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CANTRK

A Canadian Ring Study to Optimize Detection of NTRK *Gene Fusions by Next-Generation RNA Sequencing*

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The Canadian *NTRK* (CANTRK) is a ring study to optimize testing for neurotrophic receptor tyrosine kinase (*NTRK*) fusions in Canadian laboratories. Sixteen diagnostic laboratories used next-generation sequencing (NGS) for *NTRK1*, *NTRK2*, or *NTRK3* fusions. Each laboratory received 12 formalin-fixed, paraffin-embedded tumor samples with unique *NTRK* fusions and two control non-*NTRK* fusion samples (one *ALK* and one *ROS1*). Laboratories used validated protocols for NGS fusion detection. Panels included Oncomine Comprehensive Assay v3, Oncomine Focus Assay, Oncomine Precision Assay, AmpliSeq for Illumina Focus, TruSight RNA Pan-Cancer Panel, FusionPlex Lung, and QIAseq Multimodal Lung. One sample was withdrawn from analysis because of sample quality issues. Of the remaining 13 samples, 6 of 11 *NTRK* fusions and both control fusions were detected by all laboratories. Two fusions, *WNK2::NTRK2* and *STRN3::NTRK2*, were not detected by 10 laboratories using the Oncomine Comprehensive or Focus panels, due to absence of *WNK2* and *STRN3* in panel designs. Two fusions, *TPM3::NTRK1* and *LMNA::NTRK1*, were challenging to detect on the AmpliSeq for Illumina Focus panel because of bioinformatics issues. One *ETV6::NTRK3* fusion at low levels was not detected by two laboratories using the TruSight Pan-Cancer Panel. Panels detecting all fusions included FusionPlex Lung, Oncomine Precision, and QIAseq Multimodal Lung. The CANTRK study showed competency in detection of *NTRK*

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fusions by NGS across different panels in 16 Canadian laboratories and identified key test issues as targets for improvements. *(J Mol Diagn 2023, ■: 1–7; https://doi.org/10.1016/j.jmoldx.2022.12.004)*

Rearrangement involving the neurotrophic tyrosine receptor kinase (*NTRK*) family genes (*NTRK1*, *NTRK2*, and *NTRK3*) generate fusion proteins that are oncogenic drivers in many cancer types across adult and pediatric populations.[1–3] Recently, larotrectinib and entrectinib have received clinical approval in the United States, Canada, Europe, and other countries for the treatment of adult patients with metastatic or locally advanced unresectable disease, and in some countries, entrectinib also in pediatric patients (≥12 years old), with solid tumors bearing the *NTRK* fusion.[4–7] Health Canada approved larotrectinib in 2019 and entrectinib in 2020 for adult and pediatric patients. Many methods may be used to screen for the presence of *NTRK* fusions in the tumor, including immunohistochemistry (IHC), fluorescent *in situ* hybridization, RT-PCR, and massively parallel next-generation sequencing (NGS). However, with three *NTRK* genes and >80 possible fusion partners to consider for testing, IHC and NGS have emerged as the preferred methods for screening and/or diagnosis of *NTRK* fusion tumors and patients in the clinic.[2,8,9] Screening using IHC prior to NGS is especially applicable for cancers with a low incidence of *NTRK* gene fusions.[2,8–11]

NGS panel testing is now a standard platform in clinical molecular diagnostic laboratories within Canada.[12] However, multiple NGS sequencing platforms and commercially developed test panels are available. Except for large referral centers or commercial laboratories, most institutional laboratories adopt one NGS platform and one or a small number of commercial targeted panels to validate and use in their routine clinical work. In this context, the Canadian *NTRK* (CANTRK) ring study was initiated with the goal to optimize and enable testing protocols to screen for *NTRK* fusion cancers in pathology and molecular diagnostic laboratories across Canada, using pan-TRK IHC for screening and RNA-based NGS targeted panels for confirming the presence of *NTRK* gene fusions as currently used in Canada. The current article describes the design, execution, and results of the NGS part of the CANTRK project and discusses issues and possible solutions that may be useful for institutions and/or laboratories that also plan to establish *NTRK* testing programs.

