FREQUENTLY ASKED QUESTIONS

TruSight™ Oncology Comprehensive (EU)

Learn more about this CE-marked *in vitro* diagnostic, next-generation sequencing solution for comprehensive genomic profiling

illumina®

General information

What is TruSight Oncology Comprehensive (EU)?

As a global leader in next-generation sequencing (NGS) and microarray-based solutions, Illumina is dedicated to improving human health by unlocking the power of the genome. Illumina continues to innovate by offering TruSight Oncology Comprehensive (EU), a CE-marked *in vitro* diagnostic (IVD), pan-cancer comprehensive genomic profiling (CGP) panel. TruSight Oncology Comprehensive (EU) can generate a broad molecular profile of solid tumor patient samples, including formalin-fixed, paraffin-embedded (FFPE) tissue, maximizing a lab's ability to find actionable alterations that can help inform therapy decisions according to clinical guidelines.

Additional key points:

- TruSight Oncology Comprehensive (EU) can be implemented easily in house, features a streamlined workflow that proceeds from sample to final clinical report in 4-5 days, and requires as few as five FFPE slides
- TruSight Oncology Comprehensive (EU) reliably detects all DNA and RNA variant categories, including single nucleotide variants (SNVs), insertions/deletions (indels), amplifications, fusions, and splice variants and enables analysis of genomic signatures such as tumor mutational burden (TMB) and microsatellite instability (MSI)
- TruSight Oncology Comprehensive (EU) is indicated as a companion diagnostic (CDx) test to identify cancer patients with solid tumors who are positive for ***NTRK1***, ***NTRK2***, or ***NTRK3*** gene fusions for treatment with VITRAKVI (larotrectinib) in accordance with the approved therapeutic labeling
- A roadmap of additional CDx claims will add multiple indications linked to breakthrough therapies that can improve patient outcomes

Generate a CGP report for a patient sample

- Detect DNA plus RNA variants and genomic signatures for multiple solid tumor types, generate a CGP report for a patient's tumor, and increase confidence in correct treatment decisions

Enable targeted therapies and clinical trials

- Leverage content that includes key biomarkers associated with drug labels, ESMO guidelines, and clinical trials for multiple solid tumor types
- Deliver results that inform therapy decisions according to clinical guidelines

Perform IVD testing in-house

- Implement a streamlined workflow, going from sample to report in 4-5 days
- Offer precision oncology research, keep data in your institution, and avoid losing samples to send-out services

Who is the target customer?

The target customers for TruSight Oncology Comprehensive (EU) are molecular pathologists and lab directors in European countries who routinely run solid tumor tissue biomarker tests to aid oncologists with therapy selection for cancer patients. Types of institutions include:

- Academic medical centers
- Large to medium hospitals
- Independent commercial labs

TruSight Oncology Comprehensive (EU) will be available in the following countries:

- Austria
- Belgium
- Bulgaria
- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Iceland
- Ireland
- Israel
- Italy
- Liechtenstein
- Luxembourg
- Malta
- Netherlands
- Norway
- Poland
- Spain
- Sweden
- Switzerland
- UK
- Greece
- Portugal
- Romania
- Slovenia
- South Africa
- UAE
- Qatar

What are the IVD claims?

TruSight Oncology Comprehensive (EU) is an *in vitro* diagnostic test that uses targeted next-generation sequencing to detect variants in 517 genes using nucleic acids extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms using the Illumina NextSeq™ 550Dx instrument. The test can be used to detect single nucleotide variants, multinucleotide variants, insertions, deletions and gene amplifications from DNA, and gene fusions and splice variants from RNA. The test also reports a Tumor Mutational Burden (TMB) score and Microsatellite Instability (MSI) status.

The test is intended as a companion diagnostic to identify cancer patients for treatment with the targeted therapy listed in Table 1, in accordance with the approved therapeutic product labeling. In addition, the test is intended to provide tumor profiling information for use by qualified healthcare professionals in accordance with professional guidelines and is not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 1: CDx indication

Tumor type	Biomarkers	Targeted therapy
Solid tumors	*NTRK1*, *NTRK2*, *NTRK3* gene fusions	VITRAKVI (larotrectinib)

Read the TruSight Oncology Comprehensive Package Insert to learn more, support.illumina.com/sequencing/sequencing_kits/trusight-oncology-comprehensive.html

What gene/biomarker content is included?

TruSight Oncology Comprehensive (EU) includes key biomarkers in clinical guidelines, drug labels, and clinical trials across multiple solid tumor types and histologies (Figure 1). Content includes small DNA variants (Table 2) as well as fusions (RNA), splice variants (RNA), amplifications (DNA), and complex genomic signatures (Table 3). These lists are based on content that has been validated as part of the Illumina IVD regulatory submission.

Pan-cancer: *BRAF, NTRK1, NTRK2, NTRK3, RET*, MSI, TMB													Genes with biomarkers of potential clinical significance†
		Genes with biomarkers of clinical significance*											
	Breast	*BRCA1*	*BRCA2*	*ERBB2*	*ESR1*	*PALB2*	*PIK3CA*						**180**
	Colorectal	*ERBB2*	*KRAS*	*NRAS*									**166**
	Bone	*EGFR*	*ERG*	*ETV1*	*ETV4*	*EWSR1*	*FEV*	*FLI1*	*FUS*	*H3F3A*	*HEY1*	*IDH1*	**140**
		MDM2	*NCOA2*	*SMARCB1*									
	Lung	*ALK*	*EGFR*	*ERBB2*	*KRAS*	*MET*	*NUTM1*	*ROS1*					**223**
	Melanoma	*KIT*	*NRAS*	*ROS1*									**172**
	Ovarian	*BRCA1*	*BRCA2*	*FOXL2*									**149**
	CNS‡	*APC*	*ATRX*	*CDKN2A*	*CDKN2B*	*EGFR*	*H3F3A*	*HIST1H3B*	*HIST1H3C*	*IDH1*	*IDH2*	*MYCN*	**140**
		PTCH1	*RELA*	*TERT*	*TP53*								
	Prostate	*AR*	*ATM*	*BARD1*	*BRCA1*	*BRCA2*	*BRIP1*	*CDK12*	*CHEK1*	*CHEK2*	*FANCL*	*FGFR2*	**151**
		FGFR3	*PALB2*	*PTEN*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD54L*					
	Thyroid	*HRAS*	*KRAS*	*NRAS*	*TERT*								**165**
	Uterine & cervical	*BRCA2*	*EPC1*	*ERBB2*	*ESR1*	*FOXO1*	*GREB1*	*JAZF1*	*NCOA2*	*NCOA3*	*NUTM2A*	*NUTM2B*	**138**
		PAX3	*PAX7*	*PHF1*	*POLE*	*SMARCA4*	*SUZ12*	*TP53*	*YWHAE*				
	Other solid tumors	*ALK*	*APC*	*ARID1A*	*ASPSCR1*	*ATF1*	*ATIC*	*BAP1*	*BCOR*	*BRCA1*	*BRCA2*	*CAMTA1*	**152**
		CARS	*CCNB3*	*CDK4*	*CDKN2A*	*CIC*	*CITED2*	*CLTC*	*COL1A1*	*COL6A3*	*CREB1*	*CREB3L1*	
		CREB3L2	*CSF1*	*CTNNB1*	*DDIT3*	*DDX3X*	*DNAJB1*	*DUX4*	*EED*	*EGFR*	*ERBB2*	*ERG*	
		ETV1	*ETV4*	*ETV6*	*EWSR1*	*FEV*	*FGFR2*	*FGFR3*	*FLI1*	*FOXL2*	*FOXO1*	*FOXO4*	
		FUS	*GLI1*	*HEY1*	*HGF*	*HMGA2*	*IDH1*	*KRAS*	*LEUTX*	*MAML3*	*MDM2*	*MYB*	
		MYOD1	*NAB2*	*NCOA2*	*NF1*	*NFATC2*	*NFIB*	*NR4A3*	*NRAS*	*NUTM1*	*NUTM2A*	*NUTM2B*	
		PALB2	*PATZ1*	*PAX3*	*PAX7*	*PDGFB*	*PDGFRA*	*PRKACA*	*PRKD1*	*RANBP2*	*ROS1*	*SDHA*	
		SDHB	*SDHC*	*SDHD*	*SMARCB1*	*SS18*	*SSX1*	*SSX2*	*SSX4*	*STAT6*	*SUZ12*	*TAF15*	
		TCF12	*TERT*	*TFE3*	*TFEB*	*TFG*	*TP53*	*TPM3*	*TPM4*	*TRAF7*	*TSPAN31*	*VGLL2*	
		WT1	*WWTR1*	*YAP1*	*YWHAE*	*ZC3H7B*							

