Resistance to TRK inhibition mediated by convergent MAPK pathway activation

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TRK fusions are found in a variety of cancer types, leading to oncogenic addiction, and strongly predict tumor-agnostic efficacy of TRK inhibition1–5. With the recent approval of the first selective TRK inhibitor, larotrectinib, for patients with any TRK-fusion-positive adult or pediatric solid tumor, to identify mechanisms of treatment failure after initial response has become of immediate therapeutic relevance. So far, the only known resistance mechanism is the acquisition of on-target TRK kinase domain mutations, which interfere with drug binding and can potentially be addressable through second-generation TRK inhibitors6–8. Here, we report off-target resistance in patients treated with TRK inhibitors and in patient-derived models, mediated by genomic alterations that converge to activate the mitogen-activated protein kinase (MAPK) pathway. MAPK pathway–directed targeted therapy, administered alone or in combination with TRK inhibition, re-established disease control. Experimental modeling further suggests that upfront dual inhibition of TRK and MEK may delay time to progression in cancer types prone to the genomic acquisition of MAPK pathway-activating alterations. Collectively, these data suggest that a subset of patients will develop off-target mechanisms of resistance to TRK inhibition with potential implications for clinical management and future clinical trial design.

To identify mechanisms of resistance to TRK inhibition in patients with TRK-fusion-positive cancers, tumor biopsies and circulating cell-free DNA (cfDNA) were collected prospectively from patients treated with a variety of TRK inhibitors as part of prospective clinical trials and compassionate use programs. Paired sequencing was conducted (see Methods) to identify patients in whom TRK kinase domain mutations were not detected or did not entirely explain resistance to the TRK inhibitor used. Acquired alterations that involved upstream receptor tyrosine kinase or downstream MAPK pathway nodes were identified in six patients, prompting further analysis of these cases.

In the first patient (Patient 1), who had a CTRC–NTRK1 fusion-positive pancreatic cancer that developed resistance to larotrectinib, targeted sequencing of paired pre-treatment and post-progression tumor biopsies revealed an acquired BRAFV600E mutation (Fig. 1a and Extended Data Fig. 1a). Sequencing of serial cfDNA samples orthogonally confirmed the acquisition of BRAFV600E along with a subclonal KRASG12D mutation (Extended Data Fig. 1b). Patient-derived xenografts (PDXs) established from this patient’s tumor and treated with larotrectinib over time similarly demonstrated outgrowth of a BRAFV600E-positive subclone at the time of acquired resistance (Fig. 1b and Extended Data Fig. 1c). Consistent with the hypothesis that downstream MAPK pathway activation was responsible for TRK-independent bypass resistance, the disease progressed rapidly on subsequent treatment with LOXO-195, a second-generation TRK inhibitor designed to maintain potency in the setting of TRK kinase domain mutations9. In addition, the ectopic expression of BRAFV600E in a NTRK1 fusion-positive pancreatic cancer cell line (TPR–NTRK1, NTRK1G595R) conferred resistance to LOXO-195 (Fig. 1c), which further supports the idea that this alteration has a causative role in mediating resistance.

In the second patient (Patient 2), who had a LMNA–NTRK1 fusion-positive colorectal cancer (CRC) that developed acquired resistance to LOXO-195, sequencing of tumor and serial cfDNA samples revealed emergence of multiple KRAS mutations consistent with polyclonal resistance mediated by a convergent mechanism (Fig. 1d and Extended Data Fig. 1d,e). This patient previously had a prolonged response to larotrectinib followed by resistance driven by acquisition of an NTRK1G595R solvent-front mutation (the resultant substitution prevents drug binding10). Consistent with on-target NTRK-dependent resistance, subsequent treatment with LOXO-195 achieved a second response, eventually followed again by solitary site progression in the liver. Genomic analysis of the liver metastasis biopsy and serial cfDNA revealed the emergence of a KRASG12D substitution (Fig. 1d and Extended Data Fig. 1d,e). This mutation disappeared in cfDNA after ablation of the liver metastasis.

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and LOXO-195 continuation; however, a new KRAS<sup>G12D</sup> mutation emerged upon further disease progression (Fig. 1d). Consistent with this clinical observation, chronic treatment of a LMNA–NTRK1-positive, NTRK1<sup>G96R</sup> CRC cell line (LMNA–NTRK1, NTRK1<sup>G96R</sup>) with LOXO-195 likewise demonstrated KRAS<sup>G12D</sup> acquisition (Extended Data Fig. 1f,g). In further support of the causative nature of these alterations in mediating resistance, ectopic expression of both KRAS<sup>G12A</sup> and KRAS<sup>G12D</sup> in TRK fusion-positive CRC cell lines was sufficient to increase MAPK pathway activation and confer resistance to both larotrectinib and LOXO-195 (Fig. 1e,f).

In the third patient (Patient 3), who had a PLEKHA6–NTRK1 fusion-positive cholangiocarcinoma that developed acquired resistance to the first-generation TRK inhibitor entrectinib, sequencing of both tissue and cfDNA identified an acquired high-level
focal amplification of \( \text{MET} \) (Fig. 1g). Acquisition of \( \text{MET} \) high-level amplification and protein overexpression was orthogonally confirmed by \( \text{MET} \) fluorescence in situ hybridization (FISH) and immunohistochemistry, respectively (Fig. 1h,i), and sequencing of \( \text{NTRK1} \) did not identify a kinase domain point mutation. Of note, \( \text{MET} \) amplification has been observed as a mechanism of off-target resistance in other oncogene-addicted cancers\(^{12-16}\). Consistent with the hypothesis that \( \text{MET} \) amplification drove TRK-independent resistance, the disease progressed immediately despite subsequent treatment with \( \text{LOXO-195} \).

