Pan-TRK Immunohistochemistry

An Example-Based Practical Approach to Efficiently Identify Patients With *NTRK* Fusion Cancer

Esther Conde, MD, PhD; Susana Hernandez, PhD; Elena Sanchez; Rita Maria Regojo, MD; Carmen Camacho, MD; Marta Alonso; Rebeca Martinez; Fernando Lopez-Rios, MD, PhD

• ***Context.***—**Food and Drug Administration–approved TRK inhibitors with impressive overall response rates are now available for patients with multiple cancer types that harbor *NTRK* rearrangements, yet the identification of *NTRK* fusions remains a difficult challenge. These alterations are highly recurrent in extremely rare malignancies or can be detected in exceedingly small subsets of common tumor types. A 2-step approach has been proposed, involving a screening by immunohistochemistry (IHC) followed by a confirmatory method (fluorescence in situ hybridization, reverse transcriptase–polymerase chain reaction, or next-generation sequencing) in cases expressing the protein. However, there is no interpretation guide for any of the available IHC clones.**

Objective.—**To provide a pragmatic update on the use of pan-TRK IHC. Selected examples of the different IHC staining patterns across multiple histologies are shown.**

Data Sources.—**Primary literature review with PubMed, combined with personal diagnostic and research experience.**

Conclusions.—**In-depth knowledge of pan-TRK IHC will help pathologists implement a rational approach to the detection of *NTRK* fusions in human malignancies.**

(*Arch Pathol Lab Med*. 2021;145:1031–1040; doi: 10.5858/arpa.2020-0400-RA)

Several TRK inhibitors with impressive overall response rates in patients with *NTRK* rearrangements are currently available or under clinical development.[1,2] The search for *NTRK* fusions should benefit from what we have learned in recent years about identifying other druggable rearrangements, mainly in lung cancer (*ALK*, *ROS1*, etc).[3–7] Therefore, *NTRK* fusions can be detected with immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse transcriptase–polymerase chain reaction, or next-generation sequencing (NGS).[8] If NGS is not routinely performed in all advanced malignant tumors, most proposed algorithms use IHC as a screening method, followed by orthogonal confirmation of all positive IHC cases (mainly using FISH or NGS).[8–11] To further enrich for *NTRK* fusions, both histology-based and genomic-based triaging approaches have been proposed.[12] A summary of the available evidence is presented in the Table. Until NGS becomes the main testing methodology on all advanced cancers, algorithm considerations should include feasibility, cost, sample size, and pretest probability of *NTRK* fusions. A review of the frequencies of *NTRK* fusions highlights the need to be aware of these strategies. *NTRK* fusions have been observed in 0.31% of adult tumors and 0.34% of pediatric tumors.[13] In clinical series, the most common partners have been *NTRK1* and *NTRK3*.[14–16] The most frequent fusion is *ETV6-NTRK3*.[16] With the exception of gliomas,[13,15] *NTRK2* fusions appear to be restricted to isolated examples of sarcomas or lung adenocarcinomas.[15,17–19] Although it may be too soon to draw definitive conclusions on the positive and negative predictive value of pan-TRK IHC by partner and cancer type, the largest series to date showed an overall sensitivity of 87.9% and specificity of 81.1%.[14] Decreased sensitivity was reported for *NTRK3* fusions and sarcomas, and lower specificity

Accepted for publication August 20, 2020.
Published online October 28, 2020.

From Pathology and Laboratory of Therapeutic Targets, Hospital Universitario HM Sanchinarro, HMHospitales, CIBERONC, Madrid, Spain (Conde, Lopez-Rios); Pathology and Laboratory of Therapeutic Targets, Hospital Universitario HM Sanchinarro, HMHospitales, Madrid, Spain (Hernandez, Sanchez, Alonso, Martinez); Pathology, Hospital Universitario La Paz, Madrid, Spain (Regojo); and Pathology, Complejo Hospitalario Universitario Insular Materno-Infantil, Las Palmas de Gran Canaria, Spain (Camacho). Conde and Hernandez contributed equally as co–first authors.

This study was mainly funded by Roche Spain. We also thank Instituto de Salud Carlos III (ISCIII) (Fondos FEDER and Plan Estatal I+D+I 2008–2011 [PI11/02866] and 2013–2016 [PI14-01176 y PI17-01001]) and the iLUNG Program (B2017/BMD-3884) from the Comunidad de Madrid. Thermo Fisher Scientific provided an unrestricted grant for the optimization of the next-generation sequencing.

Conde receives honoraria from Roche and Pfizer; travel expenses were covered by Roche, Pfizer, and Merck Sharp & Dohme. Lopez-Rios received honoraria from Lilly, Roche, Thermo Fischer Scientific, Pfizer, Bristol-Myers Squibb, Bayer, AstraZeneca, Merck Sharp & Dohme; research funding was received from Lilly, Roche, and Thermo Fischer Scientific. The other authors have no relevant financial interest in the products or companies described in this article.

Corresponding author: Fernando Lopez-Rios, MD, PhD, Pathology-Laboratory of Therapeutic Targets, Hospital Universitario HM Sanchinarro, HMHospitales, C/Oña, 10. 28050 Madrid, Spain (email: flopezrios@hmhospitales.com).

Multiparametric Approach to Identify Patients With *NTRK* Fusion Cancer Using Pan-TRK Immunohistochemistry (IHC)

Organ	% of *NTRK* Fusions	Triaging Strategies		Typical Pan-TRK IHC Pattern	Frequent *NTRK* Fusions	Practical Comments
		Histology	Genomics			
Soft tissue[16,18,19,24,33–39,58–68]						
Children	Unknown	Infantile fibrosarcoma (not exclusively[a])	NA	Cytoplasmic + nuclear (diffuse)	*ETV6-NTRK3* (not exclusively)	Perform pan-TRK IHC in all pediatric aggressive mesenchymal tumors[a] *NTRK3* FISH preferable to *ETV6* FISH for confirmation[a]
Adults	<2%	Multiple[a]	No driver gene alterations	Cytoplasmic (alone or combined with membrane/nuclear membrane staining[a])	*TPM3-NTRK1*, *LMNA-NTRK1*, *TPR-NTRK1*	Screen for *NTRK* fusions in sarcomas located in the gynecologic tract Screen for *NTRK* fusions in sarcomas with S-100 and CD34 coreactivity
Breast[14,28,40–43]	<0.05% of invasive carcinomas	Secretory carcinoma	Triple negative (usually[a])	Cytoplasmic + nuclear (diffuse)	*ETV6-NTRK3* (pathognomonic)	Can hide under triple-negative breast carcinoma diagnoses Nonsecretory breast carcinomas can show pan-TRK IHC positivity[a] Expect heterogeneous pan-TRK IHC staining in resection specimens[a]
Salivary gland[29,44–47]	<2.8% of salivary gland tumors	Secretory carcinoma	NA	Cytoplasmic + nuclear (diffuse)	*ETV6-NTRK3* (not exclusively)	Other malignant salivary gland tumors can show pan-TRK IHC staining[a] Expect heterogeneous pan-TRK IHC staining in resection specimens[a]
Lung[15–17,21,24,50]	<1% of NSCLCs	Adenocarcinoma (usually[a])	Without driver gene alterations[a]	Cytoplasmic (alone or combined with membrane staining if *TPM3-NTRK1*)	*TPM3-NTRK1*, *IRF2BP2-NTRK1*, *SQSTM1-NTRK1*, *MPRIP-NTRK1*	Persevere in driver-negative lung adenocarcinomas: Trust intense and diffuse pan-TRK IHC staining in lung adenocarcinomas Make sure the NGS panel is broad enough and RNA based[a]
Colon[14,16,26,27,53,54]	0.02%–0.3% of carcinomas	Adenocarcinoma	*BRAF/RAS* wild type, MSI high	Cytoplasmic (alone or combined with membrane/nuclear membrane/nuclear staining[a])	*TPM3-NTRK1*, *LMNA-NTRK1*, *TPR-NTRK1*, *ETV6-NTRK3*	Organize pan-TRK IHC simultaneously to MMR IHC
Thyroid[13–16,35,55–57]	2.3% of PTC	PTC	*BRAF* wild type	Cytoplasmic (alone or combined with nuclear staining if *ETV6-NTRK3*)	*ETV6-NTRK3*, *TPR-NTRK1*	Perform pan-TRK IHC in higher-stage and radiation-induced PTC Expect heterogeneous pan-TRK IHC staining in resection specimens[a]

Abbreviations: IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NA, not applicable; NGS, next-generation sequencing; NTRK, neurotrophic receptor kinase; NSCLC, non–small cell lung carcinoma; PTC, papillary thyroid carcinoma.

