SUPPLEMENT FOR: A Second Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to First Generation TRK Inhibitors in Patients with *NTRK*-Rearranged Solid Tumors

AUTHORS

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SUPPLEMENTARY METHODS

Modeling inhibitor binding

Structural models of various TRKA and TRKC mutants were derived from an in-house X-ray crystal structure of TRKA in complex with larotrectinib. For the substitution of an arginine

residue for glycine at position 595 in TRKA (G595R) and a cysteine for glycine at 667 (G667C), the mutations were carried out in Maestro (Schrodinger Release 2016-3). For both mutants, the likely side chain conformations (illustrated in **Figure 1** of the manuscript) were predicted with the Prime module (Schrodinger Release 2016-3), using the default settings (1). No modifications to the backbone conformations were considered during this procedure. A homology model of TRKC was produced from the X-ray crystal structure of LOXO-101 bound to TRKA, also with the Prime module[2] and default settings. The TRKA and TRKC sequence alignment was derived from published X-ray structures (2). The corresponding G623R and G696A TRKC mutants were produced from the TRKC homology model utilizing the identical procedure described above for the TRKA mutants.

LOXO-195 synthesis

Please see US Patent 8,933,084 B2, Column 67, Example 33 for LOXO-195 and references therein (3).

Enzyme assays

TRK binding assays

The affinity of a compound binding to the TRK kinase was measured using LanthaScreen™ Eu Kinase Binding technology (Invitrogen, Inc.). Briefly, His-tagged recombinant human TRK cytoplasmic domain (5 nM TRKA or 5 nM TRKC) was incubated with 5 nM Alexa-Fluor® Tracer 236, 2 nM biotinylated anti-His antibody and 2 nM europium-labeled Streptavidin along with test compound in a buffer consisting of 25 mM MOPS, pH 7.5, 5 mM $MgCl_2$, 0.005% Triton X-100, and 2% DMSO. Compounds were prepared in a three-fold serial dilution in DMSO and added to the assay to give the appropriate final concentration. After a 60-minute incubation at 22 °C, the reaction was measured using an EnVision multimode plate reader (PerkinElmer, Inc.) via TR-FRET dual wavelength detection, and the POC was calculated using a ratio metric emission factor. 100 POC was determined using no test compounds and 0 POC was determined using a concentration of control compound that completely inhibits the enzyme. The POC values were fit to a 4-parameter logistic curve and the IC_{50} value is the point where the curve crosses 50 POC.

TRK enzyme radioactive activity assay

The activity of compounds against wild type and mutant TRKA and TRKC was determined by monitoring the incorporation of $[^{33}P]PO_4$ from $[\gamma\text{-}^{33}P]$-ATP into poly-EAY. The assays were conducted in 96-well polypropylene V-bottom microtiter in a total volume of 50 µL. Reaction mixtures typically contained 25 mM Na^+MOPS, pH 7.4, 5 mM $MgCl_2$, 0.005% Triton X-100, 2% DMSO, 1 mM DTT, 5 µM $[^{33}P]$-ATP (40 µCi/mL), 100 µg/mL poly-EAY, TRKA or TRKC (wild-type or mutant at an appropriate concentration ranging from 0.25-10 nM, depending on the construct) and compound varying over a 10-point, three-fold dilution series ranging from 2000 to 0.1 nM. Incubations were conducted at 22 °C for 60 min and quenched by the addition of 100-µL aliquots of 25% trichloroacetic acid. The radiolabeled product was then captured on glass fiber filter plates using a MACH III Harvester 96® (Tomtec, Inc.). After adding 35 µL/well of

liquid scintillation cocktail, the plates were counted in a TopCount NXT (PerkinElmer, Inc.) using a counting time of 30 s/well.

The wild type and mutant enzymes of TRKA contain amino acids 486-786, and TRKC, amino acids 506-829. All enzymes contain an N-terminal six histidine tag, were expressed in *E. coli* and then were purified using nickel-NTA resin.

K_m estimates for the TRK enzymes
The K_m's for ATP of TRKA and TRKA-G595R was determined using the assay described above in the absence of compound, except that the concentration of non-radiolabeled ATP was varied over a construct-dependent 8-dose range. To ensure that the determinations were performed under steady-state conditions, progress curves were generated by quenching specific wells at various times during the 60-min incubation period. Initial velocities (slopes) at each concentration of ATP were estimated using linear fits to the product versus time plots, and the K_m's were determined from a hyperbolic fit to the initial velocity versus [ATP] plots. The K_m is defined as the concentration of ATP at half-maximal velocity.

Broad kinase panel profiling
LOXO-195 was tested with the KinaseProfiler™ service (Millipore, Inc.) against 228 kinases in duplicate at a LOXO-195 concentration of 1 μM and an ATP concentration near the K_m of each individual kinase. Data is reported as percent of control and is the average of the two replicates.

