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NTRK GENE FUSIONS AND TRK INHIBITORS

NOVEMBER 2019

NTRK, neurotrophic tyrosine receptor kinase

TRK, tropomyosin-receptor kinase

STEERING COMMITTEE

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These slides **summarise the BluePrint document '*NTRK* Gene Fusions and TRK Inhibitors' dated November 2019**

- https://ntrkconnect.info/

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FUNDING

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KEY CLINICAL POINTS

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- *NTRK* (**N**eurotrophic **T**yrosine **R**eceptor **K**inase) gene fusions are **targetable genetic alterations** that drive tumourigenesis
- ***NTRK* gene fusions** can occur either at a high frequency in some rare cancers (**high-prevalence tumours**) or at a low frequency in more common cancer types (**low-prevalence tumours**)
- **Tumour-agnostic treatment** is a genetically informed treatment strategy that enriches for novel targets regardless of tumour histology
- **TRK inhibitors** are a form of tumour-agnostic treatment
  - Two TRK inhibitors are currently approved for use in clinical practice (larotrectinib and entrectinib)
  - Other TRK inhibitors are in clinical development
- A **testing algorithm for *NTRK* gene fusions** has been proposed to identify patients who would benefit from therapies targeting TRK fusion proteins
- Once a patient has been identified as having a ***NTRK* gene fusion, treatment with an approved TRK inhibitor is recommended**

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GENE FUSIONS

GENE FUSIONS: INTRODUCTION

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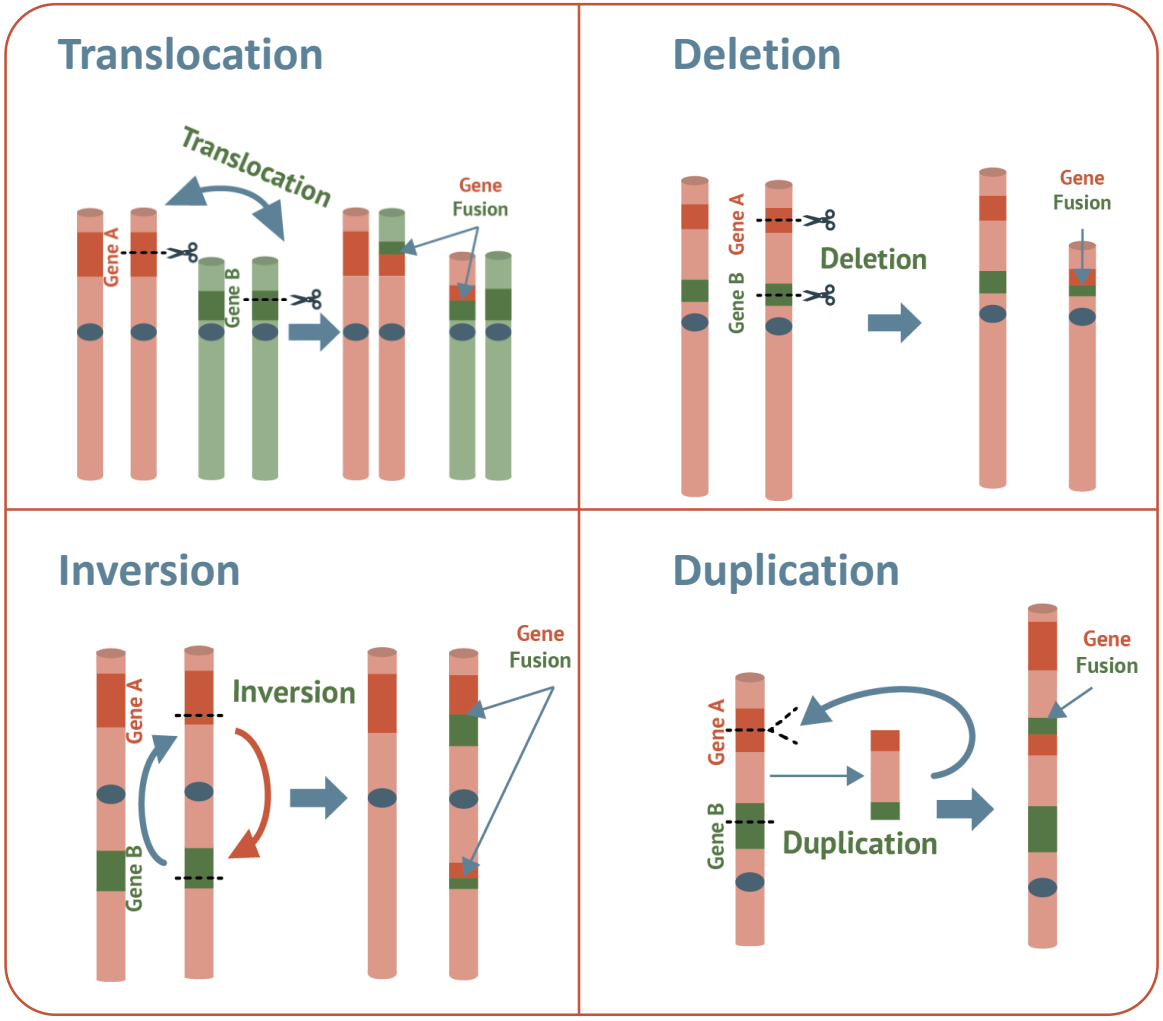

- **Gene fusions** are 2 or more genes that form **chimeric genes** involving parts of each gene
- They can form as a result of **chromosomal rearrangements** or abnormal transcription*

*In the context of NTRK gene fusions, only chromosomal rearrangement is illustrated and presented

GENE FUSIONS: ONCOGENIC DRIVERS

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Gene fusions:

- Constitute **strong driver alterations** in the initial steps of tumour development
- May play important **roles in tumour progression**
- Recently, gene fusions have emerged as **oncogenic drivers** in some solid tumours, including the *NTRK* family, the *FGFR* family, *ROS1*, and *BRAF*
- **Used as diagnostic and prognostic markers** to confirm diagnosis and monitor response to molecular therapies