[a] See text for details.

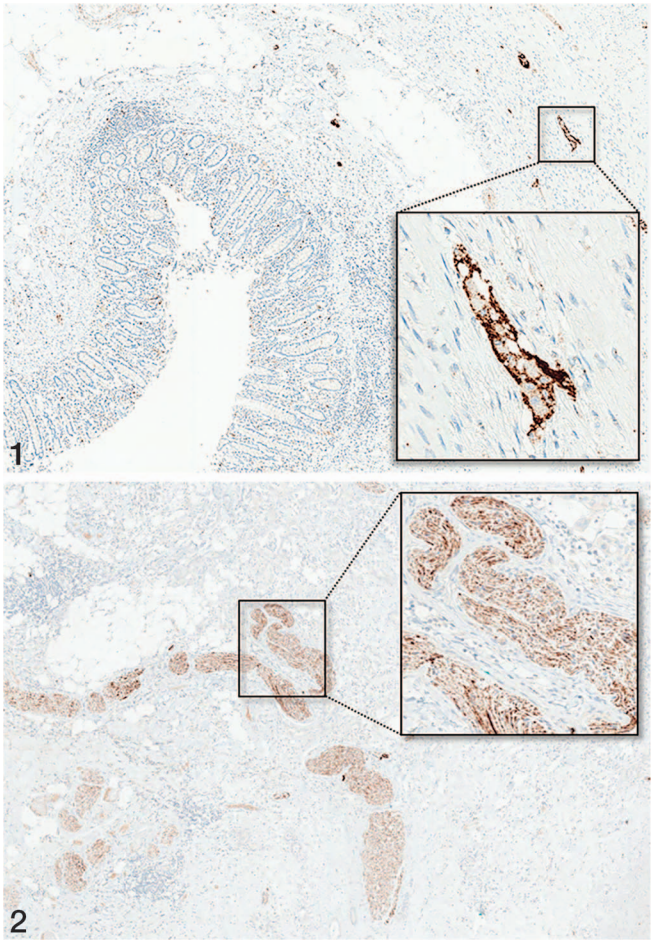


Figure 1. *Pan-TRK immunohistochemistry expression in the appendix. Absence of staining in epithelial cells and muscle and lymphoid tissue, and presence of a strong granular cytoplasmic staining in ganglion cells (inset) (Ventana Medical Systems, Tucson, Arizona) (original magnifications ×40 and ×400 [inset]).*

Figure 2. *Example of pan-TRK immunohistochemistry expression in peripheral nerves in a section from a Whipple resection. Nerves show strong granular cytoplasmic staining (Ventana Medical Systems, Tucson, Arizona) (original magnifications ×40 and ×200 [inset]).*

involved sarcomas, breast carcinomas, and salivary gland carcinomas.[14]

PREANALYTIC AND ANALYTIC PRECAUTIONS

Because IHC is considered a screening method, we must use the highest-sensitivity assays. This is particularly relevant in this setting because of the low prevalence of *NTRK* fusions: patients with a negative IHC result are unlikely to be tested again for this biomarker. Therefore, the universal preanalytic recommendations for IHC are also valid here.[20] A properly fixed tissue block should be selected to avoid heterogeneous staining. In therapy-naïve patients, intertumor heterogeneity should not be an issue, because *NTRK* fusions are founder alterations.[13] The expression of the 3 TRK proteins (TRKA, TRKB, and TRKC) must be identified simultaneously using a pan-TRK antibody. The clone EPR17341 is the most frequently used and well characterized.[21–23] A positive control must be included in all the slides to ensure the proper functioning of the analytic phase, minimizing the risk of a false-negative result. The most accessible positive control is the appendix.[22] The neural structures (ganglion cells) in its wall should be positive (positive control), in contrast to the rest of the completely blue tissue (negative control) (Figure 1). To facilitate the presence of a relevant number of these nerves, there are 2 measures that help: (1) do not select an appendix with appendicitis and (2) include 2 sections of the positive control in each slide to increase the likelihood of having these neural cells present in the section. The staining of the external positive control does not guarantee the absence of preanalytic problems. The only reliable pan-TRK IHC in situ positive controls are peripheral nerves (Figure 2). Because most slides will lack a pan-TRK IHC in situ positive control, pathologists should continue their search for *NTRK* fusions whenever there is a reason to question the optimal preanalytic conditions of the specimen (eg, decalcified samples or large surgical resections) or in specific circumstances that increase the likelihood of finding an *NTRK* fusion (see Table; eg, pathognomonic/characteristic histology, nuclear IHC staining, and solid tumors in therapy-naïve patients without driver gene mutations/fusions/amplifications after limited biomarker testing).[10,11,16,24]

POSTANALYTIC PHASE (INTERPRETATION)

The interpretation of pan-TRK IHC may be more challenging than that of other IHC-based biomarkers. Findings from a literature review are difficult to harmonize because of the different preanalytic and analytic conditions of the published series. Although there is no interpretation guide for any of the available IHC clones, the literature does suggest the level of IHC positivity that requires genomic confirmation. A positive cutoff has been defined in large pan-tumor series as staining above background in at least 1% of tumor cells.[14,15]

Cytoplasmic staining is seen in most *NTRK* fusion–positive tumors,[21,25] but it is also the staining pattern most frequently observed in false-positive cases.[14] In addition, 3 different subcellular staining patterns have been described with a certain degree of fusion partner specificity: nuclear (eg, *ETV6*), perinuclear/nuclear membrane (eg, *LMNA*, *MUC2*), and membranous (eg, *TPM*, *TPR*, *TRAF2*).[21,25–27] The reduced IHC sensitivity that has been reported for the detection of *NTRK3* fusions[14,15] could be due to the overrepresentation of *ETV6-NTRK3* fusions, as this nuclear pattern of pan-TRK IHC staining can be quite heterogeneous (see below: secretory breast carcinoma, secretory carcinoma of the salivary gland, and papillary thyroid carcinoma). Conversely, to the best of our knowledge, relevant false-positive pan-TRK IHC nuclear staining has been described in only a minority (8%–10%) of nonsecretory breast and salivary gland carcinomas,[28,29] always in a subdiagnostic manner (ie, focal and/or weak). Finally, it must be emphasized that there is pan-TRK physiologic cytoplasmic expression in neural and smooth muscle tissue and their malignant counterparts.[25] Therefore, tumors with these differentiations (for example, gastrointestinal stromal tumors, neuroblastomas, leiomyosarcomas, or glioblastomas) should not be screened with pan-TRK IHC.[11,25]

ORTHOGONAL CONFIRMATION

Pan-TRK IHC is not entirely specific for *NTRK* fusions, so positive pan-TRK IHC should always be followed by a second assay. It must be emphasized that even the most expensive and sophisticated methodologies have their own sensitivity

