V SIMPOSIO GETHI 18/19
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Ilustre Colegio Oficial de Médicos de Madrid. Aula Jiménez Díaz. Madrid

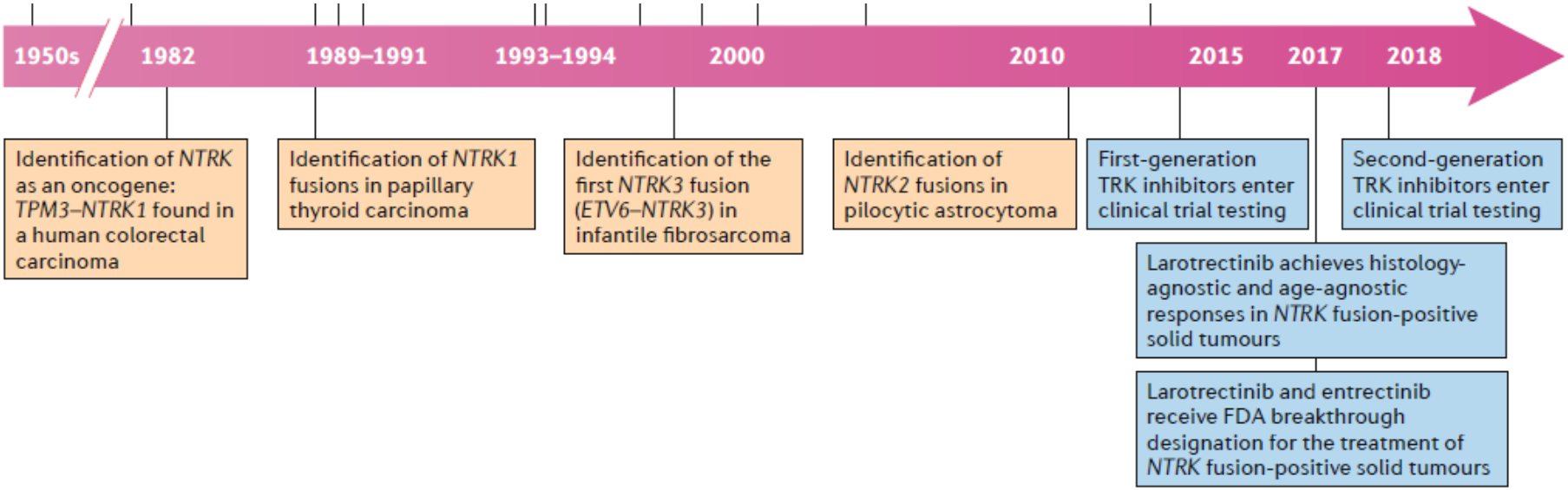
NTRK: ¿Qué alteraciones presentan y en qué tumores?

Hospital Universitario 12 de Octubre
SaludMadrid

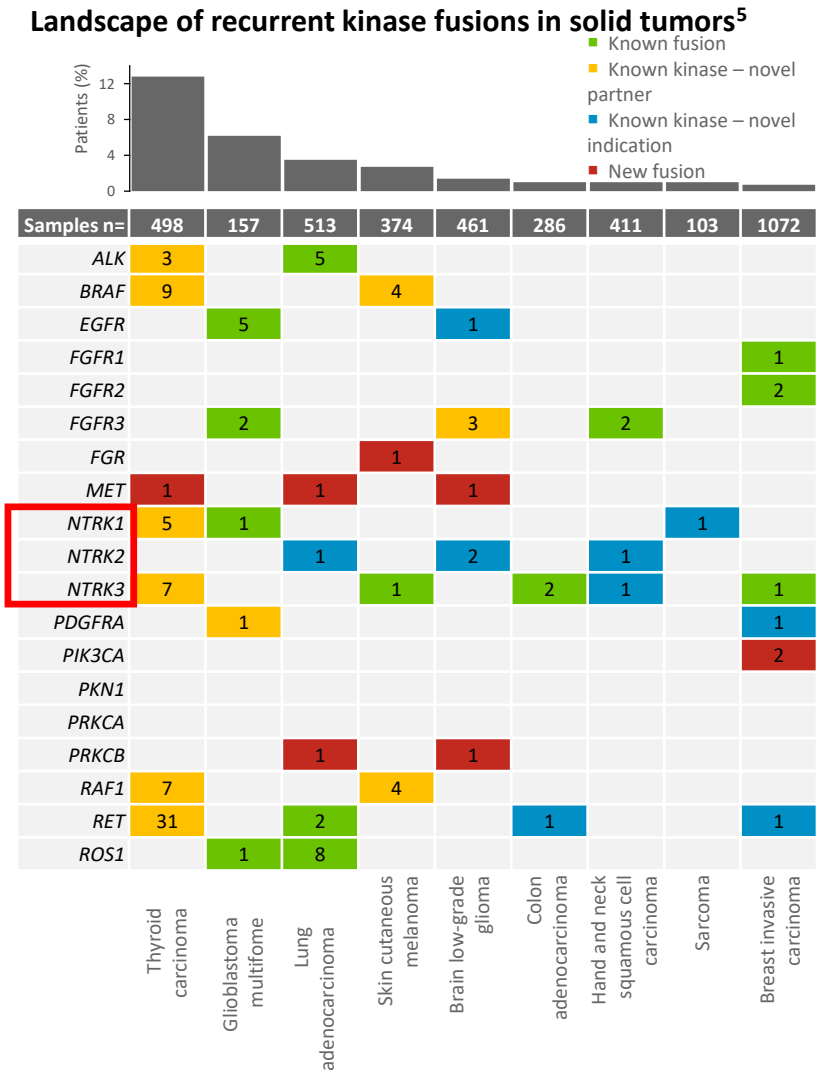
Dra. Lara Iglesias

Oncología Médica

HISTORY OF ONCOGENIC *NTRK* GENE FUSIONS



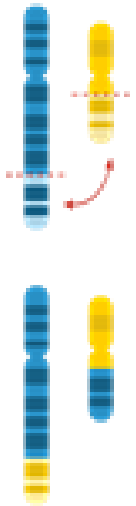
- Associated with a diverse range of solid tumors and hematologic malignancies[1,2]
- Neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions are associated with many human cancers
  - Associated with ≥19 tumor types[2,3]
  - Implicated in up to 1% of all solid tumors[4]



1. Khotskaya YB, *et al*. *Pharmacol Ther*. 2017;173:58-66
2. Vaishnavi A, *et al*. *Cancer Discov.* 2015;5:25-34.
3. Amatu A *et al*. *ESMO Open*. 2016;1:e000023.
4. Drilon A, *et al*. *N Engl J Med.* 2018;378:731-739.
5. Stransky N, *et al*. *Nat Commun*. 2014;5:846.

