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NTRK gene amplification in patients with metastatic cancer

Su Jin Lee[1]*, Nayoung K. D. Kim[2]*, Se-Hoon Lee[1]*, Seung Tae Kim[1], Se Hoon Park[1], Joon Oh Park[1], Young Suk Park[1], Ho Yeong Lim[1], Won Ki Kang[1], Woong Yang Park[2], Hee Jin Bang[3], Kyoung-Mee Kim[3], Keunchil Park[1], Jeeyun Lee[1]

[1]Division of Hematology-Oncology, Department of Medicine, Sungkyunkwan University School of Medicine, Seoul, Korea
[2]Samsung Genome Institute, Samsung Medical Center, Seoul, Korea
[3]Department of Pathology, Sungkyunkwan University School of Medicine, Seoul, Korea

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Corresponding author:
Jeeyun Lee
Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-1779
E-mail: jyunlee@skku.edu

ABSTRACT

Purpose: Neurotropic tropomyosin receptor kinase (*NTRK*) fusions have been identified in a variety of cancers, and tyrosine kinase inhibitors targeting the tropomyosin receptor kinase (TRK) receptor are currently in clinical trials. However, no reports are available on the effects of *NTRK* gene amplification.

Methods: Samples from patients enrolled in the sequencing program were analyzed using a next-generation sequencing (NGS) cancer panel. For cases in which *NTRK* amplification (defined as ≥4.0 copies) was identified, panTRK immunohistochemical (IHC) staining of tissue microarrays was performed.

Results: A total of 1,250 tumor specimens collected between February 2014 and January 2016 were analyzed using the NGS cancer panel. *NTRK* amplification was detected in 28 cases of various types of cancer. Among 27 cases, only four were positive for panTRK IHC. These four cases were melanoma, sarcoma, lung cancer, and gastric cancer. We found that 2.2% of cancer patients showed *NTRK* amplification using NGS cancer panel and *NTRK* amplification resulted in protein overexpression in 14.8% of these patients.

Conclusion: Patients with *NTRK* amplification and increased TRK protein expression may be considered for inclusion in clinical trials for *NTRK* inhibitors.

Keywords: NGS cancer panel; *NTRK* gene amplification; TRK immunohistochemistry

INTRODUCTION

Tropomyosin receptor kinase (TRK) is a receptor in the tyrosine kinase family that is activated by neurotrophins, a family of nerve growth factors [1-4]. Three members of the TRK family have been described: TRKA, TRKB, and TRKC, encoded by neurotropic tropomyosin receptor kinase 1 (*NTRK1*), *NTRK2*, and *NTRK3*, respectively. Trk family members play important roles in nervous system development through regulation of cell proliferation, differentiation, apoptosis, and sur-

vival of neurons in both the central and peripheral nervous system. Trk receptors are expressed not only in the nervous system, but also in many other non-neuronal cell types and tissues, including monocytes, lung, bone, and pancreatic β-cells [5,6].

In 1982, the first *NTRK1* gene fusion was identified in a colon cancer specimen; it contained sequence from tropomyosin 3 (*TPM3*; non-muscle tropomyosin) [7,8]. Subsequently, *NTRK1* fusions have been detected at a frequency of 12% in papillary thyroid cancers, with *TPM3-NTRK1* being the most common gene rearrangement [9-11]. In addition, TRKC and very recently TRKB have also been shown to form oncogenic chimeras in multiple tumor types [12,13]. Aside from gene fusions, only an in-frame deletion of *NTRK1* in acute myeloid leukemia and a splice variant of *NTRK1* in neuroblastoma have been functionally characterized as oncogenic to date [14-18]. However, no reports are available on the effects of NTRK gene amplification (defined as ≥4.0 copies).

In the past several years, DNA sequencing technology has evolved dramatically. Next-generation DNA sequencing (NGS) has brought genome sequencing to clinical laboratories. The huge reduction in sequencing cost and the increase in sequencing efficiency, and the incomparable sequencing throughput, sensitivity, and accuracy all make NGS the most promising technology for cancer genomics and personalized cancer therapy. In this study, we investigated the prevalence of *NTRK* gene amplification using targeted sequencing and analyzed the association between gene amplification and TRK protein expression.

