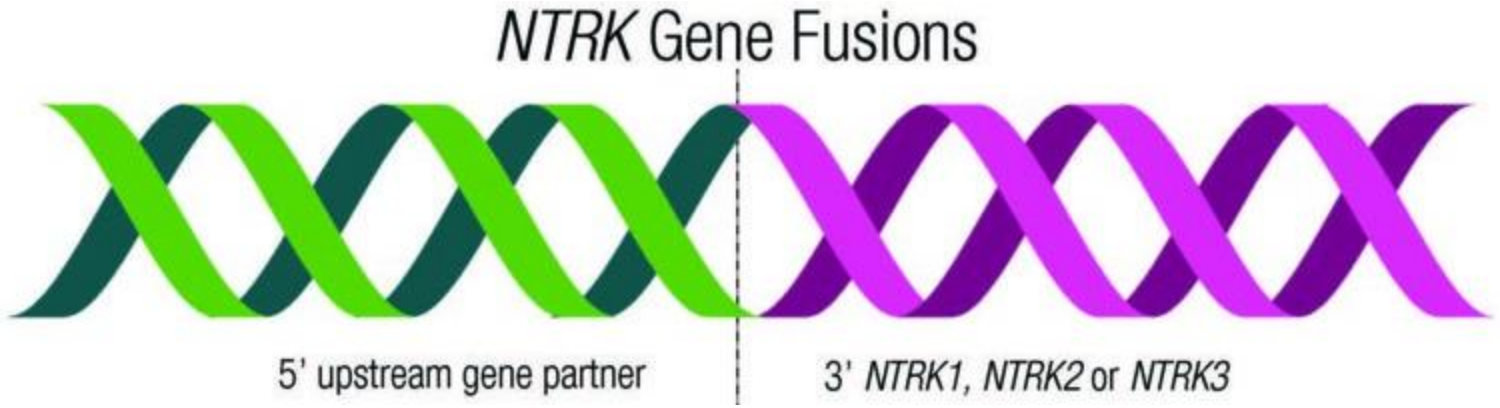
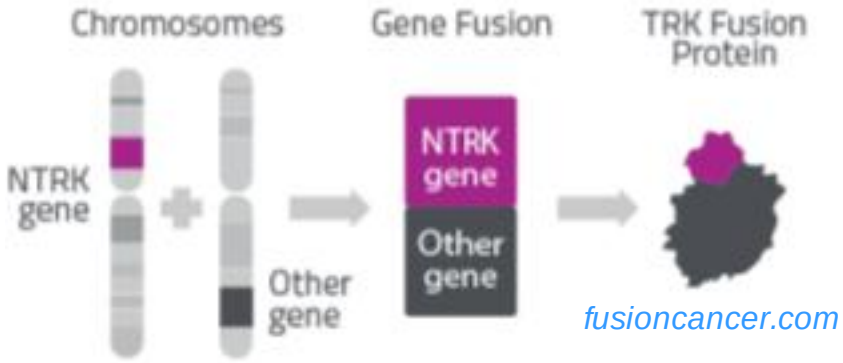
Medimail
May 2021

NTRK Gene Fusion Testing



NTRK Gene Fusions

- *Neurotrophic tyrosine receptor kinase (NTRK)* gene fusions involving either *NTRK1*, *NTRK2*, or *NTRK3* (encoding the neurotrophin receptors TRKA, TRKB, and TRKC, respectively) are oncogenic drivers of various adult and paediatric tumour types.
- Treatment of patients with *NTRK* fusion-positive cancers with first-generation TRK inhibitors, such as larotrectinib or entrectinib, is associated with high response rates (>75%), regardless of tumour histology.



