TRK fusion positive cancers: From first clinical data of a TRK inhibitor to future directions

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A B S T R A C T

Genetic alterations of neurotrophic tropomyosin or tyrosine receptor kinase (NTRK) 1/2/3 genes generate TRK fusion proteins that have been reported in a variety of adult and child cancers from diverse cell/tissue lineages. Larotrectinib, a tumour-agnostic TRK inhibitor, has shown remarkable efficacy in a novel “basket” study which has enrolled patients from infants to elderly with different TRK fusion-positive cancers. In this review, we focus on the challenges and expectations on the development of “tumour-agnostic” targeted therapies in rare malignancies.

1. Introduction

Cancer is normally classified and treated according to its pathologic features and the tissue/organ of origin. Recently, thanks to the new sequencing technologies and large-scale genomics approaches such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), several driver genetic alterations have been identified as useful biomarkers and targets for innovative cancer therapeutics. These discoveries have driven cancer sub-classification to a genetic alteration-based categorization (Zhang et al., 2019; Weinstein et al., 2013).

In agreement with this approach, lung adenocarcinoma has been subclassified taking into consideration possible translocations of ALK and ROS genes or mutations of KRAS and EGFR genes (Chang et al., 2015). The discovery of these genetic alterations has also brought to a substantial change toward precision cancer therapeutic strategies. More recently several other driver genetic alterations in this disease have been identified as a well as in a variety of other common and less common tumours (Chen and Chi, 2018).

For example, the BRAFV600 mutation is frequently observed in melanoma, colorectal cancer, hairy cell leukaemia and thyroid cancer, whereas it has been rarely reported in other cancers including lung adenocarcinoma (Hyman et al., 2015). For this particular mutation, variable objective response rates have been achieved ranging from 0% in colorectal cancer to more than 40% in mutant melanoma and non-small cell lung cancer (Hyman et al., 2015; Flaherty et al., 2010). Thus, despite a hypothesized uniform activity of potent selective BRAFV600 inhibitors in different tumour types harbouring the aforementioned mutated oncoprotein, as a consequence of their identical site of action, the variable tumour-specific cellular context may markedly impact on tissue lineage-specific primary resistance to treatment (Prahallad et al., 2012).

More recently, a novel therapeutic opportunity has emerged from the presence of oncogenic rearrangements of neurotrophic tropomyosin receptor kinase (NTRK) genes 1, 2, and 3. These genes encode for NTRKA, B, and C proteins that have been first found, although rarely, in common tumours such as colorectal, glioblastoma, lung and breast cancers, and may appear more frequently in a large variety of rare tumours (Cocco et al., 2018), and represent novel biomarkers and targets for cancer therapy. The response observed in diverse cancers to TRK inhibition has provided the first example of the histology-independent activity in a molecularly defined subset of cancers (Drilon et al., 2017, 2018).

This mini-review aims to summarize the role of NTRK gene fusions as an emergent target of cancer treatment.
2. TRK gene and signalling

Tropomyosin receptor kinases (TRK) including TRKA, TRKB and TRKC are tyrosine kinases receptors encoded by NTRK1, NTRK2 and NTRK3 genes usually expressed in the nervous system (Chao, 2003). Fig. 1, NTRK1 is mapped on chromosome 1q21-q22 (Weier et al., 1995) and encodes for a 796 residue protein (TRKA receptor) with an intracellular domain containing a short C-terminal tail, a TK domain and a juxtamembrane region (Mardy et al., 1999). Mutations altering the function of TRKA protein are reported in patients affected by the congenital insensitivity to pain with anhidrosis syndrome (CIPA) (Mardy et al., 1999). NTRK2 is located on chromosome 9q22.1 (Nakagawara et al., 1995) and includes 24 exons (Yeo et al., 2004) and encodes for an 822-residue protein (TRKB receptor) made of 2 immunoglobulins (Ig)-like domains, followed by a cysteine-rich domain, a leucine-rich domain, a second cysteine-rich domain and an N-terminal signal sequence. These sequences assemble the C-terminal PLCγ-docking site, a TK domain close to the C-terminus, an Src homology 2 domain, a transmembrane domain and the BDNF-binding region. The NTRK3 gene is found on chromosome 15q25 (Valent et al., 2020) and consist of a 145 kD glycoprotein mainly expressed in the human granular cell layer of cerebellum, hippocampus and cerebral cortex (Lamballe et al., 1991).