METHODS

Patients

This investigation was conducted in accordance with the ethical standards of the Declaration of Helsinki as well as national and international guidelines, and was approved by the Institutional Review Board at Samsung Medical Center, Seoul, Korea. Between October 2013 and January 2016, 1,250 patients with gastrointestinal cancer, lung cancer, and rare forms of cancer were prospectively enrolled in the NEXT-1, VIKTORY (NCT#02299648), or LUNG PERSEQ (NCT#02299622) trials at Samsung Medical Center that employed targeted sequencing (other cancer panels, such as Ion Torrent [Thermo Fisher, Waltham, MA, USA], were excluded from this analysis). Patient inclusion criteria were as follows: age ≥18 years, pathologically confirmed cancer, and availability of resection/biopsies of the primary or metastatic site and data on clinicopathologic characteristics.

Targeted exome sequencing

Genomic DNA was extracted, and a SureSelect customized kit (Agilent Technologies, Santa Clara, CA, USA) was used to capture 83 or 379 cancer-related genes, depending on the sequencing panel version. An Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) was used for sequencing, and 100-bp paired-end reads were obtained. The sequencing reads were aligned to the human genome reference sequence (hg19) using BWA-mem (v0.7.5), SAMTOOLS (v0.1.18), Picard (v1.93), and GATK (v3.1.1) for sorting SAM/BAM files, duplicate marking, and local realignment, respectively. Local realignment and base recalibration were carried out using dbSNP137, Mills indels, HapMap, and Omni. Single nucleotide variants and indels were identified using Mutect (v1.1.4) and Pindel (v0.2.4), respectively. ANNOVAR was used to annotate the detected variants. Only variants with an allele frequency >1% were included in the results. Copy number variation was calculated for the targeted sequencing regions by dividing read depth per exon by the normal reads per exon using an in-house reference. Translocations in the target region were identified using an in-house algorithm (in preparation).

PanTRK immunohistochemistry

For tissue microarray construction, all H&E stained slides were reviewed and the representative area was carefully selected and marked on all paraffin blocks. A 3-mm tissue core was taken from the representative region of each tumor specimen using Accumax (ISU Abxis, Seoul, Korea). Immunohistochemistry (IHC) was performed under various conditions using five primary antibodies; panTRK (C17F1) from Cell Signaling (Danvers, MA, USA) was identified as a highly sensitive and specific primary antibody and was adopted for screening [19]. Mild cytoplasmic or membranous staining was considered to indicate a weakly positive result, and moderate to strong cytoplasmic staining was considered to indicate a positive result.

Statistical analysis

Student t-test was used to compare the means of continuous variables between the TRK-IHC-positive and -negative groups. P values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients' characteristics

Between October 2013 and January 2016, 1,250 patients with gastrointestinal cancer, lung cancer, or rare cancers were prospectively enrolled in the NEXT-1, VIKTORY (NCT#02299648), or LUNG PERSEQ (NCT#02299622) trials using targeted sequencing at Samsung Medical Center, Seoul, Korea. Among these 1,250 samples, we identified 28 cases with *NTRK* amplification (2.2%), defined as ≥4.0 copies (Fig. 1). Of 28 cases, almost all cases were *NTRK1*-amplified except for two cases of *NTRK3* amplification and one case of *NTRK2* amplification. The median age was 59 years (range, 23 to 74 years), and 60% of patients were male. These cases included lung cancer (n=6), gastric cancer (n=5), biliary tract cancer (n=3), melanoma (n=3), sarcoma (n=3), pancreatic cancer (n=2), hepatocellular carcinoma (n=2), renal cell carcinoma (n=2), bladder cancer (n=1), and ovarian cancer (n=1). The most common site of metastases was a non-regional/distant lymph node (LN; n=14) followed by lung, liver, peritoneal seeding, bone, pleura, pancreas, and brain. Baseline characteristics are listed in Table 1 and individual patients' information, with concomitant genetic aberrations, are also shown in Table 2. The median *NTRK* copy number of all patients was 4.95 (range, 4.2 to 7.8).

