A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion–Positive Solid Tumors

Alexander Drilon1,2, Ramamoorthy Nagasubramanian3, James F. Blake4, Nora Ku5, Brian B. Tuch5, Kevin Ebata5, Steve Smith6, Veronique Lauriault5, Gabrielle R. Kolakowski4, Barbara J. Brandhuber1, Paul D. Larsen4, Karyn S. Bouhana4, Shannon L. Winski4, Robyn Hamor4, Wen-I Wu4, Andrew Parker4, Tony H. Morales4, Francis X. Sullivan4, Walter E. DeWolf4, Lance A. Wollenberg4, Paul R. Gordon3, Dorothea N. Douglas-Lindsay3, Maurizio Scaltriti6,7, Ryma Benayed7, Sandeep Raj7, Bethany Hanusch1, Alison M. Schram1, Philip Jonsson6, Michael F. Berger2,6,9, Jaclyn F. Hechtman2,7, Barry S. Taylor6,8,9, Steve Andrews4, S. Michael Rothenberg5, and David M. Hyman1,2

ABSTRACT
Larotrectinib, a selective TRK tyrosine kinase inhibitor (TKI), has demonstrated histology-agnostic efficacy in patients with TRK fusion–positive cancers. Although responses to TRK inhibition can be dramatic and durable, duration of response may eventually be limited by acquired resistance. LOXO-195 is a selective TRK TKI designed to overcome acquired resistance mediated by recurrent kinase domain (solvent front and xDFG) mutations identified in multiple patients who have developed resistance to TRK TKIs. Activity against these acquired mutations was confirmed in enzyme and cell-based assays and in vivo tumor models. As clinical proof of concept, the first 2 patients with TRK fusion–positive cancers who developed acquired resistance mutations on larotrectinib were treated with LOXO-195 on a first-in-human basis, utilizing rapid dose titration guided by pharmacokinetic assessments. This approach led to rapid tumor responses and extended the overall duration of disease control achieved with TRK inhibition in both patients.

SIGNIFICANCE: LOXO-195 abrogated resistance in TRK fusion–positive cancers that acquired kinase domain mutations, a shared liability with all existing TRK TKIs. This establishes a role for sequential treatment by demonstrating continued TRK dependence and validates a paradigm for the accelerated development of next-generation inhibitors against validated oncogenic targets.

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1Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. 2Weill Cornell Medical College, New York, New York. 3Nemours Children’s Hospital, Orlando, Florida. 4Array BioPharma, Boulder, Colorado. 5Loxo Oncology, Inc., Stamford, Connecticut. 6Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York. 7Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, New York.

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S. Andrews, S.M. Rothenberg, and D.M. Hyman contributed equally to this article.

Corresponding Authors: David M. Hyman, Memorial Sloan Kettering Cancer Center, 300 E 66th Street, Room 1353, New York, NY 10065. Phone: 646-888-4544; Fax: 646-888-4270; E-mail: hymand@mskcc.org; and S. Michael Rothenberg, Loxo Oncology, Inc., 281 Tresser Boulevard, 9th Floor, Stamford, CT 06901. Phone: 203-653-3880; Fax: 203-724-2481; E-mail: rothenberg@loxooncology.com
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INTRODUCTION

Chromosomal rearrangements involving the NTRK1, NTRK2, or NTRK3 genes (encoding the TRKA, TRKB, or TRKC receptor tyrosine kinases) produce oncogenically activated fusion kinases in a wide variety of adult and pediatric malignancies (1). These fusions have been identified at varying frequencies in a multitude of adult and pediatric solid and hematologic malignancies. Rearrangements produce an in-frame fusion of the TRK kinase domain downstream of diverse 5’ gene partners (2). TRK fusions possess ligand-independent constitutive kinase activity, activate canonical downstream signaling pathways involved in growth and survival, transform primary cells in vitro and in vivo, and can occur in human cancers in a mutually exclusive pattern from other oncogenic drivers (2–5).

Recent data demonstrate NTRK fusions are highly therapeutically actionable drivers of tumor growth. In an integrated analysis of three studies of larotrectinib, the confirmed overall response rate in 50 evaluable patients was 76% (95% confidence interval, 62–87; ref. 6). A total of 17 unique tumor types were treated, and efficacy was observed regardless of patient age, tumor type, NTRK gene fusion, and upstream fusion partner. Responses have generally been long-lasting, with the median duration of response not yet reached and 91% of all responding patients remaining progression free at a 6-month landmark analysis.

