Identification of NTRK gene fusions in lung adenocarcinomas in the Chinese population

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Abstract

The molecular profile of neurotrophic tyrosine kinase receptor (NTRK) gene fusions in lung adenocarcinoma (LUAD) is not fully understood. Next-generation sequencing (NGS) and pan–tyrosine kinase receptor (TRK) immunohistochemistry (IHC) are powerful tools for NTRK fusion detection. In this study, a total of 4,619 LUAD formalin-fixed, paraffin-embedded tissues were collected from patients who underwent biopsy or resection at the Shanghai Chest Hospital during 2017–2019. All specimens were screened for NTRK1 rearrangements using DNA-based NGS. Thereafter, the cases with NTRK1 rearrangements and cases negative for common driver mutations were analyzed for NTRK1/2/3 fusion events using total nucleic acid (TNA)-based NGS and pan-TRK IHC. Overall, four NTRK1/2 fusion events were identified, representing 0.087% of the original sample set. At the DNA level, seven NTRK1 rearrangements were identified, while only two TPM3–NTRK1 fusions were confirmed on DNA-based NGS as functional. In addition, two NTRK2 fusions (SQSTM1–NTRK2 and KIF5B–NTRK2) were identified by TNA-based NGS in 350 'pan-negative' cases. Two patients harboring NTRK1/2 fusions were diagnosed with invasive adenocarcinoma, while the other two were diagnosed with adenocarcinoma in situ and minimally invasive adenocarcinoma. All four samples with NTRK1 fusions were positive for the expression of pan-TRK. The two samples with NTRK2 fusions showed cytoplasmic staining alone, while the other two samples with NTRK1 fusions exhibited both cytoplasmic and membranous staining. In summary, functional NTRK fusions are found in early-stage LUAD; however, they are extremely rare. According to this study’s results, they are independent oncogenic drivers, mutually exclusive with other driver mutations. We demonstrated that NTRK rearrangement analysis using a DNA-based approach should be verified with an RNA-based assay.

Keywords: LUAD; NTRK; gene fusion; NGS; pan-TRK

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Introduction

The development of non-small-cell lung cancer (NSCLC) treatment has dramatically progressed since the introduction of epidermal growth factor receptor (EGFR) gene mutation and anaplastic lymphoma kinase (ALK) gene fusions [1,2]. Recently, the neurotrophic tyrosine kinase receptor (NTRK) gene, as an uncommon therapeutic target, has gained more attention. Generally, NTRK genes NTRK1, NTRK2, and NTRK3 encoding for proteins TRKA, TRKB, and TRKC, respectively, express and function in neuronal development, while gene fusion events have been identified in different types of cancer in both adults and children [3,4]. Tyrosine kinase receptor (TRK) inhibitors such as larotrectinib and entrectinib targeting NTRK fusion events have been recently developed and evaluated for treatment efficacy in patients of different ages with various types of cancer [5,6].

NTRK fusion-positive lung adenocarcinoma (LUAD) cases are rare, with an incidence of 0.1–3.3% depending on the analytical setup. Among the three aforementioned TRK genes, NTRK2 has been rarely reported [6–8]. Nevertheless, these genes represent a
novel ‘tissue-agnostic’ target, whose therapeutic inhibitors could be potentially applied for the treatment of a broad range of tissue malignancies in clinical trials.

Multiple approaches have been developed to identify NTRK fusion at the DNA, RNA, and protein levels. Fluorescence in situ hybridization (FISH) [7] with break-apart probes can be employed to detect the occurrence of gene fusions regardless of their fusion partners; however, the application of this approach is constrained by its poor throughput and resolution. Moreover, FISH provides no identity of fusion partners or RNA-level information for therapeutic guidance. In contrast, pan-TRK immunohistochemistry (IHC) has been applied for efficient and reliable NTRK fusion screening [9]. The sensitivity and specificity of pan-TRK IHC generally depend on the specific tumor type and gene; a high sensitivity is observed for NTRK1 and NTRK2 fusions, and a high specificity for common tumors (lung and colorectal cancers) [10]. Furthermore, next-generation sequencing (NGS) with different targeted gene enrichments can identify divergent genetic events during clinical diagnosis. Hybridization capture-based large DNA panels that simultaneously survey hundreds of genes are particularly powerful in identifying localized mutations; however, they fail to investigate complex genomic translocations with diverse partners and dispersed breakpoints. Amplification-based approaches that utilize sequence-specific primer pairs to enrich targeted regions are challenging; however, they can only detect gene fusions with known fusion partners. RNA-based anchored multiplex polymerase chain reaction (PCR) [11] is specifically tailored for detecting gene fusions through ligation-mediated amplification. Therefore, it is capable of identifying novel fusion events with unknown fusion partners or breakpoints.