Reported functions of tyrosine kinases receptors are the promotion of synaptic plasticity and neuronal survival in the central nervous system once activated by tethering to complete neurotrophins such as the nerve growth factor (NGF) for TRKA, brain-derived growth factor (BDGF) for TRKB and Neurotrophin-3 (NT3) for TRKC (Chao, 2003). Some genetic alterations in NTRK genes such as translocations can cause TRK fusion proteins, leading to ligand-independent activation of TRK proteins and thus promoting the oncogenic process (Amatu et al., 2016; Martin-Zanca et al., 1986; Vaishnavi et al., 2015a). All TRK re-
domain, a transmembrane domain and an extracellular domain for ligand binding. The ligand binding initiates the process of oligomerization which if followed by the specific tyrosine residues phosphorylation in the intracytoplasmic kinase domain. This step leads to the activation of the pathway for proliferation, differentiation and survival in normal and malignant cells (Nakagawara, 2001).

The NGF binding to TRKA receptor triggers the activation of the Ras/Mitogen-activated protein kinase (MAPK) signalling pathway, leading to a higher rate of proliferation and cell growth via the Extracellular Signal-Regulated Kinase (ERK) pathway. TRKB triggers the BDNF signal through the PLCγ, PI3K and Ras-ERK pathway, turning into survival and neuronal differentiation whereas the coupling of NT3 with TRKC leads to the activation of the PI3/AKT pathway, increasing cell survival and precluding apoptosis (Nakagawara, 2001).

So far, more than 20 different types of cancer are supposed to be promoted by TRK fusion proteins. Except for some uncommon cancers such as congenital infantile fibrosarcoma, salivary gland mammary analog secretory carcinoma, congenital mesoblastic nephroma and se- cretory breast carcinoma, TRK genetic alterations has been observed in common cancers such as colorectal cancer, non-small cell lung cancer, breast ductal carcinoma, acute myeloid leukaemia and soft tissue sar- comas, although with low frequency (Amatu et al., 2016; Vaishnavi et al., 2015a; Knezevich et al., 1998; El Demellawy et al., 2016; Frattini et al., 2013; Zheng et al., 2014; Shi et al., 2016; Brenca et al., 2016; Wu et al., 2014; Bourgeois et al., 2000; Ross et al., 2014; Vaishnavi et al., 2013; Jones et al., 2013; Prasad et al., 2016; Wajjwalku et al., 1992; Braghiroli et al., 2016; Stransky et al., 2014; Tognon et al., 2002; Krings et al., 2017; Créancier et al., 2015; Ardini et al., 2014; Rubin et al., 1998) (Table 1). Thus, the clinical assessment of TRK inhibition still represents a great challenge mainly due to the heterogeneity of tumour types and the low frequency of TRK fusions.

3. TRK inhibitor larotrectinib demonstrates efficacy in a basket trial

Larotrectinib (ARRY470, Vitrakvi, LOXO101) is a new highly selective small agent and a potent inhibitor of the three TRK fusion proteins which has been recently assessed by Drilon and his colleagues (Drilon et al., 2018). Larotrectinib selectively blocks the ATP-binding site of TRK-family receptors with a 50 % inhibitory constant of 5−11 nmol/L in vivo (Vaishnavi et al., 2015b). The drug did not show activity when assessed on a panel of 226 non-TRKs whereas G1 cell-cycle arrest and apoptotic ability were observed when evaluated in vitro assay of TRK-expressing tumours (Doebel et al., 2015). Moreover, larotrectinib corroborated the in vivo dose-dependent tumour inhibi- tion on athymic nude mice (Doebel et al., 2015). In terms of phar- macokinetics, larotrectinib is rapidly absorbed after oral administration with a plasma concentration peak of 0.5−2 hours and an average half- life of 1.5−2 hours in both children and adults (Laetsch et al., 2018). However, the nasogastric and gastrostomic administration were also allowed for patients unable to orally take the drug and no food re- strictions were required in clinical trials. Although its linear pharma- cokinetic pattern with different dosing approaches, no drug accumu- lation was reported. The CYP3A4 is the key player in terms of drug metabolism while its elimination is performed by biliary and renal apparatus (Laetsch et al., 2018).

