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Neurotrophic Tyrosine receptor Kinase (*NTRK*) gene fusion in CNS tumours: A diagnostic key for a novel targeted therapy

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The identification of dominant oncogenic mutations with the capability to inhibit their genetic aberrations by specific targeted inhibitors have improved the strategic approach of human cancer diagnosis and management, particularly in those patients who previously received ineffective conventional therapies. Neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion is one of the newly detected gene mutations at significant frequencies in several solid body tumors and was rarely identified in central nervous system (CNS) tumours. *NTRK*-gene is a main member of three different genetic subtypes (*NTRK1-3*), which produce tyrosine kinase proteins (Trk-A, B, and C). The protein receptor expression (Pan-Trk) can generally be detected by immunohistochemistry (IHC) technique however, its confirmation always requires advanced molecular methodologies. Although Pan-Trk immuno staining has been used as an efficient assesing tool for most of non-CNS cancers to detect *NTRK*-fusion, its utilization in CNS tumour is questionable due to the normal physiological presence of TK receptors in CNS neuropil. This diagnostic pitfall is important as the detection of *NTRK*-fusion may reflect patient response to Trk-inhibitor. In this review, we described the relationship between *NTRK*-fusions and Pan-Trk protein in CNS and non-CNS tumours, aided with the previous studies in the literature.

Keywords: CNS tumours, *NTRK*-fusion, Pan-Trk, immunohistochemistry

INTRODUCTION

Over the last few years, the identification of oncogenic mutations and the ability to specifically suppress these genetic abnormalities with targeted inhibitors have modified the treatment approach of many cancer patients **(**Vaishnavi et al. 2015**).** Clinical trials have been shifted away from histological subtype models to become more focused on basket trials that were planned to test targeted therapies for specific molecular mechanisms. These molecular mechanisms included chromosomal, genomic, epigenetic, and transcriptomic aberrations, that identify mutant genes and core pathways in different types of body tumours, which also played significant roles in the molecular classification of CNS tumour **(**Weller et al. 2021**).** One of these common molecular changes is the gene fusion. A fused gene is a hybrid gene created by combining two previously unrelated independent genes after structural rearrangements in form of translocation, duplication, interstitial deletion, or chromosomal inversion. The fused genes produce active aberrant proteins which can promote tumorigenesis. Several techniques have been used to detect gene fusions in body tumours which included immunohistochemistry (IHC), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), Fluorescent in-situ Hybridization (FISH), and Loss of Heterozygosity (LOH) analysis which all can provide molecular information. High-throughput Next Generation Sequencing (NGS) is a recent advanced technique where multiple mutations can be detected in a single analysis **(**Priesterbach-Ackley et al. 2018; Capper et al. 2018**)**.

Despite the improved understanding of the molecular subtypes of CNS tumours and the underlying alterations in

different signaling pathways, these observations have failed to result in successful targeted therapies, as has occurred in non-CNS tumors. Although radiotherapy is so far the best treatment modality for CNS tumours, to date, few targeted therapies have been approved by the Food and Drug Administration (FDA) of United States, which targets specific receptors in CNS tumours **(**Chen et al. 2017**)**. Despite the scarcity, *NTRK*-fusions recently gained additional care because of the impressive results achieved through their therapeutic targeting receptors. Successful TK-inhibitor (larotrectinib) received fast track approval from FDA and, as a result, the standard of care currently requires precise identification of patients benefit from this challenging therapy **(**Solomon et al. 2019**;** Elfving et al. 2021**).** Since *NTRK*-fusions have been discovered at substantial frequencies in some non-CNS tumors, their detection is a rail stay in the diagnostic assessment of these tumors, and specific expertise in this era would become required.