Cell lines

Sources of cell lines and methods of authentication are described in the main **Methods** section of the manuscript. Cell lines were tested regularly for Mycoplasma (MycoAlert™, Lonza, Inc. or STAT-Myco, Idexx BioResearch, Inc.). Frozen stocks prepared after ~1-2 passages were thawed ~3-6 days (~2-3 passages) before use.

In Vitro cell assays

Generation of NIH 3T3-ΔTRKA cell lines and assessment of phosphorylated-TRKA.
NIH-3T3 cell lines stably expressing wild-type and mutant versions (i.e. G595R, G667C, V573M, F589L) of a constitutively active form of human TRKA ("TRKA delta Ig2" or "ΔTRKA", (4)) were generated using either lentiviral transduction or standard transfection methods.

For assessment of cellular inhibition potency, cells were harvested per a standard protocol, counted and added to flat-bottom, collagen I-coated 96-well assay plates at 3X104 cells/well (wild-type cell line) or 5X104 cells/well (mutant cell lines) in 100 μL/well of DMEM growth medium containing 10% FBS. Plates were then incubated at room temperature for 30 minutes prior to an overnight incubation at 37°C with 5% C02. Next, cells were treated for 1 hour at 37°C, 5% C02 with TRK inhibitor compounds, each prepared as a 10-point, 1:3 dilution series with final concentrations ranging from 51 pM-1 μM and a constant DMSO concentration of 0.25%. Control wells contained either 0.25% DMSO alone (no inhibition control) or 1 μM

larotrectinib or LOXO-195 (complete inhibition control). Following compound incubation, growth medium was discarded and cells were lysed by addition of 60 µL/well of ice-cold lysis buffer containing protease and phosphatase inhibitors. To determine levels of phosphorylated TRKA (phospho-TRKA or pTRKA), lysates were tested using a phospho-TRKA ELISA kit (Cell Signaling Technologies) per the manufacturer's instructions. ELISA plates were analyzed by reading optical density at 450 nm using either a Versamax (Molecular Devices, Inc.) or Cytation 5 (Biotek, Inc.) microplate reader. IC_{50} values were calculated using Graphpad Prism 5 software.

Generation of ETV6-NTRK3 cell lines and assessment of phosphorylated-ERK

NIH-3T3 cells *ETV6-NTRK3* plasmids encoding N-terminal 3X FLAG-tagged wild-type or mutant (G623R or G696A) versions of the ETV6-TRKC fusion protein were transiently transfected using Lipofectamine 2000 reagent per standard methods. Transfected cells were harvested, washed, and transferred to a polypropylene, round-bottom 96-well plate at a density of 5X 10e5 cells/well in complete growth medium. Cells were treated with test compounds for one hour in a 12-point, 1:3 dilution series at concentrations ranging from 56 pM-10 µM. DMSO concentration was held constant at 0.25% and control wells contained either 0.25% DMSO alone (no inhibition control) or 1 µM LOXO-195 (complete inhibition control). Following compound treatment, cells were fixed in 2% formaldehyde for 10 minutes at 37°C. Fixed cells were washed twice and re-suspended in a saponin-based permeabilization buffer containing purified rabbit anti-phospho-ERK (pERK) 1/2 antibody (T202/Y204) (Cell Signaling Technology). ERK1/2 phosphorylation was used as a downstream indicator of TRKC activity due to a lack of commercially available anti-pTRKC antibodies suitable for use in flow cytometry. After a 30 minute, room temperature incubation, cells were washed twice with permeabilization buffer and re-suspended in permeabilization buffer containing APC-conjugated mouse anti-FLAG (Biolegend, Inc.) and PE-conjugated goat anti-rabbit lgG (Thermo Fisher Scientific, Inc.) antibodies. After a 30-minute incubation at room temperature, cells were washed twice with permeabilization buffer and re-suspended in 1 X PBS containing 0.2% BSA and 0.1 % sodium azide. Following sample analysis by flow cytometry, data were analyzed using FlowJo 7 flow cytometry analysis software. By comparing the PE vs. APC data plot for transfected cells to that of mock-transfected cells, a gate was created to encompass the transgene-expressing cell population. The gate was drawn to exclude a consistently observed small population of cells with apparent FLAG-positive staining that did not show an elevated level of ERK.1/2 phosphorylation in the DMSO-treated control sample. PE channel mean fluorescence intensity data of the gated population was used to generate POC data, which were then plotted versus compound concentration using GraphPad Prism 5 software to generate IC_{50} data using a 3-parameter curve fit.

ADME studies

Oral bioavailability was determined in male animals. Intravenous doses were formulated in 20% hydroxypropyl-β-cyclodextrin. Oral doses were formulated as shown in **Supplementary Table 1**. Samples of blood were collected at various time after dosing for both routes of administration.