The safety and efficacy profile of larotrectinib was evaluated in the novel phase I/II “basket” clinical trial which enrolled patients based on their NTRK genetic alterations, regardless of their tumour type or age (Drilon et al., 2018). The trial involved 55 patients, aged between 4 months and 76 years old with 16 different tumour types, who were treated complying with three different protocols and the results were pooled. Drilon and colleagues showed that larotrectinib was overall well-tolerated with grade 3 or 4 adverse events (AE) reported by less than 5% of patients. Generally, the Response Evaluation Criteria in Solid Tumours (RECIST) response rate was 80 % (95 % confidence
interval, 61–85) by investigator assessment and 75 % (95 % confidence interval, 61–85) by independent review. 75 % of patients were on a continuous response to the treatment and 55 % of patients were progression-free after 1 year. However, after a median follow-up of 8.3 and 9.9 months, the average duration of response and progression-free survival (PFS) were both not achieved, even though effective responses were reported for most patients regardless of age group and tumour type. Despite the molecular identification of TRK fusion at a screening level, three out of six patients did not show any response to larotrectinib. This failure could be due to primary drug-resistance since no TRK proteins were detected by immunochemistry (IHC).

Secondary mutations in NTRK1 or NTRK3 genes including the DFG position (NTRK1 G667S or NTRK3 G696A), gatekeeper mutation (NTRK1 F589 L) and substitutions in the front position of solvent (NTRK1 G295R or NTRK3 G623R) were detected in nine out of ten patients who progressed after 6 months initial response. This acquired mechanism of resistance to larotrectinib is known to be shared by other oncogenic kinase-targeted therapies (Katayama et al., 2012; Kobayashi et al., 2005; Core et al., 2014) and, to overcome this issue, a new generation of TRK inhibitors are under development (Mardy et al., 1999; Lamballe et al., 1991; Amatu et al., 2016; Martin-Zanca et al., 1986; Nakagawara, 2001; Jones et al., 2013) (Table 2).

