

***NTRK* and *NRG1* gene fusions in advanced non-small cell lung cancer (NSCLC)**

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Abstract: Personalized medicine has revolutionized the therapeutic landscape of most solid tumors, including advanced non-small cell lung cancer (NSCLC), with unprecedented results in molecularly defined patient subgroups. Recently, the identification of rare oncogenic drivers with very low frequency in multiple solid tumors has further changed the drug development process leading to the approval of targeted therapies after only phase I studies and independently of tumor histology. *Neurotrophic receptor tyrosine kinase (NTRK)* and *neuregulin-1 (NRG1)* gene fusions represent two of the most compelling examples of tumor agnostic biomarkers and, although uncommon in NSCLC, constitute two clinically relevant subgroups of patients that can derive benefit from matched targeted drugs. Here we provide a comprehensive overview of the biological and clinicopathological characteristics of *NRG1*- and *NTRK*-rearranged NSCLC and the available data on therapeutic exploitation of these targets.

Keywords: Non-small cell lung cancer (NSCLC); *neurotrophic receptor tyrosine kinase (NTRK)*; *neuregulin-1 (NRG1)*; rearrangements; larotrectinib; entrectinib; afatinib; targeted therapy

Received: 31 October 2019; Accepted: 24 February 2020.

doi: 10.21037/pcm.2020.03.02

View this article at: <http://dx.doi.org/10.21037/pcm.2020.03.02>

Introduction

Gene rearrangements are one of the most commonly observed genetic aberrations found in solid tumors and have growing diagnostic and therapeutic relevance. These genetic events were initially identified in hematological malignancies; *breakpoint cluster region/Abelson murine leukemia viral oncogene homolog (BCR-ABL)* rearrangement in chronic myeloid leukemia was the most prominent example. However, over the past several years, a multitude of gene rearrangements have been described in various solid tumors, in part due to the increased access to highly advanced

sequencing technologies (1). Furthermore, implementing effective targeted drugs in rationally-designed, molecularly-selected clinical trials has prompted the rapid approval of some of these agents after only phase I studies either in specific histologies (for example crizotinib for *anaplastic lymphoma kinase (ALK)* and *c-ros protooncogene 1 (ROS1)* rearrangements in non-small cell lung cancer (NSCLC) (2,3) or in tumor agnostic patients [such as larotrectinib and entrectinib in *neurotrophic receptor tyrosine kinase (NTRK)* rearranged tumors] (4).

During the last two decades, breakthrough discoveries in lung cancer biology launched a new era in advanced

NSCLC with the rise of personalized medicine. Since the discovery of *epidermal growth factor receptor (EGFR)* mutations in 2004, the list of molecularly defined subgroups of patients that can derive benefit from targeted therapies has grown considerably and current international guidelines recommend molecular testing for all patients with a newly diagnosed locally advanced or metastatic non-squamous NSCLC for at least 5–8 biomarkers for optimal patient selection (5–9). The incredible story of *ALK* rearrangements in NSCLC with the approval of the first-in-class inhibitor, crizotinib, only 4 years after the identification of *ALK* fusions in NSCLC (10) prompted the search for other oncogenic rearrangements potentially exploitable with targeted therapies. *Neuregulin-1 (NRG1)* and *NTRK* fusions are two of the most recently discovered rearrangements in NSCLC and represent two brilliant examples of tumor agnostic biomarkers. Although relatively rare, these two genetic aberrations represent two clinically relevant subgroups of NSCLC that can derive benefit from targeted therapies. Here we provide a comprehensive overview of the biological and clinicopathological characteristics of *NRG1*- and *NTRK*-rearranged NSCLC and the available data on the therapeutic exploitation of these targets.

NRG1 fusion genes

The epidermal growth factor (EGF) family plays an important role in both carcinogenesis and resistance to targeted therapy in NSCLC. NRGs are a group of growth factors for EGF receptor (11). The *NRGs* family of genes comprises four members (*NRG1*, *NRG2*, *NRG3* and *NRG4*), and *NRG1* is the most well studied. *NRG1* has an essential role in normal physiology of the nervous system, heart and breast, in addition to a pathologic role in some diseases, including cancer (12). *NRG1* presents three major isoforms, type I (heregulin), type II [glial growth factor-2 (GGF2)] and type III [sensory and motor neuron-derived factor (SMDF)], and six minor isoforms with specific function and expression (13).

All NRGs serve as ligands for the human epidermal growth factor receptor 4 (HER4), while *NRG1/2* binds also for HER3 and are synthesized as transmembrane molecules. In addition, they can also act as soluble ligands after their release by membrane metalloproteases of the ADAM (a disintegrin and metalloproteinase) subfamily, such as tumor necrosis factor-alpha converting enzyme (TACE/ADAM17). NRGs binding, in an autocrine and juxtacrine manner, causes a conformational change of

HER3, with exposition of the dimerization arm which can interact with other HER receptors, especially with HER2, leading to activation of the intracellular signaling cascade influencing critical cell processes such as growth and proliferation (14–16). Although HER3 lacks significant tyrosine kinase activity, its activation by NRGs is important in carcinogenesis by promoting heterodimerization with other HER receptors, leading to inappropriate activation; disrupting the interaction of NRGs with HER3 and HER4 receptors may be a potential therapeutic target (Figure 1).

Beyond its role in carcinogenesis, NRGs are also involved in resistance to targeted therapy. Zhou and colleagues observed in NSCLC cell lines resistant to gefitinib that a selective ADAM 17 inhibitor (INCB3619) was able to reverse resistance to gefitinib, highlighting the contribution of NRG-dependent HER3 activation that contributes to gefitinib insensitivity in NSCLC (17). Upregulation of *NRG1* has also been described as a mechanism of resistance to ALK inhibitors in NSCLC and systemic therapy in melanomas and HER2 inhibitor in breast cancer (18,19) and *NRG1* mutations have been described in genetic disorders, such as the Hirschsprung disease.