As all three index cases involved tumors of gastrointestinal origin, we next broadened our analysis to all patients with TRK fusion-positive gastrointestinal cancer for whom we had serial cfDNA samples (excluding gastrointestinal stromal tumors). Five additional patients were identified, three of whom developed emergent MAPK alterations while on TRK inhibitors (Supplementary Table 1). One patient with an \( \text{ETV6} \text{-NTRK3} \) fusion-positive pancreatic cancer had a prolonged response to the multikinase TRK inhibitor \( \text{PLX7486} \). No mechanism of resistance was identified at progression and he was subsequently treated with \( \text{LOXO-195} \) with no response. At the time of progression on \( \text{LOXO-195} \), cfDNA demonstrated acquisition of a hotspot \( \text{MEK1} (\text{MAP2K1}) \) \( \text{P124S} \) mutation. Although testing within the context of a TRK fusion suggests that this mutation has weak oncogenic potential, this alteration has previously been proposed to confer resistance to targeted therapy in patients with \( \text{BRAF}^{\text{V600E}} \) melanoma\(^{17}\). A second patient with \( \text{TPR} \text{-NTRK1} \) fusion-positive pancreatic cancer had a prolonged response to entrectinib followed by resistance driven by acquisition of \( \text{NTRK1}^{\text{G595R}} \). This patient was subsequently treated with \( \text{LOXO-195} \) with a transient decline in tumor markers and resolution of tumor foci, followed quickly by clinical deterioration and radiologic progression. Serial cfDNA sequencing on \( \text{LOXO-195} \) revealed loss of the \( \text{NTRK1}^{\text{G595R}} \) mutation but emergence of the known activating \( \text{ERBB2}^{\text{S310F}} \) mutation\(^{18}\). Lastly, a \( \text{TPM3} \text{-NTRK1} \) fusion-positive CRC patient developed polyclonal resistance to larotrectinib through parallel activation of upstream receptor tyrosine kinase domains. These alterations are all predicted to restore MAPK signaling of (6 out of 8) TRK-fusion positive gastrointestinal cancers analyzed. These alterations are all predicted to restore MAPK signaling through parallel activation of upstream receptor tyrosine kinase and downstream MAPK signaling nodes. We therefore reasoned that these alterations represent a recurrent and convergent mechanism of treatment failure of TRK inhibitors and that a subset of these alterations may be pharmacologically actionable. To evaluate this hypothesis in the clinic, we treated Patients 1 and Patient 3 with targeted therapy directed at their respective acquired resistance mechanisms.

Patient 1, who had pancreatic cancer and the emergent \( \text{BRAF}^{\text{V600E}} \) mutation, was treated with a combination of \( \text{RAF} \) and \( \text{MEK} \) inhibitors (dabrafenib and trametinib), resulting in early tumor regression accompanied by a slight decrease in the allele frequency of the \( \text{NTRK} \) fusion and a tenfold decrease in the mutant allele frequency of \( \text{BRAF}^{\text{V600E}} \) detected in cfDNA (Fig. 2a and Extended Data Fig. 2).
Fig. 3 | Dual TRK and MEK blockade is required to inhibit tumor growth in TRK fusion-positive models that acquired MAPK alterations. **a**. Western blots from the two CRC cell lines LMNA–NTRK1, NTRK1G595R and LMNA–NTRK1, NTRK1G595R, KRASG12D, treated as indicated. LOXO-195 (50 nM), trametinib (10 nM), or the combination of both drugs (195 + tram) were administered at the indicated time and protein lysates were probed with the indicated antibodies. Although LOXO-195 was sufficient to inhibit both phosphorylated TRK (pTRK) and pERK in the wild-type cell line, the combination of both drugs was required for this dual inhibition in the KRASG12D cell line. Three biological replicates were performed. **b**, Proliferation assays on the same cell lines (labeled NTRK1G595R and KRASG12D) treated for 72 h with LOXO-195 (125 nM), trametinib (2 nM), or their combination. Data are presented as mean ± s.d. of four biological replicates. Two-tailed unpaired t-test was used to evaluate significant differences in percentage of viability cells. P values < 0.05 were considered statistically significant. **c**, In vivo efficacy of LOXO-195 (100 mg kg−1 BID, 5 days a week), trametinib (1 mg kg−1, 4 days a week), or their combination on xenografts established from the LMNA–NTRK1, NTRK1G595R, KRASG12D cell line (vehicle n = 5, LOXO-195 n = 6, trametinib n = 5, LOXO-195 + trametinib n = 6). **d**, In vivo efficacy of LOXO-195 (100 mg kg−1 BID, 5 days a week), trametinib (3 mg kg−1, 4 days a week) or their combination on PDXs established from the KRASG12D-positive liver biopsy collected at the time of LOXO-195 progression from Patient 2 (Fig. 1d; vehicle n = 4, LOXO-195 n = 5, trametinib n = 5, LOXO-195 + trametinib n = 5). **e**, In vivo efficacy of larotrectinib (200 mg kg−1 daily 5 days a week), trametinib (1 mg kg−1 daily 4 days per week) and the combination of both drugs in larotrectinib-resistant PDXs established from Patient 1 (Patient 1-derived PDX, BRAFV600E, Fig. 1a; vehicle n = 8, larotrectinib n = 8, trametinib n = 8, larotrectinib + trametinib n = 7). **f**, In vivo efficacy of larotrectinib (200 mg kg−1 daily, 5 days a week), trametinib (1 mg kg−1, 4 days a week) and the combination of both drugs in larotrectinib-sensitive PDXs established from Patient 1 (Patient 1-derived PDX, Fig. 1a; note that trametinib was also tested in combination with larotrectinib at half of the dose: 0.5 mg kg−1, 4 days a week, orange line; vehicle n = 6, larotrectinib n = 8, trametinib n = 7, larotrectinib + trametinib (1 mg kg−1) n = 7, larotrectinib + trametinib (0.5 mg kg−1) n = 7). Combination therapy prevents the development of primary or acquired resistance (ongoing at 3 months). Two-tailed unpaired t-test was used to evaluate significant differences in the tumor volumes. Data are presented as mean ± s.e.m. P values < 0.05 were considered statistically significant.
Simultaneously, a subclonal but preexisting KRASG12D mutation arose in cfDNA, followed by radiographical progression shortly thereafter, which suggests that outgrowth of this alteration may have been responsible for the acquired resistance to RAF and MEK inhibition (Fig. 2a). However, it is likely that this tumor was still partially driven by the TRK fusion. Although a combination including a TRK inhibitor as a third agent was favored initially, it could not be secured in time. We therefore tested whether the addition of a TRK inhibitor to the combination of dabrafenib and trametinib would enhance anti-tumor activity in TRK fusion-positive preclinical models transduced with sequences encoding BRAFV600E. Triple therapy (l retailotinib, dabrafenib, and trametinib) was significantly more effective than dabrafenib and trametinib at suppressing tumor growth and deeply inhibiting TRK-mediated signaling (AKT, ERK, MEK; Extended Data Fig. 3a,b). Interestingly, post-progression cfDNA demonstrated reappearance of focal MET amplification in addition to 13 emergent missense mutations in MET (Fig. 2b and Extended Data Fig. 4a,b). Several of which are known to impair crizotinib binding12,13. Although multiple resistance mechanisms were observed, presumably secondary to intertumoral or intratumoral heterogeneity, these alterations were remarkably convergent on MET reactivation. This on-target resistance to crizotinib further supports the mechanistic role for MET as an acquired driver of resistance to prior TRK inhibitor therapy.