Materials and Methods

Participating Laboratories

The CANTRK study included two components, one evaluating pan-TRK IHC and the other evaluating NGS-based testing for *NTRK* gene fusions (*NTRK1*, *NTRK2*, and *NTRK3*). Details on the IHC study will be reported separately. In the NGS study, 16 clinical molecular diagnostic laboratories from seven provinces (Alberta, British Columbia, Manitoba, Nova Scotia, Ontario, Quebec, and Saskatchewan) participated. Two additional laboratories were invited but were unable to complete testing. Laboratories were selected based on accreditation and licensing as a clinical molecular diagnostic laboratory in their province, as well as on existing ability to perform NGS using methods that detected fusions in *NTRK1*, *NTRK2*, or *NTRK3* genes. All laboratories used various methods based on RNA sequencing for gene fusion detection. Results were returned to the project leader, and meetings were held to review and discuss results and discrepancies. The project and study protocol received approval by the Research Ethics Board at the University Health Network (19-5438).

Samples

Before the start of the ring study, a commercial formalin-fixed, paraffin-embedded (FFPE) control sample containing multiple gene fusions including four *NTRK* gene fusions (Seraseq FFPE Tumor Fusion RNA Reference Material v4, DMark Biosciences, Toronto, ON, Canada) was distributed to all participating molecular laboratories as an aid to NGS validation. Twelve FFPE patient tumor samples with known *NTRK1*, *NTRK2*, or *NTKR3* gene fusions, as well as two non-*NTRK* fusion cases (*ALK* and *ROS1*), were used as study materials (Table 1, Supplemental Table S1). The *NTRK* fusion cases were collected from CANTRK participant laboratories following approval by local institutions as required. Tumor sites included lung adenocarcinoma, atypical nevus, papillary thyroid carcinoma (follicular variant, classical variant, metastatic), infantile fibrosarcoma, salivary gland secretory carcinoma, glioneuronal tumor, and breast secretory carcinoma. Pathology review and assessment of the tumor cellularity were performed by board-certified study pathologists (M.S.T. and S.N.M.F.). All samples had tumor cellularity >90% in the tumor-rich regions. The presence of the *NTRK* gene fusions and two control non-*NTRK* gene fusions (*ALK* and *ROS1*) were confirmed at two reference laboratories by study participants (B.L., The Ottawa Hospital, Ottawa, ON, Canada; T.L.S., University Health Network, Toronto, ON, Canada). Reference laboratories were chosen based on their use of two different NGS library methods, one using multiplex PCR with primers designed to detect known fusions [Oncomine Comprehensive Assay v3 (OCAv3), Thermo Fisher Scientific, Waltham, MA; used at University Health Network] and one using anchored multiplex PCR that can detect *NTRK* gene fusions in a partner-agnostic manner [FusionPlex Lung Panel (FPL) ArcherDX, Boulder, CO; used at The Ottawa Hospital].

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Table 1 Validation Samples Used in the CANTRK Study

Sample	Gene fusion (exon)	Disease site or diagnosis
CTRK-02	*TPR*(21)::*NTRK1* (10)	Lung adenocarcinoma
CTRK-06	*ALK*(20)::*EML4* (20)	Lung adenocarcinoma
CTRK-07	*TPM3*(7)::*NTRK1* (12)	Lung adenocarcinoma
CTRK-09	*LMNA*(3)::*NTRK1* (11)	Atypical nevus
CTRK-12	*TPM3*(7)::*NTRK1* (10)	PTC, follicular variant
CTRK-13	*ETV6*(5)::*NTRK3* (15)	Salivary gland, MASC
CTRK-14	*WNK2*(23)::*NTRK2* (16)	Glioneuronal tumor
CTRK-15	*STRN3*(7)::*NTRK2* (16)	Glioneuronal tumor
CTRK-18	*ROS1*(34)::*CD74* (6)	Lung adenocarcinoma
CTRK-20	*ETV6*(4)::*NTRK3* (14)	PTC, classical variant
CTRK-22	Not tested	Infantile fibrosarcoma
CTRK-23	*ETV6*(4)::*NTRK3* (14)	Salivary gland
CTRK-24	*ETV6*(5)::*NTRK3* (15)	Breast secretory carcinoma
CTRK-28	*TPM3*(7)::*NTRK1* (10)	PTC, metastatic

The expected fusion result for each sample was determined by using the Oncomine Comprehensive Assay v3 (Thermo Fisher Scientific) next-generation sequencing panel. Numbers in parentheses represent exons determined by reference laboratory testing [FusionPlex Lung (ArcherDX) or Oncomine Comprehensive Assay v3] and confirmed by consensus among other laboratory exon results. The disease site/diagnosis was determined by one of the study board-certified pathologists. One sample (CTRK-22) of infantile fibrosarcoma was withdrawn from analysis due to poor sample quality.