To date, 18 different fusion partners for *NRG1* have been reported in NSCLCs, although this list is destined to grow as many other variants have been described in other solid tumors (20–22). The *CD74-NRG1* fusion variant is the most common in NSCLC and was first described in 2014 by Fernandez-Cuesta *et al.* (23). It consists of the first six exons of *CD74* linked to the exons encoding the EGF-like domain β of the *NRG1* isoform III (23). This gene fusion leads to extracellular expression of the EGF-like domain of *NRG1* III- β , which interacts with EGF receptors enabling heterodimerization of HER2-HER3 and subsequent activation. In lung cancer cell lines, ectopic expression of *CD74-NRG1* showed a potential to promote cell tumor proliferation by activation of PI3K-AKT pathway through HER2 and HER3 receptors (23). This gene fusion seems particularly associated with a rare histological subtype that has been described in the 2015 WHO Classification of Lung Tumors (24), known as invasive mucinous adenocarcinoma (IMA) (23,25–27), which accounts for approximately 5% of all lung adenocarcinomas and harbors *KRAS* mutations in ~40–60% of the cases (25,28). IMA is associated with a poorer prognosis than other common subtypes of lung adenocarcinoma, including lepidic and acinar subtypes, and is rarely associated with *EGFR* mutations and *ALK* rearrangements (25,29,30).

In an analysis of 21,858 solid tumor specimens analyzed using RNA sequencing, 41 *NRG1* fusions were identified.

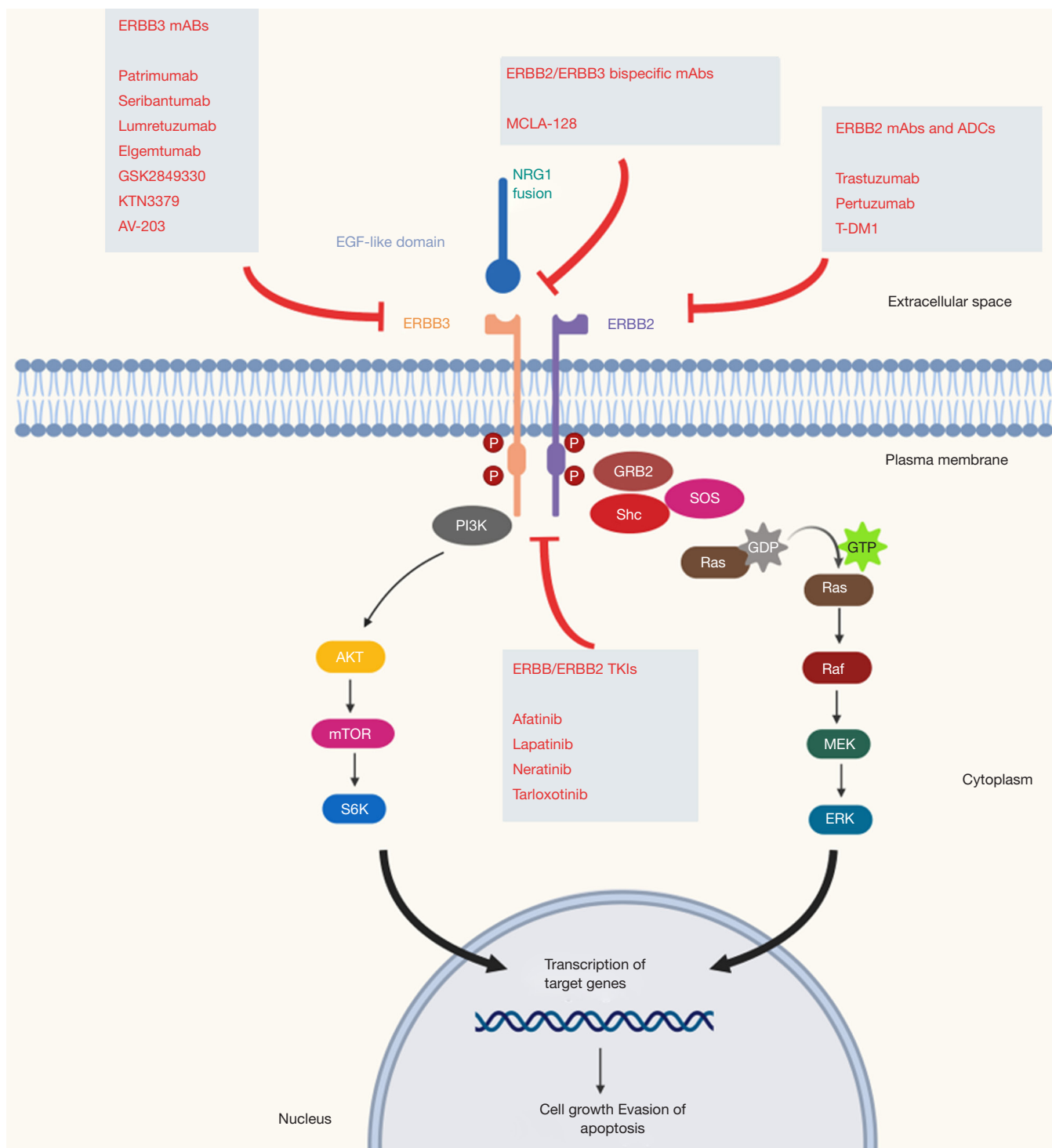


Figure 1 NRG1 fusion as therapeutic target (Created with BioRender). ADCs, antibody drug conjugates; AKT, v-akt murine thymoma viral oncogene homologue; GDP, guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; GTP, guanosine-5'-triphosphate; mAbs, monoclonal antibodies; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; P, phosphate; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; Raf, rapidly accelerated fibrosarcoma kinase; Ras, rat sarcoma kinase; SHC, Src homology 2 domain containing; S6K, ribosomal protein S6 kinase; TKIs, tyrosine kinase inhibitors.

