Spotlight on NTRK Gene Fusions:

Tumor-Agnostic Cancer Therapies and the Importance of Testing

NTRK Gene Fusions

As knowledge of the role gene mutations and alterations play in oncogenesis has grown, so has our understanding of cancer development and strategies for its treatment. Cancer cells typically harbor genomic alterations, or DNA changes; for purposes of this discussion, these may be broadly characterized as passenger or driver mutations.[1] Passenger mutations are not responsible for cancer progression. Rather, they are seen as occurring as a result of the underlying genomic instability of cancer, and as having limited impact on cancer development.[2] Conversely, oncogenic drivers initiate and promote tumor growth and can include mutations, amplifications, and gene fusions.[1]

Although a review of the role of gene fusion in oncology management is the primary objective of this discussion, a brief characterization of genomic alterations is in order. Gene mutations are the result of the incorrect placement of one of the four nucleotides comprising DNA. Gene amplifications occur when excess copies of a gene cause an abnormal level of protein production, ultimately resulting in abnormal cell growth and survival behaviors that lead to cancer.[1] This protein overexpression occurs when cells produce abnormally high amounts of protein as a result of genomic alterations.[1]

By definition, a gene fusion is a hybrid gene formed through the combination of two normally separated genes.[3] Gene fusions are a result of genomic rearrangements that may occur through a variety of mechanisms, including chromosomal rearrangements such as inversions, duplications, deletions, and translocations, as well as through transcription read-through or messenger RNA (mRNA) splicing.[3–5] The fusion of genes that occurs from chromosomal rearrangements can involve a protein kinase, which controls many cellular processes including cell proliferation and survival.[3–5] Since their initial discovery nearly 40 years ago, more than 11,000 gene fusions have been identified and implicated in more than 68,000 patient cases of malignancy.[4,5] Gene fusions were first discovered in hematologic malignancies; however, they have subsequently been implicated in a broad range of solid tumors. Some gene fusions are specific to a certain cancer type, while others may be common to a variety of malignancies.[6]

The neurotrophic tropomyosin receptor kinase (NTRK) gene family encodes the tropomyosin receptor kinase (TRK) family of proteins.[7] These are transmembrane proteins and function through the ligand-dependent transmission of extracellular signals to the nucleus, activating cell growth, proliferation, and survival pathways.

Table 1: Incidence of NTRK Gene Fusion by Tumor Type[7]

Tumor Type	Frequency of NTRK Gene Fusion
Infantile sarcoma	91% to 100%
Thyroid cancer	2% to 12%
High-grade gliomas (pediatric)	10%
Lung cancer	0.2% to 3%
Colon cancer	1%
Sarcoma	1%
Glioblastoma	1%
Head and neck squamous cell carcinoma	0.5%

Normally functioning TRK proteins are expressed in neuronal cells and each subtype plays a critical role in the development and maintenance of the nervous system.[7]

However, NTRK gene fusions are pathologic, leading to an overexpression of TRK proteins and promoting oncogensis.[1] Unless silenced, NTRK gene fusion leads to the expression of a chimeric protein, which retains the TRK kinase domain, but not the ligand-binding domain[1] NTRK genes, which result in constitutive activation of the downstream pathways (NTRK1, NTRK2, or NTRK3) and tend to fuse with housekeeping genes. Once fused, they are continuously turned on due to the genetic alteration. Therefore, NTRK gene fusions can lead to the development of solid tumors in a variety of tissue types.[3,4]

Incidence of NTRK Gene Fusions

NTRK gene fusions are extremely rare, occurring in 1% to 3% of all solid tumors, which extrapolates to approximately 1,500 to 5,000 patients annually in the U.S.[4] Although they occur infrequently in many of the common adult and pediatric cancers, NTRK gene fusions are found at high frequencies in certain rare pediatric tumors, including infantile fibrosarcoma and papillary thyroid cancer. Furthermore, NTRK gene fusions have been associated with at least 19 different tumor types, in both adults and pediatrics, including colorectal cancer, melanoma, and lung cancer.[4,7]