CANTRK, Canadian *NTRK*; MASC, mammary analogue secretory carcinoma; PTC, papillary thyroid carcinoma.

Ring Study Protocol

For the ring study, each participating laboratory received three 7 μm thick sections for each study case. Laboratories also received an image of a hematoxylin and eosin—stained slide with the tumor-rich area marked with ink, for use in macro-dissection if required by standard practice for the participating laboratories. Laboratories were blinded to the known gene fusion in each sample. Each laboratory used their own validated protocols for RNA extraction and *NTRK* fusion detection (Supplemental Table S2).[13−18] The NGS panels used were capable of detecting gene fusions in *NTRK1*, *NTRK2*, and *NTRK3*. NGS panels included OCAv3 (four laboratories), Oncomine Focus Assay (OCF; Thermo Fisher Scientific; two laboratories), Oncomine Precision Assay (OPA; Thermo Fisher Scientific; one laboratory), AmpliSeq for Illumina Focus Panel (ASIFP: Illumina, San Diego, CA; four laboratories), TruSight RNA Pan-Cancer Panel (TS; Illumina; two laboratories), FPL (two laboratories), and QIAseq Multimodal Lung (QMML; Qiagen, Germantown, MD; one laboratory). RNA input amounts were validated by each laboratory, with 10 to 40 ng RNA input used for libraries for the OCAv3, OCF, OPA, and ASIFP panels, and 30 to 250 ng RNA for the TS, FPL, and QMML panels. Laboratories used their own bioinformatics tools for analysis, ranging from analysis tools supplied by panel manufacturers to custom laboratory-developed analyses (Supplemental Table S2). Participating laboratories reported back the results to the study lead and reference laboratory lead (M.S.T. and T.L.S.) who assessed the reported fusions against expected results. Discussions were held with participating laboratories to present results and discuss any discordant findings.

Sample Exchange for TruSight RNA Pan-Cancer Panel

For the two-way sample exchange between the two laboratories using the TS panel and the reference laboratory (University Health Network), extracted RNA from CTRK-20 in which the *ETV6::NTRK3* fusion was sent from the reference laboratory to each laboratory using TS. In return, each laboratory using the TS panel sent extracted RNA to the reference laboratory. All three laboratories tested the received samples, and results of all exchanged samples were reported to the reference laboratory lead for comparison.

Results

Overall Results

The average RNA yield of each sample is shown in Supplemental Table S3. In the confirmation of *NTRK* fusion cases by the reference laboratories, two cases, CTRK-14 (*WNK2::NTRK2*) and CTRK-15 (*STRN3::NTRK2*), were detected by one reference laboratory using the anchored multiplex PCR-based FPL assay but not by the second reference laboratory using the amplicon-based OCAv3 due to the absence of primers for the partner genes *WNK2* and *STRN3* in the panel designs. One sample (CTRK-22; infantile fibrosarcoma with *ETV6::NTRK3* fusion) was withdrawn from analysis due to a quality issue with the FFPE tumor tissue block (no laboratories generated data for the CTRK-22 sample). Histology review of the sample revealed evidence of autolysis and poor tissue preservation, which could contribute to the inadequacy for NGS. Of the 13 remaining samples, 8 of the fusions were detected by all 16 laboratories, including 6 samples with *NTRK* gene fusions (6 of 11 *NTRK* fusion samples, 55%) and 2 control samples with *ALK* or *ROS1* fusions (Figure 1).

Amplicon Library Panels: OCAv3, OCF, OPA, and ASIFP

Amplicon panels have the limitation of detecting only fusions for which primers are included in the panel design. Of the four amplicon library panels used in this ring study, one panel detected all fusions (OPA). Two *NTRK* fusions in glioneuronal tumor, *WNK2::NTRK2* (CTRK-14) and *STRN3::NTRK2* (CTRK-15), were not detected by the 10 laboratories using the OCF, ASIFP, or OCAv3 panels; this was due to the absence of primers for the partner genes *WNK2* and *STRN3* in the panel designs for the OCF and ASIFP assays (includes a total of 13 isoforms present for 9 partner genes, does not include *WNK2* and *STRN3*; information supplied by manufacturer) and in the OCAv3 assay (includes a total of 18 isoforms present for 13 partner genes;

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does not include *WNK2* and *STRN3*; information supplied by manufacturer). The more recent OPA panel from the same manufacturer includes primers for a larger number of *NTRK2* gene partners (total of 89 isoforms present for 37 partner genes, including *WNK2* and *STRN3*), and the *WNK2::NTRK2* and *STRN3::NTRK2* fusions were both detectable on the OPA panel.