Identification of panTRK IHC-positive cases

Tissue staining with TRK IHC was performed for 27 of the 28 cases (tissue was not available for the remaining case). Four of the 27 cases (14.8%) were positive for TRK IHC (Fig. 2), and these four cases were all *NTRK1*-amplified. The first was a patient with acral melanoma, with an *NTRK* copy number of 6 and moderate to strong cytoplasmic TRK IHC staining. The second was a patient with sarcoma, with an *NTRK* copy number of 7.8 (the highest) and cytoplasmic TRK staining. The third was a patient with non-small cell lung cancer, with an

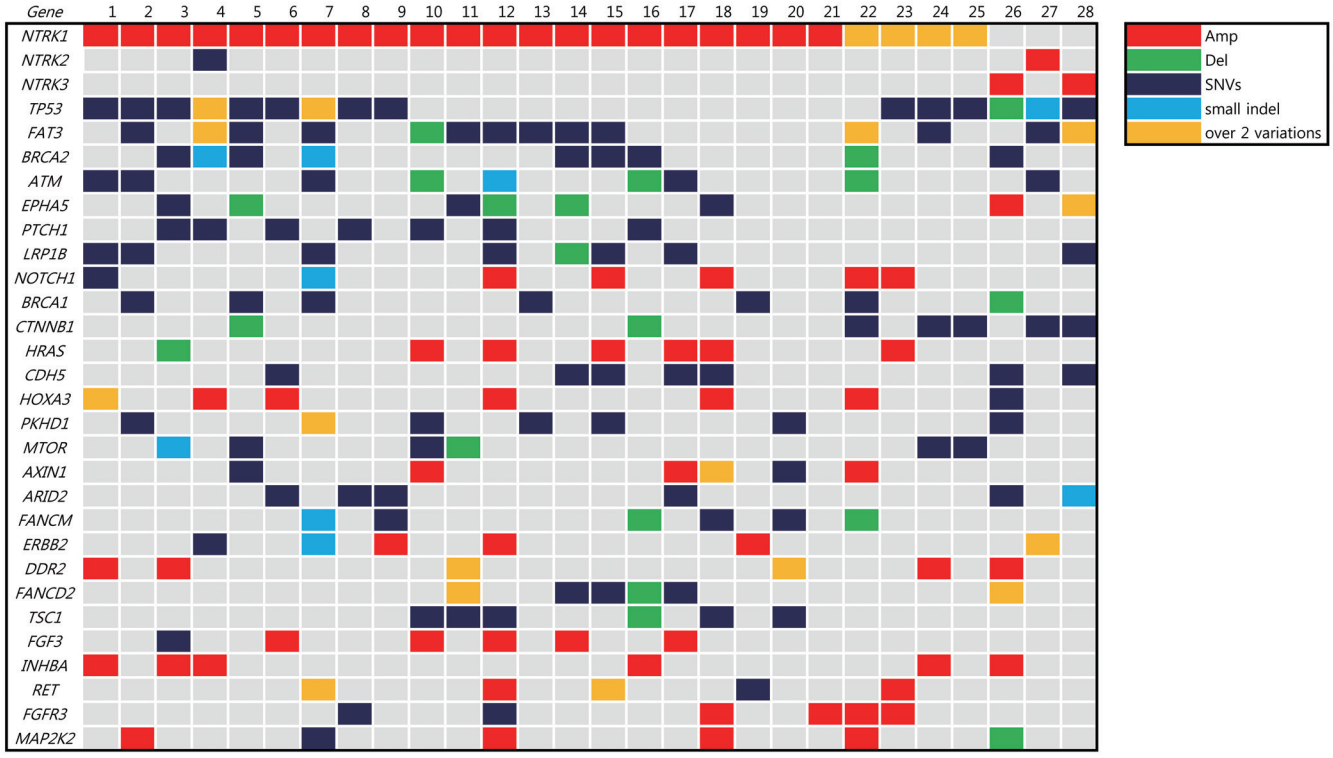

Fig. 1. Genomic landscape of cancer patients with neurotropic tropomyosin receptor kinase 1 (*NTRK1*) amplification. TP53, tumor protein p53; FAT3, FAT atypical cadherin 3; BRCA, breast cancer gene; EPHA5, EPH receptor A5; PTCH1, protein patched homolog 1; LRP1B, low-density lipoprotein receptor-related protein 1B; NOTCH, neurogenic locus notch homolog protein; CTNNB1, catenin beta-1; CDH5, cadherin 5; HOXA3, homeobox A3; PKHD1, polycystic kidney and hepatic disease 1; MTOR, mammalian target of rapamycin; AXIN1, axin 1; ARID2, AT-rich interactive domain-containing protein 2; FANCM, Fanconi anemia, complementation group M; *ERBB2*, erb-b2 receptor tyrosine kinase 2; DDR2, discoidin domain receptor tyrosine kinase 2; FANCD2, Fanconi anemia complementation group D2; TSC1, tuberous sclerosis 1; FGF3, fibroblast growth factor 3; INHBA, inhibin, beta A; FGFR3, fibroblast growth factor receptor 3; MAP2K2, mitogen-activated protein kinase kinase 2; SNV, single nucleotide variant.

