SAPIENZA
UNIVERSITÀ DI ROMA

Site- and histology-independent indications of medicinal products in oncology

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Summary

List of abbreviations

ADR: adverse drug reaction

AE: adverse event

AIFA: Agenzia Italiana del Farmaco (Italian Medicines Agency)

ALK: anaplastic lymphoma kinase

ALT: alanine aminotransferase

APC: antigen-presenting cells

AST: aspartat aminotransferase

ATP: adenosine triphosphate

BDNF: brain-derived growth factor

BICR: blinded independent central review

BRAF: Raf Murine Sarcoma Viral Oncogene Homolog B

BCRP: breast cancer resistance protein

CDF: Cancer Drug Fund

CDx: companion diagnostics

CHF: congestive heart failure

CHMP: Committee for Medicinal Products for Human Use

CI: confidence interval

CMA: conditional marketing authorization

Cmax: maximum concentration

CNS: central nervous system

CPR: Comitato Prezzi e Rimborso (Price and Reimbursement Committee)

CR: complete response

CRC: colorectal cancer

CTS: Commissione Tecnico Scientifica (Scientific Technical advisory Committee)

CUP: cancer of unknown primary

CYP: cytochrome P450

dMMR: mismatch repair deficient

DNA: deoxyribonucleic acid

DOR: duration of response

EC: European Commission

EEA: European Economic Area

EGFR: epidermal growth factor receptor

EMA: European Medicines Agency

EPAR: European Public Assessment Report

ERBB2: human epidermal growth factor receptor 2

ERK: extracellular signal-regulated kinase

ESCAT: ESMO Scale of Clinical Actionability of molecular Targets

ESMO: European Society of Medical Oncology

ETV6: translocation-Ets-leukemia virus

EU: European Union

FFPE: formalin-fixed paraffin-embedded

FISH: fluorescence in situ hybridisation

FDA: Food and Drug Administration

GDNF: glial cell line-derived neurotrophic factor

GEJ: gastro-esophageal junction

GIST: gastrointestinal stromal tumor

HTA: health technology assessment

IC50: inhibitory concentration 50

IC: Iimmune-checkpoint inhibitor

IFS: infantile fibrosarcoma

Ig: immunoglobulin

IHC: immunohistochemistry

IRC: Independent Review Committee

IVD: *in vitro* diagnostic

IVDR: In Vitro Diagnostic Regulation

MA: marketing authorization

MAPK: mitogen-activated protein kinase

MASC: mammary analogue secretory carcinoma

Mb: megabase

MEK: mitogen-activated protein kinase

MET: mesenchymal-epithelial transition factor

MSI: microsatellite instability

MSI-H: microsatellite instability-high

MTC: medullary thyroid cancer

NGF: nerve growth factor

NGS: next generation sequencing

NHS: national health system

NICE: National Institute for Health and Care Excellence

NSCLC: non-small cell lung cancer

NT: neurotrophin

NTRK: neurotrophic tropomyosin-receptor kinase

OATP: organic anion transporting polypeptides

ORR: overall response rate

OS: overall survival

P-gp: permeability glycoprotein

PD: progressive disease

PD-1: programmed cell death-1

PD-L1: programmed death-ligand 1

PFS: progression-free survival

PR: partial response

PRIME: Priority Medicine

RCT: randomised clinical trial

RECIST: Response evaluation criteria in solid tumors

RET: rearranged during transfection

RMP: Risk Management Plan

RNA: ribonucleic acid

ROS1: ROS proto-oncogene 1

RT-PCR: reverse transcriptase polymerase chain reaction

SAG: Scientific Advisory Group

SAE: serious adverse event

SBC: secretory breast cancer

SmPC: Summary of Product Characteristics

SOB: specific obligation

SSN: sistema sanitario nazionale (national health system)

STR: short tandem repeats

TEAE: treatment emergent adverse event

Tmax: time-to-maximum plasma concentration

TMB: tumor mutational burden

TME: tumor microenvironment

TRK: tropomyosin receptor kinases

UGT: UDP-glucuronosyltransferase

UK: United Kingdom

US: United States

WES: Whole Exome Sequencing

Introduction

The development of drugs approved for a site and histology-independent indication is based on the identification of biological drivers that define cancer course across anatomical sites and histologies. Therefore, the selection of patients responsive to a specific drug occurs by identify a specific molecular alteration in the tumor, and not based on the site and/or histology of the tumour, the latter being the common way to develop the anticancer treatments so far. When cancer cells harbour a specific molecular alteration which is believed acting as an oncogenic driver, it is anticipated that molecularly targeted treatments would be effective against a spectrum of biomarker-defined tumour types, rather than being restricted to the site of tumour origin. This revolutionary approach to drug development has thus led to a paradigm shift in some cancer therapies: from drug indicated for a tumor originating in a specific part of the body (e.g. lung, breast, colon etc.) to drugs indicated for a multitude of tumour types as long as they express a specific driver mutation. However, such new type of indication is challenging the existing diagnostic, drug development, regulatory and reimbursement frameworks worldwide.

This thesis is meant to describe the state of art of drugs approved based on a site and histology independent indication, which have been granted in the European Union (EU) and in the United States (US) so far.

The primary focus will be to review and discuss the processes that led to the clinical development, approval, national market entry, and post-marketing monitoring of the first two medicinal products which were granted a site and histology independent indication in the European Union, namely the NTRK inhibitors larotrectinib and entrectinib.

Additional drugs received site and histology independent indications in the US, including immune-checkpoint inhibitors. The differences in the regulatory approach between the two health authorities, the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) related to the assessment of site and histology-independent indication for anti-PD1 drugs in microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR) solid tumors will be analysed, as well as the FDA indication granted to pembrolizumab in solid tumors based on Tumor Mutational Burden (TMB). The peculiarity of such molecular alterations, together with the controversies around those approvals, will be discussed.

Finally, the situations where an indication of a targeted therapy, initially approved from one tumor type, was then extended to a site and histology-independent one will be presented, with reference to the FDA approvals of the combination dabrafenib plus trametinib for BRAF V600E positive solid tumors, and selpercatinib in solid tumors with RET gene fusion.

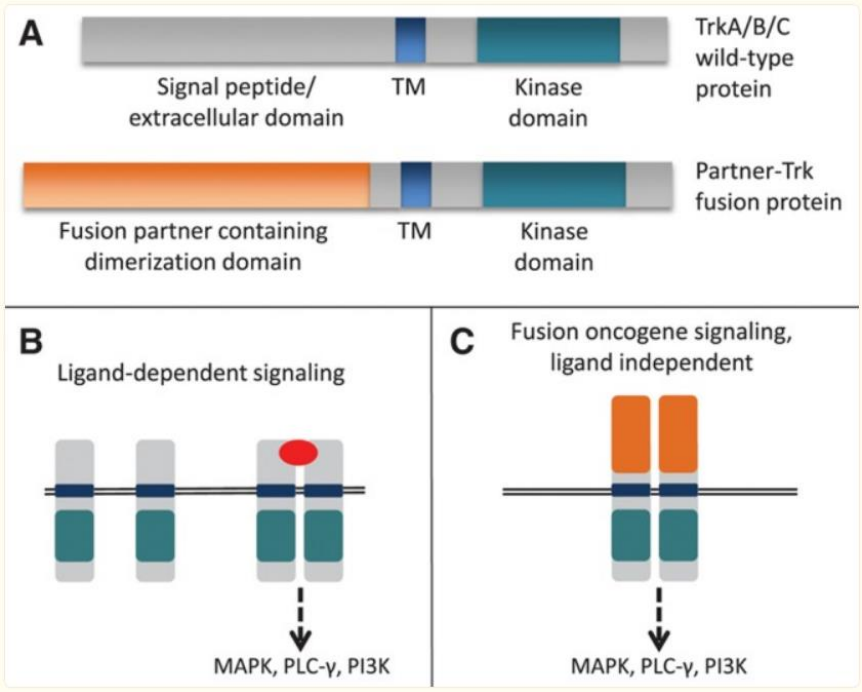
The first site and histology independent approvals in EU: the NTRK inhibitors

NTRK genes and gene fusions

The neurotrophic receptor tyrosine kinase genes NTRK1, NTRK2, and NTRK3 are a family of genes that encode the tropomyosin receptor kinases A, B and C (TRKA, TRKB and TRKC), respectively. Those are transmembrane tyrosine kinases receptors responsible for neuronal development which are expressed in neuronal tissue, acting as high affinity signal transducing receptors for neurotrophins. Specifically, TRKA binds nerve growth factor (NGF), TRKB binds brain-derived growth factor (BDNF) and neurotrophin-4 (NT4, also called NTF5) with high affinity and neurotrophin-3 (NT3) with lower affinity, finally TRKC binds NT3. After a neurotrophin binds its specific TRK receptor, homodimerization of receptors occurs, followed by autophosphorylation and activation of downstream intracellular signalling pathways that transduce the signals within the cell. Transduction pathways activated by TRK receptors include C-gamma phospholipase, mitogen activated protein kinase and phosphatidylinositol 3 kinase. TRK signalling is involved in cell proliferation, apoptosis, and survival of neurons and other cell types. In normal tissue, such signalling pathway has an essential physiological role in development and function of central and peripheral nervous systems. [1] [2] [3]

NTRK 1, 2 and 3 genes are located in chromosomes 1, 9 and 15, respectively. Molecular alterations involving NTRK genes can induce carcinogenesis both in neurogenic and non-neurogenic cells. Indeed, NTRK gene was identified as an oncogene initially around 1980 in a human colorectal carcinoma. [4] [5] In particular, the molecular alteration relevant in tumors is represented by NTRK gene fusions, arising from intra- or inter-chromosomal rearrangements that juxtapose the 3' NTRK gene sequences encoding the catalytic tyrosine kinase domain in-frame with various 5' partner gene sequences. [6] The gene fusion lead to the synthesis of a chimeric TRK protein which has a constitutive ligand-independent kinase activation. Such chimeric protein showed to be oncogenic, as can promote tumorigenesis by leading to tumour cell proliferation, differentiation, and/or apoptosis.

Figure: TRK signalling (figure from [7])



The number of NTRK gene fusions and fusion partners identified has progressively increased. Westphalen et al [8] in 2021 analised the prevalence of NTRK gene fusions from the FoundationCORE® database (Foundation Medicine Inc., Cambridge, MA, USA) including almost 296,000 cancer patients, representing to date one of the largest studies on NTRK rearrangements in solid tumors. A total of 88 fusion partner pairs were identified in this study. The most common gene fusion found is ETV6-NTRK3. ETV6 gene on chromosome 12 encodes an ETS family transcription factor, and it is known to be involved in a large number of chromosomal rearrangements. ETV6-NTRK3 fusion is indeed also the most common gene rearrangement observed in the pivotal studies of approved NTRK inhibitors. [9] [10] Second and third most common gene fusions are TPM3-NTRK1 and LMNA-NTRK1, respectively.

NTRK mutations have been found rarely coexisting with mutations such as KRAS, APC, TP53, and PIK3CA, although this may occur. [10] [11] In particular, NTRK gene fusions appeared to be mutually exclusive with the most common tumor's driver mutations (EGFR, ERBB2, RET, ALK, MET). [8]

Epidemiology of NTRK fusion positive solid tumors

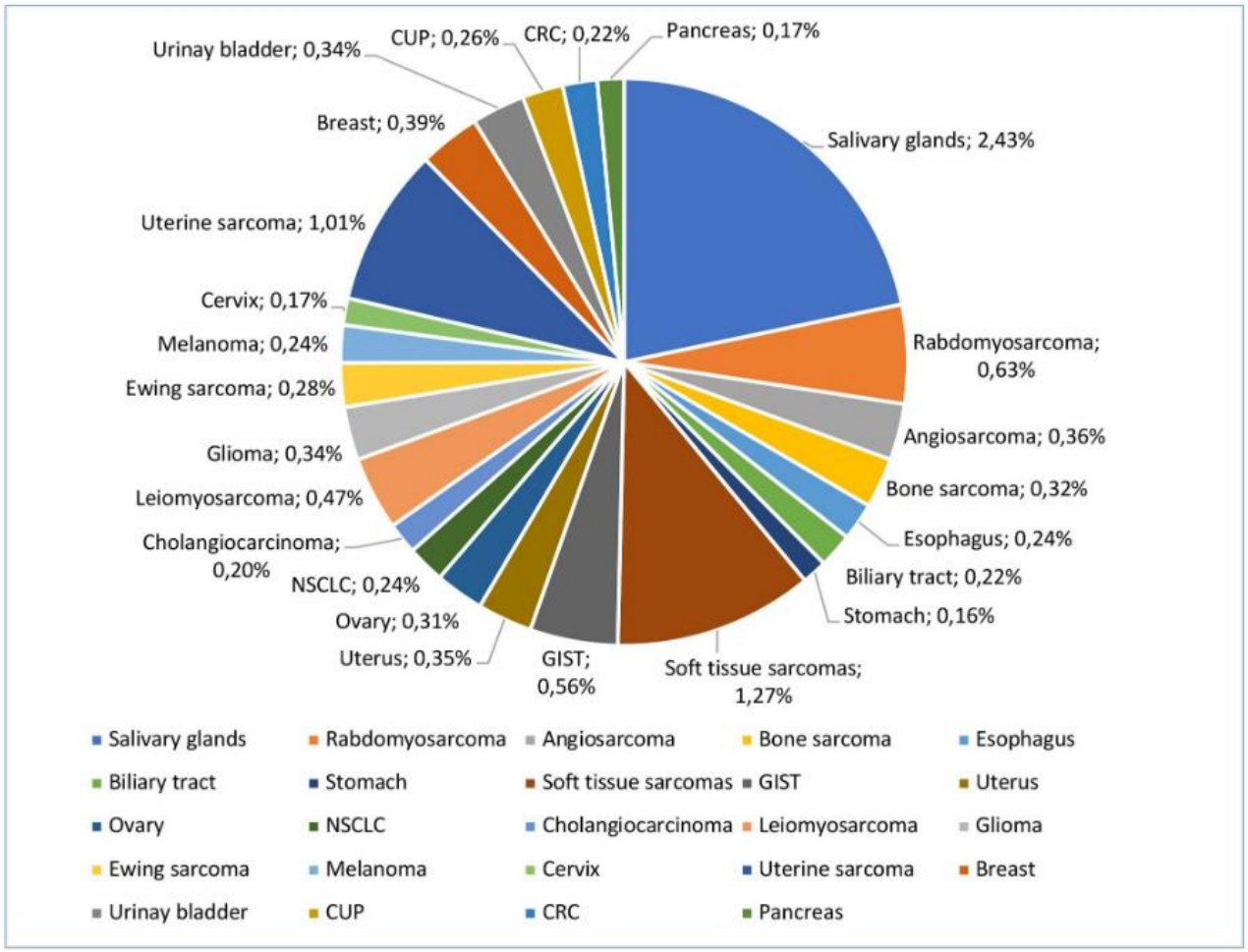
The overall prevalence of NTRK fusions in all cancer patients has been estimated to be around 0.3%. [8] [12] [13] In more details, those gene rearrangements have a particular distribution: NTRK fusions are extremely rare events in common cancers, while they can present at high frequency in some very rare tumors.

In adults, frequency of NTRK fusions in very common cancers, such as lung or colorectal cancer, is very low, usually <1%. On the contrary, NTRK fusions are observed with a frequency up to 90-100% in very rare diseases such as mammary analogue secretory carcinoma (MASC), a rare form of salivary gland cancer, and secretory breast cancer (SBC), where ETV6-NTRK3 fusion is a pathognomonic hallmark for both diseases.

With regard to children, NTRK fusions have been described in several tumours in the paediatric age. ETV6-NTRK3 fusion is a characteristic feature of infantile fibrosarcoma (IFS) as well as in congenital mesoblastic nephroma. NTRK fusion have also been observed with high frequency (i.e. about 40%) in high grade glioma in children. [2] [12] [14]

A recent literature review summarise the available large-scale molecular analyses conducted to understand prevalence, distribution, and genomic context of NTRK fusions in different tumor forms. [15]

Figure: Prevalence of NTRK gene fusions in tumors in the adult population (data obtained from Westphalen et al, Rosen et al, Solomon et al.) (figure from [15])



Abbreviation: CRC, colorectal cancer; CUP, cancer of unknown primary; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer.

Little is known about the overall prognostic relevance of NTRK gene fusion. Some information regarding the natural history and prognosis of rare tumor types characterized by high prevalence of NTRK alteration can be found in literature. For example, secretory breast cancer is a very rare type of breast cancer, generally associated with a favourable prognosis, although having triple-negative phenotype. [16] Mammary Analogue Secretory Carcinoma (MASC) is a rare salivary gland malignancy: while usually low grade, high-grade transformation of MASC has been described, thus aggressive salvage surgery is recommended, with modest response rate to various chemotherapy regimens. [17] Congenital Infantile fibrosarcoma is a rare mesenchymal tumour primarily developing in the soft tissue of distal extremities, accounting for 10% of STS in children, and usually

occurring in the first year of life. Surgery is the treatment of choice for the majority of cases where IFS remains localised, and it is associated with good prognosis. However, complete non-mutilating resection is rarely feasible, and chemotherapy in the neoadjuvant setting can reduce tumor size allowing for conservative surgery. Despite good initial control, the clinical course can be aggressive for some patients with local recurrences and metastatic spread requiring multiple additional surgeries and adjuvant chemotherapy or radiotherapy. [18]

Methods for the detection of NTRK fusions

Several methods can be used to identify NTRK fusions in solid tumors. Those are pan-TRK immunohistochemistry (IHC), fluorescence in situ hybridisation (FISH), reverse transcriptase polymerase chain reaction (RT-PCR), and sequencing methods (next generation sequencing, NGS). Each method has its advantages and disadvantages. In addition, various testing algorithms have been conceived and described in literature to be applied in the real-world setting, and recommendation have been also incorporated in international guidelines.