BRAF, v-Raf murine sarcoma viral oncogene homolog B; CNS, central nervous system; FGFR, fibroblast growth factor receptor; GBM, glioblastoma multiforme; NTRK, neurotrophic tyrosine receptor kinase; ROS1, ROS proto-oncogene 1

Brastianos PK. et al. Oncologist. 2018;23(9):1063-1075

GENE FUSIONS: PRECISION MEDICINE

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- Recurrent gene fusions are **present across multiple cancer types**
- Therefore a type of targeted therapy named **Tumour-agnostic therapy** was designed
  - To treat cancer based on the cancer's genetic and molecular features, regardless of the cancer type or location
  - To use the same drug to treat all cancer types that have the genetic mutation or biomarker targeted by the drug
- Clinical investigation of such drug is conducted through **basket design trials**: patients with tumours harbouring a particular genomic alteration are treated with a matched therapeutic regardless of tumour histology

Offin M. et al. Am Soc Clin Oncol Educ Book. 2018;38:184-187

GENE FUSIONS: TUMOUR-AGNOSTIC THERAPY VS TRADITIONAL CANCER THERAPY

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	Tumour-agnostic treatment	Traditional cancer therapies
Basis of approval	• **Biomarker** present across many tumour types • Biomarkers of response, establishing the effects of context, and deciphering mechanisms of treatment resistance across **a variety of tumour types**	• Consider heterogeneity of drug effects in different biomarker-positive tumour types • Can be directed toward a specific genomic abnormality in **a specific tumour type**
Patient population	• Small patient cohorts with **diverse tumour types** and a **common genomic alteration**	• Large patient cohorts with **tumours originating from a single anatomic site** • Tumours that commonly have **heterogeneous genomic alterations**

GENE FUSIONS: DETECTION METHODS

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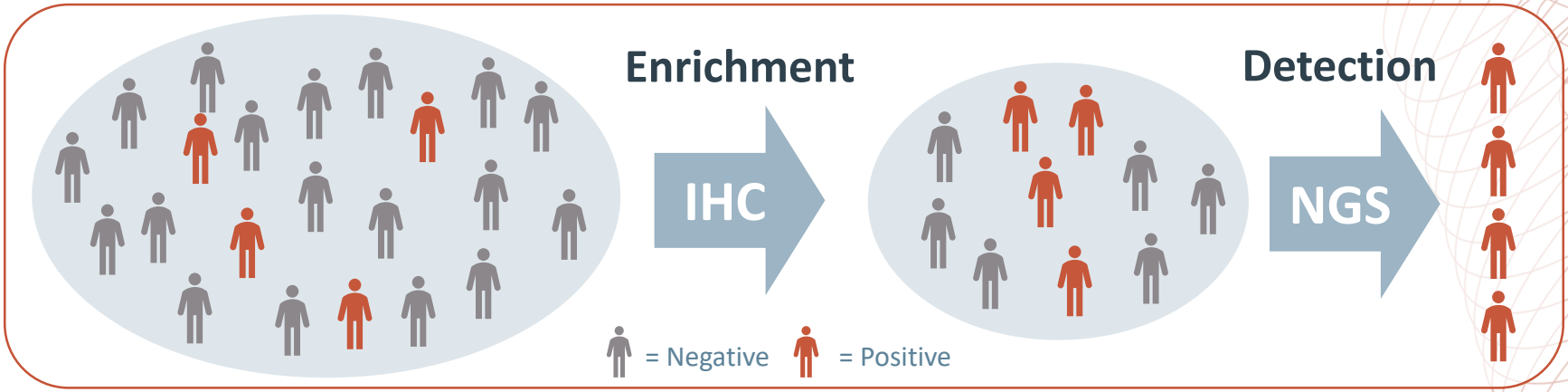
- Testing for gene fusions allows for the **identification of patients** who may benefit from gene-specific inhibitor therapy
- **Target enrichment strategies**: allows for the rapid screening of a large patient population to identify a small percentage who have gene fusions
- Techniques used include DNA-based NGS, RNA-based NGS, RT-PCR, FISH, and IHC
- **Each technique detects the gene fusions either at the DNA, RNA, or protein level**
- Over **22,091 gene fusions** in cancer have been **found and indexed** in the Mitelman Database of Chromosome Aberrations and Gene Fusions in cancer[1]



FISH, fluorescence *in situ* hybridisation; IHC, immunohistochemistry; NGS, next-generation sequencing; RT-PCR, reverse-transcriptase PCR

[1]last update 15 October 2019; https://mitelmandatabase.isb-cgc.org/about

GENE FUSIONS: DETECTION METHODS DNA LEVEL

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Detection methods	Use	Advantages	Disadvantages
DNA-based NGS	Detects known and novel fusions with breakpoints in DNA	• **Assesses molecular information** such as mutations, copy number changes, and microsatellite instability status with high resolution • **Detects rearrangements** in multiple genes and introns • Can **identify translocation** partners without direct targeting of these partners	• **High cost** per sample compared with other methods • **Long turnaround time** of approximately 1 to 3 weeks • Potentially **lower sensitivity** due to difficulty covering long introns that often harbour breakpoints • DNA-based platforms must tile over introns, requiring more probes, more space on the sequencer, and more starting material • **Lack of confirmation** at the RNA transcription or protein expression level • **May not work** well if specimen quality, quantity, or tumour **purity is poor** • The amount of data produced places **substantial demands on bioinformatics** and information technologies
FISH	Detects gene rearrangements in DNA that may generate a fusion transcript	• Relatively **quick** • Does **not require much tumour tissue** or high tumour content for a single test • Usually **high specificity and sensitivity** for DNA level rearrangements within a single given gene • Can be **performed on many types of biopsy specimens** including formalin-fixed paraffin-embedded tissue sections	• **Multiple tests are required** as most commercial labs perform single gene, break-apart FISH • DNA level rearrangements are not confirmed to be transcribed (RNA) or expressed (protein) fusions (e.g. may detect 'passenger' alterations) • Requires that the **target gene is known**