Table 1. The characteristics of patients with NTRK amplification (n=28)

Characteristic	Value
Age (yr)	59 (2–74)
Sex	
Male	17 (60.7)
Female	11 (39.3)
Primary cancer site and histology	
Lung cancer	6 (21.4)
Adenocarcinoma	3 (10.7)
Squamous cell carcinoma	1 (3.6)
Large cell neuroendocrine carcinoma	1 (3.6)
Small cell lung cancer	1 (3.6)
Gastric cancer	5 (17.9)
Adenocarcinoma	2 (7.1)
Signet ring cell carcinoma	2 (7.1)
Poorly differentiated neuroendocrine carcinoma	1 (3.6)
Biliary tract cancer (all adenocarcinoma)	3 (10.7)
Hilar cholangiocarcinoma	1 (3.6)
Intrahepatic cholangiocarcinoma	1 (3.6)
Ampulla of Vater cancer	1 (3.6)
Melanoma	3 (10.7)
Mucosal	2 (7.1)
Acral	1 (3.6)
Sarcoma	3 (10.7)
Uterine leiomyosarcoma	1 (3.6)
PEComa	1 (3.6)
Unspecified	1 (3.6)
Pancreatic cancer	2 (7.1)
Acinar cell carcinoma	2 (7.1)
Adenocarcinoma	2 (7.1)
Hepatocellular carcinoma	2 (7.1)
Renal cell carcinoma (all clear cell)	2 (7.1)
Bladder cancer, TCC	1 (3.6)
Ovarian cancer	1 (3.6)
Initial stage	
Loco-regional disease	15 (5.6)
Metastatic disease	1 (46.4)
No. of prior systemic treatment regimen	
1	9 (2.1)
2	10 (5.7)
3	4 (14.3)
≥4	5 (17.9)
Site of distant metastasis	
Lymph node	14 (50.0)
Lung	12 (42.9)
Liver	8 (28.6)
Peritoneal seeding	7 (25.0)
Bone	4 (14.3)
Pleura	3 (10.7)
Pancreas	2 (7.1)
Brain	2 (7.1)

Values are presented as median (range) or number (%).
NTRK, neurotropic tropomyosin receptor kinase; PEComa, perivascular epithelioid cell tumor; TCC, transitional cell carcinoma.