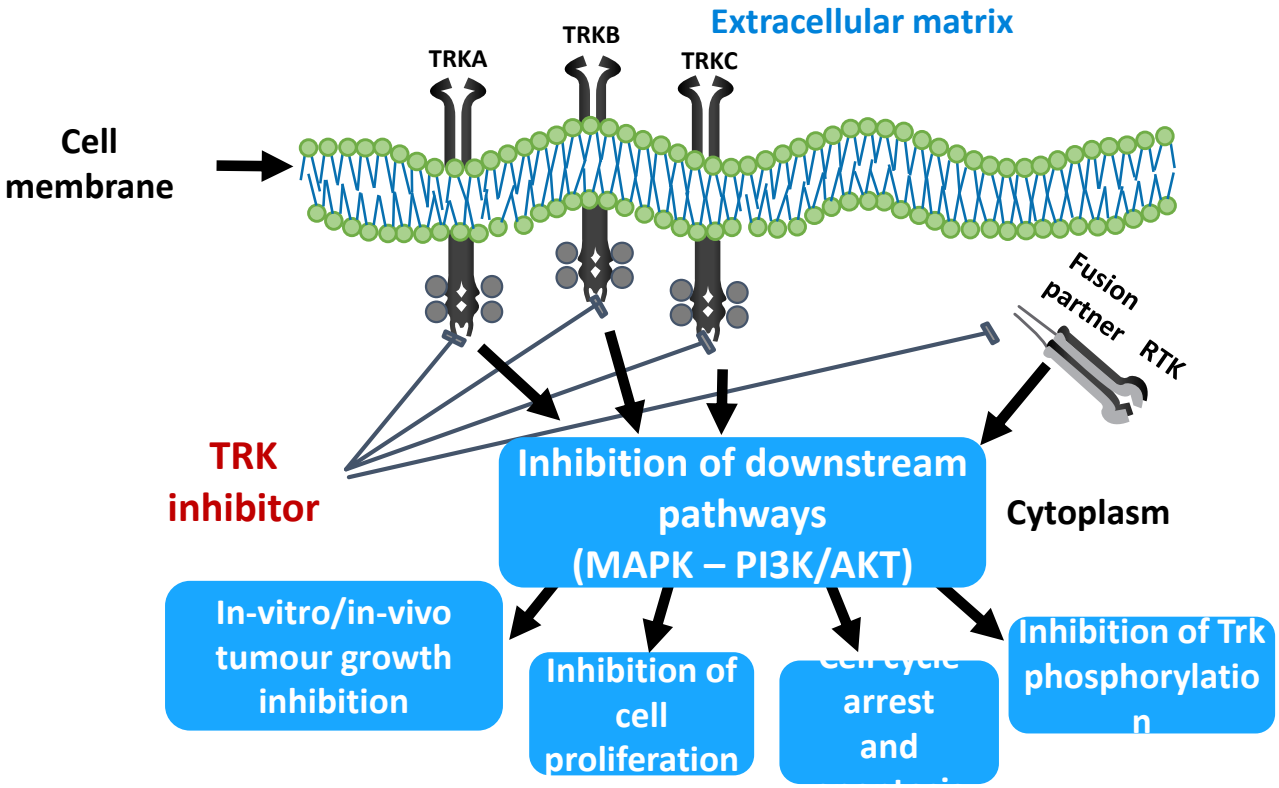
NTRK: Rearrangements/ fusions

- Neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions :
  - involving either *NTRK1*, *NTRK2* or *NTRK3*
  - (encoding the neurotrophin receptors
  - TRKA , TRKB and TRKC, respectively)



2. Vaishnavi A, *et al. Cancer Discov.* 2015;5:25-34.
3. Amatu A *et al. ESMO Open.* 2016;1:e000023.
4. Drilon A, *et al. N Engl J Med.* 2018;378:731-739.
5. Stransky N, *et al. Nat Commun.* 2014;5:846.

Tyrosine kinase domain of TrkA, TrkB, TrkC receptors[1]



