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Official Title:	EPI VITRAKVI: A comparison of clinical outcomes in Infantile Fibrosarcoma (IFS) patients treated with larotrectinib in the phase I/II SCOUT study versus (an) external historical cohort(s)
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Observational Study Information

Acronym/Title	EPI VITRAKVI: A comparison of clinical outcomes in Infantile Fibrosarcoma (IFS) patients treated with larotrectinib in the phase I/II SCOUT study *versus* (an) external historical cohort(s)
Protocol version and date	v 1.0, 27 JULY 2021
IMPACT study number	21767
Study type / Study phase	Retrospective, observational, externally-controlled study / Phase IV
Medicinal product	Larotrectinib (BAY 2757556)
Comparator / Reference therapy	Standard of care, external historical control
Study Initiator and Funder	Bayer HealthCare SAS
Research question and objectives	The *Haute Autorité de Santé* (HAS), French National Authority for Health, gave a positive opinion for the reimbursement of larotrectinib for the treatment of pediatric patients with IFS or another Soft Tissue Sarcoma (STS), harboring a Neurotrophic Tyrosine Receptor Kinase (*NTRK*) gene fusion, which is locally advanced or metastatic, and refractory or in relapse. The HAS granted a moderate clinical benefit (*service médical rendu* [SMR]) with no improvement in clinical benefit (*amélioration du service médical rendu* [ASMR V]). This positive opinion is conditional and will be reassessed upon the provision of comparative data of larotrectinib treatment *versus* standard of care at least *versus* an external control arm, in particular in IFS. This study is designed to address this request. Primary objective: The primary objective of the present study is to compare the time to medical treatment failure (defined as: next systemic treatment or mutilating surgery or radiation therapy or death due to any cause) between larotrectinib and standard of care in IFS patients using externally-controlled comparison performed with phase I/II SCOUT study and eligible external historical cohort(s). Secondary objectives: The secondary objectives are to compare: • Treatment outcomes (next systemic treatment, mutilating surgery, radiation therapy, death due to any cause), • Treatment discontinuation rates due to toxicity.

Countries of study	Countries involved in SCOUT study (13 countries worldwide including France) and eligible external historical cohort(s)
Authors	PPD Bayer HealthCare SAS, 220 Avenue de la Recherche, 59120 Loos, France PPD Bayer SAS, 10 Place de Belgique, 92257 La Garenne-Colombes, France PPD Bayer US LLC, 100 Bayer Boulevard, Whippany, 07981 NJ, USA

The study will be conducted in compliance with the protocol
and any applicable regulatory requirements.

Throughout this document, symbols indicating proprietary names (®, TM) may not be displayed. Hence, the appearance of product names without these symbols does not imply that these names are not protected.

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2. List of abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
AKT	Protein Kinase B
ALT	Alanine Aminotransferase
ANSM	*Agence Nationale de Sécurité du Médicament et des Produits de Santé* (French National Agency for Drug and Health Product Safety)
ASMR	*Amélioration du Service Médical Rendu* (Added medical value)
AST	Aspartate Aminotransferase
ATE	Average Treatment Effect
ATT	Average Treatment in the Treated
ATU	*Autorisation Temporaire d'Utilisation* (Temporary Authorization for Use)
BDNF	Brain-Derived Neurotrophic Factor
BTBD1	BTB Domain Containing 1
CESREES	*Comité Ethique et Scientifique pour les Recherches, les Etudes et les Evaluations dans le domaine de la Santé* (French Ethic and Scientific Committee for the Researches, Studies and Evaluations in the Health Domain)
CI	Confidence Interval
CMN	Congenital Mesoblastic Nephroma
CNIL	*Commission Nationale de l'Informatique et des Libertés* (French National Committee of Information Technology and Civil Liberty)
CNS	Central Nervous System
COG	Children Oncology Group
CR	Complete Response
CRF	Case Report Form
CRO	Contract Research Organization
CWS	*Cooperative Weichteilsarkom Studiengruppe*
DCR	Disease Control Rate
DoR	Duration of Response
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EpSSG	European pediatric Soft tissue sarcoma Study Group
ESCAT	ESMO Scale of Clinical Actionability for molecular Targets
ESMO	European Society for Medical Oncology
ETV6	ETS Variant Transcription Factor 6
FDA	Food and Drug Administration
FISH	Fluorescence In Situ Hybridization
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GPP	Good Publication Practice

HAS	*Haute Autorité de Santé* (French National Authority for Health)
HDH	Health Data Hub
HEOR	Health Economics and Outcomes Research
HR	Hazard Reduction
ICH	International Conference on Harmonization
ID	Ifosfamide-Doxorubicin
IEC	Independent Ethics Committee
IFS	Infantile Fibrosarcoma
IHC	Immunohistochemistry
IPTW	Inverse Probability of Treatment Weighting
IRB	Institutional Review Board
IRR	Incidence Rate Ratio (underlying hazard ratio)
IRS	Intergroup Rhabdomysosarcoma Study
IVA	Ifosfamide-Vincristine-Actinomycin D
IVADo	Ifosfamide-Vincristine-Actinomycin D-Doxorubicin
MA	Marketing Authorization
MAH	Marketing Authorization Holder
MAPK	Mitogen-Activated Protein Kinase
N/A	Not Applicable
NGF	Nerve Growth Factor
NGS	Next Generation Sequencing
NT	Neurotrophin
NTRK	Neurotrophic Tyrosine Receptor Kinase
ORR	Overall Response Rate
OS	Overall Survival
PFS	Progression-free survival
PH	Proportional Hazard
PICO	Patient, Intervention, Comparison, Outcome
PR	Partial Response
PT	Preferred Term
RECIST	Response Evaluation Criteria in Solid Tumors
RMS	Rhabdomyosarcoma
RR	Risk Ratio
RTK	Receptors Tyrosine Kinase
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SEER	Surveillance of Epidemiology and End Results
SLR	Systemic Literature Review
SMR	*Service Médical Rendu* (Medical value quotation)

STROBE	The Strengthening the Reporting of Observational Studies in Epidemiology
STS	Soft Tissue Sarcoma
TEAE	Treatment Emergent Adverse Event
TPM3	Tropomyosin 3
TRK	Tropomyosin Receptor Kinase
TRKi	TRK Inhibitor
VA	Vincristine-Actinomycin D
VAC	Vincristine-Actinomycin D-Cyclophosphamide
VAC/VI	Vincristine-Actinomycin D-Cyclophosmamide/Vincristine-Irinotecan
VAdriaC	Vincristine-Doxorubicin (Adriamycine)-Cyclophosphamide
VDC/IE	Vincristine-Doxorubicin-Cyclophosphamide/Ifosfamide-Etoposide
VOD	Veno-Occlusive Disease

3. Responsible parties

3.1 Study initiator and funder

Role:	PPD
Name:	PPD
E-mail:	PPD
Role:	PPD
Name:	PPD
E-mail:	PPD
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E-mail	PPD
Role:	PPD
Name:	PPD
E-mail:	PPD

Contact details of the responsible parties at Bayer HealthCare SAS, data controller of the study, are available upon request.

Data privacy aspects of the study are managed by the data protection officer of the study initiator who can be reached at the following contact details:

Bayer SAS
Délégué à la Protection des Données – DPO
Direction Juridique
16 Rue Jean-Marie Leclair
69009 Lyon
DPO-BayerFrance@bayer.com

3.2 External partners/Scientific Committee

The Principal Investigator of this observational study is Dr. Daniel Orbach, MD, Pediatric Oncologist of Institut Curie, SIREDO Oncology Center (Care, Innovation and Research for Children, Adolescents and Young Adults with Cancer, Paris, France).

A Scientific Committee was constituted for this study. Its role is to help to define and validate the methodologies and the modalities of the data analyses, to review and validate content of the study protocol, the study-related documents, the study results and the study report. The Scientific Committee can be solicited during the study course to obtain an independent evaluation and/or advices as needed. The Scientific Committee will participate in the communication/dissemination of the results and is composed of:

- Dr. Daniel Orbach, MD, Pediatric Oncologist of Institut Curie, SIREDO Oncology Center (Care, Innovation and Research for Children, Adolescents and Young Adults with Cancer),
- Dr. PPD MD, PhD, PPD of Institut Curie, Biometry Unit.

The writing of the study protocol, the statistical analyses and the writing of the study report are subcontracted to a Contract Research Organization (CRO) (Keyrus Life Science [previously Keyrus Biopharma]).

Two Systematic Literature Reviews (SLRs) will be performed to identify: (1) appropriate non-biased data sources to constitute the external control arm and (2) the important prognostic factors to be taken into account in adjusted analysis. The 2 SLRs are subcontracted to IQVIA Operations France. SLR protocol and results will be kept as stand-alone documents.

Contact details of the Principal Investigator, members of the Scientific Committee, and the CROs are kept as stand-alone documents (see Table 2, Annex 1) which are available upon request.

Administrative changes of responsible persons and/or the composition of the committee will be documented by updating the respective lists but will not require formal protocol amendments.

4. Abstract

Acronym/Title	EPI VITRAKVI: A comparison of clinical outcomes in Infantile Fibrosarcoma (IFS) patients treated with larotrectinib in the phase I/II SCOUT study *versus* (an) external historical cohort(s)
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Rationale and background	Although very rare (less than 1% of all childhood cancers), IFS is the most common Soft Tissue Sarcoma (STS) in infants under one year of age, commonly located in extremities. IFS tumors are typically characterized by a fusion transcript constituted of Neurotrophic Tyrosine Receptor Kinase (*NTRK*) and ETS Variant Transcription Factor 6 (*ETV6*) (*ETV6-NTRK*). *ETV6-NTRK3* transcript was detected in 87.2% of IFS patients, which makes this specific gene fusion nearly pathognomonic for IFS (Orbach 2016). Conservative surgery remains the primary treatment for patients with IFS. When upfront resection is not feasible, treatment must be chosen to minimize acute and chronic/long term toxicities, due to the very young age of patients. Chemotherapy is generally indicated as first-line treatment and usually shows a good response, notably the Vincristine-Actinomycin-D (VA) regimen which if the preferred first line option, or other regimens including Vincristine-Actinomycin-D-Cyclophosphamide (VAC) and Vincristine-doxorubicin (Adriamycine)-Cyclophosphamide (VAdriaC) in case of insufficient response to VA (Loh 2002; Orbach 2016; Orbach 2020; Weiss 2014).

	However, central venous administration is challenging to manage in such young patients. Because of the risk of sequelae, extensive and mutilating surgery, as well as radiotherapy, should only be performed after failure of salvage therapies but were discouraged and not recommended in these young patients (Orbach 2010; Orbach 2016). In case of failure of chemotherapy, alkylating agents and anthracyclines can be prescribed. However, because of their acute and chronic/long-term toxicities, their use is limited, raising a need for alternative medical treatments with less toxicity. The clinical development program of the first-in-class selective Tropomyosin Receptor Kinase inhibitor (TRKi) larotrectinib, including the pediatric Phase I/II SCOUT study[1], to date has shown that the tumor response observed under treatment with the drug, is pronounced and durable even if no comparator is used in the ongoing clinical studies. Larotrectinib presents high potency and high specificity for TRKA, TRKB and TRKC receptors inhibition and has the benefit to be provided in 2 formulations: a liquid oral formulation mainly for the pediatric population and capsules with 2 different doses mainly for adults. In addition, it is generally well tolerated. In September 2019, larotrectinib was granted a conditional Marketing Authorization (MA) in Europe for the treatment of adult and pediatric patients with solid tumors that display a *NTRK* gene fusion: • who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and • who have no satisfactory treatment options. In France, a dossier was submitted to the National Authority for Health (*Haute Autorité de Santé* [HAS]) in October 2019 to obtain the reimbursement of larotrectinib in the country in line with the population described in the MA. In July 2020, the HAS's final appraisal was a positive opinion for the reimbursement of larotrectinib only in pediatric patients with IFS or another STS harboring a *NTRK* gene fusion, which is locally advanced or metastatic, and refractory or in relapse. The HAS granted a moderate clinical benefit (*service médical rendu* – SMR) with no improvement in clinical benefit (*amélioration du service médical rendu* – ASMR V) mainly due to the lack of comparative evidence. In this context, the positive opinion is conditional and will be reassessed upon the provision

[1] SCOUT study, a Phase I/II study of the oral TRK inhibitor larotrectinib in pediatric patients with advanced solid or primary central nervous system tumors – international clinical study.

	of comparative data of larotrectinib treatment *versus* standard of care at least *versus* an external control arm, in particular in IFS. This study is designed to address this request. Performing a comparison of the available data from the ongoing SCOUT study with data from an external historical cohort reflecting standard of care, constitutes a relevant approach to assess the efficacy of larotrectinib and to contextualize its therapeutic benefit over current therapies which may be associated with detrimental outcomes. Given the rarity of the condition and ethics consideration, the use of an external control arm allows for the timely generation of comparative data.
Research question and objectives	The aim of this study is to assess the therapeutic benefit of larotrectinib over the current standard of care in pediatric patients with locally advanced or metastatic IFS. <u>Primary objective</u>: The primary objective of the present study is to compare the time to medical treatment failure (defined as: next systemic treatment or mutilating surgery or radiation therapy or death due to any cause) between larotrectinib and standard of care in IFS patients using externally-controlled comparison performed with phase I/II SCOUT study and eligible external historical cohort(s). <u>Secondary objectives</u>: The secondary objectives are to compare: • Treatment outcomes (next systemic treatment, mutilating surgery, radiation therapy, death due to any cause), • Treatment discontinuation rates due to toxicity.
Study design	This study will be a retrospective observational externally-controlled study. Data of patients with IFS in the eligible external historical cohort(s) will serve as control for the comparison with data of patients with IFS who have been enrolled in the SCOUT study. Data which will be used for the analyses will come from: • SCOUT study: o At least 27 sites across 13 countries, including France. • External cohort(s) meeting inclusion and exclusion criteria described below (see Population section), including at least the *Institut Curie* database.