Despite durable responses to TRK kinase–directed therapy in patients with NTRK-rearranged tumors, it is expected that acquired resistance to therapy will ultimately emerge in most patients. Consistent with this expectation, previous reports have described the acquisition of secondary mutations in the TRK kinase domain after treatment with entrectinib, a multikinase inhibitor with activity against TRK, in 2 patients. Specifically, TRKA G595R and G667C substitutions were identified in independent resistant clones from a patient with LMNA–NTRK3 fusion–positive colorectal cancer, and a TRKC G623R substitution (homologous to TRKA G595R) was identified in a patient with ETV6–NTRK3 fusion–positive mammary analogue secretory carcinoma (7, 8).

These mutations are homologous to resistance mutations previously identified in other kinase drivers such as ALK and ROS1 and affect the kinase solvent front (e.g., G595R and G623R in TRKA/C, G1202R in ALK, G2032R in ROS) and the xDFG motif (e.g., G667C in TRKA, G1269A in ALK; refs. 9, 10). Structural modeling suggests that it would have similarly negative effects on drug binding.

We describe the identification, preclinical characterization, and accelerated early clinical development of LOXO-195, a compound that is structurally distinct from existing TRK TKIs. LOXO-195 is an orally available, highly potent, and selective TRK kinase inhibitor designed to overcome resistance mediated by acquired kinase domain mutations.

RESULTS

Preclinical Development of LOXO-195

The preclinical development of LOXO-195 paralleled the early clinical development of larotrectinib in the hope that a clinical candidate could be ready in time for the first patients who progress on larotrectinib. Although a clinical case series of patients with acquired resistance on larotrectinib was not available, we performed directed mutagenesis experiments (12) and extrapolated from precedent mechanisms of resistance characterized for structurally similar oncogenic kinases (e.g., ALK, ROS1; refs. 9, 10) and published case studies (7, 8) to anticipate the structural basis of acquired resistance in the setting of larotrectinib exposure.

We used X-ray crystallography to understand whether solvent front or xDFG substitutions involving the ATP-binding site of TRK might impair larotrectinib binding potency (Fig. 1A, top). The glycine-to-arginine substitutions G595R and G623R in the solvent front of TRKA and TRKC, respectively, introduce steric clashes between the arginine side chain and the difluorophenyl group of larotrectinib. Similarly, the TRKA G667C or TRKC G696A xDFG substitutions create steric clashes between the cysteine or alanine side chain and the difluorophenyl group of larotrectinib. Although the TRKC G696A substitution at the homologous position to TRKA G667C (and ALK G1269A) has not yet been observed in patients, structural modeling suggests that it would have similarly negative effects on drug binding.

On the basis of these structural insights, we performed an in silico evaluation of a collection of structurally distinct, potent, and specific TRK kinase inhibitors to identify compounds that could best accommodate these acquired resistance mutations. From this screen, LOXO-195 was selected for further study. LOXO-195 is a low molecular weight macrocycle predicted to potently inhibit the clinically observed TRK resistance mutations (Supplementary Fig. S1A). In contrast to larotrectinib, structural modeling suggests that LOXO-195 can accommodate the bulky, positively charged arginine side chain in the solvent front without any steric clashes (Fig. 1A, bottom). Similarly, LOXO-195 is predicted to better accommodate TRKA and TRKC xDFG substitutions (G667C and G696A, respectively) than larotrectinib (Fig. 1A, bottom). Additional biochemical characterization revealed that TRKA G595R (but not TRKA G667C) increases the ATP affinity of the kinase (Km = 6 μmol/L for TRKA G595R vs. 51 μmol/L for TRKA), resulting in an intrinsically more active kinase reminiscent of the EGFR T790M gatekeeper substitution (16). Such dual effects on intrinsic kinase activity and inhibitor binding may be more challenging to overcome therapeutically. Finally, LOXO-195 showed high oral exposure across

in predominantly the same population used to validate and approve the preceding inhibitor.
preclinical species and exhibited absorption, distribution, metabolism, and excretion (ADME) properties favorable for projected human dosing (Supplementary Table S1).