Immunohistochemistry (IHC) aims to examine the TRK protein expression on cells. The three TRK proteins, A, B and C, can be simultaneously identified by using a pan-TRK antibody. The most frequently used clone is EPR17341. [19] Positive staining to NTRK is defined as staining in at least 1% of tumour cells above the background. [11] In most of the cases NTRK-fusion positive tumors are characterised by cytoplasmic staining. However, in addition to cytoplasmic immunoreactivity, different gene fusion partners may change such appearance, leading to different subcellular staining patterns, with some specificity. In contrast to the native TRK expressed on the cell membrane, the fusion partner can in fact direct the fusion protein to localise to other cellular compartments. Indeed, nuclear staining can be observed in case of ETV6 fusion partner, perinuclear/membrane staining is detected if NTRK gene is fused with e.g. LMNA, while partners like TPM or TPR displayed membranous immunostaining. [20]

In order to minimize the risk of false-negative, a positive control must be included in the slides, such as appendix. As discussed above, TRK proteins are physiologically expressed in neuronal tissue, thus peripheral nerves can act as *in situ* positive controls. [19] Further, due to the physiologic cytoplasmic expression of pan-TRK, tumors such as gastrointestinal stromal tumors (GIST), neuroblastomas, leiomyosarcomas, or glioblastomas should not be screened with IHC. [21]

IHC presents several advantages: it is widely available and usually routinely used in local laboratories, it is affordable due to its low cost as compared to other methods, it requires limited amount of tumor materials, and has a fast turn-around time. Further, IHC has high sensitivity: for NTRK1 and NTRK2 fusions, sensitivity has been estimated to be approximately 96% and 100%, respectively. However, recent studies showed reduced sensitivity for NTRK3 fusions, being around 79%. This could be related to an over-representation of ETV6-NTRK3 fusion, due to heterogeneity in its nuclear staining pattern. [11] [19] [22] Therefore, predictive value of pan-TRK may be related to NTRK fusion partner as well as tumor type. The main con of IHC is specificity, as pan-TRK IHC is not entirely specific for NTRK fusions.

Fluorescence in situ hybridization (FISH) is a commonly used method for detecting chromosomal rearrangements in solid tumors. To investigate for the presence of NTRK gene fusions, either fusion or break-apart probes can be used. Scoring is also made according to what is generally accepted for detection of other gene fusions by FISH. FISH can be used to identify ETV6–NTRK3 gene fusion in tumor types where this alteration is typically observed (e.g. infantile fibrosarcoma, secretory carcinoma). Low amount of material and short turnaround time are some of the advantages of FISH method. However, fusions can involve any of the NTRK protein as well as a high number of fusion partners through translocation of deletions, in such a case break-apart probes should be used. Therefore, while this is a good and useful technology to detect and/or confirm highly recurrent known fusion genes, the utility of FISH as a screening method for NTRK1/2/3 fusions is limited. [22] [23]

Reverse transcriptase polymerase chain reaction (RT-PCR) is a method that can identify transcribed RNA. This technique is useful to detect qualitatively and quantitatively the presence of a single fusion oncogene when both the NTRK gene and the fusion partner are known. The value of RT-PCR is therefore usually limited to and mainly used to detect the more common ETV6-NTRK3 fusion. [22]

Next-generation sequencing (NGS) is a technology for determining the sequence of the entire genome or of targeted regions of DNA or RNA. This massive parallel sequencing method has revolutionised the biological science. The analysis of the DNA allows to detect molecular rearrangements, while the analysis of RNA evaluate the presence of RNA-level fusion transcripts. Some recent platforms are able to analyse both DNA and RNA at the same time. For the detection of NTRK fusions, both DNA- and RNA-based NGS can be used. Tumor DNA is extracted from tissue fixed in formalin-paraffin and subsequently sequenced with NGS. DNA-based NGS can detect gene

rearrangements and predicted fusions. However, one of the limits of DNA analysis is when there are very long introns to be covered, which may decrease the panel coverage of other introns and the overall sensitivity of the technique. Also, poor mapping quality can be due to some specific alterations. As such, especially fusions involving NTRK3 with large intronic region can be too long to be accurately sequenced by DNA-based NGS. Further, if a new structural variant is identified on DNA, it should be determined whether this result in a functional expressed fusion. This is possible by analysing the RNA. Contrary to DNA sequencing, next-generation RNA sequencing eliminates the limitations of the coverage of introns, as those are DNA sequences that are eliminated by splicing thus not transcribed into RNA. Gene fusions are often highly expressed in the tissues, nevertheless RNA can be degraded and fragmented more easily, and quality control of the available tumor sample is really important, especially with old samples.

The advantage of NGS is that it allows a comprehensive analysis of NTRK fusions, with high sensitivity and specificity. However, main cons of this technique are represented by high cost, long turnaround time, requirement of relevant amount of material. In addition, as discussed above DNA-based NGS can show reduced sensitivity in some cases, while for RNA-based NGS the failure rate of this method should be considered. Therefore, in case an only DNA or only RNA-based NGS is negative, new test with different technique should be considered. [22] [23]

NTRK testing algorithms

While testing for molecular alterations usually depend on availability of various testing modalities and economic considerations for each individual laboratory, several authors and scientific societies started to elaborate testing algorithms to be implemented in the real-world setting. Larotrectinib and entrectinib can be classified as Tier 1C treatments, i.e. considered standard of care based on evidence from basket trials, according to the ESCAT [European Society of Medical Oncology ESMO) Scale of Clinical Actionability of molecular Targets]. [24] ESMO guidelines published in 2019 included a general algorithm for NTRK gene fusion testing to identify patients who would benefit from therapies targeting TRK fusion proteins. According to this guideline, where the presence of an NTRK gene fusion needs to be confirmed in patients affected by tumours in which NTRK gene fusion are highly prevalent/pathognomonic, any test may be used, considering FISH, RT-PCR or RNA-based targeted panels the best confirmatory techniques. In patients whose tumors have low prevalence of NTRK fusion, NGS targeted panel (DNA- or better RNA-based) would be ideal for screening, then,

if an NTRK gene fusion is identified, IHC can be used to confirm protein expression. Alternatively, a two-step approach with IHC first and confirmation of any positivity detected with IHC by NGS, could be considered. [23] [25]

Alternative algorithms for NTRK gene fusions testing have been proposed by other authors, starting from classifying the tumor based on the incidence of NTRK gene fusion (high or low), based then on strengths and availability of different diagnostic techniques. [26]

NTRK inhibitors: EU regulatory assessment

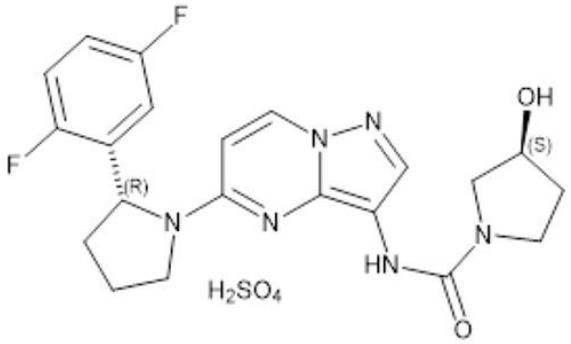
Vitrakvi (larotrectinib)

The applicant (Bayer AG) submitted on 24th August 2018 an application to obtain marketing authorisation (MA) to the European Medicines Agency (EMA) for Vitrakvi (INN larotrectinib) through the centralised procedure (Regulation (EC) No 726/2004). On 25 July 2019, the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion recommending granting of a conditional marketing authorisation (CMA) for larotrectinib for the treatment of patients with solid tumours with a NTRK gene fusion.

Pharmacological aspects

Larotrectinib is an orally bioavailable targeted agent which acts as an adenosine triphosphate (ATP) competitive, selective tropomyosin receptor kinase (TRK) inhibitor. The target for larotrectinib is the TRK proteins family TRKA, TRKB and TRKC. In a broad panel of purified enzyme assays, larotrectinib inhibited all three TRK proteins with IC50 values between 5 and 11 nM. In *in vitro* and *in vivo* tumour models larotrectinib demonstrated anti-tumour activity in cells with constitutive activation of TRK proteins resulting from gene fusions, deletion of a protein regulatory domain, or in cells with TRK protein overexpression. Acquired resistance mutations after progression on TRK inhibitors have been observed. Larotrectinib had minimal activity in cell lines with TRKA kinase domain point mutations, including the clinically identified acquired resistance mutation G595R. Point mutations in the TRKC kinase domain with clinically identified acquired resistance to larotrectinib are G623R, G696A, and F617L.

Figure: larotrectinib active substance structure (figure from [9])



Vitrakvi is available as a capsule and oral solution formulation. After oral administration, peak plasma levels (Cmax) of larotrectinib were achieved at approximately 1 hour after dosing. Half-life is approximately 3 hours and steady state is reached within 8 days. The mean absolute bioavailability of larotrectinib was 34% following a single 100 mg oral dose. Larotrectinib has pH-dependent solubility, however it is unlikely to be affected by pH-modifying agents. The mean volume of distribution of larotrectinib in healthy adult subjects was 48 L. Binding to human plasma proteins *in vitro* was approximately 70%. Larotrectinib was metabolised predominantly by CYP3A4/5 *in vitro*. The excreted larotrectinib is found in faeces and urine. Larotrectinib was shown to be a substrate of CYP3A4, P-gp and BCRP. Larotrectinib is an inducer of both CYP3A4 and CYP2B6 *in vitro*, a weak inducer of PXR regulated enzymes and an inhibitor of OATP1B1. [9] [27]

Clinical data

A summary of the clinical data (efficacy and safety) submitted to the EU regulatory authorities by the applicant in the context of the request for marketing authorization in the EU is provided below. Data are extracted from the European Public Assessment Report (EPAR) publicly available on the EMA website. [9]

Clinical efficacy

Three clinical studies represented the primary efficacy basis for larotrectinib. Those were: the phase 1 study in adult patients LOXO-TRK-14001, the phase 1/2 study in paediatric (from 1 month to 21 years) patients LOXO-TRK-15003 (“SCOUT”), and the phase 2 basket study LOXO-TRK-15002

("NAVIGATE") in patients aged 12 years and older. The efficacy dataset presented in the application was based on interim data that have been pooled from the three studies above.

The primary endpoint for the pooled efficacy analyses was overall response rate (ORR) based on Independent Review Committee (IRC) assessment, according to RECIST v1.1 criteria. ORR was defined as the proportion of patients with best overall response of confirmed complete response (CR) or confirmed partial response (PR). All patients (pediatrics and adults) included in the analysis set met the following criteria: documented NTRK fusion as determined by local testing; non-CNS primary tumour with 1 or more measurable lesions at baseline as assessed by RECIST 1.1; received 1 or more doses of larotrectinib.

The pooled primary analysis set initially submitted included a total of 55 patients. During the evaluation procedure, results from two subsequent data cut-offs with extended primary analysis sets have been presented, including 73 and then 93 patients. The pooled primary analysis populations excluded patients from the pivotal studies who had primary CNS tumours, which were presented separately.

A summary of the efficacy data for the most updated pooled adult and paediatric population is presented in the table below (adapted from EPAR):

Table: Efficacy results of larotrectinib in adult and paediatric patients

Total no. of patients	**93**
Median duration of survival follow-up (25^{th} – 75^{th} percentiles)	16.7 (9.3 - 23)
PRIMARY ENDPOINTS (IRC-ASSESSED, RECIST 1.1)	
Objective response rate (ORR)	
No. of patients with confirmed CR or PR, n. ORR, % (95% CI) Complete Response, n (%) Surgical complete response, n(%) Partial Response, n (%) Stable Disease, n (%) Progressive Disease, n (%) Not evaluable, n (%)	67 72% (62, 81) 15 (16%) 1 (1%) 51 (55%) 14 (15%) 9 (10%) 3 (3%)

Duration of Response (DOR)	
Median, months (95% CI)	NE (17.3, NE)
Rate of duration of response (%)	
6 months or more (95% CI)	88% (80, 96)
12 months or more (95% CI)	75% (63, 87)
SECONDARY ENDPOINTS (IRC-ASSESSED, RECIST 1.1)	
Progression-Free Survival (PFS)	
No. of patients with event, n (%)	34 (37%)
Median, months (95% CI)	27.4 (13.8, NE)
Overall Survival (OS)	
No. of patients with event, n (%)	14 (15%)
Median, months (95% CI)	NE (NE, NE)
Alive at 12 months (%)	88%

NE= not estimable

In the analysis set of patients (adult and paediatric) with primary CNS tumors, a total of 9 patients were included. Of those, 1 patient was observed having and objective response (11%).

ORR and DOR were also provided by tumor type (see table below from EPAR):

Table 66. Overall Response Rate by Tumour Types - IRC Assessment (ePAS2)

Tumour type	N	CR + PR	ORR % (95% CI)
Overall	93	67	72 (62, 81)
Soft tissue sarcoma	21	17	81 (58, 95)
Salivary gland (MASC and non-MASC)	17	15	88 (64, 99)
Infantile fibrosarcoma	13	12	92 (64, 100)
Colon	6	2	33 (4, 78)
Thyroid	10	7	70 (35, 93)
GIST	4	4	100 (40, 100)
Lung	7	5	71 (29, 96)
Melanoma	7	3	43 (10, 82)
Bone sarcoma	2	1	50 (1, 99)
Cholangiocarcinoma	2	0	0 (NC)
Appendix	1	0	0 (NC)
Breast (non-secretory)	1	0	0 (NC)
Congenital mesoblastic nephroma	1	1	100 (3, 100)
Pancreas	1	0	0 (NC)

Abbreviations: CI = confidence interval; CR = complete response; ePAS = extended primary analysis set; GIST = gastrointestinal stromal tumour; IRC = Independent Review Committee; MASC = mammary analogue secretory carcinoma of the salivary glands, NC = not calculated; ORR = overall response rate; PR = partial response. (Visit Cut-off 30 JUL 2018)

Among 85 patients with wider molecular characterisation, 48 (56%) had additional oncogenic alterations and 37 (44%) had no other oncogenic alteration detected. ORR in the two subgroups showed an ORR of 58% in patients with oncogenic alterations (28 CR/PR) and an ORR of 84% in patients without (31 CR/PR).

Clinical safety

The most updated clinical safety data supporting the application of larotrectinib included a total of 208 patients, composed by 152 adults and 56 paediatrics. Almost all patients (98%) experienced at least a treatment emergent adverse event (TEAE). Half of the study population experienced Grade 3 or 4 events. In the overall safety dataset, TEAEs most commonly reported (>20%) were fatigue (36%), dizziness (29%), nausea (28%), constipation (27%), AST and ALT increase, anaemia, cough (26% each), vomiting (24%), diarrhoea (23%). The most common observed Grade 3 and 4 event was anemia. None of the cases of death were considered related to the toxicity of the drug.

The events showed in the tables below were considered adverse drug reactions of larotrectinib, and included in the Vitrakvi SmPC, separately for adults and children. The type of reported adverse events appeared to be consistent between age groups with difference in incidence.

Table 2: Adverse drug reactions reported in TRK fusion-positive cancer patients treated with VITRAKVI at recommended dose (n=125)

System organ class	Frequency	All grades	Grades 3 and 4
Blood and lymphatic system disorders	Very common	Anaemia Neutrophil count decreased (Neutropenia) Leukocyte count decreased (Leukopenia)	
	Common		Anaemia Neutrophil count decreased (Neutropenia)[a] Leukocyte count decreased (Leukopenia)
Nervous system disorders	Very common	Dizziness Paraesthesia	
	Common	Gait disturbance	Dizziness Paraesthesia
Gastrointestinal disorders	Very common	Nausea Constipation Vomiting	
	Common	Dysgeusia	Nausea
Musculoskeletal and connective tissue disorders	Very common	Myalgia Muscular weakness	
	Common		Myalgia
General disorders and administration site conditions	Very common	Fatigue	
	Common		Fatigue
Investigations	Very common	Alanine aminotransferase (ALT) increased Aspartate aminotransferase (AST) increased Weight increased (Abnormal weight gain)	
	Common	Blood alkaline phosphatase increased	Alanine aminotransferase (ALT) increased[a] Aspartate aminotransferase (AST) increased Weight increased (Abnormal weight gain)

[a] Grade 4 reactions were reported

Table 3: Adverse drug reactions reported in TRK fusion-positive paediatric cancer patients treated with VITRAKVI at recommended dose (n=37); all Grades

System organ class	Frequency	Infants and toddlers (n=14)[a]	Children (n=15)[b]	Adolescents (n=8)[c]	Paediatric patients (n=37)
Blood and lymphatic system disorders	Very common	Anaemia Neutrophil count decreased (Neutropenia) Leukocyte count decreased (Leukopenia)	Anaemia Neutrophil count decreased (Neutropenia) Leukocyte count decreased (Leukopenia)	Neutrophil count decreased (Neutropenia) Leukocyte count decreased (Leukopenia)	Anaemia Neutrophil count decreased (Neutropenia) Leukocyte count decreased (Leukopenia)
Nervous system disorders	Very common			Dizziness Paraesthesia	
	Common		Paraesthesia Gait disturbance		Dizziness Paraesthesia Gait disturbance
Gastrointestinal disorders	Very common	Nausea Constipation Vomiting	Nausea Constipation Vomiting	Nausea Vomiting	Nausea Constipation Vomiting
	Common		Dysgeusia		Dysgeusia
Musculoskeletal and connective tissue disorders	Very common			Myalgia Muscular weakness	
	Common		Myalgia		Myalgia Muscular weakness
General disorders and administration site conditions	Very common	Fatigue	Fatigue		Fatigue
Investigations	Very common	Alanine aminotransferase (ALT) increased Aspartate aminotransferase (AST) increased Weight increased (Abnormal weight gain)	Alanine aminotransferase (ALT) increased Aspartate aminotransferase (AST) increased Weight increased (Abnormal weight gain) Blood alkaline phosphatase increased	Alanine aminotransferase (ALT) increased Aspartate aminotransferase (AST) increased Blood alkaline phosphatase increased	Alanine aminotransferase (ALT) increased Aspartate aminotransferase (AST) increased Weight increased (Abnormal weight gain) Blood alkaline phosphatase increased
	Common	Blood alkaline phosphatase increased			

[a] Infant/toddlers (28 days to 23 months): one Grade 4 Neutrophil count decreased (Neutropenia) reaction reported. Grade 3 reactions included two cases Neutrophil count decreased (Neutropenia) and one case of anaemia.
[b] Children (2 to 11 years): no Grade 4 reactions were reported. One reported Grade 3 case each of Neutrophil count decreased (Neutropenia), Paraesthesia, Myalgia, Weight increased (Abnormal weight gain).
[c] Adolescents (12 to <18 years): no Grades 3 and 4 reactions were reported.

Neurologic reactions including dizziness, gait disturbance and paraesthesia were reported in patients receiving larotrectinib.

The following safety concerns were identified for larotrectinib, and included in the Risk Management Plan (table from EPAR):

Table 94 Summary of safety concerns

Important identified risks	None
Important potential risks	Severe neurologic reactions
	Severe drug-induced liver injury
	Serious infections secondary to neutropenia
	Impairment of neurodevelopment in paediatric patients
Missing information	Use in pregnancy and lactation
	Long-term safety

Benefit/Risk evaluation

The CHMP observed that there is uncertainty about the precise magnitude of effect, firstly due to the study conduct related to the limited efficacy database and the lack in prospectively studied cohorts that could provide an unbiased estimate of ORR, and since the understanding of the extent that tissue of origin is an effect modifier is incomplete. However, the observed overall ORR of 72% was considered outstanding, the consistently short median time to response and the median change in tumor size were considered of high clinical value, and an expected median response duration of around 12 months would generally be considered clinically relevant. In the context of advanced cancer that has exhausted or does not have established therapeutic options, an outstanding ORR along with clinically meaningful DOR was considered sufficient by the CHMP to establish clinical benefit. From a safety perspective, larotrectinib appeared reasonably tolerated and toxicity was considered manageable with appropriate risk minimization measure, although one main concern was the potential on-target central nervous system effects due to the mechanism of action.

