Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children


BACKGROUND
Fusions involving one of three tropomyosin receptor kinases (TRK) occur in diverse cancers in children and adults. We evaluated the efficacy and safety of larotrectinib, a highly selective TRK inhibitor, in adults and children who had tumors with these fusions.

METHODS
We enrolled patients with consecutively and prospectively identified TRK fusion–positive cancers, detected by molecular profiling as routinely performed at each site, into one of three protocols: a phase 1 study involving adults, a phase 1–2 study involving children, or a phase 2 study involving adolescents and adults. The primary end point for the combined analysis was the overall response rate according to independent review. Secondary end points included duration of response, progression-free survival, and safety.

RESULTS
A total of 55 patients, ranging in age from 4 months to 76 years, were enrolled and treated. Patients had 17 unique TRK fusion–positive tumor types. The overall response rate was 75% (95% confidence interval [CI], 61 to 85) according to independent review and 80% (95% CI, 67 to 90) according to investigator assessment. At 1 year, 71% of the responses were ongoing and 55% of the patients remained progression-free. The median duration of response and progression-free survival had not been reached. At a median follow-up of 9.4 months, 86% of the patients with a response (38 of 44 patients) were continuing treatment or had undergone surgery that was intended to be curative. Adverse events were predominantly of grade 1, and no adverse event of grade 3 or 4 that was considered by the investigators to be related to larotrectinib occurred in more than 5% of patients. No patient discontinued larotrectinib owing to drug-related adverse events.

CONCLUSIONS
Larotrectinib had marked and durable antitumor activity in patients with TRK fusion–positive cancer, regardless of the age of the patient or of the tumor type. (Funded by Loxo Oncology and others; ClinicalTrials.gov numbers, NCT02122913, NCT02637687, and NCT02576431.)
THE NEUROTROPHIC RECEPTOR TYROSINE KINASE GENES NTRK1, NTRK2, AND NTRK3 Encode the Tropomyosin Receptor Kinase (TRK) Proteins TRKA, TRKB, and TRKC, Respectively. After Embryogenesis, TRK Expression Is Limited Primarily to the Nervous System, Where These Kinases Help Regulate Pain, Proprioception, Appetite, and Memory. Recurrent Chromosomal Fusion Events Involving the Carboxy-terminal Kinase Domain of TRK and Various Upstream Amino-terminal Partners Have Been Identified Across Diverse Cancers That Occur in Children and Adults. TRK Fusions Lead to Overexpression of the Chimeric Protein, Resulting in Constitutively Active, Ligand-independent Downstream Signaling. Biologic Models and Early Clinical Evidence Suggest That These Fusions Lead to Oncogene Addiction Regardless of Tissue of Origin and, in Aggregate, May Be Implicated in Up to 1% of All Solid Tumors.

We Evaluated the Efficacy of Larotrectinib (Table S1 in the Supplementary Appendix, Available with the Full Text of This Article at NEJM.org), a Potent and Highly Selective Small-molecule Inhibitor of All Three TRK Proteins, in a Development Program That Encompassed Patients of Any Age and with Any Tumor Type (an “Age- and Tumor-agnostic” Therapy). The Program Involved Three Clinical Studies: a Phase 1 Study Involving Adults, a Phase 1–2 Study Involving Children, and a Phase 2 “Basket” Study Involving Adolescents and Adults. Here We Report an Integrated Safety and Efficacy Analysis of the First 55 Consecutively Enrolled Patients (a Sample Size That Was Established with Input from Global Regulators and That Was Designed to Rule Out a Lower Estimate of 30% for the Overall Response Rate) with Prospectively Identified TRK Fusion–positive Cancers Treated Across These Studies.