Of the six laboratories using the Focus panel from either of the two manufacturers (four laboratories using ASIFP, Illumina; two laboratories using OFA, Thermo Fisher Scientific), three laboratories (laboratories 1, 2, and 3) (Figure 1) were not able to detect the expected fusion in two lung adenocarcinomas samples, *TPM3::NTRK1* (CTRK-07) and *LMNA::NTRK1* (CTRK-09). The three laboratories that did not detect these fusions used ASIFP. One of the three laboratories using ASIFP did obtain a partial result detecting a fusion involving *NTRK1*, without identifying the fusion partner, by using an analysis for an imbalance in the difference in expression between the 5′ assay and the 3′ assay of each gene in the fusion (no other laboratories assessed the expression imbalance assay results in routine use). Because the ability of three other laboratories to detect these fusions using the ASIFP or OFA ruled out an issue with primers in the panel design (as both ASIFP and OFA panels use the same design), it suggested the issue was due to either a lack of tumor in the FFPE material sent to these three laboratories (could not be investigated further due to lack of residual material) or due to a difference in the bioinformatics analysis in these laboratories. Further analysis identified that using a different bioinformatics tool (STAR-Fusion) (Supplemental Table S2) with the ability to align against a broader range of possible fusion targets enabled the detection of the *TPM3::NTRK1* and *LMNA::NTRK1* gene fusions in these two samples.

Hybridization Capture, Single Primer Extension, and Anchored Multiplex PCR Assays: TS, QMML, and FPL

Use of library methods that can select gene fusions for sequencing without prior knowledge of each partner gene or the specific fusion isoform can theoretically improve detection of rare or novel fusions. In the ring study, five laboratories used partner-agnostic methods (ie, able to detect *NTRK* fusions partners without prior knowledge of the partner gene), including two laboratories using hybridization capture libraries (TS, Illumina), two laboratories using anchored multiplex PCR libraries (FPL), and one laboratory using a single primer extension and amplification library (QMML, Qiagen). Both laboratories using the FPL panel detected all fusions.

For the two laboratories using the TS panel, an *ETV6::NTRK3* fusion in a papillary thyroid carcinoma (CTRK-20) was not detected, although a similar *ETV6::NTRK3* fusion in a salivary duct carcinoma sample (CTRK-23) was detected by both laboratories (Figure 1). It was also noted that the results of CTRK-20 testing by other

Samples	CTRK-02	CTRK-06	CTRK-07	CTRK-09	CTRK-12	CTRK-13	CTRK-14	CTRK-15	CTRK-18	CTRK-20	CTRK-23	CTRK-24	CTRK-28
Expected Fusion Result	*TPR::NTRK1*	*ALK::EML4*	*TPM3::NTRK1*	*LMNA::NTRK1*	*TPM3::NTRK1*	*ETV6::NTRK3*	*WNK2::NTRK2*	*STRN3::NTRK2*	*ROS1::CD74*	*ETV6::NTRK3*	*ETV6::NTRK3*	*ETV6::NTRK3*	*TPM3::NTRK1*

Lab:	Test Panel:
1	AmpliSeq Focus panel, Illumina
2	AmpliSeq Focus panel, Illumina
3	AmpliSeq Focus panel, Illumina
4	AmpliSeq Focus panel, Illumina
5	Oncomine Focus Assay, TF
6	Oncomine Focus Assay, TF
7*	Oncomine Comprehensive Assay v3, TF
8	Oncomine Comprehensive Assay v3, TF
9	Oncomine Comprehensive Assay v3, TF
10	Oncomine Comprehensive Assay v3, TF
11*	FusionPlex Lung, ArcherDX
12	FusionPlex Lung, ArcherDX
13	TruSight RNA Pan-Cancer, Illumina
14	TruSight RNA Pan-Cancer, Illumina
15	Oncomine Precision Assay, TF
16	QIAseq Multimodal Lung, Qiagen

Legend:
Fusion detected
Fusion not detected (inadequate assay design)
Fusion not detected (bioinformatic failure)
Fusion not detected (detected *NTRK1* fusion only by imbalance assay)
Fusion not detected (low fusion variant level)
Sample not tested
Inconclusive result

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Figure 1 The result from each laboratory for the 13 CANTRK (CTRK) samples in the ring study are shown. Expected results are shown as columns and the 16 unique laboratories as rows. Two control fusions are shown as white text on black (sample CTRK-06, *ALK::EML4*; CTRK-18, *ROS1::CD74*). As per the figure color key, the results are as follows: **green**, fusion detected; **gray**, sample not tested; **white**, inconclusive result; **red**, fusion not detected due to inadequate assay design; **blue**, fusion not detected due to bioinformatics failure; **purple**, fusion not detected but detected *NTRK1* fusion only by imbalance assay; and **yellow**, fusion not detected due to low variant level. TF, Thermo Fisher Scientific. *Indicates reference laboratories.