Notwithstanding the above, the overall available data were considered non-comprehensive (i.e. explorative and adaptive nature of the development program, immaturity of DOR, single arm design, limited understanding of the extent that tissue origin might be an effect modifier). As such, granting a conditional marketing authorisation, subject to submission of post-approval data, was considered relevant. [9]

Post-approval data

The main areas that were considered non-comprehensive at the time of approval were: the benefit based on histology, the need for unbiased estimates of ORR and DOR, the benefit in terms of time-

dependent outcomes (i.e. OS, PFS), resistance mechanisms as well as the impact of concomitant oncogenic drivers, long-term safety including impact on the neurodevelopment of paediatric patients.

It was therefore agreed with the Applicant the submission of specific obligation measures to obtain further clinical efficacy and safety data, which included a pooled analysis for the increased sample size (approximately 200 patients) for the study LOXO-TRK-15002 (NAVIGATE) by 2024, and the submission of the final report including long-term follow-up of the paediatric study LOXO-TRK-15003 (SCOUT) by 2027. [9]

Rozlytrek (entrectinib)

On 7th January 2019, the applicant (Roche Registration GmbH) submitted to the European Medicines Agency (EMA) an application to obtain a marketing authorisation (MA) in EU for the drug Rozlytrek (INN entrectinib) through the centralised procedure pathway. The type of marketing authorization requested was a so-called conditional marketing authorisation (CMA) (Regulation (EC) No 726/2004). On 28th May 2020, the CHMP adopted a positive opinion recommending the granting of a CMA for this medicinal product.

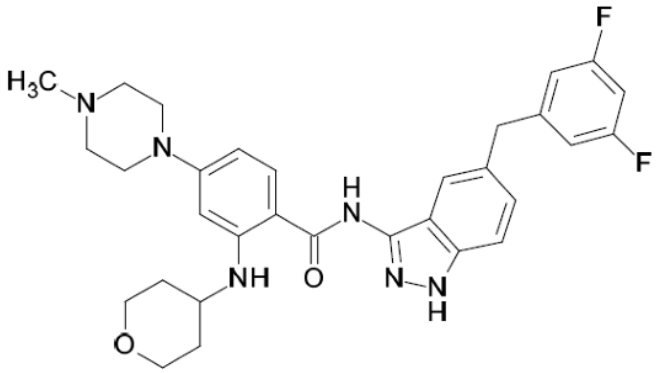
It is to note that, prior to the submission of the MA, entrectinib was granted PRIME eligibility in October 2017. PRIME means “Priority Medicine”. This is a voluntary scheme run by the EMA which has the scope of enhancing a tailored regulatory support for the development of potentially promising medicines that target an unmet medical need. The optimization of development plans and acceleration of evaluation may allow promising medicines to reach patients earlier. [28]

Pharmacological aspects

Entrectinib is an adenosine triphosphate (ATP) competitive inhibitor of receptor tyrosine kinases TRKA, TRKB, and TRKC, tyrosine-protein kinase ROS1, and anaplastic lymphoma kinase (ALK). Inhibition by entrectinib of the TRK, ROS1, and ALK kinase fusion activity leads to inhibition of downstream signalling pathways, such as phospholipase C gamma, mitogen activated protein kinase

(MAPK), and phospho-inositol-3-kinase (PI3K)/protein kinase B (PI3K/AKT), which in turn leads to inhibition of cell proliferation, and induction of tumor cell apoptosis. Entrectinib inhibits TRKA, TRKB, TRKC, ROS1, and ALK with IC50 values at low or sub nanomolar level (1.7, 0.1, 0.1, 0.2, and 1.6 nM, respectively). M5, its major active metabolite, showed similar IC50 values.

Figure: entrectinib active substance structure (figure from [10])



The pharmacokinetics of entrectinib and M5 are linear and are not dose- nor time-dependent. Steady state is achieved within one week for entrectinib and two weeks for M5 following daily administration. Following a single oral administration, entrectinib was rapidly absorbed reaching time-to-maximum plasma concentration (Tmax) after approximately 4-6 hours. Steady-state was achieved within 5 days. Entrectinib and M5 have >99% binding to human plasma proteins. After a single oral dose, the geometric mean volume of distribution was 600 L. Entrectinib is metabolised predominantly by CYP3A4, minor contributions also from other CYPs and UGT1A4. The active metabolite M5 (formed by CYP3A4) and the direct N-glucuronide conjugate, M11, (formed by UGT1A4) are the two major circulating metabolites identified. The elimination half-lives of entrectinib and M5 were estimated to be 20 and 40 hours, respectively. Most excreted entrectinib/M5 is found in faeces, minimal in the urine. Entrectinib is a weak P-gp substrate *in vitro*, while M5 is a P-gp substrate, while only M5 is a substrate of BCRP. [10] [29]

Clinical data

A summary of the clinical data (efficacy and safety) submitted to the EU regulatory authorities by the applicant in the context of the request for MA in EU, is provided below. Data are extracted from the European Public Assessment Report (EPAR) publicly available on the EMA website. [10]

Clinical efficacy

The applicant submitted the clinical results of entrectinib from three phase I studies, ALKA and STARTRK-1 in adult patients, and STARTRK-NG in paediatric patients, and from one phase II basket study STARTRK-2 in adults. All studies included patients with NTRK1/2/3, ROS1 or ALK molecular alterations. ALKA was a first in human dose escalation study, STARTRK-1 and STARTRK-NG both included a dose escalation and dose expansion phase in adult and paediatric subjects, respectively. The dose indicated in adult patients (600 mg once daily) was selected based on clinical data from ALKA and STARTRK-1 studies, supported by graphical PK/PD analysis of dose ranging data and exposure response analyses.

The efficacy dataset used to support an indication in NTRK fusion positive solid tumors was made by pooling a sample of patients from the three adult studies mentioned above (ALKA, STARTRK-1, STARTRK-2). All patients included in the pooled analysis were adults (≥18 years) having an extra-cranial solid tumour that harbour an NTRK gene fusion, who received at least one dose of entrectinib, and not previously treated with a TRK inhibitor. This dataset, called "NTRK Efficacy-Evaluable Analysis Set", initially was submitted including a total of 54 adult patients. Following CHMP request during the procedure, the number of patients included in the dataset was expanded to 74 adult patients which were followed for more than 6 months of follow-up.

Primary CNS tumors, as well as paediatric patients, were analysed and reported separately.

The primary endpoints of this pooled analysis were overall response rate (ORR) and duration of response (DOR), as assessed by blinded independent central review (BICR) according to international response criteria called Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

ORR was defined as the proportion of patients with confirmed complete response (CR) or partial response (PR) (i.e. responders) per RECIST 1.1 by BICR. A confirmed response was defined as a response that persisted on repeat-imaging ≥4 weeks after initial documentation of response.

DOR was defined as the time from the date of first objective response (either CR or PR) to first documentation of radiographic disease progression or the date of death due to any cause, whichever occurred first. DOR was calculated only for responders.

A summary of the efficacy data for the most updated pooled adult population is presented in the table below (adapted from EPAR):

Table: Efficacy results of entrectinib in adult patients

Total no. of patients	74
Median duration of survival follow-up (range)	14.2 (0.1*-29.7)
PRIMARY ENDPOINTS (BIRC-ASSESSED, RECIST 1.1)	
Objective response rate (ORR)	
No. of patients with confirmed CR or PR, n.	47
ORR, % (95% CI)	63.5% (51.5, 74.4)
Complete Response, n (%)	5 (6.8%)
Partial Response, n (%)	42 (56.8%)
Stable Disease, n (%)	9 (12.2%)
Progressive Disease, n (%)	6 (8.1%)
non CR/non-PD, n (%)	3 (4.1%)
Missing or unevaluable, n (%)	9 (12.2%)
Duration of Response (DOR)	
No. of patients with events, n (% of responders)	21/47 (44.7%)
Median, months (95% CI)	12.9 (9.3, NE)
Event-free probability (95% CI)	
6 months	0.71 (0.58, 0.85)
12 months	0.55 (0.39, 0.72)
SECONDARY ENDPOINTS (BIRC-ASSESSED, RECIST 1.1)	
Progression-Free Survival (PFS)	
No. of patients with event, n (%)	29 (53.7%)
Median, months (95% CI)	11.2 (8.0, 14.9)
Overall Survival (OS)	
No. of patients with event, n (%)	16 (29.6%)
Median, months (95% CI)	20.9 (14.9, NE)

NE= not estimable

Confidence Interval (CI) for ORR are calculated with the Clopper-Pearson method.

Median and percentiles for time-to-event analyses based on Kaplan-Meier estimates. Confidence Intervals (CI) for the median were computed using the method of Brookmeyer and Crowley.

Event-Free Probabilities are Kaplan-Meier estimates and confidence intervals were calculated using the method of Kalbfleisch and Prentice.

The pooled NTRK Efficacy-Evaluable Analysis Set included patients having 12 different tumor types, all harbouring an NTRK-fusion. The ORR and DOR were therefore also presented according to tumor type (see table below, from EPAR):

Table 62: ORR and DOR (BICR Assessment) by Tumour Type, (NTRK-Efficacy Evaluable Population) – high level grouping - CCOD: 31 OCT 2018

Tumour Type	Patients (N = 74)	ORR		DOR
		n (%)	95% CI	Range (months)
Sarcoma	16	9 (56.3)	(29.9, 80.3)	2.8, 15.1
Non-small cell lung cancer	13	9 (69.2)	(38.6, 90.9)	1.4*, 25.9*
Salivary (MASC)	13	12 (92.3)	(64.0, 99.8)	2.8, 22.1*
Breast cancer (secretory)	4	4 (100)	(39.8, 100)	5.5, 20.2*
Breast cancer (non-secretory)	2	NE, PR	NA	4.2
Thyroid cancer	7	3 (42.9)	(9.9, 81.6)	5.6, 10.9*
Colorectal cancer	7	2 (28.6)	(3.7, 71)	7.9*, 15.2
Neuroendocrine cancers	4	2 (50.0)	(6.8, 93.2)	1.9*, 9.2*
Pancreatic cancer	3	2 (66.7)	(9.4, 99.2)	7.1, 12.9
Ovarian cancer	1	Non CR/PD	NA	26.0*
Endometrial carcinoma	1	PR	NA	26.0*
Cholangiocarcinoma	1	PR	NA	9.3
Gastrointestinal cancer (other)	1	PR	NA	5.6*
Neuroblastoma	1	NE	NA	NA

*Censored
ORR: Objective Response Rate; DOR: Duration of Response; MASC: mammary analogue secretory carcinoma; NA: not applicable due to small number or lack of response; CR: complete response; PR: partial response; PD: progressive disease; NE: not estimable.

As noted above, patients having a primary central nervous system (CNS) tumor with an NTRK gene fusion were provided separately. Among the 8 subjects with primary CNS tumors, only one achieved an objective response according to RANO criteria.

Paediatric population: during the procedure, the available data in paediatric subjects (<18 years of age) included a total of 7 paediatric patients with NTRK fusion positive solid tumors treated within the STARTRK-NG clinical study. A summary of the efficacy data in this group is provided in the table below (adapted from EPAR):

Table: efficacy results in pediatric patients with NTRK fusion positive tumors from STARTRK-NG

Tumor type	**Best overall response (BOR)**	**Duration of response (DOR)**
Infantile fibrosarcoma	Partial Response	9,265 months
Epithelioid glioblastoma	Complete Response	3,713 months
Anaplastic ganglioglioma	Partial Response	1,840 months
CNS primary ganglioneuroblastoma	n/a	n/a

Metastatic melanoma	Partial Response	6,472
High grade glioma	Partial Response	6,439
Infantile fibrosarcoma	Complete Response	4,698

BOR and DOR were retrospectively evaluated by blinded independent central review assessment (confirmed responses)

n/a not available, as patient did not yet have first post-tumor assessment at the data cut-off.

Clinical safety

The clinical safety data supporting the application of Rozlytrek derived from the three adult studies ALKA, STARTRK-1, and STARTRK-2 and from the paediatric study STARTRK-NG. Safety data from all those studies were pooled and collectively analysed as an integrated safety population. The analysis set of the safety population initially submitted included 355 patients. During the procedure, an updated larger safety population including 504 patients was presented. For the paediatric setting, a final safety dataset including 32 patients aged <18 years was provided. Of those, 7 were adolescent (age between 12 and 18 years).

In the overall integrated safety population, almost all patients (99.4%) experienced at least one AE (all grade) during treatment. Grade ≥3 AEs and serious AEs (SAEs) were experienced by 61.1% and 39.9% of patients, respectively. AEs led to discontinuation of entrectinib in 9.1% (46/504) of patients. Overall, fatal AEs were reported in the context of advanced cancer/ progressive disease, no cluster or specific pattern with respect to the type of Grade 5 AEs was observed.

The most common adverse reactions (≥ 20%) were fatigue, constipation, dysgeusia, oedema, dizziness, diarrhoea, nausea, dysaesthesia, dyspnoea, anaemia, increased weight, increased blood creatinine, pain, cognitive disorders, vomiting, cough, and pyrexia. The most frequent (≥ 2%) serious adverse reactions were lung infection (5.2%), dyspnoea (4.6%), cognitive impairment (3.8%), and pleural effusion (2.4%).

The below table, extracted from EPAR, shows the events that have been considered adverse drug reactions (ADRs) to entrectinib. These data were included in the Summary of Product Characteristics for Rozlytrek.

Table: Summary of adverse drug reactions in patients treated with entrectinib in clinical trials

System Organ Class	Adverse Reaction	Entrectinib N=504		
		All grades (%)	Frequency category (all grades)	Grade 3-5 (%)
Infections and infestations	Lung Infection[1]	13.1	very common	6.0[19]
	Urinary tract infection	12.7	very common	2.6
Blood and lymphatic system disorders	Anaemia	28.2	very common	9.7
	Neutropenia[2]	11.3	very common	4.4
Metabolism and nutritional disorders	Hyperuricaemia	9.1	common	1.8
	Weight increased	26.4	very common	7.3
	Decreased appetite	11.9	very common	0.2
	Dehydration	7.9	common	1.0
	Tumor lysis syndrome	0.2	uncommon	0.2[19]
Nervous system disorders	Dysgeusia	42.3	very common	0.4
	Dizziness[3]	39.7	very common	1.2
	Dysaesthesia[4]	29.0	very common	0.2
	Cognitive disorders	24.2	very common	4.4
	Headache	17.5	very common	1.0
	Peripheral Sensory Neuropathy[5]	15.7	very common	1.0
	Ataxia[7]	15.7	very common	0.8
	Sleep disturbances[16]	13.5	very common	0.4
	Mood disorders	9.1	common	0.6
	Syncope	4.6	common	3.0
Eye disorders	Vision Blurred[8]	11.9	very common	0.4
Cardiac disorders	Congestive Heart Failure[9]	3.0	common	2.2
	Electrocardiogram QTc prolonged	2.0	common	0.6
Vascular disorders	Hypotension[10]	16.5	very common	2.4
Respiratory, thoracic and mediastinal disorders	Dyspnoea	27.0	very common	5.8[19]
	Cough	21.4	very common	0.6
	Pleural effusion	6.9	common	2.8
Gastrointestinal disorders	Constipation	42.9	very common	0.4
	Diarrhoea	33.5	very common	2.6
	Nausea	32.1	very common	0.8
	Vomiting	23.2	very common	1.2
	Abdominal pain	11.1	very common	0.6
	Dysphagia	10.1	very common	0.4
Hepatobiliary disorders	AST increased	17.5	very common	3.6
	ALT increased	16.1	very common	3.4
Skin and subcutaneous tissue disorders	Rash[11]	11.5	very common	1.4
Musculoskeletal and connective tissue disorders	Myalgia	19.6	very common	0.6
	Arthralgia	19.0	very common	0.6
	Muscular weakness	12.3	very common	1.2
	Fractures[12]	6.2	common	2.4
Renal and urinary disorders	Blood creatinine increased	25.4	very common	0.6
	Urinary retention[17]	10.9	very common	0.6
General disorders and administration site conditions	Fatigue[13]	45.0	very common	5.0
	Oedema[14]	37.3	very common	1.4
	Pain[15]	24.4	very common	1.6
	Pyrexia	20.0	very common	0.8

[1] Lung infection includes the PTs bronchitis, lower respiratory tract infection, lung infection, pneumonia, respiratory tract infection, upper respiratory tract infection.

[2] Neutropenia includes the PTs neutropenia, neutrophil count decreased.

[3] Dizziness includes the PTs dizziness, vertigo, dizziness postural.

[4] Dysaesthesia includes the PTs paraesthesia, hyperaesthesia, hypoaesthesia, dysaesthesia.

[5] Cognitive impairment includes the PTs cognitive disorder, confusional state, disturbance in attention, memory impairment, amnesia, mental status changes, hallucination, delirium, 'hallucination visual' and mental disorder.

[6] Peripheral sensory neuropathy includes the PTs neuralgia, neuropathy peripheral, peripheral motor neuropathy, peripheral sensory neuropathy.

[7] Ataxia includes the PTs ataxia, balance disorder, gait disturbances.

[8] Vision blurred includes the PTs diplopia, vision blurred, visual impairment.

[9] Congestive heart failure includes the PTs acute right ventricular failure, cardiac failure, cardiac failure congestive, chronic right ventricular failure, ejection fraction decreased,pulmonary oedema.

[10] Hypotension includes the PTs hypotension, orthostatic hypotension.

[11] Rash includes the PTs rash, rash maculopapular, rash pruritic, rash erythematous, rash popular.

[12] Fractures includes the PTs humerus fracture, foot fracture, ankle fracture, femoral neck fracture, stress fracture, fibula fracture, fracture, rib fracture, spinal fracture, wrist fracture, femur fracture, pathological fracture.

[13] Fatigue includes the PTs fatigue, asthenia.

[14] Oedema includes the PTs face oedema, fluid retention, generalized oedema, localized oedema, oedema, oedema peripheral, peripheral swelling.

[15] Pain includes the PTs back pain, neck pain, musculoskeletal chest pain, musculoskeletal pain, pain in extremity.

[16] Sleep disturbances includes the PTs hypersomnia, insomnia, sleep disorder, somnolence.

[17] Urinary retention includes the PTs urinary retention, urinary incontinence, urinary hesitation, micturition disorder, micturition urgency.

[18] Mood disorders includes the PTs anxiety, affect lability, affective disorder, agitation, depressed mood, euphoric mood, mood altered, mood swings, irritability, depression, persistent depressive disorder, psychomotor retardation.