NTRK was first identified as an oncogene in 1982 by Barbacid et al **(**Pulciani et al. 1982**)**. *NTRK*-gene is a major member of three genetic phenotypes (*NTRK1-3*), which produce tyrosine kinase proteins (Trk-A, B, C) **(**Vaishnavi et al. 2015; Amatu et al. 2016; Hechtman et al. 2017; Lange and Lo, 2018; Hsiao et al. 2019; Märkl et al. 2019; Solomon et al. 2019; Torre et al. 2020**)**. These TK receptors are normally expressed in CNS tissue which play an essential role in brain development, cellular adaptation, and plasticity **(**Torre et al. 2020**)**. They have a parallel composition; each one resides an extracellular binding domain, a transmembrane region, and an intracellular domain. Trk-A has the greatest affinity for neurotrophin nerve growth factor, Trk-B for neurotrophic factor and neurotrophin-4 while Trk-C has an affinity to neurotrophin-3 **(**Solomon et al. 2019**)**. Normally, the ligand that binds to the extracellular region activates the kinase domain of the Trk receptor, resulting in homodimerization, phosphorylation, and signaling cascade activation **(**Hsiao et al. 2019**) [Figure 1]**.

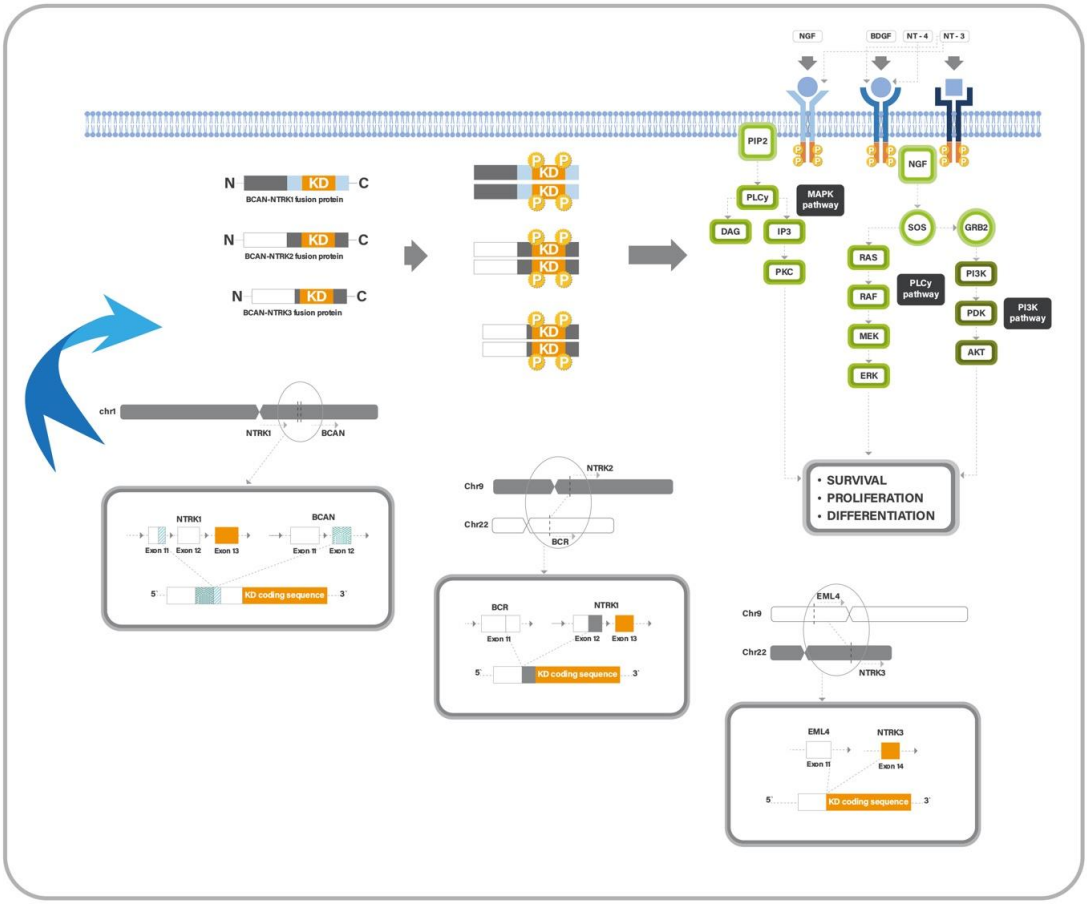

Figure 1: A diagram describing the NTRK gene and their receptors.

Rearrangements in the *NTRK* gene may lead to two genes fusions at the C-terminal and linked with N-terminal fusional partner to make altered Trk proteins. The fusion may cause uncontrolled proliferation of the cells (Solomon et al. 2019). This process is considered a part of oncogenic drive pathway which leads to an abnormal Trk receptor dimerization, upregulation and apoptosis

resistance (Torre et al. 2020).