**LOXO-195 Potently Inhibits Diverse Activated TRK Kinases In Vitro**

To determine the impact of TRK kinase resistance mutations on inhibitor activity, larotrectinib and LOXO-195 were tested against purified kinase domains in vitro. Both agents demonstrated strong binding to the wild-type TRKA, TRKB, and TRKC kinase domains (Supplementary Fig. S1B). Both drugs also had potent (i.e., IC<sub>50</sub> < 3 nmol/L) inhibitory activity in kinase enzyme assays (Fig. 1B). Importantly, LOXO-195 achieved low nanomolar inhibitory activity against TRKA G595R, TRKC G623R, and TRKA G667C, with IC<sub>50</sub> ranging from 2.0 to 9.8 nmol/L (Fig. 1B). In contrast, larotrectinib
displayed significantly reduced inhibitory activity in cells expressing TRKA G595R and G667C mutations as well as TRKC G623R (between 17- and 77-fold higher IC50 than for the wild-type kinases; Fig. 1B).

To verify that these in vitro findings translated into cell models, we assessed the activity of LOXO-195 and larotrectinib against activated TRK kinases in NIH-3T3 cells expressing TRK kinase acquired resistance mutations. We studied both ΔTRKA, a constitutively activating in-frame deletion of the TRKA extracellular domain (17), and the ETV6–NTRK3 fusion that has been identified in diverse human cancers. LOXO-195 displayed significant inhibitory activity in cells across all resistance mutations previously identified in patients (Fig. 2A) and preclinically (Supplementary Fig. S2A).

**Figure 2.** Effect of LOXO-195 and larotrectinib on TRK cellular activity. A, NIH 3T3 cells expressing the indicated TRK proteins were treated with LOXO-195 or larotrectinib for 1 hour, followed by quantification of cellular phospho-TRK (pTRK) levels by ELISA (for ΔTRKA, ΔTRKA G595R, and ΔTRKA G667C) or cellular phospho-ERK (pERK) levels by flow cytometry (for ETV6-TRKC, ETV6-TRKC G623R, and ETV6-TRKC G696A). IC50 (nmol/L) values are displayed as mean ± SD of at least three replicates. WT, wild-type. B, KM12 (TPM3-TRKA) xenografts or NIH 3T3 ΔTRKA (−/−, G595R or G667C) allografts following oral treatment with larotrectinib or LOXO-195 at the indicated doses (n = 7 animals/treatment group). Data are displayed as mean tumor volume ± SEM. QD, daily; BID, twice daily.
caused inhibition of tumor growth relative to vehicle at all doses in four TRKA-dependent tumor models (NIH 3T3 ΔTRKA, ΔTRKA G595R, ATRK G667C, and ΔTRK3–NTRK1 fusion–positive KM12 colorectal cancer cells; Fig. 2B). Larotrectinib inhibited KM12 and NIH 3T3 ΔTRKA tumors to a similar degree, but as expected had only a minimal effect on NIH 3T3 ΔTRKA G595R and ΔTRKA G667C tumors. Group mean body weight loss did not exceed 5% for any agent (Supplementary Fig. S2C).

**LOXO-195 Displays High Selectivity for the TRK Proteins**

To determine LOXO-195 selectivity for the TRK kinases, we profiled 228 individual kinases in vitro at a LOXO-195 concentration of 1 μmol/L, which is approximately 1,667-fold higher than its IC50 for TRKA (0.6 μmol/L). LOXO-195 was more than 1,000-fold selective for 98% of non-TRK kinases tested (Supplementary Table S3).

We extended our study of LOXO-195 selectivity in 87 human cancer cell lines. LOXO-195 demonstrated potent inhibition of cell proliferation in TRK fusion–containing KM12, CUTO-3, and MO-91 cell lines (IC50 ≤ 5 μmol/L; Supplementary Fig. S3). In contrast, LOXO-195 treatment with concentrations up to 10 μmol/L had no inhibitory effect on the growth of the 84 cell lines that did not contain a TRK fusion.

