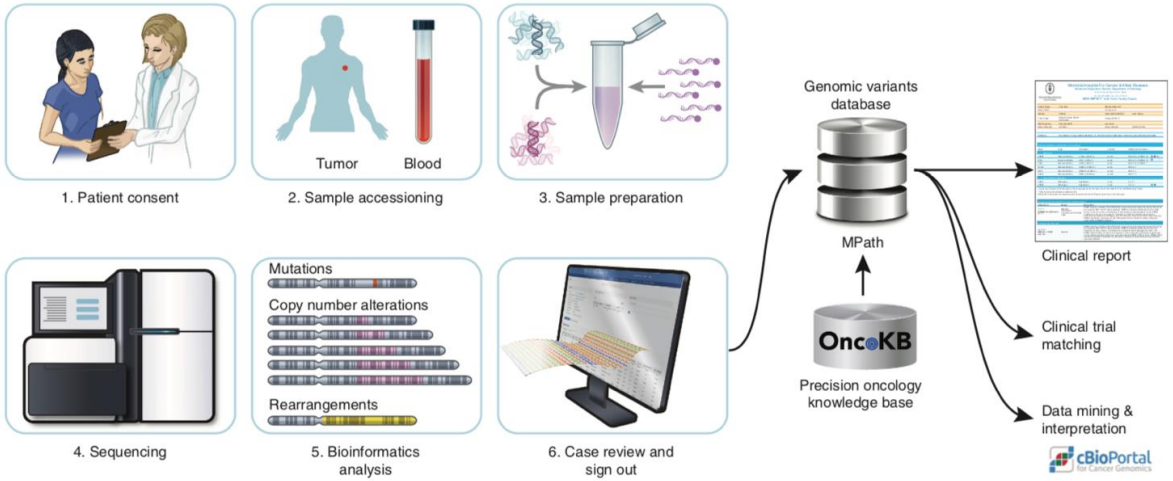
Images from https://archerdx.com

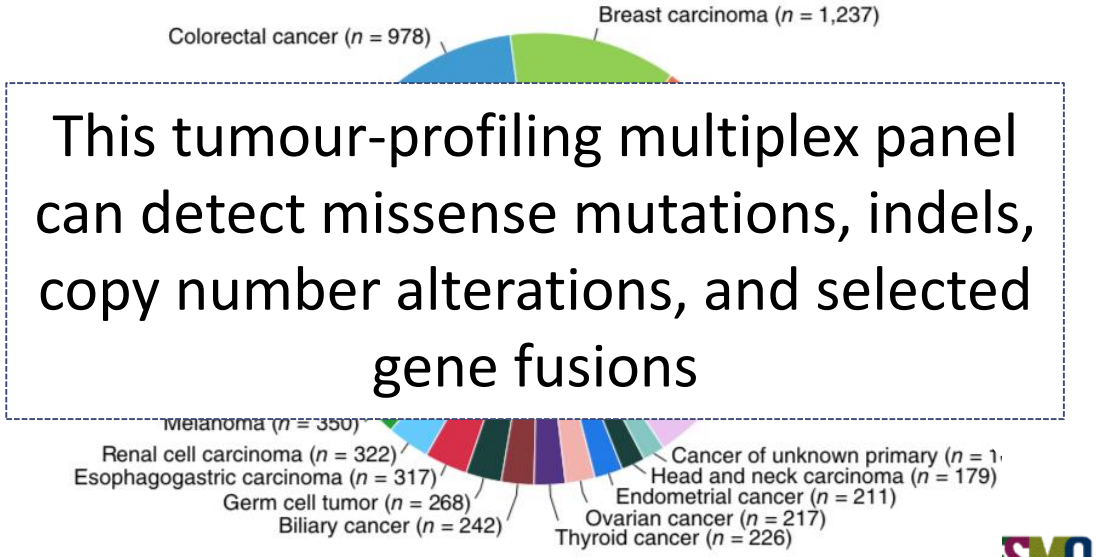
NGS

Targeted next generation DNA sequencing assays

Memorial Sloan Kettering Cancer Center

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

NGS

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)