[19] Grade 5 (fatal) ADRs were reported for dyspnea (2 patients), pneumonia (2 patients) and tumor lysis syndrome (1 patient).

Some AEs were considered of special interest for entrectinib. First of all, neurological toxicity, which was an on-target off-tumor toxicity in accordance with the entrectinib mechanism of action and the widespread expression of TRK receptors in nervous tissues. Neurological toxicity was heterogeneous and involved both central and peripheral nervous systems, encompassing adverse events like cognitive disorders (including confusion, mental status changes, memory impairment, and hallucinations), peripheral neuropathy, dysesthesia, ataxia, syncope, and seizure. Also, the AE weight increase, due to deregulated food intake, was considered a consequence of TRK inhibition by entrectinib.

Cardiovascular toxicity was another event of special interest. More specifically, congestive heart failure (CHF) was reported across the clinical trials with entrectinib, although in this case no potential mechanism of entrectinib elucidating/contributing to CHF was identified. QTc interval prolongation was also observed.

Gastro-intestinal (GI) toxicity was not negligible, with a significant fraction of subjects experiencing constipation, diarrhoea, nausea, and vomiting, although GI events were usually mild or moderate in grade.

Pneumonitis (also including interstitial lung disease, alveolitis and radiation pneumonitis) was observed in about 2% of patients. Eye disorder events and (non-cytotoxic) myelotoxicity were also reported.

Abnormal liver function tests together with blood creatinine increase were among the laboratory abnormalities.

An event of interest was bone fractures, which were observed at a higher rate in paediatric as compared to adult patients. Again, an on-target off tumor toxicity was suspected, due to a potential impact of TRK/ROS1 inhibition in physiological bone remodelling processes.

The following safety concerns were identified for entrectinib, and included in the Risk Management Plan (table from EPAR):

Summary of safety concerns	
Important identified risks	• Congestive Heart Failure • QT Prolongation • Fractures
Important potential risks	• Severe neurologic reactions • Neuro-developmental impairment in paediatric patients
Missing information	• Use in Patients with Hepatic Impairment • Safety in long term use

Benefit/Risk evaluation

Based on the data provided, entrectinib showed antitumor activity by inducing objective and durable responses in adult patients with solid tumors harbouring NTRK gene-fusion. The precise magnitude of effect was however uncertain, firstly because the results were based on pooled data containing a mix of data intended as pivotal or not, therefore lacking a confirmatory element.

However, the observed overall ORR of 63.5% was in the end considered outstanding. Further, responses appeared durable, with median >12 months of duration.

Given that the indication was sought for all solid tumors NTRK positive, i.e. based on the presence of the target but regardless tumor site/histology, another source of uncertainty was that, due to the limited efficacy database, the extent to which the tissue of origin of the tumor or concomitant genetic alterations could impact efficacy was not fully clear. Indeed, CIs for ORR and DOR were generally wide due to the small sample size, thus making efficacy estimates imprecise and

hampering the possibility to draw conclusions regarding efficacy in subgroups. Although far from definitive conclusion, the ORR in the overall population does not appear to represent the expected response in each tumour type. Indeed, higher response rates were observed in rare tumors characterized by NTRK-gene fusion such as MASC (90%) and breast secretory (100%). High response rate was also seen in NSCLC (70%), where although NTRK fusions are rare, several targeted therapies have shown high response rate in NSCLC with molecular alterations. On the contrary, quite low ORR is seen in CRC, and even lower response rate was achieved in the CNS primary tumour (ORR 10%). Such uncertainties around the drug effect according to tumour type have been reflected in the product information. Further, the possibility to extrapolate activity in all clinical TRK fusions and tumour histologies not represented in the clinical sample was hampered by a limited non-clinical pharmacological package.

The safety database was overall considered limited, although acceptable in the context of a rare condition. The safety profile of entrectinib in adult patients was considered overall manageable.

A limitation of single arm non-controlled data was that the limit in interpreting time-to-event endpoints. From a safety perspective, the uncontrolled design did not allow to clearly disentangle signs/symptoms of the underlying malignancy from entrectinib-related adverse events.

Regarding the paediatric population, the CHMP concluded that an indication could be granted in adolescents (≥ 12 years). Indeed, the dose recommendation in these subjects was based on population PK analysis considered acceptable based on simulation, while the activity of entrectinib in adolescents was considered established based on extrapolation from adults with NTRK fusion positive solid tumours, with an overall safety profile yet limited but in line with what was observed in adults.

The conclusion was that the overall activity of entrectinib in NTRK-positive tumours was deemed clinically meaningful in a setting where non-targeted therapies are either not established, or where such treatment options have been exhausted, and a positive benefit/risk ratio was concluded by majority of the CHMP. [10] [30]

Post-approval data

In the context of a CMA, the overall data provided in the submission for entrectinib was considered to be non-comprehensive. Therefore, additional data were requested post-approval in the form of

Specific Obligation (SOB), and they were agreed at the time of the marketing authorization with specific timelines. As discussed above, main uncertainty was the impact of tissue of origin and concomitant genetic alterations on entrectinib activity. The first SOB was aimed at confirming the histology-independent efficacy of entrectinib. The applicant agreed to submit a pooled analysis of efficacy and safety for an increased sample size of NTRK fusion-positive patients from the ongoing studies STARTRK-2, STARTRK-NG and any additional agreed clinical trial. The target sample is a total of about 200 patients with NTRK fusion positive solid tumors, and efficacy data will be presented overall as well as by histology/tumor site. The second SOB was aimed at further investigate the impact of the presence/absence of other molecular alteration on the efficacy of entrectinib. Therefore, the results of tumour genomic profiling by plasma and/or tissue (when possible) at baseline and progression together with clinical outcomes association per tumour histology for the patients from the updated pooled analysis described above will be presented. The target date for those SOBs is 2027.

In addition, based on the evaluation of the safety profile of entrectinib, additional pharmacovigilance activities (Category 3) were requested to further investigate two safety concerns of the drug, namely the risk of congestive heart failure and the risk of fracture. The first analysis on CHF was concluded, leading to specific changes in the SmPC [31], while the analysis on fractures is awaited by 2025. [10]

NTRK inhibitors: national market entry in EU

The EMA is responsible for the scientific evaluation of drugs submitted for centralised marketing authorisation applications. After the assessment of the benefits and risks of a certain drug for an intended therapeutic indication, the role of EMA, through its Committee for Medicinal Products for Human Use (CHMP), is to make a recommendation to the European Commission (EC) about granting a marketing authorization to such medicinal product. Within 67 days of receipt of EMA's recommendation, the EC then takes a final legally binding decision on whether the medicine can be marketed in the EU. Such decision is valid over all EU member states and the EEA (EU countries plus Iceland, Norway and Liechtenstein). Once a medicinal product has received an EEA-wide marketing

authorisation, any decisions about pricing and reimbursement take place at national and regional level, and choices are made in the context of the national health system of each country. [32]

Pricing and reimbursement measures affect the possibility to place a medicinal product onto the national markets, and impact on the access of patients in each country to a certain drug. Therefore, as the decisions are taken at the national level, a drug that has been centrally approved may be prescribed to widely varying degrees within each EU countries. In general, health technology assessment (HTA) bodies of the respective European country are responsible to provide recommendations on medicines and other health technologies that can be financed or reimbursed by the national/regional healthcare system. HTA is a scientific evidence-based procedure for the assessment of the added value, effectiveness, costs and broader impact of a health care intervention. HTA focuses in determining the relative effectiveness and additional therapeutic benefit of a new drug as compared to existing alternative treatments in a certain setting. [33]

Challenges of HTA assessment of site and histology independent indications

As for the process leading to marketing authorization, also oncology-related HTA assessments have historically focused on the evaluation of scientific evidence generated as histology-specific data. Therefore, drugs having histology independent indications, i.e. for several histologies but with the same molecular alteration, turned out to be a new challenge for HTA bodies when demonstrating value to payers within the existing appraisal framework.

Clinical evidence supporting site and histology independent indications is mostly based on pooled analyses from various trials, usually single arm trials, or basket trials of relatively limited sample size, generally including a limited number of patients per tumor histology or even including none for certain tumor types. Indeed, because the prevalence of the biomarkers that are targeted by histology independent drugs can be low, conducting randomised controlled trials is not always feasible. As such, the clinical evidence is coming from uncontrolled single arm study, meaning that no randomized comparison is generally performed. Further, usually main endpoints are measure of the drug activity on the tumor in terms of objective response rate, while time-related endpoints like OS and PFS are usually hardly interpretable for two main reasons: the single arm design with no comparison treatment, and the mixed type of tumors included which may have different prognosis and natural behaviour. Thus, the main body of evidence available at the time of HTA assessment,

which is usually the same that already supported the marketing authorization, has multiple consequences on the evaluation by HTA bodies.

Added value is indeed evaluated by HTA based on comparative evidence with respect to available treatment options. Being however clinical data essentially uncontrolled, there is the question of the appropriate control. The lack of direct comparison in a properly designed RCT lead to the need of using external control. However, comparison with historical data has various methodological limitations that can result in biased effectiveness estimates. External controls are also usually not selected for a specific biomarker. Further, information on the prognosis of patients with a certain tumor type harbouring a certain molecular alteration may lack. Unavailable prognostic information led to difficulties determining the burden of illness, the behaviour of the disease under standard treatment, and finally the positioning of the histology-independent drug within existing treatment pathways. Even if external data are identified, due to the limited sample size, lack of statistical power e.g. within the individual tumor entities can prevent the use of historical data for an indirect comparison. The choice of the optimal comparator arm for benefit assessment can also be an issue, as the appropriate comparator might vary with the tumor entity or even with the patient. So far, tumour-agnostic licences have only been granted in a last-line setting, i.e. when patients have no other satisfactory treatment options (as larotrectinib and entrectinib indications). However, defining an end-of-line patient population in clinical trials can be complicated, and may vary considerably across tumour types according to the availability of alternative treatments. Line of therapy may be a significant prognostic factor, and failure to adjust for this may affect estimates of effectiveness. Some histologies/tumor types have not been included so no data at all is available, which might be an additional challenge. [34] [35]

As discussed above, the efficacy evaluation of targeted drug in single arm trial is mostly based on the assessment of tumor response. However, HTA assessment of effectiveness is usually based on health outcomes that are important to patients as to determine clinical benefit, being overall survival (or in some cases progression free survival) preferred by HTAs. When only response data are available, assumptions have to be made on the drug's clinical benefit, which increases the uncertainty in the HTA assessment. [36] [37]

Regardless the outcome evaluated (response or survival), when considering how effective tumour-agnostic therapies are expected to be in clinical practice, an additional relevant issue arising for the HTA assessment is heterogeneity, i.e. whether it can be expected that outcomes will differ across

patient subgroups, such as tumor histologies, or adult vs paediatric patients. Some statistical techniques can be useful in data interpretation in this regard, e.g. Bayesian hierarchical model (BHM) framework has been applied as an approach to characterise heterogeneity. [38]

Strictly related to heterogeneity, the generalizability of the study results should be evaluated, meaning how well patients and results of the clinical trial reflect those patients expected to be treated in clinical practice. If homogeneity cannot be assumed, and large differences is expected between study patients and real-life patients, there is high chance that the study results are not representative of real-world results. Immaturity of data together with lack of surrogacy between response and survival may further introduce uncertainties in the extrapolability of trial results. [39]

Mitigate the uncertainties of HTA assessment of site and histology independent indications

In order to handle the uncertainties and challenges encountered in the assessment of site and histology independent indications by HTA bodies, some proposals and solutions have been discussed. Those may be implemented at various levels. Indeed, manufacturers may mitigate payer uncertainties around products aimed at site and histology-independent indications. HTA bodies may also introduce new tools in the evaluation of drugs approved with site and histology-independent indications.

One of the methods used by payers to manage decision uncertainty is by negotiating price discounts. This is often preferred to more complex arrangements that may require, for example, further data collection. This is however highly dependent on processes and infrastructures available at national level. The use of conditional HTA/reimbursement decisions and of systematic reassessments have been suggested as one of the useful tools that can be applied to site and histology indications. For example, conditional reimbursement with systematic reassessments of evidence is used in some countries. Outcome-based reimbursement models may be another useful assessment methods. The collection of real-world data may be requested before definitive decision on reimbursement or for reassessment of initial decisions or as a pre-condition to reimbursement. [36]

The collection of post-approval evidence has been suggested as a solution by several authors for benefit assessment in the HTA evaluation of site and histology-independent indications. Data collection can be made through post-marketing studies or in the real world/clinical practice. The

additional data collected may cover for example the lack of evidence of one or more tumor site/histology not included in the pivotal trial. [34] Some publications suggested that data collection should be managed on an EU-wide basis. [40] Collection of additional data might help reducing clinical uncertainty and may enable more informed recommendations to be made.

As discussed above, one of the problems for HTA is the lack of randomized controlled trials as supporting evidence for site and histology independent development. However, demonstrating an effect on survival or health-related quality of life is usually needed for HTA approval. Therefore, approaches to generate appropriate comparative evidence should be implemented, which are described in the literature and also proposed by HTA bodies. [41] [42] Each approach has strengths and weaknesses and further research in needed in this regard. [41]

Manufacturers may also mitigate payer uncertainties around site and histology-independent products through for example a careful planning of comparator arm(s), clear definition of patient population, ensure the inclusion of a0n adequate number of patients, statistically sound use of RWE in the analyses. Data collection is of importance also from the manufacturer side. Indeed, relevant data that may be useful at the time of HTA submission includes information on the prevalence of a certain biomarker across tumor types, prognostic value of the biomarker, treatment used and natural history outcomes of patients with tumors harbouring a specific biomarker, as well as data on the validity of any surrogate outcomes planned to be used for economic analysis. The company itself should make plan in advance to collect data after approval/reimbursement. [39]

Close collaboration between all relevant stakeholders and early engagement between manufacturers and payers may be extremely helpful in planning in advance all aspects of the development programme, from the type and amount of pivotal evidence as well as the collection of the right data to supplement clinical trials, in order to help regulatory approval first and HTA assessment after, to allow real access to patients on new drugs.

HTA assessment/ price and reimbursement decision on NTRK inhibitors across EU

In the table below, a summary of the outcome of national HTA assessment and price and reimbursement decisions across some European countries is presented.

	Vitrakvi (larotrectinib)	Rozlytrek (entrectinib)
Belgium	Reimbursed [43]	Reimbursed [43]
Finland	Reimbursed [44]	Reimbursed [44]
France	Favourable opinion for reimbursement only in the treatment of paediatric patients with refractory or relapsed, locally advanced or metastatic infantile fibrosarcoma or another soft tissue sarcoma, with NTRK gene fusion. Unfavourable opinion for reimbursement in the rest on indication [45] [46]	Unfavourable opinion for reimbursement [47]
Germany	An additional benefit is not proven [48]	An additional benefit is not proven [49]
Ireland	The NCPE recommends larotrectinib (Vitrakvi) not be recommended for reimbursement, unless cost effectiveness can be improved relative to existing treatments [50]	A full HTA is recommended to assess the clinical effectiveness and cost effectiveness of entrectinib compared with the current standard of care, on the basis of the proposed price relative to currently available therapies [51]
Italy	Reimbursed by National Health System [52]	Reimbursed by National Health System [53]
Netherlands	Larotrectinib meets the extended criteria for VT (conditional inclusion) [54] [55]	Entrectinib should be designated as a potential candidate for VT (conditional inclusion) [56] [57]
Norway	Larotrektinib (Vitrakvi) is not indicated as monotherapy for the treatment of patients over 18 years of age with solid tumors with NTRK fusion gene. There are no documented benefits which	Entrektinib (Rozlytrek) is temporarily introduced as monotherapy for the treatment of adult and pediatric patients over 12 years who have solid tumors with NTRK fusion. The decision

	could indicate that this preparation may have a higher price than other approved alternatives. [58]	is linked to an alternative price agreement which means that the price of the medicine starts at a reduced level and can then follow the documented effect. [59]
Portugal	-	No added therapeutic value of entrectinib has been demonstrated over best supportive care. Public funding rejected. [60]
Spain	Non-financing resolution [61]	Non-financing resolution [62]
Sweden	Vitrakvi is included in the high-cost protection with a general subsidy [63] [64]	Rozlytrek is included in the high-cost protection with a limited subsidy [65] [66]
UK	Larotrectinib is recommended for use within the Cancer Drugs Fund [38]	Entrectinib is recommended for use within the Cancer Drugs Fund [67]

Additional information on assessments and decisions across Europe are summarized below:

Belgium: larotrectinib and entrectinib can be used within the approved NTRK indication in patients after approval by a multidisciplinary oncology consultation. The reimbursement can be granted based on an electronic application via the eHealth platform, and it is subject to a mandatory registration of tumor and outcome-specific variables via the Belgian Cancer Registry. [43]

France: for Vitrakvi (larotrectinib), the maintenance of the initial opinion (i.e. paediatric patients with infantile fibrosarcoma or another soft tissue sarcoma, with NTRK gene fusion) was subject to submission of comparative data for larotrectinib versus the standard of care in these patients within a maximum period of 12 months, as well as the implementation of an exhaustive registry identifying all children treated with Vitrakvi in France. A re-evaluation was carried out in 2023 confirming favourable opinion for such indication. A new assessment will be carried out in 2025 based on the data from the SACHA registry and additional data from SCOUT clinical study. [45] [46]

The Netherlands: in the Netherlands, the procedure for VT (conditional inclusion) states that an obligation to do research is linked to the entitlement to VT in the care package. This means that patients are only entitled to reimbursement for care with larotrectinib or entrectinib if they participate in further research into its effectiveness (or cost-effectiveness) by entering a registry. It was agreed that additional data collection was to be done through the DRUG Access platform, which allow to collect data on Dutch patients about the effectiveness of the NTRK inhibitors (including overall response percentage, duration of the response, time until response, progression-free survival and the overall survival rate and safety). Additionally, information about the diagnostics and previous treatment guidelines will be recorded. The centres participating in DRUG Access will ensure that an explanation is given to patients about the fact that treatment with the medicine involves temporary reimbursement in a research context. Progresses will be reviewed annually during the conditional period and will be re-assessed within 3.5 years using the new assessment framework for tumour-agnostic medicinal products. [54] [55] [56] [57]

Sweden: Vitrakvi was initially included since 1 November 2020 in the pharmaceutical benefits with a limited subsidy for patients who start their treatment before the age of 18. Subsequently, the company applied for an extended subsidy to include also adult patients with NTRK fusion positive solid tumors, and contextually a price reduction was requested. [64] Currently, both drugs can be used in Sweden.