Tumor entities with *NTRK*-fusions can be clustered into two groups at which levels these fusions are detected. The first group includes tumors that are extremely rare and are identified by a specific *NTRK*-fusion, which is generally diagnosed in these cases. The second category includes a variety of tumors that are more common, but rarely include *NTRK* fusions (Märkl et al. 2019). *NTRK*-fusions were detected in low frequencies in both solid body tumours and CNS tumours. This detection was performed based on both molecular and non-molecular techniques.

In this review, we describe in details the common solid body tumours and CNS tumours detected with *NTRK*-fusions. We also compare the efficacy of different detection technique used in the previous studies and how these methodologies affect the *NTRK*-fusions detection in CNS tumours.

DISCUSSION

The strategic approach to develop targeted therapies for specific oncogenic driver-causing cancers was conventionally based on tissue diagnosis. This strategy approved multiple monoclonal immunotherapies for the treatment of patients with different cancer types or, more commonly, a variant that anchorages several molecular alterations. With advances in sequencing technology, new and known oncogenic drivers continue to be explored across a variety of tumours. One of these discovered oncogenic drivers is *NTRK*-gene rearrangement. The abundant isoform of Trk is expressed in CNS tissues and is efficiently activated by neurotrophin. The gene-expression itself is largely transcribed in the adult CNS tissues, as well as during embryonic development **(**Barbacid et al. 1991**)**. Loss of function in this gene may lead to neurodevelopmental disorders; conversely, activating alterations harbor oncogenic prospective, stimulating cellular proliferation and tumorigenesis. Because of the substantial role of *NTRK* in the CNS development, the level of expression has been extensively studied in tumoural and non-tumoral CNS conditions **(**Farhang et al. 2014**)**.

The prevalence of *NTRK*-fusion in all body tumours is less than 2%, approximately around 1500-5000 children and adults annually **(**Amatu et al. 2016**)**. Although *NTRK*-fusions have been detected in a common tumour, they had also been identified in other rare types cancers **(**Hsiao et al. 2019**)**. Around eighty different *NTRK*-gene fusion partners in different tumour types were discovered **(**Hsiao et al. 2019**)**. The first *NTRK*-fusion protein was initially reported in a colorectal adenocarcinoma cell line **(**Solomon et al. 2020**).** *NTRK*-fusions were also detected in broad range of adult solid tumours including salivary gland cancer, thyroid carcinoma, breast adenocarcinomas, non-small cell carcinoma of lung, and cancers affecting gynecological organs, and rarely soft tissue sarcomas, melanoma, and acute myeloid leukemia **(**Tomasson et al. 2008; Geiger et al. 2011; Harada et al. 2011; Miranda et al. 2014; Amatu et al. 2016**;** Lange and Lo, 2018**).** In pediatric-age groups, *NTRK*- usions were identified in rare cases of diffuse CNS gliomas, melanoma, sarcomas, myofibroblastic tumours, and congenital fibrosarcoma (Lange and Lo, 2018). Less than 2% of all neuroepithelial tumours contain *NTRK*-fusions (Torre et al. 2020).