1. Rolfo, et al. Expert Opin Investig Drugs 2015

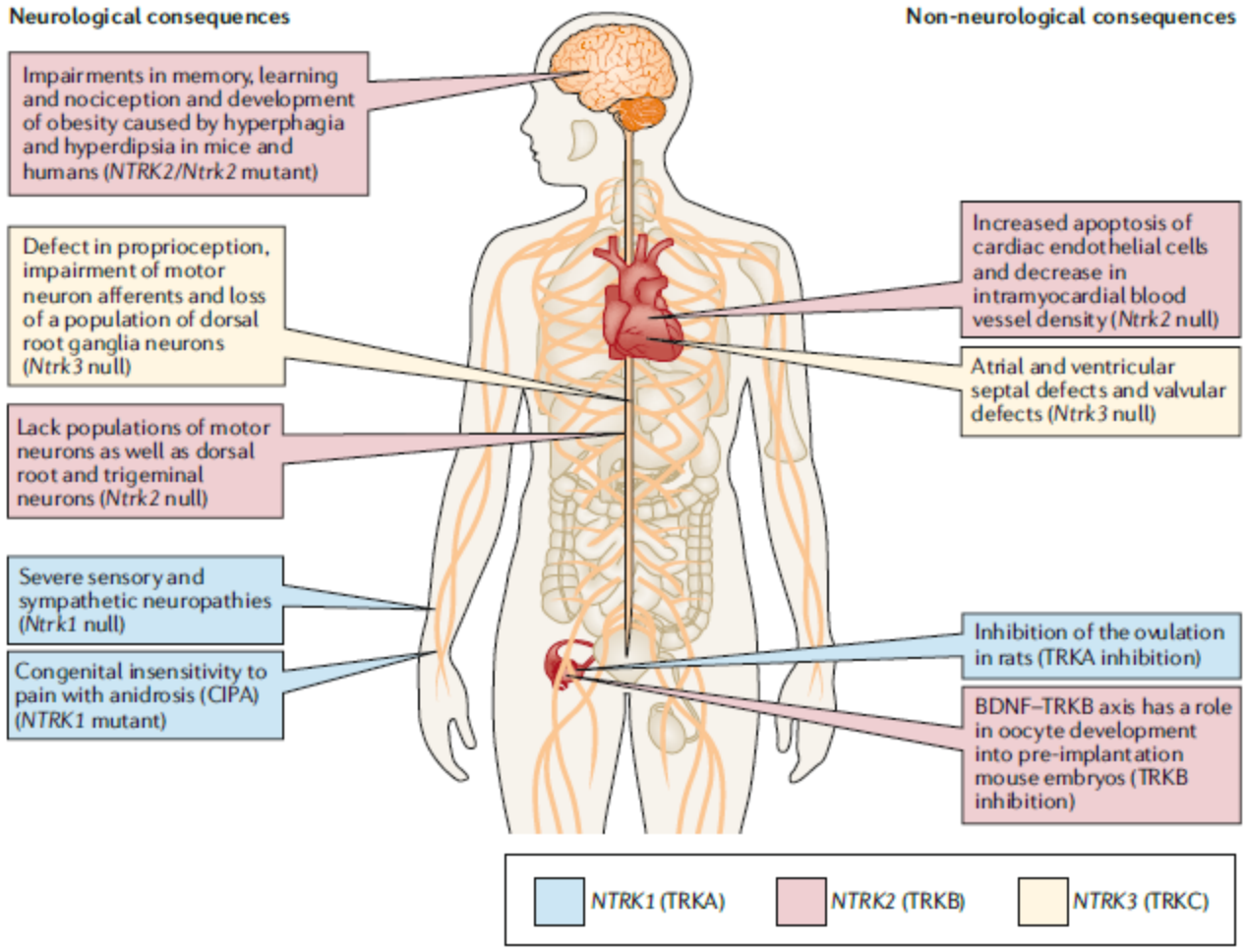


Fig. 6 | **Consequences of loss, decreased activity or inhibition of TRK.** Genetic or pharmacological disruption of TRK

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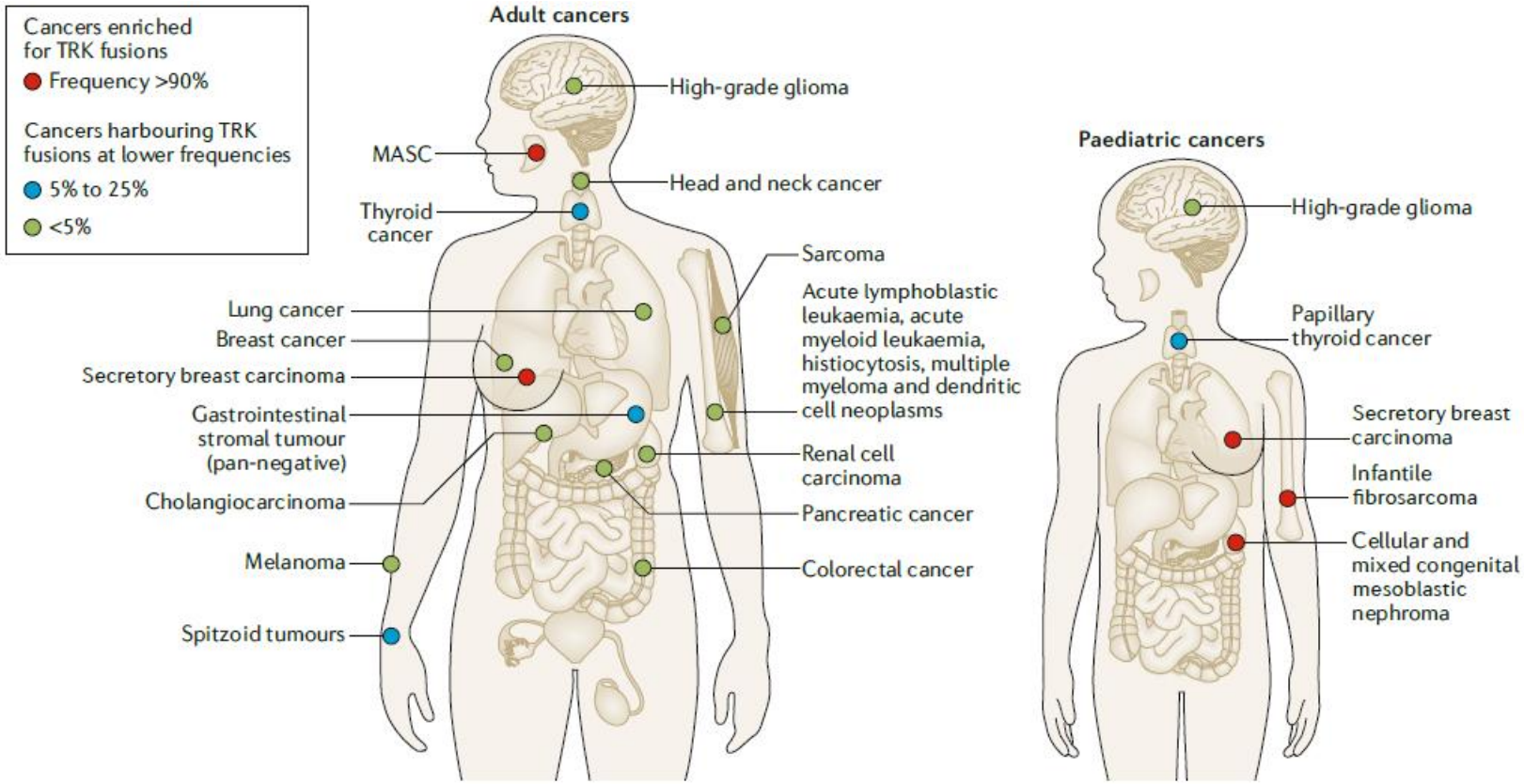
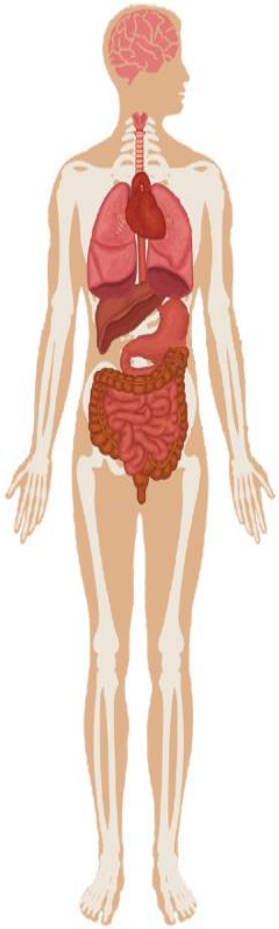
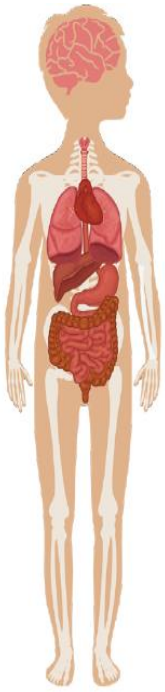