METHODS

PATIENTS

Specific Eligibility Criteria Varied According to Study Protocol (All Three Protocols and the Statistical Analysis Plan Are Available at NEJM.org). In General, Eligible Patients Had a Locally Advanced or Metastatic Solid Tumor, Had Received Standard Therapy Previously (If Available), Had an Eastern Cooperative Oncology Group Performance-status Score of 0 to 3 (on a Scale from 0 to 5, with Higher Scores Indicating Greater Disability), and Had Adequate Major Organ Function. An Early Amendment to the Phase 2 Study Involving Adolescents and Adults Prohibited Previous Treatment with Kinase Inhibitors with Anti-TRK Activity, Although One Such Patient Was Enrolled Before This Amendment.

All Three Protocols Were Approved by the Institutional Review Board or Independent Ethics Committee at Each Site, and All the Protocols Complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice Guidelines, the Declaration of Helsinki, and Local Laws. All the Patients, or Guardians for Patients Younger than 18 Years of Age, Provided Written Informed Consent.

STUDY DESIGN AND TREATMENT

All the Patients Who Enrolled in the Phase 1 Studies Involving Adults and Children Were Treated During the Dose-escalation Portion of Those Studies. At the Time That the 55th Patient with a TRK Fusion–Positive Cancer Was Enrolled Across the Program, the Phase 2 Portion of the Phase 1–2 Study Involving Children Had Not Yet Started; Thus, No Patient From That Phase 2 Study Is Included in the Current Report.

The Phase 2 Study Involving Adolescents and Adults Used the Recommended Dose of 100 mg of Larotrectinib Twice Daily, Administered Orally Continuously. Although a Maximally Tolerated Dose of Larotrectinib Was Not Defined, a Dose of 100 mg Twice Daily Was Selected for Adults and Children Who Had a Body-surface Area of at Least 1 m². For Children Who Had a Body-surface Area of Less Than 1 m², a Twice-daily Dose of 100 mg per Square Meter Was Selected. A Liquid Formulation Was Available for Patients Who Were Unable to Swallow Capsules. The Drug Was Administered Continuously Until Disease Progression, Withdrawal of the Patient from the Study, or the Occurrence of an Unacceptable Level of Adverse Events.

The Presence of a TRK Fusion Before Enrollment Was Mandated in the Phase 2 Basket Study But Was Not Required in the Phase 1 Studies That Involved Adults and Children, Although Patients with Prospectively Identified TRK Fusions in the Phase 1 Studies Were Included in This Integrated Efficacy Analysis. TRK Fusions Were Identified by Next-generation Sequencing, According to the Procedures and Analytic Pipelines Established by Each Laboratory, or by Fluorescence In situ
hybridization. All the testing was performed in a Clinical Laboratory Improvement Amendments–certified (or equivalent) laboratory. Details of the assays that were used to identify patients with TRK fusion–positive cancer are provided in the Supplementary Appendix.

The primary end point for the combined analysis was the overall response rate, as assessed by an independent radiology review committee according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Secondary end points included the overall response rate according to the investigator’s assessment, duration of response, progression-free survival, and safety.

**STUDY ASSESSMENTS**

Tumor assessments were performed by means of computed tomography, magnetic resonance imaging, and clinical measurement with electronic calipers (when appropriate), in the case of cutaneous lesions, at baseline and every 8 weeks for 1 year and every 12 weeks thereafter until disease progression. All tumor responses were confirmed at least 4 weeks after the initial response. Adverse events were assessed from the date that informed consent was obtained until at least 28 days after the last dose of larotrectinib was administered. Adverse events were classified and graded according to the Common Terminology Criteria for Adverse Events, version 4.0.

**STUDY OVERSIGHT**

The phase 1 study involving adults was designed by the sponsor, Loxo Oncology. The phase 1–2 study involving children was designed jointly by five of the authors and the sponsor. The phase 2 study involving adolescents and adults was designed jointly by the first and last authors and the sponsor. The sponsor collected and analyzed the data. The first and last authors had access to all the data and wrote the first draft of the manuscript. All the authors were involved in the data analysis and manuscript preparation. All the authors vouch for the completeness and accuracy of the data and analyses and for the adherence of the studies to the protocols. All the authors made the decision to submit the manuscript for publication. Editorial support, which did not include writing, was provided by Miller Medical Communications with funding from the sponsor.