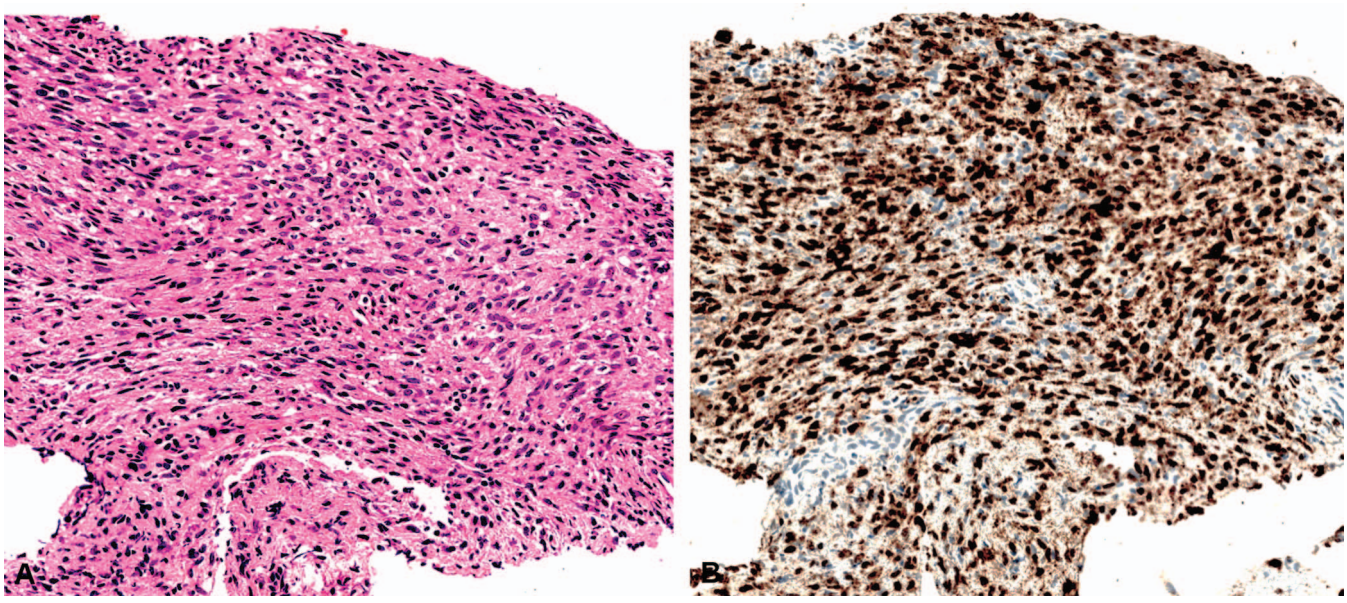


Figure 3. *Infantile fibrosarcoma with an* ETV6-NTRK3 *fusion (Oncomine Comprehensive Assay v3, Thermo Fisher Scientific, Waltham, Massachusetts). A, The tumor exhibits fascicles of ovoid and spindle cells with moderate cytologic atypia. B, Pan-TRK immunohistochemistry shows a diffuse and strong nuclear staining associated with a weaker cytoplasmic granular positivity (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B]).*

and specificity issues.[12] A clinically concerning situation would be a true-positive pan-TRK IHC result not confirmed by the orthogonal method. Knowing which positives are true for *NTRK* fusions is not intuitive, but certain histologic (ie, rare tumors with a very high incidence of *NTRK* fusions), immunohistochemical (ie, nuclear pan-TRK IHC) and molecular features (for example, solid tumors without driver gene mutations/fusions/amplifications or triple-negative colorectal carcinomas; see Table) can identify good candidates. Although it is beyond the scope of this paper to describe the advantages and disadvantages of the different methodologies (reviewed elsewhere[8–12,25,30–32]), some comments are helpful. Break-apart FISH can give false-negative results in *NTRK1* fusions because of insufficient separation of the signals.[12] The performance of reverse transcriptase–polymerase chain reaction can be limited because of the large number of partners that have been described.[12] In routine clinical work, the use of FISH or reverse transcriptase–polymerase chain reaction is restricted to 3 situations: (1) confirming the alteration in those rare tumors where *NTRK* fusions are pathognomonic/very frequent, (2) confirming an *NTRK3* fusion after a nuclear IHC result, and (3) addressing DNA/RNA failure of the NGS assay if no additional tissue is available.[12,25,30] Finally, even the most comprehensive NGS panel might not detect all *NTRK* fusions, so a precise knowledge of the width of the assay and its real-world performance can help rule out a false-negative NGS result.[9,12,30–32] Along those lines, it has been reported that there is a significant risk of false negatives when using a DNA-based NGS approach.[5,16,30,31] Conversely, the RNA quality of formalin-fixed tissue can influence the analysis of any PCR-based assay.[12] An unbiased review[12] of the different NGS approaches was recently released.

In summary, there are 3 questions that pathologists ask most frequently when scoring pan-TRK IHC: (1) Do I need to trigger orthogonal testing based on this level of positivity? (2) The confirmatory method result came back negative after a clearly positive pan-TRK IHC; is there something else I need to do? and (3) Are there any histologic or molecular features that can help me suspect a pan-TRK IHC false-negative result? In other words, when do we need to persevere after a negative or inconclusive pan-TRK IHC result? To further help pathologists answer these questions, illustrative examples are shown below, classified using a 3-tier approach: (1) rare tumors with a very high incidence of *NTRK* fusions (infantile fibrosarcomas and secretory carcinomas of the breast and salivary glands), (2) common carcinomas with an extremely low probability of harboring *NTRK* fusions (lung adenocarcinomas, colorectal carcinomas, and papillary thyroid carcinomas), and (3) tumor types with frequent and relevant TRK protein expression not associated with *NTRK* fusions (leiomyosarcomas, olfactory neuroblastomas, and adenoid cystic carcinomas).

RARE TUMORS WITH A VERY HIGH INCIDENCE OF *NTRK* FUSIONS

Infantile Fibrosarcoma

Infantile fibrosarcoma (Figure 3, A) is a very rare pediatric tumor that is characterized by an *ETV6-NTRK3* fusion in 80% to 90% of cases.[33–36] Accordingly, most cases show a characteristic pan-TRK IHC nuclear staining in addition to diffuse cytoplasmic positivity, given that *ETV6* encodes a transcription factor (Figure 3, B).[34] However, diffuse pan-TRK staining can also be found in many other pediatric spindle cell tumors, either *NTRK* rearranged (even outside lipofibromatosis-like neural tumors; see below)[18,37] or not harboring any of the *NTRK* fusions (eg, primitive myxoid mesenchymal tumors of infancy, fibrous hamartomas of infancy, fibrosarcomatous dermatofibrosarcoma protuberans, synovial sarcomas).[33,34] Taking into account the overlapping clinical and pathologic attributes of *NTRK*-rearranged and nonrearranged tumors in this population, it is sensible to perform pan-TRK IHC in all pediatric patients with mesenchymal tumors.[18,34,38] Likewise, the expanding

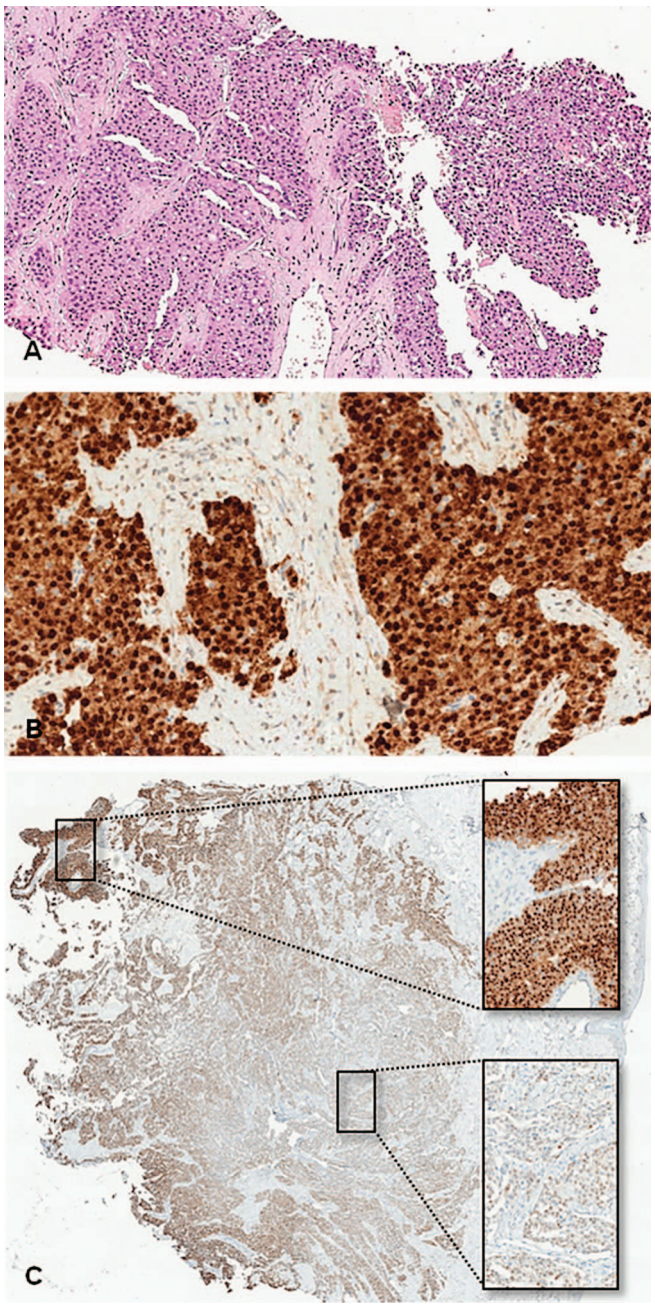


Figure 4. *Secretory breast carcinoma with an* ETV6-NTRK3 *fusion (Foundation One CDx, Foundation Medicine, Cambridge, Massachusetts). A, The tumor shows solid nests composed of cells with abundant eosinophilic cytoplasm. B, A diffuse and strong nuclear positivity associated with a cytoplasmic granular staining for pan-TRK immunohistochemistry is seen in the core needle biopsy (Ventana Medical Systems, Tucson, Arizona, original magnification ×200). C, A heterogeneous staining for pan-TRK immunohistochemistry is present in the excisional biopsy: diffuse and strong nuclear staining (TTF-1–like pattern) in the periphery of the tissue (upper inset), and focal and punctiform nuclear staining (silver in situ hybridization–like pattern) in the center of the biopsy (lower inset) (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×20 [C] and ×200 [B and C insets]).*

number of *NTRK* partners described in such tumors (and for this matter also in congenital mesoblastic nephroma, an *NTRK*-rearranged renal tumor of infancy) argues against the traditional use of *ETV6* FISH as an orthogonal method, particularly if pan-TRK IHC nuclear staining is not clearly identified.[18,34,39] Therefore, the use of *NTRK3* FISH or NGS has been recommended.[39]