Adult



- Brain cancers (0.4–3.1%)[1]
- Salivary (MASC; 90–100%)[1]
- Secretory breast cancer (92%)[2*]
- Pancreatic cancer (<1%)[3,4]
- Cholangiocarcinoma (3.6%)[1]
- Thyroid cancer (1.5–14.5%)[1]
- Lung cancer (0.2–3.3%)[1]
- GIST (3.2%)[5]
- Colon cancer (1.5%)[1]
- Melanoma (0.3%)[1,6]
- Sarcomas (1%)[6*]

Pediatric



- Brain cancers (~10%)[7]
- Spitzoid neoplasms (16.4%)[8]
- Gliomas (7.1%)[1]
- Thyroid (9.4–25.9%)[9,10]
- Infantile fibrosarcoma (91–100%)[11]
- Congenital nephroma (83%)[1]
- Secretory breast cancer (92%)[2*]
- Sarcomas (1%)[6*]

- *Frequency in adult vs. pediatric patients not specified;
GIST, gastrointestinal stromal tumor;
MASC, mammary analogue secretory carcinoma.

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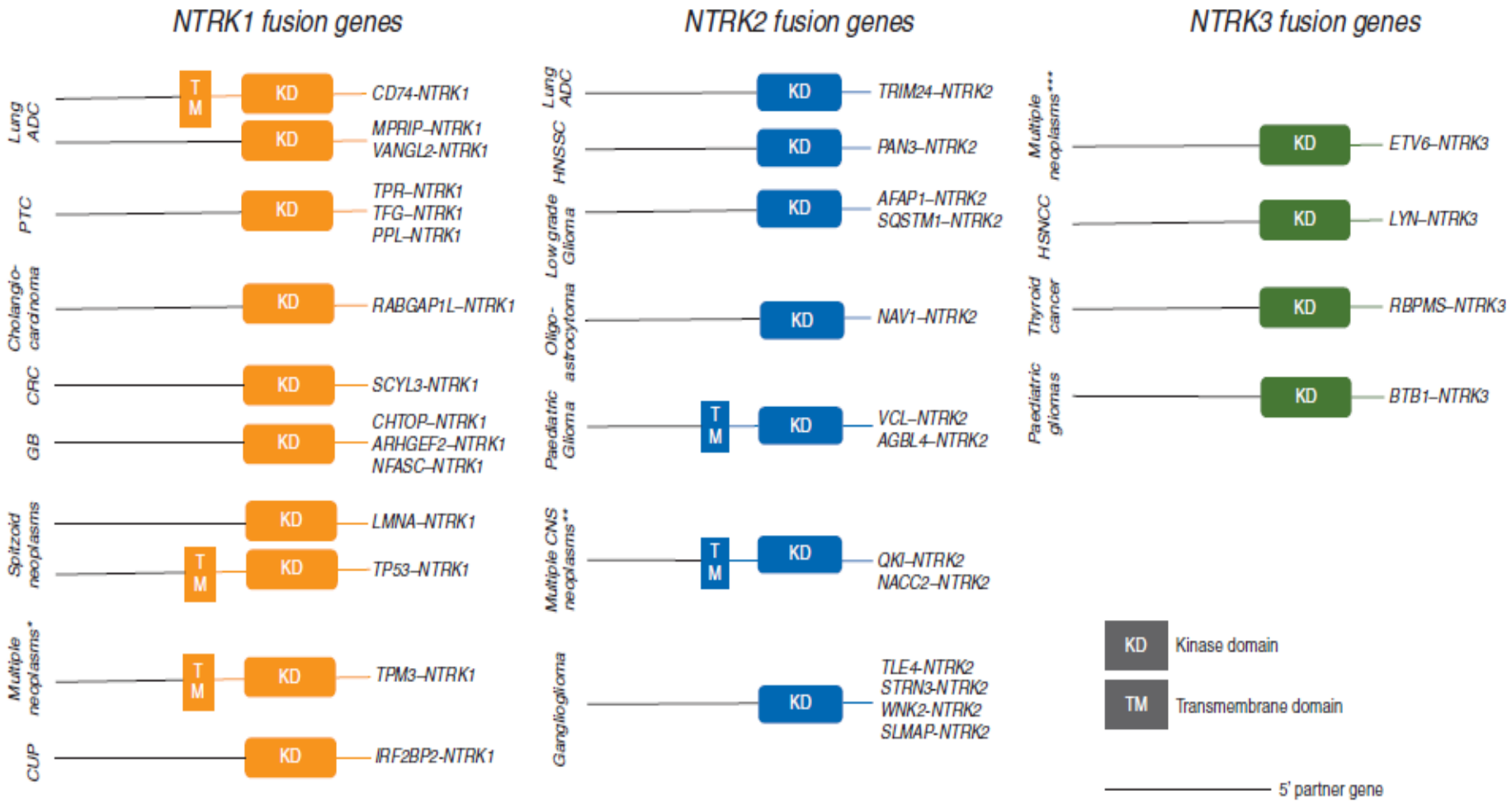