	Primary endpoint: • *Time to medical treatment failure:* defined as the time (months) from the start of treatment to the date of the following events, whichever comes first: subsequent systemic treatment, radiation therapy, mutilating surgery or death due to any cause. Secondary endpoints: • Time to subsequent systemic treatment. • Time to mutilating surgery including limb amputation. • Time to radiation therapy. • Time to complete surgical resection (excluding amputation). • Overall Survival (OS): defined as the time (months) from the start of treatment to the date of death due to any cause. Patients alive or lost to follow-up at the time of analysis will be censored at their last date of follow-up. • Incidence of patients with treatment discontinuation due to treatment-related adverse events (AEs). Exploratory endpoints: • Progression-free survival (PFS): defined as the time (months) from the start of treatment to the date of first observed disease progression (radiological or clinical, whichever is earlier) or death due to any cause, if death occurs before progression is documented. Patients alive without documented progression at the time of analysis will be censored at the date of last tumor assessment. • Overall response rate (ORR): defined as the proportion of patients with a best overall response of complete response (CR) or partial response (PR) assessed by investigators. • Disease Control Rate (DCR): defined as the proportion of patients with a best overall response of CR, PR, or Stable Disease (SD).
Population	Selection criteria for the sources of the external historical control cohort(s) The choice of the control cohort(s) constituting the comparator arm of the study is not arbitrary. The non-arbitrary choice of the data sources to constitute the control cohort will be ensured by a comprehensive review of the existing relevant databases in France and internationally (notably based on a Systematic Literature Review [SLR]). Data sources will be selected upon the following eligibility and feasibility criteria:

	Inclusion criteria: ✓ Cohorts with prospective enrollment and with retrospective and prospective data collection from 2000 to present, ✓ Cohorts containing at least clinical data allowing to assess the efficacy of the treatment and the main prognostic factors as follows: o Diagnosis and stage of the disease (locally advanced or metastatic), o Type of treatments (chemotherapy, radiotherapy, surgery: mutilating yes/no) and date of the initiation or of the procedure, o Death and date, o Localization of the tumor (axis versus limb), o Size of the tumor (< 5 cm versus > 5 cm). Exclusion criteria: ✓ Databases not containing patients with locally advanced or metastatic IFS, ✓ Medico-administrative databases or absence of data allowing the assessment of the efficacy of the treatment and main prognostic factors, or high rate of missing data (>10% on outcome and >25% on covariates), ✓ Cohorts with retrospective enrollment and case report, ✓ Cohorts with prospective enrollment for which all patients were included before 2000. Based on a preliminary search of databases and the selection criteria described above, in addition to the database of the *Institut Curie* which is the only database confirmed, the following data sources are theoretically eligible to be used to constitute (an) external control cohort(s) for the present study: • Databases found in the literature (preliminary search that will be completed by the SLR): ✓ Database of the European pediatric Soft tissue Sarcoma Study Group (EpSSG, a consortium housed in the University of Padua, Italy, European data), ✓ Database of the *Cooperative Weichteilsarkom Studiengruppe* (CWS, Germany). • Commercial databases with electronic medical data: ✓ No eligible databases were found. The SLR that will be performed might identify additional eligible cohorts. The availability, the access, and the feasibility of use of the different eligible databases identified will be then

	investigated. Further details will be described in the final study report. *** <u>Selection criteria for the patients</u> The study population will comprise all patients in the SCOUT study and the eligible external historical cohort(s) with a diagnosis of locally advanced or metastatic IFS, regardless of their refractory or relapsed status, i.e. including treatment-naive patients to avoid further reducing the sample size. The choice of the study population has mainly been driven by feasibility/sample size considerations, in order to be able to perform a comparison based on a minimal number of patients. <u>Inclusion criteria</u>: The inclusion criteria listed below are in line with those of the SCOUT study in terms of patients and disease characteristics: • Age ≤ 21 years old. • Locally advanced or metastatic IFS. • Patients with available information on clinical, radiological characteristics of their tumor, therapies administered and outcomes. • Patients receiving larotrectinib in the SCOUT trial. • Patients receiving at least one chemotherapy-based regimen[2] in the external historical control cohort(s). • No opposition from the patients and/or representatives for data use. <u>Exclusion criteria</u>: • Patients treated with TRKi in the external historical control cohort(s). • Patients with documented absence of *NTRK* gene fusion. • Patients participating in an investigational program with interventions outside of routine clinical practice.
Variables	To determine the different endpoints, the variables described below will be collected from the Case Report Forms (CRFs) of the patients eligible for the present observational study of the SCOUT study and from the databases of the external historical control cohort(s).

[2] In order to preserve the sample size, patients will be included regardless of the type of chemotherapy they have received.

	Variables to determine the primary endpoint: For the *time to medical treatment failure* endpoint: • Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and whichever comes first: o Start date of a post-treatment[3] systemic anti-cancer therapy, if any, or, o Start date of a post-treatment[3] radiation therapy, if any, or, o Date of a post-treatment[3] mutilating surgery, if any, or, o Date of death due to any cause, if applicable. Variables to determine the secondary endpoints: For the *time to subsequent systemic treatment* endpoint: • Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and, • Start date of a post-treatment[3] systemic anti-cancer therapy, if any. For the *time to mutilating surgery including limb amputation* endpoint: • Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and, • Date of a post-treatment[3] mutilating surgery including limb amputation, if any. For the *time to radiation therapy* endpoint: • Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and, • Start date of a post-treatment[3] radiation therapy, if any. For the *time to complete surgical resection (excluding amputation)* endpoint: • Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and, • Date of a post-treatment[3] complete surgical resection (excluding amputation), if any.

[3] After the start of larotrectinib treatment for the SCOUT study or after the start of chemotherapy (first line) for the external historical control cohort(s).

	For the *OS* endpoint:
	• Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
	• Date of death due to any cause, if applicable, or,
	• Last date of patient follow-up for patients alive and lost to follow-up, if applicable.
	For the *Incidence of patients with treatment discontinuation due to treatment-related AEs* endpoint:
	• Record of treatment[4] discontinuation due to treatment-related AEs, if applicable.
	
	<u>Variables to determine the exploratory endpoints</u>:
	For the *PFS* endpoint:
	• Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for locally-advanced/metastatic disease for the external historical control cohort(s), and,
	• Date of first post-treatment[5] disease progression/relapse assessed by radiological or clinical examination by investigator, if any, or,
	• Date of death due to any cause, if applicable, or,
	• Censored at last date of tumor assessment. A complete list of censoring rules will be provided in the statistical analysis plan (SAP).
	For the *ORR* and *DCR* endpoints:
	• Patient response to treatment[4] (CR, PR and SD) assessed by investigator.
	
	<u>Potential confounders</u>:
	The following baseline variables will be considered when balancing the two cohorts: patients with IFS enrolled in SCOUT and the external historical control cohort(s).
	• Patients and disease characteristics:
	  ○ Sex,
	  ○ Age,
	  ○ Disease history:
	  ▪ Date of initial diagnosis,

[4] Larotrectinib treatment for the SCOUT study and chemotherapy (first line) for the external historical control cohort(s).
[5] After the start of larotrectinib treatment for the SCOUT study or after the start of chemotherapy (first line) for the external historical control cohort(s).

	▪ Anatomical disease site(s) at diagnosis, ▪ Stage of disease at diagnosis (local versus locally advanced versus metastatic), ▪ Primary tumor size (< 5 cm or > 5 cm), ▪ Date of advanced disease stage evolution (locally advanced, metastatic), ▪ Time from locally advanced/metastatic disease diagnostic to initiation of larotrectinib (SCOUT) or first line of chemotherapy (external historical control cohort(s)), ▪ Intergroup Rhabdomyosarcoma Study (IRS) group, ▪ Anatomical site(s) of metastases, if any. • Collection of treatment-related information: o Prior/current surgical treatment(s) for cancer: ▪ Anatomical site(s), ▪ Date(s) of surgery, ▪ Best resection (R0, R1, R2), ▪ Nature of intervention(s) (e.g. amputation or other disfiguring procedures), ▪ Surgery for a curative intent (excluding amputation) because of the use of systemic treatment. o Prior/current radiation treatment(s) for cancer: ▪ Anatomical sites, ▪ Date(s) of radiation therapy. o Systemic treatment(s), including: ▪ Treatment regimen, ▪ Date of initiation, ▪ Date of radiological or clinical progression, ▪ Reasons for discontinuation, as appropriate.
Data sources	• SCOUT database (Bayer-sponsored study), • The *Institut Curie* database and the other eligible external historical control cohort(s) identified after SLR.
Study size	All patients meeting the selection criteria will be included in the study. Statistical power will be computed retrospectively according to the available sample size.
Data analysis	<u>Statistical analyses</u> A comparison of patients in the larotrectinib group with patients from the external historical control cohort(s) who received systemic therapy (chemotherapy-based regimen) will be conducted by Bayer, at least with the *Institut Curie* database. In the event that other external historical control cohorts are eligible and accessible, the databases will be pooled if feasible.

	<u>Index Date and Endpoints</u> The comparison of the two cohorts will be considered for the primary composite endpoint: • Time-to-treatment-failure (earliest of next systemic therapy, radiotherapy, mutilating surgery or death due to any cause). In addition, all secondary and exploratory endpoints detailed above will be analyzed: • Time to subsequent systemic treatment, • Time to mutilating surgery or limb amputation, • Time to radiation therapy, • Time to complete surgical resection (excluding amputation), • OS, • PFS, • Incidence of treatment discontinuation due to treatment-related AEs, • ORR, • DCR. The analysis will include patients on both first and second line (or higher) therapy, though the index date will be initiation of first line chemotherapy for the external historical control cohort(s). For SCOUT patients, the index date will be defined as the start date of larotrectinib, regardless of the line of treatment. Therefore, the index date will represent first or second line in larotrectinib patients, and first line in control subjects. Given the expected small sample size, this will avoid discarding events on key endpoints above, while the initial imbalance on lines of therapy between treatment groups will be corrected in the statistical modeling, as detailed later in this document. Given approximately 70% of SCOUT patients received larotrectinib as second line of treatment, while in the external historical control cohort(s) all patients will have received chemotherapy in first line of treatment, the proposed primary analysis is expected to provide a rather conservative estimate of treatment effect. <u>Patient matching methodology</u> Patients who received larotrectinib in the SCOUT trial will likely differ in various underlying characteristics from those who are recorded in the historical control cohort(s). These characteristics are listed above as potential confounders (see Variables section).

	The analyses will need to be adjusted for these patient characteristics to avoid biased results. The Inverse Probability of Treatment Weighting [IPTW] propensity method will be employed to reduce the effects of measured confounding variables in the interpretation of the treatment effect (larotrectinib versus control group). A propensity score can be seen as an overall "balancing score", that is calculated for each patient based on his or her measured underlying characteristics. <u>IPTW Diagnostics and Sensitivity Analyses</u> For all baseline covariates included in the propensity score model, standardized differences between larotrectinib and comparator groups will be computed. A graph will show the absolute standardized difference for each of the covariates, comparing larotrectinib and comparator, for both the unweighted and weighted samples. A standardized difference of 10% is commonly considered the threshold below which demonstrates reasonable balance between the two groups. Sensitivity analyses include trimming the IPTW weights and truncating large weights. Trimming at 5% level amounts to dropping the individuals with the most extreme PS values in both the treatment and control groups, as they may lack a match in the other group. Weight truncation reduces any 'large' weight down to a maximum weight. <u>Outcome model</u> **Time-to-event variables** The primary endpoint, time to medical treatment failure, and other time-to-event endpoints detailed above will be investigated by plotting Kaplan-Meier survival curves for the original unweighted samples. Also included on the survival graphs will be adjusted Kaplan-Meier curves for the two groups that incorporate the IPTW balancing weights. Summary statistics using the Kaplan-Meier method will be reported for both the unweighted and weighted samples. Additionally, a hazard ratio will be computed using a weighted Cox Proportional Hazards model, regressing survival time on an indicator variable denoting treatment status (larotrectinib / external historical control) and incorporating the IPTW weights. Hazard ratios will also be reported for the unweighted samples.

	Response variables The response endpoints, ORR and DCR, will be summarized for both groups in both the original and weighted samples. Incidence of treatment discontinuation due to treatment-related AEs will be summarized descriptively in the original unweighted sampled only. Bias Analysis for Primary Endpoint A bias analysis will investigate the effect of an unmeasured covariate for the primary endpoint, time to medical treatment failure. Sample Size and Power considerations Given the expected small sample size and the rarity of the events observed in the SCOUT trial, it is very unlikely that the required number of events will occur: the study is only powered to detect a very large treatment effect, e.g. with a risk reduction of 75% (Hazard Reduction [HR]=0.25) we cannot exceed 49% power (this assumes 35 IFS subjects and very low probabilities of failure in SCOUT [0.04] and the external historical control cohort [0.19]). As the study is underpowered, a formal hypothesis cannot be formulated, and the results should only be interpreted as exploratory. The matching methodology described above will attempt to balance the two groups of patients. However, considering that all SCOUT patients had either relapsed or were refractory to previous treatment (or at risk of mutilating surgery), which might not be the case in the external historical control cohort(s), the SCOUT population might be more severe. Therefore, the results of the comparison in the original unweighted sample are unlikely to be biased in favor of larotrectinib. Sensitivity Analyses An exploratory analysis will define the index date as: the start of larotrectinib, regardless of line of therapy; or for the external historical control cohort(s) the start date of chemotherapy. However, control patients who take a second line of therapy will have their index date set at the start of second line, in order to provide a closer comparison with the larotrectinib group prior to any adjustment using IPTW. As the majority of larotrectinib patients will be receiving 2nd line therapy, the analysis attempts to maximize consistency between

	treatment groups in terms of line of therapy while committing to the inclusion of all available patients. Sensitivity analyses will be detailed in the SAP. Subgroup analyses will also be performed for: • Patients treated with second line or more systemic therapy (with index date set at start of second line for both groups). <u>Complementary Analyses</u> The following complementary analyses will be conducted on the primary, secondary and exploratory endpoints: • Patients with IFS or other STS with *NTRK* gene fusion. Number of events and censored patients will be reported for all time-to-event endpoints referenced above, and Kaplan-Meier plots of the survival curves will be produced showing median survival and 95% CI, and also survival probabilities at 12 and 24 months. Frequency tables (n, %) will be produced for discrete endpoints, such as ORR, DCR and incidence rate of treatment discontinuation due to treatment-related AEs.
Milestones	• Approval of the Observational Study protocol: Q2 2021 • Submission to Health Data Hub (HDH): Q3 2021 • *Comité Ethique et Scientifique pour les Recherches, les Etudes et les Evaluations dans le domaine de la Santé* (CESREES) opinion: Q3 2021 • *Commission Nationale de l'Informatique et des Libertés* (CNIL) authorization (decision within 2 months – can be renewed 2 months): Best case: Q4 2021/Worst case: Q1 2022 • Start of analyses: Best case: Q4 2021/Worst case: Q1 2022 • End of analyses – Minimum 2 months of analyses – Best case: Q1 2022/Worst case: Q2 2022 • Observational Study report – Minimum 2 months of writing – Best case: Q2 2022/Worst case: Q3 2022 <u>Note</u>: these milestones are only valid for the comparison with the *Institut Curie* database. The use of any additional databases constituting the external historical control cohort(s) can have an impact on the calendar since it will require to set-up a data transfer agreement between Bayer and the data source owner(s).