**Clinical Experience with LOXO-195**

We treated 2 patients with LOXO-195 whose tumors developed solvent front substitution-mediated acquired resistance to larotrectinib. These are the first 2 patients to develop acquired resistance to larotrectinib. The first patient was a 55-year-old woman with heavily pretreated advanced LMNA–NTRK1 fusion–positive colorectal cancer who achieved a rapid confirmed partial response to larotrectinib treatment but progressed after 6 months of treatment. Whole-exome sequencing of the pre- and post-larotrectinib treatment biopsies demonstrated a clonal TRKA G595R–driven recurrent tumor (Supplementary Fig. S4). The second patient was a 2-year-old girl with an ETV6–NTRK3 fusion–positive recurrent infantile fibrosarcoma of the right neck and base of skull who had progressed despite numerous attempted surgical resections and multiple lines of combination chemotherapy. A greater than 90% tumor regression was achieved in response to larotrectinib (18), followed by progression after 8 months, upon which repeat biopsy revealed an acquired TRKC G623R mutation.

Both patients were treated with LOXO-195 under FDA-allowed single-patient protocols (Fig. 3A). Because of clinical urgency and the desire to achieve biologically relevant clinical exposures, a real-time intrapatient pharmacokinetic-guided dose escalation approach was undertaken. By integrating predicted ADME data for LOXO-195 with in vitro and in vivo TRK-mutant potency estimates and preclinical toxicology studies, we established target pharmacokinetic thresholds (i.e., Cmax, Cmin, and AUC) thought to be consistent with significant TRK target coverage: at least 90% inhibition of TRK target coverage at Cmax, 50% inhibition at Cmin, and AUC that provides target inhibition for the duration of the dosing interval (Fig. 3B).

**Case 1: Adult LMNA–NTRK1-Rearranged Colorectal Cancer**

The adult patient with recurrent LMNA–NTRK1–rearranged colorectal cancer and a G595R acquired resistance mutation initiated treatment with LOXO-195 at a dose of 50 mg twice daily. Although real-time pharmacokinetic analysis revealed significant TRKA G595R target coverage at Cmax, Cmin, levels fell below the TRKA G595R IC50 (Fig. 3B, left; Supplementary Table S3). Therefore, on day 14, the dose was increased to 100 mg twice daily. This higher dose resulted in an approximately dose-proportional increase in exposure predicted to result in continuous TRKA G595R target inhibition with twice-daily dosing (Fig. 3B, left). The patient tolerated therapy well. Initial grade 2 dizziness and grade 1 diarrhea both resolved completely despite continued dosing of LOXO-195.

This patient experienced a rapid clinical response to therapy soon after starting LOXO-195, with decreasing abdominal pain and fullness. Repeat imaging after 4 weeks of therapy demonstrated a 38% decrease in measurable tumor burden that was accompanied by resolution of FDG-avidity in previously hypermetabolic liver, peritoneal, and adrenal metastases (Fig. 3C, top). Tumor burden continued to decrease with subsequent therapy, achieving a 58% decrease compared with baseline to date (a confirmed RECIST partial response). Concurrent profiling of tumor-derived cell-free DNA from plasma specimens collected longitudinally throughout LOXO-195 therapy detected the known G595R mutation at baseline. Consistent with the patient’s clinical and radiologic response, the G595R allele fraction decreased to undetectable levels after 2 weeks of treatment and has remained undetectable since this time (Fig. 3C, bottom). The patient remains on LOXO-195 at more than 6 months from initiation and is tolerating treatment well.

**Case 2: Pediatric ETV6–NTRK3-Rearranged Infantile Fibrosarcoma**

The pediatric patient with recurrent ETV6–NTRK3–rearranged infantile fibrosarcoma and a G623R acquired resistance mutation initiated treatment with a liquid formulation of LOXO-195 at a dose of 20 mg twice daily. The dose was increased to 60 mg twice daily after 7 days of treatment, and to 100 mg twice daily after 21 days on the 60 mg dose. These dose escalations produced approximate dose-proportional increases in exposure and resulted in significant TRKC G623R target coverage over the dosing interval (Fig. 3B, right; Supplementary Table S3). The patient tolerated LOXO-195 treatment well, with the only LOXO-195–related adverse event being grade 2 dizziness that did not interfere with dosing.