FISH, fluorescence *in situ* hybridization; NGS, next-generation sequencing

GENE FUSIONS: DETECTION METHODS

RNA LEVEL

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Detection methods	Use	Advantages	Disadvantages
RNA-based NGS	Detects known and novel fusions with breakpoints in RNA	• **Screens multiple genes** for fusions • Confirms the presence of transcription • Allows large expansion (multiplexing) of the number of targets and throughput of diagnostic testing • **Meets or exceeds the sensitivity** of other methods (i.e. FISH) for detecting gene fusions • Allows **detection of novel partners** • Requires **no prior knowledge** of fusion partners or of intron/exon breakpoints	• **High cost** per sample compared with other methods; however, targeted sequencing of a subset of genes or transcripts can reduce costs • Turnaround time of approximately **1 to 3 weeks** • **Labile nature of RNA**: RNA quality in formalin-fixed paraffin-embedded tissue may be too poor for clinical testing, especially in older blocks • The amount of data produced places **substantial demands on bioinformatics** and information technologies
Anchored Multiplex PCR (AMP)	Detects known and novel fusions	• Can **detect partner genes** in fusions even when the partner gene is not targeted by the panel • Targets RNA • Has demonstrated **diagnostic utility** for detection of gene fusions	• Does not detect other genomic events • **Cost and turnaround time are higher and longer** than FISH, IHC, or RT-PCR • **Uses RNA**, which is sometimes not of sufficient quality
RT-PCR	Detects known fusion transcripts in RNA	• **High sensitivity and specificity** for a known fusion transcript • Relatively **fast** • **Low cost**	• RT-PCR uses RNA, which may not always be of **sufficient quality** due to its labile nature • There is limited multiplexing ability as RT-PCR can only look at **detect a few targets**, whereas NGS can look at hundreds of targets at the same time
Nanostring	Detects the expression of known gene fusions	• There is no need to run multiple individual FISH or RT-PCR tests for single genes or single fusion variants • Has **high specificity** • Provides **rapid results** • Provides **easier data analysis** compared with NGS	• Requirement for **more input nucleic acids** than some other methods • **Inability to detect novel gene fusions** variants

FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; RT-PCR, reverse transcriptase-polymerase chain reaction.

GENE FUSIONS: DETECTION METHODS PROTEIN LEVEL AND OTHER METHODS

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Detection methods	Use	Advantages	Disadvantages
IHC	Useful in detecting fusion protein	• **Useful for screening** as it is sensitive • **Low cost** • Can be used even **in low-prevalence tumours** • **Fast turnaround time**	• Confirmation of fusion by a **second assay is recommended**, particularly when cytoplasmic staining is present without nuclear, peri-nuclear, or membranous staining • Similarly, **confirmation of fusion by a second assay is recommended** if there is a possibility of smooth muscle or neurologic differentiation
Liquid biopsy	Sampling and analysis of non-solid biological tissue (primarly blood)	• **Minimally invasive** technique • Used to **detect a range of gene fusions** (*ALK*, *RET*, *ROS1* and *NTRK1* gene fusions) from plasma, serum, circulating tumour cells, platelets and exosomes • **Can be performed over time during treatment** and therefore can be used more easily than tissue biopsy for detecting molecular alterations arising during tumour evolution and those driving acquired resistance	• Are **not as sensitive** as IHC or FISH for testing gene fusions • If circulating DNA and RNA volume is low (e.g. when the tumour volume is low), a **test failure or false negative** may result • **Not available for routine clinical** use as further development is required

ALK, Anaplastic lymphoma kinase; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; RT-PCR, reverse transcriptase-polymerase chain reaction.

NTRK FAMILY

NTRK FAMILY: 3 GENES

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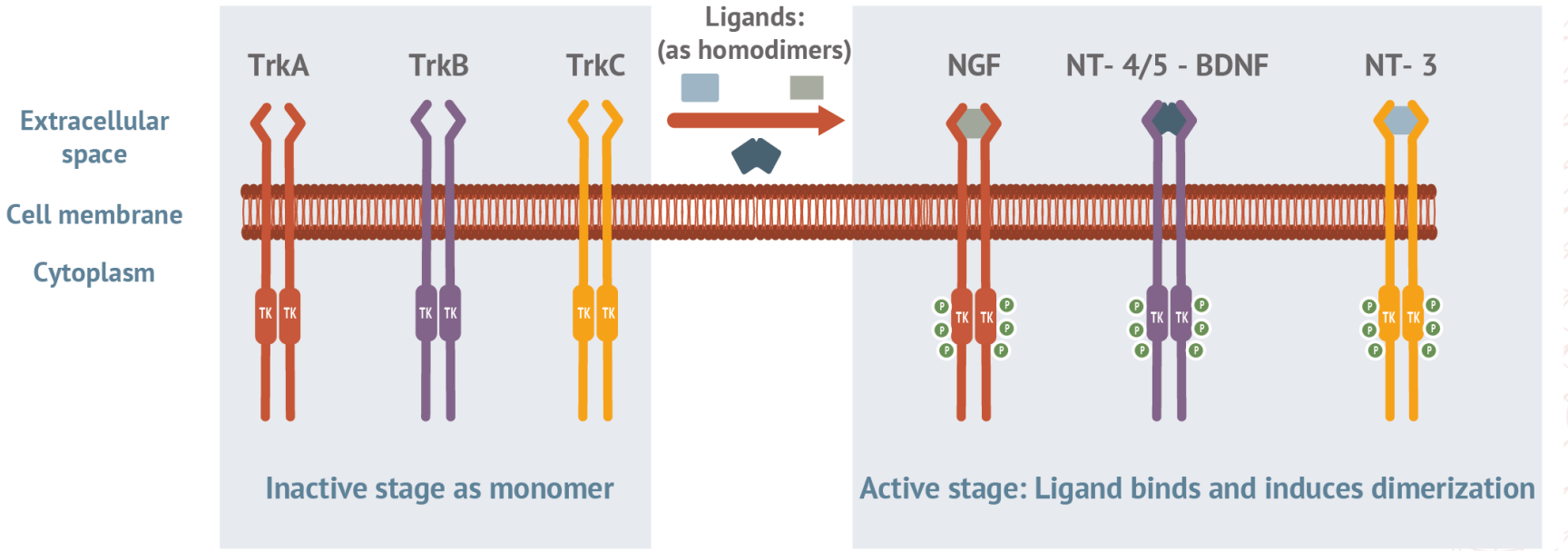
- NTRK1, NTRK2 and NTRK3 (Neurotrophic Tyrosine Receptor Kinase)
- Encoding for transmembrane Tropomyosin Receptor Kinase: TRKA, TRKB, and TRKC



BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NT-3, neurotrophin 3; NT-4/5, neurotrophin 4/5; P, autophosporylation; TK, tyrosine kinase domain

Cocco E. et al. Nat Rev Clin Oncol. 2018;15(12):731-747

TRK FAMILY: PHYSIOLOGICAL ROLE

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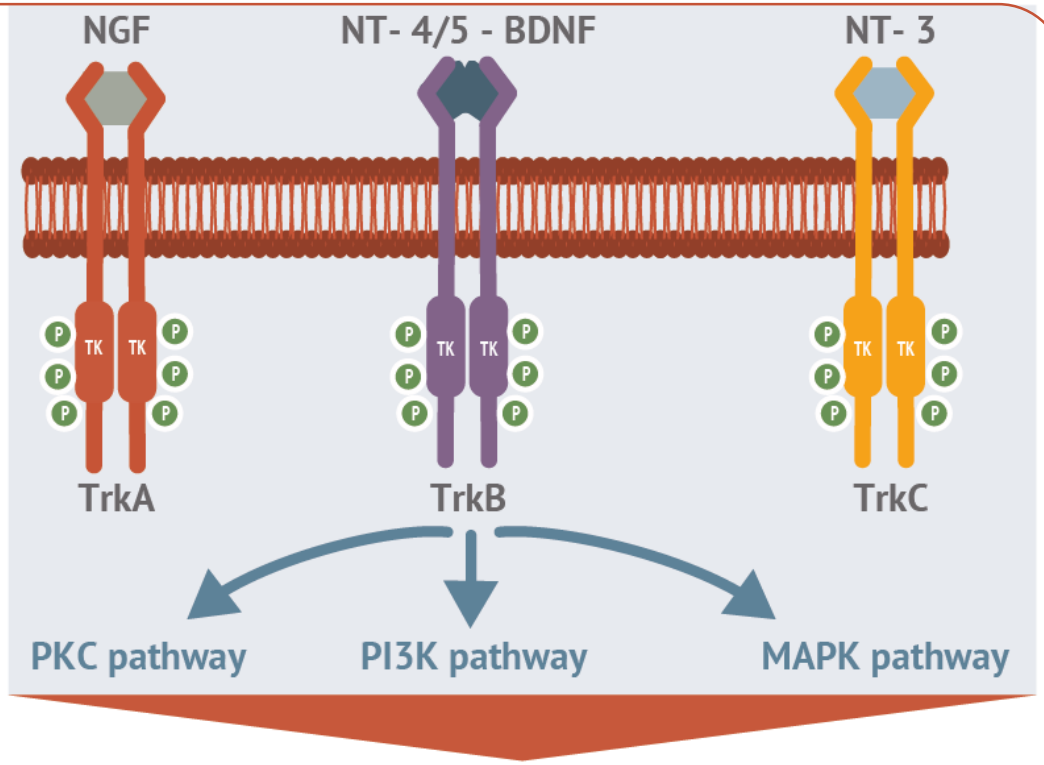


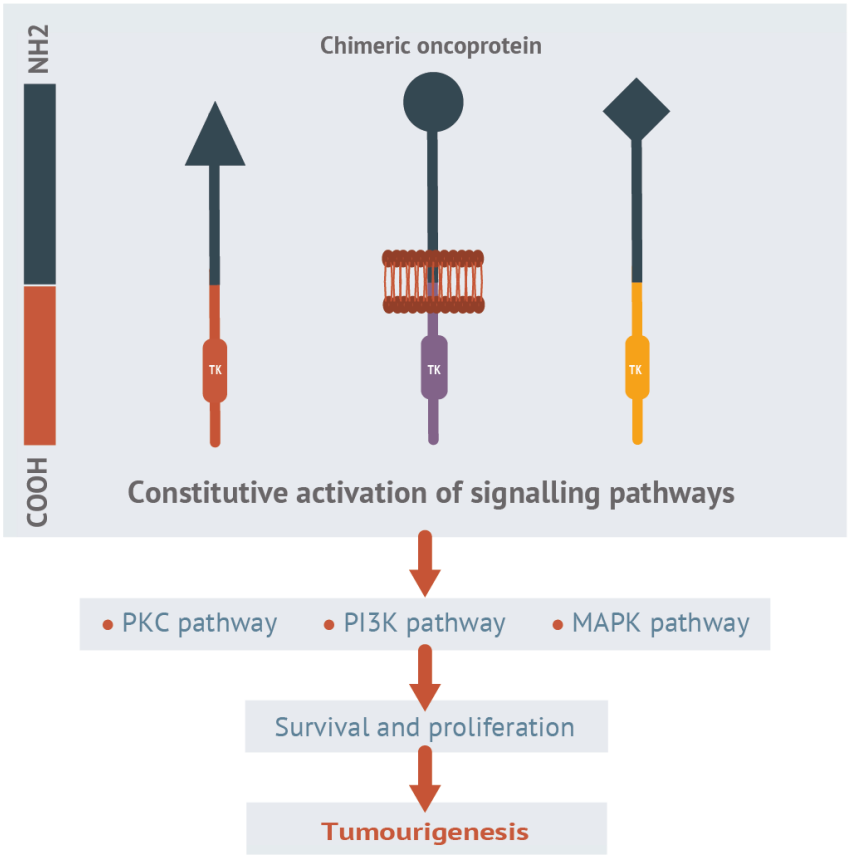
Physiological functions

1. Differentiation and survival of neurons
2. Synapse formation and plasticity
3. Membrane trafficking
4. Formation of axons and dendrites

Activation of 3 signalling pathways involved in **proliferation**, **differentiation** and **survival**