NTRK Inhibitors and Pipeline

Larotrectinib

Larotrectinib (Vitrakvi®, Loxo Oncology and Bayer) is a potent, highly selective small molecule inhibitor of TRKA, TRKB, and TRKC.[8] NTRK fusions can occur in a wide variety of tumor types. When larotrectinib binds to the kinase domain to inhibit the constitutive activation, its pharmacological effects and responses can be seen regardless of the tumor types. The safety and efficacy of larotrectinib was evaluated in a Phase I/II study that included 55 patients, ranging in age from 4 months to 76 years, with 17 unique TRK fusion-positive tumor types. The most common tumor types that were included in the study were salivary gland tumors (n=12), other soft tissue sarcoma (n=11), and infantile fibrosarcoma (n=7).[8]

Based on an independent review of the data, the overall response rate achieved was 75% (95% confidence interval [CI], 61 to 85). At one year, 71% of responses were ongoing and 55% of patients who achieved a response remained free of disease progression. In addition, after a median follow-up of 9.4 months, 86% of

patients who had achieved a response (38/44) were continuing treatment or had undergone surgery with curative intent.[8]

One-year data for larotrectinib was presented at the European Society of Medical Oncology (ESMO) 2018 Congress.[9] The update included data for the 55 patients included in the initial report discussed above, as well as 67 additional patients who had subsequently enrolled in the clinical development program. At the time of the data cut-off, the median duration of response had not yet been reached in either dataset, with median follow-up of 17.6 months in the primary dataset and 7.4 months in the supplementary dataset. In the primary dataset (n=55), the overall response rate was 80% (95% confidence interval [CI], 67% to 90%), with a 62% partial response rate and an 18% complete response rate.[9]

The Food and Drug Administration (FDA) granted larotrectinib the Breakthrough Therapy, Orphan Drug, and Rare Pediatric Disease designations, and granted accelerated approval in November 2018.[10–12] Larotrectinib (Vitrakvi) is indicated for the treatment of adult and pediatric patients with solid tumors that have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation, are metastatic, or where surgical resection is likely to result in severe morbidity and have no satisfactory alternative treatments or have progressed following treatment.[12]

Entrectinib

Entrectinib is an ATP-competitive TKI that has demonstrated in vitro and in vivo activity against NTRK1,2,3-, ROS1-, and ALK-rearranged cancers.[13] Similar to larotrectinib, entrectinib exerts its pharmacologic effect in a tumor-agnostic manner, based on the presence of NTRK gene fusions.[10,13]

An analysis of data integrated across three Phase I/II clinical trials (ALKA, STARTRK-1, and STARTRK-2) was presented at the ESMO 2018 Congress.[14] The studies enrolled patients with metastatic and/or locally advanced solid tumors with NTRK-fusions. The primary endpoints across the three trials were overall response rate and duration of response, with secondary endpoints that included safety, progression-free survival, and overall survival in patients with and without baseline central nervous system (CNS) disease.[14]

The evaluation of tumor-agnostic efficacy included 54 adults with at least six months of follow-up of NTRK-fusion positive tumors comprising more than 19 histopathologies involving 10 general tumor types.[14] Among these patients, the overall response rate was 57.4% (95% CI, 43.2% to 70.8%), which included four complete responses (7.4%). Furthermore, the median duration of response was 10.4 months (95% CI, 7.1 to not reached (NR)), median progression-free survival was 11.2 months (95% CI, 8.0 to 14.9), and median overall survival was 20.9 months (95% CI, 14.9 to NR). The median follow-up for survival was 12.9 months.[14]

The FDA granted entrectinib the Breakthrough Therapy designation in May 2017 and the Phase II STARTRK-2 basket trial evaluating entrectinib in solid tumors with NTRK, ROS1, or ALK fusions is currently ongoing.[13]

LOXO-195

LOXO-195 is an investigational, next-generation, selective TRK inhibitor specifically designed to address potential mechanisms of acquired resistance that may emerge in patients treated with larotrectinib or other multi-kinase inhibitors with anti-TRK activity.[15] In a small case report, two patients who had developed solvent-front substitution-mediated acquired resistance to larotrectinib were treated with LOXO-195.[16]