Table 1 Prevalence of *NRG1* fusions in NSCLC

Ref.	Ethnicity	Cohort	<i>NRG1</i> fusions (%)	Concomitant oncogene drivers	Detection methods
(34)	Caucasian	51 IMAs and 34 non-IMA cases	31% IMAs and 3% non-IMAs	11% (<i>KRAS</i> mutations)	FISH and RNA sequencing
(23)	Asian	102 pan-negative LUAD NS	3.9% (27% IMAs)	None	RT-PCR
(36)	Asian	4,874 non-SqCC NSCLC	0.3%	None	RT-PCR and NGS
(20)	NR	9,592 NSCLC	0.3%	None	NGS RNA-sequencing
(25)	Asian	34 <i>KRAS</i> -negative IMAs	17.6%	None	NGS RNA-sequencing
(26)	Asian	13 IMAs	8%	None	Direct RNA-sequencing
(27)	Asian	59 IMAs	27%	62% (<i>KRAS</i> mutations)	NGS RNA-sequencing
(35)	Caucasian	404 NSCLC	0.5%	None	NGS RNA sequencing
(22)	Asian	1681 LUAD	0.36%	None	NGS RNA sequencing
(37)	Caucasian and Asian patients	25 IMAs	4%	None	FISH
(21)	Caucasian	2,079 LUAD	1.14%	None	NGS DNA-sequencing

NRG1, *neuregulin-1*; LUAD, lung adenocarcinoma; IMA, invasive mucinous adenocarcinoma; NS, never smoker; non-SqCC, non-squamous; RT-PCR, reverse transcriptase polymerase chain reaction; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; NR, not reported.

CD74-NRG1 was the most common variant (29%), followed by *AT1P1-NRG1* (10%) and *SDC4-NRG1* (7%) (20). Other fusion partners have been described at lower frequency and include *TNC*, *MDK*, *DIP2B*, *KIF13B*, *RBPMS*, *MRPL13*, *ROCK1*, *DPYSL2*, *SLC3A2*, *VAMP2*, *WRN*, *ITGB1* and *PARP8* in NSCLC (20-22,25,27,31,32). As commonly observed in other oncogene-addicted tumors, *NRG1* fusions are usually mutually exclusive with other oncogenic drivers, such as *EGFR*, *BRAF*, and *KRAS* mutations or *ALK*, *ROS1*, and *RET* rearrangements (20). However, in selected cases, *NRG1* fusions can coexist with other oncogenic drivers, such as *ALK* rearrangements (27,33) and *KRAS* amplification/mutations (27,32,34).

The reported incidence of *NRG1* fusions is of ~0.2–0.5% in unselected NSCLCs (35,36) (Table 1).

The gold standard for detection of *NRG1* gene fusions is RNA sequencing in comparison with DNA sequencing, although fluorescence *in situ* hybridization (FISH) can be used as a pre-screening method for its detection, but only genetic sequencing will allow the identification of the gene fusion (13). In a recent multicenter registry of 117 *NRG1*-positive lung cancers, RNA-based assays (anchored multiplex PCR, nCounter, RT-PCR, and transcriptome) were the most common detection methods (79.5%),

followed by FISH (12%) and DNA-based methods (hybrid capture-based NGS and amplicon-based NGS) (9.4%) (38). RNA-sequencing is associated with higher sensitivity for genetic rearrangements and can increase the detection of *NRG1* gene fusions compared with DNA-based methods, which often do not cover the large introns in *NRG1* (21).

The clinicopathological characteristics of *NRG1* fusion-positive NSCLCs were recently analyzed in a large multicenter retrospective study evaluating 117 patients. *NRG1* fusions were more frequently associated with female sex (54.7%) and never smoking history (43.6%), adenocarcinoma histology (94.9%), mostly of mucinous subtype (71%), and lung metastases (80% in stage IV patients). Treatment with platinum-based chemotherapy in 18 evaluable patients was associated with an 11% overall response rate (ORR) and a 61% disease control rate (DCR), whereas no responses were observed in patients treated with PD-1/PD-L1 inhibitors either as monotherapy (n=6) or in combination with chemotherapy (n=5) (38). This data, albeit limited by the small sample size, suggests that *NRG1* fusion-positive NSCLCs, consistent with other oncogene-addicted subgroups, is associated with low response to immune checkpoint inhibitors and other treatment strategies should be pursued.

Preclinical studies demonstrated that NRG1 signals through induction of HER2–HER3 heterodimers, leading to subsequent PI3K-AKT pathway activation and stimulation of oncogenic growth, and that the downstream signaling is inhibited by HER2/HER3 blockage (23,39). Different HER2/HER3 inhibitors are approved in other clinical indications (such as afatinib, pertuzumab, and neratinib) or are under active clinical development. Several case reports and small retrospective studies provided clinical evidence of activity of these agents (Table 2).

Afatinib is a potent and selective pan-inhibitor of HER family blocker, that covalently binds to and irreversibly blocks signaling from all homo- and heterodimers formed by the HER family members, including EGFR (*HER1*), HER2, *HER3* and *HER4*. It is currently Food and Drug Administration (FDA) approved for the treatment of *EGFR* mutated NSCLCs based on the results of the phase III trials LUX-Lung-3 and -6 (44,45) and for the treatment of squamous cell lung cancer after prior platinum-based chemotherapy based on the results of the LUX-Lung-8 study (46). A growing body of evidence suggest that afatinib is a potential treatment option for patients with *NRG1* fusion-positive tumors across multiple cancer types, including NSCLC, as reported in multiple case reports (Table 2). Recently a multicenter global registry of 117 *NRG1*-positive cases described afatinib activity in 12 patients with stage IV NSCLC harboring an *NRG1* gene fusion. In these heavily pretreated patients (line of treatment ranging from 1 to 15), afatinib was associated with a 33% ORR, a 50% DCR and a median PFS of 2.0 months. In a few patients, long term responses to afatinib were observed. However, the presence of *NRG1* gene fusion was associated with a favorable OS (4.83 months in stage IV patients) and was not significantly influenced by treatment with afatinib (38). Prospective studies are ongoing in the Drug Rediscovery Protocol trial (DRUP) (NCT02925234) and the Targeted Agent and Profiling Utilization Registry study (TAPUR) (NCT02693535) (42).