Although the tumor regressions observed in patients with acquired BRAFV600E and MET amplifications were ultimately transient, they provide further clinical validation that the putative bypass mechanisms identified in these patients were biologically relevant. Given the convergence of these alterations on MAPK pathway activation, we explored the utility of combination TRK and MEK inhibition preclinically and clinically. Combinatorial treatment with LOXO-195 and a MEK inhibitor (trametinib or MEK–162) was more effective than either single agent alone in suppressing TRK and ERK activation and cell viability in the LMNA–NTRK1, NTRK1G19R, KRASG12D—LOXO-195-resistant model (Fig. 3a,b and Extended Data Fig. 5a,b). Furthermore, xenografts derived from this cell line were more sensitive to combinatorial therapy compared to each of the single agents (Fig. 3c), and similar results were obtained from PDXs established from the LOXO-195-resistant tumor collected from Patient 2 (KRASG12A, LMNA–NTRK1-positive CRC; Fig. 3d). Despite these observations, Patient 2 was treated with the combination of LOXO-195 and trametinib and experienced rapid disease progression (Extended Data Fig. 6). Although the KRAS mutations in the patient and the PDX had different G12 substitutions (a factor that can affect GTPase activity and consequent response to MEK inhibition11), this outcome was also consistent with prior clinical experience showing that KRAS mutations are insensitive to MEK inhibition at exposures achievable in people12,13.

However, in multiple oncogene-addicted cancers, the management of acquired resistance with next-generation inhibitors or combinatorial therapy has generally been less efficacious than upfront use of these agents14–18. We therefore reasoned that the upfront dual targeting of TRK and MEK might delay the emergence of off-target resistance that converges on downstream MAPK pathway activation in TRK fusion–positive models. To test this hypothesis, we treated the larotrectinib-resistant and sensitive PDXs established from Patient 1 with larotrectinib, trametinib, or a combination of both. In larotrectinib-resistant PDXs, the combination of larotrectinib and trametinib delayed but did not prevent tumor growth compared to single agent treatments (Fig. 3e). In larotrectinib-sensitive PDXs, single-agent larotrectinib effectively controlled tumor growth for approximately 1 month, but the combination resulted in complete and durable tumor regression (ongoing response at 3 months; Fig. 3f). Droplet digital PCR on residual responding tumors collected at the end of the experiment from mice treated with the combination found that the BRAFV600E mutation was indeed present, albeit at low variant allele frequency (Supplementary Table 2), suggesting that concomitant TRK and MEK inhibition limited the emergence of this resistant cell population. If recapitulated in additional models, these data suggest that the upfront combination may further delay the emergence of MEK-sensitive resistance mechanisms such as the BRAFV600E mutation, compared to sequential TRK inhibitor monotherapy followed by the combination upon development of clinical resistance19.

Together, our data suggest that a subset of TRK fusion–positive cancers will develop off-target resistance to TRK inhibition that will not be adequately addressed by next-generation TRK inhibitors alone. Intriguingly, although TRK fusions seem to predict an initial response to TRK inhibition in a tumor-agnostic manner, early clinical evidence suggests that the durability of response may be more limited in gastrointestinal cancers11,12. Our findings provide potential mechanistic insight into why this may be the case and show similarities to prior experience with targeted therapy in BRAFV600E or EGFR-amplified CRCs15–18. The bypass mechanisms we identified demonstrate remarkable convergence on the ERK signaling. A portion of these resistance mechanisms may be managed successfully with simultaneous TRK and MEK inhibition, drugs that have largely non-overlapping toxicity in patients, although upfront treatment with the combination may confer more durable responses.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0542-z.