NGS

Targeted next generation DNA sequencing assays – key concepts

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

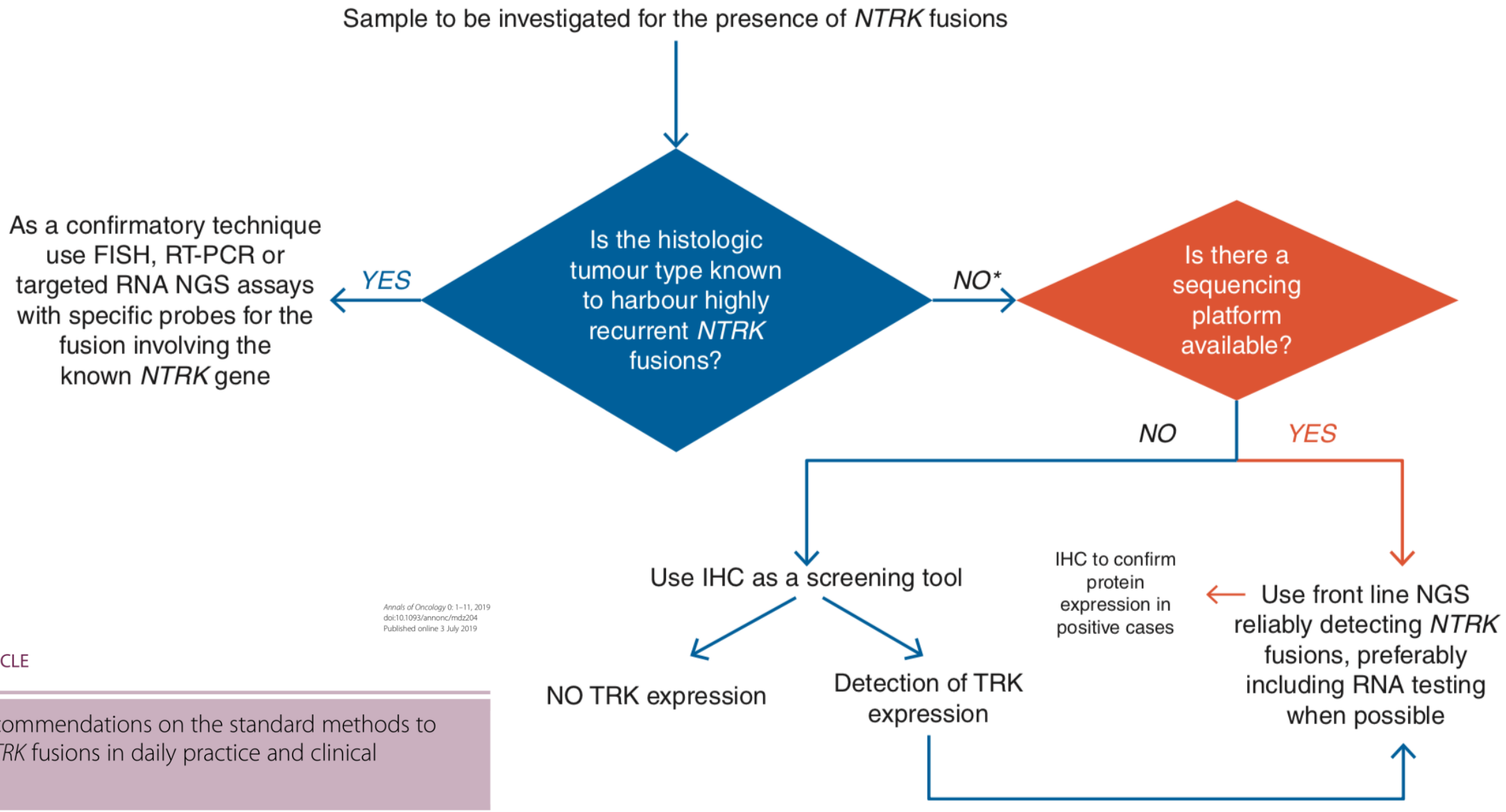
2) Detected rearrangements by DNA-based assays may not result in fusions

3) NOT ALL of the rearrangements can be practically detected using targeted assays: for instance, those fusions involving *NTRK2* and *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*)