Concentrations in plasma were determined by liquid chromatography with detection by mass spectrometry. Pharmacokinetic parameters were calculated by non-compartmental analysis.

Xenograft studies

The ΔTRKA, ΔTRKA-G595R, and ΔTRKA -G667C NIH-3T3 tumor cell lines (~2-3 x 10e6 cells) and KM12 cells (5 x 10e6 cells) were injected subcutaneously into the right flank of female nu/nu NCr mice. Tumors were allowed to grow to ~ 100 - 200 mm3 (for efficacy analysis) or ~500 mm3 (for PK-PD analysis), and animals were randomized by tumor size into dosing groups of 5 (KM12), 7 (NIH 3T3 ΔTRKA variants) or 3-4 (for PK-PD) animals. Animals were dosed by oral gavage with vehicle, LOXO-195 in 1% carboxymethylcellulose/ 0.5% Tween-80 or larotrectinib in 100% Labrafac lipophile.

All animals were obtained at 6-8 weeks of age, housed in groups of 5 and allowed a one-week acclimation period before cancer cell injection. Food, water, temperature and humidity were prepared per Pharmacology Testing Facility performance standards (SOP's) which are in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NRC) and AAALAC-International.

PK-PD studies
Animals were dosed with vehicle twice daily, LOXO-195 at 10 mg/kg, 30 mg/kg and 100 mg/kg twice daily and larotrectinib at 60 mg/kg daily for 3 days. Two hours after the last dose, animals were euthanized by CO2 inhalation and tumors were excised, flash-frozen in liquid nitrogen and stored at -80 C. Plasma was collected into tubes containing 10% v/v EDTA, centrifuged at 14000 rpm to separate plasma from packed blood cells and stored at -80C.

To determine the levels of pTRKA in tumors, tumor tissue was homogenized in lysis buffer (1% NP-40, 20 mM Tris ph 8.0, 137 mM NaCl, 10% glycerol, 2mM EDTA, protease and phosphatase inhibitors) and centrifuged twice to separate insoluble material. Protein levels in each lysate were determined using Comassie protein reagent (Pierce, Inc.). pTRK levels were determined in 250 μg each sample by ELISA assay (R & D Systems, Inc.). pTRK levels were normalized to vehicle-treated controls, and results were expressed as POC pTRKA levels in tumors.

The concentrations of larotrectinib and LOXO-195 in mouse plasma was determined by LC-MS/MS following protein precipitation with acetonitrile and comparison to a calibration curve derived from spiking serially dilutions of each agent into naïve mouse plasma.

Efficacy studies
Animals were dosed with vehicle twice daily, LOXO-195 at 30 mg/kg, 100 mg/kg and 300 mg/kg twice daily and larotrectinib at 60 mg/kg daily for 9-12 days. Body weight and tumor size were monitored after cell implantation and at regular intervals during dosing. Tumor diameters were measured with digital calipers, and the tumor volume in mm3 was calculated by the formula: Volume = ((width)2 x length)/2.

Tumor next-generation sequencing

Next-generation sequencing was performed utilizing both targeted and whole-exome sequencing on DNA from formalin-fixed paraffin embedded tumor and matched blood specimens. Specifically, sequencing of tumor specimens was performed using 410-gene exon-capture and deep targeted sequencing assay ("MSK-IMPACT") per previously published methods(5). Additionally, whole-exome sequencing (WES) was performed by recaptured from the remaining MSK-IMPACT library generated for the adult patient and sequenced and analyzed as previously described (6).

Genomic sequencing data and analysis

All mutation calls across platforms were post-processed and annotated to ensure cross-comparison. Briefly, mutations were annotated with VEP (version 85) and common variants identified by the Exome Aggregation Consoritum (ExAC) (7) as having a minor allele frequency greater than 0.0004 in any subpopulation were excluded as presumed germline (https://github.com/mskcc/vcf2maf). Furthermore, only mutations with a variant allele frequency greater than 5% were considered (as per New York State clinical testing standards for IMPACT sequencing), except for known hotspot mutations that were reported regardless of allele frequency in the sequenced specimen. Hotspot mutations were those identified by an adaptation of methodologies described previously (8) applied to a cohort of ~24592 sequenced human cancers (Chang MT et al. *submitted*). Mutations with low numbers of supporting reads or those called in repeat regions were also flagged for manual inspection. The clonality of all somatic mutations was inferred in MSK-IMPACT or WES samples using overall and local tumor ploidy and purity estimates derived using FACETS for allele-specific copy number (ref) (https://github.com/mskcc/facets). Briefly, for each somatic mutation, cancer cell fractions (CCFs) were estimated as previously described (9) and for those mutations in regions of genomic gains, two CCFs were calculated, assuming the minimum and maximum possible number of copies. The probability of a mutation's CCF was calculated with a binomial distribution using maximum likelihood (ML) estimation, which we normalize to produce posterior probabilities. Confidence intervals (CI) for the CCF are calculated as the full-width-at-half-maximum of the ML value. Mutations were defined clonal if the upper confidence interval overlapped 0.85; otherwise they were defined as subclonal.