UK: The National Institute for Health and Care Excellence (NICE) concluded that larotrectinib and entrectinib could not be recommended for routine use, because of uncertainties about clinical or cost effectiveness. However, the committee concluded that the NTRK-inhibitors can be recommended for treating NTRK fusion-positive solid tumours within the Cancer Drugs Fund (CDF), through a Managed Access Agreement. In UK, during managed access, more evidence is collected to address uncertainties about a treatment using a Managed Access Agreement. For larotrectinib and entrectinib, it was considered that some of the inherent uncertainty in appraising a histology-independent treatments could be resolved through further data collection. As part of the managed access agreement, the treatments continue to be available through the Cancer Drugs Fund until the data are collected and reviewed as a full technology appraisal. The evidence collected during this period will be indeed used to assess whether the treatment should be made available for routine use on the NHS.

About larotrectinib, primary source of data collection includes pooled analysis from the ongoing clinical studies NAVIGATE, SCOUT and LOXO-TRK-14001, in accordance with the conditional marketing authorization granted by EMA and FDA, but also additional data including Public Health England Systemic Anti-Cancer Therapy data, Public Health England molecular data set and Genomics England analysis. Secondary source will be ON-TRK (PrOspective Non-interventional study in patients with locally advanced or metastatic TRK fusion cancer treated with larotrectinib), a company global non-interventional study (NIS) planned as result of post-marketing commitments made to EMA and FDA. The purpose of this study is to describe the safety and effectiveness of larotrectinib under real-world conditions. UK treatment centres will be eligible to participate once larotrectinib is available via the CDF. Access to EURACAN registry is another secondary source of evidence. [68]

About entrectinib, sources of data collection are the ongoing STARTRK-2 and STARTRK-NG, according to the conditional marketing authorization conditions agreed with EMA at the time of approval. Additional sources of data collection include Public Health England (PHE)'s Systemic Anti-Cancer Therapy (SACT) dataset, PHE's Molecular dataset, NHS England's Blueteq data, Flatiron real-world data and Foundation Medicine genomic database from US and Intergroup non-interventional study (NIS) sponsored by European Thoracic Oncology Platform (ETOP) in Europe. [69]

HTA evaluation of larotrectinib and entrectinib: the Italian case

In Italy, the Italian Medicines Agency (AIFA) and its technical and advisory committees, Scientific Technical advisory Committee (CTS) and Price and Reimbursement Committee (CPR), are responsible for defining the system of eligibility for reimbursement and supply for all authorized medicinal products, as well as negotiating the price of those to be paid by the National Health Service, after negotiation with pharmaceutical companies. On 7 September 2021, official decisions were published in the "Gazzetta Ufficiale" on both Vitrakvi (larotrectinib) and Rozlytrek (entrectinib). [52] [53] Both drugs were included in the "Class H" medicinal products, meaning that both drugs are reimbursed and dispensed by the National Health Service (SSN) and can be distributed only through hospitals, for the entire indication in NTRK-fusion positive solid tumors. Only medical doctors specialist in medical oncology are allowed by law to prescribe larotrectinib and entrectinib.

In addition, both NTRK inhibitors were granted the status of “innovative medicinal products”. The definition of innovation, its assessment, and the granting of the status of innovative medicine are the responsibility of AIFA and its Scientific-Technical Committee. This status implies the assessment of three elements: therapeutic need, added therapeutic value and robustness of the scientific evidence submitted by the company together with the request for innovation. This status allowed both drugs to be included in a list of “Innovative medicinal products”, thus having access to a state Fund for innovative oncological medicines, having economic benefits, and being automatically included into regional therapeutic schedules. [70]

In order for larotrectinib and entrectinib to be prescribed and to be paid by the Italian National Health Service, it is mandatory to register patients into the AIFA Monitoring Registers. This web-platform allows access to treatment in a homogeneous manner throughout the country. A patient can thus receive an NTRK inhibitor only if satisfy certain eligibility criteria within the approved indication. In addition, information such as tumor reassessments throughout the treatment, and end of treatment data, must be entered in the IT platform. [71]

The official decision also included recommendation regarding the management of diagnostic aspects. Indeed, it was recommended that, within each Italian Region, the diagnostic evaluation occur in a limited number of centres where appropriate competencies and diagnostic instruments are available. Ideally, a “Hub & Spoke” organization is suggested in this regard. It was considered needed to identify centres to perform appropriate molecular assays and to implement within those centres “molecular tumor boards”: those are multidisciplinary groups that should include experts like medical and radiation oncologists, surgeons, radiologists, pathologists, pharmacists, and even bioinformatics, molecular biologists and clinical pharmacologists. The aim is that the expertise of these centres with molecular tumor boards in place can guarantee the best diagnostic and therapeutic approach for each patient with regard to the use of molecular targeted drugs. [52] [53]

The approach followed by HTA in Italy leading to the above decisions for NTRK inhibitors has been discussed in a recent article. [72] A traditional HTA approach would have allow for reimbursement of the drug only for the types of tumor studied, even if only in a very small number of patients. However, the approach taken from the AIFA CTS was to accept the idea of a tissue-independent strategy and to manage the drug accordingly. In addition to the evaluation of the “place-in-therapy” of the medicinal products, this “adaptive” approach aimed to define the diagnostic-therapeutic management on its entirety, meaning that the drug is part of an overall management strategy. As a

result, centres that can use the drug should be defined, multidisciplinary teams should be built which have to decide which patients to test and using which assays. Ideally, those teams should follow common algorithms, otherwise such kind of drugs increase the risk of dis-homogeneity and disparity in treatment access by patients across Italian Regions. [72]

The site and histology independent approval of immune-checkpoint inhibitors

Immunotherapy in MSI-H cancer

The mismatch repair (MMR) system is a highly conserved DNA repair mechanism protecting the integrity of the genome against mismatching errors arising during DNA replication. Impaired DNA mismatch repair can cause accumulation of mutations in microsatellites (MSI). MSI can occur in a wide variety of tumor types. MSI has emerged as a predictive biomarker for response to immune checkpoint inhibitor (ICI) therapy.

On May 23, 2017, the FDA granted accelerated approval to pembrolizumab (Keytruda) for adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options or with MSI-H or dMMR colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. This authorization had high coverage at that time, as this was the first FDA's tissue/site-agnostic approval, and first in the regulatory space worldwide.

Few years later in 2021, another anti-PD1 antibody, dostarlimab (Jemperli), received accelerated approval by FDA for adult patients with dMMR recurrent or advanced solid tumors, as determined by an FDA-approved test, that have progressed on or following prior treatment and who have no satisfactory alternative treatment options.

While dostarlimab is currently not approved in EU for the dMMR istology independent indication, pembrolizumab is approved but only in some selected MSI-H/dMMR tumor types.

The regulatory story in US and EU of anti-PD1 drugs in MSI-H/dMMR cancers is described below.

MSI-H/dMMR molecular aspects

The DNA mismatch repair (MMR) is a highly conserved system responsible for the recognition and correction of mismatched nucleotides: MMR system repairs DNA erroneously damaged during DNA replication, playing a key role in maintaining genomic stability. Four major proteins encoded by the MLH1 (mutL homologue 1), MSH2 (mutS homologue 2), MSH6 (mutS homologue 6), and PMS2

(postmeiotic segregation increased 2) genes are known MMR gene product. The MMR system functions through the formation of heterodimers, called hMutS and hMutL. hMutS consists of MSH2 and a one secondary proteins (either MSH6 or MSH3) and recognizes mismatched nucleotides and small indels. The hMutL heterodimers, formed by MLH1 and one secondary proteins (PMS2, PMS1, or MLH3), participate in MMR reactions. hMutL deficiency leads to increased DNA excision. The inactivation of at least one of the following genes, MLH1, MSH2, MSH6, or PMS2, due to germline and/or somatic mutations, or epigenetic silencing, results in the MMR system deficiency (dMMR). Alteration of MMR genes lead to dysfunctional MMR proteins incapable of recognising DNA mismatch in coding regions of repetitive nucleotide sequences called microsatellites; as a result, DNA damage fails to be repaired and may lead to generation of non-functional protein. This form of genomic instability is called microsatellite instability (MSI). MSI resulting from impaired DNA MMR causes an accumulation of mutations in microsatellites (MS), also called short tandem repeats (STRs). STRs consist of repeated sequences of 1 - 6 nucleotides and account for 3% of the genome, in coding and noncoding regions. The nature of MS determines its tendency to accumulate errors. Due to DNA slippage in the process of DNA replication, this usually leads to a change in MS length.

The inactivation of MMR gene can either be somatic (sporadic) or germline (e.g. Lynch syndrome). Lynch syndrome is a hereditary disorder with autosomal dominant transmission that predisposes primarily to colorectal and endometrial cancer, and it is also associated with other extra-colonic malignancies (e.g. gastric, small bowel, pancreatic, bladder, prostate, and biliary tract cancers). [73] [74] [75]

The prevalence of MSI-H across different tumours varied widely by tumour type and by disease stage. Several tumour types, including endometrial, colorectal, and gastric cancers were consistently found to have the highest MSI-H prevalence. The incidence of MSI-H or dMMR has been reported to be nearly 30% for endometrial cancer, 20% for colon or gastric cancer. For most of the other cancers, MSI-H prevalence was less than 5%. In addition, the prevalence of MSI-H in late-stage disease is generally lower than in earlies cancer stages. [76] The prognostic effect of MSI-H or dMMR appears to depend on the stage of the cancer, mostly more favourable in early-stage disease.

MSI-H cancers are usually characterized by a high mutational burden and tumour-specific neoantigen load mediated by MSI and common defects in MMR, and can demonstrate highly upregulated expression of PD-1 and PD-L1, as well as other immune checkpoints, thereby providing a scientific rationale for PD-1 blockade with immune-checkpoint inhibitors for the treatment of

patients with MSI-H cancer. However, not all patients with MSI-H tumours respond to immunotherapy, suggesting that a deeper understanding of immune-related mechanisms in MSI-H is required. [77]

MMR or MSI status can be determined by various methods. In general, tumours are classified as MSI-high (MSI-H) (including MMR deficient) when the expression of at least 1 out of 4 MMR proteins is not detectable by IHC, or when at least 2 allelic size shifts among 3 to 5 analyzed microsatellite markers are detected by PCR. The assays that can be used to assess MSI/MMR status are listed below: [78]

- analysis of protein expression in tumor cell nuclei of 4 MMR proteins (MLH1, MSH2, MSH6, PMS2) by immunohistochemistry (IHC). IHC is a fast and cheap technique, however it does not cover all MMR genes and require large tissue sample, with an error rate of 10%. In case of intact expression of MMR proteins detected by IHC, the tumor sample is MMR proficient. When heterogeneous staining or loss of expression in one or more MMR protein is detected, molecular analysis by PCR/NGS is to be performed.
- MSI testing by PCR, it is considered the gold standard for MSI diagnostics. Usually, 3 to 5 tumour microsatellite loci are analysed by PCR assay.
- Next generation sequencing (NGS), this can analyse about 100 loci. NGS was useful to shows the complexity of MSI biology.

For the two anti-PD1 drug approved by FDA for MSI-H/dMMR solid tumors, the FDA approved a test to be companion diagnostic for each drug. Specifically, with pembrolizumab FDA approved FoundationOne CDx, which is an NGS-based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations in 324 genes and select gene rearrangements, as well as genomic signatures including MSI and TMB using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens. [79] The test approved for dostarlimab was instead an IHC assay, VENTANA MMR RxDx Panel. This is a qualitative immunohistochemistry test intended for use in the assessment of mismatch repair proteins (MLH1, PMS2, MSH2 and MSH6) in FFPE tissue specimens by light microscopy. [80]

Pembrolizumab for MSI-H solid tumors (FDA)

Pharmacological aspects

Pembrolizumab is a humanised monoclonal antibody of the IgG4/kappa isotype, with a stabilising sequence alteration in the Fc region. The target of pembrolizumab is the programmed cell death-1 (PD-1; CD279), which is a protein member of the immunoglobulin (Ig) superfamily. PD-1 is expressed on CD4+ and CD8+ T cells, natural killer T cells, B cells, and monocytes, and also some dendritic cell (DC) subsets upon their activation. [81]

PD-1 is an inhibitory receptor: PD-1 engagement by its ligands, PD-L1 (CD274; also known as B7-H1) and PD-L2 (CD273; also known as B7-DC), has a role in regulating the immune system's response by down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. The PD-1 ligands are expressed in cancer cells and antigen-presenting cells (APCs) of the tumor microenvironment (TME), and the role of PD-1 as T cell inhibitory receptor led to the targeting of PD-1 and its ligands for induction of antitumor T cell responses. Blocking antibodies against PD-1, like pembrolizumab, or its ligands, have revolutionized cancer treatment. Pembrolizumab is currently approved worldwide as monotherapy and in combination with chemotherapy and/or with other agents (such as monoclonal antibodies, tyrosine kinase inhibitors) for the treatment of different types of cancer.

Clinical data

Data supporting the FDA approval of pembrolizumab for the treatment of MSI-H or dMMR cancers is adult and pediatric patients was derived by pooling data from patients with metastatic previously treated solid tumors, enrolled in five single-arm, multicenter trials and selected for inclusion in the pooled dataset based on MSI-H or dMMR tumor testing. The studies differed in eligibility criteria [(pre-specified requirement for MSI-H or dMMR tumor vs. pre-specified testing for PD-L1 status/retrospective testing for MSI-H or dMMR); extent of prior therapy (≥ 1 prior line of therapy vs. specified prior treatment regimens for a specific cancer type); primary cancer (limited to colorectal cancer vs. multiple primary cancers)], use of local vs. central laboratory to determine MSI-H/dMMR status, pembrolizumab dosage regimen (10 mg/kg every 2 weeks vs. 200 mg every 3 weeks). Details on the trials and on the patients submitted as pivotal evidence to the FDA are in the table below: (from [82])

Table 3: Table of trials with subjects submitted to sBLA

KN	Design/Eligibility/Pop	N	MSI-H	Dose	Prior therapy
016	Single arm, **prospective,** 6 sites, activity finding	28 CRC 30 non CRC	PCR IHC Local	10 mg/kg q2w	**CRC**: received ≥ 2 prior therapy regimens **nonCRC**: ≥1 prior therapy regimen
164	**CRC, prospective** single arm, multi-center Merck trial	61	PCR IHC local	**200mg q3w**	Prior fluoro+ox, fluoro+irino +/- anti-VEGF/EGFR mAb
012	PD-L1 TNBC, gastric, urothelial, H & N. PDL1+. Measurable disease.	6	**Retro** PCR central	10 mg/kg q2w	Previously treated; no standard therapy
028	Multi-disease cohorts PD-L1+. Measurable disease.	5	**Retro** PCR central	10 mg/kg q2w	Previously treated; no standard therapy
158	**Prospective,** MSI-H multi-cohort rare tumor trial: **Cohort K Retrospective: Cohort B, D**	19	PCR IHC local	**200mg q3w**	≥1 prior therapy regimen
Total	5 trials	**149**			

Key: "Retro" (MSI-H) were identified retrospectively, KN=KEYNOTE trial number, PCR=polymerase chain reaction, IHC=immunohistochemistry, q3w=every 3 weeks, q2w=every 2 weeks, fluoro=fluorouracil, irino=irinotecan, ox=oxaliplatin.

Of those pooled sample of 149 subjects, 90 had colorectal cancer (CRC) and 59 had other cancer types overall. Except of CRC, overall 14 type of tumors were represented, most common being endometrial cancer (n=14), biliary cancer (n=11) and gastric/GEJ cancer (n=9). All patients received at least one prior systemic treatment. The major efficacy outcome measures were objective response rate (ORR) as assessed by blinded independent central radiologic review according to RECIST 1.1, and response duration. A pooled analysis of the overall 149 patient sample showed an ORR of 39.6% (95%CI 31.7, 47.9), with 11 complete responses and 48 partial responses. The duration of response ranged from 1.6+ months to 22.7+ months, with 78% of responses lasting ≥6 months. [83]

ORR was quite similar in patients diagnosed with CRC (36%) or with a different non-CRC cancer type (46% across the 14 other cancer types). When analysed by each tumor type represented in the sample, ORR and DOR breakdown is presented in the table below: (from [82])

Table 8: ORR by Tumor type across all trials

	N	Response (ORR)	95% of ORR	DOR
GI Tumor				
BILIARY	11	3 (27%)	(6.0%, 61.0%)	(11.6, 19.6)
COLORECTAL	90	30 (33%)	(23.7%, 44.1%)	(1.6, 22.7)
GASTRIC	8	4 (50%)	(15.7%, 84.3%)	(2.0, 22.1)
PANCREATIC	6	5 (83%)	(35.9%, 99.6%)	(2.0, 9.1)
SMALL INTESTINAL	8	3 (38%)	(8.5%, 75.5%)	(1.9, 6.2)
ESOPHAGEAL	1	PR		18.2, on-going
GE JUNCTION	1	PD		
Non-GI Tumor				
ENDOMETRIAL	14	5 (36%)	(12.8%, 64.9%)	(1.9, 17.3)
BREAST	2	PR, PR		7.6, 15.9, on-going
PROSTATE	2	PR, SD		9.8, on-going
BLADDER	1	Missing		
SARCOMA	1	PD		
THYROID	1	NE		
RETROPERITONEAL	1	PR		2.1, on-going
SMALL CELL LUNG	1	PR		2.2, on-going
RENAL CELL	1	PD		

Key: GE=Gastroesophageal tumor, PR=partial response; PD=progressive disease; NE=non-evaluable

The safety of pembrolizumab in patients with MS-H/dMMR tumors was consistent with the known safety profile of the drug, associated with immune-mediated side effects including pneumonitis, colitis, hepatitis, endocrinopathies, and nephritis.

The FDA reviewers identified four major issues during review of this application:

- whether the presence of MSI-H or dMMR in tumors predicted similar efficacy across different primary tumors, such that this phenotype identified a “tissue agnostic” phenotype sufficient to identify patients who will derive similar benefit (overall response rate of sufficient magnitude and durability) from treatment with pembrolizumab;

- whether one or more companion diagnostic devices were required to select the indicated patient population in order to ensure safe and effective use of pembrolizumab;

- whether the observed differences in response rate observed in subgroups defined by the pembrolizumab dosage regimen administered provided evidence of a differential dose-response relationship.

- Extrapolation of the efficacy results to pediatric patients with MSI-H cancers.