Figure 1: Key actionable biomarkers—Genes listed represent a subset of genes present in the TSO Comprehensive (EU) panel. Content analysis provided by Velsera based on IVD software Knowledge Base v8.5 (February 2023).

* Genes linked to current drug labels or guidelines.

† Based on evidence in scientific literature, presence in clinical trials, or linked to labels in other histologies.

‡ CNS, central nervous system.

Table 2: TruSight Oncology Comprehensive (EU) panel content

Small variants - 517 genes (from DNA)											
ABL1	*BCR*	*CREBBP*	*ERBB4*	*FGFR4*	*HIST1H3G*	*KEAP1*	*MST1*	*PDCD1*	*PTPRS*	*SH2D1A*	*TFRC*
ABL2	*BIRC3*	*CRKL*	*ERCC1*	*FH*	*HIST1H3H*	*KEL*	*MST1R*	*PDCD1LG2*	*PTPRT*	*SHQ1*	*TGFBR1*
ABRAXAS1	*BLM*	*CRLF2*	*ERCC2*	*FLCN*	*HIST1H3I*	*KIF5B*	*MTOR*	*PDGFRA*	*QKI*	*SLIT2*	*TGFBR2*
ACVR1	*BMPR1A*	*CSF1R*	*ERCC3*	*FLI1*	*HIST1H3J*	*KIT*	*MUTYH*	*PDGFRB*	*RAB35*	*SLX4*	*TMEM127*
ACVR1B	*BRAF*	*CSF3R*	*ERCC4*	*FLT1*	*HIST2H3A*	*KLF4*	*MYB*	*PDK1*	*RAC1*	*SMAD2*	*TMPRSS2*
ADGRA2	*BRCA1*	*CSNK1A1*	*ERCC5*	*FLT3*	*HIST2H3C*	*KLHL6*	*MYC*	*PDPK1*	*RAD21*	*SMAD3*	*TNFAIP3*
AKT1	*BRCA2*	*CTCF*	*ERG*	*FLT4*	*HIST2H3D*	*KMT2A*	*MYCL*	*PGR*	*RAD50*	*SMAD4*	*TNFRSF14*
AKT2	*BRD4*	*CTLA4*	*ERRFI1*	*FOXA1*	*HIST3H3*	*KRAS*	*MYCN*	*PHF6*	*RAD51*	*SMARCA4*	*TOP1*
AKT3	*BRIP1*	*CTNNA1*	*ESR1*	*FOXL2*	*HNF1A*	*LAMP1*	*MYD88*	*PHOX2B*	*RAD51B*	*SMARCB1*	*TOP2A*
ALK	*BTG1*	*CTNNB1*	*ETS1*	*FOXO1*	*HNRNPK*	*LATS1*	*MYOD1*	*PIK3C2B*	*RAD51C*	*SMARCD1*	*TP53*
ALOX12B	*BTK*	*CUL3*	*ETV1*	*FOXP1*	*HOXB13*	*LATS2*	*NAB2*	*PIK3C2G*	*RAD51D*	*SMC1A*	*TP63*
AMER1	*CALR*	*CUX1*	*ETV4*	*FRS2*	*HRAS*	*LMO1*	*NBN*	*PIK3C3*	*RAD52*	*SMC3*	*TRAF2*
ANKRD11	*CARD11*	*CXCR4*	*ETV5*	*FUBP1*	*HSD3B1*	*LRP1B*	*NCOA3*	*PIK3CA*	*RAD54L*	*SMO*	*TRAF7*
ANKRD26	*CASP8*	*CYLD*	*ETV6*	*FYN*	*HSP90AA1*	*LYN*	*NCOR1*	*PIK3CB*	*RAF1*	*SNCAIP*	*TSC1*
APC	*CBFB*	*DAXX*	*EWSR1*	*GABRA6*	*ICOSLG*	*LZTR1*	*NEGR1*	*PIK3CD*	*RANBP2*	*SOCS1*	*TSC2*
AR	*CBL*	*DCUN1D1*	*EZH2*	*GATA1*	*ID3*	*MAGI2*	*NF1*	*PIK3CG*	*RARA*	*SOX10*	*TSHR*
ARAF	*CCND1*	*DDR2*	*FAM46C*	*GATA2*	*IDH1*	*MALT1*	*NF2*	*PIK3R1*	*RASA1*	*SOX17*	*U2AF1*
ARFRP1	*CCND2*	*DDX41*	*FANCA*	*GATA3*	*IDH2*	*MAP2K1*	*NFE2L2*	*PIK3R2*	*RB1*	*SOX2*	*VEGFA*
ARID1A	*CCND3*	*DHX15*	*FANCC*	*GATA4*	*IFNGR1*	*MAP2K2*	*NFKBIA*	*PIK3R3*	*RBM10*	*SOX9*	*VHL*
ARID1B	*CCNE1*	*DICER1*	*FANCD2*	*GATA6*	*IGF1*	*MAP2K4*	*NKX2-1*	*PIM1*	*RECQL4*	*SPEN*	*VTCN1*
ARID2	*CD274*	*DIS3*	*FANCE*	*GEN1*	*IGF1R*	*MAP3K1*	*NKX3-1*	*PLCG2*	*REL*	*SPOP*	*WISP3*
ARID5B	*CD276*	*DNAJB1*	*FANCF*	*GID4*	*IGF2*	*MAP3K13*	*NOTCH1*	*PLK2*	*RET*	*SPTA1*	*WT1*
ASXL1	*CD74*	*DNMT1*	*FANCG*	*GLI1*	*IKBKE*	*MAP3K14*	*NOTCH2*	*PMAIP1*	*RHEB*	*SRC*	*XIAP*
ASXL2	*CD79A*	*DNMT3A*	*FANCI*	*GNA11*	*IKZF1*	*MAP3K4*	*NOTCH3*	*PMS1*	*RHOA*	*SRSF2*	*XPO1*
ATM	*CD79B*	*DNMT3B*	*FANCL*	*GNA13*	*IL10*	*MAPK1*	*NOTCH4*	*PMS2*	*RICTOR*	*STAG1*	*XRCC2*
ATR	*CDC73*	*DOT1L*	*FAS*	*GNAQ*	*IL7R*	*MAPK3*	*NPM1*	*PNRC1*	*RIT1*	*STAG2*	*YAP1*
ATRX	*CDH1*	*E2F3*	*FAT1*	*GNAS*	*INHA*	*MAX*	*NRAS*	*POLD1*	*RNF43*	*STAT3*	*YES1*
AURKA	*CDK12*	*EED*	*FBXW7*	*GPS2*	*INHBA*	*MCL1*	*NRG1*	*POLE*	*ROS1*	*STAT4*	*ZBTB2*
AURKB	*CDK4*	*EGFL7*	*FGF1*	*GREM1*	*INPP4A*	*MDC1*	*NSD1*	*PPARG*	*RPS6KA4*	*STAT5A*	*ZBTB7A*
AXIN1	*CDK6*	*EGFR*	*FGF10*	*GRIN2A*	*INPP4B*	*MDM2*	*NTRK1*	*PPM1D*	*RPS6KB1*	*STAT5B*	*ZFHX3*
AXIN2	*CDK8*	*EIF1AX*	*FGF14*	*GRM3*	*INSR*	*MDM4*	*NTRK2*	*PPP2R1A*	*RPS6KB2*	*STK11*	*ZNF217*
AXL	*CDKN1A*	*EIF4A2*	*FGF19*	*GSK3B*	*IRF2*	*MED12*	*NTRK3*	*PPP2R2A*	*RPTOR*	*STK40*	*ZNF703*
B2M	*CDKN1B*	*EIF4E*	*FGF2*	*H3F3A*	*IRF4*	*MEF2B*	*NUP93*	*PPP6C*	*RUNX1*	*SUFU*	*ZRSR2*
BAP1	*CDKN2A*	*ELOC*	*FGF23*	*H3F3B*	*IRS1*	*MEN1*	*NUTM1*	*PRDM1*	*RUNX1T1*	*SUZ12*	
BARD1	*CDKN2B*	*EML4*	*FGF3*	*H3F3C*	*IRS2*	*MET*	*PAK1*	*PREX2*	*RYBP*	*SYK*	
BBC3	*CDKN2C*	*EMSY*	*FGF4*	*HGF*	*JAK1*	*MGA*	*PAK3*	*PRKAR1A*	*SDHA*	*TAF1*	
BCL10	*CEBPA*	*EP300*	*FGF5*	*HIST1H1C*	*JAK2*	*MITF*	*PAK5*	*PRKCI*	*SDHAF2*	*TBX3*	
BCL2	*CENPA*	*EPCAM*	*FGF6*	*HIST1H2BD*	*JAK3*	*MLH1*	*PALB2*	*PRKDC*	*SDHB*	*TCF3*	
BCL2L1	*CHD2*	*EPHA3*	*FGF7*	*HIST1H3A*	*JUN*	*MLLT3*	*PARP1*	*PRKN*	*SDHC*	*TCF7L2*	
BCL2L11	*CHD4*	*EPHA5*	*FGF8*	*HIST1H3B*	*KAT6A*	*MPL*	*PAX3*	*PRSS8*	*SDHD*	*TERC*	
BCL2L2	*CHEK1*	*EPHA7*	*FGF9*	*HIST1H3C*	*KDM5A*	*MRE11*	*PAX5*	*PTCH1*	*SETBP1*	*TERT*	
BCL6	*CHEK2*	*EPHB1*	*FGFR1*	*HIST1H3D*	*KDM5C*	*MSH2*	*PAX7*	*PTEN*	*SETD2*	*TET1*	
BCOR	*CIC*	*ERBB2*	*FGFR2*	*HIST1H3E*	*KDM6A*	*MSH3*	*PAX8*	*PTPN11*	*SF3B1*	*TET2*	
BCORL1	*COP1*	*ERBB3*	*FGFR3*	*HIST1H3F*	*KDR*	*MSH6*	*PBRM1*	*PTPRD*	*SH2B3*	*TFE3*	