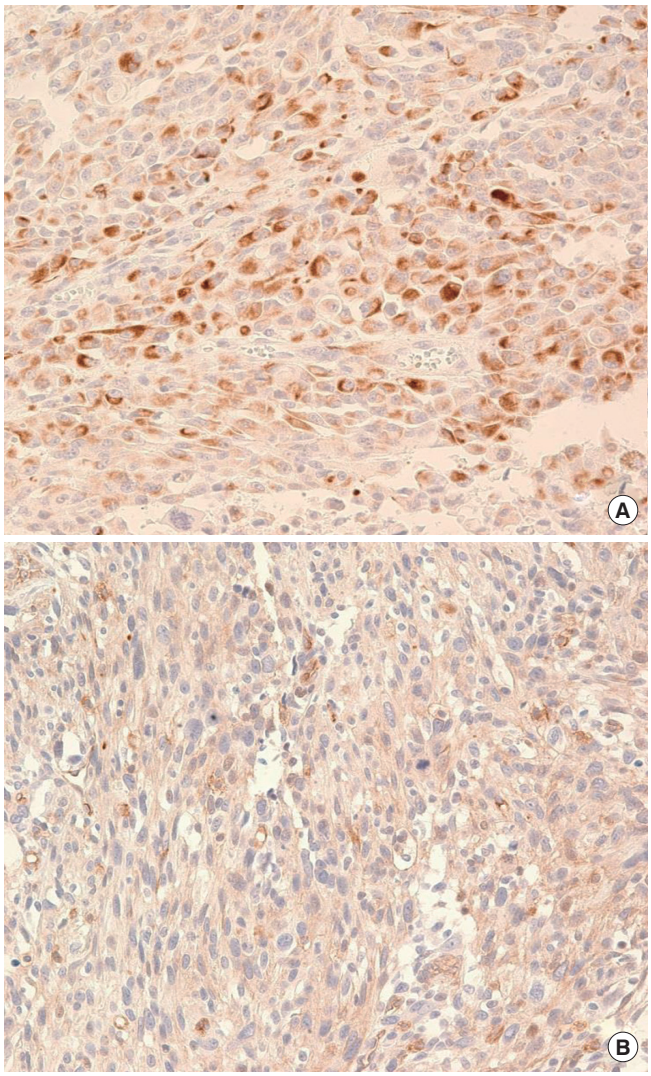

Fig. 2. Representative tropomyosin receptor kinase immunohistochemical staining (×400). (A) Acral melanoma with moderate to strong cytoplasmic immunohistochemical staining. (B) Sarcoma with mild cytoplasmic staining.

NTRK copy number of 4.6 and mild cytoplasmic staining. The last case was a patient with gastric cancer, with an *NTRK* copy number of 4.6. The median *NTRK* copy number in TRK IHC-negative versus positive cases was 4.95 vs. 5.3, but the difference was not significant (P=0.509)

Characteristics of patients showing *NTRK* amplification and overexpression

The first case that showed *NTRK* amplification is a 60-year-old male patient who initially presented with stage II acral melanoma of the big toe and underwent a curative resection in 2012. He recurred with inguinal LN metastasis and underwent an inguinal LN dissection in 2014. One year later, new inguinal, external iliac, and obturator LN metastases devel-