Plasma cfDNA extraction and analyses

Whole blood was collected for MSKCC patients in 10-ml Cell-Free DNA BCT tubes (STRECK) was centrifuged in two steps to separate plasma from cells. Initially, whole blood was centrifuged at 800 X g for 10 min (ambient temperature). Plasma was then separated from red blood cells. In the second phase, separated plasma was further centrifuged in a high-speed micro-centrifuge at 18,000 X g for 10 min (ambient temperature). Cell-free plasma was aliquoted and frozen at minus 80°C until extraction. Extraction of cfDNA was performed using a fully automated QIAGEN platform, QIAsymphony SP, and QIAsymphony DSP Virus/Pathogen Midi Kit (catalog #937055). This bead-based custom protocol was optimized to work with 3ml of

plasma as starting material. The extraction process includes lysis, binding, wash, and elution steps. The final product is a 60µl elution of cfDNA with an average size ~170-200 bp. Quality and quantity of cfDNA was evaluated with automated electrophoresis using either TapeStation with High Sensitivity D1000 ScreenTape and Reagents (Agilent Technologies) or Fragment Analyzer with High Sensitivity genomic DNA Analysis Kit (Advanced Analytical). Plasma extraction for non-MSK patients was as follows. In total, 10 or 20mL of whole blood were collected at individual local study sites into venous blood collection tubes containing EDTA as anti-coagulant. Within 1 hour, plasma was processed by centrifugation at approximately 2,000 X g for 10 min using a pre-chilled centrifuge set to 4°C. Plasma was then transferred to a 15mL Falcon tube, centrifuged as above at approximately 2,000 X g for 10 min, aliquoted, and frozen at minus 80°C until extraction.

Digital droplet PCR profiling of tumor-derived cell-free DNA

The NTRK1 G595R mutation was detected via custom assays designed and ordered through BioRad, and were as follows:

NTRK1 G595R:
forward primer 5’ CCTGCTCATGGTCTTTGA 3’
reverse primer: 5’ GTGCTGGTACCGGAG 3’
mutation-specific probe: 5’ CGGCACAGGGACCT 3’
Fluorophore: FAM

wild type:
forward primer 5’ CCTGCTCATGGTCTTTGA 3’
reverse primer: 5’ GTGCTGGTACCGGAG 3’
probe: 5’ CGGCACGGGGACC 3’
Fluorophore: HEX

Cycling conditions were tested to ensure optimal annealing/extension temperature as well as optimal separation of positive from empty droplets. All reactions were performed on a QX200 ddPCR system (Biorad) for which we load 9ul cfDNA per ddPCR reaction or for gDNA, first dilute to 1ng/ul. Each sample was evaluated in technical duplicates. Each PCR reaction contains primers, probes, DNA, and digital PCR Supermix for probes (no dUTP). Reactions were partitioned into a median of ~16,000 droplets per well using the QX200 droplet generator. Emulsified PCRs were run on a 96-well thermal cycler using cycling conditions identified during the optimization step (95°C 10’;40 cycles of 94°C 30’’ 55°C 1’, 98°C 10’, 4°C hold). In every run, wells of water, gDNA, and a mutation-positive control are included (for manual processing: 1 of each, for robotic processing, two of each). Plates were read and analyzed with the QuantaSoft software to assess the number of droplets positive for mutant DNA, wild-type DNA, both, or neither. The assay threshold sensitivity was set at 1 mutant droplets.

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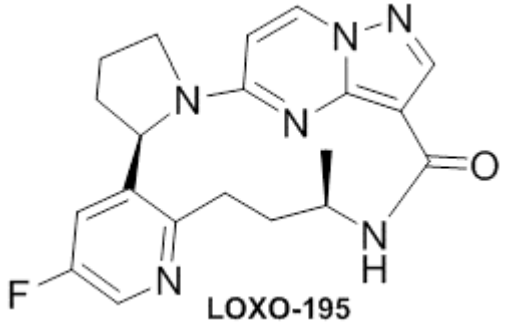
A