Secretory Breast Carcinoma

Secretory carcinoma (Figure 4, A) is a rare type of breast carcinoma (<0.05% of all invasive breast carcinomas) that is characterized by an *ETV6-NTRK3* fusion.[40] In clinical practice, these carcinomas are frequently underdiagnosed because they are almost always triple negative or at most weakly estrogen receptor (ER)/progesterone receptor (PR) positive.[28,41,42] Because the identification of all breast secretory carcinomas in each surgical pathology practice seems unlikely, the use of pan-TRK IHC in both triple-negative breast carcinomas and invasive carcinomas with eosinophilic secretions could be a good strategy to identify these patients. Secretory breast carcinomas typically show strong cytoplasmic and nuclear pan-TRK staining (Figure 4, B).[28] However, because of suboptimal fixation, heterogeneous staining for pan-TRK IHC can be seen in resection specimens (Figure 4, C). Nonsecretory breast carcinomas can exhibit either faint cytoplasmic staining (7%–18%)[14,43] or barely visible nuclear staining in less than 5% of the cells (10% of cases in another series).[28] To rule out the influence of preanalytics on the intensity or the percentage of the nuclear staining, it is useful to select the best-fixed tissue block or compare the result of the pan-TRK IHC in the core needle biopsy versus the resection specimen if both are available.

Secretory Carcinoma of the Salivary Gland

Secretory carcinoma of the salivary gland (Figure 5, A), initially described as mammary analogue secretory carcinoma, usually contains an *ETV6-NTRK3* fusion.[44,45] Pan-TRK IHC usually shows nuclear staining with a TTF-1–like or silver in situ hybridization–like staining pattern, associated with a weak cytoplasmic positivity in 100% of the cells (Figure 5, B). Although nuclear pan-TRK IHC is very specific,[29,46] pathologists should be aware of several facts: (1) cytoplasmic staining alone has also been described at a lower frequency in larger series,[46,47] and unfortunately this cytoplasmic pattern can also be found in many other benign and malignant salivary gland neoplasms (see below)[29,46–48]; and (2) the nuclear pan-TRK staining can be weak and/or focal,[29,46,47] and a suboptimal sensitivity of pan-TRK IHC for *NTRK3*-rearranged tumors has been suggested.[14]

COMMON CARCINOMAS WITH AN EXTREMELY LOW PROBABILITY OF HARBORING *NTRK* FUSIONS

Lung Adenocarcinoma

The estimated frequency of *NTRK* fusions in non–small cell lung carcinomas is less than 1%.[14,16,17] Most cases are adenocarcinomas (Figure 6, A), but squamous cell carcinomas and carcinomas with neuroendocrine differentiation have also been described.[17] Signet ring cells can also be found, as in other rearranged lung adenocarcinomas.[4,17,49] Pan-TRK IHC–positive cases usually show diffuse strong cytoplasmic staining in tumor cells (Figure 6, B). *NTRK* fusions are usually mutually exclusive with other known oncogenic drivers, so frequencies are higher in therapy-naïve patients without driver gene mutations/fusions/amplifications.[16,50] If all advanced non–small cell lung carcinomas are not routinely screened for *NTRK* fusions using a large NGS panel, the use of pan-TRK IHC is particularly useful in such patients after the initial limited

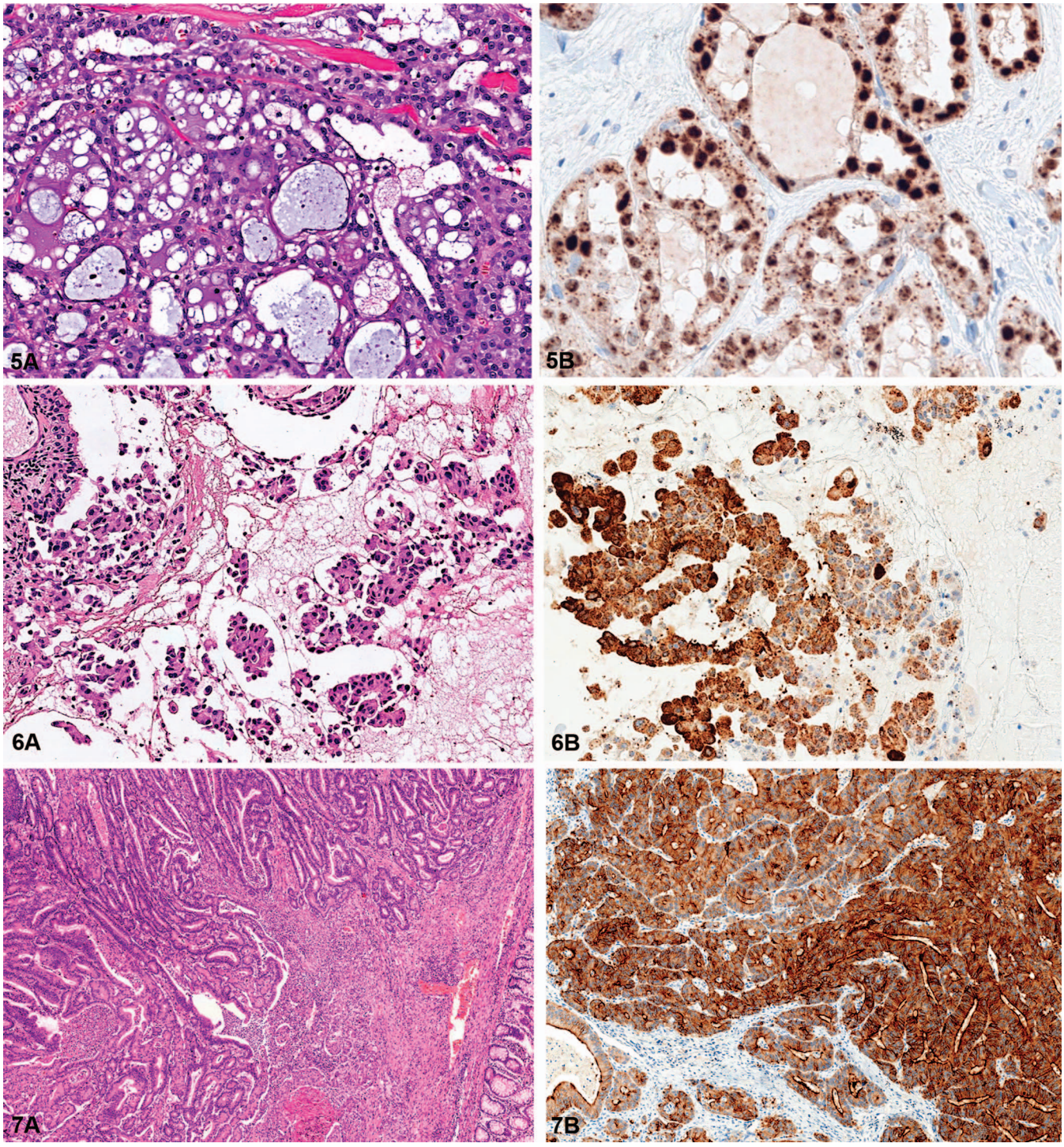


Figure 5. *Secretory carcinoma of the salivary gland with an* ETV6-NTRK3 *fusion (Oncomine Comprehensive Assay v3, Thermo Fisher Scientific, Waltham, Massachusetts). A, The tumor exhibits a microcystic growth pattern with luminal secretion. B, Pan-TRK immunohistochemistry shows a nuclear staining with a TTF-1–like (top part of the figure) or silver in situ hybridization–like (bottom part of the figure) staining patterns, associated with a diffuse but weak cytoplasmic positivity (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×400 [B]).*

Figure 6. *Lung adenocarcinoma with an* KIF5B-NTRK1 *fusion (Foundation One CDx, Foundation Medicine, Cambridge, Massachusetts). A, The adenocarcinoma exhibits a micropapillary growth pattern. B, The tumor cells show a pan-TRK diffuse and strong cytoplasmic granular staining (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B]).*