To improve NTRK fusion detection in LUAD, we conducted this large-scale LUAD cohort study on NTRK fusions. Gene fusion events were identified using NGS at both DNA and RNA levels. Meanwhile, pan-TRK IHC was also employed for protein-level gene fusion identification.

**Materials and methods**

**Patient enrollment and sample collection**

A total of 4,619 consecutive formalin-fixed, paraffin-embedded (FFPE) LUAD tissues were collected from patients who underwent biopsy or resection at the Shanghai Chest Hospital during 2017–2019 (Figure 1). These samples consisted of 2,651 surgical specimens and 1,968 small biopsies or cell blocks (703 transbronchial biopsies [TBBs], 442 endobronchial ultrasound transbronchial needle aspirations [TBNA], and 823 pleural effusion cell blocks).

The age, sex, specimen types, and histotypes of the 4,619 patients who provided specimens are shown in Table 1. The histotypes were classified according to the 2015 World Health Organization classification scheme for lung carcinoma.

All procedures involving human participants were performed as per the ethical standards of the National Research Committee and the 1964 Helsinki Declaration (including its follow-up amendments and comparable ethical standards). Informed consent was obtained from all the enrolled participants. This study was approved by the Ethics Committee of Shanghai Chest Hospital (#IS2001).

**Extraction of DNA/RNA**

Genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions. Quantification of the DNA was performed with the Qubit 3.0 dsDNA assay (Life Technologies, Carlsbad, CA, USA).
USA). The total nucleic acids (TNAs) for the RNA- and DNA-based NGS were prepared differently (see the following subsection ‘Unified RNA and DNA NGS based on PANO-Seq’).

DNA hybridization capture-based NGS

DNA fragmentation was performed using Covaris M220 (Covaris Inc., Woburn, MA, USA), followed by end repair, phosphorylation, and adaptor ligation. Fragments measuring 200–400 bp in length were selected for further hybridization using capturing probes, magnetic bead enrichment, and PCR amplification.

All samples were subjected to a DNA hybridization capture-based NGS assay as a routine test. The assay is a 68-gene panel covering NTRK1/2/3 for all exons and selected introns (Burnin Rock Biotech Ltd, Guangzhou, PR China; see supplementary material, Table S1) and is capable of detecting the rearrangement of NTRK1. DNA quality and size distribution were assessed using a bioanalyzer. All indexed libraries were sequenced using the NextSeq 550 sequencer (Illumina Inc., San Diego, CA, USA) with paired-end sequencing.

The sequence data were mapped to the human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization, variant calling, and annotation were performed with GATK 3.2, MuTect, and VarScan, respectively. The variants were filtered using the VarScan filter pipeline, with loci with depths <200 filtered out, and then annotated using ANNOVAR and SnpEff v4.3. DNA genomic rearrangement analysis was performed using Factera 1.4.3.

Table 1. Relationships between NTRK fusions and clinicopathological factors.

| Factor | Total | NTRK fusion |
|--------|-------|-------------|
| Age (years) |       |             |
| Mean     | 58.6  | 39.25       |
| Median   | 61    | 37.5        |
| Range    | 17–88 | 31–51       |
| Sex      |       |             |
| Male     | 2,439 | 2           |
| Female   | 2,180 | 2           |
| Specimen type | |             |
| Surgical | 2,651 | 4           |
| Biopsies/cell blocks | 1,968 | 0           |
| Histotype |       |             |
| AIS      | 247   | 1           |
| MIA      | 412   | 1           |
| IA       | 1,992 | 2           |

AIS, adenocarcinoma in situ; IA, invasive adenocarcinoma; MIA, minimally invasive invasive adenocarcinoma.

Thereafter, 357 samples were analyzed using TNA-based NGS and pan-TRK IHC, including the cases that carried NTRK1 rearrangements (seven cases, see Results section). Generally, driver gene mutations occur in LUAD in a mutually exclusive manner. Therefore, for further testing, we selected 350 ‘pan-negative’ samples that did not have common driver gene mutations. The strategy employed to detect NTRK fusions is illustrated in Figure 1.

