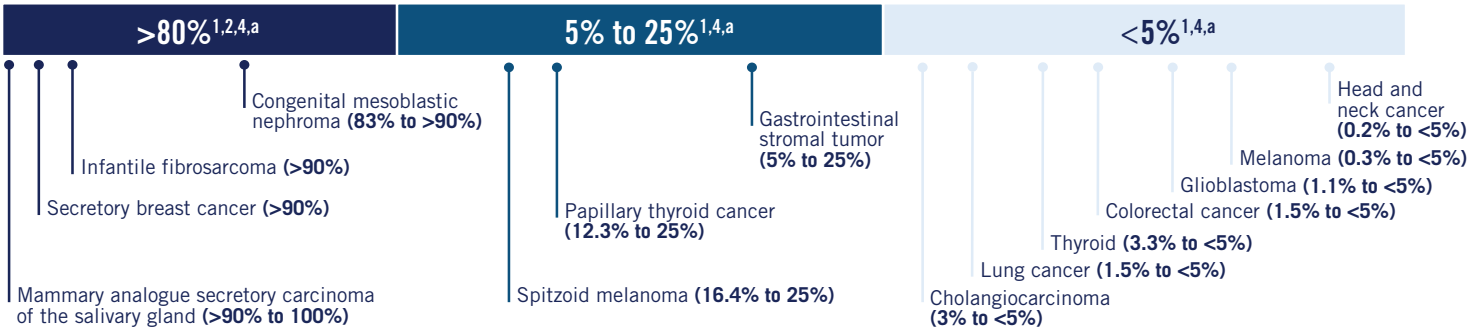
DETECTING *NTRK* GENE FUSIONS

Oncogenic fusions involving neurotrophic tyrosine receptor kinase (*NTRK*) are oncogenic drivers across a wide range of pediatric and adult cancer types that can be treated with targeted therapies[1-3]

- The tropomyosin receptor kinase (TRK) family contains 3 members—TRKA, TRKB, and TRKC—and these proteins are encoded by the genes *NTRK1*, *NTRK2*, and *NTRK3*, respectively[2]

NTRK gene fusions are most common in rare tumors, but have also been detected less frequently in more common cancers[1]



[a]Tumor types for each prevalence block (>80%, 5% to 25%, and <5%) are organized according to their prevalence in descending order, from left to right. The location of each tumor type on each discrete frequency block is not indicative of its absolute (ie, raw) frequency.

Prevalence of *NTRK* gene fusions across cancer types has been reported to be between ~0.2% to 100%[1,5]

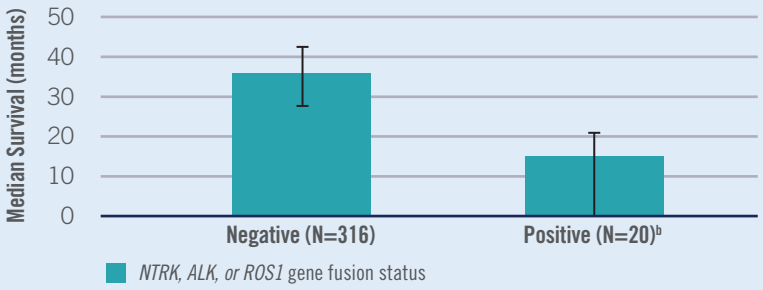
NTRK gene fusions drive cancer through aberrant signaling[1,3,6,7]

- *NTRK* gene fusions create an oncogenic chimera protein that activates a signaling cascade implicated in cell proliferation, survival, and angiogenesis[1,3]
- *NTRK* gene fusions may be mutually exclusive to other oncogenic drivers[1,2,8]
- Each *NTRK* gene can combine with multiple fusion partners; at least 25 distinct *NTRK* gene fusions have been identified to date[1-3,8]

NTRK gene fusion testing is not routinely incorporated into diagnostic workups, but comprehensive molecular profiling using next-generation sequencing (NGS) can identify *NTRK* gene fusion patients and support optimal patient care[9]

The presence of *NTRK* gene fusions may confer a worse prognosis[10]