Figure 7. *Colorectal adenocarcinoma with an* TPM3-NTRK1 *fusion (Oncomine Comprehensive Assay v3, Thermo Fisher Scientific, Waltham, Massachusetts). A, The tumor is composed of complex tubular structures that invade the wall of the bowel. B, Pan-TRK immunohistochemistry shows a diffuse cytoplasmic and membranous (with luminal accentuation) staining pattern (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×40 [A]; original magnification ×100 [B]).*

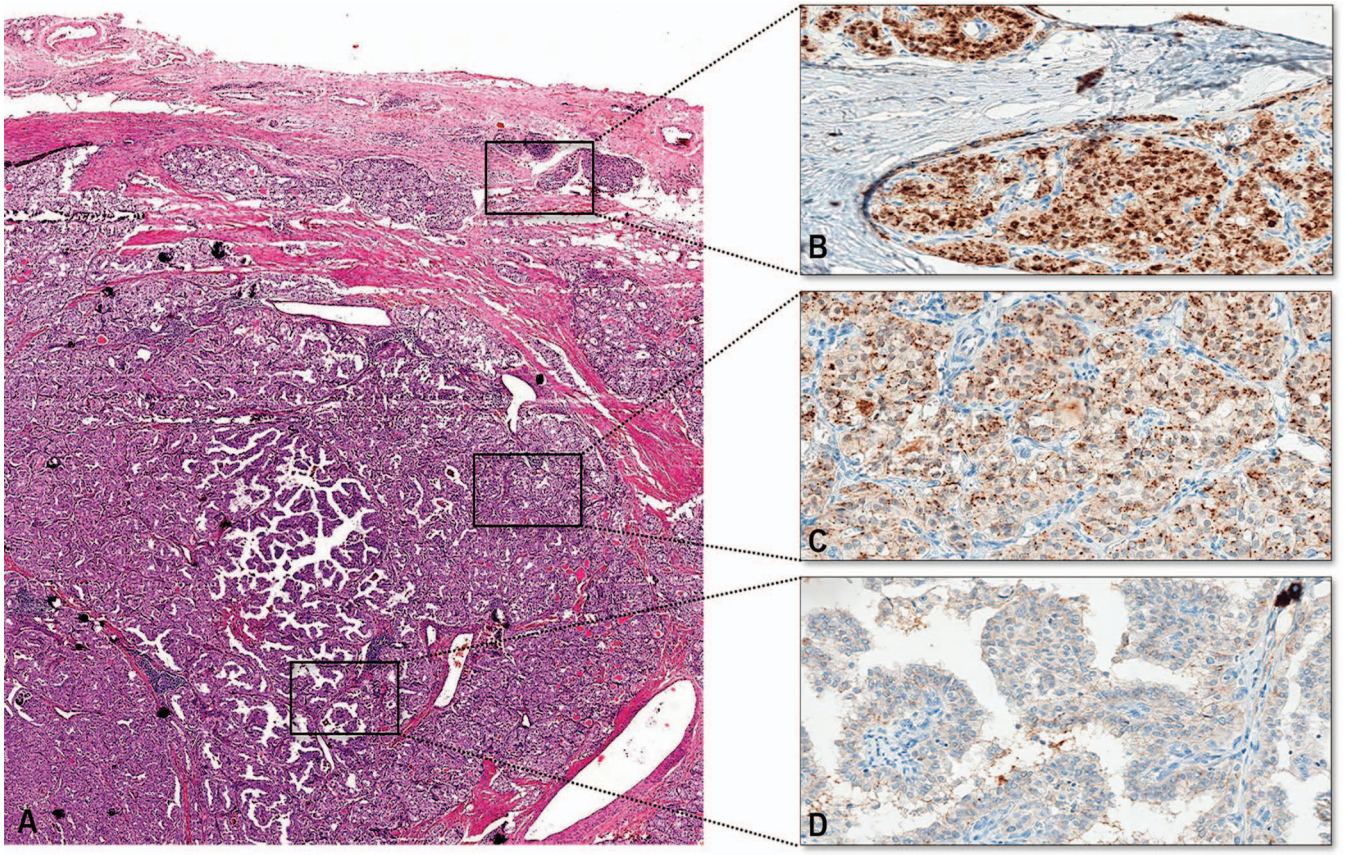


Figure 8. *Papillary thyroid carcinoma with an* ETV6-NTRK3 *fusion (Oncomine Comprehensive Assay v3, Thermo Fisher Scientific, Waltham, Massachusetts). A, A papillary thyroid carcinoma with a classic and follicular growth pattern was diagnosed in the resection specimen. Pan-TRK immunohistochemistry shows a heterogeneous staining result: B, TTF-1–like nuclear positivity, and C, silver in situ hybridization–like nuclear positivity, combined with D, subdiagnostic areas lacking nuclear staining (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×20 [A]; original magnification ×200 [B through D]).*

testing (ie, only results from main drivers or from a small NGS panel are available). A positive pan-TRK IHC result in lung adenocarcinomas is an indication for further NGS testing.[24,51] Nonetheless, given the large number of therapeutic targets in this tumor type, NGS should always be prioritized over IHC if tissue is limited. Finally, *NTRK1* fusions have been proposed as a resistance mechanism to EGFR tyrosine kinase inhibitors.[52]

Colorectal Adenocarcinoma

Screening all colorectal carcinomas (Figure 7, A) with pan-TRK IHC may not be feasible (0.02%–0.3% positivity in series with several thousand patients),[14,16,27] but a higher frequency of *NTRK* fusions (~5%) in *BRAF/RAS* wild-type, microsatellite instability–high colorectal carcinoma provides a rationale for a more sensible approach.[27,53,54] Therefore, a molecular testing workflow for colorectal carcinomas has been proposed.[53] After the initial microsatellite instability/mismatch repair assessment with IHC, deficient patients are sequentially tested for *MLH1* promoter hypermethylation and *BRAF* status before fusion testing is considered.[53] Pan-TRK IHC–positive cases usually show diffuse strong cytoplasmic staining in all tumor cells (Figure 7, B).[27] In addition, there could be positivity in other cell compartments in a partner-specific manner: membrane (eg, *TPM3*, *TPR*), nuclear membrane (eg, *LMNA*, *MUC2*), or nuclear (eg, ETV6).[21,26,27,54]