BDNF, brain-derived neurotrophic factor; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NT-3, neurotrophin 3; NT-4/5, neurotrophin 4/5; P, autophosporylation; PI3K, Phosphoinositide 3-kinase; PKC, protein kinase C; TK, tyrosine kinase domain

NTRK GENE FUSIONS: CHIMERIC ONCOPROTEINS



NTRK gene fusions are targetable genetic alterations that code for fusion proteins and drive tumourigenesis

MAPK, mitogen-activated protein kinase; PI3K, Phosphoinositide 3-kinase; PKC, protein kinase C; TK, tyrosine kinase domain

Chen Y. and Chi P. J Hematol Oncol. 2018;11(1):78; Cocco E. et al. Nat Rev Clin Oncol. 2018;15(12):731-747; Stransky N. et al. Nat Commun. 2014;5:4846; Vaishnavi A. et al. Cancer Discov. 2015;5(1):25-34

NTRK GENE FUSIONS STRUCTURE

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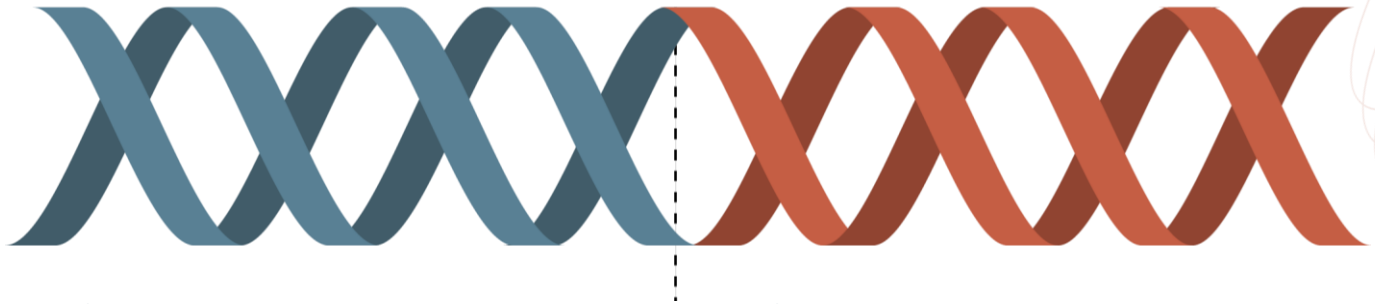
Characteristics of all identified *NTRK* gene fusions:

1. Created by chromosomal rearrangement
2. Fusion of the **3' region of the *NTRK* gene** with the 5' region of a different gene (= the **fusion partner**)
3. The **tyrosine kinase (TK) domain** is **always present**
4. The transmembrane domain is not present in all

NTRK fusion



5' upstream gene partner

3' *NTRK1*, *NTRK2* or *NTRK3*

MAPK, mitogen-activated protein kinase; PI3K, Phosphoinositide 3-kinase; PKC, protein kinase C; TK, tyrosine kinase domain

Chen Y. and Chi P. J Hematol Oncol. 2018;11(1):78; Cocco E. et al. Nat Rev Clin Oncol. 2018;15(12):731-747; Stransky N. et al. Nat Commun. 2014;5:4846; Vaishnavi A. et al. Cancer Discov. 2015;5(1):25-34

NTRK GENE FUSIONS: TUMOURS ACCORDING TO FREQUENCY OF DETECTION

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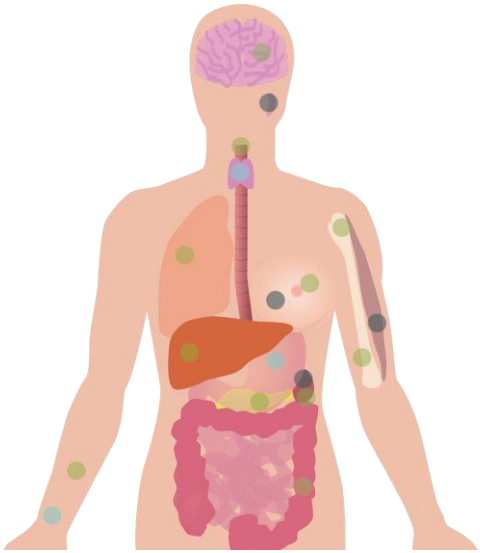
- *NTRK* gene fusions can occur either at a high frequency in some rare cancers (high-prevalence tumours) or at a low frequency in more common cancer types (low-prevalence tumours)[a]

High-prevalence tumours
- Frequency of *NTRK* gene fusions >90%

Low-prevalence tumours
- Frequency 5% to 25%
- Frequency <5%

Cancers enriched for TRK fusions

Frequency >90%
- MASC
- Secretory breast carcinoma[b]
- Cellular and mixed congenital mesoblastic nephroma[d]
- Infantile fibrosarcoma[d]



Cancers harbouring TRK fusions at lower frequencies

Frequency 5% to 25%
- Gastrointestinal stromal tumour (pan-negative)
- Thyroid cancer[c]
- Spitzoid tumours

Frequency <5%
- Acute lymphoblastic leukaemia, acute myeloid leukaemia,histiocytosis, multiple myeloma and dendritic cell neoplasms
- Sarcoma[d]
- Breast cancer
- Colorectal cancer
- Cholangiocarcinoma
- High-grade glioma[b]
- Head and neck cancer
- Lung cancer
- Pancreatic cancer
- Melanoma
- Renal cell carcinom[a]
- Sarcoma

[a]Found in adult cancers only, unless indicated; [b]found in adult and paediatric cases; [c]found in adult cases as thyroid cancer and papillary thyroid cancer in paediatric cases; [d]found in paediatric cases only.