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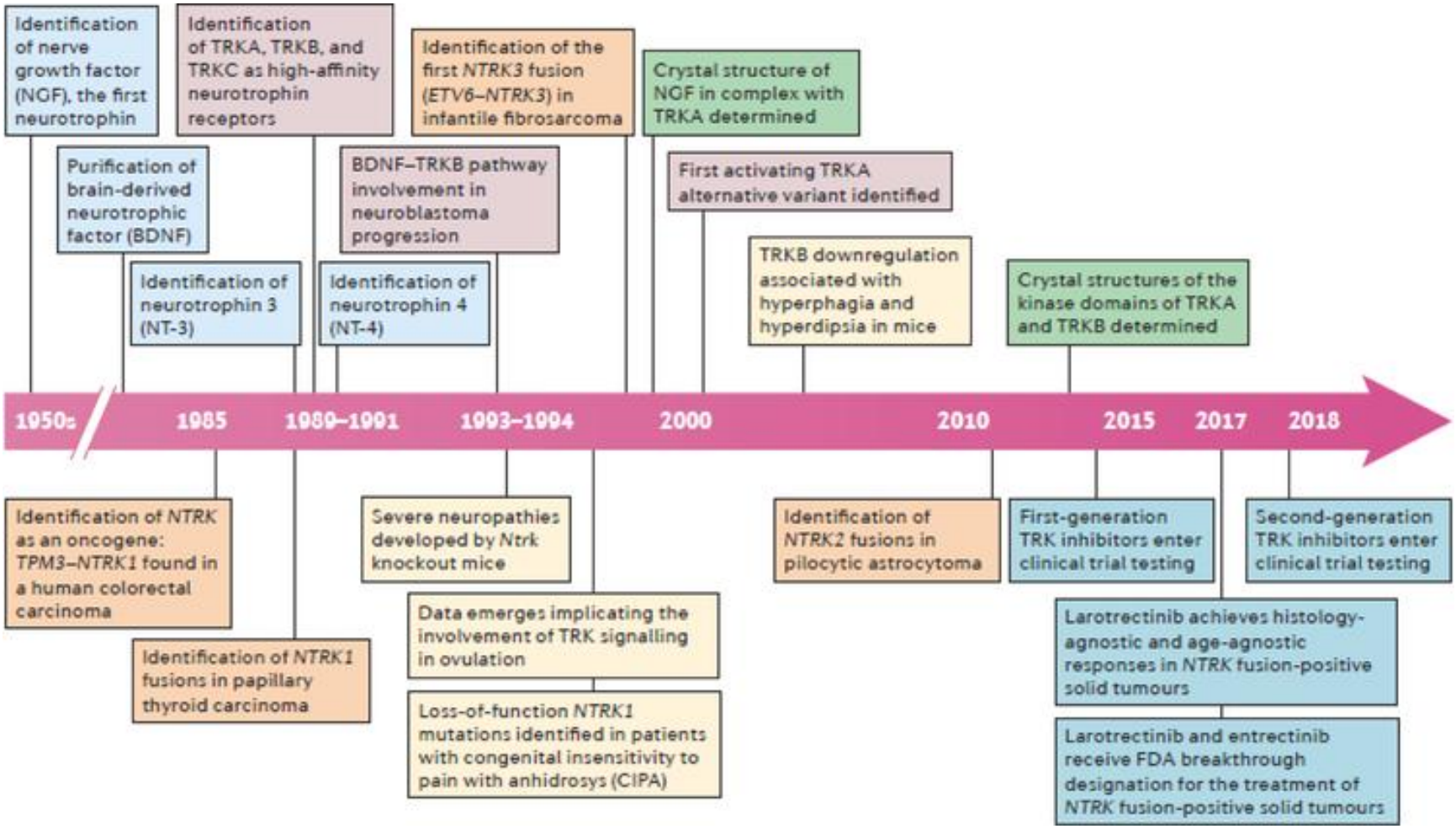
Timeline of Key Advances in TRK Signaling