5. Amendments

None.

6. Milestones

Table 1 presents planned milestones for the project. These milestones are based on a timely review and approval of the project. Administrative changes to milestones due to delays in study preparation, data release and analysis do not require amendments to the protocol. Revised study timelines and milestones which do not constitute a need for a formal protocol amendment are kept as stand-alone document (Table 2, Annex 1) that is available upon request.

Table 1: Milestones

Milestone	Planned date
Approval of the Observational Study Protocol	Q2 2021
Submission to HDH	Q3 2021
CESREES opinion	Q3 2021
CNIL authorization (decision within 2 months – can be renewed 2 months)	Best case: Q4 2021/Worst case: Q1 2022
Start of analyses	Best case: Q4 2021/Worst case: Q1 2022
End of analyses (minimum 2 months of analyses)	Best case: Q1 2022/Worst case: Q2 2022
Observational Study Report (minimum 2 months of writing)	Best case: Q2 2022/Worst case: Q3 2022

CESREES: Comité éthique et Scientifique pour les Recherches, les Etudes et les Evaluations dans le domaine de la Santé; CNIL: Commission Nationale de l'Informatique et des Libertés; HDH: Health Data Hub.

Note: these milestones are only valid for the comparison with the *Institut Curie* database. The use of any additional databases constituting the external historical control cohort(s) can have an impact on the calendar since it will require to set-up a data transfer agreement between Bayer and the data source owner(s).

7. Rationale and background

7.1 *NTRK* gene family and *NTRK* gene fusion-associated cancers

Neurotrophin (NT) receptors also known as Tropomyosin Receptor Kinase (TRK) are a family of transmembrane receptors tyrosine kinases (RTKs) which specifically bind to neurotrophic factors and with important physiological roles in neurodevelopment (Nakagawara 2001). The 3 main TRK receptors are TRKA, TRKB, and TRKC encoded by the *NTRK* genes *NTRK1*, *NTRK2* and *NTRK3*, respectively. The main ligands of these receptors are Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and Neurotrophin-3 (NT-3).

The first *NTRK* oncogene that was identified as a fusion with the tropomyosin gene in a colorectal cancer, follows the well-established paradigm of other oncogenic fusions by driving the growth of tumors via constitutive activation of its tyrosine kinase activity, thereby inducing cell proliferation and initiating cancer-related downstream signaling pathways (Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PH. A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet. 1998b; 18(2):184-187.

Kremer, LC, van Dalen EC, Offringa M, Ottenkamp J, Voûte PA. Anthracycline-induced clinical heart failure in a cohort of 607 children: long-term follow-up study. J. Clin. Oncol. 2001. 19, 191-196.

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Loh ML, Ahn P, Perez-Atayde AR, Gebhardt MC, Shamberger RC, Grier HE. Treatment of infantile fibrosarcoma with chemotherapy and surgery: results from the Dana-Farber Cancer Institute and Children's Hospital, Boston. J Pediatr Hematol Oncol. 2002; 24(9):722-726.

Manck E. Institut Curie. Epidemiology of sarcomas: who is affected ? 2017. https://institut-curie.org/dossier-pedagogique/epidemiology-sarcomas-who-affected.

Martin-Zanca 1986; Vaishnavi 2013).

The role of *NTRK* genes in pediatric cancer was initially reported in 1998, with the identification of the fusion between ETS variant gene 6 (*ETV6*) and *NTRK3* (*ETV6-NTRK3*), as the predominant genetic feature of both Infantile Fibrosarcoma (IFS) and cellular Congenital Mesoblastic Nephroma (CMN) (Knezevich 1998). In a recent study, *ETV6-NTRK3* transcript was detected in 87.2% of the IFS patients tested in the study (Orbach 2016), which makes this specific gene fusion nearly pathognomonic for IFS. However, it is important to note that the *NTRK* gene fusions are not specific to IFS. Other studies have implicated the *ETV6-NTRK3* gene fusion as one of a number of kinase alterations in children with Philadelphia chromosome positive (Ph+)-like acute lymphoblastic lymphoma (Roberts 2014). *NTRK* gene fusions have been incriminated in pediatric astrocytoma, pontine glioma, as well as other Central Nervous System (CNS) primary tumors (Wu 2014). In addition, in vivo testing of human Tropomyosin 3 (*TPM3*)-*NTRK1* or BTB Domain Containing 1 (*BTBD1*)-*NTRK3* transduced into the mouse brain induced high-grade gliomas which showed high levels of phosphorylated protein kinase B (AKT) and Mitogen-Activated Protein Kinase (MAPK) (Wu 2014).

The analysis of the transcriptomes of nearly 7,000 tumors also identified *NTRK* gene fusions in various adult cancers including thyroid cancer, glioblastoma multiforme, lung adenocarcinoma, colon adenocarcinoma, head and neck squamous cell carcinoma, as well as Soft Tissue Sarcoma (STS) (Stransky 2014). *NTRK* gene fusions are rare. It is estimated that such fusions are present in less than 1% of solid tumors (Stransky 2014). For example, in lung cancers, it represents 0.23% of the cancers (Farago 2018). However, this gene fusion is not tested *in routine* for all tumors. Therefore, the epidemiological data are likely not accurate.

The identification of *NTRK* gene fusions has recently come into focus due to the availability of targeted treatment modalities (TRK inhibitors [TRKi]). *NTRK* gene fusions can be detected indirectly or directly by multiple methods (Prasad 2016; Brenca 2016; Hechtman 2017), including:

- Next Generation Sequencing (NGS),
- Fluorescence In Situ Hybridization (FISH),
- Reverse Transcription-Polymerase Chain Reaction (RT-PCR),

- Immunohistochemistry (IHC).

7.2 IFS and current standard of care

IFS is a rare tumor, representing less than 1% of childhood cancers. IFS is a subtype of STS that primarily affects children below 2 years old. An incidence peak has been reported before the first year of age, diagnosed at birth or in the neonatal period. *NTRK* gene fusion is considered pathognomonic for IFS (Orbach 2016). Surveillance of Epidemiology and End Results (SEER) data suggest an incidence of 5 per one million infants born (Ries 1999). IFS accounts for 5-10% of all sarcoma diagnoses in children less than 1 year of age (Orbach 2005; Orbach 2010). IFS most commonly arises in the extremity (almost 50% of cases) and often presents with rapid initial growth sometimes followed by a more indolent course. Metastatic spread is uncommon (1-13%) (Loh 2002; Mertens AC, Yutaka Yasui, Liu Y, Stovall M, Hutchinson R, Ginsberg J, Sklar C, Robison LL. Pulmonary Complications in Survivors of Childhood and Adolescent Cancer A Report from the Childhood Cancer Survivor Study. Cancer. 2002; volume 95, number 11.

Miettinen 2019; Orbach 2010; Orbach 2016). Overall, IFS has a good prognosis and life expectancy is rarely impacted. A retrospective study analyzing clinical features and results of treatment of 56 infants under the age of 2 years between 1979 and 2005 from 6 European studies reported a 5-year overall survival rate of 89% (Orbach 2010). The objective is to provide these patients with treatment inducing minimal sequelae. At diagnosis, primary tumors are considered to be unresectable in 48-62% of the cases and, therefore, require a multidisciplinary strategy with preoperative cytoreductive treatment and local therapy including conservative surgery (Demetri 2020; Orbach 2016; Orbach 2020; Weiss 2014). Because of the very young age of patients, special attention must be paid to minimizing both the acute and chronic toxicities of therapy, including delivering drugs with minimal long-term consequences.

Chemotherapy plays a major role in the treatment strategy for unresectable tumors. The Vincristine-Actinomycin-D (VA) regimen is the preferred first line option and has shown good response rates in IFS. For those patients with insufficient response to VA, other effective regimens include Vincristine-Actinomycin-D-Cyclophosphamide (VAC) and Vincristine-doxorubicin (Adriamycine)-Cyclophosphamide (VAdriaC) (Loh 2002; Orbach 2016; Orbach 2020; Weiss 2014).

Almost 17% of patients with IFS have experienced significant negative long-term effects with conventional therapies. In addition, 5-10% of treated patients died, mostly as a consequence of a tumor which was refractory to multiple lines of therapy (Loh 2002; Orbach 2016; Orbach 2020). Chemotherapy drug administration is challenging to manage in such young patients (because of the need for central venous catheter insertion, risk of systemic infections, weekly hospital visits for chemotherapy administration, potential acute toxic side effects such as Veno-Occlusive Disease [VOD] or neuropathy). VA is preferred over VAC chemotherapy (cyclophosphamide) or anthracycline-containing regimens which were previously used in 53-87% of IFS patients, as it does not have gonadal, mutagenic or cardiac long-term toxicities (Sadurska 2015). Because of their acute and long-term toxicities, the use of alkylating agents and anthracyclines is limited.

7.3 STS and current standard of care

Although the main analysis focuses on IFS patients, this section is provided in order to contextualize the complementary analyses focusing on the wider STS population (including IFS and other NTRK fusion positive STS).

STSs are a heterogeneous group of relatively rare mesenchymal tumors, representing 1% to 2% of all cancers, with 4,000 to 5,000 new cases per year reported in France. The tumors develop mainly in adults around the age of 50, but may occur at any age: 10% of patients are children and adolescents (Manck 2017). STSs represent about 8% of all pediatric cancers, with about 120 cases per year reported in France (Regnault 2017). Two main types of STSs have been characterized: rhabdomyosarcomas (RMS) and non-RMS STSs. RMS are more frequent in young children while non-RMS STSs are more frequent in adolescents. These tumors can be localized or metastatic (St. Jude Children's Research Hospital 2018)St. Jude .

The 5-year relative survival rate for STS is 65% for all SEER stages combined; 81%, 56%, and 15% for localized, regional and distant tumors, respectively (American Cancer Society 2021).

Treatment of these tumors has not dramatically changed over the last two decades. It relies on knowledge of their natural history and tumor biology as this information is used to categorize STSs according to their risk. Pre-treatment clinical staging aims to categorize the tumors according to their site, size, local invasion, regional lymph node involvement and distant metastasis (Orbach 2005; Orbach 2016).

The aim of any standard STS treatment should be to achieve adequate local and systemic tumor control while minimizing the long-term sequelae. Chemotherapy, surgery and irradiation are used in the treatment of STS. Although surgery is the main treatment in localized low-risk tumors, good outcomes are not achieved without adjuvant radiation and chemotherapy aiming to reduce the risk of relapse and improve overall survival. In addition, upfront chemotherapy reduces the aggressiveness of the required surgery, especially in younger children with growing bodies, and helps preserve organ function in a number of cases (Orbach 2005; Orbach 2016).

Chemotherapy is an essential component of the multimodal treatment of RMS. The standard regimen in non-metastatic RMS in Europe, is a combination of Ifosfamide, Vincristine and Actinomycin-D (IVA) associated with Doxorubicine (IVADo) in case of metastases (Bisogno 2018). For intermediate- and high-risk patients, successive Children's Oncology Group (COG) trials have failed to improve the survival outcome by incorporating novel agents, such as etoposide (Vincristine-Doxorubicin-Cyclophosphamide alternating with Ifosfamide-Etoposide combination [VDC/IE]), and irinotecan (Vincristine-Actinomycin D-Cyclophosphamide alternating with Vincristine-Irinotecan [VAC/VI]), with the aim of reducing the cumulative cyclophosphamide dose (Ruymann 2003). In non-RMS, chemotherapy provides a poorer response than in RMS. In such tumor group, the therapeutic regimen typically comprises Ifosfamide/Doxorubicin (ID) (Ferrari 2015).

7.4 Toxicities associated with chemotherapy and radiotherapy

Chemotherapy is associated with toxicities. The onset of these side effects can be acute or chronic, having the potential of severe disabling, life-threatening or fatal illness, such as cardiovascular disease, kidney failure, or even a second malignancy (Sadurska 2015; Oberlin 2009).

Among the most significant complications of pediatric cancer treatment is the occurrence of cardiac morbidity, occurring in up to 57% of survivors treated with anthracycline chemotherapy (Ramjaun 2015RamjaunRamjaunRamjaun). Moreover, chemotherapy combined with the administration of other cardiotoxic agents increases toxic effect of anthracyclines on the cardiovascular system (Sadurska 2015). A high cumulative dose of anthracycline is the greatest risk factor for cardiotoxicity, although, it has also become evident that there is no 'safe' dose of anthracyclines; patients have experienced cardiac damage even at doses < 240 mg/m^2 (Lipshultz 2013; Lipshultz 2014). In addition, the prevalence of myocardial impairment due to anthracycline increases

with a longer follow-up (Lipshultz 2013). In fact, childhood cancer survivors exposed to 250 mg/m^2 of anthracyclines prior to age 5 have an ongoing risk of developing sustained echocardiographic abnormalities for up to 25 years following treatment (Ramjaun 2015). After cancer recurrence and secondary malignancies, the leading cause of morbidity and mortality in survivors of childhood cancer is cardiovascular-related disease (Lipshultz 2014).