This patient demonstrated visible tumor regression in a previously palpable mass in the head and neck region within 15 days of initiating LOXO-195 treatment. Although a definitive assessment was limited by anatomic distortion from prior therapies, MRI after 28 days of treatment revealed a partial response, with tumor regression by at least 30% (Fig. 3D). Notwithstanding the clinical and radiographic response, the dose of LOXO-195 was increased to 100 mg twice daily with the goal of maximizing tumor regression prior to possible surgical re-section. Repeat MRI after 66 days of treatment confirmed an ongoing response (Fig. 3D). The patient continued LOXO-195 at the same dose for an additional 1 month, after which she was hospitalized for respiratory distress. Imaging revealed a new mediastinal mass and a pleural effusion. Despite further palliative chemotherapy, her condition worsened, and she succumbed to her cancer.
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**Figure 3.** Clinical testing strategy and proof of concept of LOXO-195 activity against acquired resistance mutations. **A,** Design of single-patient clinical protocols. AE, adverse event; PK, pharmacokinetics. **B,** Measured \( C_{\text{max}} \) and \( C_{\text{min}} \) (upper and lower black symbols, respectively) for larotrectinib and LOXO-195 for each patient are displayed relative to patient-specific TRK protein target coverage: TRKA/TRKC (green rectangles), TRKA G595R/TRKC G623R (red rectangles). The lower edge of each rectangle = IC_{50} for the indicated inhibitor/target, the upper edge = IC_{90}. xDFG (i.e., TRKA G667C, TRKC G696A) mutant target coverages (blue rectangles) are included for comparison. **C,** Top, CT-PET axial images from adult patient with LMNA1–NTRK1 G595R colorectal cancer demonstrating reduction in hypermetabolic liver and adnexal masses with LOXO-195 treatment; bottom, decrease in NTRK1 (TRKA) G595R cell-free DNA allelic fraction preceded the decrease in tumor burden by RECIST 1.1. **D,** Post-contrast axial T2 MRI images from the pediatric patient with ETV6–NTRK3 G623R infantile fibrosarcoma before (left) and after 28 (right) and 66 (bottom) days of LOXO-195 treatment. Arrows in **C** and **D** indicate disease sites.
DISCUSSION

For actionable molecular targets such as ABL, EGFR, and ALK, next-generation kinase inhibitors designed to overcome acquired resistance have extended the period of durable disease control in affected patients (9, 13, 15, 19–22). Historically, it has taken years to bring these next-generation inhibitors into the clinic, even after the predominant resistance mechanisms are identified in patients. Rarely, if ever, have some of the first patients participating in the initial studies of a TKI had immediate access to a next-generation inhibitor designed to overcome bona fide resistance mechanisms. Here, we report on the feasibility and utility of a paradigm for drug development that combines predictive models such as directed mutagenesis, crystallography-informed structural modeling, and a rapid rebiopsy approach in patients, utilizing both tissue and plasma. A dynamic medicinal chemistry collaboration can translate this structure–activity relationship knowledge into analogues worthy of clinical advancement, while a dynamic clinical collaboration can implement a pharmacologically guided dose escalation schema that delivers early clinical validation of the next-generation agent, and hopefully benefit to patients.

LOXO-195 was identified as a potent and selective TRK inhibitor with preclinical activity against all NTRK resistance mutations identified in patients to date, including solvent-front (e.g., TRKA G595R, TRKC G623R) and xDFG (e.g., TRKA G667C) substitutions. Importantly, solvent-front mutations have been described in every clinical case of acquired TRK inhibitor resistance described to date (including those herein), in contrast to other mutational sites that have only been shown in vitro or together with solvent front substitutions at low frequency. Notably, the solvent-front motif is a frequent site of acquired resistance mutations to TKIs in other kinases involved in recurrent rearrangements, including ALK (e.g., G1202R, G1202del, D1203N) and ROS1 (e.g., G2032R, D2033N), and it has also been challenging to overcome therapeutically (9, 10). The substitution of basic (e.g., arginine) and bulky (e.g., asparagine) residues in this motif directly interferes with inhibitor binding to the kinase ATP site. Moreover, TRKA G595R increases the ATP affinity of the kinase, resulting in intrinsically more kinase activity.