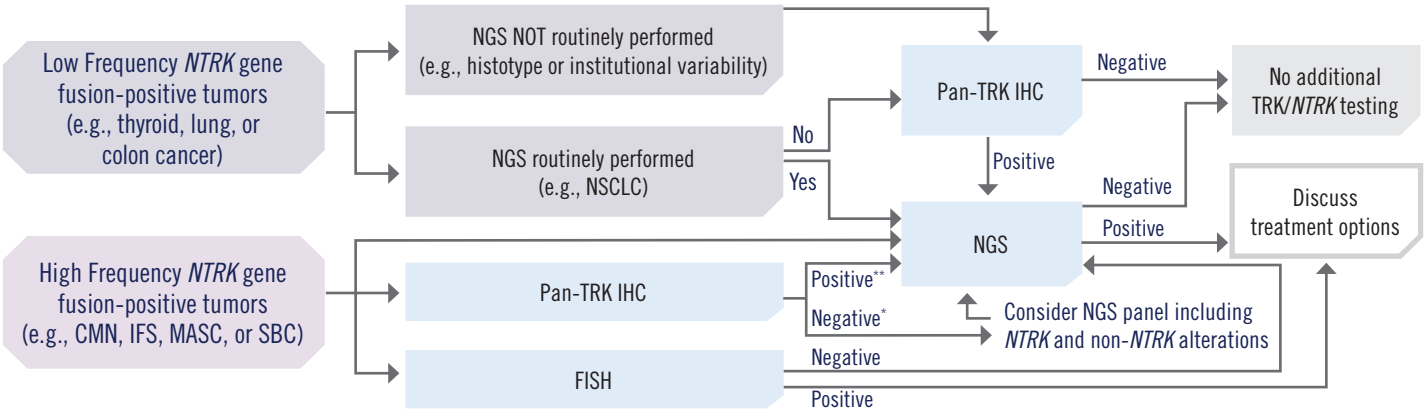
- An example is a retrospective study of 27 metastatic CRC patients with *NTRK* (n=13), *ALK* (n=11), or *ROS1* (n=3) gene fusions were compared to a cohort of *ALK*, *ROS1*, and *NTRK* negative mCRC patients (n=316)
- HR=2.17[a] (95% CI 1.03-4.57), *P*<0.001



[a]Univariate analysis was used; [b]Survival data available for 20 out of 27 patients
ALK = anaplastic lymphoma kinase; CI = confidence interval; CRC = colorectal cancer; HR = hazard ratio; ROS1 = c-ros oncogene 1.

Incorporating *NTRK* gene fusion testing into diagnostic workup will help advance personalized health care for cancer patients[10-14]

Example *NTRK* gene fusion-detection algorithm[15]



*If histology is typical, then confirmation by NGS is recommended.
**Treatment may be considered concurrently with confirmatory NGS testing.
CMN = congenital mesoblastic nephroma; FISH = fluorescence *in situ* hybridization; IFS = infantile fibrosarcoma; IHC = immunohistochemistry; MASC = mammary analogue secretory carcinoma; NSCLC = non-small cell lung carcinoma; SBC = secretory breast cancer.

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NTRK1, 2, 3 Gene Fusions Can Be Detected Using Various Methods[1]

	NGS	IHC	FISH	RT-PCR
Ability to detect different fusions	• Can detect fusions in all 3 *NTRK* genes, as well as fusion partner and position[1] • Note difference between DNA and RNA[a]	• Pan-TRK antibodies may detect presence of protein encoded by any of the 3 *NTRK* genes[26,27]	• Different probes needed for each gene[36]	• Multiple primer sets are needed for each gene, since the location of the gene rearrangement is not known[44,45]
Reliability	• Varies based on the assay, depth of coverage, tumor content, and design of the assay (ie, regions of the sequence targeted by the NGS panel)[16]	• Pan-TRK antibodies have been reported to have 95% to 100% sensitivity and up to 100% specificity[26,27]	• Depends on the probes used; can be used to confirm other results[37,38] • May miss variant *NTRK* rearrangements[27,29]	• Reliable for known fusion variants. Requires multiple reactions with specific primers for known variants and can miss detection of unknown/ untested variants[45]
Tissue processing	• Routinely processed, FFPE samples[17-19] • Avoid DNA-damaging fixatives or acidic decalcifying agents • RNA samples are more susceptible to degradation over time	• Routinely processed, FFPE samples[27,28]	• Routinely processed, FFPE samples[26] • Avoid DNA-damaging fixatives or acidic decalcifying agents	• RNA can be successfully extracted from nearly all types and ages of fixed tissues; yield and quality will vary with sample quality and age[44,45]
Specimen requirements	• Amount required varies, depending on the NGS platform • Samples with greater viable tumor content will return more reliable results[17]	• Requires dedicated tissue and limits multiplexing[25,29,30]	• Requires dedicated tissue[25,39]	• Requires dedicated tissue • Poor quality and quantity of RNA risks false-negative results[45,46]
No. of FFPE sections[b]	>5-10 (4-5 µm)[20-22]	≥2-3 (4-5 µm)[31-35]	≥4 (4-5 µm)[34,35,40,41] 1 for each *NTRK* gene fusion	>8-10 (7-30 µm)[41]
Cells/DNA/RNA	≥20% tumor cells[20]	>50 tumor cells[28,33]	>50 tumor cells[42]	0.1-0.5 µg RNA[25,46]
TAT	2-21 days[21,23,24]	0.5-2 days[25,31]	2-10 days[25,43]	1 day[25]
Multigene testing beyond *NTRK* gene fusions	YES[25]	NO[25]	NO[25]	NO[25]

[a]RNA sequencing may be able to better detect *NTRK* fusion partners and the position of gene rearrangement.
[b]Depends on the tumor content in FFPE sections and the assay being used.

Comprehensive testing via NGS simultaneously provides information about other rare and common mutations without requiring additional tissue[26,29]

It is important to ensure that the selected testing method includes all 3 *NTRK* gene fusions (*NTRK1*, *NTRK2*, and *NTRK3*)

This material does not constitute medical advice. The decision to implement these practice suggestions should be made by each laboratory in conjunction with its clinical care team to balance resources and clinical needs with the individual health care setting.