Cocco et al. suggested to divide the *NTRK*-fusions group, which was raised by Markel et al. into two subgroups with approximated rates of 25% and less than 5%. Melanomas, gastrointestinal stroma tumors and thyroid cancers are all included in the first sub-group. Colorectal cancers and non-small cell lung cancers (NSCLCs) have been detected in a wide range of tumors with a frequency less than 5% (Cocco et al. 2018; Märkl et al. 2019; Gambella et al. 2020). Cocco et al. also reported *ETV6–NTRK3* fusion in more than 90% of cases of mammary salivary gland cancers, mesoblastic nephroma and breast adenocarcinoma (Cocco et al. 2018). The same fusion has also been found in cases of secretory carcinoma of the skin, thyroid gland cancers, and infantile fibrosarcoma (Bourgeois et al. 2000; Sheng et al. 2001; Wong et al. 2015; Amin et al. 2016; Dogan et al. 2016; Church et al. 2018). Agaram et al reported *TPM3-NTRK1* fusion in lipofibromatosis-like neural tumor with a very high frequency of *TPR*- and *TPM3-NTRK1* fusions (Agaram et al. 2016). Moreover, two cases published by Haller et al were found to have similar clinicopathological features of pediatric spindle cell tumors with *LMNA-TRK1* fusion (Haller et al. 2016). In 2018, a study done by Okamura et al have assessed *NTRK*-fusions prevalence in 9,966 adult tumors and 3,501 pediatric tumours from the Cancer Genome Atlas. *NTRK3*-fusions were found in 0.16% of adult tumors and *NTRK1* in less than 0.2% of pediatric tumors. They also reported that 93% of *NTRK*-fusions in the literature was *ETV6-NTRK3* in salivary gland cancers. About 92% of cases were found to have *ETV6-NTRK3* fusion in breast carcinoma while 91% of *ETV6-NTRK3* fusion were found in congenital fibrosarcoma. On the other hand, around 40% of fusions in *NTRK1-3* were found in pediatric high-grade gliomas (in those age < 3 years) (Okamura et al. 2018). *EML4-NTRK3* and *LMNA-NTRK1* fusions have also been described in other types of infantile fibrosarcoma (Hsiao et al. 2019). In a large study done by Hsiao et al in 2019 included 390 adult tumours with predominant 373 gliomas, the *NTRK*-fusions were identified in 2% of the tumors while the *NTRK2*-fusions were identified in 6 tumors (Hsiao et al. 2019). Elfving et al (2021) reported 359 out of 617 lung adenocarcinomas with *NTRK*-fusions (Elfving et al. 2021).

The prevalence of *NTRK*-fusions in CNS tumours has been estimated to be less than 5% of all body tumours (Gatalica et al. 2019) [Table 1]. Although high-grade gliomas are rarely reported with *NTRK*-fusions, around 11% of glioblastomas showed to have *NTRK2*-fusions, 1% *NTRK1*-fusions and only single case have reported

glioblastoma with *NTRK3*-fusions **(**Zheng et al. 2014; Schram et al. 2017; Cocco et al. 2018; Ferguson et al. 2018; Gatalica et al. 2019; Torre et al. 2020; Mohamed et al. 2022**)**. Recently, Mohamed et al (2022) detected two glial tumours (glioblastoma, liponeurocytoma) cases with *NTRK2*-fusions (*SLCO5A1-NTRK2, AGBL4-NTRK2, BEND5-NTRK2*) **(**Mohamed et al. 2022**)**. In 2020. Torre et al. reported in their study that *NTRK*-fusions is prevalent in 86% of adult gliomas and 46.2% in pediatric gliomas or pediatric CNS tumours of uncertain grading. About 69% of pediatric gliomas had *NTRK2*-fusions while 68% of adult gliomas were found to have *NTRK1*-fusions **(**Torre et al. 2020**)**. Gambella et al. also reported that *NTRK* fusions have been found in both low-grade and high-grade pediatric gliomas. *NTRK2* seems to be the most commonly involved gene followed by *NTRK1* fusions **(**Gambella et al. 2020**)**. In 2019, Gatalica et al. reported some cases of glioblastoma with *NTRK2*-fusions (*GKAP-NTRK2, TBCID2-NTRK2, KCTD8-NTRK2*) **(**Gatalica et al. 2019**)**. Okamura et al (2018) reported in their study of unselected cohorts about 4% of pediatric gliomas have *NTRK*-fusions **(**Okamura et al. 2018**)**. Indeed, supratentorial PA was the most common reported tumours (Okamura et al. 2018). In 2017, Hechtman et al. explored 4 cases of glioblastomas with *NTRK*-fusions (Hechtman et al. 2017). Before 2017, Wu et al were the only team who reported high frequency of *NTRK*-fusions [*ETV6-NTRK3 and TPM3-NTRK1*] in up to 40% of children less than 3 years old (Wu et al. 2014). There were no reported cases of ependymoma, schwannoma, meningioma, neurocytoma, or medulloblastoma with *NTRK*-fusions (Grotzer et al. 2000; Nobusawa et al. 2014).