Early clinical proof-of-principle data demonstrated activity with HER3-directed targeted therapy in patients with advanced *NRG1*-rearranged cancers. GSK2849330 is an HER3 monoclonal antibody (mAb) that blocks the binding of NRG1 to HER3 and inhibits receptor heterodimerization. Drilon *et al.* reported a dramatic (32% tumor reduction) and durable response (19 months) to this HER3 mAb in an 86-year-old male with IMA harboring a *CD74-NRG1* gene fusion enrolled in an NSCLC expansion cohort of a phase I trial (NCT01966445). This trial included

more 28 patients with similar and higher HER3 expression, but without *NRG1* gene fusions and none of these patients responded to therapy. This clinical data is supported by preclinical evidence of antiproliferative activity in *NRG1* fusion-positive cell line MDA-MB-175-VII and durable tumor regression in a patient-derived xenograft (PDX) mouse model (21). In contrast, afatinib was associated with a significant reduction in tumor growth compared with vehicle, but no tumor regression was observed in the PDX model and no responses were observed in three patients harboring *NRG1* rearrangements (21). Several other HER3 inhibitors have been evaluated in clinical trials, such as patritumab (U3-1287, AMG-888), seribantumab (MM-121/SAR256212), lumretuzumab (RG7116), AV-203 and elgemtumab (LJM 716), these studies did not focus on *NRG1* gene fusions. The HER2/HER3 bispecific antibody MCLA-128, which blocks both NRG1 binding *in vitro* and *in vivo* activity in *NRG1* fusion-positive models (47). A global phase II basket trial (NCT02912949) is ongoing and will evaluate the safety and activity of this compound in three *NRG1* fusion-positive cohorts: pancreatic cancer (n=25), NSCLC (n=25), and other solid tumors (n=40). *NRG1* gene fusion assessment can be done with different molecular assay such as PCR, NGS (RNA or DNA) or FISH (48).

NTRK fusion genes

NTRK genes encode tropomyosin receptor kinases (Trk) family proteins, which includes three members (TRKA, TRKB and TRKC, encoded by *NTRK1*, *NTRK2*, and *NTRK3*, respectively). These receptor tyrosine kinases play a physiologic role in central and peripheral nervous system development and are activated by different ligands, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 4 (NT-4), and neurotrophin 3 (NT-3) (49-51). Once activated, TRKs signal through three main downstream pathways (MAPK, PI3K and PLC- γ), resulting in neuronal development and differentiation (52) (Figure 2).

Different mechanisms can be responsible of TRK oncogenic activation, although gene fusions involving *NTRK1*, *NTRK2* or *NTRK3* are the most commonly observed in solid tumors, including NSCLC. Several gene partners have been described to date and the majority harbor oligomerization domains that can constitutively activate the kinase domain of TRK (51).

Table 2 Case reports and retrospective case series of *NRG1* gene fusions positive NSCLC treated with HER2/HER3 inhibitors

Age (yrs)	Sex	Smoking habits	Histology	NRG1 Fusion	Treatment	Line(s) of therapy	Response	PFS (mos)	Treatment duration (mos)	Ref.
Case reports with HER2/HER3 inhibitors										
43	F	Never smoker	Adenocarcinoma	SDC4-NRG1	Afatinib	3 rd	PR	12	12	(40)
62	F	Never smoker	IMA	CD74-NRG1	Afatinib	2 nd	PR	6	6	(41)
42	M	Never smoker	Adenocarcinoma	SLC3A2-NRG1	Afatinib	2 nd	PR	12	18	(35)
62	M	Never smoker	Mucinous adenocarcinoma	CD74-NRG1	Afatinib	1 st	PR	10	>10	(35)
70	F	Never smoker	Non-mucinous adenocarcinoma	NR	Afatinib	15 th	PR	24	24	(42)
66	F	Never smoker	Non-mucinous adenocarcinoma	CD74-NRG1	Afatinib	5 th	PR	19+	19+	(42)
68	M	Former smoker	Non-mucinous adenocarcinoma	SDC4-NRG1	Afatinib	3 rd	SD	4	4	(42)
43	F	Never Smoker	IMA	CD74-NRG1	Afatinib	4 th	PR	18+	18+	(42)
81	M	Former cigar use	IMA	CD74-NRG1	Afatinib	1 st	SD	3	3	(21)
52	F	Current smoker	IMA	SDC4-NRG1	Afatinib	2 nd	PD	1	1	(21)
51	M	Former smoker	IMA	CD74-NRG1	Afatinib	1 st	PD	2	2	(21)
86	M	N.R.	IMA	CD74-NRG1	GSK2849330	5 th	PR	19	N.R.	(21)
55	F	Never Smoker	IMA	SLC3A2-NRG1	Lumretuzumab + erlotinib	6 th	SD	3.8	3.8	(43)
42	F	Never Smoker	IMA	SLC3A2-NRG1	Lumretuzumab + erlotinib	6 th	SD	3.8	3.8	(43)
Retrospective studies with HER2/HER3 inhibitors in <i>NRG1</i> gene fusions positive NSCLC										
12 (number of pts)					Afatinib*	1–15	ORR 33%, DCR 50%	PFS 2.0 months, OS not reached		(38)

*, one patient was treated with afatinib in combination with docetaxel-ramucirumab. *NRG1*, *neuregulin-1*; NR, not reported; mos, months; yrs, years; ORR, overall response rate; DCR, disease control rate; PFS, progression free survival; OS, overall survival; pts, patients; IMA, invasive mucinous adenocarcinoma; NSCLC, non-small cell lung cancer; PR, partial response; SD, stable disease; PD, progressive disease.