4. Future perspectives

The aforementioned study by Drilon and colleagues (Drilon et al., 2018) comes on the heels of several basket trials that specifically investigated, although reporting controversial clinical results the pan-HER kinase inhibitor neratinib in HER2 and HER3 mutant neoplasms (Hyman et al., 2018), the PDI inhibitor pembrolizumab in mismatch repair deficient neoplasms (Le et al., 2017) and the AKT inhibitor capivasertib (AZD5363) in AKT I17 K mutant neoplasms (Hyman et al., 2017). Drilon’s study represents a novel intriguing and efficient molecular-driven “basket” approach investigating a different group of tumours with uncommon driver mutations. The aim of the study is to open a window on a new set of treatments for patients with rare driver mutations in tumours. However, Drilon and colleagues did not report data on median duration of response, progression-free survival and long-term safety with several cancers types such as colon, lung, breast and melanoma lacking in the study. Therefore, subsequent analysis has been performed to evaluate the efficacy and long-term safety of larotrectinib in a larger population of patients with TRK fusion-positive solid tumours (Hong et al., 2020). A total of 159 patients with TRK fusion-positive cancer, including the previously evaluated 55 patients by Drilon, were enrolled and treated with larotrectinib. Of note, the
Actively assessing TRK inhibitors.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Targets</th>
<th>Stage</th>
<th>Trial Identifier</th>
<th>Company</th>
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</thead>
<tbody>
<tr>
<td>MCCD516 (Sitravatinib)</td>
<td>MET, AXL, RET, NTRK1/2/3, DDR2, KDR, PDGFRA, KIT or CBL</td>
<td>Phase I/II</td>
<td>NCT02219711</td>
<td>Mirati Therapeutics</td>
</tr>
<tr>
<td>RXDX-101 (Entrectinib)</td>
<td>NTRK1/2/3, ROS1, ALK</td>
<td>Phase I</td>
<td>NCT02097930</td>
<td>Hoffmann-La Roche</td>
</tr>
<tr>
<td>LOXO-101 (Larotrectinib)</td>
<td>NTRK1/2/3</td>
<td>Phase I</td>
<td>NCT02650401</td>
<td>Hoffmann-La Roche</td>
</tr>
<tr>
<td>LOXO-195 (Selretcinib)</td>
<td>NTRK1/2/3 (resistant)</td>
<td>Phase I/II</td>
<td>NCT02122913</td>
<td>Bayer</td>
</tr>
<tr>
<td>DS-6051b</td>
<td>NTRK1/2/3, ROS1</td>
<td>Phase I</td>
<td>NCT02637687</td>
<td>Bayer</td>
</tr>
<tr>
<td>LY2801653 (Merefitinib)</td>
<td>NTRK1/2/3, ROS1, TEK, MERTK, MSTRK, FLT3, AXL, DDR1/2, MKNN1/2</td>
<td>Phase I</td>
<td>NCT03834961</td>
<td>Children's Oncology Group</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>RET, NTRK1/2/3, MET, AXL, ROS1</td>
<td>Phase II</td>
<td>NCT02121704</td>
<td>National Cancer Institute (NCI)</td>
</tr>
<tr>
<td>VMO-908</td>
<td>NTRK1/2/3, ALK, ROS1</td>
<td>Phase I</td>
<td>NCT02576431</td>
<td>Bayer</td>
</tr>
<tr>
<td>TPX-0005 (Repotrectinib)</td>
<td>NTRK1/2/3, ALK, ROS1</td>
<td>Phase I/II</td>
<td>NCT03215531</td>
<td>Loxo Oncology</td>
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Proportion of patients with an objective response was approximately 80% according to investigator assessment (similar to the data previously reported by Drilon et al.) with 16% of patients who achieved a complete response. In addition, the median duration of response for larotrectinib was 35.2 months and the median progression-free survival was 28.3 months. Finally, the proportion of patients with TRK fusion-positive cancer who had a dose reduction because of adverse events was 8% with only 2% of patients who discontinued due to a drug-related adverse event (Hong et al., 2020). In 2019, Rosen et al. identified 76 cases with confirmed TRK fusions (0.28% overall prevalence) from a centre-wide screening program involving more than 26,000 prospectively sequenced patients (Rosen et al., 2020). The authors showed that the presence of a TRK fusion is negatively correlated with the presence of simultaneous concurrent oncogenic drivers mutation but positively associated with a lower tumour mutation burden with the only exception the concomitant presence of TRK fusion and microsatellite instability (MSI-H) in colorectal cancer. Besides, the authors reported that alternative standards of care, excluding immunotherapy, are effective for TRK fusion-positive cancers.

Entrectinib is an inhibitor of TRKA, B, C and ROS1, with demonstrated activity against central nervous system (Liu et al., 2018). More recently, data on the integrated efficacy and safety from two phase 1 trials (ALKA-372-001 and STARTRK-1) and one phase 2 trial (STARTRK-2), which have enrolled NTRK fusion-positive patients with metastatic or locally advanced solid tumours including patients with gastrointestinal cancers, confirmed the safety and efficacy of entrectinib for patients with NTRK fusion-positive solid tumours (Doebele et al., 2020; Siena et al., 2020).

Although all together these data suggest positive outcomes for the use of TRK inhibitors in cancer treatment, there are important issues and questions that should be addressed. In diagnostic pathology, NTRK gene fusions can be detected in tissue specimens by a variety of methods, including next-generation sequencing (NGS), targeted RNA sequencing, reverse transcriptase PCR (RT-PCR), fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). Each of these approaches shows specific advantages and drawbacks (Table 3).

Initially, FISH and RT-PCR have been widely used to achieve a low-cost detection of NTRK fusions in tumours with a high TRK prevalence and known NTRK fusions involving recurrent partners. For tumour types such as secretory breast cancer (with the ETV6-NTRK3 gene fusion with pathognomonic significance) and congenital infantile fibrosarcoma, a single gene testing approach could be more suitable.

Indeed, the FISH method has the advantage of being able to detect novel fusion partners if break-apart probes are used. The technique per se, however, does not allow the use of three probes at the same time for the same specimen. Therefore, for unknown fusions, three sets of break-apart FISH probes (i.e. one for each NTRK gene) are required.