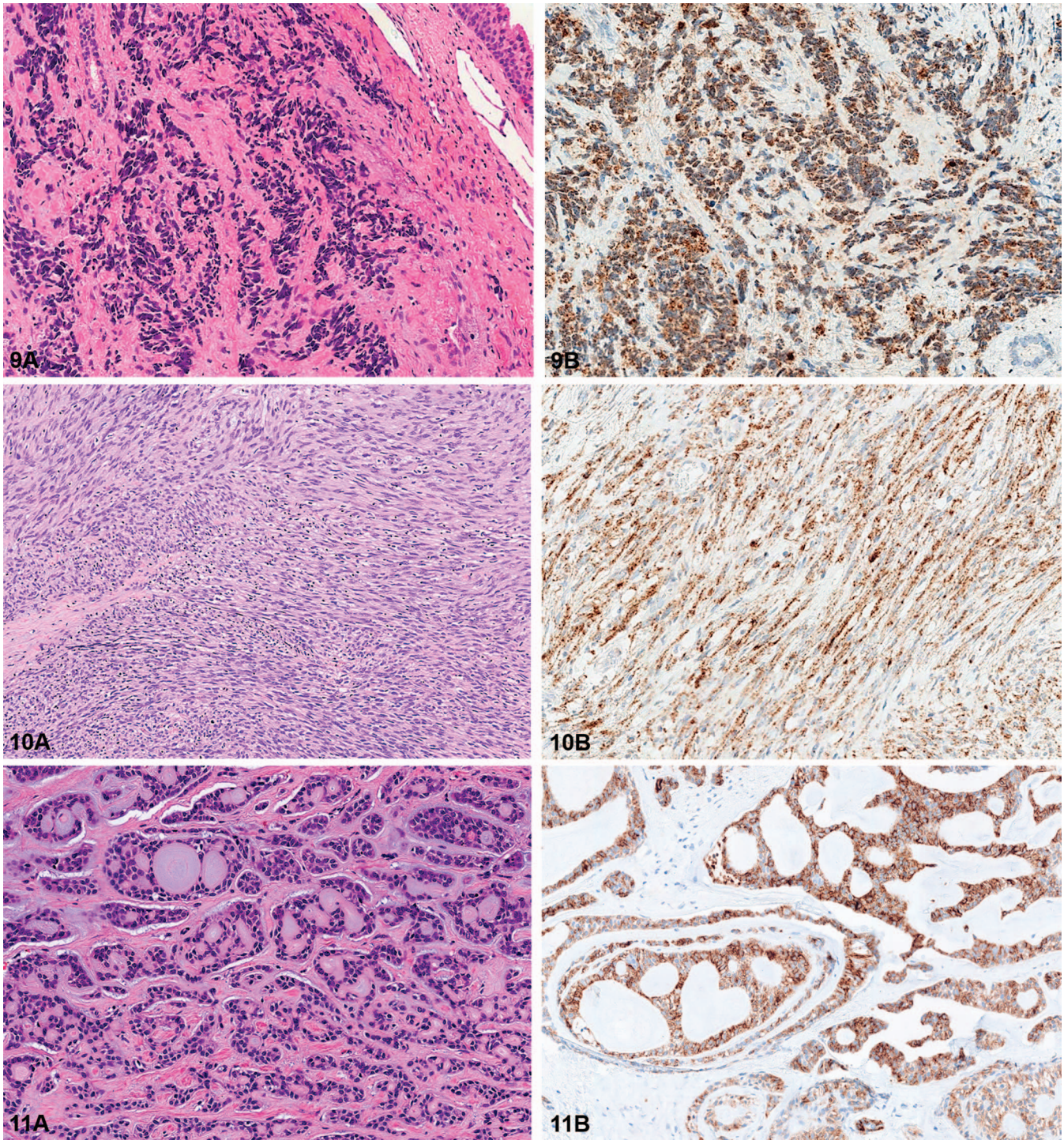
Papillary Thyroid Carcinoma

The overall frequency of *NTRK* fusions in thyroid carcinomas is around 2.3%,[13,14,16] but this figure can be much higher in children (26%).[35] In fact, kinase fusions have been linked to radiation-induced thyroid carcinomas in pediatric patients.[35] Most *NTRK*-positive thyroid cases are papillary carcinomas (Figure 8, A), but poorly differentiated and anaplastic carcinomas have also been described.[15,55] In fact, *NTRK* fusions may be associated with higher stage,[56,57] so a recommendation could be made to start by screening those papillary thyroid carcinomas with lymph node metastases. In addition to the characteristic cytoplasmic and nuclear positivity of the *ETV6-NTRK3* fusion, the patterns of IHC expression include multiple nonnuclear *NTRK1* and *NTRK3* partners.[13,55,57] It is worth remembering that this nuclear staining can be quite focal, as in the case reported herein (Figure 8, B through D), but it is extremely specific for this rearrangement.

TUMOR TYPES WITH FREQUENT AND RELEVANT PAN-TRK PROTEIN EXPRESSION NOT ASSOCIATED WITH *NTRK* FUSIONS

Olfactory Neuroblastoma

Olfactory neuroblastoma (Figure 9, A) is a malignant neuroectodermal tumor, and as such it can show very intense and diffuse pan-TRK IHC staining (Figure 9, B) not



Figures 9. *Olfactory neuroblastoma without* NTRK *fusions (Foundation One CDx, Foundation Medicine, Cambridge, Massachusetts). A, The tumor shows nests of small, blue, and round cells infiltrating the subepithelial connective tissue. B, A diffuse strong cytoplasmic staining, with dotlike accentuation, was seen with pan-TRK immunohistochemistry (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B]).*

Figure 10. *Uterine leiomyosarcoma without* NTRK *fusions (Foundation One CDx, Foundation Medicine, Cambridge, Massachusetts). A, The tumor is composed of fascicles of atypical spindle cells with frequent mitoses. B, The tumor cells are diffusely positive for pan-TRK immunohistochemistry (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×200 [B]).*

Figure 11. *Adenoid cystic carcinoma without* NTRK *fusions (Foundation One CDx, Foundation Medicine, Cambridge, Massachusetts). A, The tumor is composed of tubular and cribriform structures with sharply defined round spaces filled with a basophilic matrix. B, Pan-TRK immunohistochemistry shows a moderate to strong membranous and cytoplasmic staining (Ventana Medical Systems, Tucson, Arizona) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×200 [B]).*

associated with an *NTRK* fusion. In fact, several lines of evidence involve the sonic hedgehog (SHH) signaling pathway in its pathogenesis.[44] Because *NTRK* fusions are particularly common in pediatric tumors, the frequent and intense pan-TRK IHC positivity of *NTRK* fusion–negative neuroectodermal tumors (eg, Ewing sarcomas or neuroblastomas) should caution pathologists against overexpectations when waiting for the NGS results.[14,58]

Uterine Leiomyosarcoma

Strong and diffuse cytoplasmic pan-TRK IHC expression has been described in 2% of bona fide uterine spindle cell leiomyosarcomas (Figure 10, A and B) without *NTRK* rearrangements.[59] Because *NTRK* fusions seem to be singularly prevalent in gynecologic sarcomas,[59–63] and leiomyosarcomas are the most frequent uterine malignant mesenchymal tumors, it seems plausible that practicing pathologists will encounter this scenario. Identifying *NTRK*-rearranged soft-tissue and visceral sarcomas is extremely difficult for a number of reasons: (1) less than 2% of sarcomas contain an *NTRK* fusion[64]; (2) in most centers diagnosis is not systematically interrogated in sarcomas through NGS, given the traditional lack of targeted therapies in these tumor types[65]; (3) *NTRK* fusion–positive sarcomas display a very wide clinical, morphologic, and IHC spectrum[58,65]; and (4) the sensitivity and specificity of pan-TRK IHC to detect fusions in sarcomas has been considered the lowest for all tumor types, because of the significant expression of the wild-type TRK protein in many of the mesenchymal lines of differentiation, including smooth muscle, as shown above.[14,58,59] Therefore, awareness that several morphologic patterns have been linked with kinase fusions in sarcomas can help pathologists narrow their investigation of these patients[65]: (1) lipofibromatosis-like neural tumors[37]; (2) spindle cell tumors with S-100 and CD34 coexpression, sometimes resembling malignant peripheral nerve sheath tumors[19,63]; (3) adult-type fibrosarcomas located in the uterus without desmin, ER, and PR expression[59,60]; and (4) spindle cell sarcomas with an hemangiopericytic or myopericytoma-like pattern.[66] Along these lines, a provisional category encompassing lipofibromatosis-like neural tumors and tumor that closely resemble peripheral nerve sheath tumors has been included in the recently released World Health Organization[36] classification of soft tissue and bone tumors as *NTRK*-rearranged spindle cell neoplasm (emerging). Accordingly, it may be wise to screen for *NTRK* fusions in all adult sarcomas without obvious differentiation or driver gene mutations/fusions/amplifications, particularly those with S-100 and CD34 coreactivity and/or located in the gynecologic tract.[24,60–62,67,68]

Adenoid Cystic Carcinoma

Because of the perception that *NTRK* fusion–positive tumors are frequent in the salivary glands and the relative abundance of relentless adenoid cystic carcinomas (Figure 11, A) in most institutions (<10% of all salivary gland neoplasms),[44] it is plausible that pathologists will be asked to perform and score pan-TRK IHC in these tumors.[14] At least 40% of adenoid cystic carcinomas can show moderate to strong cytoplasmic staining in the outer layer of tumor cells (Figure 11, B), which never corresponds to an *NTRK* fusion.[14,48] Interestingly, adenoid cystic carcinomas can also show overexpression of KIT and EGFR, which are rarely mutated or amplified, further preventing druggable opportunities.[44]

CONCLUSIONS

Through this paper we have emphasized the key role of pan-TRK IHC in the identification of *NTRK* fusions. The application of these histology-based and genomic-based triaging strategies is illustrated with 9 examples from our daily practice and is summarized in the Table. Although NTRK inhibitors are being approved in a tumor-agnostic manner, pathologists should take the clinical, histologic, and molecular context into consideration when scoring this marker.

This work is dedicated to all the COVID-19 victims. Dr Lopez-Rios thanks T. Crean for his constant support.