Regarding the last point, the approved indication indeed included also pediatric patients (all ages), although the patients sample included only adult subjects. The FDA clinical review team considered that MSI-H/dMMR solid tumors can occur in children, particularly those with Lynch syndrome or with rare congenital bi-allelic genetic defects, and extended the indication to these patients by extrapolation of the efficacy in adults to children with MSI-H/dMMR tumors. The recommended dose in children was based on the results of studies in pediatric patients (previously reviewed by FDA for the classic Hodgkin lymphoma indication). However, in the extrapolation to children, the only caveat was the specific situation of CNS malignancies with mismatch repair deficiencies which are more likely to occur in children. The potential risks of lymphocytic infiltration (the suspected cause of "tumor flare" with these immunologic agents) occurring in a closed space are likely to increase the risk of herniation, based on literature data. Thus, pending additional clinical data to be collected in the post-marketing setting (described in the paragraph below), FDA requested that the Keytruda product labelling includes a "Limitation of Use" stating that the safety and effectiveness of pembrolizumab in pediatric patients with MSI-H central nervous system cancers have not been established. [82]

Post-approval data

The indication for pembrolizumab in MSI-H/dMMR solid tumors was approved under accelerated approval based on tumor response rate and durability of response. Being an accelerated approval, FDA required the company to conduct trials to further evaluate ORR and DOR in additional patients with different tumor types in a non-randomized setting. Indeed, FDA authors underlined the possibility of differential activity (ORR and DOR) in different tumor types based on e.g. extent of prior treatment, disease burden, or other factors, providing uncertainty regarding the generalizability of clinical benefit across this indication. [84] While most of the accelerated approvals require randomized trials after approval, in this case, FDA agreed for post-approval commitment based on single arm studies; this was justified by FDA given the limited number of patients and the "unprecedented effects" on response rate and response duration in some diseases such as MSI-H or dMMR endometrial, biliary, or pancreatic cancer, therefore randomized trials were not believed by the FDA to be feasible as equipoise may no longer exist. Despite this, the company later conducted a randomized trial (KEYNOTE-177) in a single tumor type, comparing single-agent pembrolizumab with standard cytotoxic chemotherapy in the first-line treatment of metastatic MSI-

H/dMMR colorectal cancer. This controlled study was not part of the post-approval agreement with FDA, though. [84]

Further, FDA considered that response rates can be assessed in non-randomized trials because, in general, tumors do not decrease in size in the absence of therapy. [85]

The post-marketing clinical trials should have included patients with MSI-H/dMMR tumors including at least 124 patients with colorectal cancer and at least 300 patients with non-colorectal cancer, including a sufficient number of patients with prostate, thyroid, small cell lung and ovarian cancer, and also 25 children. In order to characterize response rate and duration, patients were to be followed for at least 12 months from the onset of response. It was expected the final report to be submitted by March 2023. [86]

An additional uncertainty arising in the FDA assessment was related to the safety of pembrolizumab in children with MSI-H/dMMR primary central nervous system tumors given the potential risks of increased intracranial pressure arising from an immune response to treatment in the brain (e.g., edema or lymphocytic infiltration). Indeed, based on this concern, the FDA included a limitation of use in product labeling that safety and effectiveness of pembrolizumab in this population was not established, as mentioned above. Therefore, FDA requested that this aspect should have been evaluated in the context of clinical trial, thus asking to assess the safety of ascending dose of pembrolizumab to determine a reasonably safe dosage regimen in an adequate number of children affected by MSI-H/dMMR CNS tumors. The due date to submit the final report of this study was March 2023. [85] [86]

As agreed with FDA, the company submitted both the final study report regarding patients with MSI-H/dMMR tumors as well as children with MSI-H/dMMR primary CNS tumors. After reviewing those data, the FDA considered the post-marketing requirements fulfilled, thus leading to the conversion from accelerated to regular approval in March 2023. [87]

In details, the conversion from an accelerated to a full (regular) approval for pembrolizumab in adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors progressed following prior treatment and who have no satisfactory alternative treatment options, was based on results from the phase 2 KEYNOTE-158, KEYNOTE-164 and KEYNOTE-051 trials and included data for a total of 504 adult and pediatric patients across more than 30 types of cancer. KEYNOTE-164 enrolled 124 patients with advanced MSI-H/dMMR CRC progressed after prior therapy. KEYNOTE-158 enrolled 373 patients with advanced MSI-H/dMMR non-colorectal cancers who had disease

progression following prior therapy, and KEYNOTE-051 enrolled 7 pediatric patients with MSI-H/dMMR cancers. A pooled analysis of these three trials showed an ORR of 33.3% (95% CI 29.2-37.6), including 10.3% of complete responses. ORR for patients with MSI-H/dMMR CRC was 34% (95% CI, 26-43). At a median follow-up time of 20 months, 77% of the responding patients had responses lasting 12 months or longer, and 39% 36 months or longer, for a median DOR of 63.2 months (range 1.9+ to 63.9+ months). [88]

Another consideration made by FDA was the potential absence of accurate and reproducible molecular assay to identify patients with MSI-H/dMMR cancers in real world, as a companion in vitro diagnostic (IVD) device was not contemporarily available and approvable with the drug. However, in order not to delay approval in case where the benefit of the product outweighs the risks from the lack of an approved or cleared companion IVD, the FDA agreed with the company that companion diagnostic tests for detection of MSI-H/dMMR across cancers may be developed post-marketing as agreed-upon commitments. The Applicant should therefore support the availability through an appropriate analytical and clinical validation study using clinical trial data that support labeling of an IHC based and of a nucleic acid-based in vitro diagnostic devices essential to the safe and effective use of pembrolizumab for patients with tumors that are MSI-H/dMMR and submit the final report by June 2019. [85] [86] In 2022, two tests were approved by FDA as companion diagnostics for Keytruda to identify patients with MSI-H/dMMR status. One was an immunohistochemistry test to identify protein of the mismatch repair system (dMMR status) from Ventana, [89] the second one was an expansion of the intended use of the next generation sequencing oncology panel (somatic or germline variant) FoundationOne CDx (F1CDx) for the detection of MSI-H status. [90]

Pembrolizumab for MSI-H selected solid tumors (EMA)

Clinical data

Contrary to the US, in the EU the marketing authorization holder of Keytruda did not apply for a site and histology independent indication based on the MSI/MMR biomarker. Indeed, in June 2021 the company submitted a marketing application only for selected tumor types harbouring MSI-H/dMMR status. In details, an indication was requested for pembrolizumab as monotherapy for six solid tumours (namely CRC, endometrial, gastric, small intestine, biliary, and pancreatic cancer) with

microsatellite instability-high (MSI-H) or mismatch repair deficiency (dMMR) in advanced stage following prior treatments. These indications were requested based on the single arm clinical studies KEYNOTE-164 (for CRC) and KEYNOTE-158 (for non-CRC tumors). Those studies were the same trials based on which few years earlier FDA approved the site and histology independent MSI-H/dMMR pembrolizumab indication, although, for each of the six tumour types, more patients were included in the EMA rather than in the FDA dossier. [91]

KEYNOTE-164 was a phase II single arm study of pembrolizumab as monotherapy in subjects with previously treated locally advanced unresectable or metastatic (Stage IV) dMMR or MSI-H colorectal carcinoma. A total of 124 patients were enrolled and received at least one dose of pembrolizumab, included in two different cohorts based on the number of previous treatments received: at least 2 lines of standard of care therapies in Cohort A (n=61), at least 1 line of systemic standard of care therapy in Cohort B (n=63). KEYNOTE-158 was an open-label, single-arm, multicenter, multicohort, Phase 2 study of pembrolizumab in previously treated participants with advanced solid tumours evaluated for predictive biomarkers. Cohort K enrolled any participant with an advanced solid tumour that was MSI-H (except for CRC, evaluated in KEYNOTE-164). The data presented for centralized assessment were however, as said above, only related to patients affected by one of the 5 tumour type proposed for the indication, i.e. MSI-H endometrial (n=83), gastric (n=51), small intestine (n=27), biliary (n=22), and pancreatic cancer (n=22). Both KEYNOTE-164 and KEYNOTE-158 studies had ORR based on RECIST 1.1 as assessed by independent central radiologic review as primary endpoint. Duration of response (DOR) was also an endpoint. Enrolment in both studies was based on MSI-H/dMMR testing which was performed locally. About 70% of the patients were tested by IHC, followed by PCR (about 30%) and a minority by other testing, such as NGS.

Clinical efficacy results for each of the six tumor types included in the sought indication are presented in the tables below (adapted from EPAR and SmPC). [91] [92]

Table: summary of efficacy results in KEYNOTE-164

Endpoint	n=124
Objective response* rate	
ORR % (95% CI)	34% (25.6, 42.9)
Complete response	10%
Partial response	24%
Response duration*	
Median in months (range)	NR (4.4, 58.5+)
% with duration ≥ 36 months#	92%

* Based on patients with a best objective response as confirmed complete or partial response

\# Based on Kaplan-Meier estimation

\+ Denotes there is no progressive disease by the time of last disease assessment

NR = not reached

Table: summary of efficacy results in KEYNOTE-158

	Database Cutoff Date (15-OCT-2021)
Endometrial	**n=83**
ORR, % (95% CI)	50.6 (39.4, 61.8)
Median DOR, months (range)	NR (2.9 - 60.4+)
Extended DOR, % at ≥24 months	65.4
Median PFS, months (95% CI)	13.1 (4.9, 25.7)
PFS rate, % at 24 Months	39.0
Median OS, months (95% CI)	NR (48.0, NR)
OS rate, % at 24 Months	67.2
Gastric	**n=51**
ORR, % (95% CI)	37.3 (24.1, 51.9)
Median DOR, months (range)	NR (6.2 – 63.0 +)
Extended DOR, % at ≥24 months	81.3
Median PFS, months (95% CI)	4.1 (2.1, 24.6)
PFS rate, % at 24 Months	38.5
Median OS, months (95% CI)	26.9 (6.6, NR)
OS rate, % at 24 Months	50.0
Small Intestine	**n=27**
ORR, % (95% CI)	55 (35.3, 74.5)
Median DOR, months (range)	NR (3.7+ - 57.3+)
Extended DOR, % at ≥24 months	73.1
Median PFS, months (95% CI)	23.4 (4.3, NR)
PFS rate, % at 24 Months	49.8
Median OS, months (95% CI)	NR (16.2, NR)
OS rate, % at 24 Months	62.7
Biliary Cancer	**n=22**
ORR, % (95% CI)	40.9 (20.7, 63.6)
Median DOR, months (range)	30.6 (6.2 - 46.0+)
Extended DOR, % at ≥24 months	62.2
Median PFS, months (95% CI)	4.2 (2.1, 24.9)
PFS rate, % at 24 Months	31.8
Median OS, months (95% CI)	19.4 (6.5, 44.8)
OS rate, % at 24 Months	50.0
Pancreatic Cancer	**n=22**
ORR, % (95% CI)	18.2 (5.2, 40.3)
Median DOR, months (range)	NR (8.1 - 24.3+)
Extended DOR, % at ≥24 months	50.0
Median PFS, months (95% CI)	2.1 (1.9, 3.4)
PFS rate, % at 24 Months	10.4
Median OS, months (95% CI)	3.7 (2.1, 9.8)
OS rate, % at 24 Months	22.7

From the safety perspective, toxicity was as expected for pembrolizumab and comparable with the known safety profile of pembrolizumab monotherapy reference safety dataset. Pembrolizumab is characterised by immune-related adverse reactions. [92] No new safety signals were identified during this assessment.

To further support the indication, the company submitted a systematic literature review (SLR) and meta-analysis of each of the tumor types in an MSI unselected population due to the general lack of data in this particular subset, and in MSI selected population where data were available (i.e. colon and endometrial cancer). In addition, intrapatient comparison between response to prior treatment and response to subsequent pembrolizumab in each tumor type was provided. Overall, those data were considered useful to contextualize the results of pembrolizumab within each tumour type. [91]

Benefit/Risk evaluation

The MAH explained that the six tumour types with MSI-H/dMMR applied for in the indication were chosen based on a combination of factors, including unmet need, MSI-H prevalence, enrolled participant numbers, and antitumour activity observed with anti-PD-1 immunotherapy. This application was thus somewhat based on a "hybrid" strategy, where approval was sought for a selected subset of cancer types justified based on a histology-independent approach. However, limitations and uncertainties were highlighted related to the overall exploratory nature of the data provided, coming from single arm trials and from post-hoc selected subset of single arm trial, limited number of patients, no pre-specified hypotheses, lack of multiplicity control and no confirmatory data sets. While it was acknowledged that MSI-H status has generally shown to be predictive of increased activity of checkpoint inhibitors relative to non MSI-H tumours of the same origin, MSI-H is not a driver mutation and substantial heterogeneity of response depending on tumour type was seen, therefore the benefit risk ration was assessed by the CHMP separately by tumour type, as a fully tissue agnostic approach was not considered warranted.

Therefore, the CHMP conclusions in each of six the tumor types were the following:

- approval granted for pembrolizumab in MSI-H colon and endometrial cancer, for which larger evidence was available, supported also by external controlled data (KEYNOTE-177 for MSI-H colon cancer, indirect comparison with KEYNOTE-775 for endometrial cancer).

- for gastric, small intestine and biliary MSI-H cancer, an indication was considered acceptable based on the overall data provided in each tumour type, but due to the limited available evidence, the MAH was requested to provide additional data post-approval to confirm the results in those 3 tumour types.

- in pancreatic cancer, the CHMP concluded that efficacy was not established. Indeed, acknowledging the unmet need, and the possibility to have long responses, the likelihood of achieving a response was very low and not predictable, with an ORR estimate evaluated in a very limited number of patients and lower than what generally considered to indicate clinically relevant activity. [91]

Post-approval data

As compared to the submission of the site and histology independent pembrolizumab indication in MSI-H tumors in 2017 in US, at the time when the indication for pembrolizumab was granted in the EU for selected tumor types in 2021, data accumulated, and more were available on anti-PD(L)1 monoclonal antibodies in MSI-H/dMMR tumors. In particular, it is of note that in the EU, before the approval of pembrolizumab for MSI-H selected tumor types, the indication for MSI-H colorectal cancer as first line treatment was already granted in January 2021 on the basis of the phase III randomized clinical trial KEYNOTE-177 comparing pembrolizumab monotherapy versus standard chemotherapy. [93] [94] Further, other immune-checkpoint inhibitors were already approved in MSI-H/dMMR colon cancer, namely dostarlimab in MSI-H/dMMR endometrial cancer, and the combination nivolumab and ipilimumab in MSI-H/dMMR CRC. Those external data were considered supportive for the later-line use of pembrolizumab in CRC and endometrial cancer, for which no further post-approval measures were considered required in the EU.

On the contrary, for the other three tumor types approved (MSI-H/dMMR gastric, biliary and small intestine cancers), additional measures were considered necessary to address issues related to efficacy, included in Annex II. As post authorisation efficacy study (PAES), to further characterise the efficacy of Keytruda in patients with MSI-H/dMMR gastric, biliary and small intestine cancers, the MAH was requested to submit the results including ORR data from Cohort K and L of the phase II - single arm study KEYNOTE-158 (about 23 participants in gastric, 7-10 in small intestine and 8-11 in biliary cancer). Results are expected for the first quarter of 2025. [91]

Dostarlimab for MSI-H solid tumors (FDA)

In August 2021, the FDA granted an indication to dostarlimab for the treatment of adult patients with mismatch repair deficient (dMMR) recurrent or advanced solid tumors, as determined by an FDA-approved test, that have progressed on or following prior treatment and who have no satisfactory alternative treatment options. This indication was overall similar to the one granted to pembrolizumab in MSI-H/dMMR solid tumors. Prior to this extension of indication, the initial marketing authorization for dostarlimab was granted in a specific dMMR tumor type, namely endometrial cancer.

Pharmacological aspects

Dostarlimab (Jemperli) is a humanised monoclonal antibody of the IgG4 isotype that binds to PD-1 receptors and blocks the binding with its ligands PD-L1 and PD-L2. This drug is thus of the same class and has the same mechanism of action of pembrolizumab, described above. The inhibition of PD-1 pathway by dostarlimab potentiates T-cell anti-tumor responses. [95]

Clinical data

The clinical data supporting the FDA application for dostarlimab in MSI-H/dMMR solid tumors was derived from the phase 1, dose escalation/cohort-expansion, non-randomized, open-label, multicenter clinical trial GARNET that enrolled patients with recurrent or advanced solid tumors who have limited available treatment options. Efficacy was based on a total of 209 patients included into two of the expansion cohorts of the GARNET study: Cohort A1 enrolling dMMR endometrial cancer (N=103), and Cohort F including patients with non-endometrial dMMR solid tumors (N=106).

In the overall pooled population of 209 patients, the ORR was 41.6% (95%CI 34.9, 48.6), with 9.1% of complete response rate. Median duration of response was almost 3 years (34.7 months) ranging from 2.6 to 35.8+ months; almost all responding patients (95.4%) have response duration of at least 6 months. [96] ORR and DOR analysed by tumor type is presented in the table below (from FDA label [97])

Table 8. Efficacy Results in GARNET dMMR Tumor Types

Tumor Type	Patients N	Confirmed ORR (per RECIST v 1.1) n (%)	95% CI[a]	DOR Range (months)
EC	103	46 (44.7)	(34.9, 54.8)	2.6, 35.8+
non-EC	106	41 (38.7)	(29.4, 48.6)	5.6, 30.1+
CRC	69	25 (36.2)	(25.0, 48.7)	5.6, 30.1+
Small intestinal cancer	12	4 (33.3)	(9.9, 65.1)	11.1+, 28.0+
Gastric cancers	8	3 (37.5)	(8.5, 75.5)	8.4+, 17.5
Pancreatic carcinoma	4	0 (0.0)	(0.0, 60.2)	NA
Biliary neoplasm	2	CR, CR	NA	8.4+, 13.5+
Liver cancer	2	PR, PD	NA	13.8+
Ovarian cancer	2	PR, SD	NA	25.1+
Adrenal cortical	1	PR	NA	19.5+
Breast cancer	1	CR	NA	16.8+
Esophageal cancer	1	PD	NA	NA
Genital neoplasm malignant female	1	PR	NA	22.2+
Pleural	1	PR	NA	15.2+
Renal cell carcinoma	1	SD	NA	NA
Unknown origin	1	PR	NA	20.4+

+ = ongoing at last assessment.

dMMR = Mismatch Repair Deficient, ORR = Overall Response Rate, DOR = Duration of Response, CI = Confidence Interval, EC = endometrial cancer, CRC = colorectal cancer, PR = partial response, PD = progressive disease, CR = complete response, SD = stable disease.

[a] Exact, 2-sided 95% CI for binomial proportion.