Cocco E. Nat Rev Clin Oncol. 2018 Dec; 15(12): 731–747

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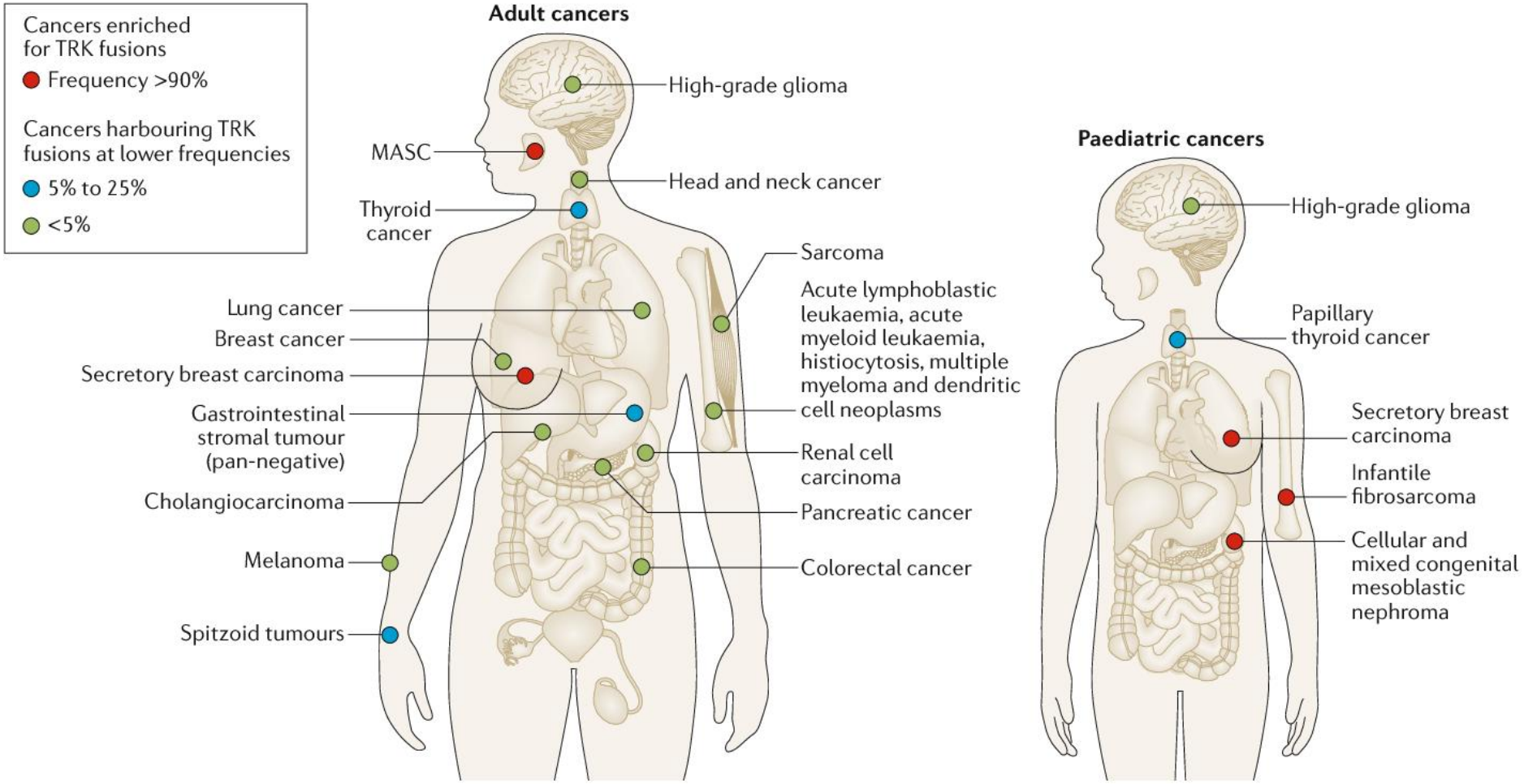
Sites of *NTRK* Gene Fusions in Adult and Pediatric Cancers



Cocco E. Nat Rev Clin Oncol. 2018 Dec;15(12): 731–747

Frequency and Types of *NTRK* Gene Fusion in Adult and Pediatric Cancers

Tumour Type	*NTRK* gene fusions involved	Frequency
Breast secretory carcinoma	*NTRK3*	96%
Infantile fibrosarcoma	*NTRK3*	95.5%
MASC ~90%	*NTRK3*	89.1%
Congenital mesoblastic nephroma	*NTRK3*	72.0%
Spitz tumours and spitzoid melanoma	*NTRK1*	16.4%
Papillary thyroid carcinoma	*NTRK1,3*	8.8%
Intrahepatic cholangiocarcinoma	*NTRK1*	3.6%
Astrocytoma	*NTRK2*	3.1%
High-grade glioma	*NTRK1,2,3*	2.1%
Uterine sarcoma	*NTRK1,3*	2.1%
GIST	*NTRK3*	1.9%
Lung cancer	*NTRK1,2*	1.7%
Thyroid carcinoma	*NTRK1,3*	1.2%
Glioblastoma	*NTRK1,2*	1.2%
Sarcoma	*NTRK1*	1.0%
Ph-like ALL	*NTRK3*	0.7%
Colorectal cancer	*NTRK1,3*	0.61%
Melanoma	*NTRK3*	0.3%
Head and neck cancer	*NTRK2,3*	0.24%
Invasive breast cancer	*NTRK3*	<0.1%

https://oncologypro.esmo.org/
Chen and Chi. 2018

ALL, acute lymphoblastic leukaemia, GIST, gastrointestinal stromal tumours; MASC, mammary analogue secretory carcinoma