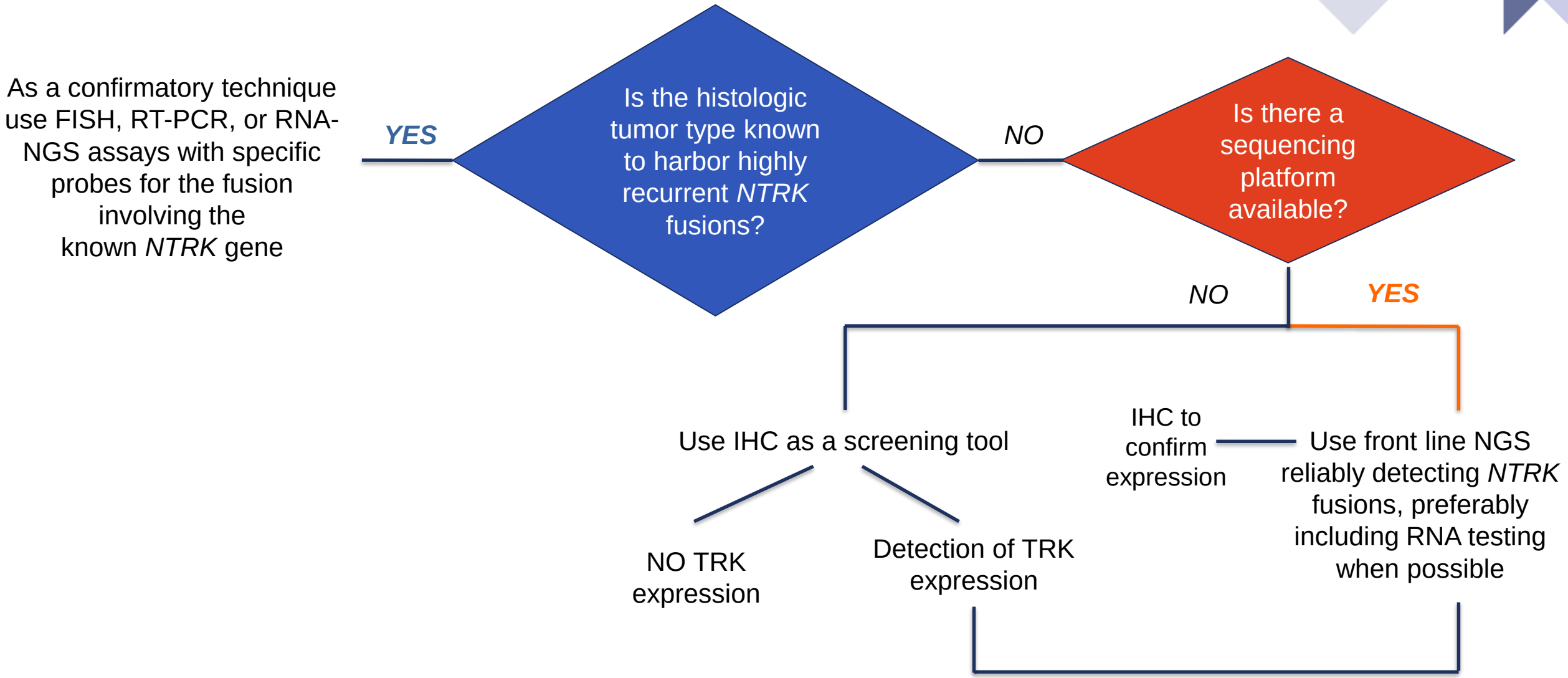


Annals of Oncology 0: 1–11, 2019
doi:10.1093/annonc/mdz204
Published online 3 July 2019