For FDA's analysis of safety, data from 515 patients with a variety of advanced solid tumors in the expansion cohorts of GARNET were submitted to support the application. FDA concluded that dostarlimab demonstrated an acceptable safety profile with manageable toxicities. The most common adverse reactions (≥20%) were fatigue/asthenia, anemia, diarrhea, and nausea. The most common Grade 3 or 4 adverse reactions (≥2%) were anemia, fatigue/asthenia, increased transaminases, sepsis, and acute kidney injury. Serious adverse reactions occurred in 34% of patients receiving dostarlimab, and one patient experienced a fatal adverse reaction due to respiratory failure. Immune-related adverse events were reported in 34%, the majority being Grade 1 or 2 in severity, and manageable by treatment interruptions or discontinuations, and administration of steroids and other immunomodulators, overall is consistent with those reported with other approved anti-PD1 class of drugs. [96]

Post-approval data

Full approval of the site and histology independent indication for dostarlimab remained contingent on accumulation of supportive data from additional studies. The FDA requested the company to conduct a clinical trial evaluating ORR and DOR, to verify and describe the clinical benefit of Jemperli in patients with dMMR recurrent or advanced solid tumors, including at least 300 patients across all tumor types, and including a sufficient number of patients and representation of tumor types (other than endometrial and gastrointestinal tumors). Following patients for at least 12 months from the onset of response to characterize response duration. The final report submission was agreed for October 2022. [98] At the time of writing, no information regarding conversion of the approval has been provided.

In addition, the commitment to establish and support the availability of a nucleic acid based in vitro diagnostic device essential to support the safe and effective use of the drug for patient selection was also taken with due date December 2025. [98]

TMB and immunotherapy

Tumor mutational burden (TMB) is defined as the number of non-synonymous mutations within coding regions across the genome, which has emerged as a predictive marker for responsiveness to immune checkpoint inhibitors (ICI) in multiple tumour types. The proposed underlying principle is that higher TMB is likely to generate more neoantigens that the human immune system can recognise, thereby eliciting an anti-tumour immune response.

The history of TMB as a biomarker started in 2014, when some researchers suggested for the first time a correlation between high TMB/high neoantigens load and response to ICI in melanoma and in NSCLC. [99] [100] Subsequently, several retrospective studies have been conducted, which however showed variable association of TMB and clinical benefit, depending on the cancer type, clinical end points assessed, and TMB thresholds. For example, a recent large analysis based on data from over 10 000 patient tumors included in the Cancer Genome Atlas, failed to support application of TMB-H as a biomarker for treatment with ICI across all solid cancer types, concluding that further tumor type-specific studies are warranted. [101]

As described in more details below, in 2020 the FDA granted accelerated approval to the anti-PD1 monoclonal antibody pembrolizumab (Keytruda) for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors with high TMB (TMB-H) defined as ≥10 mutations/megabase (mut/Mb), that have progressed following prior treatment and who have no satisfactory alternative treatment options. [102] This indication is not available in the EU.

TMB molecular aspects and diagnostic assays

Tumor mutational burden (TMB) is defined as the total number of somatic mutations per megabase (per 106 bases) in a tumor tissue. TMB is calculated from somatic mutations in coding regions. The number of mutations is highly variable across cancer entities.

TMB reflects the overall burden of tumor antigens, thus it has been identified as a potential biomarker for immune-checkpoint inhibitor (ICI) response in patients with cancer. Indeed, the potential clinical benefit of TMB as biomarker is based on the hypothesis that highly mutated tumors can produce a high number of neoantigens (i.e. mutations are antigens) that may increase T-cell reactivity and the chance for the immune system to detect the tumor, which then leads to improved response to treatment with ICI. [103] [104]

Testing methodologies for TMB should be discussed. The gold standard for TMB assessment is Whole Exome Sequencing (WES) as it allows a comprehensive genomic approach: WES include testing of about 30Mb, 22k Genes, 1% of the genome. However, factors like high costs and availability limit the widespread use of WES, supporting the use of gene panel sequencing for approximating TMB in routine diagnostics. There are various commercial panels on the market. However, panels may underestimate or overestimate TMB, and some studies suggested that, to optimize cost-benefit ratio, panels between 1.5 and 3 Mbp are ideally suited to estimate TMB with small confidence intervals, whereas smaller panels tend to deliver imprecise TMB estimates for low to moderate TMB. [105] The abundance of available assays has led to questions surrounding standardization of TMB estimation across different assays and tumor types, and this may be one of the contributing factors to the heterogeneity of the study results regarding correlation between TMB and response to ICI treatment, as discussed above. Efforts have been therefore made to harmonise TMB evaluation, for example Friends of Cancer Research established a TMB Harmonization Consortium of stakeholders (pharmaceutical and diagnostic companies, scientific organizations, FDA) to conduct a comprehensive review of available data regarding TMB and

response to immunotherapy. They developed consensus recommendations for standardization of NGS panels for TMB estimation and built a calibration tool to enhance the comparability of assays. [106] [107] In US, FDA approved the FoundationOneCDx assay (Foundation Medicine, Inc.) as companion diagnostic for pembrolizumab: the indication for pembrolizumab specifies that high-TMB is defined as 10 or more mut/Mb as determined by an FDA approved test. [102]

Another challenge to the clinical application of TMB assays is how to define an appropriate cut-off for high TMB. In general, patients with a TMB of at least 10 mut/Mb had better outcomes than those with a TMB below 10 mut/Mb; however, the universal cut-off did not reliably predict improved survival after immunotherapy across all tumor types. Many investigators thus argue that a cancer type-specific threshold may be better. Other researchers suggested that an arbitrary threshold of high TMB is inappropriate and that the use of ICI should instead be considered in the context of the cause of the TMB. [101] [108]

Pembrolizumab for TMB-H solid tumors (FDA)

Clinical data

On June 16 2020, FDA approved pembrolizumab for adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) (≥10 mutations/megabase) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. FoundationOneCDx assay (Foundation Medicine, Inc.) was also approved as a companion diagnostic test for pembrolizumab to determine the TMB biomarker.

The US prescribing information for pembrolizumab includes a “Limitation of Use”, where it is stated that the safety and effectiveness of pembrolizumab in pediatric patients with TMB-H central nervous system tumors was not established.

The efficacy of pembrolizumab in the above indication was investigated in a prospectively-planned retrospective analysis of ten cohorts of patients including various previously treated unresectable or metastatic TMB-H solid tumors enrolled in the multicenter, non-randomized, open-label trial KEYNOTE-158. ORR and DoR assessed by blinded independent central review according to modified RECIST v1.1 were the primary efficacy outcome measures. The efficacy analysis population consisted of 1050 patients who received at least one dose of pembrolizumab. Of those, TMB score was missing in about 25% of the patients, leaving a total of 790 patient who met criteria for TMB assessment. Of

those 790, a total of 102 patients (13%) had tumors identified as TMB-H (TMB ≥10 mut/Mb). In this subset, the ORR was 29% (95% CI: 21, 39), with 4% complete response and 25% partial response. The median DoR was not reached, with 57% of patients having response durations ≥12 months.

The efficacy of pembrolizumab was supported by the results of retrospective WES analyses of TMB in additional patients enrolled across multiple pembrolizumab clinical trials, conducted using pooled data from 12 studies across 24 tumor types with tissue available for TMB assessment. A total of 2234 patients had available WES TMB results: 1772 pembrolizumab-treated patients and 462 chemotherapy-treated patients (enrolled in KEYNOTE-010, -045, or -061). Among the 1772 pembrolizumab-treated patients, 433 (24%) had WES TMB ≥175 mut/exome (approximately equivalent to ≥10 mut/Mb by F1CDx). The response to pembrolizumab was higher in the 433 patients with WES TMB ≥175 mut/exome cancer (ORR 31.4%) compared to <175 mut/exome population (ORR 9.5%). FDA also reviewed the results of supportive exploratory post-hoc analyses of PFS and OS by TMB in the randomized clinical trials KEYNOTE-010 (NSCLC), KEYNOTE-045 (urothelial), and KEYNOTE-061 (gastric), showing numerically lower hazard ratios for PFS and OS compared to those observed in the WES TMB < 175 mut/exome subgroups.

From a safety perspective, the adverse reactions occurring in patients with TMB-H cancer enrolled in KEYNOTE-158 were similar to that already described in the Keytruda product labelling. [102]

Post-approval data

The indication for pembrolizumab in TMB high solid tumors was approved under the FDA accelerated approval programme, which is subject to the submission of data post-approval to confirm benefit. The FDA requested to submit by December 2025 the final report and datasets from clinical trials evaluating ORR and DOR (based on independent central review and follow-up of at least 12 months from the onset of response), to verify and describe the clinical benefit of pembrolizumab in adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥10 mutations/megabase (mut/Mb)] solid tumors (as determined by an FDA approved test) that have progressed following prior treatment and who have no satisfactory alternative treatment options. The FDA requested that a sufficient number of patients and representation of tumor types (other than lung cancers, MSI-H/ dMMR cancers, or melanoma; and including CNS tumors TMB-H based on tissue collected before temozolomide chemotherapy), and

with cancers having a TMB of 10 to <13 mut/Mb, should be evaluated to characterize response and duration of response, including a minimum of 20 pediatric patients. [109]

Controversies around the indication based on TMB

The tumor "agnostic" nature of the pembrolizumab approval based on TMB generated considerable debate within the oncology community.

In an article published by FDA describing the approval of pembrolizumab for TMB-H solid tumors, FDA authors stated that, although they considered the results across tumors as generally consistent, a limitation is that much of the data was derived from select tumor types. In assessing the appropriateness of the 10 mut/Mb cut-point, FDA recognized that TMB is a continuous biomarker with the limit of cut-point selection. However, the cut-point of 10 mut/Mb was based on available data from the company, high unmet need, biological rationale, pre-specified analysis plan, clinical results, and overall risk-benefit considerations, and also previously identified through the multi-stakeholder TMB harmonization project (described above). FDA concluded that uncertainties regarding tissue agnostic accelerated approvals, including limited numbers of patients in certain tumor types needs to be balanced against the context of unmet medical need (e.g., patients without satisfactory available therapy), while additional data is going to be obtained via a post-marketing requirement, and potential exists for future modification or withdrawal of the indication as with any accelerated approval. [110]

Some authors expressed their agreement with FDA decision, and believed that the pembrolizumab approval in TMB-H tumors was based on a strong biological rationale. It was underlined that this was an expansion of the label of a drug already authorized, which is known to be effective for many indications and with a widely known safety profile. This FDA approval would facilitate access to a therapy that can result in significant benefit for this molecularly defined subset of patients with no effective alternative treatment options, and especially patients with rare cancer types which are less likely to access to tumor molecular profiling or off-label therapies. [111] On the contrary, other authors questioned this FDA approval for several reasons. One was that the TMB cut-off of 10 was considered arbitrary. Indeed, also results based on the cut-point 13 were provided to FDA, enriching further the population for response. This cut-off was questioned also in light of literature data failing to show predictive value of TMB for overall survival across cancers. Data supporting the approval were considered weak and limited, uncontrolled, with no evidence that the pembrolizumab in this

indication may improve clinically relevant outcomes of survival or quality of life. Although agnostic approval, tumor seems to matter, but no data were available on some common tumors types. Reasoning about cost-effectiveness and the need for “good” therapeutic options were also raised by these authors. [112]

Targeted therapies' indications extended to site and histology independent

After the approval of pembrolizumab in MSI-H solid tumors by FDA, followed by the two NTRK targeted inhibitors entrectinib and larotrectinib for NTRK fusion positive solid tumors, FDA granted new site and histology independent indications. Differently to what happened with NTRK inhibitors, both obtaining a site and histology independent indication at their first marketing authorization, the most recent FDA approvals of this kind were granted to targeted therapies already on the market, which were initially approved in tumors expressing a specific molecular alteration hit by the drug, but only in specific tumor type/site. These new approvals include BRAF/MEK inhibitors dabrafenib/trametinib in BRAF-V600E solid tumors, followed by RET inhibitor selpercatinib in solid tumors harbouring a RET fusion. Of particular interest is the BRAF case, as, despite a site-independent indication, colon cancer is specifically excluded in the product label. Such latest FDA approvals are described and discussed below.

At the time of writing, none of the indications above are available in the EU.

Dabrafenib/trametinib for BRAF-V600E solid tumors (FDA)

In June 2022 the FDA granted accelerated approval to the combination of the BRAF inhibitor dabrafenib (Tafinlar) with the MEK inhibitor trametinib (Mekinist) for the treatment of adult and pediatric patients ≥ 6 years of age with unresectable or metastatic solid tumors with BRAF V600E mutation, who have progressed following prior treatment and have no satisfactory alternative treatment options. Dabrafenib in combination with trametinib is not indicated for patients with colorectal cancer, because of known intrinsic resistance to BRAF inhibition. [113] [114]

The combination dabrafenib plus trametinib is currently not approved in the EU for the indication above.

On the one hand, the site-independent indication of dabrafenib/trametinib partly built on the previous experience of BRAF and MEK inhibitors combinations showing clinically relevant activity in specific histologies harbouring a driver BRAF-V600E mutation. Indeed, according to FDA official information, this approval was supported by results in COMBI-d, COMBI-v, and BRF113928, which are the clinical studies in melanoma and lung cancer, which are already described in product

labelling as leading to prior approval in those tumor types. [113] Prior efficacy demonstration, especially if based on controlled randomized comparisons, may indeed increase the overall confidence on the activity of a certain drug, as well as the knowledge of the safety profile, as compared to a drug requesting *in primis* a site and histology indication based on limited uncontrolled data.

On the other hand, however, despite the dabrafenib/trametinib indication being defined as "agnostic", the FDA label include a specific limitation of use, i.e. that the combination dabrafenib with trametinib should not be used in BRAFV600E mutant colorectal cancer, because of known intrinsic resistance to BRAF inhibition. This restriction somewhat may be considered putting into question the concept of histology independent activity itself, since it is expected that the type of tumour may be an effect modifier. As described below in more details, the "BRAF history" in colon cancer is paradigmatic. First, it shows the complexity of molecular biology, and the risk that the characteristics of a certain biomarker are not totally understood when targeted drugs are available. Secondly, the site of origin (colon cancer) clearly played a relevant role as effect modifier, pointing toward the possibility of a complex interplay between molecular alteration and site/histology of the tumor.

BRAF molecular aspects

The RAS/RAF/MEK/ERK pathway is a one of the most important cell signalling pathways, which can be activated by various transmembrane receptors such as receptors tyrosine kinase (RTKs). Following the activation of this signalling pathway, this acts on cytoplasmic and nuclear substrates leading to proliferation, survival, senescence, angiogenesis, migration and invasion of normal as well as transformed cells. There are various intermediates and adaptors that can interact and modulate the signals. The molecules composing this pathway can have various isoforms: 3 RAS isoforms (H, N and K-Ras), 3 RAF isoforms (ARAF, BRAF, RAF1 (aka c-Raf), KSR1/2) each with several splice variants, 2 MEK isoforms (MEK-1 and 2 with 2 splice variants), 2 ERK isoforms (ERK-1 with 1 splice variant, ERK2). All elements can form homo- and hetero-dimers, and isoforms and dimer diversity can represent a pharmacological challenge. [115] BRAF is thus a member of the RAF family of serine/threonine kinases, which has been described in a variety of tumor types. Upstream of BRAF, growth factor binding to RTKs at the cell surface leads to phosphorylation of RAS proteins, which

then activate BRAF. Signal transduction continues downstream from BRAF to MAPK kinase and finally to ERK, which phosphorylates multiple targets.

Several mutations of the BRAF gene have been identified in cancer, which have been classified in 3 classes:

- Class I mutants: including BRAF V600D/E/K/R mutations -those results in strong activation of BRAF's kinase activity and constitutive activation of MAPK pathway;

- Class II mutants: predominantly located in the activation segment (i.e. K601, L597) or P-loop (i.e. G464, G469), with intermediate to high kinase activity;

- Class III mutants: located in the P-loop (G466), catalytic loop (N581) and DFG motif (D594, G596), with lack or low kinase activity. [116]

The most studied activating BRAF mutations occur at position V600 (V600E, V600K), resulting in constitutive activation of BRAF and downstream activation of MEK and ERK. The frequency of BRAF mutations varies by tumor type: mutations are observed in half of the patients with melanoma, about 25% of patients with anaplastic thyroid cancer, and about 2-8% of patients with NSCLC. [117]

As mentioned above, while BRAF/MEK inhibitors revolutionized the treatment of BRAF mutated melanoma, disappointing results were achieved in BRAF mutated colon cancer. Approximately 10% of colon cancer harbour a BRAF mutation, being BRAF-V600E the most common. This subgroup has morphological, clinical, and therapeutic characteristics that differ substantially from patients who do not carry this genetic alteration. BRAF-V600E mutated colon cancer are indeed characterised by a dismal prognosis, with a median overall survival of less than one year. Phenotypically, colon cancers with BRAF-V600E mutation are right sided, with mucinous and poorly differentiated histology, more frequent in female above 70 years of age. Site of metastases are more frequently peritoneum and lymph nodes and less frequently lung. An overlap between BRAF-V600E mutation and MSI was described, as half of MSI-H metastatic colorectal cancer may harbour such mutation. Other rarer BRAF mutations have on the contrary a better prognosis. [118] [119]

The activity of BRAF inhibitor as monotherapy in BRAF-V600E positive mCRC were quite disappointing, showing in small studies in advanced disease overall response rate no better than 5%, with a slight increased activity to about 10% ORR with the combination BRAF/MEK inhibitors. It was then understood that BRAF inhibition in colon cancer downregulated the negative feedback signals from ERK, resulting in the activation of the EGFR pathway with rapid EGFR-mediated

reactivation of RAS and C-RAF. The activity of BRAF inhibitors may thus be circumvented, partly explaining the modest efficacy relative for example to melanoma cells, which express low levels of EGFR. It was additionally observed that acquired oncogene mutations such as KRAS, NRAS and MAPK1, as well as copy number amplification in BRAF may be mechanism of resistance to BRAF targeted therapies in colon cancer.

Based on the findings regarding EGFR, combining EGFR targeted therapies with BRAF inhibitors +/- MEK inhibitors was attempted, in order to overcome resistance to BRAF inhibitors. In 2020, the combination encorafenib plus cetuximab (an anti-EGFR monoclonal antibody) was approved in US and in EU for patients with mCRC with a BRAFV600E mutation who have received prior systemic therapy. [120] [121]

Clinical data

The BRAF kinase inhibitor dabrafenib was approved for the first time by FDA in 2013 for the treatment of patients with unresectable or metastatic melanoma with BRAF-V600E mutation. It was after demonstrated that the association of a BRAF inhibitor with another compound inhibiting MEK may provide some advantages by preventing the aberrant activation of mutated BRAF in the context of the RAS/RAF/MEK/ERK pathway. BRAF inhibitors, by paradoxically activating the MAP kinases, favoured the development of secondary tumors, especially cutaneous squamous cell carcinomas. Also, the main cause of BRAF inhibitors monotherapy failure was represented by acquisition of resistance mechanisms mainly due to a reactivation of the MAP kinase pathway. Therefore, the association of BRAF and MEK inhibitors demonstrated improved efficacy as compared to BRAF inhibitor alone, delayed resistance as well as different safety profile in particular low risk of squamous cutaneous cancer. [122] [123] Dabrafenib in combination with trametinib was first approved in 2014 by FDA for BRAF-V600E/K mutated metastatic melanoma. Before the latest site-independent indication, the combination of dabrafenib and trametinib was approved in melanoma, NSCLC, and anaplastic thyroid cancer.