References

1. Rolfo C. NTRK gene fusions: a rough diamond ready to sparkle. *Lancet Oncol*. 2020;21(4):472–474. doi:10.1016/S1470-2045(20)30026-7

2. Pestana RC, Sen S, Hobbs BP, Hong DS. Histology-agnostic drug development—considering issues beyond the tissue. *Nat Rev Clin Oncol*. 2020; 17(9):555–568. doi:10.1038/s41571-020-0384-0

3. Kerr KM, López-Ríos F. Precision medicine in NSCLC and pathology: how does ALK fit in the pathway? *Ann Oncol*. 2016;27(suppl 3):ii16–iii24. doi:10.1093/annonc/mdw302

4. Conde E, Hernandez S, Martinez R, et al. Assessment of a new ROS1 immunohistochemistry clone (SP384) for the identification of ROS1 rearrangements in patients with non–small cell lung carcinoma: the ROSING study. *J Thorac Oncol*. 2019;14(12):2120–2132. doi:10.1016/j.jtho.2019.07.005

5. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. *Clin Cancer Res*. 2019;25(15):4712–4722. doi:10.1158/1078-0432.CCR-19-0225

6. Doebele RC, Drilon A, Paz-Ares L, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1–2 trials. *Lancet Oncol*. 2020;21(2):271–282. doi:10.1016/S1470-2045(19)30691-6

7. Hong DS, DuBois SG, Kummar S, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. *Lancet Oncol*. 2020;21(4):531–540. doi:10.1016/S1470-2045(19)30856-3

8. Marchiò C, Scaltriti M, Ladanyi M, et al. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. *Ann Oncol*. 2019;30(9):1417–1427. doi:10.1093/annonc/mdz204

9. Pfarr N, Kirchner M, Lehmann U, et al. Testing NTRK testing: wet-lab and in silico comparison of RNA-based targeted sequencing assays. *Genes Chromosomes Cancer*. 2020;59(3):178–188. doi:10.1002/gcc.22819

10. Penault-Llorca F, Rudzinski ER, Sepulveda AR. Testing algorithm for identification of patients with TRK fusion cancer. *J Clin Pathol*. 2019;72(7):460–467. doi:10.1136/jclinpath-2018-205679

11. Hsiao SJ, Zehir A, Sireci AN, Aisner DL. Detection of tumor NTRK gene fusions to identify patients who may benefit from tyrosine kinase (TRK) inhibitor therapy. *J Mol Diagn*. 2019;21(4):553–571. doi:10.1016/j.jmoldx.2019.03.008

12. Solomon JP, Benayed R, Hechtman JF, Ladanyi M. Identifying patients with NTRK fusion cancer. *Ann Oncol*. 2019;30(suppl 8):viii16–viii22. doi:10.1093/annonc/mdz384

13. Okamura R, Boichard A, Kato S, Sicklick JK, Bazhenova L, Kurzrock R. Analysis of NTRK alterations in pan-cancer adult and pediatric malignancies: implications for NTRK-targeted therapeutics. *JCO Precis Oncol*. 2018;2018. doi:10.1200/PO.18.00183

14. Solomon JP, Linkov I, Rosado A, et al. NTRK fusion detection across multiple assays and 33 997 cases: diagnostic implications and pitfalls. *Mod Pathol*. 2020;33(1):38–46. doi:10.1038/s41379-019-0324-7

15. Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol*. 2019;32(1):147–153. doi:10.1038/s41379-018-0118-3

16. Rosen EY, Goldman DA, Hechtman JF, et al. TRK fusions are enriched in cancers with uncommon histologies and the absence of canonical driver mutations. *Clin Cancer Res*. 2020;26(7):1624–1632. doi:10.1158/1078-0432.CCR-19-3165

17. Farago AF, Taylor MS, Doebele RC, et al. Clinicopathologic features of non–small-cell lung cancer harboring an NTRK gene fusion. *JCO Precis Oncol*. 2018; 2018(2):1–12. doi:10.1200/po.18.00037

18. Davis JL, Lockwood CM, Stohr B, et al. Expanding the spectrum of pediatric NTRK-rearranged mesenchymal tumors. *Am J Surg Pathol*. 2019;43(4):435–445. doi:10.1097/PAS.0000000000001203

19. Suurmeijer AJH, Dickson BC, Swanson D, et al. A novel group of spindle cell tumors defined by S100 and CD34 co-expression shows recurrent fusions involving RAF1, BRAF, and NTRK1/2 genes. *Genes Chromosomes Cancer*. 2018; 57(12):611–621. doi:10.1002/gcc.22671

20. Lantuejoul S, Sound-Tsao M, Cooper WA, et al. PD-L1 testing for lung cancer in 2019: perspective from the IASLC Pathology Committee. *J Thorac Oncol*. 2020;15(4):499–519. doi:10.1016/j.jtho.2019.12.107

21. Hechtman JF, Benayed R, Hyman DM, et al. Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. *Am J Surg Pathol*. 2017;41(11):1547–1551. doi:10.1097/PAS.0000000000000911

22. pan-TRK (EPR17341) Assay, VENTANA. http://reagent-catalog.roche.com/product/1909?type=2442. Accessed May 27, 2020.

23. Recombinant anti-pan Trk antibody [EPR17341] (ab181560). Abcam. https://www.abcam.com/pan-trk-antibody-epr17341-ab181560.html?productWallTab=ShowAll. Accessed May 27, 2020.

24. Yoshino T, Pentheroudakis G, Mishima S, et al. JSCO/ESMO/ASCO/JSMO/TOS: international expert consensus recommendations for tumour-agnostic treatments in patients with solid tumours with microsatellite instability or NTRK fusions. *Ann Oncol*. 2020;31(7):861–872. doi:10.1016/j.annonc.2020.03.299

25. Solomon JP, Hechtman JF. Detection of NTRK fusions: merits and limitations of current diagnostic platforms. *Cancer Res*. 2019;79(13):3163–3168. doi:10.1158/0008-5472.CAN-19-0372

26. Lasota J, Chłopek M, Lamoureux J, et al. Colonic adenocarcinomas harboring NTRK fusion genes: a clinicopathologic and molecular genetic study of 16 cases and review of the literature. *Am J Surg Pathol*. 2020;44(2):162–173. doi:10.1097/PAS.0000000000001377

27. Chou A, Fraser T, Ahadi M, et al. NTRK gene rearrangements are highly enriched in MLH1/PMS2 deficient, BRAF wild-type colorectal carcinomas—a study of 4569 cases. *Mod Pathol*. 2019;33(5). doi:10.1038/s41379-019-0417-3

28. Harrison BT, Fowler E, Krings G, et al. Pan-TRK immunohistochemistry: a useful diagnostic adjunct for secretory carcinoma of the breast. *Am J Surg Pathol*. 2019;43(12):1693–1700. doi:10.1097/PAS.0000000000001366

29. Hung YP, Jo VY, Hornick JL. Immunohistochemistry with a pan-TRK antibody distinguishes secretory carcinoma of the salivary gland from acinic cell carcinoma. *Histopathology*. 2019;75(1):54–62. doi:10.1111/his.13845

30. Kummar S, Lassen UN. TRK inhibition: a new tumor-agnostic treatment strategy. *Target Oncol*. 2018;13(5):545–556. doi:10.1007/s11523-018-0590-1

31. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol*. 2018;15(12):731–747. doi:10.1038/s41571-018-0113-0

32. Kirchner M, Glade J, Lehmann U, et al. NTRK testing: first results of the QuiP-EQA scheme and a comprehensive map of NTRK fusion variants and their diagnostic coverage by targeted RNA-based NGS assays. *Genes Chromosomes Cancer*. 2020;59(8):445–453. doi:10.1002/gcc.22853

33. Hung YP, Fletcher CDM, Hornick JL. Evaluation of pan-TRK immunohistochemistry in infantile fibrosarcoma, lipofibromatosis-like neural tumour and histological mimics. *Histopathology*. 2018;73(4):634–644. doi:10.1111/his.13666

34. Rudzinski ER, Lockwood CM, Stohr BA, et al. Pan-Trk immunohistochemistry identifies NTRK rearrangements in pediatric mesenchymal tumors. *Am J Surg Pathol*. 2018;42(7):927–935. doi:10.1097/PAS.0000000000001062

35. Albert CM, Davis JL, Federman N, Casanova M, Laetsch TW. TRK fusion cancers in children: a clinical review and recommendations for screening. *J Clin Oncol*. 2019;37(6):513–524. doi:10.1200/JCO.18.00573

36. WHO Classification of Tumours Editorial Board. *Soft Tissue and Bone Tumours*. 5th ed. Lyon, France: International Agency for Research on Cancer; 2020. *WHO Classification of Tumours*; vol 3.

37. Agaram NP, Zhang L, Sung YS, et al. Recurrent NTRK1 gene fusions define a novel subset of locally aggressive lipofibromatosis-like neural tumors. *Am J Surg Pathol*. 2016;40(10):1407–1416. doi:10.1097/PAS.0000000000000675

38. Warren M, Anselmo D, Takeda M, Shillingford N, Hiemenz MC, Shah R. NTRK-rearranged mesenchymal tumour in a 3-year-old female: a diagnostic quandary. *Histopathology*. 2019;75(5):772–775. doi:10.1111/his.13920

39. Church AJ, Calicchio ML, Nardi V, et al. Recurrent EML4-NTRK3 fusions in infantile fibrosarcoma and congenital mesoblastic nephroma suggest a revised testing strategy. *Mod Pathol*. 2018;31(3):463–473. doi:10.1038/modpathol.2017.127

40. WHO Classification of Tumours Editorial Board. *Breast Tumours*. 5th ed. Lyon, France: International Agency for Research on Cancer; 2019. *WHO Classification of Tumours*; vol 2.

