Supplementary Methods

Detection of the ETV6-NTRK3 fusion

After extraction of ribonucleic acid (RNA) from the leukemic cells, 1385 cancer-associated target genes were amplified via a Next Generation Sequencing-based RNA panel (TruSight RNA Pan-Cancer Panel, Illumina) and sequenced on an Illuina MiSeq machine. For detection of fusion genes in the measured sequences, the following BaseSpace Apps were used in the BaseSpace Sequencing Hub (Illumina): RNA-Seq alignment (Illumina) and TopHat alignment (Illumina). The ETV6 break-apart probe was obtained from Abbott.

MRD analysis

MRD measurements in bone marrow samples were performed using real-time quantitative PCRs for three Ig/TCR markers with the following sensitivities and quantitative ranges (QR): 1) Vd2-Dd3, sensitivity and QR 1 x 10^{-4}; 2) Vk2D26-Kde, sensitivity 1 x 10^{-4} and QR 5 x 10^{-4}; 3) VH1.2*02-JH3b, sensitivity 1 x 10^{-5} and QR 1 x 10^{-4}. For MRD assessments in the cerebrospinal fluid (CSF), genomic desoxyribonucleic acid (DNA) was prepared using the NucleoSpin Plasma XS-Kit (Macherey-Nagel) suitable for small sample sizes. MRD was analyzed by PCR of the three Ig/TCR-genes after normalization with albumin. Data interpretation was based on the Euro-MRD ALL guidelines[1], however these were not formally applied due to a lower DNA input. MRD values in the CSF were therefore expressed as "negative" or "positive" and not as quantitative values.

Preclinical in vivo experiment with larotrectinib

NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Charles River, bred and maintained in accordance with governmental regulations. After informed consent, secondary NSG xenograft mice were generated by intravenous injection of 1 x 10^6 leukemic cells derived from primary PDX material from this patient and flow cytometric monitoring of human blasts in the murine peripheral blood was performed as previously described[2]. Endpoints for terminating an experiment were defined using clinical scores as approved by authorities ("MELUND" of Schleswig-Holstein, Germany, V242–45475/2019(54-5/16)).

Larotrectinib was obtained from Hycultec, dissolved as previously described[3] and applied at a dose of 200 mg/kg daily by oral gavage[3].

References for Supplementary Methods

1. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21(4):604-611. Prepublished on 2007/02/09 as DOI 10.1038/sj.leu.2404586.
2. Schewe DM, Alsadeq A, Sattler C, et al. An Fc engineered CD19 antibody eradicates MRD in patient-derived MLL-rearranged acute lymphoblastic leukemia xenografts. *Blood*. 2017. Prepublished on 2017/07/13 as DOI 10.1182/blood-2017-01-764316.
3. Roberts KG, Janke LJ, Zhao Y, et al. ETV6-NTRK3 induces aggressive acute lymphoblastic leukemia highly sensitive to selective TRK inhibition. *Blood*. 2018;132(8):861-865. Prepublished on 2018/06/09 as DOI 10.1182/blood-2018-05-849554.

Supplemental Figure 1

Bone marrow (BM) and CSF minimal residual disease (MRD) status during the treatment course as assessed by polymerase chain reaction (PCR) for patient-specific Ig/TCR rearrangements. Day 0 is the day of first relapse post-HSCT. †One target. ‡Two targets. BM, bone marrow; CSF, cerebrospinal fluid; MRD, minimal residual disease; PCR, polymerase chain reaction.

Days since first relapse	BM-MRD by PCR (3 targets)	CSF-MRD by PCR (3 targets)
First relapse		
0	Morphological blasts	Morphological blasts
16	-	No signal
44	-	Positive
50	Negative	Morphological blasts
Second relapse		
64	10^{-3}	-
72	10^{-4} / $10^{-6\dagger}$	-
79	-	No signal
91	10^{-4}	No signal
106	10^{-3}	Positive
113	Negative / $10^{-6\dagger}$	Positive
121	Negative	No signal
128	Negative	No signal
134	Negative / $10^{-6\dagger}$	No signal
143	Negative	No signal
156	Negative	No signal
163	Negative / $10^{-6\ddagger}$	No signal
170	Negative	No signal
Third relapse		
207	-	Morphological blasts
211	Negative / $10^{-6\ddagger}$	Positive
219	Negative / $10^{-6\dagger}$	-
232	Negative / $10^{-6\ddagger}$	-
240	10^{-6}	Positive
246	-	Positive
249	-	Positive
253	Negative	Positive
261	-	No signal‡
267	-	positive†
268	-	-
274	-	No signal
281	Negative	No signal
295	Negative	No signal
309	Negative	No signal
319		No signal

Supplemental Figure 2

Original flow cytometry plots from the xenograft experiment depicted in Figure 1C.