Table 3: Additional content in TruSight Oncology Comprehensive (EU)

Fusions: 23 genes (from RNA)					
ALK	*EGFR*	*ETV1*	*FGFR3*	*NTRK2*	*RET*
AXL	*EML4*	*ETV4*	*KIF5B*	*NTRK3*	*ROS1*
BCL2	*ERG*	*FGFR1*	*NRG1*	*PAX3*	*TMPRSS2*
BRAF	*ESR1*	*FGFR2*	*NTRK1*	*RAF1*	
Splice variants: 2 genes (from RNA)					
MET				*EGFR*	
Amplifications: 2 genes (from DNA)					
ERBB2				*MET*	
Complex genomic signatures					
TMB				MSI	

What are the key attributes of the TruSight Oncology Comprehensive (EU) assay?

TruSight Oncology Comprehensive (EU):

- Provides a kitted solution that can be implemented in house by any lab
- Includes both DNA and RNA content and detects all classes of variants plus genomic signatures such as TMB and MSI; of note fusions are called from RNA to maximize sensitivity for detection
- Enables CGP test results to be generated in only 4-5 days

How is TruSight Oncology Comprehensive (EU) different from current research use only (RUO)-labeled CGP kit offerings, eg, Oncomine Comprehensive Assay Plus (Thermo Fisher Scientific, Catalog no. A48578)?

- IVD label: As a CE-marked IVD test, TruSight Oncology Comprehensive (EU) is compliant with European IVD Directive (IVDD) requirements and is on course to comply with stricter IVD Regulation (IVDR) legislation; the IVD label provides labs with the benefits of IVDR preparedness and reduced liability risk and enables easier implementation with significantly reduced test validation efforts as compared to RUO tests
- Pipeline of companion diagnostic claims: Multiple pharmaceutical partnerships have been established around TruSight Oncology Comprehensive (EU) with the goal of having many companion diagnostic claims; these are not in the roadmap for RUO-labeled products
- Underlying technology drives high level of reliability: TruSight Oncology Comprehensive (EU) is based on proven Illumina technology for library preparation, sequencing, and bioinformatics, maximizing data quality and accuracy; library prep uses hybrid–capture chemistry, enabling full detection and characterization of fusion events not possible with amplicon-based techniques (Figure 2)

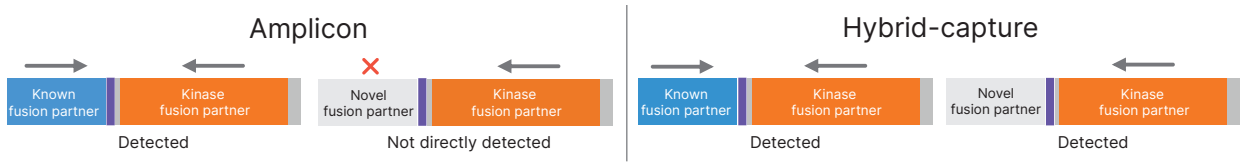

Figure 2: Hybrid–capture chemistry detects novel fusions missed by amplicon-based approaches— Amplicon-based approaches typically require confirmatory testing and do not detect fusions with novel partners. Hybrid–capture chemistry can identify fusions with both known and novel partners.