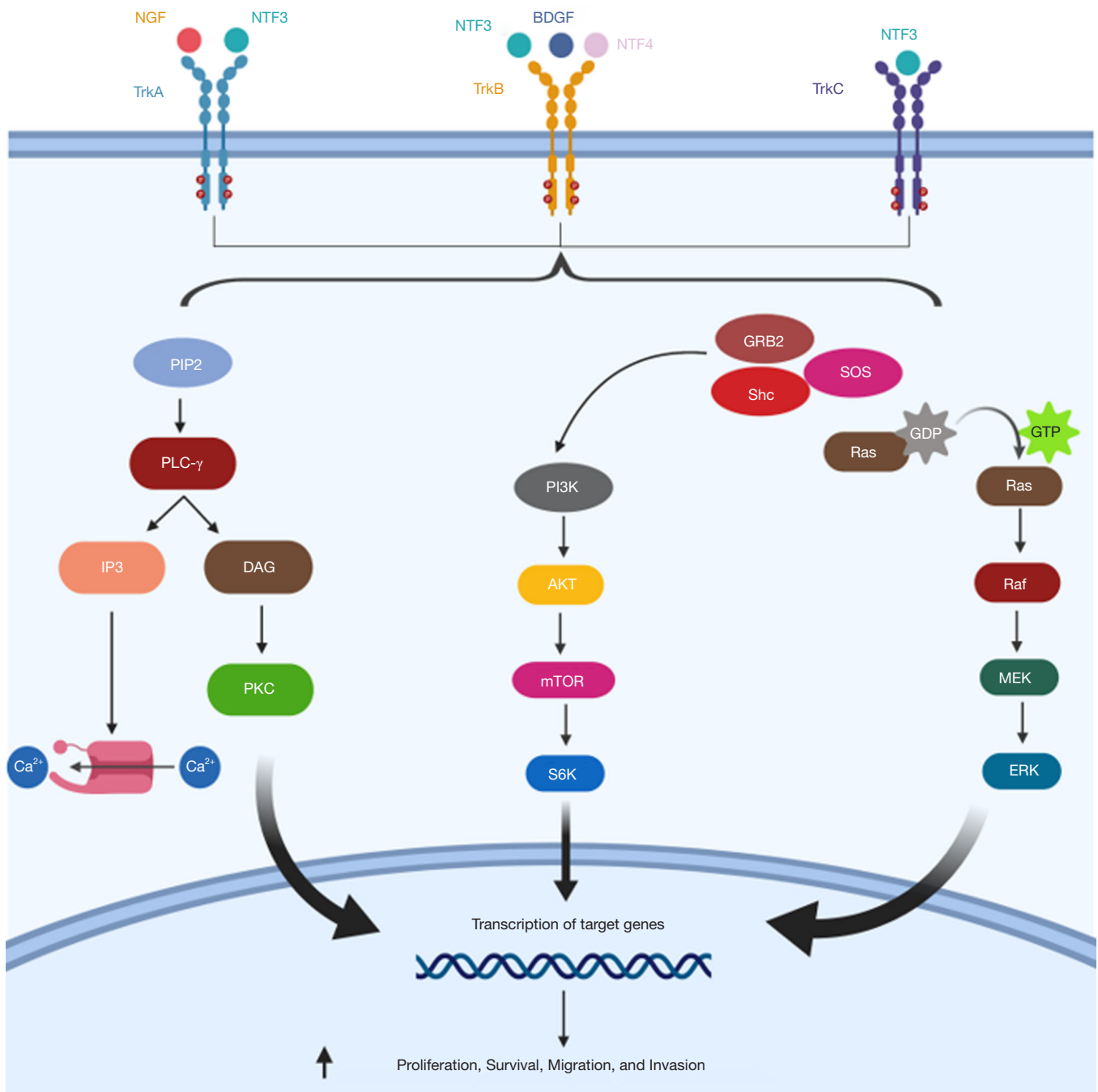


Figure 2 Schematic overview of Trk receptors signaling (*Created with BioRender*). AKT, v-akt murine thymoma viral oncogene homologue; BDGF, brain-derived growth factor; Ca²⁺, calcium ions; DAG, diacyl-glycerol; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; GTP, guanosine-5'-triphosphate; IP3, inositol trisphosphate; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NGF, nerve growth factor; NTF-3, neurotrophin 3; NTF-4, neurotrophin 4; P, phosphate; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C γ; PLC, phospholipase C γ; Raf, rapidly accelerated fibrosarcoma kinase; Ras, rat sarcoma kinase; SHC, Src homology 2 domain containing; S6K, ribosomal protein S6 kinase.

Table 3 Reported frequency of *NTRK* gene fusions in NSCLC

Study	Population [n]	Frequency (%)	NTRK	Fusion partner(s)	Detection methods
Farago, 2018 (53)	NSCLC [4,872]	0.23%	NTRK1, NTRK3	SQSTM1, TPR, IRF2BP2, TM3, MPRIP, ETV6	DNA NGS, RNA NGS or FISH
Vaishnavi, 2013 (50)	LUAD without oncogenic drivers [91]	3.3%	NTRK1	MPRIP, CD74	DNA NGS or FISH
Stransky, 2014 (54)	LUAD [513]	0.19%	NTRK2	TRIM24	RNA sequencing
Miyamoto, 2019 (36)	Non-SqCC NSCLC [4,874]	0.05%	NTRK3	NR	RT-PCR and NGS
Gatalica, 2018 (55)	LUAD [4,073]	0.1%	NTRK1-3	TPM3, SQSTM1, ETV6	DNA and RNA NGS & IHC
Ou, 2019 (56)	NSCLC [42,791]	0.1%	NTRK1-3	IRF2BP2, TPM3, and others	DNA NGS
Xia, 2019 (57)	NSCLC [21,155]	0.056%	NTRK1	CD74, IRF2BP2, LMNA, PHF20, SQSTM1, TPM3, TRP	DNA NGS

NTRK, neurotrophic receptor tyrosine kinase; NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; non-SqCC, non-squamous; RT-PCR, reverse transcriptase polymerase chain reaction; NGS, next generation sequencing; NR, not reported.

NTRK gene fusions were first identified in NSCLC in 2013 by Vaishnavi *et al.* through a targeted DNA NGS assay on tumor samples from 36 lung cancer patients without known oncogenic alterations, identifying two *NTRK1* gene fusions with *MPRIP* and *CD74*, respectively. They also developed a FISH assay to detect *NTRK1* rearrangements, reporting another additional case in a cohort of 56 lung adenocarcinoma samples without detectable oncogenic alterations (overall frequency 3.3%) (50). Since this initial report, subsequent studies have identified *NTRK* gene fusions in NSCLC with a frequency <1% in unselected patients (Table 3).