LOXO-195

B

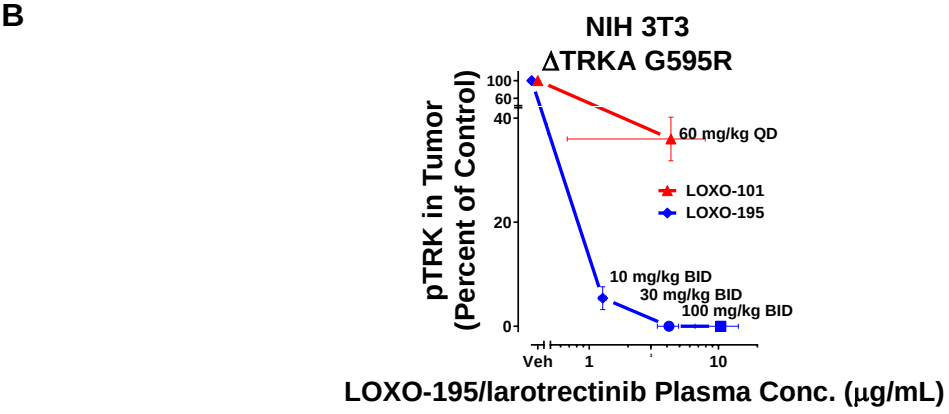
Kinase	Larotrectinib	LOXO-195
	IC_{50} ± SD (nM)	**IC_{50} ± SD (nM)**
TRKA	9.1 ± 3.8	3.7 ± 1.3
TRKB	4.2 ± 1.5	1.9 ± 0.6
TRKC	4.2 ± 1.8	1.4 ± 0.4

Supplementary Figure 1. Effect of acquired resistance mutations on TRK inhibitor activity. A. chemical structure of LOXO-195. **B,** the binding affinities of larotrectinib and LOXO-195 for the wild-type TRKA, TRKB and TRKC kinase domains were determined as described in **Supplementary Methods**. Data are displayed as mean +/- SD of ≥10 replicates.

A

Kinase	Resistance mutation	class	IC_{50} ± SD (nM)	
			Larotrectinib	LOXO-195
NIH 3T3 ΔTRKA	WT		5 ± 1	1.6 ± 0.5
	V573M	ATP site	1018 ± 708	29 ± 9
	F589L	gatekeeper	47 ± 7	9 ± 3
	G667S	xDFG	70 ± 13	25 ± 8

B



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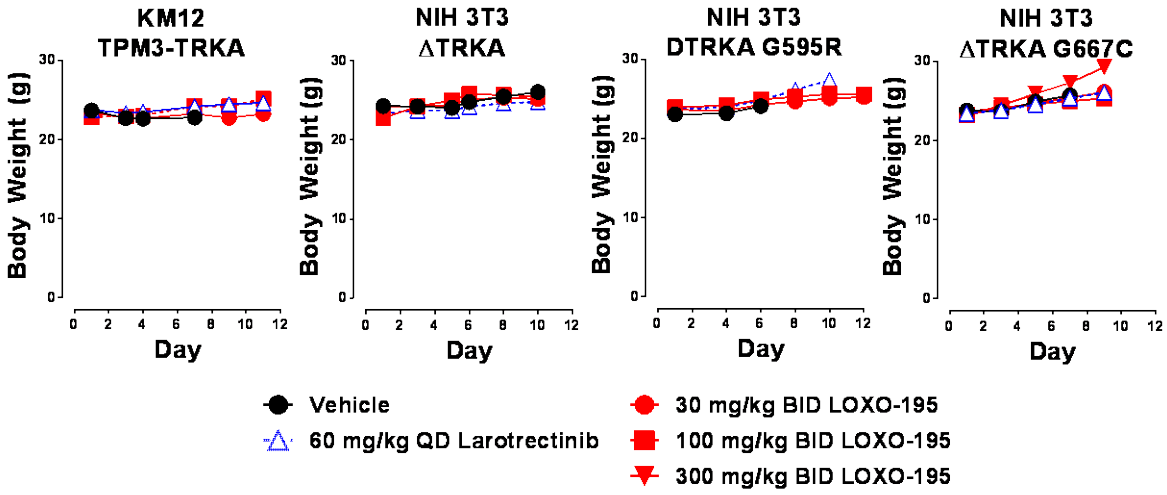
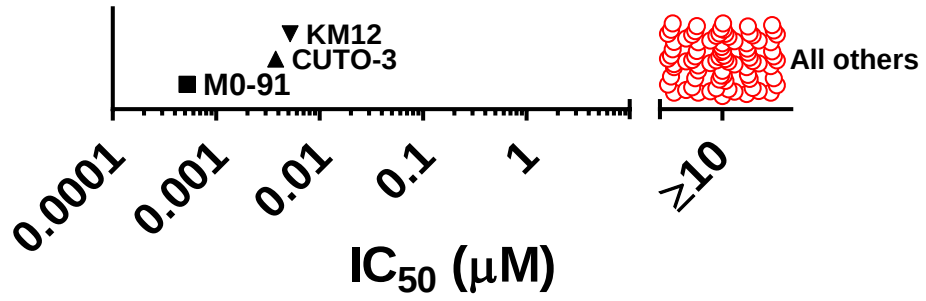