Alkylating agent toxicities and especially those related to cyclophosphamide exposure are multiple. Their related gonadotoxicity has been widely described in the literature (Ridola 2009). Beside acute toxicities such as hematopoietic or gastrointestinal, it is to be noted that alkylating agents are associated with direct damage to the gonads and a significant risk of infertility (Oberlin 2009). Long-term male gonadal damage following cyclophosphamide-containing regimens has been shown to be dose-dependent with up to 70% or 80% of patients being affected by abnormal Follicle Stimulating Hormone (FSH) levels after treatment with a cumulative dose of the drug exceeding 9 g/m^2 (Ridola 2009). Elevated serum FSH is consistently associated with an abnormal sperm count. However, normal FSH was not found to be predictive of a normal semen analysis. Furthermore, cyclophosphamide exposure prior to the onset of puberty did not appear to protect males from subsequent gonadal damage (Kenney 2001).

A study aiming at comparing gonadal toxicity of ifosfamide versus cyclophosphamide during childhood showed that ifosfamide was associated with a lower risk of gonadal damage than cyclophosphamide and that the risk of abnormal FSH increased with the cumulative dose of cyclophosphamide (Ridola 2009). In addition, ifosfamide may cause renal failure and an increased risk of second malignancies (tubular and glomerular) (Oberlin 2009).

Although radiation therapy is very effective to control tumor growth and prolong overall survival, it has adverse effects on healthy tissue within the field of radiation. In addition, the combination with chemotherapy increases normal-tissue toxicity and therefore leads to even further reduction of the tolerable maximum dose. While acute radiation toxicity is marked by acute cell death, the processes in chronic toxicity are generally characterized by fibrogenesis and extracellular matrix deposition (Klaus 2021; Palmer 2021). A publication focusing on the risk assessment of radio-chemotherapy in 106 pediatric STS highlighted acute and delayed toxicity in the form of hematological, gastrointestinal toxicity and alopecia that occurred in all patients. In addition, hepatic, genitourinary toxicities, cardiotoxicity, neurotoxicity and skin complications could be seen in 13.2%, 11.3%, 1.9% and 4.7% and 28.3% of patients respectively. Mucositis was noticed in 42.5% of patients, and for 15.1% of them it was due to radiotherapy, which also caused dysphagia and dysphonia, impaired taste sensation and transient conjunctivitis in 4.7%, 1.9% and 6.6% of patients respectively (Abaza 2015). Additionally, 46.7% of post-pubertal patients were found to be azoospermic more than 5 years after the end of treatment. Respectively, 3.8% and 6.6% of patients developed ototoxicity and skin fibroses due to local irradiations (Abaza 2015). Furthermore, hypo- or hyperthyroidism and growth retardation was encountered in 7.5% and 6.6% of patients, respectively. Regarding late onset of toxicity, 5.7% of patients developed secondary malignancy, 7 years after the end of therapy (Abaza 2015).

Toxicity due to radiotherapy is usually irreversible and may worsen over time. The latency, or time to onset of late toxicity, is thought to be inversely related to radiation dose, whereas its progression directly depends on radiation dose (Palmer 2020).

Toxicities associated with chemotherapy and radiotherapy are summarized in Table 3, Table 4 and Table 5 (Annex 2).

7.5 Larotrectinib

7.5.1 Larotrectinib clinical development and efficacy

Larotrectinib is a first-in-class selective TRKi with high potency (IC_{50} = 5-11 nM in cellular assays) and high specificity for TRKA, TRKB and TRKC receptors (≥ 100-fold selectivity versus 229 other kinases).

Larotrectinib has the benefit to be orally bioavailable and to be provided in 2 formulations: a liquid oral formulation mainly for the pediatric population and hard gelatin capsules formulated in 2 doses (25 mg or 100 mg) mainly for adults.

Larotrectinib has been tested on patients with advanced solid tumors harboring *NTRK* gene fusion in 3 clinical trials:

- Phase I clinical trial conducted in adults (NCT02122913, "A study to test the safety of the investigational drug larotrectinib in adults that may treat cancer" [active, non-recruiting]),
- Phase I/II clinical trial conducted in the pediatric population (up to 21 years old): SCOUT study (NCT02637687, "A Phase I/II study of the oral TRK inhibitor larotrectinib in pediatric patients with advanced solid or primary central nervous system tumors [international clinical study; recruiting]),
- Phase II conducted in adults and adolescents (from 12 years old): NAVIGATE study (NCT02576431, "A study to test the effect of the drug larotrectinib in adults and children with NTRK-fusion positive solid tumors [recruiting])[6].

A first pooled analysis from these 3 clinical trials was performed with a primary data cut-off date of 17 July 2017 (Drilon 2018). The decision to pool efficacy data from patients with a *NTRK* fusion-positive tumor across all three studies was made early in the development program on the basis of the rarity of *NTRK* fusions, the inherent heterogeneity of cancer types, and global regulatory advice. The best overall response rate (ORR) derived from the time point responses using the Response Evaluation Criteria in Solid Tumors (RECIST) as determined for the first 55 patients with *NTRK* gene fusion by an independent radiology review committee (central assessment) was 75% (95% CI, 61-85%), with 13% of CR, 62% of PR and 13% of SD. By investigator assessment, the ORR was 80% (95% CI, 67-90%).

A second pooled analysis was performed on 159 patients with *NTRK* gene fusion from the same 3 clinical trials *at data cut-off date of February 19, 2019* (Hong 2020) second analysis confirmed the results of the first one, with a best ORR of 79% (95% CI, 72-85), 16% of CR, 63% of PR and 12% of SD (investigator assessment), regardless of the type of tumors, the population (adult or pediatric) and the type of gene fusion involved. The median Duration of Response (DoR) was 35.2 months, the median progression-free survival (PFS) was 28.3 months and the median overall survival (OS) was 44.4 months. In the IFS population (n=28), 27 patients had an objective response (ORR 96%, 95% CI 82–100). The median duration of response was not estimable.

Moreover, the interim clinical study report of the SCOUT study, at data cut-off date of July 15, 2019, reported 88 patients of whom 38 patients were in the Phase I portion of the study (24 in escalation and 14 in expansion) and 50 patients were in Phase II. The full analysis set included all 88 treated patients. At the time of this interim analysis, all 79 *NTRK* gene fusion patients and all 9 of the non-*NTRK* gene fusion patients were evaluable for response. Within the *NTRK* gene fusion subgroup (consisting

[6] Following a protocol amendment of the NAVIGATE study, the study now only includes patients older than 18 years.

predominantly of IFS and STS), 54 of the 79 patients with measurable disease had a confirmed objective response, yielding an ORR of 77% (95% CI: 66, 86) based on Investigator assessment. This included 21 CRs and 37 PRs (including 4 pending confirmation of a PR). Within the non-*NTRK* gene fusion subgroup, no responses were observed.

In the IFS population (n=34), the ORR was 94% (95% CI 80, 99). Among the patients with IFS, included in SCOUT at data cut-off of July, 2019, 21 patients had no other curative options besides disfiguring surgery or amputation before larotrectinib initiation. At the time of analysis, all of these patients showed responses, with 4 complete surgical responses, 7 complete responses and 9 partial responses recorded. None of these patients required an amputation or disfiguring surgery and no death was reported at the time of analysis.

As a conclusion, in these various analysis, larotrectinib has demonstrated clinically meaningful antitumor activity in pediatric patients with locally advanced or metastatic solid tumors with *NTRK* gene fusions, including IFS, that had previously progressed and had little or no effective treatment alternatives and facing limb amputation or disfiguring surgery.

According to the European Society for Medical Oncology (ESMO) Scale of Clinical Actionability for molecular Targets (ESCAT), larotrectinib was classified as “tier I-C”, which is attributed “when clinical trials in multiple tumor types, or basket clinical trials, have demonstrated a clinically meaningful benefit for the target-drug pair with similar magnitude of benefit across the different tumor types” (Mateo 2018).

In the United States, FDA approved larotrectinib (Vitrakvi) in November 2018 with an accelerated approval in the following indication:

Vitrakvi is a kinase inhibitor indicated for the treatment of adult and pediatric patients with solid tumors that:

- have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation,
- are metastatic or where surgical resection is likely to result in severe morbidity, and
- have no satisfactory alternative treatments or that have progressed following treatment.

In France, a Temporary Authorization of Use (*Autorisation Temporaire d’utilisation* [ATU]) was granted by the National Agency for Drug and Health Products Safety (*Agence Nationale de Sécurité du Medicament et des Produits de Santé* [ANSM]) in March 2019.

In September 2019, larotrectinib was granted a conditional MA in Europe, for the treatment of adult and pediatric patients with solid tumors that display a *NTRK* gene fusion:

- who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and
- who have no satisfactory treatment options.

7.5.2 Larotrectinib safety profile

At the cut-off date of July 2019, 88 pediatric patients ranging in age from less than 1 month to 19.9 years had been enrolled in the SCOUT trial and treated with larotrectinib. 85/88 patients experienced at least one Treatment Emergent Adverse Event [TEAE]. The TEAEs were usually mild. The most commonly occurring TEAEs by preferred term (PT) were pyrexia (47%), vomiting (44%), alanine aminotransferase (ALT) increase (40%), cough (39%), diarrhea (38%), aspartate aminotransferase (AST) increase (34%), neutrophil count decrease (30%), constipation (25%), upper respiratory tract

infection (25%), fatigue (22%), and nausea (20%). Sixty-two (62) patients (70%) experienced at least one TEAE attributed to larotrectinib. Forty-seven (47) patients experienced a TEAE that was Grade 3 or 4 (53%), although these were considered related to larotrectinib in only 17 patients (19%). The related Grade 3 or 4 TEAEs, occurring in more than one patient each by PT, were neutrophil count decrease (8 patients), weight increase (2 patients) and ALT increase (2 patients). Twenty-seven (27) patients experienced a TEAE that was considered serious (31%) with 6 considered related to the drug (7%). Two (2) patients (2%) experienced a TEAE with fatal outcome but none were related to larotrectinib.

Moreover, an expanded safety population analysis of 260 patients enrolled and treated in the phase I study in adults, the phase I/II pediatric (SCOUT), or the phase II adolescent and adult trial (NAVIGATE), was performed (data cut-off February 2019, Hong 2020). No new safety signals were identified from this analysis. This was also the case in the subset of pediatric patients with TRK fusion-positive tumors (n=52). To date, the known safety profile has not changed over time. The long-term toxicity and developmental effects of larotrectinib in pediatric patients are being investigated in the SCOUT trial and also via the observational post-authorization safety study. With at least 60 months of follow-up from larotrectinib initiation in pediatric patients, this non-interventional study will generate additional safety and effectiveness data in a broader population (300 patients, with a minimum of 30 pediatric patients) in a real-world setting, including long-term effects of larotrectinib on growth (height and weight), neurological outcomes, developmental milestones, and sexual development.

Larotrectinib offers a novel, well-tolerated and often highly effective treatment for infants with TRK fusion IFS. Moreover, the oral solution of larotrectinib allows for an easy administration in very young patients.

7.6 Rationale for the conduct of this observational study

The clinical development program of larotrectinib to date has shown that the tumor response observed with the drug is pronounced and durable even if no comparator is used in the ongoing clinical studies, including the pediatric Phase I/II study (SCOUT).

In France, a dossier was submitted to the National Authority for Health (*Haute Autorité de Santé* – HAS) in October 2019 to obtain the reimbursement of larotrectinib in the country, in line with the population described in the MA.

In July 2020, the HAS's final appraisal was a positive opinion for the reimbursement of larotrectinib only in the pediatric patients with IFS or another STS harboring a *NTRK* gene fusion, which is locally advanced or metastatic, and refractory or in relapse, for moderate clinical benefit (SMR) with no improvement in clinical benefit (ASMR V) mainly due to the lack of comparative evidence. Among other requests, the HAS requested post-inscription clinical data to be provided. In this context, the reimbursement remains conditional and will be reassessed based on the provision of comparative data of larotrectinib versus standard of care at least *versus* an external control arm, in particular in IFS. This study is designed to address this request but also to possibly use the data to provide comparison with historical data as agreed in the pediatric investigation plan with EMA.

7.7 Rationale for the use of an external historical control

Performing a comparison of the available data in the larotrectinib ongoing pediatric phase I/II study (SCOUT) with that of (an) external historical cohort(s) reflecting standard of care, constitutes a relevant approach to assess the efficacy of larotrectinib and to contextualize its therapeutic benefit

over current therapies which may be associated with detrimental outcomes. Given the rarity of the condition and ethics consideration, the use of an external control arm allows for the timely generation of comparative data.

To our knowledge, the database of the *Institut Curie* is the only database that is confirmed today to be eligible and accessible to be an external historical cohort for the present study (see eligibility criteria in Section 9.2.3.1). The *Institut Curie* database is the largest database available in France (including patients treated in *Institut Curie* and also treated in other hospitals) which describes French pediatric patients harboring TRK fusion cancer (especially IFS) that is able to provide patient-level data for the constitution of a historical control group. The patients constituting the *Institut Curie* database were all diagnosed with a *NTRK* gene fusion by RNAseq.

For nearly 25 years, the Somatic Genetics Unit of the *Institut Curie* has been involved in the establishment of molecular diagnoses and the classification of pediatric sarcomas and tumors with the objective to optimize patient care. This institute is a leader and can be considered as a reference in the field in France and in Europe. The care of the patients included in the *Institut Curie* database corresponds to the standard of care of the patients with the targeted indication.

Additional databases meeting the eligibility and feasibility criteria identified following a comprehensive search of databases (Systematic Literature Review [SLR]) will be used as external historical control cohorts (see details in Section 9.2.3.1).

7.8 Rationale for the choice of the primary endpoint

In oncology clinical trials, *OS*, defined as the time from randomization until death due to any cause, is generally considered as the most convincing measure for drug efficacy and benefit and represents the gold standard primary endpoint for these trials (FDA 2018).

In the present observational study, the *time to medical treatment failure* (defined as the time from the start of treatment to the date of the following events, whichever comes first: subsequent systemic treatment, radiation therapy, mutilating surgery or death due to any cause), was chosen to be the primary endpoint.