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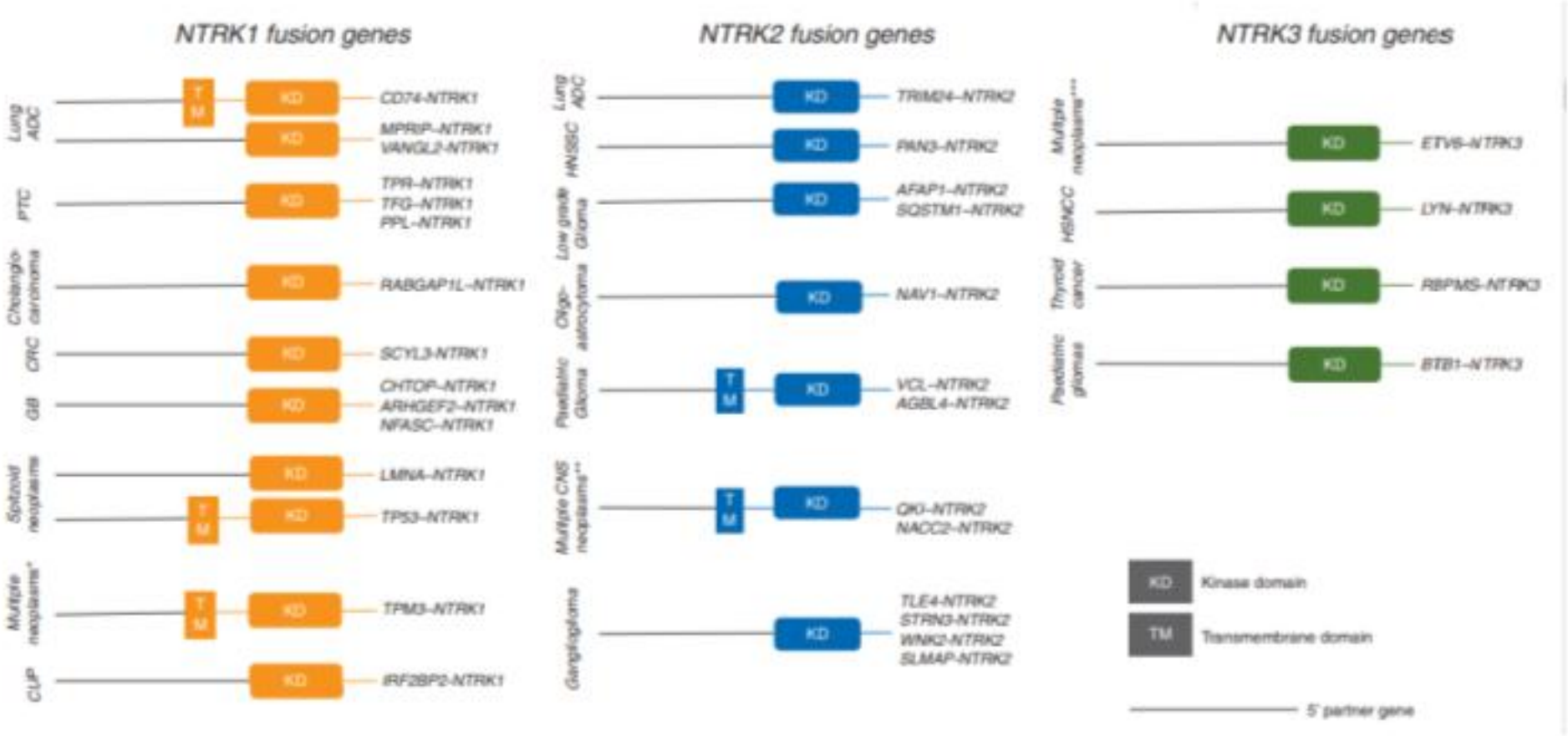
NTRK1, 2 & 3 fusion genes