The combination of high potency, selectivity, and a wide therapeutic window in preclinical toxicology studies enabled our unique initial clinical development approach. We implemented single-patient protocols that utilized “real-time” safety- and pharmacokinetics-guided dose escalation. Our ability to safely and rapidly escalate the dose of LOXO-195 within weeks for each patient permitted drug exposures to be achieved that salvaged prior responses, with minimal toxicity. In the case of the adult patient, the duration of the ongoing response to LOXO-195 has exceeded that achieved with prior larotrectinib treatment. Although the duration of response in the pediatric case was shorter, this initial proof-of-principle experience with LOXO-195 warrants continued clinical development.

The unusually rapid entry of LOXO-195 into the clinic allowed us to treat the first 2 patients ever to develop acquired resistance to larotrectinib. This feat was accomplished by accelerating preclinical in vitro testing and in vivo toxicology studies, and by utilizing FDA’s expanded access program. We are not aware of a similar, precedent drug development approach.

The traditional phase I study design was developed in the cytotoxic chemotherapy era and was predicated on the concept that the fewest number of patients should be exposed to unacceptable toxicities. The resulting first-in-human dose estimates and dose-escalation schemes were therefore conservative. Less concern was given to the “underdosed” patient, as the underlying biologic hypothesis had often yet to be validated. However, in the setting of a validated target and rationally designed agent—or, arguably, a validated patient— who has acquired resistance following an initial response to a therapeutic class—great care should be afforded to the problem of subtherapeutic dosing. Our experience described here suggests that many of the historic risk-benefit assumptions that have guided phase I study design may need to be revisited when developing an agent like LOXO-195. A personalized, real-time clinical development strategy such as the one depicted in Fig. 4 may be more broadly appropriate for highly selective agents against clearly validated targets.

In summary, the next-generation TRK kinase inhibitor LOXO-195 possesses potent and selective activity against all three TRK kinases, their fusions, and acquired resistance mutations identified both preclinically and in patients. Sequential therapy that begins with larotrectinib and is followed by LOXO-195 after the development of acquired resistance represents a potential new treatment paradigm for patients with TRK-fusion solid tumors, with the opportunity to extend the period of durable disease control in some patients. A real-time, safety- and pharmacokinetics-guided phase I study of LOXO-195 for patients with advanced TRK fusion–positive cancers whose tumors have progressed on a prior TRK inhibitor is currently accruing (NCT03215511).

METHODS

Preclinical Studies

Structural modeling. Structural models of TRKA and TRKC mutants were derived from an in-house X-ray crystal structure of TRKA in complex with larotrectinib. Amino acid substitutions were carried out in Maestro (v.11), and side chain conformation and the homology model for TRKC were carried out with the Prime module (Schrödinger Release 2016-3).

Enzyme assays. Binding affinities for each purified TRK kinase domain were measured using Lanthascreen Eu Kinase Binding technology (Invitrogen). Briefly, each donor europium antibody–labeled recombinant TRK kinase domain was incubated with the Fluor 236 probe and a serial dilution of each inhibitor, and the effect of added inhibitor on probe binding was monitored by TR-FRET. Enzyme activity was determined by monitoring the incorporation of [γ-33P]-ATP into poly-EAY peptide substrate in the presence of a serial dilution of each compound. Kinase profiling was performed using KinaseProfiler (Millipore, Inc.). See Supplementary Methods for additional details.

Cell lines and assays. KM12 cells were obtained from the NCI-Frederick Cancer DCTD Tumor Cell Repository in 2011. CUTO-3 cells were obtained from Dr. Robert Doebele, University of Colorado Cancer Center (Aurora, CO) in 2015. MO-91 cells were obtained from Dr. Stephen Nimer, University of Miami Sylvester Cancer Center (Miami, FL) in 2015. Eighty-four NTRK gene rearrangement–negative...
human cancer cell lines were selected from the Eurofins OncoPanel collection of cell lines (Eurofins) in 2015. NIH 3T3 cells were obtained by Array BioPharma from 2007 to 2016. Eurofins cell lines were authenticated by short tandem repeat analysis (Genetica DNA Laboratories, Inc., last July 2015). KM12, CUTO-3, and MO-91 cells were authenticated by confirmation of the presence of each fusion (e.g., TPM3–NTRK1, ETV6–NTRK3, or MPRIP–NTRK1, respectively) by the Oncomine Focus Assay NGS assay (Thermo Fisher Scientific, Inc., within 12 months of experiments). NIH 3T3 cells expressing ΔTRKA and ETV6-TRKC variants were engineered as described in Supplementary Methods. For cellular phospho-TRK measurement, cells were seeded into 96-well plates and incubated overnight at 37°C, incubated for 1 hour with a dilution series of each inhibitor, followed by lysis of each well monolayer in situ and quantitation of phospho-TRKA levels by ELISA assay (Cell Signaling Technologies) per the manufacturer’s instructions.