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References
1. Cocco, E., Scaltriti, M. & Drilon, A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat. Rev. Clin. Oncol. 15, 731–747 (2018).
2. Demetri, G. D. et al. LBA17 Efficacy and safety of entrectinib in patients with NTRK fusion-positive (NTRK-fp) tumors: pooled analysis of START-2, START-TRK-1 and ALKA-372-001. Ann. Oncol. 29, myd420.17 (2018).
3. Drilon, A. et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. N. Engl. J. Med. 378, 731–739 (2018).
4. Drilon, A. et al. Safety and antitumor activity of the multitargeted Pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and START-2). Cancer Discov. 7, 400–409 (2017).
5. Laetsch, T. W. et al. Larotrectinib for paediatric solid tumours harbouring NTRK gene fusions: phase 1 results from a multicentre, open-label, phase 1/2 study. Lancet Oncol. 19, 705–714 (2018).
6. Martín-Zanca, D., Hughes, S. H. & Barbacid, M. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. Nature 319, 743–748 (1986).
7. Schram, A. M., Chang, M. T., Jonsson, P. & Drilon, A. Fusions in solid tumors: diagnostic strategies, targeted therapy, and acquired resistance. Nat. Rev. Clin. Oncol. 14, 735–748 (2017).
8. Vaishnavi, A., Le, A. T. & Doebele, R. C. TRKInig down an old oncogene in a new era of targeted therapy. Cancer Discov. 5, 25–34 (2015).
9. Drilon, A. et al. A next-generation TRK kinase inhibitor overcomes acquired resistance to prior TRK kinase inhibition in patients with TRK fusion–positive solid tumors. Cancer Discov. 7, 963–972 (2017).
10. Drilon, A. et al. Retropertucinib (TPX-0005) is a next-generation ROS1/TRK/ALK inhibitor that potently inhibits ROS1/TRK/ALK solvent-front mutations. Cancer Discov. 8, 1227–1236 (2018).
11. Russo, M. et al. Acquired resistance to the TRK inhibitor entrectinib in colorectal cancer. Cancer Discov. 6, 36–44 (2016).
12. Bardelli, A. et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov. 3, 658–673 (2013).
13. Le, X. et al. Landscape of EGFR-dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-mutant NSCLC. Clin. Cancer Res. 24, 6195–6203 (2018).
14. Pietrantonio, E. et al. MET-driven resistance to dual EGFR and BRAF blockade may be overcome by switching from EGFR to MET inhibition in BRAF-mutated colorectal cancer. Cancer Discov. 6, 963–971 (2016).
15. Sanchez-Vega, F. et al. EGFR and MET amplifications determine response to HER2 inhibition in ERBB2-Amplified esophageal cancer. Cancer Discov. 9, 199–209 (2019).
16. Turke, A. B. et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 17, 77–88 (2010).
17. Carlino, M. S. et al. Preexisting MEK1P124 mutations diminish response to BRAF inhibitors in metastatic melanoma patients. Clin. Cancer Res. 21, 98–103 (2015).
18. Hyman, D. M. et al. Author correction: HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 566, E11–E12 (2019).
19. Heist, R. S. et al. Acquired resistance to crizotinib in NSCLC with MET exon 14 skipping. J. Thorac. Oncol. 11, 1242–1245 (2016).
20. Qi, J. et al. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. Cancer Res. 71, 1081–1091 (2011).
21. Hunter, J. C. et al. Biochemical and structural analysis of common cancer-associated KRAS mutations. Mol. Cancer Res. 13, 1325–1335 (2015).
22. Caunt, C. J., Sale, M. J., Smith, P. D. & Cook, S. J. MEK1 and MEK2 inhibitors and cancer therapy: the long and winding road. Nat. Rev. Cancer 15, 577–592 (2015).
23. Falchook, G. S. et al. Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. Lancet Oncol. 13, 782–789 (2012).
24. Long, G. V. et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N. Engl. J. Med. 371, 1877–1888 (2014).
25. Peters, S. et al. Alectinib versus Crizotinib in Untreated ALK-positive non-small-cell lung cancer. N. Engl. J. Med. 377, 829–838 (2017).
26. Soria, J. C. et al. Osimertinib in untreated EGFR-Mutated advanced non-small-cell lung cancer. N. Engl. J. Med. 378, 113–125 (2018).
27. Misale, S. et al. Vertical suppression of the EGFR pathway prevents onset of resistance in colorectal cancers. Nat. Commun. 6, 8305 (2015).
28. Nathenson, M., et al. O-020 Activity of larotrectinib in patients with TRK fusion GI malignancies. Ann. Oncol. 29 (Suppl. 5), O-020 (2018).
29. Misale, S. et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486, 532–536 (2012).
30. Russo, M. et al. Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. Cancer Discov. 6, 147–153 (2016).
31. Siravegna, G. et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat. Med. 21, 827 (2015).
32. Taeger, R. et al. Mechanisms of acquired resistance to BRAF V600E inhibition in colon cancers converge on RAF dimerization and are sensitive to its inhibition. Cancer Res. 77, 6513–6523 (2017).

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Author contributions
E.C., A.M.S., D.M.H., R.Y., A.D., and M.S. conceived the study. E.C., A.M.S., A.K., S.M., E.T., J.C., R.S., S.S., E.d.S., and S.G. designed and performed the experiments. J.F.H., B.B.T., M.M., R.J.N., E.d.S., and M.F.B. performed the data analysis and assisted with data interpretation. A.M.S., P.R., R.P., S.D.S., H.H.W., B.B.T., A.S., K. E., R.B.L., B.H.-L., J.A.P., M.F.B., and M.L. assisted with prospective genomic and clinical data collection and sample annotation. E.C., A.M.S., D.M.H., A.D., and M.S. wrote the manuscript with input from all authors.