Unified RNA and DNA NGS based on PANO-Seq

The selected cases were analyzed with parallel amplification numerically optimized sequencing (PANO-Seq) [12], using a modified on-shelf product (panel #022T; HeliTec Biotechnologies, Shenzhen, PR China) with atypical NTRK1/2/3 primers spiked in the multiplexed primer pools. The panel can identify all functional fusion events in multiple common genes and genetic variations in all National Comprehensive Cancer Network (NCCN)-specified biomarkers (see supplementary material, Table S2). To perform this assay, TNA was first extracted from FFPE samples using a PANO-Pure FFPE TNA extraction kit (HeliTec Biotechnologies) and quantified using the Qubit 3.0 dsDNA assay (Life Technologies). Sample quality was determined as pass if DNA was >10 ng/μl and RNA was >25 ng/μl. Then, 50 ng of TNA input was used for library construction. This single-tube library construction protocol used DNA for single nucleotide variant and indel detection, and RNA for fusion detection, as well as tiled intronic primers for DNA-based fusion detection when transcripts were unavailable. The reactions from extraction to sequencing were performed in a single tube as a unified library, without experimentally separating DNA or RNA. Sequencing was performed on an Illumina HiSeq X10 platform with PE150+PE7 (8) setting. Raw sequencing data were analyzed using the proprietary PANO-Call ver. 18.12 bioinformatics pipeline for both mutation and fusion calls (HeliTec Biotechnologies).

Pan-TRK IHC

The Ventana pan-TRK (EPR17341) assay (Ventana Medical Systems Inc., Tucson, AZ, USA), in combination with the OptiView DAB IHC Detection Kit (Ventana Medical Systems), was used on the Ventana BenchMark XT automated slide stainer for the detection of TRK A, B, and C. Cortical brain tissue was used as the positive control, while alveolar epithelium was used as a negative internal control. The test results were interpreted by well-trained senior pathologists.
IHC staining was graded according to the percentage of stained tumor cells (0, 0–49, 50–79, or 80–100%) and staining intensity (0, no staining; +, weak; ++, moderate; +++, strong). The staining pattern (cytoplasmic, nuclear, and/or membranous) was also identified. The optimal IHC cut-off was determined according to the NGS and IHC results.

Results

DNA capture-based NGS

In total, NTRK1 gene rearrangements were identified in seven (0.15%, 7/4,619) patients (cases 1–7; Table 2). Two of these seven NTRK1 rearrangements (cases 6 and 7) carried both an NTRK1 kinase domain and a 5’ portion of TPM3, indicating constitutive transcriptional functions. In contrast, the other five (cases 1–5) carried noncanonical rearrangements, which were without either an NTRK1 kinase domain or a 5’ portion of a partner gene. Moreover, the two samples carrying canonical TPM3-NTRK1 rearrangements had no detectable concurrent driver mutations, while three of the other five samples carried activating EGFR or KRAS mutations.

Notably, case 5, who had received EGFR-tyrosine kinase inhibitor (TKI) treatment, was NTRK1 rearrangement-negative before therapy. Therefore, the noncanonical NTRK1-FMN2 rearrangement might have been introduced by EGFR-TKI treatment.

Overall, 90.5% (4,180/4,619) of the adenocarcinoma samples possessed known activating mutations of EGFR (2,878, 62.31%), ALK (202, 4.37%), ROS1 (77, 1.67%), KRAS (472, 10.22%), BRAF (103, 2.23%), RET (86, 1.86%), MET (227, 4.91%), or ERBB2 (135, 2.92%). In addition to the seven NTRK1-rearranged samples, another 432 samples were pan-negative without the aforementioned driver gene mutations.

Parallel amplification numerically optimized sequencing

A total of 357 samples, including 350 pan-negative adenocarcinoma samples and the seven aforementioned NTRK1 rearranged samples, were selected for PANO-Seq, a concurrent dual-template NGS assay, to confirm the results of DNA-based NGS. The TPM3-NTRK1 chimeric transcripts of cases 6 and 7 consisted of exon 8 of TPM3 at the 5’ end and NTRK1 exon 10 at the 3’ end at the RNA level, reflecting the partial excision of NTRK1 exon 9 during splicing in case 6. The other five samples that carried noncanonical NTRK1 rearrangements were found to have no chimeric NTRK1 transcripts. In contrast, a KIF5B-RET fusion was found at the RNA level in case 1; this was not detected using the former DNA-based NGS assay.