**STATISTICAL ANALYSIS**

All the analyses were conducted in accordance with the statistical analysis plan. The decision to pool efficacy data from patients with a TRK fusion–positive tumor across all three studies was made early in the development program on the basis of the rarity of TRK fusions, the inherent heterogeneity of cancer types, and global regulatory advice. The primary analysis, presented in this article, was therefore based on the first 55 patients (children and adults) who were enrolled across the three larotrectinib studies and met the following criteria: they had a documented TRK fusion as determined by local testing; had a non–central nervous system primary tumor that could be assessed according to RECIST, version 1.1; and had received one or more doses of larotrectinib. As of the data-cutoff date, a total of 144 patients had received at least one dose of larotrectinib across the development program.

Analyses were performed according to the intention-to-treat principle. A true overall response rate of at least 50% was hypothesized, and we estimated that a sample of 55 patients would provide the study with 80% power to establish a lower boundary of 30% for a two-sided 95% exact binomial confidence interval. Ruling out a lower limit of 30% for the overall response rate was considered to be clinically meaningful and consistent with approved targeted therapies for genomically defined populations of patients who had stopped having a response to previous therapies. Confidence intervals were calculated with the use of the Clopper–Pearson method. Patients who underwent surgical resection and had no viable tumor cells and negative margins (i.e., had a pathological complete response), as well as having no remaining radiographic evidence of disease, were considered to have had a complete response, consistent with RECIST, version 1.1. Duration of response and progression-free survival were estimated by the Kaplan–Meier method according to the investigators’ assessments of response.

**RESULTS**

**PATIENTS**

From March 2015 through February 2017, we enrolled 55 consecutive patients across all studies who had TRK fusion–positive cancers that could be evaluated according to RECIST, version
The demographic characteristics of the patients, the tumor type, and fusion characteristics are summarized in Table 1, and in Table S2 in the Supplementary Appendix. Patients ranged in age from 4 months to 76 years.

This population of patients encompassed 17 unique cancer diagnoses, including mammary analogue secretory carcinoma of the salivary gland (in 12 patients), infantile fibrosarcoma (in 7), thyroid tumor (in 5), colon tumor (in 4), lung tumor (in 4), melanoma (in 4), gastrointestinal stromal tumor (in 3), and other cancers (in 16). This distribution in the enrollment of patients may reflect an increased vigilance of testing in rare tumor types that are known to be enriched for the presence of TRK fusions, such as salivary-gland cancer and infantile fibrosarcoma.6,10,11

TRK fusions involved TRKA (NTRK1) (in 45% of the patients), TRKB (NTRK2) (in 2%), and TRKC (NTRK3) (in 53%) and 14 unique upstream fusion partners. These TRK fusions were prospectively identified by means of next-generation sequencing (in 50 patients) or fluorescence in situ hybridization (in 5) at 15 laboratories. Confirmation of expression of the fusion transcript was not required or routinely performed.

**Efficacy**

At the primary data-cutoff date of July 17, 2017, the overall response rate was 75% (95% confidence interval [CI], 61 to 85), as determined by the independent radiology review committee (Table 2). A total of 13% of the patients (7 patients) had a complete response, 62% (34) had a partial response, 13% (7) had stable disease, 9% (5) had progressive disease, and 4% (2) could not be evaluated owing to early withdrawal for clinical deterioration. All the patients were accounted for in the analysis, including the 2 patients who could not be evaluated, per the intention-to-treat principle.