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laboratories using amplicon panels gave a low number of fusion reads, suggesting that the fusion in this sample was present at a low level that may not be detectable by the TS panel but that was detectable by other panel methods. To investigate, a sample swap was arranged in which one reference laboratory (University Health Network) that detected the *ETV6::NTRK3* fusion in CTRK-20 sent a sample of extracted RNA to the two laboratories using TS, who in return sent extracted RNA back to the reference laboratory for retesting. All three samples tested at the reference laboratory were confirmed to have reads supporting the presence of the *ETV6::NTRK3* fusion (albeit at very low read levels), and both laboratories using TS detected the *ETV6::NTRK3* fusion on RNA extracted at the reference laboratory (Supplemental Table S4). The sample exchange thus indicated that the TS panel was able to detect the *ETV6::NTRK3* fusion but that the low fusion level in the sample was challenging to detect by using the TS panel.

Discussion

There is an emerging group of targeted tumor-agnostic therapies in which drug selection is based on tumor molecular features found in a variety of tumor types and at various ages of cancer onset.[19] Since 2015, Health Canada has approved pembrolizumab in 19 tumor indications for patients with microsatellite instability-high or mismatch repair−deficient cancers. In 2019 and 2020, larotrectinib and entrectinib, respectively, were approved by Health Canada for the treatment of adult and pediatric patients with solid tumors carrying *NTRK* gene fusions. Because these tumor-agnostic molecular features are rare in certain cancer types, molecular methods that capture the most variants are ideal for clinical testing, thus providing drug eligibility to the largest group of patients. However, the rarity of these variants poses a problem for validation by clinical molecular laboratories, which require positive control material. This is particularly true for *NTRK* gene fusions, which have pathognomonic tumor sites where certain *NTRK* gene fusions are common (eg, *ETV6::NTRK3* fusions in salivary gland secretory carcinoma, secretory breast carcinoma, infantile fibrosarcoma, and congenital mesoblastic nephroma) yet other tumor sites where *NTRK1*, *NTRK2* or *NTRK3* fusions might exist but are very rare.[2,20−25]

NTRK gene fusions are also best detected by using RNA-based NGS, a methodology still emergent in Canadian clinical laboratories. Of note, no Canadian laboratories in this study used DNA-based NGS for detection of *NTRK* gene fusions. Compared with RNA-based NGS for detection of gene fusions, DNA-based NGS is more challenging due to the larger intronic regions that need to be effectively covered by the NGS panel and the lack of evidence of expressed gene fusions evident when using RNA-based NGS. For these reasons, DNA-based NGS reportedly has reduced sensitivity for gene fusion detection compared with RNA-based NGS due to false-negative results.[26,27]

Comparison of NGS testing for *NTRK* gene fusions in clinical laboratories in other countries, including Germany[28] and South Korea,[29] have shown that comprehensive detection of *NTRK* gene fusions is highly dependent on NGS library designs and on bioinformatics approaches for data analysis. Different diagnostic sensitivity is noted between NGS using amplicon libraries, which rely on knowledge of specific breakpoints with partner genes and thus may have reduced sensitivity for rare fusions, versus partner-agnostic library approaches (eg, hybrid capture, anchored multiplex PCR) in which prior knowledge of the fusion gene partner is not required.[30,31] A clinical laboratory external quality assessment scheme in Germany, Austria, and Switzerland, which assessed both fluorescent *in situ* hybridization and NGS by RNA sequencing in 27 centers, also showed detection challenges with certain NGS commercial panels, particularly amplicon panels in which rare targets were not included in design although with high sensitivity of 95.3% overall for RNA-based NGS analysis.[32]

Because health care delivery in Canada is funded and regulated provincially, decisions by clinical molecular diagnostic laboratories regarding specific laboratory technologies to implement for clinical testing are region specific and based on local expertise or equipment access, with minimal harmonization across the country. Although this diversity of methods is acceptable under a strong laboratory accreditation program that exists within Canada (eg, by Accreditation Canada Diagnostics) and includes molecular-specific requirements and external quality assessment participation, it remains valuable for Canadian laboratories to assess their own test approaches compared with others as a way of identifying test-specific issues or to confirm test equivalency.