Competing interests
M.S. is on the Advisory Board of the Bioscience Institute and Menarini Ricerche, has received research funds from Puma Biotechnology, Daichi-Sankyo, Targimmune, Immunomedics, and Menarini Ricerche, is a co-founder of Medendi Medical Travel, and in the past 2 years has received honoraria from Menarini Ricerche and ADC Pharma. A.D. has honoraria from Medscape, OnLive, PeerVoice, Physician Education Resources, Tyra Biosciences, Targeted Oncology, MORE Health, Research to Practice, Foundation Medicine, PeerView, AstraZeneca, Genentech/Roche, Bayer, and has consulting roles at Ightya, Loxo Oncology, TP Therapeutics, AstraZeneca, Pfizer, Blueprint Medicines, Genentech/Roche, Takeda, Helsinn Therapeutics, BeiGene, Hengrui Therapeutics, Endrana, and Bayer. D.M.H. reports personal fees from Atara Biotherapeutics, Chugai Pharma, CytoMx Therapeutics, Boehringer Ingelheim, and AstraZeneca and research funding from Puma Biotechnology, AstraZeneca, and Loxo Oncology. R.Y. has received research support from GlaxoSmithKline, Novartis and Array and consulting fees from GlaxoSmithKline. J.F.H. has received honoraria from Medscape, the European Society of Medical Oncology, and Axiom Biotechnologies, as well as research funding from Bayer. R.S. has received research funding from Helsinn Therapeutics. M.B. has received honoraria for advisory board participation from Roche and research support from Illumina. M.L. has received honoraria for ad hoc advisory board participation from AstraZeneca, Bristol-Myers Squibb, Takeda, and Bayer, and research support from LOXO Oncology (for expanded Archer targeted RNAseq testing) and Helsinn Therapeutics. P.R. has received consulting fees from Novartis.

Additional information
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Methods

Ethical compliance. We declare compliance with all relevant ethical regulations.

Patients. Patients were treated with TRK inhibitors as part of prospective IRB-approved research protocols or expanded access protocols. All patients provided written informed consent for genomic sequencing of tumor and cfDNA, and review of medical records for detailed demographic, pathological, and clinical data, and for publication of this information as part of an institutional IRB-approved research protocol (Memorial Sloan Kettering Cancer Center (MSKCC); NCT01019032). Research protocols for tumor collection and analysis were approved by the ethical committees of the MSKCC.

Compounds. Larotrectinib and LOXO-195 were obtained from Lexo Oncology. Trametinib and MEK-162 were purchased from Selleckchem. All drugs were dissolved in DMSO to yield 10 mM stocks and stored at −20 °C.

Targeted tumor sequencing. DNA from formalin-fixed paraffin-embedded tissue and matched germline DNA underwent targeted next-generation sequencing assay using MSK-IMPACT33. In brief, this assay uses a hybridization-based exon capture designed to capture all protein-coding exons and select introns of oncoproteins, tumor-suppressor genes, and key members of pathways that may be actionable by targeted therapies. In this study, either 410 or 468 key cancer-associated genes were analyzed. Sequencing data were analyzed as described previously to identify somatic single-nucleotide variants, small insertions and deletions, copy number alterations, and structural rearrangements3. In addition, hotspots alterations were identified using an adaptation of a method described previously37 applied to a cohort of 24,592 sequenced human cancers3.

Targeted plasma sequencing. Cell-free DNA was extracted from all plasma samples and sequenced using a custom, ultra-deep coverage next-generation sequencing panel (MSK-ACCESS). The custom assay includes key exons and domains of 129 genes and introns of 10 genes harboring recurrent breakpoints, and uses duplex unique molecular identifiers (UMIs) and dual index barcodes to minimize background sequencing errors and sample-to-sample contamination. Sequencing data were analyzed using a custom bioinformatics pipeline that trims the UMIs, aligns the processed reads to the human genome, collapses PCR replicates into consensus sequences, and re-aligns the error-suppressed consensus reads. Consensus reads with representation from both strands of the original cDNA duplex were used for de novo variant calling using VarDict (v1.5.1). Mutation calling required at least one collapsed read at a known cancer hotspot site or at least three collapsed reads at non-hotspot sites. All samples were sequenced to an average depth of approximately 20,000x coverage. Somatic mutations were identified and quantified as variant allele frequencies. Copy number alterations were identified across all samples using a method described previously38; NTRK fusions were identified and quantified using Manta (v1.5.0). All samples were reviewed manually to identify NTRK fusions, and cfDNA from Patient 3 was reviewed manually to identify copy number alterations, including MET. Variants were called against an unmatched healthy plasma donor to identify any specimen-type-related artifacts. Mutations called at silent, intronic, and intergenic loci were removed.

Patient-derived primary cell lines. The LMNA–NTRK1 and the derived entrectinib-resistant LMNANTRK1, NTRK1G12CDRC cell lines were obtained from A. Bardelli (Candiolo Cancer Institute, Italy). The LMNA–NTRK1, NTRK1G12CD, KRASV600E cell line was established following chronic exposure of the LMNA–NTRK1, NTRK1G12CD line to increasing concentrations of LOXO-195 (ranging from 1 to 200 nM) for 4 months. The TPR–NTRK1, NTRK1G12CD pancreatic cancer cell line was established from a PDx engrailed with a biopsy of a patient at the time of progression on entrectinib. All cell lines were plated on collagen-coated petri dishes and cultured in DMEM/F12 + 10% FBS and 1% antibiotics.

Antibodies. For western blots, total protein lysates following the indicated treatment were extracted and separated using SDS-PAGE gels according to standard methods. Membranes were probed using the following antibodies: pan Trk clone A1H6r (92991 S Cell Signaling Technology), phospho TrkA (Y767/675) clone C50F3 (6421 S Cell Signaling Technology), phospho PLCγ (Y783), 2821 L Cell Signaling Technology), phospho MEK1/2 (S217/221) clone 41G9 (9154 S Cell Signaling Technology), total MEK1/2 (91221 Cell Signaling Technology), BRAF clone D9T6s (125 nM) and trametinib (2 nM). For the colony formation assay, a pool of KO cells was generated from the TPR–NTRK1, NTRK1G12CD xenografts.