According to the investigator’s assessment, the overall response rate was 80% (95% CI, 67 to 90) (Table 2). Responses were observed regardless of tumor type (Fig. 1A), age of the patient, or TRK fusion characteristics (Fig. S1 in the Supplementary Appendix). The median time to response was 1.8 months (range, 0.9 to 6.4), a time point that was consistent with the first protocol-mandated assessment of response at 8 weeks (Fig. 1B). Two children with locally advanced infantile fibrosarcoma had sufficient tumor shrink-
age during treatment to allow for limb-sparing surgery that was intended to be curative. Pathological assessment confirmed negative margins (R0 surgery), and these two patients remain progression-free without larotrectinib treatment after 4.8 months and 6.0 months of follow-up.

The median duration of response had not been reached after a median follow-up duration of 8.3 months (range, 0.03+ to 24.9+) (plus signs indicate ongoing response at the time of data cutoff) (Fig. 2A). The median progression-free survival had not been reached after a median follow-up duration of 9.9 months (range, 0.7 to 25.9+) (Fig. 2B). At 1 year, 71% of responses were ongoing, and 55% of all patients remained progression-free. As of the data-cutoff date, 86% of the patients with a response (38 of 44 patients) were ongoing, and 55% of all patients remained progression-free. As of the data-cutoff date, 86% of the patients with a response (38 of 44 patients) were continuing to receive treatment or had undergone surgery that was intended to be curative. The patient with the longest response was the first patient with a TRK fusion–positive tumor to be treated; this patient was still receiving therapy at 27 months.12

**ADVERSE EVENTS**

Table 3 shows the adverse events, regardless of attribution, that occurred during treatment and that were seen in at least 15% of the patients, as well as adverse events of grade 3 or higher that were considered by the investigators to be related to larotrectinib. Clinically significant adverse events were uncommon, with the majority (964 of 1038 events [93%]) of all the adverse events being of grade 1 or 2. Few adverse events of grade 3 or 4, regardless of attribution, were observed. The most common were anemia (in 11% of the patients), an increase in the alanine aminotransferase or aspartate aminotransferase level (in 7%), weight increase (in 7%), and a decrease in the neutrophil count (in 7%). No grade 4 or 5 events were considered by the investigators to be related to treatment, and no treatment-related grade 3 adverse events occurred in more than 5% of the patients.

Of the 55 patients, 8 (15%) had their larotrectinib dose reduced. Adverse events leading to dose reduction included an increase in the alanine aminotransferase or aspartate aminotransferase level (in 4 patients), dizziness (in 2), and a decrease in the absolute neutrophil count (in 2). All these events were of grade 2 or 3. In all cases, patients whose doses were reduced had their best response maintained at the lower dose. No patients who had a response discontinued larotrectinib because of an adverse event.

**PRIMARY AND ACQUIRED RESISTANCE**

Given the high overall response rate, we sought to determine the potential mechanisms of primary resistance to larotrectinib, defined as a best response of progressive disease, which was observed in six patients (11%). One patient had previously been treated with another TRK inhibitor, and tumor sequencing before the administration of larotrectinib revealed an NTRK3 G623R mutation in the ATP-binding site of the kinase domain.13 The NTRK3 G623R mutation and its NTRK1 G595R parologue are termed “solvent front” mutations because they alter a hydrophilic solvent–exposed portion of the nucleotide-binding loop of the kinase domain, sterically interfere with larotrectinib binding, and reduce the inhibitory potency of larotrectinib.14 Tumor-derivative material was available for three of the five remaining patients for central analysis. In all three patients, central pan-TRK immunohistochemical testing did not confirm the presence of an expressed TRK fusion, which raises the possibility that the test performed at the local laboratory was a false positive or that the molecularly identified fusion was not expressed at the protein

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**Table 2. Overall Response Rate, According to Investigator and Central Assessment.**

<table>
<thead>
<tr>
<th>Response</th>
<th>Investigator Assessment (N = 55)</th>
<th>Central Assessment (N = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall response rate (95% CI)†</td>
<td>80 (67–90)</td>
<td>75 (61–85)</td>
</tr>
<tr>
<td>Best response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>64‡</td>
<td>62</td>
</tr>
<tr>
<td>Complete response</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Stable disease</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Could not be evaluated</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

† The best overall response was derived from the responses as assessed at specified time points according to the Response Evaluation Criteria in Solid Tumors, version 1.1.
‡ Data include one patient who had a partial response that was pending confirmation at the time of the database lock. The response was subsequently confirmed, and the patient’s treatment and response are ongoing.