The choice of the *time to medical treatment failure* as primary endpoint over *OS* was motivated by the results of recent studies (Orbach 2020) confirming the excellent OS of patients with IFS. A retrospective analysis performed in 2020 of all published data from patients with IFS of the European pediatric Soft tissue Sarcoma Study Group (EpSSG) and *Cooperative Weichteilsarkom Studiengruppe* (CWS) databases revealed that among the 172 European patients treated, 162 (94.2%) were alive at the end of the study follow-up. Among the 10 patients who died, 9 patients died because of the disease and one because of toxicity (Orbach 2020). Based on these observations, OS does not therefore represent the most relevant criterion to assess the therapeutic benefit of a treatment for IFS patients.

The challenges for IFS patients are the management of their morbidities and the long-term effects associated to the type of therapies used, which includes the resection of their tumor without anatomic and functional damage, as well as the administration of adapted therapies (alkylants, anthracyclines, radiotherapy) by minimizing acute and chronic toxicities, including minimal long-term consequences.

The retrospective analysis performed by Orbach et al. (Orbach 2020), showed that among the 172 patients included in the study, 65 patients (40% of all survivors) were treated with surgery alone and 64 (39%) with surgery combined with chemotherapy. Radiotherapy was delivered to 5 patients (3% of survivors), and 28 patients (17%) exclusively received chemotherapy. Among the 129 patients who were treated with surgery, 91% had conservative surgery (118 patients). Twenty patients (12%)

survived with major functional deficits or had mutilating surgery. In this series, almost 17% of patients may have experienced significant negative long-term effects with conventional therapies (20 patients treated with mutilating surgery and 5 with radiotherapy). In addition, 10 patients died mostly as a consequence of a tumor which was refractory to multiple lines of therapy. Altogether, these observations evidence that conventional conservative strategies demonstrate efficacy in IFS, even for the extensive tumors, but they are associated with acute and chronic side effects (Orbach 2020, see Section 7.4).

To increase the robustness of the comparison for the primary analysis, events that are considered the most relevant in view of the multidisciplinary management of these tumor types will be selected. Chemotherapy is an essential component of the multimodality treatment with multiple lines considered in case of suboptimal outcomes, while surgery and radiation therapy acting as a local control may be associated with significant morbidities, with, in the worst case, failing to preserve the affected organ, particularly in the extremities. Three European studies analyzing the total burden of therapy for patients with IFS treated with conventional treatments showed that radiotherapy was used in 2.9% of patients, mutilating surgery was performed in 6.9% of patients and 5.2% of patients died of disease. Moreover, in main recent large series published in IFS, mutilating surgery, radiotherapy, alkylating chemotherapy and anthracycline agents were performed or used in 6-13%, 2-20%, 20-87% and 0-13% of cases, respectively (Orbach 2020).

The systemic treatment administered to the patient population targeted in this observational study is expected to either allow a complete response or to qualify the patient for non-mutilating complete surgical resection. Based on the experience of the experts of the field, tumor responses after conventional chemotherapy generally occur slowly over several months, whereas larotrectinib may lead to an early response (median time to response 1.8 months, with clinical improvement noted within days to weeks), which may be more important when there are life-threatening symptoms including tumor bleeding or organ compression. In cases of refractory tumors, life-threatening complications, or of metastatic tumors, TRKi should be immediately considered (Orbach 2020).

Events that constitute the primary endpoint (subsequent systemic treatment, radiation therapy, mutilating surgery or death due to any cause) were chosen based on the following rationales:

- They are all considered as markers of treatment failure from a clinical perspective and it is clinically relevant to avoid the occurrence of any of these events, because of associated morbidities and long-term toxicities:
    - Use of a subsequent treatment is considered when treatment response is not pronounced enough to qualify a patient for non-mutilating surgical resection or upon progression,
    - Radiation therapy is considered when systemic treatment fails as it can cause significant morbidity,
    - Mutilating surgery (at the investigator's discretion in SCOUT and external historical control cohorts): clinicians aim at avoiding mutilating surgery, it is the very last option in case of previous treatment failure to prevent evolution to the metastatic stage and associated risk of shortened survival,
    - Death.
- Measuring methods for those events in the SCOUT and external historical control cohort(s) seem more reliable than the evaluation of the PFS or tumor response, which are dependent upon local methods of reading radiological images.

- Due to the small patient population, the comparison with (an) external historical control cohort(s) and the anticipated rarity of events of clinical interest, the choice of a composite endpoint sounds the most appropriate approach.

8. Research questions and objectives

8.1 Primary objective

The aim of the study is to assess the therapeutic benefit of larotrectinib over the current standard of care in patients with locally advanced or metastatic IFS.

The primary objective of the present study is to compare the time to medical treatment failure (defined as: next systemic treatment or mutilating surgery or radiation therapy or death due to any cause) between larotrectinib and standard of care in IFS patients using externally-controlled comparison performed with phase I/II SCOUT study and eligible external historical cohort(s).

8.2 Secondary objectives

The secondary objectives in this study include the comparison of:

- Treatment outcomes (next systemic treatment, mutilating surgery, radiation therapy, death due to any cause),
- Treatment discontinuation rates due to toxicity.

9. Research methods

9.1 Study design

This study is a retrospective observational externally-controlled study. Data of patients with IFS in the eligible external historical cohort(s) will serve as control for the comparison with data of patients with IFS who have been enrolled in the SCOUT study. Data which will be used for the analyses will come from:

- SCOUT study:
  - At least 27 sites across 13 countries, including France.
- Eligible external historical control cohort(s) (see inclusion and exclusion criteria of databases in Section 9.2.3.1), including at least the *Institut Curie* database.

9.1.1 Primary endpoint

The primary endpoint of the study is the *time to medical treatment failure:* defined as the time (months) from the start of treatment to the date of the following events, whichever comes first: subsequent systemic treatment, radiation therapy, mutilating surgery or death due to any cause.

The rationale for the choice of this variable as primary endpoint, notably over OS variables is detailed in Section 7.8.

9.1.2 Secondary endpoints

The secondary endpoints of the study are:

- Time to subsequent systemic treatment.
- Time to mutilating surgery including limb amputation.

- Time to radiation therapy.
- Time to complete surgical resection (excluding amputation).
- OS, defined as the time (months) from the start of treatment to the date of death due to any cause. Patients alive or lost to follow-up at the time of analysis will be censored at their last date of follow-up.
- Incidence of patients with treatment discontinuation due to treatment-related adverse events (AEs).

9.1.3 Exploratory endpoints

The following exploratory endpoints will also be investigated:

- Progression-free survival (PFS): defined as the time (months) from the start of treatment to the date of first observed disease progression (radiological or clinical, whichever is earlier; assessed by the treating physician in the external historical control cohort(s)) or death due to any cause, if death occurs before progression is documented. Patients without documented progression at the time of analysis will be censored at the date of last tumor assessment.
- Overall response rate (ORR): defined as the proportion of patients with a best overall response of complete response (CR) or partial response (PR), as assessed by investigators.
- Disease Control Rate (DCR): defined as the proportion of patients with a best overall response of CR, PR, or Stable Disease (SD).

9.2 Setting

9.2.1 Study population

The study population will comprise all patients of the SCOUT study and of the external historical control cohort(s) (including at least the *Institut Curie* database) with a diagnosis of locally advanced or metastatic IFS. Inclusion and exclusion criteria for patients are described in Section 9.2.3.2.

9.2.2 Study time frame

This study, being a retrospective observational study, does not require diagnostic or therapeutic procedures nor the respect of a specific calendar for patient visits.

The detailed planned dates of the different milestones of the study are presented in Section 6 in Table 1.

9.2.3 Selection criteria

9.2.3.1 Selection criteria for the sources of the external historical control cohort(s)

The choice of the external control cohort(s) to be included in the comparator arm is not arbitrary. In order to ensure a non-biased selection and to maximize as possible the sample size and power of the comparator group, the following actions were or will be undertaken:

- A comprehensive assessment of the existing databases in France and internationally will be performed from the following sources:
  - ✓ Databases found in the literature,

- ✓ Databases from clinical studies sponsored by Bayer or known by Bayer, notably through its interactions with experts in the field,
- ✓ Commercial databases with electronic medical data.

- A SLR will be conducted to ensure completeness of the identification of all databases that could be used as external historical control cohorts. This SLR will be based on a protocol of research including the following Patient, Intervention, Comparison, Outcome (PICO) criteria:
  - Population: IFS,
  - Intervention: N/A,
  - Comparison: N/A,
  - Type of studies: all interventional and observational studies except case reports,
  - Period: from 2000 to present,
  - Scope: international.

The SLR protocol and report will be annexed to the final study report.

The selection of data sources will be performed upon the following eligibility and feasibility criteria, with the following justifications:

- Inclusion criteria:
  - ✓ Cohorts with prospective enrollment and with retrospective and prospective data collection from 2000 to present.

    This criterion was defined based on the following rationales:
    - The strong evolution of histological diagnostic in the 2000's,
    - The risk of classification bias if databases prior to 2000's were considered (e.g., with our current knowledge and the current classification, nowadays, some patients would not be diagnosed with IFS but with spindle cells rhabdomyosarcoma [Orbach 2020]),
    - The development and the use of biomolecular diagnostic since the 2000's: IFS diagnosis is no more considered in absence of *NTRK* gene fusion,
    - The absence of significant evolution in the IFS diagnosis and therapeutic approaches from 2000 to present: Chemotherapy (VA regimen) has been the standard of care for this indication for several decades (*reference: HAS opinion on larotrectinib*).
  - ✓ Cohorts containing at least clinical data allowing to assess the efficacy of the treatment and the main prognostic factors as follows:
    - Diagnosis and stage of the disease (locally advanced or metastatic),
    - Type of treatments (chemotherapy, radiotherapy, surgery: mutilating yes/no) and date of the initiation or of the procedure,
    - Death and date,
    - Localization of the tumor (axis versus limb),
    - Size of the tumor (< 5 cm versus > 5 cm).
- Exclusion criteria:
  - ✓ Databases not containing patients with locally advanced or metastatic IFS,

- ✓ Medico-administrative databases or absence of data allowing the assessment of the efficacy of the treatment and main prognostic factors, or high rate of missing data (>10% on outcome and >25% on covariates),
    - The use of these databases, especially medico-administrative databases, is not feasible because of missing data and the limited information available on the clinical, biological, diagnostic and treatment data, notably for rare diseases like IFS. These limitations would be an issue for the prognostic, outcomes and selection criteria of patients.
- ✓ Cohorts with retrospective enrollment and case report,
    - Cohorts with retrospective enrollment or case report are subject to a greater risk of selection bias than those with prospective enrollment and with retrospective and prospective data collection, given that all patients who should have been considered to be included might have not been included a posteriori.
- ✓ Cohorts with prospective enrollment for which all patients were included before 2000,
    - There is a risk of classification bias for cohorts constituted before 2000 because of the evolution of the histological and molecular diagnosis of IFS (see details in the inclusion criteria).

Based on a preliminary search of databases and the selection criteria described above, in addition to the database of the *Institut Curie* which is the only database confirmed, the following data sources are theoretically eligible to be used to constitute (an) external historical control cohort(s) for the present study:

- Databases found in the literature (preliminary search that will be completed by the SLR):
    - ✓ Database from the European pediatric Soft tissue Sarcoma Study Group (EpSSG, a consortium housed in the University of Padua, Italy, European data),
    - ✓ Database from the *Cooperative Weichteilsarkom Studiengruppe* (CWS, Germany).
- Commercial databases with electronic medical data:
    - ✓ No eligible databases were found.

The comprehensive review of the literature (SLR) that will be performed might identify additional eligible cohorts. The availability, the access, and the feasibility of use of the different eligible databases identified will be then investigated. Further details will be described in the final study report.

9.2.3.2 Selection criteria for patients

- Inclusion criteria:

  The inclusion criteria listed below are in line with those of the SCOUT study in terms of patients and disease characteristics:

  - Age ≤ 21 years old.
  - Locally advanced or metastatic IFS.
  - Patients with available information on clinical, radiological characteristics of their tumor, therapies administered and outcomes.
  - Patients receiving larotrectinib in the SCOUT trial.

- Patients receiving at least chemotherapy-based regimen in the external historical control cohort(s).
- No opposition from the patients and/or representatives for data use.

- Exclusion criteria:
  - Patients treated with TRKi in the external historical control cohort(s).
  - Patients with documented absence of *NTRK* gene fusion:
    - Formal identification of *NTRK* gene fusion has not been retained as an inclusion criteria as the information might be missing from the databases used to constitute the external historical control cohort(s). Given the rarity of the condition and the anticipated small sample size, the study will authorize the inclusion of patients with unknown *NTRK* gene fusion status to maximize sample size while there is a limited risk of introducing a bias, as *NTRK* fusion is almost pathognomonic in IFS (Orbach 2016).
  - Patients participating in an investigational program with interventions outside of routine clinical practice.
- Population sampling strategy:

The study population will comprise all IFS patients in the SCOUT study and the eligible external historical cohort(s) (including at least the *Institut Curie* database) with a diagnosis of locally advanced or metastatic IFS regardless of their refractory or relapsed status, i.e. including treatment-naive patients to avoid further reducing the sample size.

The choice of the study population has mainly been driven by feasibility/sample size considerations, in order to be able to perform a comparison based on a minimal number of patients.

In addition, the IFS population will also include patients with advanced or metastatic CMN since the histopathological characteristics of these tumors are very close to those of IFS, as mentioned in the WHO sarcomas classification (Kallen and Hornick 2020). Moreover, the presence of the same fusion transcript as in IFS is identified in the cellular form of CMN (Thebaud 2012).

9.2.4 Representativeness

Larotrectinib as monotherapy is indicated for the treatment of adult and pediatric patients with solid tumors that display a *NTRK* gene fusion,

- who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and
- who have no satisfactory treatment options.

The positive opinion of the HAS for the conditional reimbursement of larotrectinib was given only for the pediatric patients with a diagnosis of locally advanced or metastatic IFS or another STS harboring an *NTRK* gene fusion, refractory or in relapse. Therefore, the data that will be used for the analyses of this observational study are data from IFS patients who are in line with the scope of the conditional reimbursement of larotrectinib, broaden to IFS regardless of their refractory or relapsed status, i.e. including treatment-naive patients, in order to limit the further reduction of the sample size. Data will be extracted from the following sources:

- The SCOUT study, which is an international clinical trial conducted in 27 centers in 13 countries including France, started in December 2015,
- The *Institut Curie* database and the eligible databases identified through a SLR with an international scope according to the selection criteria described in Section 9.2.3.1. The choice of using cohorts with prospective enrollment and with retrospective and prospective data collection from 2000 to present will avoid the risk of selection bias that could have occurred if cohorts with retrospective enrollment would have been included and the risk of classification bias if cohorts constituted before 2000 would have been included, notably because of the evolution of histological and molecular diagnosis of IFS in the 2000's. Therefore, the control cohort(s) that will be identified using this systematic approach with an international scope and the chosen selection criteria can be considered representative.