How is TruSight Oncology Comprehensive (EU) different from other IVD CGP kit offerings in Europe?

Most IVD kit offerings for biomarker testing to aid in therapy selection in Europe are based on single-gene or small hotspot panels. In contrast, TruSight Oncology Comprehensive (EU) assays over 500 genes in a single test. Content comprises actionable biomarkers, including key immunotherapy biomarkers such as TMB and MSI and emerging biomarkers that can help find eligible patients for clinical trials. In addition, TruSight Oncology Comprehensive (EU) provides full coding sequence coverage of all genes (except for *TERT*) and wide coverage of variant types. Most currently available tests cover one variant type or provide hotspot coverage, which can lead to missed alterations.

The Personal Genome Diagnostics (PGDx) IVD CGP test offered in Europe does not include RNA and might miss important fusion events, especially when it comes to *NTRK*, since these genes can fuse with multiple partners, many of them unknown.

In addition, Illumina has an extensive sales, service, and support infrastructure in Europe and offers a comprehensive support program to help labs expedite implementation and certification. Illumina also supplies IVD users with access to ready-to-use marketing and educational assets to share with their local health care providers and help them understand the value of CGP testing.

Learn more about the potential of CGP, illumina.com/cgp

NTRK fusions performance

Panel content in TruSight Oncology Comprehensive (EU) includes *NTRK1*, *NTRK2*, and *NTRK3* genes. The advanced hybrid–capture chemistry enables unbiased and broad detection of *NTRK* gene fusions in RNA, agnostic of the fusion partner.

How does TruSight Oncology Comprehensive (EU) detect *NTRK* gene fusions?

TruSight Oncology Comprehensive (EU) is an NGS-based test that uses a hybrid–capture chemistry to prepare libraries from DNA and RNA extracted from patient tumor samples. This strategy uses a set of probes that align and capture the genes of interest from the extracted nucleic acids. Genes are then sequenced in both the 3′ and 5′ directions. This approach can sequence fusion breakpoints and identify the partner gene that formed the fusion with the target gene. The RNA hybrid–capture method for fusion detection has several advantages:

- Eliminates the need to sequence intronic regions and maximizes fusion detection
- Detects fusions agnostic of the fusion partner (both known and novel fusions); as the fusion partner is fully characterized, there is no need for a confirmatory test in contrast to some amplicon-based approaches that use an indirect method, based on 5′/3′ imbalance, to detect novel fusion events; these methods can't characterize the fusion partner and typically require confirmatory testing (Figure 2)

What is the benefit of testing for *NTRK* gene fusions as part of a CGP test?

Including *NTRK* gene fusion testing as part of a CGP assay enables generation of a comprehensive biomarker profile of the patient tumor using a single biopsy specimen and a single test. This saves time, tissue specimen, and cost, while reducing the complexity associated with running multiple biomarker test workflows in the lab. Furthermore, combining less prevalent biomarkers with more prevalent ones in the same test helps ensure that patients are tested for all relevant biomarkers, ultimately increasing the chances of identifying an actionable alteration that can lead to a targeted therapy or clinical trial.

What data is available to support the ability of TruSight Oncology Comprehensive (EU) to detect *NTRK* fusions?

Several studies were performed to assess TruSight Oncology Comprehensive (EU) performance with *NTRK* gene fusions. These include reproducibility, within-lab precision, limit of detection (LoD), and clinical validation studies. See page 13 for details.

Workflow

What is the recommended sample input?

TruSight Oncology Comprehensive (EU) requires 40 ng RNA and/or 40 ng DNA extracted from FFPE tissue.

What are the workflow steps from sample preparation to final report?

The TruSight Oncology Comprehensive (EU) workflow includes four steps: sample acquisition and processing, DNA and RNA extraction, library preparation, and fully automated sequencing, analysis, and report generation (Figure 3).

Additional details:

- Specimen tissue should be fixed using formalin fixative suitable for molecular analyses; a minimum of 20% tumor cell content is recommended and ≥ 30% is optimal
- DNA and RNA extraction can be performed using commercially available extraction kits
- Library preparation takes approximately two days
- After loading the libraries on the NextSeq 550Dx instrument, the workflow is fully automated, including sequencing, base calling and QC, variant calling, interpretation, and generation of the final clinical report

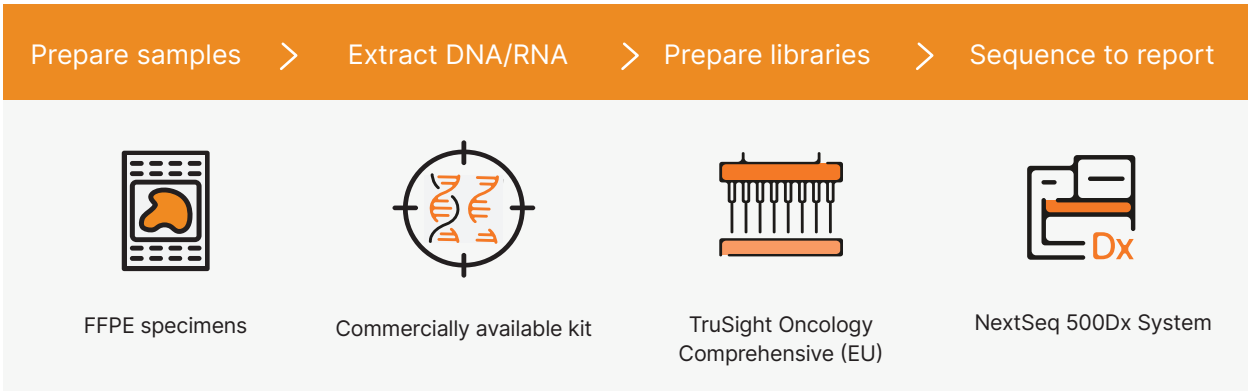

Figure 3: TruSight Oncology Comprehensive (EU) sample-to-report workflow

How long is the turnaround time (TAT) from sample to report?

The TAT is 4-5 days from extracted DNA-RNA to final clinical report.

What sequencing platform is needed?

TruSight Oncology Comprehensive (EU) is run on the NextSeq 550Dx instrument, an FDA-regulated and CE-marked high-throughput sequencing platform.

What is the workflow for data analysis?

TruSight Oncology Comprehensive (EU) offers a streamlined, automated workflow from sequencing to final clinical report. Simply set up the sequencing run using Local Run Manager software. After the sequencing run is complete, secondary, and tertiary analysis kickoff automatically and are performed on-instrument. The output is a final clinical report.