Supplementary Figure 2. Further characterization of LOXO-195 inhibitory activity against acquired resistance mutations. A, Effect of larotrectinib and LOXO-195 on cellular activity of TRK resistance mutations identified pre-clinically (Estrada-Bernal 2015). See legend to **Figure 2A** for details. **B,** LOXO-195 caused dose-dependent inhibition of TRKA phosphorylation in ΔTRKA-G595R tumors, while larotrectinib was much less effective despite similar drug plasma concentrations. Data are displayed as mean +/- SD (for plasma concentration) or SEM (for pTRK levels) of 3-4 animals/group. **C,** Body weight measurements for the efficacy analysis shown in **Figure 2B** are displayed as mean (g) +/- SEM.

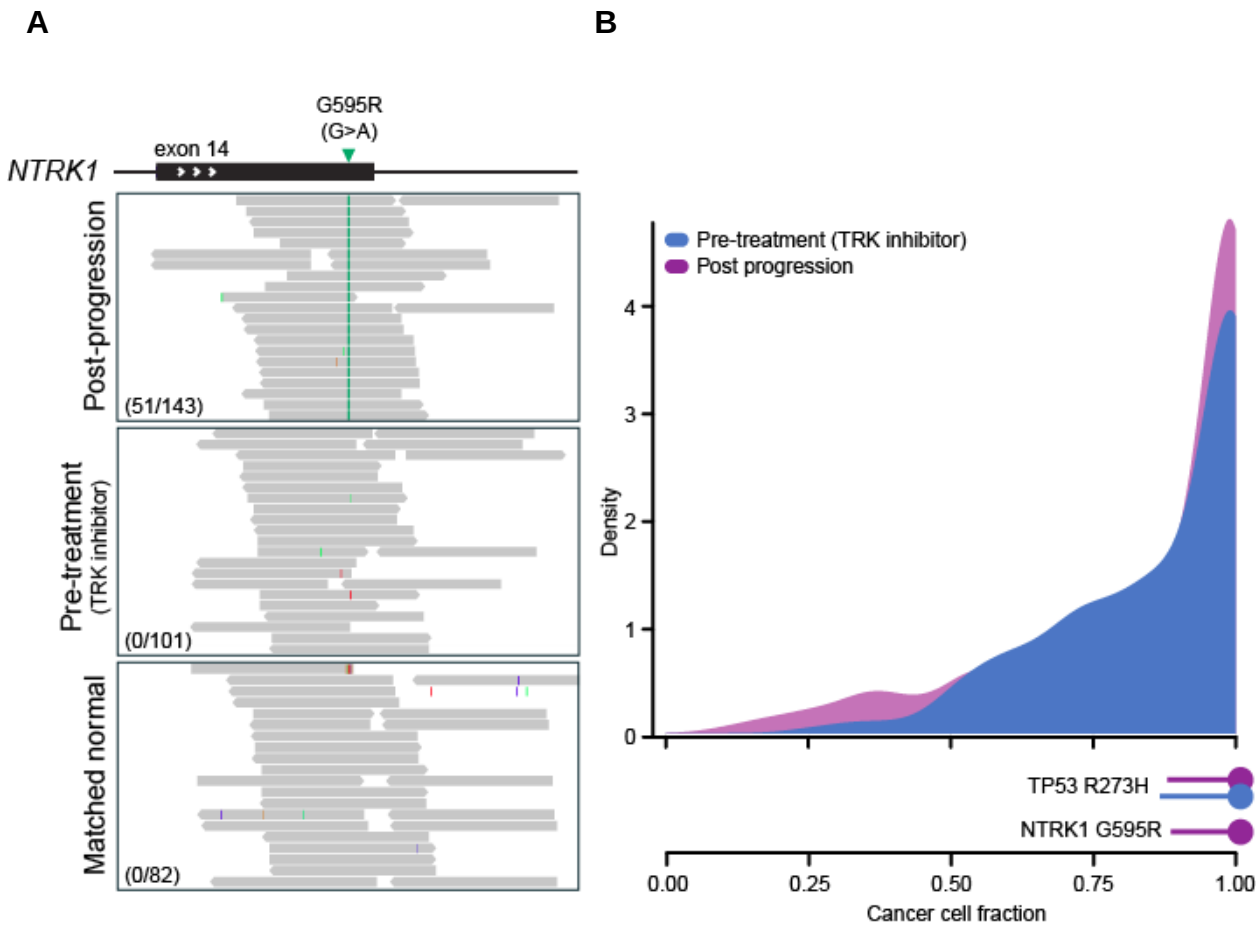
A



B

M0-91	CMLT1	L428	SH4
CUTO-3	CORL105	LC2	SHP77
KM12	D283Med	MCF7	SJRH30
5637	DBTRG05MG	MDAMB231	SKBR3
647V	Detroit562	MDAMB453	SKLMS1
A101D	DKMG	MDAMB468	SKMEL28
A172	DMS114	MG63	SKNDZ
AN3CA	DMS53	MiaPaCa2	SKNFI
AU565	DU145	MV411	SNB19
BFTC905	EFM19	MZCRC1	SNU16
BHT101	FaDu	NCIH292	SUDHL10
BT20	HEL9217	NCIH441	SW1353
BT474	HMCB	NCIH520	SW579
BT549	Hs578T	NCIH661	T24
BxPC3	HT1376	OE19	T47D
C32	HT29	REC1	TCCSUP
Cal27	J82	RT11284	TF1
CAL62	JeKo1	SaOS2	TPC1
CAMA1	K562	SCaBER	TT
CaOV3	KATOIII	SCC25	U138MG
CGTHW1	KG1	SCC4	UMUC3
CHL1	KPL1	SCC9	

Supplementary Figure 3. LOXO-195 is selectively cytotoxic to TRK-fusion cell lines. A, Each cell line was treated with 10 concentrations of LOXO-195 in triplicate for 72 hours, followed by DAPI staining and cell counting. ▼ KM12 (*TPM3-NTRK1),* ▲ CUTO-3 (*MPRIP-NTRK1*), ■ MO-91 (*ETV6-NTRK3*), ◦ n=84 TRK-fusion negative cells lines. **B,** Cell lines analyzed.



Supplementary Figure 4. Identification of a clonal *NTRK1 (*TRKA)-G595R mutation after larotrectinib treatment. A, Allele frequency of G to A transition mutation in a tumor biopsy from a patient with *LMNA-NTRK1* fusion colorectal cancer obtained after progression on larotrectinib, compared to the pre-larotrectinib biopsy and matched normal control DNA. **B,** The density of sequencing reads for the *NTRK1* (TRKA) G595R substitution mutation in the post-progression biopsy sample is similar to the *TP53* (P53) R273H mutation present in all tumor cells in both the pre-treatment and post-larotrectinib samples.