MASC, mammary analogue secretory carcinoma. See appendix section for more details about *NTRK* gene frequency and involvement in each tumour

Amatu A. et al. ESMO Open. 2016;1(2):e000023; Cocco E. et al. Nat Rev Clin Oncol. 2018;15(12):731-747; Stransky N. et al. Nat Commun. 2014;5:4846;

NTRK GENE FUSIONS: RECOMMENDED TESTING ALGORITHM[a]

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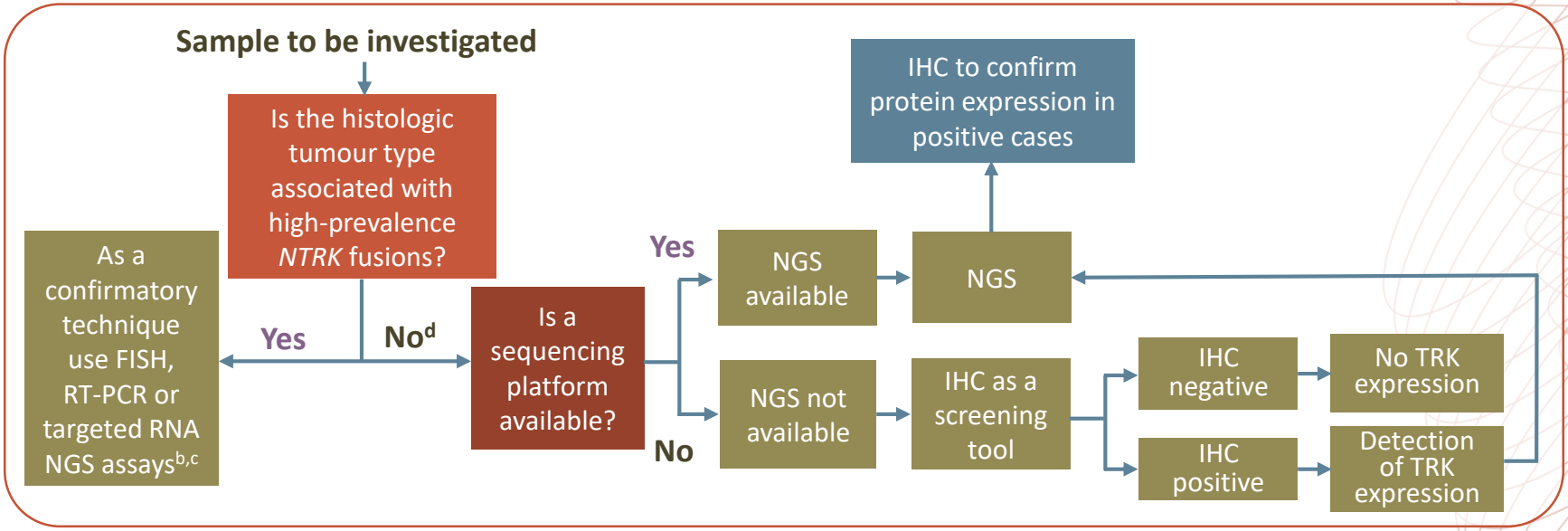
- In **high-prevalence tumours:** best options as confirmatory techniques are **FISH, RT-PCR or RNA-based targeted panels**
- In **low-prevalence tumours:** ideal option is a **DNA- or RNA-based NGS** targeted panel that reliably detects *NTRK* gene fusions



[a]Based on ESMO 2019 guidelines for NTRK fusion detection and guidelines for TRK fusion cancer in children by Albert et al. 2019; [b]Using specific probes for the rearrangement involving the known NTRK gene; [c]Albert et al., note that RT-PCR is not routinely used in clinical practice and limited data are available using this technique for NTRK fusion detection; [d]ESMO guidelines note that this population would be likely represented by "any malignancy at an advanced stage, in particular if it has been proven wild type for other known genetic alterations tested in routine practice, and especially if diagnosed in young patients".

FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; RT-PCR, reverse transcriptase-polymerase chain reaction.; Albert C.M. et al. J Clin Oncol. 2019;37(6):513-524; Marchiò C. et al. Ann Oncol 2019 [Epub ahead of print]; Mateo J. et al. Ann Oncol. 2018;29(9):1895-1902

TRK INHIBITOR THERAPY

TRK INHIBITORS: APPROVED PRODUCTS

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Drug (company)	Larotrectinib (Bayer)	Entrectinib (Roche / Genentech)
Approved US indication	For the treatment of adult and pediatric patients with solid tumors that: • have a neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion without a known acquired resistance mutation, • are metastatic or where surgical resection is likely to result in severe morbidity, and • have no satisfactory alternative treatments or that have progressed following treatment.	For the treatment of: 1. Adult patients with metastatic non-small cell lung cancer (NSCLC) whose tumours are ROS1-positive. 2. Adult and pediatric patients 12 years of age and older with solid tumours that: • have a neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion without a known acquired resistance mutation, • are metastatic or where surgical resection is likely to result in severe morbidity, and • have progressed following treatment or have no satisfactory alternative therapy.
Formulation	• Capsules: 25mg or 100mg • Oral solution: 20mg/ml	Capsules: 100mg or 200mg
Mechanism of Action	• Selective and potent inhibitor of TRKA, TRKB and TRKC	Activity against ALK, ROS1, and TRKA, TRKB, and TRKC proteins
Status	• US FDA accelerated approval: 26 November 2018 • Approval in Brazil: 9 July 2019 • Canada NOC/c: 16 July 2019 • EU conditional approval: 19 September 2019	• Japan MHLW approval: 18 June 2019 • US FDA approval: 15 August 2019

ALK, anaplastic lymphoma kinase; NOC/c, Notice of compliance with conditions; FDA; Food and Drug Administration; MHLW, Ministry of Health, Labour and Welfare; ROS1, ROS proto-oncogene 1

TRK INHIBITORS: APPROVED PRODUCTS - CLINICAL EFFICACY RESULTS

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- **The efficacy of larotrectinib**: evaluated in paediatric and adult patients with unresectable or metastatic solid tumours with a *NTRK* gene fusion enrolled in one of three multicenter, open-label, single-arm clinical trials: Study LOXO-TRK-14001 (NCT02122913), SCOUT (NCT02637687), and NAVIGATE (NCT02576431). (Cut off date July 2017 for the NDA approval)
- **The efficacy of entrectinib**: evaluated in adult patients with unresectable or metastatic solid tumours with a *NTRK* gene fusion enrolled in one of three multicenter, single-arm, open-label clinical trials: ALKA, STARTRK-1 (NCT02097810) and STARTRK-2 (NCT02568267)