In contrast to other oncogenic rearrangements, such as *ALK* and *ROS1* translocations, that are associated with peculiar clinic-pathological characteristics (58,59), *NTRK* gene fusions seem not limited to specific subgroups of NSCLC patients and can occur in both squamous and non-squamous histology, including neuroendocrine carcinomas, independently of sex and smoking status (53). A large retrospective study analyzed data from 166,067 real world solid tumor samples sequenced by Foundation Medicine (FMI), showing that *NTRK* gene fusions do not co-occur with clinically actionable drivers in solid tumors, present a tumor mutational burden (TMB) generally similar to *NTRK* fusion-negative solid tumors, and occur at a slightly higher frequency in patients with Asian ancestry (0.46% in East Asian, 0.37% in South Asian, 0.34% in American, 0.29% in European and 0.32% in African) (60). Furthermore, *NTRK*

gene fusions in NSCLC seemed associated with levels of TMB and frequencies of PD-L1 expression higher than other molecularly defined subgroups (*EGFR*, *ALK* and *ROS1* altered cases) and co-exist with *STK11* mutations, which have been associated with decreased efficacy to immune checkpoint inhibitors (61), at frequencies similar to NSCLC in general but lower than the frequency in lung adenocarcinoma only (56). This data suggests that *NTRK* fusion positive NSCLCs might benefit from immunotherapy than is usually associated with lower efficacy in other oncogene addicted NSCLC subgroups (62). A recent retrospective study in a Chinese population showed that *NTRK1* fusions may coexist with *EGFR* mutations in *EGFR* tyrosine kinase inhibitor (TKI)-pretreated patients and might represent a potential mechanism of acquired resistance to these agents (57).

Besides NSCLC, *NTRK* fusions have been found in multiple tumors types that can be grouped according to the frequency at which these fusions are detected in:

- (I) Rare cancer types that present *NTRK* fusions with a prevalence >90%, including secretory breast carcinoma, mammary analogue secretory carcinoma (MASC), congenital mesoblastic nephroma (cellular or mixed subtypes) and infantile fibrosarcomas;
- (II) Common solid tumors with a prevalence of *NTRK* fusions of 5–25%, such as papillary thyroid cancers, spitzoid neoplasms, gastrointestinal stromal tumors (GIST) lacking canonical *KIT*, *PDGFRA* or *RAS*

alterations, and certain pediatric gliomas;

- (III) Common solid tumors with low *NTRK* gene fusions prevalence (<5%, but predominantly <1%), such as lung or pancreatic adenocarcinomas, head and neck squamous cell, biliary duct, breast, colorectal and renal cell carcinomas, melanomas, primary brain tumors of adulthood (such as astrocytomas or glioblastomas) and non-GIST soft-tissue sarcomas (51,52).

Different detection methods have been reported to date for *NTRK* fusions, including immunohistochemistry (IHC), FISH, reverse transcription PCR (RT-PCR), DNA-based NGS, RNA-based NGS, and DNA/RNA hybrid sequencing assays. Each of these methodologies is associated with different sensitivity and specificity for *NTRK* fusions as well as different turnaround time and cost (63). Selection of the appropriate assay for *NTRK* fusion detection seems to be influenced by tumor type and genes involved, as well as other factors such as available material, accessibility of various clinical assays, and whether comprehensive genomic testing is needed concurrently. Indeed, a recent retrospective analysis of 87 patients with oncogenic *NTRK1-3* fusions with various solid tumors identified by a targeted DNA-based NGS (MSK-IMPACT) or an RNA-based sequencing assay (MSK-Fusion) were tested with pan-Trk IHC. DNA-based sequencing showed an overall sensitivity and specificity of 81.1% and 99.9%, respectively, for the detection of *NTRK* fusions compared to RNA-based sequencing, where false negatives occurred when fusions involved breakpoints not covered by the assay. IHC showed overall sensitivity of 87.9% and specificity of 81.1%. Sensitivity was different according to the fusion type (96% for *NTRK1*, 100% for *NTRK2* fusions, and 79% for *NTRK3* fusions) and specificity differed by tumor histology (100% for carcinomas of the colon, lung, thyroid, pancreas, and biliary tract, but decreased to 82% and 52% for breast and salivary gland carcinomas, respectively) (64). A reasonable approach is to consider FISH or, if not available, pan-Trk IHC as the diagnostic test for rare tumors with high *NTRK* fusion prevalence (>90%), such as mammary analogue secretory carcinoma, congenital mesoblastic nephroma, infantile fibrosarcoma or secretory breast carcinoma. NGS confirmation of pan-Trk IHC positive cases can be conducted concurrently with treatment decision and should be considered in FISH/IHC negative cases. For tumors with lower frequency of *NTRK* fusions (5–25%) or rarely associated with these oncogenic drivers (<5%), such as lung cancer, the diagnostic algorithm depends on the use of

NGS as diagnostic tool in routine clinical practice. If NGS is routinely performed for molecular testing, *NTRK* fusions should be incorporated in NGS analysis. Alternatively, if NGS is not routinely performed for that specific tumor histology type or institutional unavailability, pan-Trk IHC can be used as screening test, followed by confirmatory NGS in positive cases (65). Similarly, the European Society for Medical Oncology (ESMO) recommendations for *NTRK* testing incorporated the use of FISH, RT-PCR or targeted RNA NGS assays for solid tumors known to harbor highly recurrent *NTRK* fusions, while upfront use of NGS (preferably RNA-based) followed by IHC to confirm positive cases or alternatively IHC as screening tool followed by NGS for tumors harboring *NTRK* fusions with lower frequency, as NSCLC (66).

Several TKIs with various degrees of activity against TRKA, TRKB and/or TRKC have been developed and two (larotrectinib and entrectinib) have been recently approved by the US FDA.

Larotrectinib (also known as LOXO-101 and ARRY-470) is a potent and highly selective pan-TRK (TRKA, TRKB, and TRKC) ATP-competitive inhibitor with a >100-fold selectivity for inhibition of TRK versus other kinases and >1,000-fold selectivity for tested non-kinase targets. In addition, larotrectinib was designed to have limited central nervous system penetration to reduce the potential risk of neurological toxicity due to the inhibition of TRK receptors normally expressed in the brain (67). The development program of larotrectinib in *NTRK* fusion-positive tumors included three studies: a phase I study involving adults, a phase I/II study involving children, and a phase II basket trial involving adolescents and adults (NAVIGATE). The preliminary analysis of the first 55 patients with 17 unique *TRK* fusion-positive tumor types (including 7% lung cancer patients) enrolled in the phase I studies reported a 75% ORR by independent central review (80% per investigators) and an 80% DCR. The recommended phase II dose (RP2D) was 100 mg twice daily for adults and children with a body surface area (BSA) ≥ 1 m² of and 100 mg/m² for children with a BSA <1 m² (4). Larotrectinib was well tolerated with adverse events (AE) mainly of grade 1–2, with 13% of patients developing a grade 3–4 event and only one patient discontinued due to an AE related to larotrectinib (68). Based on this preliminary data, larotrectinib was the first in class highly selective pan-TRK inhibitor to gain FDA approval and European Medicine Agency (EMA) conditional approval, independently of tumor histology.