Even though the sample size is small (which is inherent to the very low incidence of the targeted indications), these sources can be considered as reliable and representative of the population targeted in the present observational study, given the time windows and the sources from which the data will be extracted (e.g. the *Institut Curie* database regroups data from French centers from all over the regions of France).

9.3 Variables

9.3.1 Variables to determine the primary endpoints

To determine the primary endpoint (*time to medical treatment failure*), the following variables will be collected from the Case Report Forms (CRFs) of the eligible patients for the present observational study from the SCOUT study and from the database(s) of the eligible external control cohort(s):

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and whichever comes first:
- Start date of a post-treatment[7] systemic anti-cancer therapy, if any, or,
- Start date of a post-treatment[6] radiation therapy, if any, or,
- Date of a post-treatment[6] mutilating surgery, if any, or,
- Date of death due to any cause, if applicable.

9.3.2 Variables to determine the secondary endpoints

To determine the secondary endpoints, the following variables will be collected from the CRFs of the SCOUT study and from the external control database(s) eligible for the present observational study:

For the *time to subsequent systemic treatment* endpoint:

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Start date of a post-treatment[7] systemic anti-cancer therapy, if any.

For the *time to mutilating surgery including limb amputation* endpoint:

[7] After the start of larotrectinib treatment for the SCOUT study or after the start of chemotherapy (first or second line) for the external historical control cohort(s).

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Date of a post-treatment[7] mutilating surgery including limb amputation, if any.

For the *time to radiation therapy* endpoint:

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Start date of a post-treatment[8] radiation therapy, if any.

For the *time to complete surgical resection (excluding amputation)* endpoint:

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Date of a post-treatment[7] complete surgical resection (excluding amputation), if any.

[8] After the start of larotrectinib treatment for the SCOUT study or after the start of chemotherapy (first line) for the external historical control cohort(s).

For the *OS* endpoint:

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Date of death due to any cause, if applicable, or,
- Last date of patient follow-up for patients alive and lost to follow-up, if applicable.

For the *Incidence of patients with treatment discontinuation due to treatment-related AEs* endpoint:

- Record of treatment[9] discontinuation due to treatment-related AEs, if applicable.

9.3.3 Variables to determine the exploratory endpoints

To determine the exploratory endpoints, the following variables will be collected from the CRFs of the SCOUT study and the external historical control cohort(s) of the patients eligible for the present observational study:

For the *PFS* endpoint:

- Start date of larotrectinib treatment for the SCOUT study and start date of chemotherapy (first line) for the external historical control cohort(s), and,
- Date of first post-treatment[7] disease progression/relapse assessed by radiological or clinical examination, if any, or,
- Date of death due to any cause, if applicable, or,
- Last tumor assessment date for patients alive without documented progression.

For the *ORR* and *DCR* endpoints:

- Patient response to treatment[8] (CR, PR and SD) assessed by investigator.

9.3.4 Potential confounders

The following baseline variables will be considered when balancing the two cohorts: IFS patients enrolled in SCOUT study and the external historical control cohort(s).

- Patients and disease characteristics:
  - Sex,
  - Age,
  - Disease history:
    - Date of initial diagnosis,
    - Anatomical disease site(s) at diagnosis,
    - Stage of disease at diagnosis (local versus locally advanced versus metastatic),
    - Primary tumor size (< 5 cm or > 5 cm),
    - Date of advanced disease stage evolution (locally advanced, metastatic),
    - Time from locally advanced/metastatic disease diagnostic to initiation of larotrectinib (SCOUT) or first line of chemotherapy (external historical control cohort(s)),
    - Intergroup Rhabdomyosarcoma Study (IRS) group,
    - Anatomical site(s) of metastases, if any.
- Collection of treatment-related information:
  - Prior/current surgical treatment(s) for cancer:
    - Anatomical site(s),

[9] Larotrectinib treatment for the SCOUT study and chemotherapy (first line) for the external historical control cohort(s).

- Date(s) of surgery,
- Best resection (R0, R1, R2),
- Nature of intervention(s) (e.g. amputation or other disfiguring procedures),
- Surgery for a curative intent (excluding amputation) because of the use of systemic treatment.

- Prior/current radiation treatment(s) for cancer:
  - Anatomical sites,
  - Date(s) of radiation therapy.
- Systemic treatment(s), including:
  - Treatment regimen,
  - Date of initiation,
  - Date of radiological or clinical progression,
  - Reasons for discontinuation, as appropriate.

A SLR will be conducted to identify and quantify prognostic factors which could impact the study primary endpoint. This SLR is based on a protocol of research including the following PICO criteria:

- Population: IFS locally advanced or metastatic.
- Intervention: no restriction.
- Comparison: no restriction.
- Outcomes: prognostic factors.
- Type of studies: no restriction except case reports.
- Period: no limit of time.
- Scope: international.

The SLR protocol and report will be annexed to the final study report.

9.4 Data sources

Data used in this study will come from the following different sources:

- From the international phase I/II clinical trial called 'SCOUT' conducted in a pediatric population (up to 21 years old) with advanced solid or primary CNS tumors.

  For the SCOUT study, data are collected via electronic CRFs (eCRFs) completed by the investigators during the study conduct in each site.
- From the *Institut Curie* database and the other eligible external historical control cohort(s), data are collected from the respective databases. The definitive choice of the external historical control cohort(s) will be done after a comprehensive assessment of the existing French and international databases and SLR. The characteristics of the control cohorts that will be identified as eligible will be described in the final study report.

9.5 Study size

All patients meeting the selection criteria will be included in the study. Statistical power will be computed retrospectively according to the available sample size.

9.6 Data management

9.6.1 Data access rights and confidentiality

For the larotrectinib arm of the study, data that will be used for the analyses of this observational study are secondary data that were originally pseudonymized during the conduct of the SCOUT study. Each patient was identified by a unique central patient identification code, which was only used for the purpose of this study. The patient's treating physician or authorized site personnel are the only persons able to identify the patient, based on the patient's identification code. Uncoded patient data (which allows to directly identify a person) as well as the list allowing to do the link between the patient and the unique central patient identification code are only and securely stored at study sites.

For the eligible control cohorts, grant access to Bayer to patient-level raw data will be requested to the corresponding databases. A data transfer agreement will be agreed between Bayer and the databases owners to set up requirements in terms of data transfer, transfer and storage. The transfer of external data to Bayer will be performed via CLIXX (Clinical Information Exchange Platform for External Partners). The purpose of this secured platform is to facilitate the exchange of personal data between Bayer and external partners. After successful download, the data will be removed from CLIXX and deposited into TOSCA, which is a globalized tool, developed internally, which combines a central location for clinical data, standards and program repository, and a SAS programming environment for storing, programming managing and analyzing data. Bayer data center is located in Leverkusen, Germany.

In compliance with the laws and regulations in application and in particular with the General Data Protection Regulation (GDPR, EU 2016/679 of 27 April 2016), the persons having a direct access to the source data will take the necessary precautions to ensure the confidentiality of the information relative to the experimental medicinal products, to the clinical studies, and to the individuals who participated to the studies. These persons will be bound by professional secrecy.

9.6.2 Document and data archiving

Data will be accessible when CNIL authorization is obtained until the end of statistical analysis. The data will be then stored during a 2-year period after finalization of the publication. Any data as well as programs from statistical programming performed to generate results will be archived within the programming system for at least 25 years.

Bayer will ensure that all relevant documents related to this observational study will be archived after the end or discontinuation of the study for at least 25 years.

9.7 Data analysis

A comparison of patients in the larotrectinib group with patients from the external historical control cohort(s) who received systemic therapy (chemotherapy-based regimen) will be conducted by Bayer, at least with the *Institut Curie* database. In the event that other external historical control cohorts are eligible and accessible, the databases will be pooled if feasible.

The data and statistical analyses that will be performed are summarized in the following sections. Further details will be described in the statistical analysis plan (SAP), including methods for handling missing data.

9.7.1 Index Date and endpoints

The comparison of the two cohorts will be considered for the primary composite endpoint:

- Time-to-treatment-failure (earliest of next systemic therapy, radiotherapy, mutilating surgery or death due to any cause).

In addition, all secondary and exploratory endpoints detailed in Section 9.1.2 and Section 9.1.3 will be analyzed:

- Time to subsequent systemic treatment,
- Time to mutilating surgery or limb amputation,
- Time to radiation therapy,
- Time to complete surgical resection (excluding amputation),
- OS,
- PFS,
- Incidence of treatment discontinuation due to treatment-related AEs,
- ORR,
- DCR.

The analysis will include patients on both first and second line (or higher) therapy. For SCOUT patients, the index date will be defined as the start date of larotrectinib. For patients of the external historical control cohort(s), the index date will be the start date of the first line of chemotherapy. The initial imbalance on lines of therapy between treatment groups will be corrected in the statistical modeling, in particular the variable defined by time from locally advanced/metastatic disease to index date will be a key variable in the propensity score model. Given approximately 70% of SCOUT patients received larotrectinib as second line of treatment, while in the external historical control cohort(s) all patients will have received chemotherapy in first line of treatment, the proposed primary analysis is expected to provide a rather conservative estimate of treatment effect.

9.7.2 Adjustment methodology

Patients who received larotrectinib in the SCOUT trial will likely differ in various underlying characteristics from those who are recorded in the external historical control cohort(s). These characteristics are listed above as potential confounders (See Section 9.3.4). The analyses will need to be adjusted for these patient characteristics to avoid biased results.

An adjustment methodology using a propensity score will be employed to reduce the effects of measured confounding variables in the interpretation of the treatment effect (larotrectinib versus historical group). A propensity score can be seen as an overall “balancing score”, that is calculated for each patient based on his or her measured underlying characteristics.

The propensity score will be obtained from a logistic regression model with dependent variable: larotrectinib group (yes/no). Covariates will be taken from the list of potential baseline confounders above (see Section 9.3.4). The predicted probabilities of receiving larotrectinib from this logistic regression model will form the propensity scores for each patient.

The most widely used propensity score is 1:1 matching, which involves selecting treated and control patients without replacement to form matched pairs. However, this typically requires a larger group of control patients than within the treated group, so that each treated patient can find a satisfactory matching control.

Because cases of IFS are exceedingly rare, resulting in small sample sizes, we favor IPTW as it is easy to implement and retains data from all patients (it does not discard the unmatched patients), which

makes it closer to the global population (Austin 2014; Thoemmes 2016; Cenzer 2020). In addition, the IPTW method allows one to estimate the average treatment effect (ATE). In this sense, it can mimic a randomized trial, which is designed to provide an unbiased estimation of the ATE (Austin 2014). In contrast, a conventional matching approach (e.g. matching pairs of treated and control subjects on the logit of the propensity score) is restricted to estimating the average treatment effect in the treated (ATT). ATT would only be expected to equal ATE in a randomized control trial setting. The IPTW method makes it possible to reduce bias by correcting each patient's contribution by a weight equal to the reciprocal of the probability of receiving the treatment that was actually received. For larotrectinib patients, this is the reciprocal of the propensity score, while for comparator patients this is the reciprocal of one minus the propensity score. These weights are stabilized by multiplying by the marginal probability of treatment that was actually received, which avoids very large weights being assigned to larotrectinib patients with a very low propensity score or to comparator patients with a propensity score close to 1. Austin and Stuart recommend keeping "design" and "analysis" separate, and not using the outcome data in the propensity score process. Therefore, the balancing and analysis steps will be two completely distinct tasks (Austin and Stuart 2015).

9.7.3 IPTW method

The choice of covariates for the propensity score model will be finalized at a later date. IPTW analysis will be adopted for all time-to-event endpoints, and also for response endpoints (ORR, DCR). If possible, all available covariates will be included in the propensity score model. A SLR will be conducted to identify and quantify the known prognostic factors (see details in Section 9.3.4). If possible, the analyses will be adjusted based on all those prognostic factors. Every effort will be made to decrease bias by including covariates that are associated with outcome, or are true confounders (associated with both exposure and outcome), as demonstrated by Piracchio et al. (Pirracchio 2012) in simulations involving small sample sizes (Cenzer 2020).

For all baseline covariates included in the propensity score model, standardized differences between larotrectinib and comparator groups will be computed. A graph will show the absolute standardized difference for each of the covariates, comparing larotrectinib and comparator, for both the unweighted and weighted samples. A standardized difference of 10% is commonly considered the threshold below which demonstrates reasonable balance between the two groups. Greater imbalance may be more acceptable for covariates that are weakly prognostic than for covariates that are strongly prognostic (Austin and Stuart 2015). The list of covariates with strong prognostic impact will be refined based on a SLR.

For continuous covariates, side-by-side boxplots comparing the distribution of the covariate between the two groups will be presented for both unweighted and weighted samples. After weighting, the boxplots for larotrectinib and comparator should appear similar.

The distribution of the propensity score will be graphed for both larotrectinib and control groups. Ideally, there will be no extreme propensity scores and good overlap of treatment and control. If there are markedly different propensity score distributions between the two groups, then it may be more challenging to provide valid treatment group comparisons.

9.7.4 Outcome model

Time-to-event variables

The primary endpoint, time to medical treatment failure, and other time-to-event endpoints detailed above will be investigated by plotting Kaplan-Meier survival curves for the original unweighted samples. Superimposed on these survival plots (e.g. with dashed lines) will be adjusted Kaplan-Meier

curves for the two groups that incorporate the IPTW balancing weights. Therefore, the dashed lines will represent the survival curves after balancing the larotrectinib and external cohort databases. Median estimates of survival and survival probabilities at meaningful timepoints determined by the data, e.g. 6 and 12 months, will be displayed using Kaplan-Meier method for both original unweighted and weighted samples. 95% confidence intervals will also be reported.