Species	Oral Vehicle	Oral Bioavailability (10 mg/kg dose)	Clearance (L/h/kg, 1 mg/kg IV Dose)
Mouse	1% carboxymethylcellulose 0.5% Tween 80	74%	0.29
Rat	1% carboxymethylcellulose 0.5% Tween 80	24%	0.35
Dog	0.5% carboxymethylcellulose 0.02% sodium dodecyl sulfate	42%	0.77
Monkey	20% hydroxypropyl-β-cyclodextrin	2%	1.4

Supplementary Table 1. LOXO-195 ADME properties.

Kinase Class	Kinase	Percent of Control	Kinase Class	Kinase	Percent of Control
AGC	AKT1	120.5	CAMK	DAPK1	120.5
	AKT2	121		DAPK2	94
	AKT3	104		DAPK3	119.5
	DMPK	106.5		DCAMKL2	140.5
	GRK5	110		DRAK1	113
	GRK6	106		LKB1	108.5
	GRK7	70.5		MAPKAP-K2	102
	MRCKalpha	138		MAPKAP-K3	102.5
	MRCKbeta	128.5		MAPKAP-K5	97
	MSK1	131		MARK1	94
	MSK2	115		MARK2	107
	p70S6K	117		MELK	104
	PDK1	101		MKNK2	100
	PKAC-alpha	109		MYLK	109
	PKCalpha	107		PASK	159
	PKCbetaI	108		PhKgamma2	103.5
	PKCbetaII	90.5		Pim-1	121.5
	PKCdelta	108.5		Pim-2	120
	PKCepsilon	102.5		Pim-3	103
	PKCeta	108.5		PKD1	105.5
	PKCgamma	97.5		PKD2	110
	PKCiota	100		SIK	107
	PKCtheta	107.5		STK33	106.5
	PKCzeta	106.5		TSSK1	99
	PRK2	100		TSSK2	119.5
	PRKG1alpha	110	CK1	CK1_y	114.5
	PRKG1beta	114		CK1delta	109.5
	PrKX	118		CK1gamma1	112
	ROCK-I	105		CK1gamma2	112
	ROCK-II	105		CK1gamma3	113
	Rsk1	109		VRK2	98
	Rsk2	133.5	CMGC	CDK1/cyclinB	101.5
	Rsk3	105.5		CDK2/cyclinA	109
	Rsk4	127		CDK2/cyclinE	106.5
	SGK1	124		CDK3/cyclinE	103.5
	SGK2	135.5		CDK5/p25	98.5
	SGK3	114.5		CDK5/p35	110.5
Atypical	eEF-2K	117.5		CDK6/cyclinD3	109.5
	mTOR	95		CDK7/cyclinH/MAT1	107.5
	mTOR/FKBP12	102.5		CDK9/cyclinT1	110
CAMK	AMPK(A1/B1/G1)	96.5		CLK2	96.5
	ARK5	98		CLK3	104.5
	BrSK1	108		DYRK2	101.5
	BrSK2	123.5		ERK1	111
	CAMK1	107		ERK2	105
	CAMK1d	116.5		GSK3alpha	117
	CAMK2b	100		GSK3beta	123.5
	CAMK2d	100.5		HIPK1	106
	CAMK2g	95		HIPK2	113
	CAMK4	116.5		HIPK3	105
	CHK1	109		JNK1alpha1	106
	CHK2	99.5		JNK2alpha2	100