The first patient was a 55-year-old female with heavily pretreated advanced LMNA-NTRK1 fusion-positive colorectal cancer who had achieved a rapid partial response to larotrectinib but progressed after six months of therapy.[16] This patient achieved a rapid clinical response to LOXO-195, with a 38% decrease in measurable tumor burden. At the time of publication of the case report, the patient's tumor burden had decreased by 58% from baseline. In addition, the patient was reported as remaining on LOXO-195 at more than six months of therapy and tolerating therapy well.[16]

The second patient was a 2-year-old female with an ETV6-NTRK3 fusion-positive recurrent infantile fibrosarcoma of the right neck and base of the skull who experienced disease progression despite numerous attempted surgical resections and several lines of combination chemotherapy.[16] The patient had experienced greater than 90% tumor regression in response to larotrectinib; however, she experienced subsequent progression after eight months, and a biopsy confirmed the presence of an acquired TRKC G623R mutation. Within 15 days of initiating treatment with LOXO-195, the patient experienced visible tumor regression in a previously palpable mass in the head and neck region. A magnetic resonance imaging (MRI) after 28 days of treatment indicated a partial response, with tumor regression of at least 30%. Unfortunately, after approximately three months of therapy, the patient developed a new mediastinal mass and ultimately succumbed to her cancer.[16]

The efficacy and safety of LOXO-195 is currently being evaluated in a Phase I/II clinical trial.[15]

NTRK Gene Fusion Testing

The development of NTRK inhibitors represents a unique approach to the treatment of cancer whereby therapy is targeted toward a specific genetic marker and not the tumor type. NTRK inhibitors will only be effective in tumors that express NTRK gene fusions and not NTRK mutations or NTRK overexpression, and gene fusions are present in a small portion of solid tumors. Therefore, it will be imperative that genetic testing that can accurately identify appropriate treatment candidates be available. Genetic testing with high sensitivity and specificity for NTRK gene fusions will permit patients with such gene fusions present in their tumor to receive the most effective treatment possible and will help those who lack this genetic marker to avoid wasting time and resources on therapy that will not be effective.

As more targeted therapies become available, genetic testing has become part of routine care to help guide therapeutic selection. There are currently a handful of testing platforms available that are designed to identify the presence of NTRK gene fusions. Such testing platforms include DNA- and RNA-based next generation sequencing (NGS), DNA fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction (PCR) assays, and immunohistochemistry (IHC).[4]

NGS Testing: DNA-Based and RNA-Based

NGS testing offers a more comprehensive approach to the identification of actionable gene amplifications, mutations, or fusions (including NTRK fusions), with a definitive result.[17] In addition, NGS testing can detect multiple genes with a small tumor sample simultaneously, also referred to as a "multigene panel," which can be tested in a pathologist's laboratory or sent to outside companies for testing. As a result, the NGS test may also identify a different therapeutic option based on the presence of genetic mutations other than NTRK gene fusions.[17]

In an NGS-based screening study, NTRK1-rearranged cancer was estimated to exist in 1.5% of cases of colorectal cancer and 3.3% of lung adenocarcinoma, highlighting the importance of establishing accurate screening methods to correctly identify which patients are appropriate candidates for NTRK inhibitor therapy.[18]

Some of the disadvantages associated with the utilization of NGS testing include the high cost associated with the test itself, a previous lack of regulation and standardization, and a slower turnaround time for results compared to FISH testing.[4,17] The current Medicare allowable rates are approximately $602 and $2,919 for small and large panel tests, respectively; however, most coverage policies are restrictive beyond a small number of specific cancer types. In addition, the FDA has proposed requiring review of these tests.[4,17]

While NGS testing is the most comprehensive of the available molecular assays and can identify NTRK gene fusions and other actionable alterations all within the same test, not all NGS assays are able to detect NTRK gene fusions.[4] Among the NGS assay testing platforms that are currently available, many platforms now include RNA fusions in their NGS gene panels. Three such available, but not yet FDA-approved, NGS assay testing options include the following kits, all of which detect all three NTRK gene fusions:[4]

- Illumina TruSight Tumor 170
- Thermo Fisher OncomineTM Focus
- Archer® FusionPlex®

In addition, there are several commercial laboratories that perform NGS testing including NTRK1, NTRK2, and NTRK3, which include but are not limited to:[19-36]