FDA = US Food and Drug Administration; FFPE = formalin-fixed paraffin-embedded; TAT = turnaround time.

References: 1. Vaishnavi A, et al. *Cancer Discov.* 2015;5:25-34; **2.** Lange AM, Lo HW. *Cancers (Basel).* 2018;10: pii: E105; **3.** Amatu A, et al. *ESMO Open.* 2016;1:e000023; **4.** Cocco E, et al. *Nat Rev Clin Oncol.* 2018;15:731-747; **5.** Gatalica Z, et al. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications; October 26-30, 2017; Philadelphia, PA. Abstract A047; **6.** Khotskaya YB, et al. *Pharmacol Ther.* 2017;173:58-66; **7.** de Lartigue J. *OncologyLive.* 2017;18. https://www.onclive.com/publications/oncology-live/2017/vol-18-no-15/trk-inhibitors-advance-rapidly-in-tumoragnostic-paradigm. Accessed September 24, 2018; **8.** Stransky N, et al. *Nat Commun.* 2014;5:4846; **9.** Kumar-Sinah C, et al. *Genome Med.* 2015;7:12; **10.** Pietrantonio F, et al. *J Natl Cancer Inst.* 2017;109:doi: 10.1093/jnci/djx089; **11.** World Health Organization. https://www.who.int/cancer/prevention/diagnosis-screening/en/. Accessed March 21, 2019; **12.** FDA. https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/precisionmedicine-medicaldevices/default.htm. Accessed March 25, 2019; **13.** Wolff AC, et al. *J Clin Oncol.* 2013;31:3997-4013; **14.** National Academics of Sciences, Engineering, and Medicine. 2017. Washington, DC: The National Academics Press. doi: 10.17226/21860; **15.** Penault-Llorca F, et al. *J Clin Pathol* 2019;72:460-467; **16.** Horak P, et al. *ESMO Open.* 2016;1:e000094; **17.** Gagan J, Van Allen EM. *Genome Med.* 2015;7:80; **18.** Hovelson DH, et al. *Neoplasia.* 2015;17:385-399; **19.** Ali SM, et al. *Oncologist.* 2016;21:762-770; **20.** Cleveland Clinic Laboratories. CC-SIGN™ NTRK Plus Gene Fusion NGS Panel https://clevelandcliniclabs.com/2019/01/31/new-cc-sign-ntrk-gene-analysis/. Accessed April 2, 2019; **21.** NeoGenomics. NTRK NGS Fusion profile. https://neogenomics.com/test-menu/ntrk-ngs-fusion-profile. Accessed April 2, 2019; **22.** University of Washington Labs. UW-OncoPlex - Cancer Gene Panel. http://tests.labmed.washington.edu/UW-OncoPlex. Accessed April 2, 2019; **23.** ThermoFisher Scientific, Oncomine NGS Assays. Product Brochure 2018; **24.** MDMolecular, Archer® FusionPlex®. Product Brochure 2016; **25.** Du X, et al. *Thorac Cancer.* 2018;9:423-430; **26.** Murphy DA, et al. *Appl Immunohistochem Mol Morphol.* 2017;25:513-523; **27.** Hechtman JF, et al. *Am J Surg Pathol.* 2017;41:1547-1551; **28.** Rudzinski ER, et al. *Am J Surg Pathol.* 2018;42:927-935; **29.** Su D, et al. *J Exp Clin Cancer Res.* 2017;36:121; **30.** Stack EC, et al. *Methods.* 2014;70:46-58; **31.** NeoGenomics. Pan-TRK. https://neogenomics.com/test-menu/pan-trk. Accessed April 2, 2019; **32.** Doebele RC, et. al. *Cancer Discov.* 2015;5:1049-1057; **33.** Hung YP, et al. *Histopathology.* 2018;73:634-644; **34.** Croce S, et al. *Mod Pathol.* 2019 Mar 16; **35.** All Common Checklist, CAP Accreditation Program. College of American Pathologists. 2015-07-28; **36.** Kummar S, Lassen UN. *Target Oncol.* 2018;13:545-556; **37.** Jensen E. *Anat Rec (Hoboken).* 2014;297:1349-1353; **38.** Wolff DJ, et al. *J Mol Diagn.* 2007;9:134-143; **39.** Knezevich SR, et al. *Nat Genet.* 1998;18:184-187; **40.** Wang L, et al. *J Mol Diagn.* 2017;19:387-396; **41.** Tognon C, et al. *Cancer Cell.* 2002;2:367-376; **42.** Luk PP, et al. *Arch Pathol Lab Med.* 2018;142:922-928; **43.** Empire Genomics. NTRK3 Break Apart FISH Probe; https://www.empiregenomics.com/fish-probes/hd-probes/NTRK3+Break+Apart+FISH+Probe. Accessed April 1, 2019; **44.** Liu M-L, et al. *Methods Mol Bio.* 2011;724:205-237; **45.** Ali G, et al. *Arch Pathol Lab Med.* 2018;142:480-489; **46.** Tatematsu T, et al. *Mol Clin Oncol.* 2014;2:725-730.

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