Efficacy Parameters	Larotrectinib (N=55)[1]	Entrectinib (N=54) [1]
Overall response rate (95% CI)	75% (61%, 85%)	57% (43, 71)
Complete response rate	22%	7.4%
Partial response rate	53%	50%
Observed duration of response[2]	**Larotrectinib (N=41)[1]**	**Entrectinib (N=31)[1]**
Range (months)	1.6+, 33.2+	2.8, 26.0+
% with duration ≥ 6 months	73%	68%
% with duration ≥ 9 months	63%	61%
% with duration ≥ 12 months	39%	45%

[1] Disclaimer: Efficacy data presentation come from the US FDA approved labels, differences in trial design do not allow for direct comparison of efficacy data (larotrectinib vs entrectinib)
[2] For larotrectinib: Median duration of response not reached at time of data cutoff
CI, confidence intervals; NDA; New drug application; Efficacy data sources: US label for larotrectinib (date: 26 November 2018) and entrectinib (date: 15 August 2019)

TRK INHIBITORS: APPROVED PRODUCTS - CLINICAL SAFETY DATA

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- **The safety profile of larotrectinib:**
  - The most common adverse reactions (≥ 20%) were fatigue, nausea, dizziness, vomiting, anaemia, increased AST, cough, increased ALT, constipation, and diarrhoea
  - The most common serious adverse reactions (≥ 2%) were pyrexia, diarrhoea, sepsis, abdominal pain, dehydration, cellulitis, and vomiting
  - Grade 3 or 4 adverse reactions occurred in 51% of patients

- **The safety profile of entrectinib:**
  - The most common adverse reactions (≥ 20%) were fatigue, constipation, dysgeusia, oedema, dizziness, diarrhoea, nausea, dysesthesia, dyspnoea, myalgia, cognitive impairment, increased weight, cough, vomiting, pyrexia, arthralgia and vision disorders
  - The most frequent serious adverse reactions (≥ 2%) were pneumonia, dyspnoea, pleural effusion, sepsis, pulmonary embolism, respiratory failure, and pyrexia
  - Grade 3 or 4 adverse reactions occurred in 60% of patients

ALT, alanine transaminase; AST, aspartate transaminase

Safety profile sources: US label for larotrectinib (date: 26 November 2018) and entrectinib (date: 15 August 2019)

TRK INHIBITORS: APPROVED PRODUCTS - ACQUIRED RESISTANCE MECHANISM

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- **Resistance to TRK inhibition** by larotrectinib and entrectinib is **mediated by recurrent mutations to the kinase domain** of *NTRK* gene at three different locations (solvent-front mutations, gatekeeper and xDFG):
- **The kinase solvent-front mutation** is mediated through G595R substitution in the TRKA protein and G623R substitution in the TRKC protein
- **The gatekeeper mutation** is found at F589L substitution in the TRKA
- **The xDFG motif mutation** is found at G667C substitution in the TRKA protein and G696A substitution in the TRKC protein

DFG motif, Asp-Phe-Gly motif

TRK INHIBITORS: IN CLINICAL DEVELOPMENT

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- **Objective**: **"Next-generation" TRK inhibitors** are being developed to overcome acquired resistance to first-generation TRK protein kinase inhibitors

Drug (company)	Objective	MoA	Development stage (Clinicaltrials.gov identifier)
Selitrectinib (formerly known as LOXO-195)* (Bayer)	To overcome resistance mediated by solvent-front mutations, and xDFG substitutions	Selectively targets *NTRK* 1/2/3 gene fusions	Phase 1/2 trial (NCT03215511)
Repotrectinib (Turning Point Therapeutics)**	To overcome resistance due to solvent-front substitutions	High selectivity for wild-type and mutated TRKA, TRKB, TRKC, ROS1, and ALK proteins	Phase 1/2 trial (NCT03093116)

*BAY 2731954; ** TPX-0005

ALK, anaplastic lymphoma kinase; DFG motif, Asp-Phe-Gly motif; ROS1, ROS proto-oncogene 1

TRK INHIBITORS: IN CLINICAL DEVELOPMENT

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- **Other TRK inhibitors** are under clinical investigation.
- These are either **multikinase inhibitors** with anti-TRK activity or drugs that are specifically TRK-directed and include:
  - cabozantinib, merestinib, sitravatinib, ONO-7579, PLX-7486, altiratinib and DS-6051b.

APPENDIX

NTRK GENE FUSIONS: TUMOURS GROUPED ACCORDING TO FREQUENCY OF DETECTION

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High-prevalence tumours : Frequency of *NTRK* gene fusions >90%		
Tumour Type	***NTRK* gene fusions involved**	**Frequency**
Breast secretory carcinoma	*NTRK*3	96%
Infantile fibrosarcoma	*NTRK*3	95.5%
MASC ~90%	*NTRK*3	89.1%
Congenital mesoblastic nephroma	*NTRK*3	72.0%

MASC, mammary analogue secretory carcinoma

NTRK GENE FUSIONS: TUMOURS GROUPED ACCORDING TO FREQUENCY OF DETECTION

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Low-prevalence tumours: Frequency 5% to 25% or Frequency < 5%

Tumour Type	*NTRK* gene fusions involved	Frequency
Spitz tumours and spitzoid melanoma	*NTRK*1	16.4%
Papillary thyroid carcinoma	*NTRK*1,3	8.8%
Intrahepatic cholangiocarcinoma	*NTRK*1	3.6%
Astrocytoma	*NTRK*2	3.1%
High-grade glioma	*NTRK*1,2,3	2.1%
Uterine sarcoma	*NTRK*1,3	2.1%
Gastrointestinal stromal tumours	*NTRK*3	1.9%
Lung cancer	*NTRK*1,2	1.7%
Thyroid carcinoma	*NTRK1,3*	1.2%
Glioblastoma	*NTRK*1,2	1.2%
Sarcoma	*NTRK*1	1.0%
Ph-like Acute lymphoblastic leukemia	*NTRK*3	0.7%
Colorectal cancer	*NTRK*1,3	0.61%
Melanoma	*NTRK*3	0.3%
Head and neck cancer	*NTRK*2,3	0.24%
Invasive breast cancer	*NTRK*3	<0.1%

REFERENCES

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1. Cocco E, Scaltriti M, Drilon A. *NTRK* fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin Oncol 2018; 15(12): 731-747.