Due to the efficacy of FDA-approved *NTRK*-targeted therapy in cancers outside CNS, it was very essential to find the best accurate technique to detect patients with *NTRK*-fusion **(**Torre et al. 2020**)**. Several approaches may be used to detect the existence of *NTRK*- fusions in tissue samples. Selecting the optimum approach requires clinical experience, turnaround time and the cost of the test should also be considered **(**Hsiao et al. 2019) (Figure 2).

Figure 2: Different approach to detect NTRK-fusions in body tumours

Table 1: Different types of CNS gliomas with *NTRK*-fusions based on literature review in the period between 2014-2022

Tumor Type	*NTRK* gene	Fusion Partner	References
	NTRK2	AGBL4	Mohamed et al. 2022
	NTRK1	AFAP1	Schram et al. 2017
		ARHGEF2	Zheng et al. 2014
		CHTOP	Zheng et al. 2014
		NFASC	Kim et al. 2014, Drilon et al. 2017
	NTRK2	GKAP	Gatalica et al. 2019
		KCTD8	Ferguson et al. 2018, Gatalica et al. 2019
		TBC1D2	Gatalica et al. 2019
	NTRK3	AFAP1	Hechtman et al. 2017
		ZNF710	Hechtman et al. 2017
		EML4	Ferguson et al. 2018
Non-brainstem high-grade glioma	*NTRK1*	TPM3	Wu et al. 2014
	NTRK3	ETV6	Wu et al. 2014
		BTBD1	Wu et al. 2014
Diffuse intrinsic pontine glioma (DIPG)	*NTRK2*	VCL	Wu et al. 2014
		AGBL4	Wu et al. 2014
Pilocytic astrocytoma	*NTRK1*	BCAN	Ferguson et al. 2018
	NTRK2	NACC2	Jones et al. 2013
		QKI	Jones et al. 2013
Anaplastic astrocytoma	*NTRK2*	NOS1AP	Ferguson et al. 2018
Glioma not otherwise specified (Glioma NOS)	*NTRK2*	SQSTM1	Ferguson et al. 2018
Low-grade glioma	*NTRK2*	AFAP1	Stransky et al. 2014
		VCAN	Ferguson et al. 2018
	NTRK3	ETV6	Zhang et al. 2013
High-grade glioneuronal tumor	*NTRK1*	ARHGEF2	Kurozumi et al. 2019
Ganglioglioma	*NTRK2*	TLE4	Prabhakaran et al. 2018
Glioma of different grades	*NTRK2*	SLCO5A1	Mohamed et al. 2022
	NTRK1	MEF2D	Gatalica et al. 2019
	NTRK2	AGBL4	Wu et al. 2014
		BCR	Gatalica et al. 2019, Hechtman et al. 2017
		GKAP1	Gatalica et al. 2019
		KCTD8	Ferguson et al. 2018, Gatalica et al. 2019
		PRKAR2A	Gatalica et al. 2019
		SQSTM1	Ferguson et al. 2018, Stransky et al. 2014
		VLC	Wu et al. 2014
		VCAN	Ferguson et al. 2018, Gatalica et al. 2019
	NTRK3	AKAP13	Yoshihara et al. 2015
		BTBD1	Wu et al. 2014
		EML4	Ferguson et al. 2018, Gatalica et al. 2019

FISH and NGS can be used to analyze DNA status, whereas RT-PCR and RNA-based NGS studies can be used to evaluate the RNA transcription **(**Gambella et al. 2020**)**. Because molecular studies are expensive, timewasting, and associated with high level of nucleic acid degradation, IHC technique (Pan-Trk staining) was the best alternative technique to detect the NTRK-fusional protein. The IHC is less costive and associated with a fast turnaround time **(**Hechtman et al. 2017**)**. Anti-Pan-Trk immunostaining has been commonly used in non-CNS cancers to detect TK protein-associated *NTRK*-fusions because of its extreme accuracy. The clone reacts with the TK terminus in a tumour having *NTRK*-fusion. The major limitation is acknowledged here is that the anti-Pan-Trk antibody is restricted to wildtype epitope, thus not specific to detect *NTRK*-fusions. Moreover, IHC cannot detect the fusion partner. Solomon et al. found that Pan-Trk is overexpressed in cancers of lung, pancreatic, colon, and thyroid **(**Solomon et al. 2019**)**. The specificity is less with salivary gland cancers as well as breast adenocarcinoma **(**Solomon et al. 2019**)**. Hechtman et al. have tested Pan-Trk on some cases of non-CNS carcinomas with identified *NTRK*-fusions, and they found that the twenty cases showed Pan-Trk expression that were consonant with Archer RNA however, two cases with *NTRK*-fusions showed unremarkable expressions. Single case of colorectal cancer was proven to have *ETV6-NTRK3* fusion but with no expression on IHC **(**Hechtman et al. 2017**)**.