Updated data of this primary cohort with additional 98

patients (153 in total) enrolled in an expanded patient cohort were recently presented at the 2019 ESMO meeting (68), confirming the impressive activity of larotrectinib [79% ORR, 95% confidence interval (CI): 72–85%] in *NTRK* fusion-positive patients. Responses were durable with a median duration of response of 35.2 months (95% CI: 22.8–NE) and a reported median PFS of 28.3 months (95% CI, 22.1–NE) and a median OS of 44.4 months (95% CI, 36.5–NE) in the integrated dataset of the expanded cohort (n=159, including 12 lung cancer patients) (68). Intracranial activity of larotrectinib has been reported recently in two *NTRK* fusion-positive patients with lung adenocarcinoma and triple negative breast cancer enrolled in the NAVIGATE study (69).

Preliminary data of the mechanisms of resistance to larotrectinib have also been reported in the primary cohort. Interestingly, of six patients with primary resistance, one had been pretreated with entrectinib and harbored a *NTRK3* G623R mutation, which is associated with steric interference of drug binding, and three of five had an unconfirmed TRK IHC expression. Furthermore, preliminary data of the mechanisms of acquired resistance to larotrectinib were reported, including substitution in the solvent front position (*NTRK1* G595R and *NTRK3* G623R mutations) or in the gatekeeper position (*NTRK1* F589L mutations) or the xDFG (a portion of the kinase-activation loop) position (*NTRK1* G667S and *NTRK3* G696A mutations) (4). In order to overcome acquired resistance mediated by recurrent kinase domain (solvent front and xDFG) mutations, a next generation TRK inhibitor, known as LOXO-195 (BAY 2731954), was designed. This compound demonstrated potent and selective activity against all three TRK kinases, their fusions, and acquired resistance mutations both preclinically and in patients (70). Preliminary safety and efficacy data of the phase I study (NCT03215511, n=20) and the FDA expanded access single patient protocol (n=11) were recently presented. LOXO-195 reported an ORR of 34% with a promising 45% ORR in 20 patients with TRK kinase mutations (50% in both solvent front and xDFG mutations and 25% in gatekeeper mutations) and lower ORR among those with unknown mechanisms of resistance (17%) and with by-pass track mechanism (0%) (71).

Entrectinib (RXDX-101, NMS-E628) is a pan-TRK, ROS1 and ALK ATP-competitive inhibitor with ability to cross the blood-brain-barrier (BBB) (72) that was recently FDA approved the treatment of patients with metastatic or unresectable solid tumors harboring a *NTRK* gene fusion without a known acquired resistance mutation and also

for the treatment of metastatic *ROS1* positive NSCLC. The combined analysis of two phase I trials of entrectinib (ALKA-372-001 and STARTRK-1) in 119 patients with various solid tumors, including 71 NSCLC (60%), reported a relatively safe toxic profile with the majority of AEs grade 1–2 and dose reduction required in only 15% of the patients. The RP2D was 600 mg daily. Preliminary data of efficacy were reported. No responses were observed in patients without genetic rearrangements of *NTRK1-3*, *ROS1* or *ALK*, with the exception of one patient with an *ALK F124SV* mutant neuroblastoma, and in *ROS1/ALK* fusion-positive patients who had been pretreated with one or more previous TKIs. The analysis of patients harboring *NTRK1-3*, *ROS1* or *ALK* rearrangements and no previous TKIs (“phase II eligible population”, n=25) showed a 100% ORR in three *NTRK* fusion positive patients with measurable disease, including one NSCLC, and a 60% disease reduction in an additional patient with a glioneuronal tumor. Promising activity was also seen in TKI-naïve *ROS1* (ORR 86%) and *ALK* (ORR 57%) rearranged tumors (73). An updated integrated analysis of phase I/II studies with entrectinib (ALKA-372-001, STARTRK-1, and STARTRK-2) was recently presented at the 2019 ESMO annual meeting (74,75). Among 54 *NTRK* fusion positive solid tumors, entrectinib reported a 59.3% ORR by blinded independent central review with a median duration of response of 12.9 months and a median OS of 23.9 months (95% CI, 16.8–NE). As expected, entrectinib was highly active even in patients with baseline brain metastases with an intracranial ORR of 54.5% and median intracranial duration of response not reached (75). The results in the NSCLC cohort (10 *NTRK* fusion positive patients) were consistent with the overall population with a 70% ORR and 10% complete response (74). Despite deep and clinically meaningful responses in many patients, resistance to entrectinib eventually occurs. The mechanisms of resistance were recently investigated in plasma samples from *NTRK* and *ROS1* fusion positive patients enrolled in the phase II basket trial STARTRK-2 using a plasma NGS platform (Foundation Medicine). Acquired resistance mutations were detected in 34% and 28% of *NTRK* and *ROS1* fusion positive patients, respectively, and off-target mechanisms of acquired resistance within the MAPK pathway were also reported in both groups (76). These data are in line with a recent report evaluating the mechanisms of resistance to various TRK inhibitors using tumor biopsies and cell free DNA and showing that MAPK signaling activation is a recurrent and convergent by-pass mediated

Table 4 Ongoing clinical trials with TRK inhibitors in *NTRK* fusion-positive solid tumors, including NSCLC