- Foundation Medicine
- Caris Life Sciences
- Cancer Genetics, Inc.
- Sirona Dx
- PathGroup
- NeoGenomics Laboratories
- OmniSeq
- Paradigm Diagnostics
- Tempus

FISH Testing

Given the lower cost and quick turnaround, FISH testing represents a common testing modality for the detection of gene fusions and is considered a high sensitivity assay.[4] However, this testing option has limitations when searching for TRK fusions.[4] FISH testing can be used to detect NTRK gene fusions; however, in order to detect fusions at multiple locations, such as the three NTRK genes, multiple FISH tests would need to be run. Hence, multiple probes are needed for gene fusions involving multiple partners — and this could add to the cost and complexity of testing.[37]

To elaborate, dual-fusion probes detect specific, common, translocations associated with cancer.[38] However, some genes are involved in many different translocations that are associated with cancer.[38] Rather than use a specific dual-fusion probe to

test for each possible translocation, a break-apart probe can be used to help determine whether a specific gene is involved in a rearrangement.[38] If there is a translocation or rearrangement, then copies of the gene break into separate signals.[38] This provides evidence of translocation but, alone, is insufficient to determine the translocation partner.[38] Additionally, the technical equipment for testing is specialized and an experienced pathologist is required to interpret FISH results as it is viewed as a relatively subjective testing methodology.[4]

Reverse Transcriptase PCR

Reverse Transcriptase PCR (RT-PCR) differs from PCR in that it allows for the use of RNA as a template. This method involves the transcription of a portion of the RNA genome into complementary DNA, which is then amplified using PCR.[39–42] RT-PCR is arguably the most sensitive technique available for detecting and quantifying mRNA .[41] One of the key advantages of RT-PCR is that it requires a much smaller sample than other common techniques, including Northern blot analysis and RNase protection assay, and has been used to quantify RNA from a single cell. However, it may be challenging to detect gene fusions that can potentially involve multiple partners, (e.g., tumors in which various NTRK gene fusions are observed). This is because the fusion partner must be known in order for it to be detected using these methodologies.

There are several real-time RT-PCR chemistries available, including TaqMan® (Applied Biosystems), Molecular Beacons, Scorpions®, and SYBR® Green (Molecular Probes).[41]

Immunohistochemistry (IHC)

IHC testing detects protein expression in tissue samples by imaging the antibodies against that protein.[4] IHC, a well-established method for screening for NTRK gene fusions, is associated with efficiency, and a lower cost when compared to other molecular tests.[4] In addition, the test can be run in virtually any laboratory. One disadvantage associated with IHC testing is that laboratories can only run a single test at a time, and with a limited amount of tumor tissue available for the tests, there is a limit to how many tests can be run. In addition, IHC does not test for the NTRK gene fusion specifically but tests for the expression of protein, so there could be other genetic alterations or tissue types that lead to a high expression of protein. As a result, additional testing is needed to confirm the presence of the NTRK gene fusion.

In November 2018, Roche launched the Ventana pan-TRK (EPR17341) Assay, the first automated in vitro IHC assay to detect TRK proteins in cancer.[43] Another such companion diagnostic is the Trailblaze Pharos Diagnostic Suite (Ignyta), which utilizes IHC testing to measure protein expression levels for NRTK1, NTRK2, NTRK3, ROS1, and ALK and NGS to confirm a gene rearrangement of interest.[17] By blending the IHC and NGS testing methodologies, this strategy is able to identify TRK fusions for entrectinib (Ignyta), the investigational, selective inhibitor for all three TRK proteins that also targets the ROS1 and ALK receptor tyrosine kinases.[17]

Summary and Future Directions

As oncology shifts toward precision therapeutics, the role of precision diagnostics will become increasingly important. The recent approval of Vitrakvi (larotrectinib) highlights the significance of appropriate diagnostic testing considerations and optimized medical policies for patient identification. Several tests may be employed for the latter, ranging from screening/nonconfirmatory (e.g., IHC) to highly sensitive and specific assays (e.g., NGS). Testing choice will ultimately be influenced by many factors, including those at the physician and laboratory level, but also reflective of patient-level considerations, including tissue availability as well as patient life expectancy. The latter dimensions should also be appreciated when developing and implementing diagnostic strategies and policy for precision oncolytic therapy.

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