Table 2. Individual patients' information and concomitant genomic alterations

Sex	Age (yr)	Cancer type	Histology	*NTRK* CNV	panTRK IHC	Other CNV	Mutation	Other biomarker
M	60	GC	ADC	4.2	Negative	*ERBB2*	KRAS, VHL, MLL3, GNAQ, TP53, NOTCH2, TGFBR2, ATR, MLL3, RB1, NCOA3, CEBPA	HER2 IHC positive
F	57	MM	Mucosal	4.2	Not done	FBXW7, NF1 deletion	MLL3, EP300, GNAQ, NOTCH2, TGFBR2, ATR, RAD50, PRKDC, CHD2, NCOA3	-
F	72	LCA	ADC	4.3	Negative	AKT2	EGFR exon 19 deletion, TP53 mutation	-
M	63	BTC	ADC (hilar CCC)	4.4	Negative	RICTOR	CTNNB1, PIK3CG, MLL3, *ERBB2*, ERBB4, GNAQ, NOTCH2, TGFBR2, ATR, RAD50, HSP90AA1, TP53, SMAD4, NCOA3, CEBPA	-
M	30	RCC	Clear cell	4.5	Negative	GNA11, STK11, AKIN1, FGF3, FGFR4, FLT4, HRAS, MEF2B	AKT1, VHL, SMO mutation	-
F	66	HCC	HCC	4.5	Negative	FANCA deletion	PRDM1, SEMA3E, MLL3, STK11, MEF2B, CHEK2, GNAQ, NOTCH2, TGFBR2, ATR, RAD50, RB1, CHD2	MET IHC positive
M	60	LCA	SCLC	4.5	Negative	CTNNB1, FBXW7, MAP2K4, MLH1 deletion	AXIN1, GNAQ, TP53, NOTCH2, TGFBR2, RAD50, MLL3, PRKDC, CHD4, HSP90AA1, BLM, NCOA3, MLL3	-
M	74	BTC	ADC (CCC)	4.6	Negative	ATM, CTNNB1, FANCD2, PTPN11, TSC1 deletion	BRAF, ARID1B, MLL3, CHEK2, GNAQ, NOTCH2, TGFBR2, RAD50	-
M	65	GC	ADC	4.6	Positive	-	PIK3CA, FBXW7, CTNNA1, WWP1, B2M, BCOR, H3F3A, TP53, NOTCH2, NCOA3	MET IHC positive
M	49	LCA	SqCC	4.6	Weak positive	JUN, NKX2-1	MLL3, *NTRK3*, GNAQ, TP53, NOTCH2, TGFBR2, NCOA3	-
F	50	LCA	LCNEC	4.7	Negative	EGFR, JUN ampli/ BRCA1, FANCD2, PBRM1, TP53, VHL deletion	MLL3, GNAQ, NOTCH2, TGFBR2, ATR, RAD50, MLL3, CHD2, CEBPA	EML4-ALK fusion
M	63	GC	Signet ring cell	4.8	Negative	*ERBB2*, GNA11, JUN, STK11	NOTCH2, MLL3, GNAQ	-
M	23	Sarcoma	PEComa	4.8	Negative	CCNE1	ARID1A, GNAQ, TP53, NOTCH2, TGFBR2, MLL3, CEBPA	-
F	52	LCA	ADC	4.9	Negative	CDKN2A deletion	CTNNB1, EGFR, exon 19 deletion, TP53	-
M	60	GC	P/D NEC	5.0	Negative	CCNE1, JUN	KRAS, KDR, MLL3, GNAQ, TP53, NOTCH2, NCOA3, CHD1	-
M	53	BTC	ADC (AOV cancer)	5.0	Negative	-	CTNNB1, KRAS, MLL3, RB1, BCR, CHEK2, GNAQ, TP53, NCOA3, NOTCH2	MET IHC positive
M	59	Pancreatic cancer	Acinar cell carcinoma	5.1	Negative	AKT3, FGFR3	SMAD3 G386V	-
F	72	RCC	Clear cell	5.2	Negative	GNA11, NKX2-1, SOX2	CDH5, NCOR1, CHEK2, NSD1, NOTCH2	-
F	52	Ovarian cancer	-	5.2	Negative	*ERBB2*	RET, BRCA1	-
M	45	GC	Signet ring cell	5.3	Negative	PTEN deletion	MLL3, RAD51, FANCI, CDH1, CHEK2, GNAQ, TP53, NOTCH2, TGFBR2, ASXL1, MLL3, CEBPA	-

(Continued to the next page)

Table 2. Continued

Sex	Age (yr)	Cancer type	Histology	*NTRK* CNV	panTRK IHC	Other CNV	Mutation	Other biomarker
F	58	Pancreatic cancer	ADC	5.9	Negative	-	KRAS, CHEK2, NOTCH2	-
M	56	Sarcoma	Unspecified	5.9	Negative	-	ARID1B, AXIN1, PIK3Rs, NCOA3, NOTCH2	-
M	59	MM	Acral	6.0	Positive	CDK4, MDM2	MLL3, GNAQ, ARID1A, NOTCH2, TGFBR2, ATR, RAD50, MLL3, CHD2	-
M	74	Bladder cancer	TCC	6.3	Negative	MET, GNA11, CDKN2A	RB1, TP53	-
F	60	LCA	ADC	6.4	Negative	EGFR, CCNE1	EGFR L858R, PIK3R2, GNAQ, TGFBR2, MLL3, CHD2, NCOA3, CEBPA	-
F	49	MM	Mucosal	7.0	Negative	ATM deletion/ JUN, MYC, SOX2 ampli	NRAS, MLL3, GATA3, ERBB4, GNAQ, NOTCH2, TGFBR2, ATR, NCOA3	KIT exon 11 mutation
M	61	HCC	HCC	7.5	Negative	ATM, PTEN, BRCA2, MDM2, MSH2, RB1 deletion/ GNA11, LMO1, NKX2-1, SOX2 ampli	CTNNB1, MLL3, SMARCB1, GNAQ, NOTCH2, TGFBR2, RAD50, NOTCH4, CHD2, CEBPA, ASXL1	-
F	49	Sarcoma	Uterine leiomyosarcoma	7.8	Weak positive	ALK ampli/ RB1 deletion	MLL3, GNAQ, TP53, MTOR, NOTCH2, TGFBR2, ATR, RAD50, NOTCH4, MLL3, CHD2, EWSR1, MLL3	-