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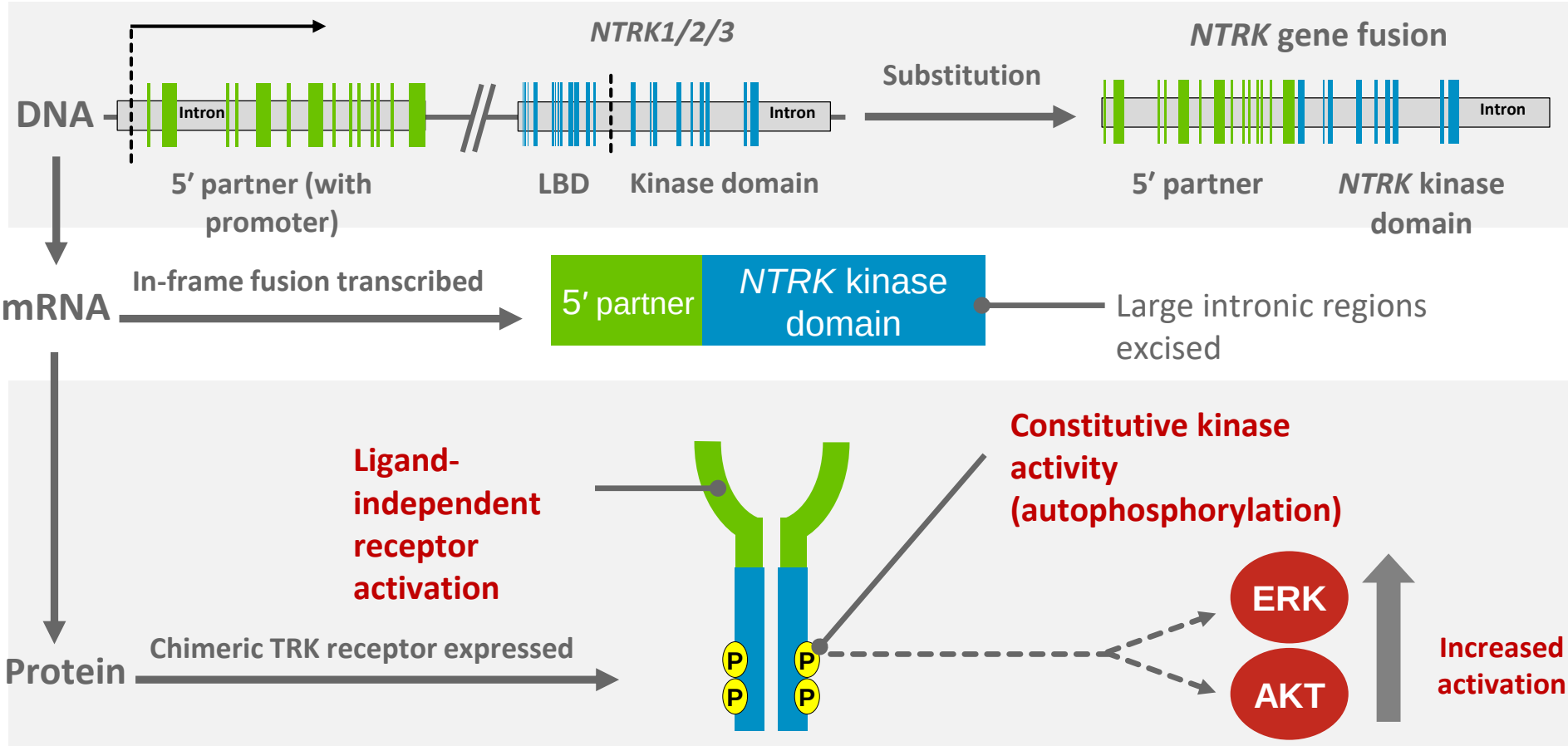
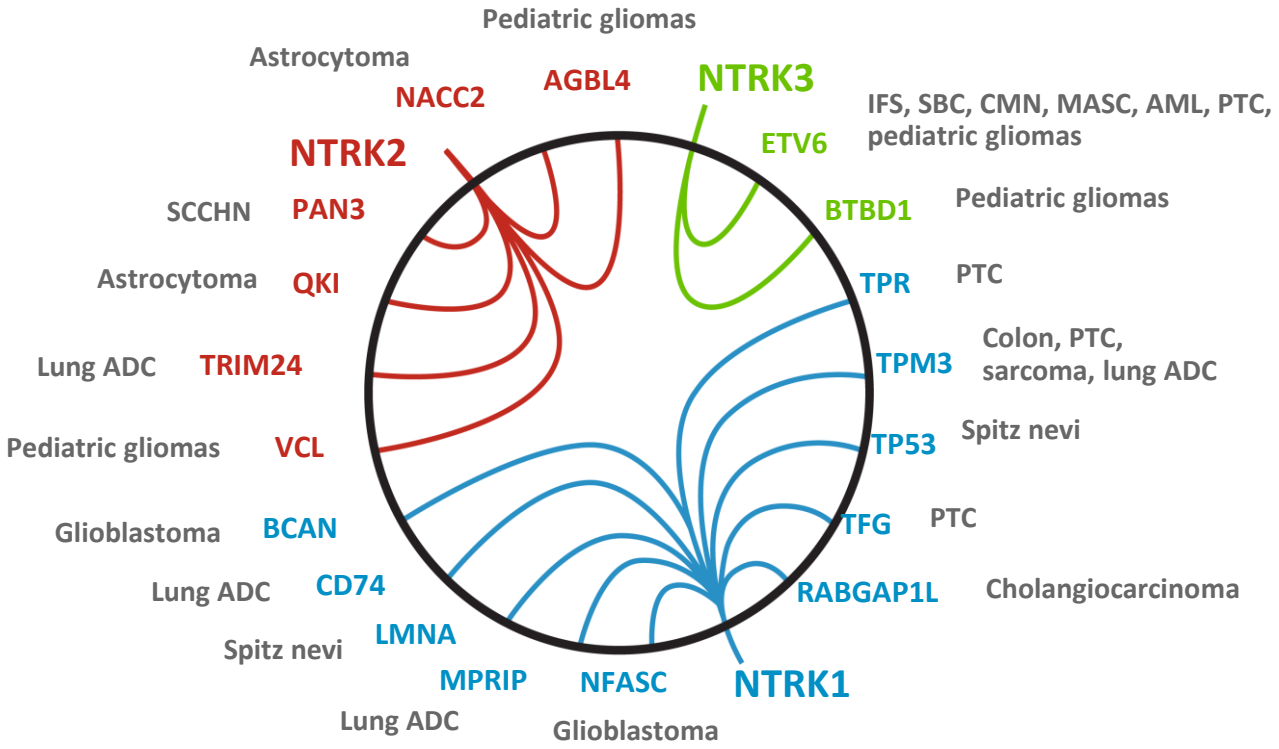
NRTK gene fusions are oncogenic – NOT SNVs, CNVs, alterations