Marchio C. Ann Oncol. 2019; 30 (9): 1417-27

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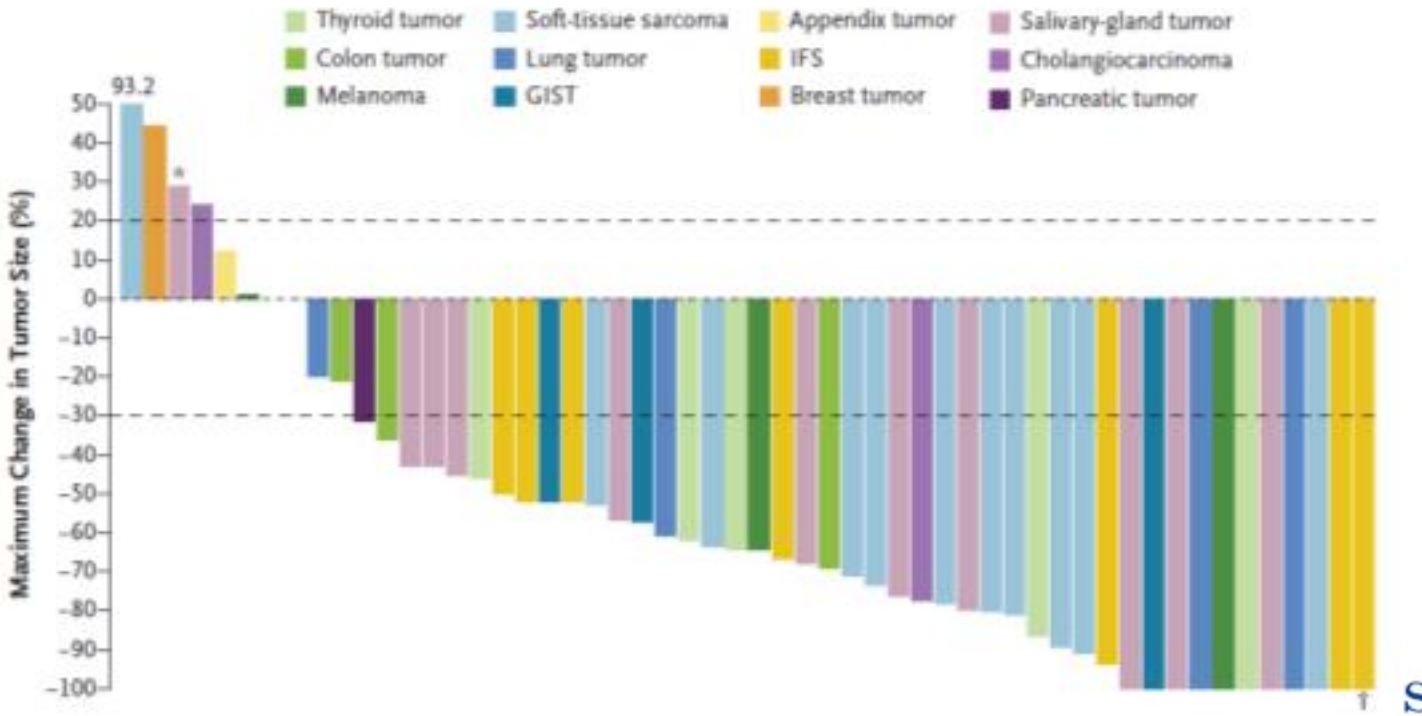
Efficacy of Larotrectinib in *TRK* Fusion–Positive Cancers in Adults and Children

N Engl J Med 2018;378:731-9

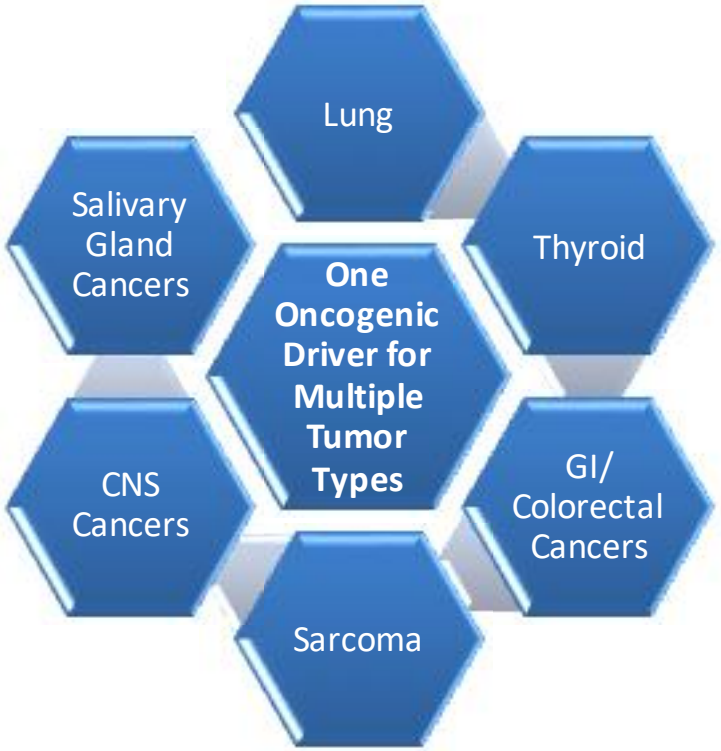
A. Drilon, T.W. Laetsch, S. Kummar, S.G. DuBois, U.N. Lassen, G.D. Demetri,