Supplementary Table 2. Selectivity of LOXO-195 for 228 kinases. Data shown are mean percent of control of two replicates. LOXO-195 was tested at a concentration of 1 μM, which is ~ 1667-fold higher than the IC_{50} for TRKA.

Kinase Class	Kinase	Percent of Control	Kinase Class	Kinase	Percent of Control
CMGC	JNK3	109	TK	BTK	87
	MSSK1	121.5		CSK	111.5
	NLK	110		DDR2	93
	p38alpha	109		EGFR	103
	p38beta	123		EphA1	74
	p38delta	110.5		EphA2	79.5
	p38gamma	104.5		EphA3	103.5
	SRPK1	103.5		EphA4	101.5
	SRPK2	93.5		EphA5	90
Other	AURKA	97		EphA7	103
	CK2alpha2	99		EphA8	81.5
	Haspin	110		EphB1	112
	IKKalpha	98.5		EphB2	81
	IKKbeta	119		EphB3	101
	NEK11	111.5		EphB4	116.5
	NEK2	117		ErbB4	116.5
	NEK3	110.5		FAK	81
	NEK6	115.5		FAK2	89
	NEK7	103		Fer	91
	Plk1	103.5		Fes	99.5
	Plk2	95		FGFR1	91.5
	Plk3	93.5		FGFR2	96.5
	TBK1	111		FGFR3	119
	TLK2	107.5		FGFR4	128.5
	ULK2	96		Fgr	96
	ULK3	95.5		Flt1	101
	WNK2	128.5		Flt3	85.5
	WNK3	110.5		Flt4	90
STE	LOK	99.5		Fms	87.5
	MAP3K5	106.5		Fyn	90.5
	MAP4K2	101.5		Hck	133.5
	MEK1	121.5		IGF-1R	96
	MINK	99.5		IGF-1R Activated	84.5
	MKK4_m	108.5		IR	92
	MKK6	100		IR Activated	97
	MKK7	115		IRR	111
	MST1	95.5		ITK	90.5
	MST2	112.5		JAK2	37.5
	MST3	125		JAK3	103
	PAK2	108.5		KDR	111.5
	PAK4	101		KIT	100.5
	PAK5	103.5		Lck	92.5
	PAK6	99		Lyn	98
	TAO1	102.5		Mer	111
	TAO2	104		Met	109
	TAO3	102.5		MuSK	89.5
TK	Abl2	105		PDGFRalpha	103.5
	Abl-P	87		PDGFRbeta	112.5
	ALK	50		PTK5	87.5
	Axl	90		PTK6	98.5
	BLK	108		Ret	80.5
	Bmx	15		Ron	88.5

Supplementary Table 2 (cont). Selectivity of LOXO-195 for 228 kinases.

Kinase Class	Kinase	Percent of Control	Kinase Class	Kinase	Percent of Control
TK	Ros	12	TK	Yes	82
	Rse	110		ZAP-70	118.5
	Src	102.5	TK-LIKE	ALK4	96
	Syk	114.5		c-RAF	92.5
	TEC Activated	111.5		IRAK1	105.5
	Tie2	119		IRAK4	95
	TNK2	10		LIMK1	85.5
	TrkA	0.5		MLK1	96.5
	TrkB	0		RIPK2	100
	Txk	14		TAK1-TAB1	100

Supplementary Table 2 (cont.). Selectivity of LOXO-195 for 228 kinases.

Patient	Dose (mg BID)	Day	C_{max} (ng/mL)	C_{min*} (ng//ml)	AUC (ng*h/mL)
Adult *LMNA-NTRK1*	50	1	350	1.86	1190
	100	15	673	7.78	2620
	100	22	800	14.7	3870
Pediatric *ETV6-NTRK3*	20	1	143	<1	615
	60	8	415	<1	2010
	100	29	776	12	4410

* 10 hours post-dose for adult patient; 12 hours post-dose for pediatric patient

Supplementary Table 3. Summary of LOXO-195 PK parameters for each patient during intra-patient dose escalation.