The clinical efficacy and safety data supporting the FDA approval of dabrafenib/trametinib in BRAF-V600E mutated solid tumors is based on an analysis including a total of 131 adult patients from the open-label, multiple cohort trials BRF117019 and NCI-MATCH, plus 36 pediatric patients from CTMT212X2101. As explained above, those evidence were supported by results in COMBI-d, COMBI-v, and BRF113928 (studies in melanoma and lung cancer already described in product labeling).

Study BRF117019 enrolled patients with BRAF V600E mutation positive specific solid tumors including high- and low-grade glioma, biliary tract cancer, adenocarcinoma of small intestine, gastrointestinal stromal tumor, and anaplastic thyroid cancer, while NCI-MATCH Subprotocol H enrolled adult patients with BRAF V600E mutation positive solid tumors except melanoma, thyroid cancer, and colorectal cancer. Parts C and D of Study CTMT212X2101 enrolled 36 pediatric patients with BRAF V600 refractory or recurrent low- and high-grade glioma. Overall response rate (ORR) was the main efficacy endpoint. ORR was 41% (95% CI: 33, 50) in 131 adult patients. A total of 24 tumor types were represented, the most numerous being biliary tract cancer (ORR 46%, 95% CI: 31, 61), high grade glioma (ORR 33%, 95% CI: 20, 48) and low-grade glioma (ORR 50%, 95% CI: 23, 77). For the 36 pediatric patients, ORR was 25% (95% CI: 12, 42) in the 36 pediatric patients. DOR was ≥6 months for 78% of patients and ≥24 months for 44% of patients.

The most common adverse reactions in adult patients were pyrexia, fatigue, nausea, rash, chills, headache, hemorrhage, cough, vomiting, constipation, diarrhea, myalgia, arthralgia, and edema, while in pediatric patients those were pyrexia, rash, vomiting, fatigue, dry skin, cough, diarrhea, dermatitis acneiform, headache, abdominal pain, nausea, hemorrhage, constipation, and paronychia. [113]

Post-approval data

The FDA granted to the combination dabrafenib/trametinib an accelerated approval, which is contingent to post-marketing studies/clinical trials that aim to verify and describe clinical benefit. In detail, FDA requested clinical trial(s) in at least 80 patients with solid tumors harbouring a BRAF-V600E mutation (including those tumor types less represented in the initial dosser), with the aim to have a more precise estimation of the ORR and mature response duration. To characterize the response rate and duration, patients should be followed for at least 6 months from the onset of response. The submission of the final report to FDA is expected by October 2028. [124]

Selpercatinib in RET fusion positive solid tumors (FDA)

FDA approved selpercatinib (Retevmo) in September 2022 for adult patients with locally advanced or metastatic solid tumors with a RET gene fusion that have progressed on or following prior systemic treatment or who have no satisfactory alternative treatment options. This was an accelerated approval; selpercatinib was already on the US market for NSCLC with RET gene fusion, and for medullary thyroid cancer (MTC) with a RET mutation. [125] Selpercatinib is currently not approved in the EU for the same indication above.

Selpercatinib is a first-in-class, small molecule inhibitor of RET. There is a second RET inhibitor drug called pralsetinib. Like selpercatinib, pralsetinib has a tumor specific indication in US for NSCLC and thyroid cancer with RET fusion and medullary thyroid cancer (MTC) with RET mutation. [126] Results of the phase 1/2 ARROW study were published in August 2022, followed closely by the LIBRETTO-001 study of selpercatinib (see below). The ARROW study showed an overall ORR of 57% (95%CI 35–77) among 23 patients with RET-fusion positive solid tumors. [127] At the time of writing, pralsetinib does not have yet an approval in US for a site/histology independent indication.

RET molecular aspects

The oncogene RET (abbreviation for “rearranged during transfection”) is located in chromosome 10, and it encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor (GDNF) family of extracellular signalling molecules. The alternative splicing of the RET gene results in the production of 3 different isoforms of the protein RET, called RET51, RET43 and RET9 (51 and 9 being the most common). Each protein is divided into an N-terminal extracellular domain, a hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase domain.

Changes in RET expression have been observed in several cancer types, from breast to pancreatic cancers and other tumors of the gastrointestinal tract, melanoma and others. RET mutations have been associated with tumor proliferation, invasion, and migration. The most relevant alterations for targeted therapies are however RET fusions or rearrangements, which are somatic juxtapositions of 5' sequences from other genes with 3' RET sequences encoding tyrosine kinase. Fusions of the RET gene have been shown to be activating genomic events leading to oncogenic addiction, causing cell transformation in *in vitro* and *in vivo* experiments and promoting cell proliferation and survival in human cancer cell lines. RET fusions in tumours appear usually mutually exclusive of other known

validated oncogenic drivers. The most common RET fusions are CDCC6-RET and NCOA4-RET (in tyroid cancers) and KIF5B-RET (in NSCLC). In particular, approximately 1-3% of all NSCLC harbour a RET-fusion, while the prevalence of the totality of patients with RET fusion-positive tumour is less known. Also, other aspects such as the natural history, and prognosis of the totality of patients with RET fusion-positive tumour is not well-described in literature. [128] [129]

Clinical data

The "agnostic" approval of selpercatinib by FDA was based on the study LIBRETTO-001, a multicenter, open-label, multi-cohort trial that evaluated 41 patients with RET fusion-positive tumors (other than NSCLC and thyroid cancer) with disease progression on or following prior systemic treatment or who had no satisfactory alternative treatment options. The efficacy evaluation was supported by data in 343 patients with RET fusion-positive NSCLC and thyroid cancer enrolled in the same trial that already are described in the product labelling and were the bases for the previous site-specific approval. Overall response rate (ORR) and duration of response (DOR) as determined by a Blinded Independent Review Committee (BIRC) were the primary efficacy outcomes. ORR was 44% (95% CI: 28, 60) among the 41 evaluable patients, DOR was 24.5 months (95% CI: 9.2, not estimable).

A total of 14 tumor types were represented in the evaluated population, although mostly with only one or 2 patients each, being pancreatic cancer (n=11) and colorectal cancer (n=10) the most common. Response rate and duration of response by tumor type is presented in the table below (from FDA label [130])

Table 15: Efficacy Results by Tumor Type in LIBRETTO-001 (Other *RET* Fusion-Positive Solid Tumors)

Tumor Type	Patients (n = 41)	ORR[1,2]		DOR Range (months)
		n (%)	95% CI	
Pancreatic adenocarcinoma	11	6 (55%)	(23, 83)	2.5, 38.3+
Colorectal	10	2 (20%)	(2.5, 56)	5.6, 13.3
Salivary	4	2 (50%)	(7, 93)	5.7, 28.8+
Unknown primary	3	1 (33%)	(0.8, 91)	9.2
Breast	2	PR, CR	NA	2.3+, 17.3
Sarcoma (soft tissue)	2	PR, SD	NA	14.9+
Xanthogranuloma	2	NE, NE	NA	NA
Carcinoid (bronchial)	1	PR	NA	24.1+
Carcinoma of the skin	1	NE	NA	NA
Cholangiocarcinoma	1	PR	NA	5.6+
Ovarian	1	PR	NA	14.5+
Pulmonary carcinosarcoma	1	NE	NA	NA
Rectal neuroendocrine	1	NE	NA	NA
Small intestine	1	CR	NA	24.5

+ denotes ongoing response.

[1] Confirmed overall response rate assessed by BIRC.

[2] Best overall response for each patient is presented for tumor types with ≤2 patients.

CI = confidence interval, CR = complete response, DOR = duration of response, NA = not applicable, NE = not evaluable, ORR = overall response rate, PR = partial response, SD = stable disease.

The most common adverse reactions of selpercatinib were edema, diarrhea, fatigue, dry mouth, hypertension, abdominal pain, constipation, rash, nausea, and headache. [125]

Post-approval data

The FDA granted to selpercatinib in RET-fusion sold tumors an accelerated approval, which is contingent to provision of further evidence to be collected post-marketing. In details, FDA requested to complete clinical trial(s) to obtain data on the clinical efficacy of selpercatinib through more precise estimation of ORR and mature DOR in at least 60 patients with locally advanced or metastatic RET-fusion positive solid tumors other than NSCLC and thyroid cancer, to be followed for at least 12 months from the onset of response. The additional data should provide a sufficient number of patients with tumor types for which responses require additional characterization (e.g., colorectal cancer, esophagogastric cancer, and glioma). Available data regarding RET fusion partners and co-occurring genetic alterations for all patients should also be provided. The submission of the final report is expected by FDA by December 2025. [131]

Discussion

In recent years, advances in our understanding of the molecular biology of cancer along with availability of high-throughput technologies have led to rapidly evolving treatment paradigm in oncology, with the development of innovative therapies to treat cancer that targets a specific molecular alteration(s), rather than the "traditional" tumor type, intended as that arising from one specific tissue/organ having a specific histology. The term "agnostic" (tumor, tissue or histology-agnostic) is often used to indicate this concept of a drug intended for various tumor types with only the molecular aberration or target in common. Years before the first "agnostic" drug approval however, this terminology was however questioned in literature: indeed, the word "agnostic" stems from the ancient Greek literally meaning "without" (a) "knowledge" (gnōsis). Strictly speaking, histology agnostic trials would imply that nothing is known about the histology of the tumor, so, pure histology agnostic trial does not exist, according to these authors. [132] [133] Therefore, "site and histology independent" can be considered better reflecting this concept of drugs developed and indicated across different tumor types.

The EU approvals of the NTRK-inhibitors larotrectinib and entrectinib showed the first opening of the European regulatory system to this new approach of drug development and tumor treatment. Although a European guideline specific on this topic is not yet available (while FDA recently released one [134]), some principles important for a biomarker-driven indication to be considered justifiable can be extrapolated from the public EMA assessment documents of larotrectinib and entrectinib.

Firstly, a clear understanding of the targeted molecular alteration, as well as full knowledge of the mechanism of action of the drug, should be established. Non-clinical data are important in this regard to define the rationale for the relevance of the molecular target, including its role in tumorigenesis and in relation to the drug activity. Targeting, by a specific inhibitor, an oncogenic driver mutation, i.e. those responsible for the initiation and maintenance of the cancer, may in theory block the relevant oncogenic pathway ultimately results in cancer cell death, regardless the type of tumor driven by such alteration. In the context of the larotrectinib procedure, the Scientific Advisory Group (SAG) agreed that available data do not support the hypothesis that NTRK gene fusions are universally oncogenic "drivers", independently of tumour type/histology and other disease. [9] In some rare tumors and some paediatric malignancies where NTRK gene fusion are

pathognomonic, preclinical and clinical data can support NTRK as an oncogenic driver, and indeed both larotrectinib and entrectinib demonstrated very high activity against those tumors (≥ 90% of ORR). The importance of a deep understanding of the biology of the targeted molecular alteration before considering a histology-independent indication is well exemplified by the BRAF history, where it was discovered that the very low activity of BRAF inhibitors in colon cancer was due to different molecular mechanisms activated by BRAF inhibition in melanoma and colon cancer, pointing toward a complex interplay between molecular alteration and primary site of the tumor. Accumulating biological data post-approval is also relevant to assess how activity of a certain drug can be modulated, indeed the evaluation of the impact of co-mutations and molecular mechanism of resistance is included as post-approval commitment of NTRK-inhibitors.

The rationale supporting the targeted molecular alteration was somewhat differently interpreted by EMA and FDA with regard to the MSI-H/dMMR biomarker to be used for a site and histology independent use of anti-PD1 treatment. While the FDA agreed with the assumption of MSI-H/dMMR as a predictive histology independent biomarker, MSI-H/dMMR has not been demonstrated being a driver mutation, and the rationale for its use is to indirectly predict a high mutational burden and tumour-specific neoantigen load. Contrary to the US, the company has not applied for a site and histology independent indication in the EU, and due to the substantial heterogeneity of response seen in the 6 tumor types presented for approval, in the EU the benefit/risk ratio was analysed in a “classical” way by tumor types. Far less “success” has been achieved by TMB as biomarker of immunotherapy, probably too early approved in the US in relation to the limited amount of data available at that time and the limited exploratory work (e.g. doubts on the cut-off chosen).

At the clinical level, what is expected if the site and histology independent concept holds, is the demonstration of homogeneity of treatment effect, i.e. reasonable consistency of activity and responses across tumor types. The absence/very limited activity in one or more specific tumor type can indeed put into question any histology independent approach. Due to the rarity of NTRK fusion-positive cancer, patients were studied across multiple tumour types with a limited number of patients in some tumour types, causing uncertainty in the ORR estimate per tumour type. In addition, some tumor types were not represented at all (in this regard, collection of non-clinical evidence where clinical data are lacking, especially in very rare setting, may be supportive). The

understanding of the extent that tissue of origin is an effect modifier was thus considered incomplete, and a warning to prescribers was included in the product information of larotrectinib and entrectinib. Again, collection of additional evidence post-approval is also aimed to answer to this uncertainty. It will be of interest to review the results of the post-approval studies requested at the time of the marketing authorizations due in the next few years.

The evidence submitted to regulatory authorities for all the (EU and US) approved site and histology independent indications are based on uncontrolled single arm trials, basket trials, even pooling of patients' data extrapolated from different phase I/II studies. While in single arm trials for oncologic products the activity of the drug can be assessed in terms of response rate and duration of response, the interpretation of survival data (e.g. progression free survival, overall survival) is hampered by the uncontrolled design, as well as, for histology independent trials, by the inclusion in the study of multiple tumor types that may have differences in natural history or aggressiveness of the disease. [135] For the NTRK inhibitors, the CHMP noted during the assessment that the pooled analyses contained a mix of data intended as pivotal or not, increasing the risk of incorrectly concluding efficacy, while those data are usually considered more appropriate for exploratory purpose. Further confirmation was indeed requested post-approval. The selection of patients e.g. in a pooled analysis may also partly impair the external validity of the trial. While it can be acknowledged that randomized clinical trials may not be always feasible due to the rarity of a molecular alterations (see NTRK), the possibility to conduct randomised trials e.g. in one or in an homogeneous selection tumor type should always be explored and strongly considered, being RCT comparing a new treatment to standard of care widely considered as the gold standard for generating regulatory pivotal evidence to assess drug efficacy and safety. In addition, the availability of randomized clinical trials previously conducted in one site/histology harbouring target biomarker, where the drug has already demonstrated benefit, may be useful to support subsequent site and histology indications, also in terms of safety data.

As reported in the EU labels of the NTRK-inhibitors, due to the above-described uncertainties larotrectinib and entrectinib should only be used if there are no satisfactory treatment options (i.e., for which clinical benefit has not been established, or where such treatment options have been exhausted), defining therefore a situation of unmet medical need. In the presence of established treatment options providing clinical benefit in the target population, a demonstration of relative

efficacy and safety (via an RCT) is expected. The overall data package was therefore considered non-comprehensive and an approval though the Conditional Marketing Authorization pathway was indeed used for both NTRK-inhibitors, subject to additional post approval data. Although not fully comparable, also all the site and histology indications authorized by FDA were accelerated approval, contingent to post-marketing studies/clinical trials that aim to verify and describe clinical benefit.

While regulatory agencies such EMA and FDA appears to have somewhat been able to partially accept some of the peculiarity of the new site and histology independent indications, much controversies has been found from the national HTA side, as demonstrating the value of site and histology independent therapies is challenged within the existing appraisal framework. Evidence is usually required by HTA on how well the drug compares with other standard treatment. However, the type of indication as well as the type of evidence available (usually the same submitted for marketing authorization) hamper such evaluation. This can lead to substantial variation in relevant outcomes that influence the clinical and cost effectiveness of the drug. Therefore, final HTA decisions can be very different in each European countries, as clearly showed by the analysis of the HTA assessment/reimbursement outcomes for the NTRK inhibitors. In some countries, reimbursement by the national healthcare system was denied, on the contrary in other countries drugs were reimbursed for their full indications. Some countries solved the challenges by allowing access only to part of the indications, while in other cases some sort of conditional reimbursement was agreed, subject for example to inclusion of treated patients' data in registries, collection of real-world evidence and planning of further reassessment of new evidence after a certain period. Introduction of new methodologies in the HTA evaluation, alternative authorization and reimbursement procedures e.g. subject to outcome of the single patients, and more importantly implementing the collection of further efficacy and safety evidence within the clinical use of drug in the real world setting, can be valid instruments to respond to the need of HTA bodies, in order to allow (and not to further delay) access to innovative drugs to patients after they have been already approved. In this regard, early dialogue between the companies and all stakeholders can be helpful to plan in advance what would be required and streamline the development of this kind of drug, for example the parallel European Scientific Advice by EMA and HTA bodies is a good available regulatory instrument that should be encouraged. [136]

One crucial aspect allowing to really implement the use in clinical practice of site and histology independent therapies is the biomarker, which goes hand in hand with the development of innovative *in vitro* diagnostic (IVD) tests. As a site and histology independent indication is only based on a molecular alteration, biomarkers should be validated to be able to correctly identify patient subgroups likely to respond to treatment and for whom a certain drug is indicated. This is relevant at the regulatory assessment stage, as the performance of the IVD may have an impact on the benefit-risk balance of the medicine. The new In Vitro Diagnostic Regulation (EU) 2017/746 (IVDR) introduces important changes in the EU legal framework for companion diagnostics (CDx) and will allow stronger connection between the assessment of a drug and of the relevant CDx. The other aspect is the implementation of molecular testing in the real clinical setting. This is a more general issues that goes beyond the HTA assessment and reimbursement processes, but without access to molecular tumor evaluation drugs having site and histology independent indication could not be used by patients in the end. A very recent ESMO study showed that precision oncology is at present out of reach of the majority of European patients, one of the problem being indeed the large-scale availability of the more advanced genetic testing/techniques. [137] The possibility to have large-scale testing in clinic might also increase the possibility to collect further data from patients treated in the clinical practice, thus increasing the knowledge about the efficacy and safety of molecularly-driven treatment that may be used both to generate post-approval evidence for regulators as well as supporting evidence for HTA. Within this context however, the collection and then the analysis of Real-World Data would be essential, e.g. through the implementation of international registries, thus ideally generating a “virtuous circle” between approval, reimbursement and clinical use.

Conclusion

Site and histology independent drug development is an innovative approach to cancer treatment, especially in the context of rare target populations. While a growing number of such programme and therapeutic indications can be expected in the future, along with the expansion of molecular tumor profiling, a deeper biological knowledge and generation of stronger and more extensive clinical evidence are essential to support regulatory decisions, along with new tools for evaluation and data collection in the HTA space, with the ultimate aim to allow patients' access to safe and effective drugs bringing real benefit in cancer treatment.

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