What is the expected analysis time for a sample batch processed in a sequencing run?

The analysis time is 8-10 hours.

What is included in the final clinical report?

One of the key concerns when using a CGP panel is how to interpret the data and filter nonsignificant variants. TruSight Oncology Comprehensive (EU) software performs analysis and filtering as part of a fully automated workflow. The final report is easy to read and highly actionable.

The IVD Clinical Report is organized into three main sections (Figure 4):

- Companion diagnostic results: A listing of any *NTRK* fusion finding for positive cases. When an *NTRK* fusion is detected and mentioned in this section, it will also note that VITRAKVI therapy regimen is indicated for this patient
- Genomic findings with evidence of clinical significance: A list of detected variants that have evidence of clinical significance (therapeutic, prognostic, or diagnostic) based on information in approved drug labels, guidelines, and clinical practice guidelines for the patient's tumor type
- Genomic findings with evidence of potential clinical significance: A list of detected variants that:

    1. Have evidence of potential clinical significance (therapeutic, prognostic, or diagnostic) based on information in drug labels, guidelines, and clinical practice guidelines in another tumor type
    2. Match genomic and tumor type eligibility criteria for a clinical trial
    3. Have evidence of potential clinical significance in the primary literature for the patient's tumor type

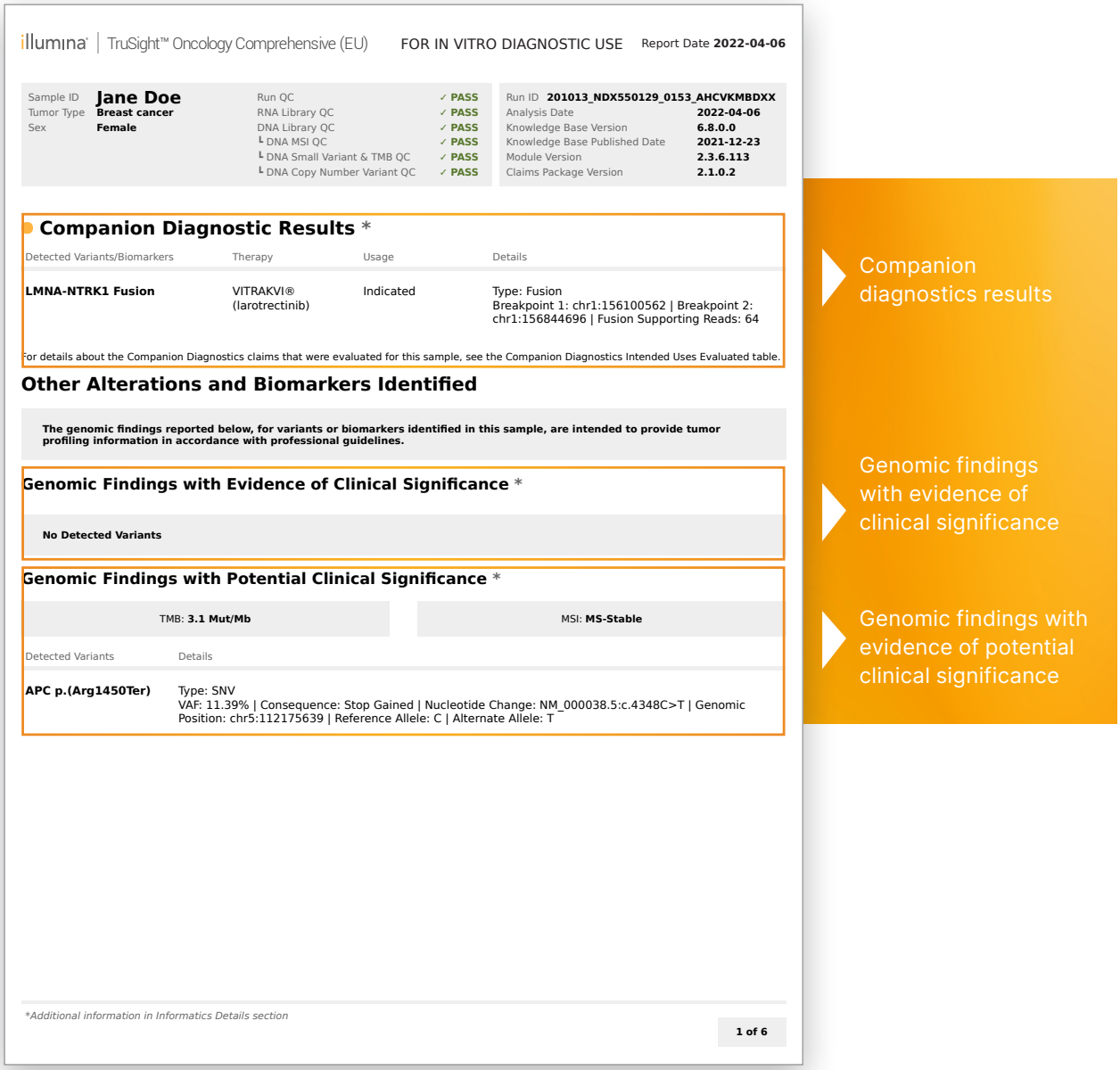

Figure 4: TruSight Oncology Comprehensive (EU) example clinical report—The clinical report includes companion diagnostic results, genomic findings with evidence of clinical significance, and genomic findings with evidence of potential clinical significance.

Reimbursement

What reimbursement is available for CGP tests in Europe? How does that vary by country?

National and/or regional funding is available in most Western European countries. Laboratory services are reimbursed in different ways depending on the country in which they are located, the clinical setting in which they work, and the services they provide (Figure 5 and Table 4). Illumina has established a dedicated Market Access team that works with Payers to further expand the reimbursement of CGP across all European countries. In addition, Illumina is working towards opening access to CGP in various major emerging markets of Europe. For further questions about the appropriate coding or reimbursement for cancer testing, we encourage customers to:

- Speak to their respective national medical society for guidance regarding how to manage reimbursement claims for testing and refer to any national or regional budget holders (Payers) for guidance on coding and reimbursement, eg, national pathology societies
- Contact their local Illumina representative who will connect them with the Illumina Market Access team. The Illumina Market Access team will provide them with guidance on where to find general reimbursement fee schedules, answer general reimbursement queries for diagnostics from customers or point customers to key local institutions they can contact. Note: as a manufacturer of IVD products, Illumina will not be able to advise customers on what specific reimbursement codes customers should bill for TruSight Oncology Comprehensive (EU)