Fluorescence in situ hybridization. MET FISH was performed using the Zytolight SPEC dual-color MET/CEN17 probe cocktail (Zytovision). MET FISH is a clinically validated assay that was performed in a CLIA accredited laboratory.

Plasms and viral particle production. pDONR223, Kras, g12A (cat. 81673) was purchased from Addgene. The pDONR223, Kras wt and pG12D were generated by site-directed mutagenesis using the Q5 Site-Directed Mutagenesis Kit (E0552S New England Biolabs) and primers pDNRunmtG12Afw F GTTGGAGCTCTTGCGATAGCC. pDNRmutG12Dfw F GTTGGAGCTGTAGGACTAGTC. The common reverse pDNRmut R TACCAAAGTGTATTACGATCTGCG. The pDONR plasmids were then subcloned in the plX302 destination lentiviral vector (Addgene, cat. 25896).

Lentiviral particles were produced in HEK 293 T cells as described previously39, and used to infect the LMNA–NTRK1 and the LMNA–NTRK1, NTRK1G12CD cell lines. pLENTi6 lentiviral plasmids encoding for wild type and BRAFV600E were obtained from N. Rosen’s laboratory and used to infect the TPR–NTRK1, NTRK1G12CD cell line. Transduced cell lines were used for western blot analyses and proliferation assays.

Proliferation assays. For cell-titer globased assays, LMNA–NTRK1 and LMNA–NTRK1, NTRK1G12CD cell lines transduced with wild-type and BRAFV600E plasmids were seeded in 96-well plates (6,000 per well). The following day larotrectinib or LOXO-195 (1:2 dilutions starting with a maximum concentration of 100 nM) was added. Cell-titer glo reagent was added 24 h later and absorbance was read at 490 nm according to the manufacturer’s protocol. Data are presented as percentage of cell viability and ± s.d. normalized to the DMSO treated control cells included 100% viable cell. Cell-titer glo was also used to test the viability of LMNA–NTRK1, NTRK1G12CD and LMNA–NTRK1, NTRK1G12CD, KRASV600E cell lines following treatment with LOXO-195 (125 nM) or the combination of LOXO-195 (125 nM) and trametinib (2 nM). For the colony formation assay, a TPR–NTRK1, NTRK1G12CD pancreatic cancer cell line transduced with sequences encoding wild-type BRAF or BRAFV600E was seeded in 6-well plates (300,000 cells per well). The following day, 50 nM of LOXO-195 were added. After 72 h of incubation cells were fixed in 4% glutaraldehyde and stained with crystal violet.

In vivo studies. Xenografts derived from the LMNA–NTRK1, NTRK1G12CD, KRASV600E cell line were generated by injecting 5 million cells into the flank of 6-week-old NSG female mice. Two weeks later, tumors were collected and expanded in additional mice. PDXs were generated in CB-17 SCID (200 nM/kg) mice with the combination of LOXO-195 (100 mg/kg) and trametinib (300 mg/kg). The following day, 50 nM of LOXO-195 were added. After 72h of incubation cells were fixed in 4% glutaraldehyde and stained with crystal violet.

Statistical analysis. Statistical analysis was conducted using GraphPad Prism 8 (GraphPad Software). Two-tailed unpaired t-tests were used to evaluate significant differences in the percentage of viable cells in cell proliferation assays. Data were presented as means ± s.d. Exact P values are indicated. Two-tailed unpaired t-tests were also used to evaluate significant differences in the tumor volumes in vivo efficacy studies. Error bars represent s.e.m. Exact P values are indicated.

Data availability
All genomic results and associated clinical data for all patients in this study are publically available in the cBioPortal for Cancer Genomics at http://cbioportal.org/
All relevant cell-free DNA sequencing data are included in the paper and/or supplementary files.