41. Laé M, Fréneaux P, Sastre-Garau X, Chouchane O, Sigal-Zafrani B, Vincent-Salomon A. Secretory breast carcinomas with ETV6-NTRK3 fusion gene belong to the basal-like carcinoma spectrum. *Mod Pathol*. 2009;22(2):291–298. doi:10.1038/modpathol.2008.184

42. Del Castillo M, Chibon F, Arnould L, et al. Secretory breast carcinoma: a histopathologic and genomic spectrum characterized by a joint specific ETV6-NTRK3 gene fusion. *Am J Surg Pathol*. 2015;39(11):1458–1467. doi:10.1097/PAS.0000000000000487

43. Zaborowski M, Gill AJ. Is secretory breast carcinoma underdiagnosed?: in the era of targeted therapy should there be a low threshold to screen for NTRK immunohistochemistry in triple negative breast cancers? *Pathology*. 2019;51(6):653–655. doi:10.1016/j.pathol.2019.04.012

44. El-Naggar AK, Chan JKC, Grandis JR, Takata T SP, ed. *WHO Classification of Head and Neck Tumours*. 4th ed. Lyon, France: International Agency for Research on Cancer; 2017. *WHO Classification of Tumours*; vol 9.

45. Skálová A, Vanecek T, Simpson RHW, et al. Mammary analogue secretory carcinoma of salivary glands. *Am J Surg Pathol*. 2016;40(1):3–13. doi:10.1097/PAS.0000000000000537

46. Xu B, Haroon Al Rasheed MR, Antonescu CR, et al. Pan-Trk immunohistochemistry is a sensitive and specific ancillary tool for diagnosing secretory carcinoma of the salivary gland and detecting ETV6-NTRK3 fusion. *Histopathology*. 2020;76(3):375–382. doi:10.1111/his.13981

47. Bell D, Ferrarotto R, Liang L, et al. Pan-Trk immunohistochemistry reliably identifies ETV6-NTRK3 fusion in secretory carcinoma of the salivary gland. *Virchows Arch*. 2020;476(2):295–305. doi:10.1007/s00428-019-02640-7

48. Guibourg B, Cloarec E, Conan-Charlet V, et al. EPR17341 and A7H6R pan-TRK immunohistochemistry result in highly different staining patterns in a series of salivary gland tumors [published online ahead of print [published online March 16, 2020]. *Appl Immunohistochem Mol Morphol*. doi:10.1097/pai.0000000000000825

49. Conde E, Suárez-Gauthier A, Benito A, et al. Accurate identification of ALK positive lung carcinoma patients: novel FDA-cleared automated fluorescence in situ hybridization scanning system and ultrasensitive immunohistochemistry. *PLoS One*. 2014;9(9):e107200. doi:10.1371/journal.pone.0107200

50. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med*. 2013;19(11):1469–1472. doi:10.1038/nm.3352

51. Gautschi O, Bubendorf L, Leyvraz S, Menon R, Diebold J. Challenges in the diagnosis of NTRK fusion-positive cancers. *J Thorac Oncol*. 2020;15(7):e108–e110. doi:10.1016/j.jtho.2019.05.001

52. Xia H, Xue X, Ding H, et al. Evidence of NTRK1 fusion as resistance mechanism to EGFR TKI in EGFR+ NSCLC: results from a large-scale survey of NTRK1 fusions in Chinese patients with lung cancer. *Clin Lung Cancer*. 2020;21(3):247–254. doi:10.1016/j.cllc.2019.09.004

53. Cocco E, Benhamida J, Middha S, et al. Colorectal carcinomas containing hypermethylated MLH1 promoter and wild-type BRAF/KRAS are enriched for targetable kinase fusions. *Cancer Res*. 2019;79(6):1047–1053. doi:10.1158/0008-5472.CAN-18-3126

54. Vaňková B, Vaněček T, Ptáková N, et al. Targeted next generation sequencing of MLH1-deficient, MLH1 promoter hypermethylated, and BRAF/RAS-wild-type colorectal adenocarcinomas is effective in detecting tumors with actionable oncogenic gene fusions. *Genes Chromosomes Cancer*. 2020;59(10):562–568. doi:10.1002/gcc.22861

55. Amatu A, Sartore-Bianchi A, Bencardino K, Pizzutilo EG, Tosi F, Siena S. Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. *Ann Oncol*. 2019;30:viii5–viii15. doi:10.1093/annonc/mdz383

56. Prasad ML, Vyas M, Horne MJ, et al. NTRK fusion oncogenes in pediatric papillary thyroid carcinoma in northeast United States. *Cancer*. 2016;122(7):1097–1107. doi:10.1002/cncr.29887

57. Chu YH, Dias-Santagata D, Farahani AA, et al. Clinicopathologic and molecular characterization of NTRK-rearranged thyroid carcinoma (NRTC) [published online May 26, 2020]. *Mod Pathol*. doi:10.1038/s41379-020-0574-4

58. Wong DD, Vargas AC, Bonar F, et al. NTRK-rearranged mesenchymal tumours: diagnostic challenges, morphological patterns and proposed testing algorithm. *Pathology*. 2020;52(4):401–409. doi:10.1016/j.pathol.2020.02.004

59. Chiang S, Cotzia P, Hyman DM, et al. NTRK fusions define a novel uterine sarcoma subtype with features of fibrosarcoma. *Am J Surg Pathol*. 2018;42(6):791–798. doi:10.1097/PAS.0000000000001055

60. Croce S, Hostein I, Longacre TA, et al. Uterine and vaginal sarcomas resembling fibrosarcoma: a clinicopathological and molecular analysis of 13 cases showing common NTRK-rearrangements and the description of a COL1A1-PDGFB fusion novel to uterine neoplasms. *Mod Pathol*. 2019;32(7):1008–1022. doi:10.1038/s41379-018-0184-6

61. Michal M, Hájková V, Skálová A, Michal M. STRN-NTRK3-rearranged mesenchymal tumor of the uterus: expanding the morphologic spectrum of tumors with NTRK fusions. *Am J Surg Pathol*. 2019;43(8):1152–1154. doi:10.1097/PAS.0000000000001292

62. Rabban JT, Devine P, Sangoi AR, et al. NTRK fusion cervical sarcoma: a report of 3 cases, emphasizing morphological and immunohistochemical distinction from other uterine sarcomas, including adenosarcoma. *Histopathology*. 2020;77(1):100–111. doi:10.1111/his.14069

63. Wells AE, Mallen AM, Bui MM, Reed DR, Apte SM. NTRK-1 fusion in endocervical fibroblastic malignant peripheral nerve sheath tumor marking eligibility for larotrectinib therapy: a case report. *Gynecol Oncol Reports*. 2019;28:141–144. doi:10.1016/j.gore.2019.04.006

64. Penel N, Lebellec L, Blay JY, Robin YM. Overview of "druggable" alterations by histological subtypes of sarcomas and connective tissue intermediate malignancies. *Crit Rev Oncol Hematol*. 2020;150:102960. doi:10.1016/j.critrevonc.2020.102960

65. Antonescu CR. Emerging soft tissue tumors with kinase fusions: an overview of the recent literature with an emphasis on diagnostic criteria. *Genes Chromosomes Cancer*. 2020;59(8):437–444. doi:10.1002/gcc.22846

66. Haller F, Knopf J, Ackermann A, et al. Paediatric and adult soft tissue sarcomas with NTRK1 gene fusions: a subset of spindle cell sarcomas unified by a prominent myopericytic/haemangiopericytic pattern. *J Pathol*. 2016;238(5):700–710. doi:10.1002/path.4701

67. So YK, Chow C, To KF, Chan JKC, Cheuk W. Myxoid spindle cell sarcoma with LMNA-NTRK fusion: expanding the morphologic spectrum of NTRK-rearranged tumors. *Int J Surg Pathol*. 2020;28(5):574–578. doi:10.1177/1066896920905888

68. Hodgson A, Pun C, Djordjevic B, Turashvili G. NTRK-rearranged cervical sarcoma: expanding the clinicopathologic spectrum [published online February 10, 2020]. *Int J Gynecol Pathol*. 2020. doi:10.1097/PGP.0000000000000669