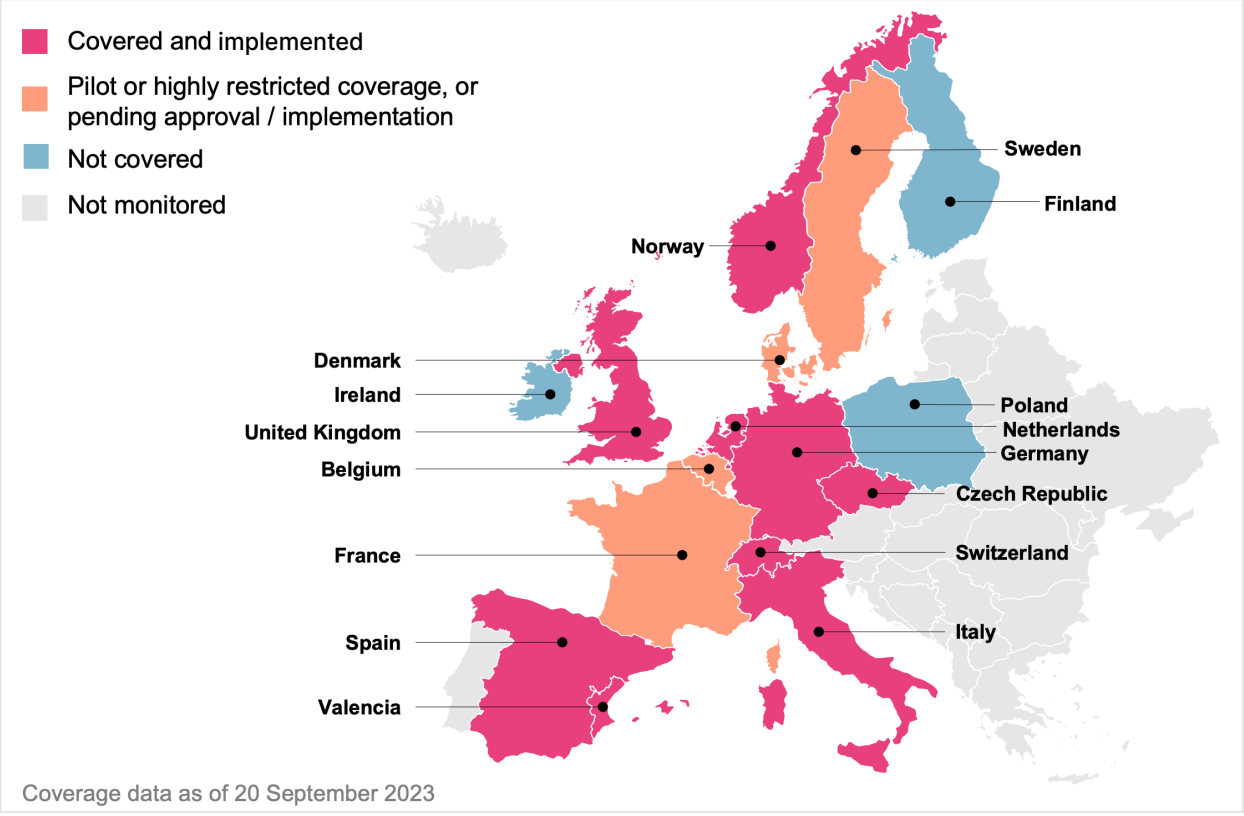

Figure 5: CGP test coverage options across Europe

Table 4: Laboratory fee schedules

Country	Relevant fee schedules
Austria	N/A
Belgium	NGS pilot study
Denmark	N/A
England	National Genomic Test Directory
Finland	N/A
France	National Reimbursement List for Innovative Technologies and National Fee Schedule
Germany	National Fee Schedule Germany
Israel	National Health Basket
Italy[a]	National Fee Schedule
Netherlands[b]	N/A
Norway	N/A
Scotland	N/A
Spain[a]	N/A
Sweden	N/A
Switzerland	National Fee Schedule for Medical Services

a. For Italy, the national fee schedule is informative only; for Italy and Spain, coverage is defined at the regional level
b. For the Netherlands, coverage is negotiated at the local level between hospitals and health insurers
N/A: Not available, ie, no fee schedule for diagnostics in the country; diagnostics are funded through global budget allocation to laboratory customers

Performance characteristics

Limit of Detection (LoD)

The LoD is defined as the lowest analyte value (eg, variant allele frequency or supporting reads) that can be detected consistently (95% detection limit or a type II error of 5%). FFPE tissues with *NTRK1-3* fusions (Low-Grade Glioma, Glioblastoma Multiforme, Myofibroblastic Sarcoma, Sarcoma, Secretory Breast Cancer, Colon Cancer) as well as an FFPE-treated cell line with *NTRK1* and *NTRK3* small DNA variants were used in the study. Eighteen observations were generated for each test level per lot per variant by three operators and three sequencing instruments initiating library preparation on three nonconsecutive days with two replicates of each sample test level (Table 5).

Table 5: LoD for NTRK fusions

Gene	Fusion	LoD (supporting reads)
NTRK1	*TPM3-NTRK1*	20.2
	BCAN-NTRK1	53.2
NTRK2	*ETV6-NTRK2*	20.3
	STRN-NTRK2	13.6
NTRK3	*ETV6-NTRK3*	16.2
	KANK1-NTRK3	13.5

Reproducibility for *NTRK* fusions

Reproducibility of the TruSight Oncology Comprehensive (EU) assay was tested across three sites (one internal, two external) with two operators per site, two within-run replicates, and three nonconsecutive testing days. RNA containing known ***NTRK*** fusions were obtained from formalin-fixed paraffin-embedded (FFPE) samples and cell lines. Testing was observed for high (two to three times the LoD) and low (approximately at LoD) variant levels. At each site, each operator tested the panel members in duplicate, three times, generating six observations per panel member (36 observations total). Percent positive calls (PPCs) and percent negative calls (PNCs) for targeted RNA fusion variants were determined as the primary endpoints. Two-sided 95% confidence intervals (CIs) associated with all endpoints were calculated using the Wilson score method (Table 6, Table 7).

Table 6: Reproducibility for NTRK fusions: PPC

Targeted fusion	No. of tests	Mean supporting reads	PPC (%)	95% CI
Variant level: High				
LMNA-NTRK1	36	37.9	100.0% (36/36)	(90.4%, 100.0%)
BCAN-NTRK1	36	33.6	94.4% (34/36)	(81.9%, 98.5%)
ETV6-NTRK2	36	24.6	100.0% (36/36)	(90.4%, 100.0%)
TRIM24-NTRK2	36	36.6	100.0% (36/36)	(90.4%, 100.0%)
ETV6-NTRK3	36	56.4	100.0% (36/36)	(90.4%, 100.0%)
BTBD1-NTRK3	35	32.9	100.0% (35/35)	(90.1%, 100.0%)
Variant level: Low				
LMNA-NTRK1	36	13.8	94.4% (34/36)	(81.9%, 98.5%)
BCAN-NTRK1	36	16.9	80.6% (29/36)	(65.0%, 90.2%)
ETV6-NTRK2	35	15.2	94.3% (33/35)	(81.4%, 98.4%)
STRN-NTRK2	36	13.6	100.0% (36/36)	(90.4%, 100.0%)
ETV6-NTRK3	36	24.8	100.0% (36/36)	(90.4%, 100.0%)
BTBD1-NTRK3	36	18.1	100.0% (36/36)	(90.4%, 100.0%)