AKT, protein kinase B; CNV, copy number variant; ERK, extracellular signal-regulated kinase; LBD, ligand-binding domain; SNV, single nucleotide variant.

1. Amatu A, *et al*. *ESMO Open*. 2016;1:e000023.
2. Vaishnavi A, *et al*. *Cancer Discov.* 2015;5:25-34.
3. Hyman DM, *et al*. *J Clin Oncol.* 2017;35:LBA2501.



- *NTRK* gene fusions occur in a tumor-agnostic manner with inconsistent break points and fusion

The optimal detection method requires no prior knowledge of fusion break points and/or fusion partner

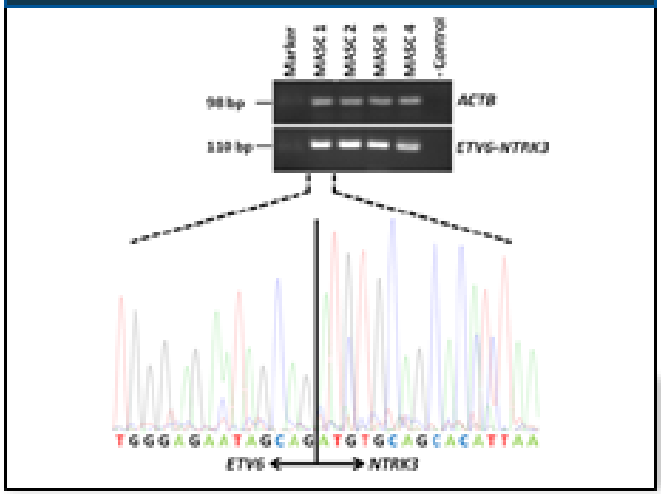
prehensive *NTRK*

The Art of Identifying *NTRK* Gene Fusions Why Not RT-PCR?

- ⊕ High sensitivity and specificity[1,2]
- ⊕ Widely available, low cost[3]

- ⊖ Specific primer sets required for each fusion – unknown fusions not detected[3-5]
- ⊖ Low-complexity, GC-rich sequences in *NTRK* genes limit useful primer selection and complicate reaction optimization[6]

Detection of *ETV6-NTRK3* fusion transcripts in MASC tumors by RT-PCR analysis[7]

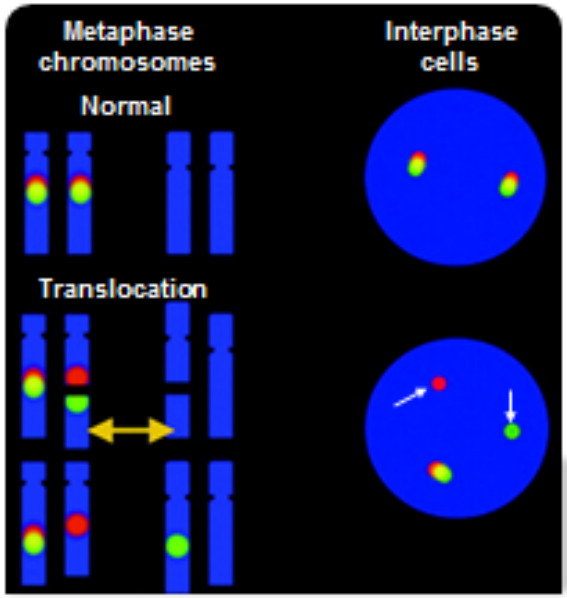


The Art of Identifying *NTRK* Gene Fusions
Why Not FISH?

- ⊕ Novel fusion partners can be detected if break-apart probes used[1]
- ⊕ High sensitivity and specificity[2]

- ⊖ Requires individual probes for each *NTRK* gene (ie, three separate FISH analyses per patient sample)
- ⊖ Break-apart FISH identifies gene disruptions in DNA but cannot confirm in-frame, functional fusions

Yellow (red/green) signals in normal interphase nuclei versus split red and green signals in cell carrying translocation



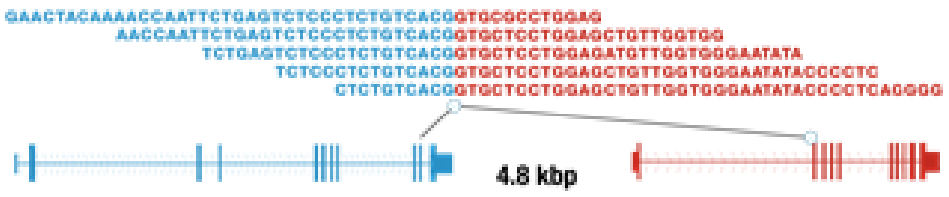
The Art of Identifying *NTRK* Gene Fusions
What Makes NGS Ideal?