Furthermore, two NTRK2 fusions were detected at the RNA level (Table 2). One of them, SQSTM1-NTRK2, was in an invasive adenocarcinoma sample, and the other KIF5B-NTRK2 in a minimally invasive adenocarcinoma (MIA) sample. No NTRK3 fusions were found in these samples.

Pan-TRK IHC

Pan-TRK IHC testing was further performed on the 357 aforementioned samples to examine TRK A, B, and C expression. The IHC results are summarized in Table 3. Weak, moderate, or strong cytoplasmic staining was observed in 13 cases, and 2 of these also had moderate membranous staining. All staining was observed in 80–100% of tumor cells. The four NTRK fusion cases detected using TNA-based NGS had moderate to strong cytoplasmic staining, and two of them also had moderate membranous staining. Therefore, the optimal cut-off was moderate to strong cytoplasmic staining, and two of them also had moderate membranous staining. The cells with NTRK2 fusions showed cytoplasmic staining only, while those with NTRK1 fusions were positive for both cytoplasmic and membranous staining (Figure 2).

Discussion

In this study, we identified four NTRK gene fusions in 4,619 LUAD samples (0.087%). Two of them were TPM3-NTRK1 fusions that were identified using DNA- and RNA-based NGS and confirmed using pan-TRK IHC. The other two were SQSTM1-NTRK2 and KIF5B-NTRK2 fusions, which were detected using RNA-based NGS and confirmed using pan-TRK IHC. However, we identified two NTRK fusions, TPM3-NTRK1 (in adenocarcinoma in situ [AIS]) and KIF5B-NTRK2 (in MIA) in the early stage of adenocarcinoma. This finding was different from previous studies that identified such fusion events in advanced adenocarcinoma, and preferentially in mucinous carcinoma or poorly differentiated cells [6,8,11]. Our findings thus extend the understanding of pathological features of NTRK gene fusions in LUAD. In addition, to the best of our knowledge, NTRK fusions in NSCLC are dominated by NTRK1 and NTRK3 fusions, with only one...
Table 2. NTRK fusion detected using NGS at DNA and RNA levels and pan-TRK IHC at the protein level.

| Case no. | Sex | Age (years) | Smoking (pack-years) | Pathological stage | Specimen type | Histotype | Tumor size (cm) | DNA rearrangement | RNA fusion | Pan-TRK | Other drivers |
|----------|-----|-------------|----------------------|--------------------|---------------|-----------|----------------|------------------|-------------|---------|---------------|
| 1        | M   | 53          | 0                    | IA                 | Small biopsy  | ADC       | 2.7            | C14orf2-NTRK1    | KIF5B-RET   | −       |              |
|          |     |             |                      |                    |               |           |                | (intergenic: intron 11) | (exon 15–exon 12) |           |           |
| 2        | M   | 41          | 0                    | IA                 | Surgical      | PPA       | 1.8            | RRNAD1–NTRK1     | −           | −       | EGFR          |
|          |     |             |                      |                    |               |           |                | (UTR3–exon 15)   |             |          | L858R         |
| 3        | F   | 64          | 0                    | IA                 | Surgical      | APA       | 1.5            | NTRK1–NBPF25P    | −           | −       | −             |
|          |     |             |                      |                    |               |           |                | (intron 8–intergenic) |             |          |               |
| 4        | M   | 54          | 30                   | IV                 | Small biopsy  | ADC       | 2              | NTRK1–ARHGEF11   | −           | −       | KRAS          |
|          |     |             |                      |                    |               |           |                | (exon 17–intron 1) |             |          | G12A          |
| 5        | F   | 56          | 0                    | IA                 | Small biopsy  | ADC       | 1              | NTRK1–FMN2       | −           | −       | EGFR          |
|          |     |             |                      |                    |               |           |                | (intron 11–intron 16) |             |          | 19del         |
| 6        | F   | 31          | 0                    | IV                 | Surgical      | AIS       | 0.8            | TPM3–NTRK1       | −           | + Cytoplasmic |
|          |     |             |                      |                    |               |           |                | (intron 8–exon 9) |             |          |               |
| 7        | M   | 51          | 0                    | IA                 | Surgical      | PPA       | 1.5            | TPM3–NTRK1       | −           | + Cytoplasmic |
|          |     |             |                      |                    |               |           |                | (intron 8–exon 10) |             |          |               |
| 8        | F   | 39          | 0                    | IIIA               | Surgical      | APA       | 2.5            | −               | SQSTM1–NTRK2     | + Cytoplasmic |
|          |     |             |                      |                    |               |           |                | (exon 4–exon 15) |             |          | membranous    |
| 9        | M   | 36          | 0                    | IA                 | Surgical      | MIA       | 1.3            | −               | KIF5B–NTRK2      | + Cytoplasmic |
|          |     |             |                      |                    |               |           |                | (exon 24–exon 15) |             |          | membranous    |