Although anti-Pan-Trk antibody has been used as an efficient tool for most body cancers to detect *NTRK*-fusions, the proficient correlation between Pan-Trk expression and *NTRK*-fusions in all CNS tumour types were not meaningfully investigated. Their utilization in CNS tumour was questionable due to the normal expression of Pan-Trk in CNS neuropil, thus the diagnostic and prognostic value of Pan-Trk was not clear

[**Figure 3**].

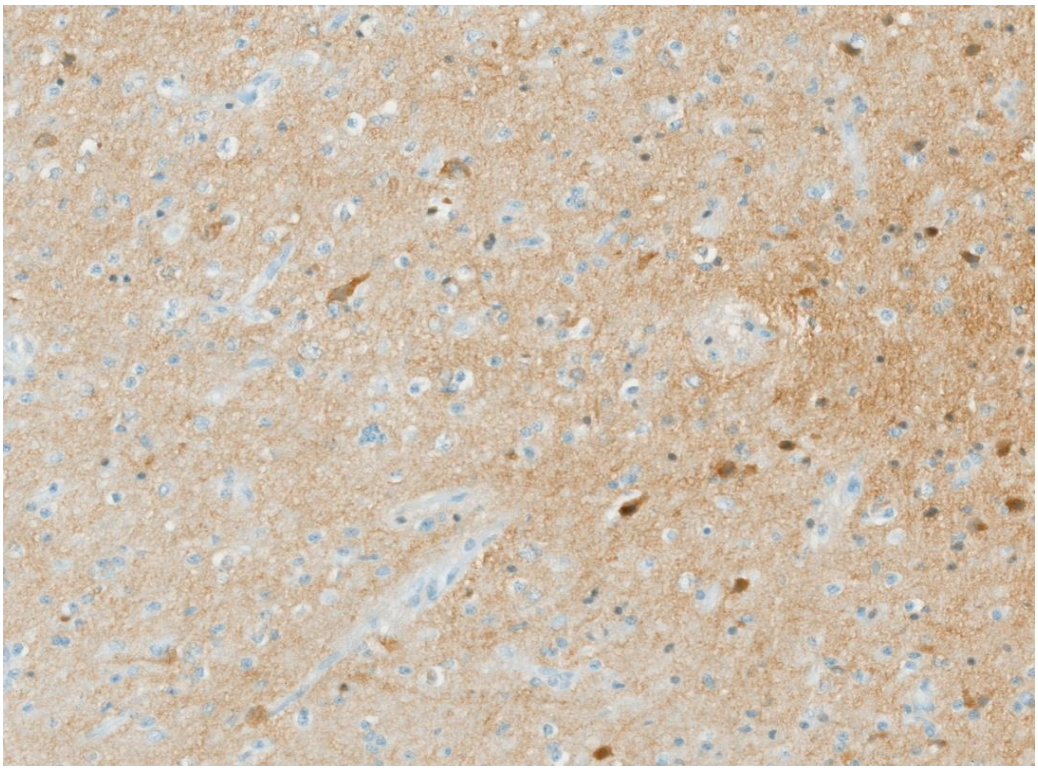

Figure 3: A Pan-Trk expression in CNS neuropil.