Statistical tables will report the log-rank test, comparing larotrectinib and comparator groups. Per Xie and Liu, an analogous log-rank test for the weighted sample will also be computed. Additionally, a hazard ratio will be computed using a weighted Cox Proportional Hazards model, regressing survival time on an indicator variable denoting treatment status (larotrectinib/comparator) and incorporating the IPTW weights. To construct the confidence interval of the hazard ratio, the bootstrap-based variance estimator will provide approximately correct estimates of standard errors as it takes into account the fact that the IPTW weights are only estimates from the available data. For each of bootstrap samples (e.g. 200 samples) drawn from the data, weights will be drawn from the propensity score model and the log-hazard ratio will be calculated from the weighted Cox model. The standard deviation of the estimated log-hazard ratios (i.e., the estimated regression coefficient for the treatment status indicator) across the 200 bootstrap samples will be used as the bootstrap estimate of the standard error of the estimated regression coefficient obtained in the original data set. Ninety-five percent confidence intervals of the hazard ratio can be constructed as:

$$\widehat{\boldsymbol{\beta}} \mp \mathbf{1.96} \times \mathrm{SE}\left(\widehat{\boldsymbol{\beta}}\right)$$

where $\widehat{\boldsymbol{\beta}}$ denotes the estimated treatment effect in the original weighted sample, and $\mathrm{SE}\left(\widehat{\boldsymbol{\beta}}\right)$ denotes the estimated standard error of the treatment effect using bootstrapping.

Hazard ratios will also be reported for the unweighted samples.

Response variables

The response endpoints, Overall Response Rate (ORR) and Disease Control Rate (DCR), will be summarized for both groups in both the original and weighted samples.

The binary response outcome is regressed on group indicator (larotrectinib, comparator) using a logistic regression model, incorporating the IPTW weights. Standard errors are calculated using a robust sandwich estimator. The odds ratios comparing larotrectinib to comparator, with 95% confidence interval, will be provided for both ORR and DCR. Odds ratios will also be reported for the unweighted samples.

Incidence of treatment discontinuation due to treatment-related AEs will be summarized descriptively in the original unweighted sampled only.

9.7.5 Sample size and power considerations

The number of patients available is quite small, and no formal hypothesis and power calculations are considered for this study.

We can calculate the power for the comparison of two groups under a Cox Proportional Hazards Model, with the following applicable formula (Rosner 2006):

$$\text{Power} = \Phi\left(\frac{\sqrt{\text{km}}|\text{IRR-1}|}{\text{k} \times \text{IRR+1}} - \text{z}_{1\text{-}\alpha/2}\right)$$

where m = expected total number of events over both groups

$= n_1 p_E + n_2 p_C$

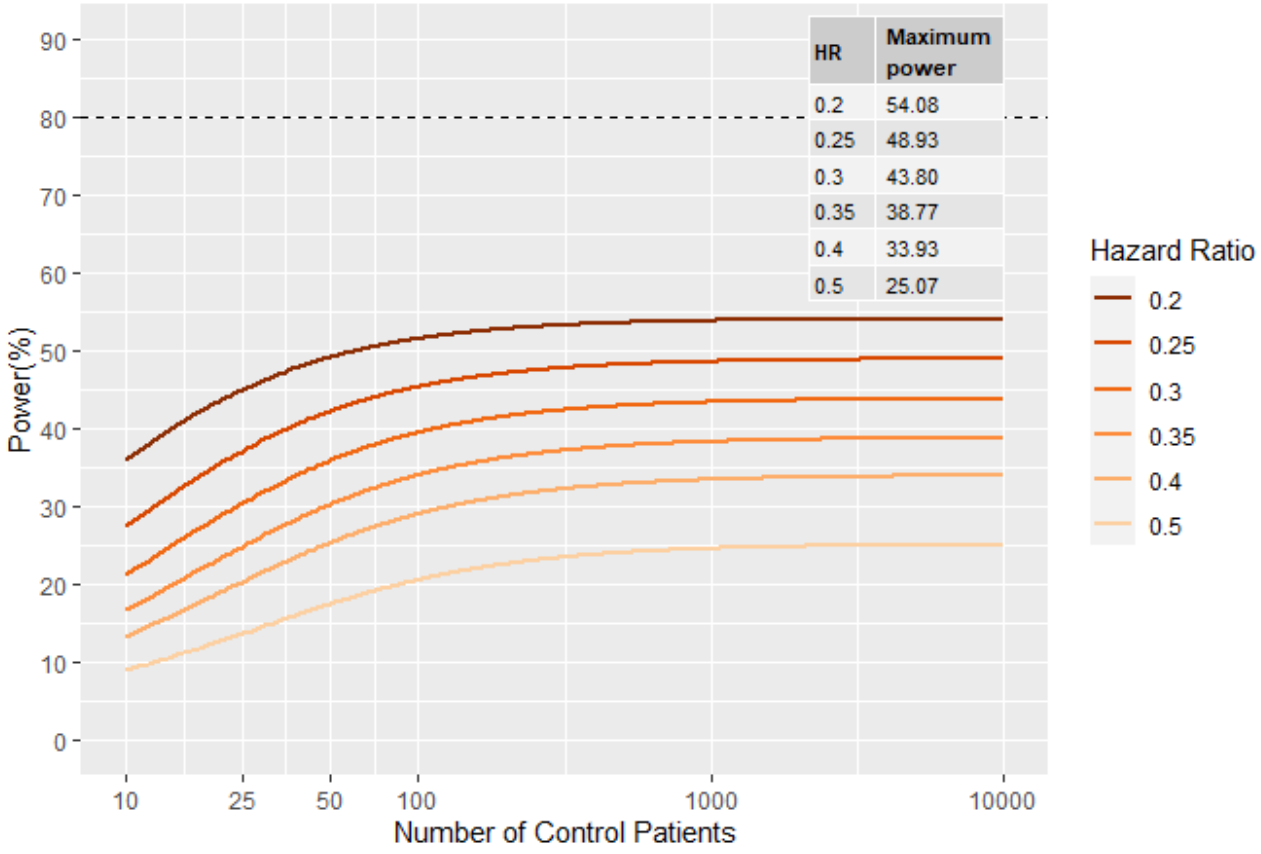
n_1, n_2 *= number of participants in experimental (E) and control (C) groups*

$k = n_1/n_2$ *(ratio of participants in E versus C groups)*

p_C = *probability of failure in group C over time period of study*
p_E = *probability of failure in group E over time period of study*
IRR = underlying hazard ratio
$z_{1-\alpha/2}$ *is the 100(1-α/2)-th percentile of the standard normal distribution*
Φ is the CDF of N(0,1)

We assume 35 IFS subjects and probabilities of failure equal to 0.04 and 0.19 in SCOUT and the external historical control cohort(s), respectively. Even with a strong treatment effect (hazard ratio=0.25), the power can only reach 49%, as further additions to the control cohort lead to smaller increases in power as the two groups become increasingly unbalanced. Of note, an effect size of 0,5 would already be considered as a valuable treatment effect (Fewell 2007). Therefore, having a powered study seems unfeasible.

Figure 1: Representation of the study power in function of the number of patients included in the comparator arm (control) for different hazard ratios



nE=35 (number of patients in the experimental group (treated with larotrectinib)
pE=0.04 (probability of failure in the experimental group during the study)
pC=0.19 (probability of failure in the control group during the study) (Orbach 2020)
RR=0.5 to 0.2 (risk ratio)
α=0.05 (type 1 error)

9.7.6 Sensitivity analysis

As the choice of index date and statistical method may affect the results, the following sensitivity analysis will be conducted in order to explore the impact of methodological choices on the results. A consistency in the findings regarding the treatment effect between the main analysis and the sensitivity

analyses should instil confidence in the primary results. Inconsistencies in the results will be explored through further analysis. Further details will be described in the SAP.

- Choice of index date:

As the majority of larotrectinib patients will be receiving 2nd line therapy, this analysis attempts to maximize consistency between treatment groups in terms of line of therapy while committing to the inclusion of all available patients. For SCOUT patients, the index date will be defined as the start date of larotrectinib, regardless of line of therapy. In the external historical control cohort(s), the index date will represent start of chemotherapy. However, control patients who take a second line of therapy will have their index date set at the start of second line, in order to provide a close comparison with the larotrectinib group prior to any adjustment using IPTW. A caveat of this analysis is that it may fail to capture some clinically relevant events, for example control subjects receiving a second line of treatment.

- Subgroup analysis:

For the patients treated with a second line or more of systemic therapies, the index date will be defined as the second line of treatment in both the treatment and control groups.

This analysis will reflect the current reimbursement scope of larotrectinib, i.e. in patients who are refractory or in relapse.

- Statistical method:

A plot of the distribution of IPTW weights by group will be used to identify very large weights. Trimming at 5% level will be considered, which amounts to dropping the individuals with the most extreme PS values in both the treatment and control groups, as they may lack a match in the other group. Weight truncation will also be explored that reduces any 'large' weight down to a maximum weight. Therefore, in addition to the main analyses, the following sensitivity analyses will also be considered:

a. IPTW (5% trimming)
b. IPTW (truncating large weights)

A consistency in the findings regarding the treatment effect between the main analysis and the sensitivity analyses should instil confidence in the primary results.

Additional details on the analyses performed and table shells will be provided in the SAP available as a stand-alone document.

9.7.7 Complementary analyses

The following complementary analyses will be conducted on the primary, secondary and exploratory endpoints:

- Patients with IFS or other STS with *NTRK* gene fusion.

IFS is a subtype of STS and the most frequent subtype harboring *NTRK* gene fusion. The SCOUT trial included pediatric patients with locally advanced and metastatic STS – other than IFS – harboring an *NTRK* gene fusion, the databases that will be used to constitute the historical external control cohort(s) may also have included such patients. Therefore we propose to analyze IFS patients jointly with other *NTRK* fusion-positive STS. The eligibility criteria for the STS patients for this analysis are detailed below, those for the IFS patients remain unchanged (see Section 9.2.3.2).

- Inclusion criteria:

The inclusion criteria listed below are in line with those of the SCOUT study in terms of patients and disease characteristics:

- Age ≤ 21 years old.
- Locally advanced or metastatic STS.
- Patients with available information on clinical, radiological characteristics of their tumor, therapies administered and outcomes.
- Patients receiving larotrectinib in the SCOUT trial.
- Patients receiving at least chemotherapy-based regimen in the external historical control cohort(s).
- No opposition from the patients and/or representatives for data use.

- Exclusion criteria:
  - Patients treated with TRKi in the external historical control cohort(s).
  - Patients with documented absence of *NTRK* gene fusion.
  - Patients participating in an investigational program with interventions outside of routine clinical practice.

Number of events and censored patients will be reported for all time-to-event endpoints referenced above, and Kaplan-Meier plots of the survival curves will be produced showing median survival and 95% CI, and also survival probabilities at 12 and 24 months.

Frequency tables (n, %) will be produced for discrete endpoints, such as ORR, DCR and incidence rate of treatment discontinuation due to treatment-related AEs.

Additional details on the analyses performed and table shells will be provided in the SAP available as a stand-alone document.

9.7.8 Bias analysis for primary endpoint

A bias analysis will investigate the effect of an unmeasured covariate for the primary endpoint, time to medical treatment failure. Per Austin (2014), pairs of larotrectinib and control subjects will be matched on the logit of the propensity score using a suitable caliper width. We define response as control time-to-event earlier than paired larotrectinib time-to-event, after removing pairs where the earlier time was censored (i.e. indeterminate response). A stratified log-rank test on the matched samples is equivalent to a 2-sided binomial test of the null proportion of responses, $p = 0.5$. If there exists an unmeasured confounding variable that increases the odds of larotrectinib exposure by Γ (e.g. 10%), then we can consider two extreme scenarios where a commensurate decrease or increase in responses occurs after adjusting for this variable in the propensity score model. Therefore the range of the true significance level can be found by repeating the binomial test using $p = \frac{\Gamma}{\Gamma+1}$ and $p = \frac{1}{\Gamma+1}$. In reality, the effect of the unmeasured covariate will lie somewhere between these 2 extremes. The effect of an unmeasured covariate will be reported for a range of values relating to increased odds of exposure. For example, with odds of 5% and 10%, we can calculate the range of true significance levels by applying null proportions: [0.4878, 0.5122] and [0.4762, 0.5238] respectively.

9.8 Quality control

Bayer Consumer Care AG, the sponsor of the SCOUT study, ensured adequate monitoring activities in accordance with applicable regulations and Good Clinical Practices (GCP, guideline for GCP (International Conference on Harmonization [ICH E6 (R2)]). The monitoring activities included: checking and assessing the progress of the study, reviewing study data collected for completeness and accuracy, conducting source document verifications by reviewing each patient's CRF against source documents, identifying any issues and addressing resolutions, recording and reporting protocol deviations not previously reported to the sponsor, and confirming that Serious Adverse Events (SAEs) were properly reported to the sponsor and submitted to Independent Ethics Committee (IEC), as appropriate.

For the *Institut Curie* database, after identification of cases of patients with *NTRK* gene fusion from the genomics platform database, all observations were collected in a paper CRF and were sent to the study sponsor for entry in the database. Data were then sent to the SIREDO service for verification of data entry and inconsistencies were corrected. This process was iterative until clean data were obtained.

All efforts will be made to check completeness, accuracy, plausibility, and validity of the data before transfer of the database to Bayer for analysis. The same approach will be followed for other eligible external historical cohorts.

Bayer HealthCare SAS will ensure that study information used for the analyses of this observational study is handled and stored to allow for accurate reporting, interpretation and verification of that information. The analytical dataset and statistical programs used to generate the data included in the final report will be kept in electronic format and available for audit and inspection.