References
33. Zehir, A. et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat. Med.* **23**, 703–713 (2017).
34. Cheng, D. T. et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J. Mol. Diagn.* **17**, 251–264 (2015).
35. Chang, M. T. et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat. Biotechnol.* **34**, 153–163 (2016).
36. Chang, M. T. et al. Accelerating discovery of functional mutant alleles in cancer. *Cancer Discov.* **8**, 174–183 (2018).
37. Tkac, J. et al. HELB Is a feedback inhibitor of DNA end resection. *Mol. Cell* **61**, 405–418 (2016).
Extended Data Fig. 1 | Hotspots mutations in KRAS and BRAF confer resistance to TRK inhibitors in patients and preclinical models. a, Representative scans of Patient 1 at baseline, 4 weeks on larotrectinib treatment (responding) and at progression. Targeted sequencing of the tumor at progression identified a BRAF V600E mutation (red square). b, cfDNA analysis confirmed the emergence of BRAF V600E and identified a subclonal KRAS G12D mutation. c, Emergence of a BRAF V600E mutation in the larotrectinib-resistant PDXs presented in Fig. 1b demonstrated by Sanger sequencing and IHC staining using a BRAF V600E specific antibody to detect the mutant protein. d, Representative scans of Patient 2 at baseline, 4 weeks on LOXO-195 treatment (responding) and at progression. Targeted sequencing of the tumor at progression identified a KRAS G12A mutation (white square). e, cfDNA analysis confirmed the emergence of KRAS G12A. f, Sanger sequencing demonstrating the emergence of a KRAS G12D mutation in a LMNA-NTRK1, NTRK1 G595R positive primary CRC cell line treated with increasing concentrations of LOXO-195 for 4 months until the development of resistance. g, Cell proliferation on the LMNA-NTRK1, NTRK1 G595R and the LMNA-NTRK1, NTRK1 G595R, KRAS G12D primary cell lines treated for 72 hours with increasing concentrations (ranging from 0 to 1,000 nM) of LOXO-195. Data are presented as mean±SD of two biological replicates.
Extended Data Fig. 2 | Radiologic response to combined RAF/MEK inhibition in Patient 1 correlates with decreased allele frequency of the TRK fusion in cfDNA. Graph depicting the allele frequencies of truncal NTRK fusion in the cfDNA of the CTRC-NTRK1 positive pancreatic adenocarcinoma patient (Patient 1) while treated with LOXO-195 and the combination of dabrafenib and trametinib. The time on treatment, best clinical response (SD: stable disease based on RECIST v1.1 criteria) and the time of progression (POD) for each of the indicated therapeutic regimens are displayed.
Extended Data Fig. 3 | TRK inhibition enhances the anti-tumor effect of the combination of RAF and MEK blockade in TRK fusion-positive preclinical models harboring a BRAF V600E mutation. a, Activity of dual RAF/MEK inhibition (dabrafenib ranging from 50 to 500 nM and trametinib 1 and 5 nM) in the absence (left panel) or presence (right panel) of the TRK inhibitor [larotrectinib or LOXO-195 (25 nM)] on the proliferation of LMNA-NTRK1 and LMNA-NTRK1, NTRK1 G595R CRC cell lines transduced with the BRAF V600E mutation. Two biological replicates were performed. b, Western blot analysis on the same cell lines treated for 4 hours as indicated (larotrectinib/LOXO-195 = 25 nM, trametinib = 5 nM, dabrafenib = 100 nM, the combination of dabrafenib = 100 nM and trametinib = 5 nM or the triple therapy at two different concentrations of larotrectinib/LOXO-195= 10 and 25 nM, respectively). The triple therapy is more potent than the combination of anti RAF/MEK alone in inhibiting MEK, ERK and AKT. Two biological replicates were performed. c, Efficacy of the triple therapy (larotrectinib + dabrafenib + trametinib) against the Patient 1-derived PDX that harbors a V600E mutation. The triple therapy is significantly more efficacious than the combination of dabrafenib and trametinib alone in inhibiting tumor growth (P = 0.000001). A minimum of six animals per group [vehicle (n = 7), larotrectinib (n = 6), dabrafenib + trametinib (n = 7) and larotrectinib + dabrafenib + trametinib (n = 6)] were used. Two-tailed unpaired t-test was used to evaluate significant differences in the tumor volumes. Data are presented as mean±SEM.
Extended Data Fig. 4 | Radiologic response to combined TRK/MET inhibition in Patient 3 correlates with decreased allele frequency of the targeted alterations in cfDNA. **a**, Graph depicting the allele frequencies of the truncal NTRK fusion in the cfDNA of the PLEKHA6-NTRK1 positive cholangiocarcinoma patient (Patient 3) while treated with LOXO-195 and the combination of LOXO-195 and crizotinib. The time on treatment, best clinical response (SD: stable disease based on RECIST v1.1 criteria) and the time of progression (POD) for each of the indicated therapeutic regimens are displayed. **b**, Copy number plots from this patient demonstrating disappearance of the MET amplification on treatment and reemergence at the time of disease progression.
Extended Data Fig. 5 | Dual TRK and MEK blockade inhibits growth of the LOXO-195 resistant LMNA-NTRK1, NTRK1 G595R, KRAS G12D cancer cell line. 

**a.** Western blot from the two colorectal cancer cell lines LMNA-NTRK1, NTRK1 G595R and LMNA-NTRK1, NTRK1 G595R, KRAS G12D, treated as indicated. LOXO-195 (50 nM), MEK-162 (50 nM) or the combination of both drugs (195 + 162) were administered at the indicated time and protein lysates were probed with the indicated antibodies. While LOXO-195 was sufficient to inhibit both phospo-TRK and phospho-ERK in the KRAS wild type cell line, the combination of LOXO-195 and MEK-162 was required for this dual inhibition in the KRAS G12D mutated cell line. Three biological replicates were performed.

**b.** Proliferation assays on the same cell lines (labeled NTRK1 G595R and KRAS G12D, respectively) treated for 72 hours with LOXO-195 (125 nM), MEK-162 (25 nM) or their combination. Data are presented as mean±SD of four biological replicates. Two-tailed unpaired t-test was used to evaluate significant differences in % of viable cells. P values < 0.05 were considered statistically significant.
Extended Data Fig. 6 | Radiologic and cfDNA correlates in a LOXO-195 resistant CRC patient treated with the combination of LOXO-195 and trametinib. Graph depicting the dynamics of select mutations detected in the cfDNA of the LMNA-NTRK1, GS95R mutated colorectal cancer patient while treated on targeted therapy (LOXO + tram: LOXO-195 + trametinib). The time on treatment, best clinical response (PR: partial response based on RECIST v1.1 criteria) and the time of progression (POD) for each of the indicated therapeutic regimens are displayed. Representative scans of Patient 2 are presented at baseline and at progression (4 weeks) with the combination of LOXO-195 and trametinib.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
An institutional IRB-approved research protocol (MSKCC; NCT01775072) was used for the collection of patients' information. No software was used to collect the data.

Data analysis
MSK-IMPACT was used as targeted next-generation sequencing platform. The MSK-IMPACT data analysis pipeline can be found here: https://github.com/rhshah/IMPACT-Pipeline. MSK-ACCESS was used as ultra-deep coverage next-generation sequencing panel for cfDNA analysis. VarDict (v1.5.1) was used for de novo variant calling and Manta (v1.5.0) for fusion calling. GraphPad Prism v. 8 was used for data analysis and statistical tests.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