Larotrectinib had marked and durable antitumor activity in patients with *TRK* fusion–positive cancer, regardless of the age of the patient or of the tumor type.



Selective TRK Inhibitor for TRK Fusion Cancer Across Solid Tumors

- *NTRK* gene fusions have been found in more than 25 tumor types
- Monotherapy (larotrectinib/entrectinib) indicated for treatment of adult and pediatric patients with solid tumors that display *NTRK* gene fusion.



	Especially in:
Lungs	• Negative for *ALK*, *EGFR*, *ROS1*; wild-type *BRAF*
Colorectal	• Positive for *MSI-H*; wild-type *BRAF*, *KRAS*, and *NRAS*
Thyroid	• Wild-type *BRAF*
Sarcoma	• Negative for *KIT* and *PDGFRA*
Those commonly driven by *NTRK*	• MASC, Secretory breast, IFS, CMN

Oncogenic TRK fusions are amenable to inhibition in hematologic malignancies

Justin Taylor,[1,2] Dean Pavlick,[3] Akihide Yoshimi,[1] Christina Marcelus,[1] Stephen S. Chung,[2] Jaclyn F. Hechtman,[4] Ryma Benayed,[4] Emiliano Cocco,[1] Benjamin H. Durham,[1] Lillian Bitner,[1] Daichi Inoue,[1] Young Rock Chung,[1] Kerry Mullaney,[4] Justin M. Watts,[5] Eli L. Diamond,[6] Lee A. Albacker,[3] Tariq I. Mughal,[3,7] Kevin Ebata,[8] Brian B. Tuch,[8] Nora Ku,[8] Maurizio Scaltriti,[1] Mikhail Roshal,[4] Maria Arcila,[4] Siraj Ali,[3] David M. Hyman,[9] Jae H. Park,[2] and Omar Abdel-Wahab[1,2]

[1]Human Oncology and Pathogenesis Program and [2]Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, New York, USA. [3]Foundation Medicine Inc., Cambridge, Massachusetts, USA. [4]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York, USA. [5]Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida, USA. [6]Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, New York, USA. [7]Tufts University Medical Center, Boston, Massachusetts, USA. [8]Loxo Oncology Inc., South San Francisco, California, USA. [9]Developmental Therapeutics, Memorial Sloan Kettering Cancer Center, New York, New York, USA.

Rearrangements involving the neurotrophic receptor kinase genes (*NTRK1*, *NTRK2*, and *NTRK3*; hereafter referred to as TRK) produce oncogenic fusions in a wide variety of cancers in adults and children. Although TRK fusions occur in fewer than 1% of all solid tumors, inhibition of TRK results in profound therapeutic responses, resulting in Breakthrough Therapy FDA approval of the TRK inhibitor larotrectinib for adult and pediatric patients with solid tumors, regardless of histology. In contrast to solid tumors, the frequency of TRK fusions and the clinical effects of targeting TRK in hematologic malignancies are unknown. Here, through an evaluation for TRK fusions across more than 7,000 patients with hematologic malignancies, we identified TRK fusions in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), histiocytosis, multiple myeloma, and dendritic cell neoplasms. Although TRK fusions occurred in only 0.1% of patients (8 of 7,311 patients), they conferred responsiveness to TRK inhibition in vitro and in vivo in a patient-derived xenograft and a corresponding AML patient with *ETV6-NTRK2* fusion. These data identify that despite their individual rarity, collectively, TRK fusions are present in a wide variety of hematologic malignancies and predict clinically significant therapeutic responses to TRK inhibition.