One pack-year is equal to smoking 20 cigarettes (1 pack) per day for 1 year.

ADC, adenocarcinoma; AIS, adenocarcinoma in situ; APA, acinar-predominant adenocarcinoma; F, female; M, male; MIA, minimally invasive adenocarcinoma; PPA, papillary-predominant invasive adenocarcinoma
Summary of pan-TRK IHC staining results.

| Subcellular Localization | Total | 0  | 1+ | 2+ | 3+ |
|--------------------------|-------|----|----|----|----|
| Cytoplasmic              | 13*   | 344| 9  | 3  | 1  |
| Membranous               | 2*    | 355| 0  | 2  | 0  |
| Nuclear                  | 0     | 357| 0  | 0  | 0  |

*Percentage of all tumor cells with staining = 80–100%.

In this study, we identified both cytoplasmic and membranous staining patterns for the two TPM3-NTRK1 fusions, while SQSTM1-NTRK2 and KIF5B-NTRK2 only exhibited cytoplasmic staining, which is partially attributed to the characteristics of the fusion partners. TPM3 encodes tropomyosins that participate in cytoskeleton formation in nonmuscle cells and localizes to the cell membrane. SQSTM1 encodes sequestosome-1, an autophagosome cargo protein localized to the cytoplasm. KIF5B encodes kinesin, which is required for normal mitochondria and lysosome distribution, and localizes to the cytoplasm. In addition, we found that the staining intensities of TPM3-NTRK1 fusions differed in different LUAD histotypes in this study and were different from that of the same fusion type in colorectal carcinoma [3]. Moreover, the staining pattern of the SQSTM1-NTRK2 fusion also differed in this study and a previous report [3]. Hence, it is suggested that the staining patterns of pan-TRK were simultaneously influenced by various factors, such as fusion partners, tumor type, and cancer histotypes.

Among the seven cases of NTRK1 rearrangement detected using DNA-based NGS, only two TPM3-NTRK1 fusions were supported by clear RNA-template evidence using TNA-based NGS, including in-frame exonic breakpoint and strong expression. Others are likely nonfunctional owing to a disrupted kinase domain and lack of RNA-level support. Driver gene mutations (KIF5B-RET fusion, EGFR L858R mutation, KRAS G12A mutation, and EGFR exon 19 deletion) with clear functional and clinical significance coexisted in four patients that carried noncanonical rearrangements, further implying that these non-canonical NTRK rearrangements are unlikely to be oncogenic. Furthermore, TNA-based NGS found two NTRK2 fusions, which were difficult to detect using DNA-based NGS because of the difficulty in tiling entire introns. Meanwhile, DNA-based NGS failed to identify canonical KIF5B-RET fusion events in case 1, which further highlights the importance of RNA-based approaches for gene fusion detection.