9.9 Limitations of the research methods

The limitations of the methods of study analyses are mainly associated to:

- The anticipated rarity of events of clinical interest (IFS are diseases with very low incidence) and consequently the lack of statistical power to pre-specify a proper hypothesis due to the small patient population. To avoid further reducing the sample size, the population was broadened to IFS regardless of their refractory or relapsed status, i.e. including treatment-naive patients. The analysis will include patients on both first and second line (or higher) therapy.
    - The primary endpoint analysis is expected to provide a rather conservative estimate of the treatment effect. Indeed, the index date for the patients in the external cohort(s) will be defined as start of chemotherapy which corresponds to first line of treatment, while for SCOUT patients, the index date will be defined as the start date of larotrectinib which corresponds to a second line of treatment for about 70% of SCOUT patients.
    - To mitigate the uncertainty induced by lack of statistical power and potentially by methodological choices such as index date or statistical model, various sensitivity analyses will be considered and consistency in the findings regarding the treatment effect between the main analysis and the sensitivity analyses should instil confidence in the primary results.
- The historical comparison with an external control arm.
  As described by Cucherat et al. (Cucherat, 2020), the comparison of results of single-arm (not randomized, not controlled) studies with an external control has potential limitations due to:
    - The *post-hoc* choice of the comparator:

- In our study, a systematic approach (SLR) will be used to ensure that the choice of the comparator reflects clinical practice and is not arbitrary or biased (see Section 9.2.3.1).
- Selection criteria were defined to avoid notably the risk of selection bias (cohorts with prospective enrollment and with retrospective and prospective data collection from 2000 to present, see details in Section 9.2.3.1).
- The choice of confounding variables will be validated by a SLR that will document the important prognostic factors in this patient population.

- The measurement bias inherent to the comparison of data from two independent studies with different settings. Notably, the SCOUT study is a very well monitored clinical trial while the external historical control cohort(s) might regroup real word data without any pre-established protocol, high regulated quality monitoring or centralized protocol of reading. In addition, results of the analyses might be biased since patients who received larotrectinib in the SCOUT trial likely differ in various underlying characteristics from those who are recorded in the external historical control cohort(s). During data analyses, statistical adjustment methods will be taken to limit bias, notably taking into account the potential cofounding variables. Propensity score methods will be employed to reduce the effects of measured confounding variables in the interpretation of the treatment effect (larotrectinib versus control group). The IPTW method makes it possible to reduce bias by correcting each patient's contribution by a weight equal to the reciprocal of the probability of receiving the treatment that was actually received (see Section 9.7.2 and Section 9.7.3). Our expectation is that there will not be large imbalance as the data for this study did not include a time when both treatments were available, which may have led to investigator bias towards one or the other treatment based on certain patient characteristics. Finally, a bias analysis will investigate, for the primary endpoint, the likelihood that a potential residual confounding bias due to an unmeasured covariate may have affected the result.

9.10 Other aspects

N/A.

10. Protection of human patients

This study is classified as "a research non-involving human beings in the health domain". These studies do not actively involved human beings, and therefore do not come under the Jardé Law but under the data protection law (chapter IX).

Since the processing of data does not comply with the MR-004 methodology (Deliberation n° 2018-155 of 3 May 2018), Bayer will submit an application dossier to the HDH ensuring a role of single secretariat. Dossier will be transmitted for opinion to the CESREES (dedicated ethical and scientific committee in charge of ruling on the public interest character of researches and studies in the health field), and then CNIL will be consulted for its authorization.

Bayer will seek an exemption from individual patient information from the CNIL. Indeed, given the international character of the SCOUT study and the different external cohorts that will be explored, the transmission of a specific information note to the patients would require an additional submission to IECs or Institutional Review Boards (IRBs) of the different locations where patients were recruited

prior to study start, and therefore could jeopardize the conduct of the study within the timeframe agreed with authorities.

However, it should be noted that patients enrolled in SCOUT where informed via the information sheet and the consent form which they signed that their data could be processed for use in future medical or pharmaceutical researches. Additional measures will be carried out by Bayer to protect the rights of the patients, by making the information on the study EPI VITRAKVI publicly available on Bayer Trial Finder page: clinicaltrials.bayer.com.

The person(s) responsible of the data processing will collect only the data relevant, appropriate and limited to the objectives of the study. The necessity of the data processing must be scientifically justified in the study protocol. The personal data information and its processing will be respected as described in the GDPR (EU 2016/679 of 27 April 2016).

11. Management and reporting of adverse events/adverse reactions

This study is a retrospective observational study using secondary data collection from a previous clinical trial (SCOUT study) and from eligible database(s) that will be used as external historical control cohort(s). Therefore, no new AEs or Adverse Drug Reactions (ADRs) are expected to be reported besides the ones already described during the conduct of the initial clinical trial.

As per the EMA Guideline on Good Pharmacovigilance Practices (Module VI – Collection, management and submission of reports of suspected adverse reactions to medicinal products [Revision 2]), individual reporting of adverse reactions is not required for non-interventional study designs that are based on secondary use of data. Reports of adverse events/reactions will be summarized in the study report (EMA 2017).

12. Plans for disseminating and communicating study results

A study report will be written and transmitted to the applicable competent authorities.

The results of this observational study are intended to be published in a peer-reviewed journal and possibly as abstracts/presentations at medical congresses under the oversight of the Market Authorization Holder (MAH). Current guidelines and recommendation on good publication practice will be followed (e.g. Good Publication Practice [GPP] 2 Guidelines [Graf 2009], The Strengthening the Reporting of Observational Studies in Epidemiology [STROBE] statement [von Elm 2008]).

Any written or oral communication of the results of the study will have to be agreed by the study initiator and funder, and, if applicable, by the Scientific Committee who participated to the observational study.

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Annex 1: List of stand-alone documents

Table 2: List of stand-alone documents

Document Name	Final version and date (if available)*
Contact details of the Principal Investigator, members of the Scientific Committee and CROs	v 1.0, 11 APR 2021
SAP	tbd
SLR	tbd

* Draft versions are indicated by <draft> in brackets and date. “tbd” indicates documents that are not available at the time of protocol creation, but will be issued at a later stage.

Annex 2: Additional information

Table 3: Long term gonadal toxicity of cyclophosphamide

Study	**Ridola (2009)**				**Kenney (2001)**			**Aubier (1989)**		
Patients number	59				17			15		
Diagnosis	Soft tissue sarcoma (n=11) Osteosarcoma (n=12) Ewing (n=5) Non hodgkin lymphoma (n=28) Other (n=3)				Rhabdomyosarcoma (n=9) Ewing (n=4) Soft tissue sarcoma (n=4)			Lymphoma (n=9) Rhabdomyosarcoma (n=3) Ewing (n=1) Pheochromocytoma (n=1) Wilm's tumor (n=1)		
Age at diagnosis	9.8 y [0-17.6]*				12 y [4-19]*			9 y [2-15]*		
Follow up intervals	8.5 y [5-16.5]*				12 y [5-22]*			9 y [1-20]*		
Cyclophosphamide cumulative doses	8.3 g/m^2 [4.6-22]*	< 9 n=28	9-11,9 n=15	> 12 n=16	20.5 g/m^2 [4.7-31.9]*	< 7.5 g/m^2 n=2	> 7.5 g/m^2 n=15	12 g/m^2 [2,6-29]*	< 9 n=2	> 9 n=13
Sperm Count Nl Oligospermia Azoospermia	Not in the scope of the study				2 5 10 (58.8%)	2 0 0	0 5 10	Gonadal function Nl Abnormal	2 0	2 11
FSH Abnormal Normal	47.4%	21.4%	53.3%	87.5%	10 5	NA 1	10 4	Not in the scope of the study		
LH Abnormal Normal	Not in the scope of the study	Not in the scope of the study	Not in the scope of the study	Not in the scope of the study	6 9	N/A 1	6 8	Not in the scope of the study		
TESTIS V Abnormal Normal	Not in the scope of the study				5 10	N/A N/A	5 10	5 5 N/A 5	0 2	5 3

* Data expressed as medians [range], Nl: normal, N/A: not available.

Table 4: Cardiotoxicities associated with anthracyclines

Study	**Ramjaun (2015)**	**Kremer (2001)**		**McCune (2019)**
Patient number	333	Total = 607	A-CHF = 17 (2.8%)	Tot: 68 Echocardiographic systolic disfunction: 15 (22%)
		Male 345 Female 262	9 (2.6%) 8(3.1%)	
Age at diagnosis	Median 8.47 1-4 y: 32.73% (n=109) > 5 y: 67.27% (n=224)	< 2 y 43 2-4 y 113 5-9 y 174 10-14 y 194 > 14 y 83	1 2.3% 2 1.8% 5 2.9% 6 3.1% 3 3.6% Cum Inc 2.8%	8.6 y [1-17]*
Time since end of treatment	15.8 y [0.9-48]*	Mean 6.3 y (0.01-21.74)		12 y [1-31]*
Doses mg/m^2	Median 212.9	< 150 70 150-300 208 300-450 183 450-600 108 > 600 20 Unknown 18 MCD 301 (14-960)	0 0% 1 0.5% 5 2.7% 7 6.5% 3 15% 1 5.6% 461 (225-803)	n=38 (55.9%): dose > 250mg/m^2 n=14 (20.6%): chest radiation n=3 (4.4%): cardioprotective agent
	Echocardiographic abnormalities Overall: 14.7% at 11.7 y 20% at 20 y	Estimated risk of A-CHF Function of follow-up time		Subclinical disfunction: Global Strain impairment+: 42/52 (80.7%)
	Probability of abnormal echo: By age < 5 / > 5; By dose < 250 / > 250 At 10 y (%): 13.6 / 10.9 / 8.3 / 23.3 At 20 y (%): 26.7 / 14.5 / 19.5 / 36.9	2y 2% 5y 2.8% 10y 3.3% 15y 4.8%		
	20 years rates abnormal echo: > 5 years / < 5 years < 250 mg/m^2: 11% / 18% > 250 mg/m^2: 29% / 49%	Function of cumulative doses 300 mg/m^2 450 mg/m^2 600 mg/m^2	1.1% 4.5% 17.8%	

* Data expressed as medians [range], A-CHF: congestive heart failure, Cum Inc: cumulative incidence, MCD: mean cumulative dose, CHF: congestive heart failure, + Global strain impairment was identified as an independent marker of future left ventricular systolic impairment.

Table 5: Toxicities associated with radiotherapy

	Acute toxicity	Late toxicity		
		SMN	Cardiac	Lung
Selo (2010) Patients number (n=690) Salivary glands (n=78) Lower GI tract (n=153) Genito-urinary (n=45) Liver (n=61) Lung (n=120) Skin (n=632)	 Total toxicities: 73% (506) 22% (17) [3.8% (3)]* 53.3% (81) [15.1% (23)]* 22% (10) [8.9%(4)]* 13% (12) [3.2%(2)]* 17% (20) [4.2%(5)]* 93% (589) [16.5% (104)]*	Not in the scope of the study		
Paulino (2005) n=429 (multiple tumors) Median follow-up: 9.6 y	Not in the scope of the study	23 SN (5.4%) 14 SMN (3%): 10 in RT field (71%) 189 dead at time of analyses: 3 (1.6%) due to RIN and 184 (97.4%) from original solid tumor	Not in the scope of the study	Not in the scope of the study
Gold (2003) n=446 (multiple tumors) Follow-up: 19.5 y [4.8-40]*	Not in the scope of the study	n=37 (8.3%) In RT field: 31 (70%) 12 died: 10 from SMN Overall standardized incidence ratio: 5.2 Cumulative probability of developing a SMN at 30 years: 13%	Not in the scope of the study	Not in the scope of the study
Mertens (2002) n=12390 (multiple tumors)/siblings cohort n=3546	Not in the scope of the study	Not in the scope of the study	Not in the scope of the study	RR Lung fibrosis 4.3[c] Abnormal chest wall 5.0[c] Pneumonia > 3 times in 2 y 2.2[c] Chronic cough, SOB > 1 month 2[c] Need supplement oxygen 1.8[c] Other respiratory problems 2.1[c]
Weiner (2006) n=30 (multiple tumors) Follow-up: 2.79 y [0-13.7]*	Not in the scope of the study	Not in the scope of the study	Not in the scope of the study	50% (15) abnormal PFT Mild, moderate and severe complications in 30%, 10% and 10% of patients
Bolling (2008) n=37 (Ewing's tumors) Follow-up: 2.1 [0-12.6]*	Not in the scope of the study	Not in the scope of the study	Not in the scope of the study	57% (21) abnormal PFT Mild, moderate, and severe pulmonary complications in 29%, 21% and 7%
Adams (2004) n=48 (Hodgkin's Disease)	Not in the scope of the study	Not in the scope of the study	100% (47) abnormal screening 42%: pathologic valvular defect	Not in the scope of the study

(4 patients had anthracyclines) Follow-up: 14.3 [5.9-27.5]*			75%: conduction defects 53%: abnormalities in stress test, decrease mean LV mass (restrictive cardiomyopathy)	
Schellong (2010) n=1132 (Hodgkin's Disease) (834 had RT / 298 no RT) Follow-up: 15.1 [3.1-29.4]*	Not in the scope of the study	Not in the scope of the study	CD Cum Inc at 20 y 36 Gy: 10.9% 21% 30 Gy: 3.7% 10% 25 Gy: 3.2% 6.4% 20 Gy: 0.6% 4.9% 0 Gy: 0.3% 3.2%	Not in the scope of the study

* Moderate to severe toxicity, ᶜ P value < 0,01, RR: rate ratio, SOB: shortness of breath, GI: gastro-intestinal, SN: secondary neoplasms, RIN: radiation induced neoplasm, SMN: secondary malignant neoplasms, RT: radiotherapy, PFT: pulmonary function test, ECG: electrocardiography, LV: left ventricle, CD: cardiac disease, Cum Inc: cumulative incidence.

Annex 3: Signature pages

Signature Page – PPD

Title	EPI VITRAKVI: A comparison of clinical outcomes in Infantile Fibrosarcoma (IFS) patients treated with larotrectinib in the phase I/II SCOUT study *versus* (an) external historical cohort(s)
Protocol version and date	v 1.0, 27 JUL 2021
IMPACT study number	21767
Study type / Study phase	Retrospective, observational, externally-controlled study / Phase IV
Medicinal product / Active substance	Larotrectinib (BAY 2757556)
Comparator / Reference therapy	Standard of care (external historical control)
Study Initiator and Funder	Bayer Healthcare SAS

The undersigned confirms that he agrees that the study will be conducted under the conditions described in the protocol.

Print Name: PPD

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Signature Page – PPD

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Last updated: April 08, 2020.