SPECIAL ARTICLE

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



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

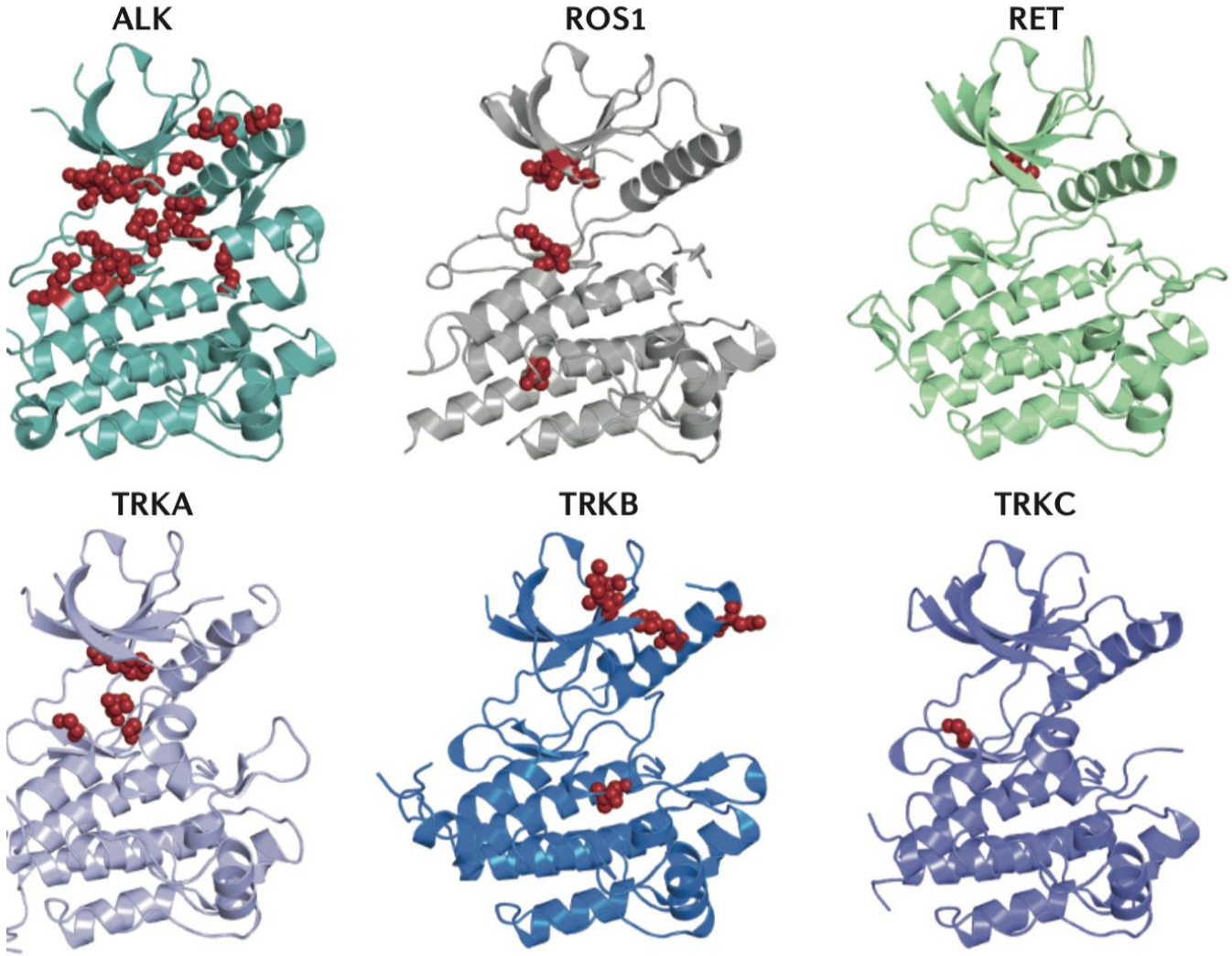
Despite durable responses to drugs targeting kinase fusions, it is expected that acquired resistance to therapy may ultimately emerge in most patients