Diagnosis

- Presence of *NTRK* gene fusion in tumor specimen should be confirmed by validated test prior to initiation of treatment.
  - **NGS** testing allows for multiplex testing to find *NTRK* gene fusions, as well as other genomic targets (ALK, BRAF, EGFR, HER2)
    - RNA is preferred over DNA testing because it offers more wide-ranging fusion identification
    - *NTRK1*, *NTRK2*, and *NTRK3* should each be included in NGS panel
  - **IHC** may be used as complementary screening test. Pan-TRK antibodies detect TRKA, TRKB, and TRKC proteins of both wild-type TRK and TRK fusion proteins with no distinction between the two. Therefore, protein expression may not be related to gene fusion.
    - Following a positive TRK IHC test, confirmation of *NTRK* gene fusion is needed prior to initiation of treatment
- Other Tests
  - **DNA FISH** can be used to detect *NTRK* gene fusions; however, in order to detect fusions at multiple locations (3 *NTRK* genes), multiple FISH tests would need to be run.
    - There is utility in using FISH in diseases such as infantile fibrosarcoma (IFS), where the predominant driver is *ETV6-NTRK3*
  - **RT-PCR** is designed to identify only known translocation partners and breakpoints and cannot identify novel breakpoints or novel fusion partners

Overview of Testing Methods for *NTRK* Gene Fusions

Assay	Advantages	Disadvantages
IHC	• Detects TRKA, B and C • Turnaround time 1–2 days	• May not be specific for *NTRK* gene fusion as it detects both wild-type and fusion proteins • Possible false positives • Possible false negatives for fusions involving TRKC • There is no standardisation of scoring algorithms
FISH	• The location of the target within the cell is visible • Several targets can be detected in one sample using several fluorophores • Requires knowledge of only one of the two fusion partners when using break-apart probes • *NTRK* gene fusions with unknown partners can be detected using breakapart FISH	• The target sequence must be known for conventional FISH otherwise three separate tests are required for *NTRK1, NTRK2* and *NTRK3* • Complex chromosomal translocations can result in false positive signals • False negative results may be above 30%
RT-PCR	• High sensitivity and specificity • Low cost per assay	• Target sequences must be known (i.e., cannot readily detect novel fusion partners) • A comprehensive multiplex RT-PCR assay might be challenging because of potentially large number of possible 5' fusion partners
NGS (Fusion Panel)	• May detect novel fusion partners (depending on the assay used) • Can be used to evaluate multiple actionable targets simultaneously while preserving limited tissue • Currently used for *NTRK* testing • RNA-NGS is preferred over DNA-NGS as sequencing for RNA-based testing is focused on coding sequences not introns	• RNA-NGS is limited by RNA quality

Penault-Llorca F, et al. J Clin Pathol 2019;72:460–467

ESMO Guidelines 2019

- When presence of an *NTRK* gene fusion needs to be confirmed, any technique could work in principle, however the best options as confirmatory techniques are FISH, RT-PCR or RNA-based targeted panels.
- For identification of *NTRK* gene fusion in an unselected population, using a DNA- or RNA-based NGS targeted panel that reliably detects *NTRK* gene fusion would be ideal.
  - Targeted RNA sequencing methods may represent gold standard for screening, if the RNA quality is optimal
  - If an *NTRK* gene fusion is identified, then the most exhaustive approach would be to include IHC to confirm protein expression of the detected NTRK fusions
- Alternatively, a “two-step approach” could be considered, which includes IHC first and confirmation of any positivity detected with IHC by NGS.