RT-PCR is another highly sensitive and widespread methodology that enables the detection of fusion transcripts with known partners, reducing the costs and the turnaround time for well-characterized NTRK-tumours that, generally, are also very rare (Bubendorf et al., 2016; Church et al., 2018).

More recently, novel NGS technologies have emerged as mainstream testing platforms with the advantage of identifying multiple potential drivers in addition to NTRK gene fusions. However, the detection of NTRK gene fusions by multiplex targeted exome capture panels (e.g., MSK-IMPACT™, Foundation One®) may be challenging due to the presence of large intronic regions. It may be considered that both DNA- and RNA-based NGS assays are relatively labour intensive and cost-prohibitive for many laboratories: the former DNA-based assays do not identify novel fusions and have a moderate false-positive and false-negative rate whereas the latter RNA-based assays, have compelling sensitivity and novel fusion detection capability. The benefit of RNA-based NGS assays is the capability to recognize fusion gene transcript variants as well as levels of fusion gene transcript expression. Examples of currently available commercial NGS assays with the capacity to detect NTRK gene fusions are the Illumina TrueSight Tumor 170, the Thermo Fisher Oncomine™ Focus and the Oncomine™ Comprehensive panels (Illumina, 2020; Thermo Fisher, 2020; Bartlett, 2020). Sample (DNA versus RNA) and bioinformatic requirements, as well as the associated enrichment methods, vary between these kits with a consequent impact on their detection capabilities (Albert et al., 2019). In the short term, approval of NGS-based tests and achievement of broad insurance coverage for NGS-based testing are major challenges.

TRK expression can be also identified with IHC with reasonable sensitivity and efficiency (Rudzinski et al., 2018; Hechtman et al., 2017). However, IHC does not easily discriminate TRK fusion proteins arising from genetic alterations that targeted by TRK inhibitors, especially in tumours where TRK function and clinical impact remain poorly known. In some cancers and tumour tissues, the detection of TRK fusion proteins with IHC is associated with false positives especially when TRK proteins may be normally expressed, e.g. in neuroblastoma (Albert et al., 2019). Unlike NGS-based assays, an IHC multiplexing approach
might be expensive and require great effort. Consequently, the application of pan-NTRK IHC as a rapid routine diagnostic screening tool for common tumours with low TRK prevalence (such as colorectal cancer) is currently under debate.

Given the variety of malignancies targeted by TRK inhibitors, in both the adult and pediatric population, and the relatively low number of patients included in the clinical trials, there is the urgent need to better identify NTRK gene fusions expression in each tumour type. As reported by Albert et al., a simultaneous approach with IHC,FISH and RT-PCR is suggested for pediatric tumours with high TRK fusion frequency, although the initial IHC screening followed by NGS testing is currently preferred (Albert et al., 2019).

Despite the identification of NTRK alterations in NGS in 3 out of 6 patients, these patients did not respond to larotrectinib and did not show a clear expression of TRK fusion proteins with the use of pan-TRK IHC (Drilon et al., 2018). Therefore, it might be speculated that TRK expression and IHC identification are both necessary for the evaluation of the clinical response. Nowadays, Illumina NGS (DNA and RNA assays in a single design) and Pan-TRK IHC are both being introduced as complementary diagnostic tests for the treatment with larotrectinib and other TRK inhibitors. Although TRK IHC would be a sensible starting diagnostic tool for rare cancers with TRK fusions and possibly for common cancers without driver mutations, the better characterization of a single assay, capable of identifying druggable genetic alterations, is urgently needed. Finally, in the foreseeable future, several trials are awaited to investigate possible mechanisms of natural and/or acquired resistance to TRK inhibitors (Passiglia et al., 2016), including possible combinations of these drugs with chemotherapy, immunotherapy or targeted therapy.

Declaration of Competing Interest

DM has received honoraria for professional services and consultancy from Novartis, Bayer HealthCare Pharmaceuticals Inc., Pierre-Fabre, Sanofi Genzyme, MSD Italia S.r.l., Roche.

The other authors declare that there are no conflicts of interest in this work.

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