Table 7: Reproducibility for *NTRK* fusions: PNC

Targeted fusion	n	PNC (%)	95% CI
Variant level: High			
LMNA-NTRK1	180	100.0% (180/180)	(97.9%, 100.0%)
BCAN-NTRK1	251	100.0% (251/251)	(98.5%, 100.0%)
ETV6-NTRK2	251	100.0% (251/251)	(98.5%, 100.0%)
TRIM24-NTRK2	216	100.0% (216/216)	(98.2%, 100.0%)
ETV6-NTRK3	144	100.0% (144/144)	(97.4%, 100.0%)
BTBD1-NTRK3	216	100.0% (216/216)	(98.2%, 100.0%)
Variant level: Low			
LMNA-NTRK1	213	100.0% (213/213)	(98.2%, 100.0%)
BCAN-NTRK1	249	100.0% (249/249)	(98.5%, 100.0%)
ETV6-NTRK2	250	100.0% (250/250)	(98.5%, 100.0%)
STRN-NTRK2	249	100.0% (249/249)	(98.5%, 100.0%)
ETV6-NTRK3	177	100.0% (177/177)	(97.9%, 100.0%)
BTBD1-NTRK3	249	100.0% (249/249)	(98.5%, 100.0%)

Within-lab precision

Within-laboratory precision was evaluated for *NTRK1-3* fusions. Each sample was tested at ~1× LoD (low variant level) and ~2-3× LoD (high variant level) and each test level was run in duplicate in each library preparation event across three operators. Each operator started library preparation on three nonconsecutive start days and sequenced on three designated NextSeq 550Dx instruments. Three reagents lots were tested generating 54 observations per level (Table 8, Table 9).

Table 8: Qualitative results for targeted *NTRK* fusions

Targeted fusions	Variant level: High			Variant level: Low		
	Mean supporting reads	PPC (95% CI)	PNC (95% CI)	Mean supporting reads	PPC (95% CI)	PNC (95% CI)
TPM3-NTRK1	57.1	100.0% (93.4-100.0%)	100.0% (98.3-100.0%)	20.2	100.0% (93.4-100.0%)	100.0% (98.6-100.0%)
BCAN-NTRK1	53.2	100.0% (93.4-100.0%)	100.0% (98.6-100.0%)	22.1	94.4% (84.9-98.1%)	100.0% (98.8-100.0%)
ETV6-NTRK2	52	100.0% (93.4-100.0%)	100.0% (98.6-100.0%)	20.3	100.0% (93.4-100.0%)	100.0% (98.8-100.0%)
ETV6-NTRK3	41.7	100.0% (93.4-100.0%)	100.0% (98.3-100.0%)	16.2	100.0% (93.4-100.0%)	100.0% (98.6-100.0%)
ETV6-NTRK3 (FFPE cell line)	28.3	100.0% (93.4-100.0%)	100.0% (98.3-100.0%)	23.1	98.1% (90.2-99.7%)	100.0% (98.6-100.0%)

Table 9: Quantitative SD and CV results for *NTRK* fusions[a,b]

Targeted fusion	Mean supporting reads	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
Supporting reads level: High							
TPM3-NTRK1	57.1	11.2 (19.6)	1.2 (2.1)	5.7 (9.9)	2.0 (3.5)	11.9 (20.8)	17.4 (30.5)
BCAN-NTRK1	53.2	8.2 (15.5)	0.8 (1.4)	5.6 (10.5)	2.9 (5.4)	11.3 (21.3)	15.4 (28.9)
ETV6-NTRK2	52	0.0 (0.0)	4.1 (7.8)	7.1 (13.6)	5.7 (11.0)	12.9 (24.9)	16.3 (31.4)
ETV6-NTRK3	41.7	7.2 (17.2)	0.4 (1.0)	6.4 (15.4)	0.0 (0.0)	10.7 (25.8)	14.4 (34.6)
ETV6-NTRK3[c]	28.3	7.9 (28.0)	1.0 (3.6)	0.0 (0.0)	0.0 (0.0)	9.1 (32.0)	12.1 (42.6)
Supporting reads level: Low							
TPM3-NTRK1	20.2	2.3 (11.5)	0.9 (4.7)	3.3 (16.4)	0.8 (4.1)	5.7 (28.2)	7.1 (35.2)
BCAN-NTRK1	22.1	3.4 (15.3)	1.4 (6.4)	1.8 (8.0)	0.0 (0.0)	6.0 (27.2)	7.3 (32.9)
ETV6-NTRK2	20.3	0.0 (0.0)	3.2 (15.7)	4.4 (21.5)	0.0 (0.0)	8.3 (40.8)	9.9 (48.7)
ETV6-NTRK3	16.2	2.3 (14.0)	2.4 (14.6)	2.2 (13.4)	0.0 (0.0)	4.7 (28.7)	6.1 (37.5)
ETV6-NTRK3[c]	23.1	4.6 (19.7)	1.2 (5.1)	0.0 (0.0)	0.0 (0.0)	6.7 (29.1)	8.2 (35.5)

a. Based on 54 valid attempts
b. SD, standard of deviation; CV, coefficient of variation
c. FFPE cell line

NRTK clinical studies

To validate the TruSight Oncology Comprehensive (EU) assay as a CDx for the selection of patients for treatment with VITRAKVI (larotrectinib; Bayer AG), samples from patients enrolled in the larotrectinib clinical trials, supplemented with commercially sourced FFPE tissue specimens, were tested to support a TruSight Oncology Comprehensive (EU) assay accuracy study and a bridging study.

Summary of the three clinical trials:

- NCT02122913 was a multicenter, open-label, Phase 1, dose escalation study in adult patients with advanced solid tumors (all-comers) unselected for *NTRK* fusion positive cancer. Following the dose escalation portion of the study, a dose expansion was initiated for patients with documented *NTRK* fusion positive cancer and for patients whom the investigator believed might benefit from a highly selective TRK inhibitor.
- NAVIGATE NCT02576431 is an ongoing, multicenter, open-label, Phase 2, "basket" study in patients age 12 and older with recurrent advanced solid tumors with a documented *NTRK* fusion as assessed by an outside laboratory.
- SCOUT NCT02637687 is an ongoing, multicenter, open-label, Phase 1/2 study in pediatric patients aged from birth to 21 years with advanced solid or primary central nervous system (CNS) tumors.

Of the *NTRK* fusion positive patients included in the TruSight Oncology Comprehensive (EU) assay study, 164 formed the larotrectinib extended primary efficacy set (ePAS4).

Data for the *NTRK* clinical studies are summarized here. For details, see the TruSight Oncology Comprehensive (EU) Product Insert at support.illumina.com/sequencing/sequencing_kits/trusight-oncology-comprehensive.html

Accuracy study for *NTRK1*, *NTRK2*, *NTRK3* fusion detection

The accuracy of the TruSight Oncology Comprehensive (EU) assay for detecting *NTRK1-3* fusions in patients with solid tumors was demonstrated by assessing the concordance of *NTRK* fusion results between the TruSight Oncology Comprehensive (EU) assay and a validated orthogonal method based on NGS (Table 10).