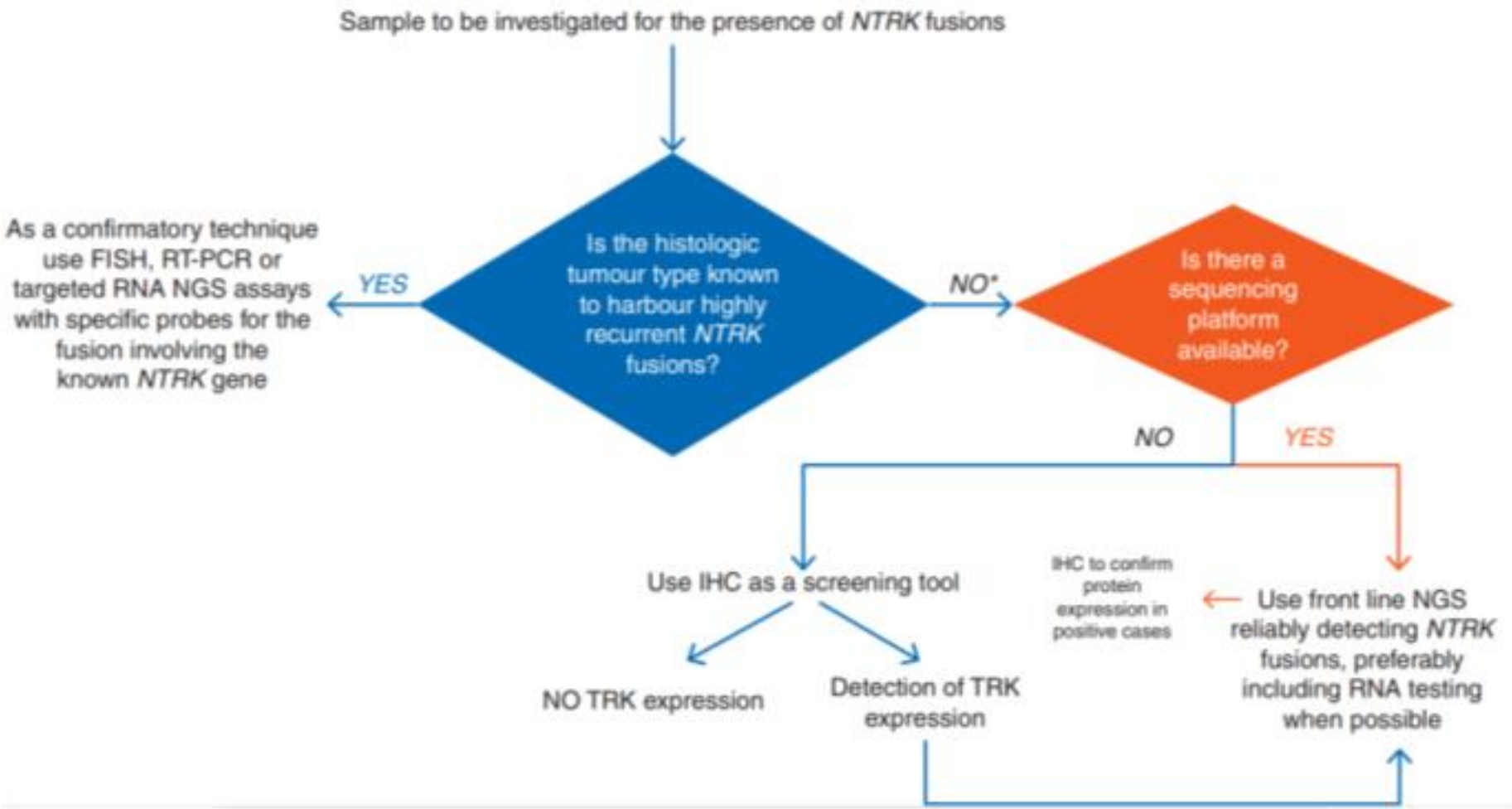
Summary of Techniques to Detect NTRK Rearrangements

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 Fusion NGS	High	High	Yes	Yes	No	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.

Marchio C. Ann Oncol. 2019; 30 (9): 1417-27

ESMO Recommendations 2019 - Algorithm



FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; MPS, massively parallel sequencing; NGS, next-generation sequencing; RT-PCR, reverse transcriptase-polymerase chain reaction.

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Tests Done at SRL

Test	Method	Specimen; TAT	Code
Solid Tumor RNA Profiler Next	NGS	Paraffin Block; 12 days	RD1505
Oncofocus Next		Tissue in 10% Formalin/ Paraffin Block with site of Biopsy	RD1499

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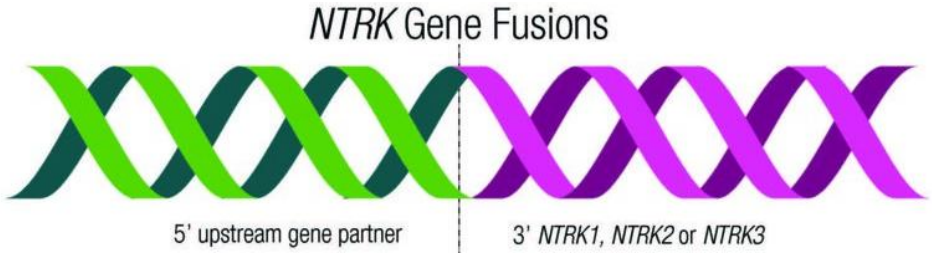
NTRK Gene Fusions
5' upstream gene partner
3' *NTRK1*, *NTRK2* or *NTRK3*

Thank You