NTRK, neurotropic tropomyosin receptor kinase; CNV, copy number variant ; panTRK, pan-tropomyosin receptor kinase; IHC, immunohistochemistry; GC, gastric carcinoma; ADC, adenocarcinoma; ERBB, erb-b2 receptor tyrosine kinase 2; VHL, von Hippel-Lindau; MLL3, mixed-lineage leukemia protein 3; GNAQ, guanine nucleotide-binding protein G(q) subunit alpha; TP53, tumor protein p53; NOTCH, neurogenic locus notch homolog protein; TGFBR, transforming growth factor beta receptor; ATR, ataxia telangiectasia and Rad3-related protein; RB1, retinoblastoma 1; NCOA, nuclear receptor coactivator; CEBPA, CCAAT/enhancer-binding protein alpha; HER2, human epidermal growth factor receptor 2; MM, ; FBXW, F-box and WD repeat domain; NF1, neurofibromatosis type 1; EP300, E1A binding protein p300; PRKDC, protein kinase, DNA-activated, catalytic polypeptide; CHD, chromodomain helicase DNA; LCA, leukocyte common antigen ; AKT, RAC-alpha serine/threonine-protein kinase; EGFR, ; BTC, ; CCC, cholangiocarcinoma; RICTOR, ; CTNNB1, catenin beta-1; PIK3CG, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma; HSP90AA1, heat shock protein 90 alpha family class A member 1; RCC, renal cell carcinoma; GNA11, G protein subunit alpha 11; STK11, serine/threonine kinase 11; FGF3, fibroblast growth factor 3; FGFR, fibroblast growth factor receptor; FLT4, fms related tyrosine kinase 4; MEF2B, myocyte enhancer binding factor 2B; SMO, ; HCC, hepatocellular carcinoma; FANCA, fanconi anemia complementation group A; PRDM1, PR domain zinc finger protein 1; SEMA3E, semaphorin 3E; CHEK2, checkpoint kinase 2; MET, mesenchymal-epithelial transition factor; SCLC, small cell cancer of the lung; MAP2K4, mitogen-activated protein kinase kinase 4; MLH1, mutL homolog 1; AXIN1, axin 1; BLM, Bloom syndrome RecQ like helicase; FANCD2, Fanconi anemia complementation group D2; PTPN11, protein tyrosine phosphatase, non-receptor type 11; TSC1, tuberous sclerosis 1; ARID1B, AT-rich interaction domain 1B; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; CTNNA1, catenin alpha 1; WWP1, WW domain containing E3 ubiquitin protein ligase 1; B2M, beta-2 microglobulin; BCOR, BCL6 corepressor; H3F3A, H3 histone family member 3A; SqCC, squamous cell carcinoma; NKX2-1, NK2 homeobox 1; LCNEC, large cell neuroendocrine carcinoma; BRCA, breast cancer gene; PBRM1, polybromo 1; EML4, echinoderm microtubule-associated protein-like 4; ALK, anaplastic lymphoma kinase; PEComa, perivascular epithelioid cell tumor; CCNE, cyclin E1; ARID1A, AT-rich interactive domain-containing protein 1A; CDKN2A, cyclin-dependent kinase inhibitor 2A; P/D NEC, ; KDR, kinase insert domain receptor ; AOV, ampulla of Vater; BCR , ; SOX2, sex determining region Y-box 2; CDH, cadherin; NCOR1, nuclear receptor corepressor 1; NSD1, nuclear receptor binding SET domain protein 1; PTEN, phosphatase and tensin homolog; FANCI, Fanconi anemia complementation group I; ASXL1, additional sex combs like 1, transcriptional regulator; PIK3R, phosphatidylinositol 3-kinase regulatory subunit alpha; CDK4, cyclin-dependent kinase 4; MDM2, mouse double minute 2 homolog; TCC, transitional cell carcinoma; NRAS , ; MSH2, mutS homolog 2; LMO1, LIM domain only 1; SMARCB1, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; MTOR, mammalian target of rapamycin; EWSR1, ewing sarcoma breakpoint region 1.

oped and he was referred to our center. After confirmation that the patient possessed wild-type BRAF and KIT, he received nine cycles of palliative dacarbazine/cisplatin/tamoxifen chemotherapy with partial response, but the disease continued to progress. We plan to treat him with second-line chemotherapy.