Study name	Phase	Drug	Population	Estimated enrollment (n)
NAVIGATE (NCT02576431)	II	Larotrectinib	Adults and children with <i>NTRK</i> -fusion positive solid tumors	320 patients
STARTRK-2 (NCT02568267)	II	Entrectinib	<i>NTRK</i> -, <i>ROS1</i> - and <i>ALK</i> -fusion positive solid tumors	300 patients
NCT01639508	I	Cabozantinib	<i>NTRK</i> fusion, or <i>MET</i> or <i>AXL</i> overexpression, amplification, or mutation (group B)	68 patients (groups A-C)
NCT03215511	I/II	LOXO-195	Adult and pediatric subjects with previously treated <i>NTRK</i> fusion cancers	93 patients
TRIDENT-1 (NCT03093116)	I/II	Repotrectinib	<i>NTRK</i> -, <i>ROS1</i> - and <i>ALK</i> -fusion positive solid tumors	450 patients
NCT02675491	I	DS-6051b	<i>NTRK</i> - or <i>ROS1</i> -fusion positive solid tumors	15 patients
NCT01804530	I	PLX7486	Solid tumors, including <i>NTRK</i> -fusion positive	59 patients-discontinued
NCT02920996	II	Merestinib	<i>NTRK</i> -fusion positive solid tumors or <i>MET</i> -mutation NSCLC	25 patients
NCT03556228	I	VMD-928	<i>NTRK1</i> alterations, including fusions, positive solid tumors	54 patients
NCT02219711	I	MGCD516	<i>NTRK</i> -fusion positive NSCLC	260 patients
ONTRK (NCT03182257)	I	ONO-7579	<i>NTRK</i> -fusion positive solid tumors	1 patient enrolled-discontinued due to commercial reasons

NTRK, neurotrophic receptor tyrosine kinase; NSCLC, non-small cell lung cancer.

resistance mechanism to various TRK inhibitors, including both first generation (larotrectinib and entrectinib) and next generation (LOXO195). The combination of TRK and MEK inhibitors has been shown to overcome these resistance mechanisms and, given the non-overlapping toxicities, might represent a promising therapeutic strategy in *NTRK* fusion positive patients (77).

Repotrectinib (TPX-0005) is next generation TRK, ALK and ROS1 TKI rationally designed to inhibit solvent-front substitutions (such as *ALK G1202R*, *ROS1 G2032R* or *ROS1 D2033N*, and *TRKA G595R*).

Repotrectinib exhibits activity against a variety of solvent front substitutions *in vitro* and *in vivo* and showed preliminary activity in patients with advanced ALK, ROS1, or *NTRK1*-3-rearranged cancers in the first-in-human dose-escalation phase I/II clinical trial (TRIDENT-1, NCT03093116), including a patient with a mammary analogue secretory carcinoma harboring an *ETV6-NTRK3* rearrangement and a *NTRK3 G623E* mutation acquired after progressing to entrectinib (78). Preliminary safety data on first 83 patients treated with various repotrectinib doses (from 40 mg daily to 200 mg twice a day under fasted/feed conditions) showed

a relatively safe toxicity profile. Most AEs were manageable and grade 1–2. The most common treatment-emergent AEs were dizziness (57%), dysgeusia (51%), dyspnea (30%), and fatigue (30%). Four dose-limiting toxicities occurred and were manageable with dose modifications: dyspnea/hypoxia G3 (n=1); G2 (n=1) and G3 (n=1) dizziness at 160 mg BID, and G3 dizziness (n=1) at 240 mg QD (79). The study is ongoing and efficacy data on *NTRK* fusion-positive patients are eagerly awaited.

Other TRK inhibitors that showed preclinical activity in *NTRK* fusion-positive models include the new selective ROS1/*NTRK* inhibitor DS-6051b (80), the IGF-1R/*NTRK* inhibitor BMS-536924 (81), the dual ALK/*NTRK* inhibitor TSR-011 (82), the multikinase inhibitor merestinib (LY2801653) (83), and the MET/TRK inhibitor altiratinib (DCC-2701) (84). Ongoing clinical trials with TRK inhibitors in *NTRK* fusion-positive solid tumor are summarized in Table 4.

Conclusions and future perspectives

Personalized medicine has revolutionized the therapeutic

approach to most of the solid tumors, including NSCLC, with unprecedented results in molecularly defined patient subgroups. This led to a dramatic shift in our vision of this disease moving from the old concept of a unique, highly frequent, indistinct entity to a multitude of several different molecular entities with peculiar clinico-pathological and therapeutic characteristics. Recently, the identification of rare genetic rearrangements at low frequency in different solid tumors has changed the old vision of drug development leading to the approval of targeted therapies after only phase I studies and independently of tumor histology. *NTRK* and *NRG1* gene fusions represent two of the most compelling examples of tumor agnostic biomarkers and, although present at very low frequency in NSCLC, constitute two clinically relevant subgroups of patients that can derive benefit from matched targeted drugs. The approval of the TRK inhibitors larotrectinib and entrectinib and the rapid clinical development of next generation TRK TKIs is reshaping the therapeutic algorithm of this small subgroups of patients, adding *NTRK* gene fusions to the list of genes that should be tested for the optimal treatment selection of NSCLC. The availability of highly potent and selective drugs directed against *NTRK* rearrangements further reinforce the utility of multiplex molecular testing in NSCLC overcoming the limits of single gene tests. *NRG1* fusion is the latest oncogene driver that has shown promising pharmacological exploitation, although the optimal therapeutic sequence should be still defined. Ongoing clinical trials with pan-HER TKIs (afatinib) or bispecific monoclonal antibodies targeting HER2/HER3 agents, such as MCLA-128, will provide definitive conclusions on the activity of targeted therapies in this rare subgroup of patients.

Acknowledgments

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure: Dr. Rolfo reports advisor board role for Inivata, ARCHER, MD Serono, Oncompass (non-financial), and Mylan; speakers' bureau for Astra Zeneca and MSD; honoraria from Elsevier; grants/research support from Lung Cancer Research Foundation (LCRF), American Cancer Society (ACS), GuardantHealth (non-financial), and Biomarkers (non-financial).

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doi: 10.21037/pcm.2020.03.02

Cite this article as: Russo A, Lopes AR, Scilla K, Mehra R, Adamo V, Oliveira J, Liu SV, Rolfo C. NTRK and NRG1 gene fusions in advanced non-small cell lung cancer (NSCLC). *Precis Cancer Med* 2020.