A data availability statement has been added to the manuscript on pages 11-12. All genomic results and associated clinical data for all patients in this study are publicly available in the cBioPortal for Cancer Genomics at the following URL: http://cbioportal.org/msk-impact.
Field-specific reporting
Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample size. Sample size was based on experimental feasibility, sample availability, and N necessary to obtain definitive results. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | none |
| Replication | All experimental findings were reproduced at least twice, with the exception of the in vivo experiments in which, however, a minimum of 5 animals per arm was utilized. Animals’ randomization was performed for all the in vivo studies just before starting the treatments to assign animals to the different arms. Tumor volume average at this time point was set at 100 mm3. Unpaired T-Test was performed to ensure that differences in tumor volume in the different groups were not significant. |
| Randomization | Randomization was not part of the design of the larotrectinib clinical trials (NCT02122913, NCT02637687 and NCT02576431) or the entrectinib clinical trials (EudraCT 2012-000148-88 and NCT02097810) or the LOXO-195 trial (NCT03215511) considered in this study. Randomization is not relevant to the current studies as the object was to decipher the mechanisms of sensitivity to targeted therapies within individual patients. |
| Blinding | Patients and clinicians were not blinded for the clinical trials referenced in this paper. Specifically, blinding was not part of the design of the larotrectinib clinical trials (NCT02122913, NCT02637687 and NCT02576431), the entrectinib clinical trials (EudraCT 2012-000148-88 and NCT02097810), or the LOXO-195 trial (NCT03215511). These studies did not involve randomization and all patients enrolled were treated with the investigational agents. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChiP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

### Antibodies

**Antibodies used**

The antibodies described in the Methods section are widely used, commercially available antibodies validated by the companies and publications cited on the company websites.

- For Western blot: pan Trk clone A7H6R (929915 Cell Signaling Technology lot: 10 1:1000; Validation: Western blot analysis of extracts from mouse neonatal and rat fetal brain using Trk (pan) (A7H6R) Rabbit mAb), phospho TrkA (Y674/675) clone C50F3 lot: 10 1:1000 (4621S Cell Signaling Technology; validation: Western blot analysis of extracts from NIH/3T3 cells stably transfected with TrkA or TrkB, and treated with NGF or BDNF, respectively, using Phospho-TrkA (Y674/675) (C50F3) Rabbit mAb), phospho MEK1/2 (S217/221) clone 41G9 lot: 18 1:1000 validation: Western blot analysis of extracts from untreated or TPA-treated HeLa and NIH/3T3 cells, using Phospho-MEK1/2 (Ser217/221) (41G9) Rabbit mAb (9154S Cell Signaling Technology), BRAF clone D9T6S lot: 1 (14814S Cell Signaling Technology; validation: Western blot analysis of extracts from various cell lines using B-Raf (D9T6S) Rabbit mAb, BRAF V600E lot: GR3235840-3 1:500 (ab228461 Abcam; validation: Immunohistochemistry (Formalin/PFA-fixed paraffin embedded sections) - Anti-BRAF (mutated V600 E) antibody [VE1] (ab228461) Image from Qiu Tet al. Sci Rep, vol 5, pg 9211, 2015), phospho p44/42 MAPK (Erk1/2; T202/Y204) clone D13.14.4E (4370S Cell Signaling Technology), phospho AKT (S473) clone D9E (4060L Cell Signaling Technology), pan RAS (BK008, part AES02 Cytoskeleton), β-actin clone 13E5 (4970S Cell Signaling Technology), For IHC: BRAF V600E (ab228461 Abcam), MET SPA4 (Ventana Medical Systems; LOT:G08106; Conc. 9.75ug/ml ready to use dilution), Trk A EP105BY (Abcam) Lot: GR179395-33; Conc |
Validation

All antibodies were purchased pre-validated from the manufacturers (specified above).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) HEK 293T cells were purchased from ATCC. LMNA-NTRK1 and LMNA-NTRK1, G595R were obtained from Dr. Alberto Bardelli (Candiolo Cancer Institute, FPO, IRCCS, Turin, Italy), the LMNA-NTRK1, G595R, KRAS G12D and the TPR-NTRK1, G595R were both established in our laboratory (patient-derived).

Authentication The cell lines obtained by Dr. Bardelli were characterized in the following publication (Russo, M., et al. Acquired Resistance to the TRK Inhibitor Entrectinib in Colorectal Cancer. Cancer Discov 6, 36-44 (2016)). The other were molecularly characterized by sequencing using MSK-IMPACT (data available under request).

Mycoplasma contamination Cells were routinely tested for mycoplasma. Cells tested mycoplasma negative.

Commonly misidentified lines (See ICLAC register) No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals six-week-old NSG female mice were used in the study. Tumors were harvested from mice.

Wild animals No wild animals were used in this study.

Field-collected samples No field-collected samples were used in this study.

Ethics oversight Six-week-old NSG female mice were implanted subcutaneously with specimens freshly collected from patients at Memorial Sloan Kettering Hospital under a MSK approved IRB biospecimen protocol (IRB # 13-040 Pi: Scaltriti). IACUC guidelines were followed during animal experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics The population included patients age 12 and above of any gender with advanced cancer harboring NTRK fusions detected by molecular profiling (DNA or RNA-based sequencing). All patients were treated with systemic, targeted therapy.

Recruitment Patients were referred for the clinical trials at the discretion of their treating oncologists. We do not believe that there is any recruitment bias that may have influenced the results of this study.

Ethics oversight All patients provided written informed consent for genomic sequencing of tumor and cfDNA, and review of medical records for detailed demographic, pathologic, and clinical data as part of an institutional IRB-approved research protocol (MSKCC; NCT01775072).

Adult and pediatric patients harboring NTRK fusion-positive tumors were consented for the following trials: larotrectinib clinical trials (NCT02122913, NCT02637687 and NCT02576431) or the entrectinib clinical trials (EudraCT 2012-000148-88 and NCT02097810) or the LOXO-195 trial (NCT03215511). Patients were separately consented to MSKCC IRB #12-245 (Pi: Hyman) for NGS of their tumors (MSK-IMPACT).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration N/A

Study protocol N/A

Data collection N/A

Outcomes N/A