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*Percentages may not total 100 because of rounding.
level; this finding potentially explains the lack of response in these patients (see the Supplementary Appendix).

We also sought to determine mechanisms of acquired resistance to larotrectinib, defined as disease progression during treatment after a documented objective response or stable disease for at least 6 months, as observed in 10 patients. Kinase domain mutations affecting the NTRK gene involved in the fusion were identified in tumor or plasma samples that were obtained after progression from all 9 patients who under-
Larotrectinib in TRK Fusion–Positive Cancers

went repeat testing (Table S3 in the Supplementary Appendix). Kinase domain mutations that were observed at progression included substitutions in the solvent front position (NTRK1 G595R or NTRK3 G623R; in 7 patients), the gatekeeper position (NTRK1 F589L; in 2), and the xDFG position (NTRK1 G667S or NTRK3 G696A; in 2). The xDFG mutations occur within a portion of the kinase-activation loop and sterically interfere with binding of the drug. All three categories of these mutations are paralogous to acquired resistance mutations that have been described for other classes of kinase inhibitors in oncogene-activated tumors.\textsuperscript{16,17} In 3 patients, more than one acquired resistance mutation was identified. Among the 10 patients in whom acquired resistance developed, 8 (80%) continued treatment with larotrectinib beyond progression because of ongoing clinical benefit, according to the judgment of their treating physicians.

**DISCUSSION**

In this series of studies, larotrectinib, a highly selective TRK inhibitor, had rapid, potent, and durable antitumor activity in children and adults with solid tumors with TRK fusions. The efficacy of larotrectinib in this diverse population compares favorably with response rates that have been seen in more clinically homogenous populations of patients receiving targeted therapy in the context of a validated oncogene. Our data not only validate TRK fusions as therapeutic targets but also show that they lead to tumor-agnostic sensitivity to larotrectinib. In contrast, for previously validated oncogenic drivers, drug responsiveness has been generally contingent on the presence of a genomic aberrancy and on tumor type.\textsuperscript{18}

Larotrectinib-related adverse events that led to dose reductions were rare, and in this sample of 55 patients with TRK fusion–positive cancer, no patients discontinued owing to drug-related adverse events. Additional data reflecting a longer follow-up and a larger patient experience may provide further insight into the safety profile of this agent. Similarly, although clinically meaningful durability of response was observed, continued follow-up will provide more information regarding the durability of larotrectinib benefit.

By sequencing tumor and plasma samples that were obtained at progression, we identified a convergent on-target mechanism of acquired drug resistance. Mutations altering the kinase domain of TRK explain most of the progression events that we observed. This finding is of immediate therapeutic relevance, given the early evidence of clinical activity that has been described with the next-generation TRK inhibitor LOXO-195.\textsuperscript{14} Specifically designed to address acquired kinase domain mutations such as solvent front substitutions, LOXO-195 is currently being evaluated in a phase 1–2 study involving children and adults (ClinicalTrials.gov number, NCT03215511).
In conclusion, TRK fusions defined a unique molecular subgroup of advanced solid tumors in children and adults in whom larotrectinib was highly active. Durable responses were observed without regard to the age of the patient, tumor tissue, and fusion status. The side-effect profile of larotrectinib suggests that long-term administration is feasible for patients. Screening strategies that include assays with the ability to detect TRK fusions will be needed in order to identify patients who may benefit from larotrectinib.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and their families.