The second *NTRK*-amplified patient is a 50-year-old female who underwent a total hysterectomy for uterine leiomyosarcoma in 2001. The tumor recurred in 2013 and she underwent radiofrequency ablation for muscle metastasis and a metastasectomy for lung metastasis. But 5 months later, multiple bone, pancreas, and lung metastases developed and she was treated with several lines of chemotherapy as well as palliative radiotherapy for bone metastasis. She has now joined a clinical trial for a new tyrosine kinase inhibitor (TKI) that targets TRK.

The third patient was a 49-year-old male with squamous cell lung cancer that was resected in 2012 after neoadjuvant chemoradiation for stage IIIA. Nine months later, supraclavicular LN metastasis developed and he underwent salvage chemoradiation followed by palliative chemotherapy and radiotherapy for progression. In 2015, he underwent a craniotomy and tumor removal for cerebellar metastasis, but died due to disease progression.

The fourth patient is a 65-year-old male with gastric adenocarcinoma who was initially diagnosed with stage IV cancer and who has been treated with third-line chemotherapy.

DISCUSSION

Gene amplification is defined as an increase in the copy number of a restricted region of a chromosome arm [20,21]. Gene amplification is an influential factor in the expression of both protein-coding and non-coding genes, affecting the activity of various signaling pathways in cancer. Gene amplification, similar to gene mutation, plays a significant role in tumorigenesis in many types of cancer, such as gastric cancer, ovarian cancer, hepatocellular carcinoma, colon cancer, and others [22,23]. Thus, targeting the "driver genes" that are amplified may provide novel opportunities for precision medicine [20].

One of the most studied gene amplifications is erb-b2 receptor tyrosine kinase 2 (*ERBB2*), an important driver oncogene for breast cancer [24]. In breast cancer, gene amplification of *ERBB2* is strongly correlated with its protein expression. Moreover, *ERBB2* amplification is also observed in gastric cancer [25,26]. Mesenchymal-epithelial transition factor (*MET*) is a proto-oncogene that encodes a receptor tyrosine kinase, and aberrant activation of *MET* signaling occurs in a subset of advanced cancers as a result of various genetic alterations, including gene amplification [27]. Recently, *MET* amplification was identified as a potential oncogenic driver for several cancers, and therapy with TKIs that target MET shows promise as an effective treatment, based on preclinical and clinical data [28].

Using gene panel analysis, we showed that 2.2% of cancer patients had *NTRK* amplification, and that *NTRK* amplification resulted in protein overexpression in 14.8% of these patients. The results of gene amplification detected frequently conflict with the results of the corresponding protein overexpression. For example in TOGA trial [29], 131 patients (22.4%) were *ERBB2* IHC 0 or 1 with gene amplification by fluorescent *in situ* hybridization. This discrepancy can be explained by decreased internalization or turnover of the HER2 (human epidermal growth factor receptor 2) protein [30] and bystander effect of *ERBB2* gene amplification [31]. Ma et al. [32] recently suggested that interactions with peripheral blood mononuclear cells in the tumor microenvironment might increase *ERBB2* and *MMP9* (matrix metallopeptidase 9) mRNA and it might be involved in the mechanism responsible for this discrepancy. In comparison with *ERBB2* amplification, protein overexpression was much lower in *NTRK*-amplified cancers. To determine whether or not *NTRK* amplification is an oncogenic driver, further preclinical study using TRK TKIs or RNA interference targeted to NTRK mRNA is needed. Even though we cannot conclude that *NTRK* amplification is an oncogenic driver, our study suggests that NTRK TKIs show promise to provide an effective treatment for cancers involving *NTRK* amplification.

Currently, several TKIs with activity against the TRK family, such as entrectinib (NCT#02097810) or LOXO-101 (NCT#02576431), are being investigated in clinical trials. Clinical efficacy has been reported in patients with well-known *NTRK* fusions, such as *TPM3-NTRK1, LMNA-NTRK1*, or *ETV6-NTRK3* fusions [33-37]. Although the clinical implications of *NTRK* amplification, in terms of responsiveness to TRK inhibitors, has yet to be demonstrated, our data indicate that patients with *NTRK* amplification that show TRK protein expression may be considered for inclusion in clinical trials for TRK inhibitors.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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