Nonetheless, few reports in the literature expressed Pan-Trk in scattered types of CNS gliomas, which were not synchronously compared with *NTRK*-fusions through molecular methods. Hechtman et al. reported some grade 4 astrocytomas with *NTRK*-fusions that showed compatibility with Pan-Trk immunostaining **(**Hechtman et al. 2017**)**. As a result, IHC sensitivity in Hechtman et al cohort was more than 90% of both specificity and sensitivity (Hechtman et al. 2017). Similarly, Solomon et al. found that the sensitivity for *NTRK1* and *NTRK2* were not less than 95% while sensitivity for *NTRK3* fusions was less than 80% (Solomon et al. 2020). It was clear that the Pan-Trk immunostaining variability correlates with fusion partners. In a recent study done by Mohamed et al (2022) on 23 CNS tumour samples, Pan-Trk was expressed in 11 CNS tumours and 12 tumours showed no Pan-Trk expression. Out of the 11 cases, 9 cases did not show any *NTRK*-fusions while 2 cases showed *NTRK2*-fusions. The remaining 12 cases with no Pan-Trk expression did not detect *NTRK*-fusions (Mohamed et al. 2022). These findings suggest that Pan-Trk cannot be used solely to detect *NTRK*-fusions in CNS tumours thus its accuracy is undervalued. We conclude here that molecular methods, particularly NGS, is considered the best molecular approach to detect *NTRK*-fusions and associated with high specificity compared to Pan-Trk IHC.

Although DNA-based NGS can detect gene fusions by targeting intronic regions, RNA-based NGS permits direct detection of exon-exon junctions indicative of a fusion. Hence, Targeted immunotherapy needs to precisely detect the mutational type between numerous subtypes that may be novel, yet few targeted drug options could benefit these patients. RNA-based NGS remains one of the most sensitive and accurate method to detect *NTRK*-fusions as it can immediately detect the transcribed gene fusion at the mRNA level (Drilon et al. 2017; Bourhis et al. 2022). In a quite fresh study that compared RNA-based NGS assay with amplicon-based multiplex PCR alone and with hybrid capture-based enrichment method with multiplex PCR, Hybrid capture-based target enrichment demonstrated that the target relevant regions of the genome allowed for more advanced detection than PCR or even Sanger sequencing. The amplicon-based PCR method showed the least limit of detection (Park et al. 2021). However, hybrid-capture or multiplex PCR method can both detect *NTRK*-fusions with novel fusion partners. For clinical sensitivity, all methods were highly matching in the detection of *NTRK*-fusions.

Several validated panels to detect solid tumours fusions and point mutations are used wisely to investigate genomic subtypes eligible for treatment. Some prearranged platforms investigate these fusions without requiring knowledge of their specific break points. A Comparison of all validated panels for detecting *NTRK*-fusions were conducted by Bormann et al to determine the specificity of panels **(**Bormann et al. 2022**)**. The panels were Archer's Fusion Plex Lung panel (AFL), Illumina's

TSO500, Thermo Fisher's On comine Precision Assay and Oncomine Focus Assay (OFA). All three panels were 100 % specific yet the quality control passing rate was variable accordingly (AFL, 43%; TSO500, 77%; and OFA, 83. The most used ready-to-use platform is Trusight oncology 500 (TSO500^{R}) high through output (Illumina, USA): 523 DNA and 55 RNA which targets immuno-oncology markers such as MSI and TMB. This platform screen gene variants including single nucleotide variants (SNVs), fusions, splice variants, copy number variants (CNVs), microsatellite instability (MSI) and tumour-burden (TMB). The libraries are sequenced on Nextseq 550 (Illumina) and the resulting data can be uploaded to the Clinical Genomics Workbench (PierianDx, France) where QC analysis, mapping to hg19, variant calling, and annotation are performed. Mohamed et al (2022) have used TSO500 in their study to detect all gene mutations including *NTRK*-fusions in the whole 23 CNS tumours (Mohamed et al. 2022).

CONCLUSION

Pan-Trk IHC is inappropriate tissue-efficient biomarker to detect *NTRK*-fusions in CNS tumours compared to non-CNS which showed that Pan-Trk had high specificity and sensitivity. IHC should be used with extreme caution, and its confirmation by other techniques requires justification. RNA-based NGS sequencing can be used as an alternative method to detect *NTRK*-fusions. TruSightOnco500 is a wide-genomic platform that can replace IHC and other molecular techniques to screen for DNA and RNA-based mutations. We recommend that NGS must be utilized as a superior method to test for *NTRK*-fusions in CNS tumours.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

FM, MK, IF, HTA, EF, AAAF, TSA, IMM and SA writing and editing. All the authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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