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



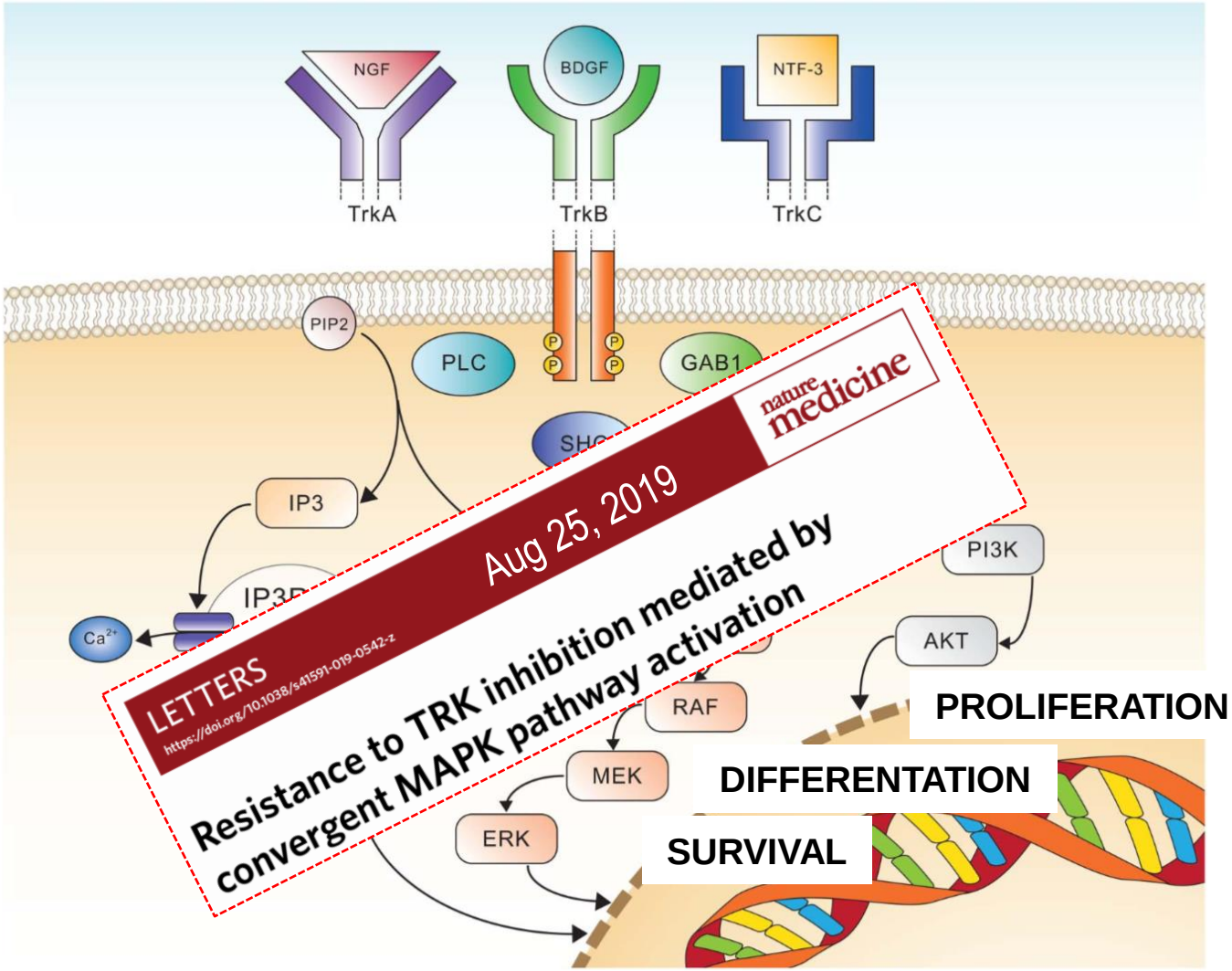
In silico structural modelling of the KD of ALK, ROS1, RET, TRKA, TRKB, and TRKC
by Schram AM et al, Nature Reviews Clinical Oncology 2017

“Off target” resistance

NTRK RECEPTOR SIGNALING

NTRK fusions lead to:

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



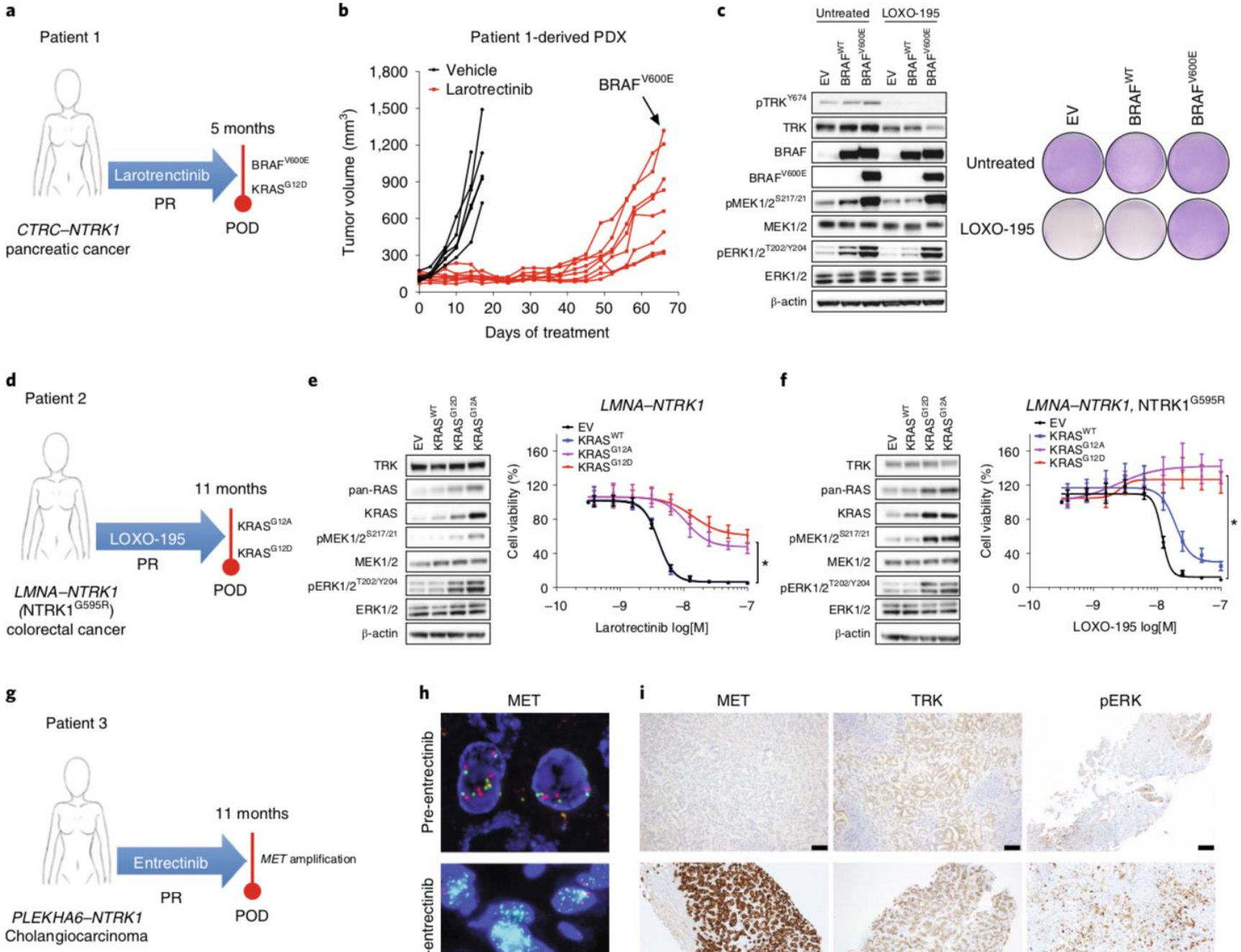
Amatu A et al, ESMO open 2016

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

Assay	Description
TruSight Tumor 170 (Illumina) TruSight Oncology 500	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. *NTRK1*, *NTRK2*, *NTRK3* genes are included in the panel for the fusions. DNA + RNA* assay targeting 523 genes for assessment of small variants, TMB, MSI, splice variants, and fusions
Oncomine Assays (ThermoFisher Scientific)	Targeted, multi-biomarker assay that enables to target hotspots, SNVs, indels, CNVs, and gene fusions from DNA and RNA in a single workflow.
FoundationOne®Heme (Foundation Medicine)	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.
Caris Molecular intelligence	Panel of 592 genes including fusion genes (*ALK*, *BRAF*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1*, *RSPO3*)
Omniseq Comprehensive	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 *NTRK1*, *NTRK2*, *NTRK3*.
Paradigm Cancer Diagnostic (PCDx)	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.
HANDLE-LCP30 panel (AmoyDx)	Multiplex and targeted deep sequencing of variants in 30 driver genes, including *NTRK1-3* fusions. The assay allows detection of SNVs, InDels, Fusions and CNVs.

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