In general, fusion events involve DNA-, RNA-, and protein-level changes. At the DNA level, gene rearrangements may be induced by genomic insertion, deletion, inversion, or amplification, and can be either intra- or inter-chromosomal [17,18]. For example, inverted translocation (in case 6) can potentially generate reciprocal gene rearrangements (Figure 3A), which may complicate the results of DNA-based NGS.
Figure 2. Histotypes of the four samples harboring NTRK fusions and the staining patterns of their corresponding pan-TRK IHC results. (A) AIS, TPM3-NTRK1, moderate cytoplasmic staining (2+). (B) Papillary-predominant invasive adenocarcinoma, TPM3-NTRK1, strong cytoplasmic staining (3+). (C) Acinar-predominant adenocarcinoma, SQSTM1-NTRK2, moderate cytoplasmic and membranous staining (2+). (D) MMIA, KIF5B-NTRK2, moderate cytoplasmic and membranous staining (2+).
Figure 3. Integrative Genomics Viewer screenshot of NTRK genes and corresponding fusion partners detected using NGS. (A) TPM3-NTRK1 rearrangement in case 6 detected using DNA-based NGS with visible bidirectional chimeric reads. (B) TPM3-NTRK1 fusion in case 6 detected using RNA-based NGS. (C) TPM3-NTRK1 fusion in case 7 detected using RNA-based NGS. (D) SQSTM1-NTRK2 fusion in case 8 detected using RNA-based NGS. (E) KIF5B-NTRK2 fusion in case 9 detected using RNA-based NGS.
Genomic alterations and transcriptional abnormalities are not always identical. Previously, EML4-ALK transcripts were identified at the RNA level in four patients harboring only nonfunctional ALK rearrangements [13], which might be formed through uncommon or unknown mechanisms [19]. At the protein level, IHC-positive results can be attributed to either gene fusion events [20] or other forms of abnormalities such as ALK alternative transcription initiation, which generates oncoproteins with kinase activities [21]. Considering the aforementioned discrepancy among genetic information, transcriptional changes, and protein functions, a combined application of DNA-, RNA-, and protein-level methods are recommended for identifying gene fusion events.

Many studies have indicated a similar testing algorithm for NTRK fusion in solid cancers; this algorithm suggested a combined strategy using IHC and NGS assays for detecting NTRK fusion in lung cancer [22–26]. For example, the European Society for Medical Oncology Translational Research and Precision Medicine Working Group recommended using targeted NGS panels (either DNA- or RNA-based) to detect NTRK fusions and confirming the positive samples using pan-TRK IHC; a reverse strategy can be adopted as well, if no frontline NGS platform is available [22]. Based on our findings, we recommend detecting NTRK fusions in LUAD FFPE samples using DNA-based NGS and identifying the nonfunctional rearrangements using RNA-based NGS or IHC. Furthermore, RNA-based approaches, followed by IHC confirmation with corresponding antibodies, or vice versa, are recommended for LUADs without common driver gene mutations. Although similar studies have been recently published [27,28], more comprehensive investigations remain essential to examine the efficiency of the recommended detection approaches.

We also performed pan-TRK IHC on 60 pan-negative lung squamous cell carcinoma cases without the aforementioned driver mutations. Four cases (6.67%, 4/60) had diffuse moderate (2+) cytoplasmic staining, which is identified as ‘pan-TRK-positive’ using the above cut-off in LUAD. Three other cases (5.0%, 3/60) had diffuse weak (1+) cytoplasmic staining. All seven of these cases were investigated using TNA-based NGS, but negative results were obtained. Therefore, pan-TRK expression in lung squamous cell carcinoma should be treated more cautiously; it tends to be negative for NTRK fusion.

In addition, among the 432 pan-negative adenocarcinoma samples, 198 were surgical tissues and 234 were small biopsies or cell blocks (83 TBB samples, 55 TBNA samples, and 96 pleural effusion cell blocks). All surgical samples had sufficient tissue to be detected using PANO-Seq and pan-TRK IHC, while 35% (82/234) of small biopsy/cell block samples had insufficient tissue for further testing. Therefore, for small specimens without enough tissue for multiple testing, an RNA- or TNA-based NGS assay should be used for fusion detection.

In conclusion, functional NTRK fusions are extremely rare in LUAD. NTRK fusions may occur at the early stage of LUAD, such as in AIS and MIA. Functional NTRK fusion is an independent driver that occurs in a mutually exclusive manner with other driver mutations. Moreover, we recommend detecting NTRK rearrangements using DNA-based NGS, followed by RNA-based verification, while pan-TRK IHC is another applicable approach for NTRK fusion detection in LUAD. IHC results with pan-TRK expression should be validated using NGS assays.

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Author contributions statement

RZ and FY were involved in study design, data analysis, data interpretation, and writing of the manuscript. CX, JZ, ZS, LG, WD, SM, AY, JS and LZ carried out the experiments and collected and analyzed the data. YH was involved in study conception and design, pathology assessment, and article revision. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.
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SUPPLEMENTARY MATERIAL ONLINE

Table S1. Gene list of DNA hybridization capture-based NGS

Table S2. Gene list of the PANO-Seq