A retrospective, noninterventional study was conducted using 170 samples from clinical trials and an additional 329 supplemental samples. Studies were conducted at one external site for the TruSight Oncology Comprehensive (EU) assay and one central laboratory for the orthogonal method (Table 11).

Read the TruSight Oncology Comprehensive (EU) Package Insert to learn more, support.illumina.com/sequencing/sequencing_kits/trusight-oncology-comprehensive.html

Table 10: Cross tabulation of TruSight Oncology Comprehensive (EU) assay vs orthogonal method results for NTRK fusion detection

		Orthogonal method			Total
		NTRK fusion positive	*NTRK* fusion negative	Invalid	
TruSight Oncology Comprehensive (EU) assay	*NTRK* fusion positive	114	16	1	131
	NTRK fusion negative	4	273	6	283
	Invalid[a]	4	28	53	85
	Total	122	317	60	499

a. TruSight Oncology Comprehensive (EU) assay invalid results come from sample and run level

Table 11: Positive percent agreement (PPA) and negative percent agreement (NPA) of the TruSight Oncology Comprehensive (EU) assay compared to orthogonal method for NTRK fusion detection

Agreement measure	Excluding invalid TruSight Oncology Comprehensive (EU) assay results		Including invalid TruSight Oncology Comprehensive (EU) assay results	
	Agreement, % (n/N)	95% CI[a]	Agreement, % (n/N)	95% CI[a]
PPA	96.6% (114/118)	91.5%-99.1%	93.4% (114/122)	87.5%-97.1%
NPA	94.5% (273/289)	91.2%-96.8%	86.1% (273/317)	81.8%-89.7%

a. 95% CI based on (exact) Clopper-Pearson method

Clinical bridging study for *NTRK1*, *NTRK2*, *NTRK3* fusion detection

The clinical bridging study was conducted to assess the clinical effectiveness of the TruSight Oncology Comprehensive (EU) assay to identify *NTRK1*, *NTRK2*, or *NTRK3* fusion positive patients for treatment with larotrectinib and to assess the concordance between the TruSight Oncology Comprehensive (EU) assay and local test (LT) methods (used to determine *NTRK* fusion status for the larotrectinib clinical trials). LT methods included NGS, fluorescent *in situ* hybridization (FISH), and PCR (Tables 12-14).

The sample set included 279 patients enrolled in the larotrectinib studies using a data cutoff of July 15, 2019 with known *NTRK* fusion status and supplemental samples determined to be fusion negative by NGS and PCR.

Table 12: Concordance between the TruSight Oncology Comprehensive (EU) assay and LT methods for detecting *NTRK* fusions

Agreement measure[a]	Excluding invalid TruSight Oncology Comprehensive (EU) assay results		Including invalid TruSight Oncology Comprehensive (EU) assay results	
	Agreement, % (n/N)	95% CI[b]	Agreement, % (n/N)	95% CI[b]
PPA	89.1% (123/138)	82.7%-93.8%	80.4% (123/153)	73.2%-86.4%
NPA	96.3% (235/244)	93.1%-98.3%	82.7% (235/284)	77.8%-87.0%
OPA	93.7% (358/382)	90.8%-95.9%	81.9% (358/437)	78.0%-85.4%

a. NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement
b. 95% CI based on (exact) Clopper-Pearson method

Table 13: Concordance between the TruSight Oncology Comprehensive (EU) assay and LT methods for detecting *NTRK* fusions, including imputed values for LT positive patients with missing TruSight Oncology Comprehensive (EU) assay results

Agreement measure	Agreement, %	95% CI
PPA	85.2%	78.6%-91.7%
NPA	96.3%	93.9%-98.7%
OPA	91.2%	87.9%-94.5%

Missing TruSight Oncology Comprehensive (EU) assay results for LT fusion negative patients were not imputed. Two-sided 95% CIs were calculated based on the multiple imputation Boot method. NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement

Table 14: Concordance between the TruSight Oncology Comprehensive (EU) assay and LT methods for detecting *NTRK* fusions by LT method

Agreement measure by LT method	Agreement, % (n/N)	95% CI
DNA NGS		
PPA	84.2% (32/38)	68.7%-94.0%
NPA	88.9% (16/18)	65.3%-98.6%
OPA	85.7% (48/56)	73.8%-93.6%
RNA NGS		
PPA	91.5% (75/82)	83.2%-96.5%
NPA	96.9% (218/225)	93.7%-98.7%
OPA	95.4% (293/307)	92.5%-97.5%
FISH		
PPA	80.0% (8/10)	44.4%-97.5%
NPA	Not calculated (1/1)	Not calculated
OPA	81.8% (9/11)	48.2%-97.7%
PCR		
PPA	100.0% (8/8)	63.1%-100.0%
NPA	Not calculated (0/0)	Not calculated
OPA	100.0% (8/8)	63.1%-100.0%

a. 95% CIs were calculated using the (exact) Clopper-Pearson method
b. Includes NGS methods that use RNA only and both DNA and RNA
Not calculated for subgroups with sample count < 5, agreement statistics were not calculated.
FISH, fluorescence *in situ* hybridization; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement

Clinical efficacy results

Within the ePAS4, the efficacy of larotrectinib in the TruSight Oncology Comprehensive (EU) assay positive, LT positive population (97 patients, ORR=78.4%, 95% CI [68.8%, 86.1%]) was similar to the efficacy of larotrectinib in the total ePAS4 population (164 patients, ORR=72.6%, 95% CI [65.1%, 79.2%]). Of the 97 TruSight Oncology Comprehensive (EU) positive patients in ePAS4, 28 (28.9%) patients had achieved a complete response/surgical complete response and 48 (49.5%) patients had achieved a partial response (Table 15).

Of the 13 TruSight Oncology Comprehensive (EU) negative, LT positive population, 1 (7.7%) showed complete response and 2 (15.4%) showed partial response with larotrectinib therapy.

Table 15: Overall response rate (ORR) for LT positive patients by LT and TruSight Oncology Comprehensive (EU) assay results in ePAS4

	LT fusion positive N = 164	TruSight Oncology Comprehensive (EU) positive and LT positive N = 97	TruSight Oncology Comprehensive (EU) negative and LT positive N = 13
Best overall response, n (%)			
Complete response (CR)	31 (18.9%)	22 (22.7%)	1 (7.7%)
Surgical complete response (sCR)	8 (4.9%)	6 (6.2%)	0
Partial response (PR)	80 (48.8%)	48 (49.5%)	2 (15.4%)
Stable disease	25 (15.2%)	13 (13.4%)	4 (30.8%)
Progressive disease	13 (7.9%)	6 (6.2%)	5 (38.5%)
Not evaluable	7 (4.3%)	2 (2.1%)	1 (7.7%)
Overall response rate			
Number of patients	164	97	13
Number of patients with CR + sCR + PR	119	76	3
ORR% (95% CI)	72.6% (65.1%, 79.2%)	78.4% (68.8%, 86.1%)	23.1% (5.0%, 53.8%)

Fifty-four patients are missing TruSight Oncology Comprehensive (EU) assay results. 95% CI was calculated using the (exact) Clopper-Pearson method.

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