General NGS

- High sensitivity and specificity potential
- Multiplexing: simultaneously queries multiple potentially actionable targets (eg, *NTRK*, *ALK*, *ROS1*, *RET*)
- Detects both known and novel fusions, regardless of break point or fusion partner (depending on library preparation method)

RNA-based NGS

- Able to distinguish in-frame, transcribed gene fusions versus out-of-frame fusions
- Avoids difficulties of sequencing large intronic regions in the *NTRK* genes



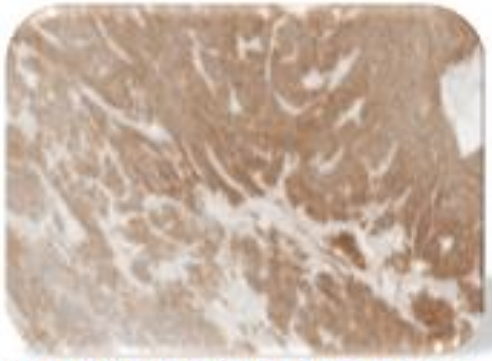
RNA-based NGS is the preferred method for detecting *NTRK* gene fusions in cancer

Pan-TRK IHC Detects Both Wild Type and Fusion TRK Protein

Fusion-driven TRK expression

Secretory carcinoma of salivary gland
95% of tumor cells staining
ETV6-NTRK3 fusion detected by NGS

Wild type TRK expression



Gastrointestinal stromal tumor (GIST)
100% tumor cells staining
No fusion detected by NGS

- 9% (n=324) of 3,574 tumors screened by pan-TRK IHC stained positive when defined as >1% of tumor cell staining (range: 0%-54% in gastric cancers and salivary gland cancers, respectively)
- Of the 324 IHC-positive tumors, 164 were confirmed by ISH and 12 fusions were detected
- The percentage of cases staining positive decreased to 5% (n=191) and 4% (n=133) if the definition of positive IHC was increased to >10% and >25% of cells staining, respectively
- At a cutoff of 25% of cells staining, two fusion-positive melanomas would have been missed

Confirmation of IHC-positive cases is REQUIRED

ISH, *in situ* hybridization
Feng J, et al. Poster presented at: ESMO Molecular Analysis for Personalised Therapy; 2015; Paris, France.

Diagnostic testing methods summary: Benefits and drawbacks

	Method			
	Next-generation sequencing (NGS)	Fluorescence *in situ* hybridization (FISH)	Polymerase chain reaction (PCR)	Pan-TRK immunohistochemistry (IHC)

Benefits	// Detection of novel fusion partners (depending on method)[1]
	// Ability to interrogate multiple actionable targets simultaneously[1]
	// Current *NTRK* testing conducted by NGS[2]
	// Relevance of NGS increases as number of actionable targets grows
	// High sensitivity and specificity potential[3]
		// Location of the target within the cell is visible[8,9]
		// High sensitivity and specificity[9]
		// Several fluorophores can be used at once to detect several targets in one sample[9]
			// High sensitivity and specificity[11,12]
			// RT-PCR assays detect fusions expressed at the RNA level (also applies to RNA-based NGS)[12]
			// Inexpensive[13]
				// Inexpensive[3,14]
				// Decentralized, available in most laboratories[11]
				// Established reimbursement codes[15]
				// Turnaround time ~2 days[14]

Drawbacks	// Turnaround time: ~1-3 weeks
	// Technically complex (high start-up cost)[4]
	// Requires specialty infrastructure[5]
	// Highly centralized testing model (academia and reference laboratories)[1]
	// Reimbursement currently restricted (although developing)[6]
	// Sensitivity and specificity of NGS assays vary widely[3,7]
		// Requires fluorescence microscopy[10]
		// Target sequence must be known (break-apart FISH may detect *NTRK* gene fusions with unknown partners, but both in-frame and out-of-frame fusions will be detected)[1]
			Target sequences must be known (ie, cannot detect novel fusion partners)[1,13]	// Detects both fusion and wild-type TRK expression[16]
				// Scoring algorithms are not standardized[11]

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

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doi:10.1093/annonc/mdz204
Published online 3 July 2019

C. Marchiò[1,2], M. Scaltriti[3,4], M. Ladanyi[3], A. J. Iafrate[5,6], F. Bibeau[7], M. Dietel[8], J. F. Hechtman[3], T. Troiani[9], F. López-Rios[10], J.-Y. Douillard[11], F. Andrè[12*] & J. S. Reis-Filho[3]

Table 1. Summary of main features, strengths and weaknesses of all available techniques to detect *NTRK* rearrangements

Method	Sensitivity	Specificity	Detection of all fusion genes	Detection of partner	Detection of expression	Screening
IHC	High[a]	High[b]	Yes	No	Yes	Yes
FISH[c]	High	High	One per probe	No	No	No
RNA seq NGS	High	High	Yes	Yes	Yes	Yes
DNA seq[c]	Moderate	High	Yes	Yes	No	Yes

[a]False negatives reported mainly in *NTRK3* fusions.

[b]In the absence of smooth muscle/neuronal differentiation.

[c]Detected rearrangements by DNA-based assays may not result in fusions, correlation with surgical pathology and predicted transcript (for sequencing) is needed.