**In vivo studies.** All animal studies were performed in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NRC) and AAALAC-International. Each cell line (2–5 × 10^6 cells) was injected subcutaneously into female nu/nu NCr mice age 7 to 9 weeks (Taconic) and allowed to grow to approximately 100 to 200 mm³ (efficacy) or approximately 500 mm³ (pharmacokinetics/pharmacodynamics) prior to randomization by tumor size and treatment with each inhibitor by oral gavage. For pharmacokinetic/pharmacodynamic analysis, animals were dosed for 3 days, followed by euthanasia, excision of tumors, and collection of plasma. Phospho-TRK levels in tumor lysates were determined by ELISA assay, whereas plasma inhibitor levels were determined by LC/MS-MS. For efficacy analysis, animals were dosed by oral gavage, and body weight and tumor size were monitored at regular intervals. See Supplementary Methods for additional details.

**Figure 4.** Traditional versus pharmacokinetics (PK)-guided, accelerated phase I design. Traditional phase I “3 + 3” dose escalation studies enroll between 3 and 6 patients at each dose level based on observed toxicity, with dose increments becoming smaller as dose increases (top). This conservative design, developed in the cytotoxic era, risks underdosing patients with validated targets. In a first-in-human phase I study of a rationally designed agent against a validated target, the risk of subtherapeutic dosing may represent a greater harm to patients. For this situation, we propose a pharmacokinetics-guided, accelerated phase I design (bottom). This design combines multiple efficiencies including: (i) biomarker selection during dose escalation; (ii) pharmacokinetics-guided rapid intrapatient dose escalation during a single cycle of therapy, (iii) sequentially escalating starting dose, and (iv) early dose expansion upon identification of a therapeutic dose.
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TREATMENT AND RESPONSE ASSESSMENT.
LOXO-195 was administered to patients as a powder-in-capule or liquid formulation. Dose escalation was allowed only after a minimum of 7 days at a specific dose level, and only in the absence of any grade 3–4 toxicity. Increases were limited to no more than three times the previous daily dose level. Dose modifications and interruptions followed a prescribed algorithm. Adverse events were captured using CTCAE version 4.03. Response was evaluated using RECIST 1.1.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
A. Drilon has provided expert testimony for Ignyta, LOXO, and TP Therapeutics. V. Laurault is a toxicologist for ToxConsult LLC. F.X. Sullivan has ownership interest (including patents) in Array BioPharma, Inc. M. Scaltriti is a consultant/advisory board member for VincentTech. D.M. Hyman reports receiving commercial research grants from AstraZeneca, Loxo Oncology, and PUMA Biotechnology and is a consultant/advisory board member for Atara Bioterapeutics, Boehringer ingelheim, Chugai, and CytoMex. No potential conflicts of interest were disclosed by the other authors.

AUTHORS’ CONTRIBUTIONS
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Drilon, R. Nagasubramanian, N. Ku, P.D. Larsen, K.S. Bouhanna, S.L. Winski, R. Hamor, T.H. Morales, F.X. Sullivan, W.E. DeWolf, P.R. Gordon, D.N. Douglas-Lindsay, M. Scaltriti, R. Benayed, A.M. Schram, M.F. Berger, B.S. Taylor, D.M. Hyman
Writing, review, and/or revision of the manuscript: A. Drilon, R. Nagasubramanian, A.M. Schram, S. Andrews, S.M. Rothenberg, D.M. Hyman
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Drilon, R. Nagasubramanian, N. Ku, G.R. Kolakowski, W.-I Wu, A. Parker, W.E. DeWolf, B. Hanusch, D.M. Hyman
Other (toxicologist for this program): V. Laurault
Other (wrote supplementary methods for cell-based assays): P.D. Larsen

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