Through the CANTRK study multicenter collaboration, tumor samples from multiple tumor sites with *NTRK1*, *NTRK2*, or *NTRK3* fusions, with six different fusion gene partners, were shared across 16 Canadian clinical laboratories from seven provinces, overcoming a major barrier of individual laboratories sourcing sufficient positive control samples for validation. Canadian laboratories in this study used seven different RNA-based NGS commercial panels for detection of *NTRK* fusions. The NGS panels that detected all fusions (11 *NTRK* fusions) included the FPL, TS, OPA, and QMML panels (all partner-agnostic, except for OPA, which is amplicon based), whereas the panel detecting the least fusions was ASIFP (6 of 11 *NTRK* fusions detected, amplicon based). Although certain NGS library methods can allow selection of fusions without *a priori* knowledge of the fusion partners, including three library methods used by participants in this study (TS, FPL, and QMML), these methods may require longer workflows and higher amounts of RNA. The TruSight hybridization capture library used in the CANTRK study encountered a challenge in detecting low levels of fusions on one sample

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by both laboratories using this panel, which may indicate a reduced sensitivity of this panel at low tumor or variant content. It has also been reported in a comparison of RNA-based NGS methods for *NTRK* gene fusion detection that the hybridization-capture NGS TruSight Oncology 500 assay (Illumina) also exhibited reduced sensitivity for low-level fusions variants.[30]

In this study, two issues came forward as having a larger impact within Canadian laboratories. The first was the lack of primers in commercial panel amplicon libraries (panels OCAv3, OFA, and ASIFP) for two *NTRK2* fusion partner genes (*WNK2* and *STRN3*). Although amplicon library methods can allow use of small amounts of RNA, by design, prior knowledge of both partner genes and the specific exons involved in the fusion are required. Although *NTRK2* fusions are the least frequent *NTRK* gene−related fusions (reported mainly in pediatric central nervous system tumors[21−25]), in the absence of frequent updates by the manufacturer to commercial NGS panel target gene content, laboratories can only increase their detection of rare fusions by moving to a library preparation with more isoform detection or switching to a fusion partner−agnostic method, all of which require extensive clinical validations. As shown in this study, the diversity of *NTRK* gene fusions leads to false-negative findings in testing in which fusions for rare or novel partner genes or isoforms are not detectable due to panel design, particularly if existing commercial panels are not updated by the manufacturer as new partner genes are identified (as is the case with the OFA and OCAv3 panels, compared with the more recent OPA panel from the same manufacturer). The findings are also supported by a recent *in silico* analysis of the bed files of current assays and coverage of various *NTRK* gene fusions[28] in which the OCAv3 and OFA panels had the lowest detection of *NTRK* fusions among nine NGS methods.

The second issue identified was bioinformatics analysis on data from the ASIFP panel. Because the manufacturer does not supply a custom bioinformatics analysis for this panel, laboratories must develop their own bioinformatics analysis. For three laboratories, their initial choice of bioinformatics analysis did not detect two *NTRK1* fusions in lung adenocarcinomas. However, one of these laboratories confirmed that these two fusions were identifiable by using an alternate fusion detection software (STAR-Fusion version 1.9.1[17]). This finding suggests that laboratories require appropriate control materials to optimize bioinformatics detection of rare events in their gene fusion detection panel data.

During the course of this Canada-wide study, several questions related to quality assurance for clinical laboratories using NGS for RNA sequencing for fusion gene testing were identified. These included the quality criteria to report a positive result for a fusion gene from RNA sequencing, the challenges for bioinformatics analysis, and the best practices for reporting gene fusions. The information gathered from this study will be used to formulate a best practice guideline for analysis and reporting of gene fusions detected by NGS methods.

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Author Contributions

M.S.T. and E.T. secured funding and designed the [Q13] CANTRK study; T.L.S. and B.L. performed reference laboratory analysis; and T.L.S. wrote the manuscript. T.L.S., B.L., A.B., A.G.C., J.D., P.D., H.F., D.G., W.G., C.H., W.Y.H., I.I., G.L., S.N.M.F., A.I.P., P.C.P., J.-B.R., B.S.S., A.S., E.S., D.T-T., S.Y., and T.Z. performed experiments and analyzed the data.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.12.004*.

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