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

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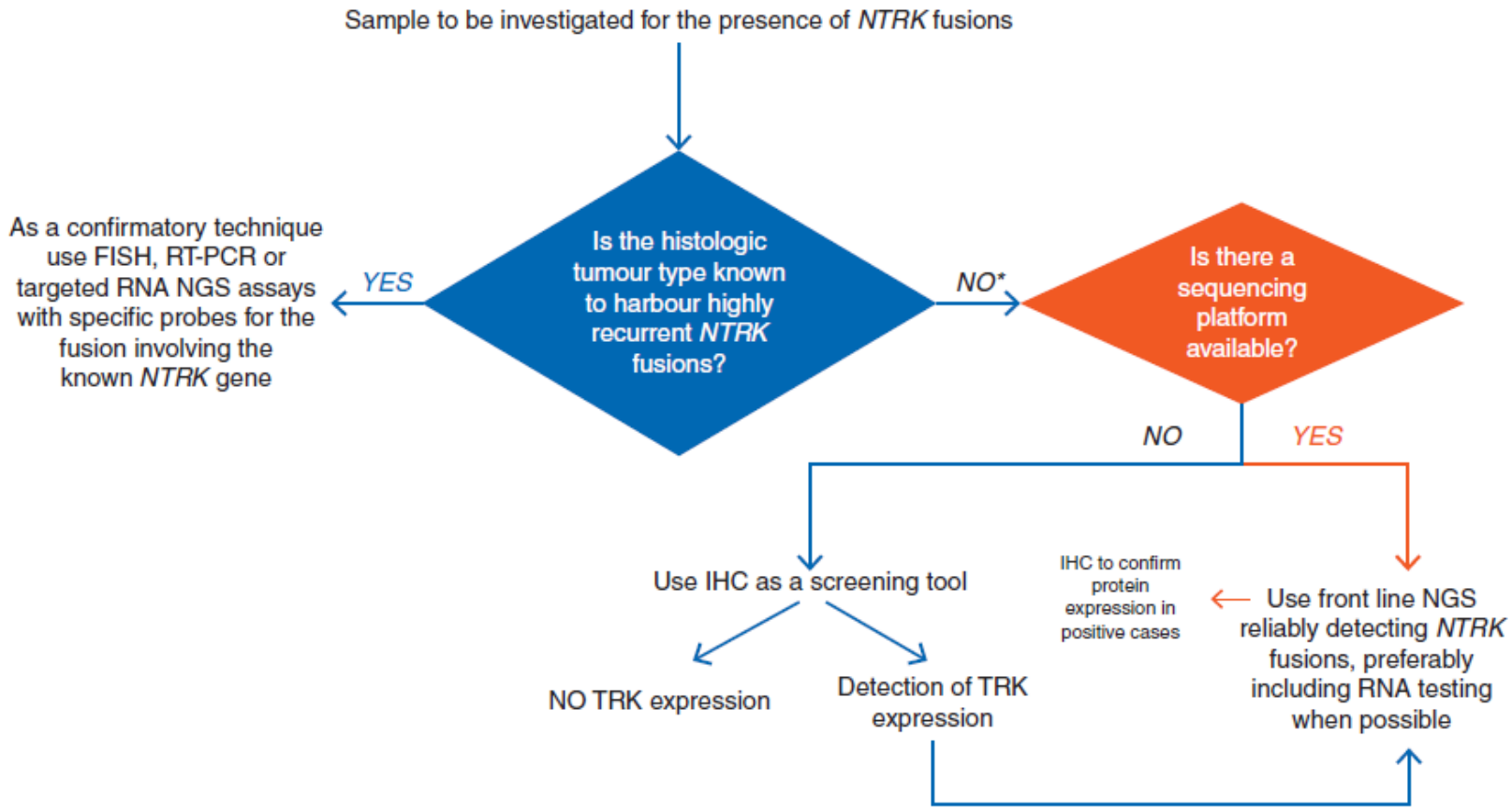
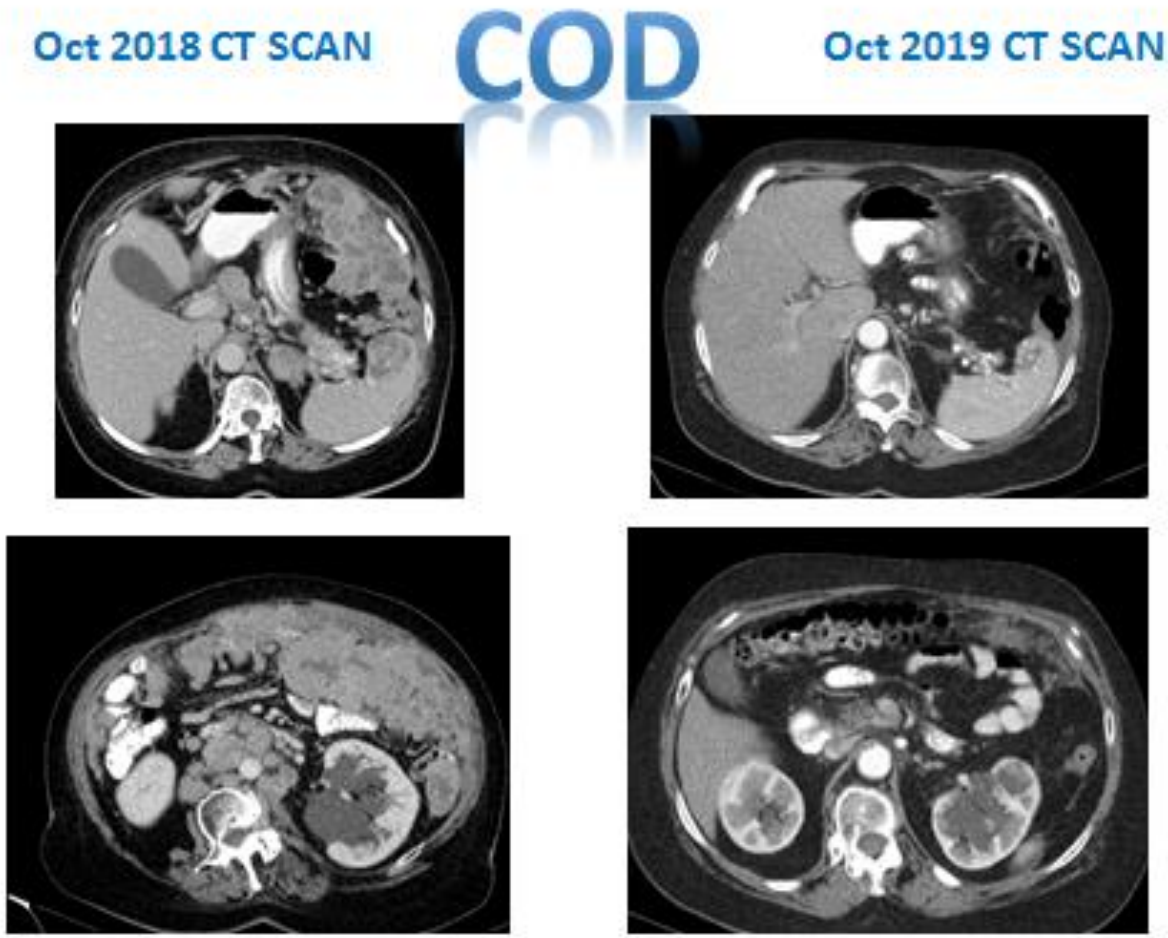


Testing for *NTRK* gene fusions is essential for identifying patients

NGS, PCR, and FISH can all identify *NTRK* gene fusions

IHC can be used as a screening tool, but results must be confirmed (preferably NGS)

NGS is a multiplex assay able to detect novel fusions and is available at several reference laboratories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laraiglesias@hotmail.com