**APPENDIX**

The authors’ full names and academic degrees are as follows: Alexander Drilon, M.D., Theodore W. Laetsch, M.D., Shivaani Kummar, M.D., Steven G. DuBois, M.D., Ulrik N. Lassen, M.D., Ph.D., George D. Demetri, M.D., Michael Nathenson, M.D., Robert C. Doebele, M.D., Ph.D., Anna F. Farago, M.D., Ph.D., Alberto S. Pappo, M.D., Brian Turpin, D.O., Afshin Dowlati, M.D., Marcia S. Brose, M.D., Leo Mascarenhas, M.D., Noah Federman, M.D., Jordan Berlin, M.D., Wafik S. El-Deiry, M.D., Ph.D., Christina Baik, M.D., M.P.H., John Deeken, M.D., Valentina Boni, M.D., Ph.D., Ramamoorthy Nagasubramanian, M.D., Matthew Taylor, M.D., Erin R. Rudzinski, M.D., Funda Meric-Bernstam, M.D., Davenandra S. Sohal, M.D., M.P.H., Patrick C. Ma, M.D., Luis E. Razez, M.D., Jaclyn F. Hechtman, M.D., Ryma Benayed, Ph.D., Marc Ladanyi, M.D., Brian B. Tuch, Ph.D., Kevin Ebata, Ph.D., Scott Crickshank, M.A., Nora C. Ku, M.D., Michael C. Cox, Pharm.D., Douglas S. Hawkins, M.D., David S. Hong, M.D., and David M. Hyman, M.D.

The authors’ affiliations are as follows: Memorial Sloan Kettering Cancer Center (A. Drilon, J.F.H., R.B., M.L., D.M.H.) and Weill Cornell Medical College (A. Drilon, D.M.H.), New York; University of Texas Southwestern Medical Center—Children’s Health, Dallas (T.W.L.); Stanford Cancer Center, Stanford University, Palo Alto (S.K.); Children’s Hospital Los Angeles, Keck School of Medicine, University of Southern California (L.M.); and UCLA David Geffen School of Medicine (N.F.), Los Angeles, and Loxo Oncology, South San Francisco (B.B.T., K.E., S.C., N.C.K., M.C.C.) — all in California; Dana-Farber–Boston Children’s Cancer and Blood Disorders Center (S.G.D.), Dana-Farber Cancer Institute (G.D.D., M.N.), Ludwig Center at Harvard (G.D.D.), and Massachusetts General Hospital...
tal (A.F.F.) — all in Boston; the Finsen Center, Rigshospitalet, Copenhagen (U.N.L.); University of Colorado, Aurora (R.C.D.); St. Jude Children’s Research Hospital, Memphis (A.S.P.); and Vanderbilt University, Nashville (J.B.) — both in Tennessee; Cincinnati Children’s Hospital Medical Center, Cincinnati (B.T.); University Hospitals of Cleveland Medical Center (A. Dowlati) and Taussig Cancer Institute, Cleveland Clinic (D.P.S.S.), Cleveland; University of Pennsylvania Perelman School of Medicine, Department of Otorhinolaryngology and Head and Neck Surgery, and the Abramson Cancer Center (M.S.B.), and Fox Chase Cancer Center (W.S.E.-D.), Philadelphia; University of Washington–Seattle Cancer Care Alliance (C.B.), Seattle Children’s Hospital (E.R.R.), and Seattle Children’s Hospital, University of Washington, Fred Hutchinson Cancer Research Center (D.S. Hawkins), Seattle; University of Texas M.D. Anderson Cancer Center, Houston (F.M.-B., D.S. Hong); Inova Schar Cancer Institute, Falls Church, VA (J.D.); START Madrid, Centro Integral Oncológico Clara Campal, Madrid (V.B.); Nemours Children’s Hospital, Orlando (R.N.), and Memorial Cancer Institute–Florida International University, Miami (L.E.R.) — both in Florida; Oregon Health and Science University, Portland (M.T.); and WVU Cancer Institute, West Virginia University, Morgantown (P.C.M.).

**REFERENCES**


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