2. Stransky N, Cerami E, Schalm S et al. The landscape of kinase fusions in cancer. Nat Commun 2014; 5: 4846.

3. Chen Y, Chi P. Basket trial of TRK inhibitors demonstrates efficacy in TRK fusion-positive cancers. J Hematol Oncol 2018; 11: 78.

4. Amatu A, Sartore-Bianchi A, Siena S. *NTRK* gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open 2016; 1: e000023.

5. Vaishnavi A, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. Cancer Discov 2015; 5: 25-34.

6. Mateo J, Chakravarty D, Dienstmann R et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018; 29: 1895-1902.

7. Albert CM, Davis JL, Federman N et al. TRK Fusion Cancers in Children: A Clinical Review and Recommendations for Screening. J Clin Oncol 2019; 37: 513-524.

8. Marchiò C, Scaltriti M, Ladanyi M et al. ESMO recommendations on the standard methods to detect *NTRK* fusions in daily practice and clinical research. Ann Oncol 2019; 2019 Jul 3. pii: mdz204. doi: 10.1093/annonc/mdz204. [Epub ahead of print].

9. Luoh SW and Flaherty KT. When tissue is no longer the issue: Tissue-agnostic cancer therapy comes of age. Ann Intern Med. 2018; 169(4):233-239

10. Offin M, Liu D, Drilon A. Tumor-Agnostic Drug Development. Am Soc Clin Oncol Educ Book 2018; 38: 184-187.

11. Loxo Oncology. FDA Approves Vitrakvi® (larotrectinib), the First Ever TRK Inhibitor, for Patients with Advanced Solid Tumors Harboring an *NTRK* Gene Fusion. 2018.

REFERENCES

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12. US prescribing information of larotrectinib, initial approval (revision: November 2018): https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210861s000lbl.pdf

13. http://portal.anvisa.gov.br/informacoes-tecnicas13?p_p_id=101_INSTANCE_WvKKx2fhdjM2&p_p_col_id=column-2&p_p_col_pos=1&p_p_col_count=2&_101_INSTANCE_WvKKx2fhdjM2_groupId=219201&_101_INSTANCE_WvKKx2fhdjM2_urlTitle=vitrakvi-larotrectinibe-e-vitrakvi-tm-larotrectinibe-novo-registro&_101_INSTANCE_WvKKx2fhdjM2_struts_action=%2Fasset_publisher%2Fview_content&_101_INSTANCE_WvKKx2fhdjM2_assetEntryId=5555812&_101_INSTANCE_WvKKx2fhdjM2_type=content

14. https://www.canada.ca/en/health-canada/services/drugs-health-products/ drug-products/notice-compliance/conditions/vitrakvi-notice-compliance-conditions-qualifying-notice.html

15. EU conditional approval of Vitrakvi® (larotrectinib), dated 19 September 2019 (https://www.ema.europa.eu/en/medicines/human/EPAR/vitrakvi#authorisation-details-section)

16. Roche. Media release, dated 18 June. 2019. https://www.roche.com/media/releases/med-cor-2019-06-18.htm (accessed 24 June 2019).

17. Food & Drug Administration. 2019. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-entrectinib-ntrk-solid-tumors-and-ros-1-nsclc (accessed 20 August 2019).

18. US prescribing information of entrectinib, initial approval (revision: August 2019): https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212725s000lbl.pdf

GLOSSARY

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Term	Definition
FISH	Also known as fluorescence *in situ* hybridisation, it detects gene rearrangements in DNA that may generate a fusion transcript
Fusion protein	A protein created by the joining of two or more genes that originally coded for separate proteins
High-prevalence tumours	Tumours with a high likelihood of *NTRK* gene rearrangements. These include tumours with certain histologies or specific molecular subgroups, such as secretory carcinoma, infantile fibrosarcoma, pan-negative lung adenocarcinoma, wild-type and MSI-high colorectal carcinomas, IDH1 wild-type gliomas, pan-negative GIST and CD34-positive fibrosarcoma of soft tissue and bone.
IHC	Also known as immunohistochemistry, it detects protein expression which may be attributable to a fusion event
Kinase domain	A structurally conserved region of protein kinases that contains the catalytic function of these enzymes
Low-prevalence tumours	Tumours with a low likelihood of *NTRK* gene rearrangements.
NGS	Also known as next-generation sequencing, it detects known and novel fusions with breakpoints in DNA or RNA
***NTRK* gene**	Gene coding for the neurotrophic tyrosine receptor kinase
***NTRK* gene fusions**	Targetable driver genetic alterations that code for aberrant fusion proteins and drive tumourigenesis; these are formed by joining of the 3' region of the *NTRK* gene, including the kinase domain, with the 5' region of a different gene (i.e. the fusion partner) by intra- or inter-chromosomal rearrangement
RT-PCR	Also known as reverse-transcriptase polymerase chain reaction, it detects known fusion transcripts in RNA
TRK fusion proteins	The protein product of *NTRK* gene fusions, these proteins are constitutively active and result in cell growth, proliferation, and survival pathway activation
TRK inhibitors	A type of tyrosine kinase inhibitor and a type of targeted therapy against TRK fusion proteins
Tumour-agnostic treatment	A drug treatment that is used to treat any kind of cancer, regardless of where in the body or what tissue it initiated from
Tyrosine kinase	An enzyme that can transfer a phosphate group from ATP to a protein in a cell, it plays a critical activation and deactivation role in many cellular functions

ATP, adenosine triphosphate; CD34, cluster of differentiation 34; IDH1, isocitrate dehydrogenase 1; GIST, gastrointestinal stromal tumour

ABBREVIATIONS

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ALT	Alanine transaminase
ANVISA	Agência Nacional de Vigilância Sanitária (Brazilian Health Regulatory Agency
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
CD34	Cluster of differentiation 34
CI	Confidence interval
DNA	Deoxyribonucleic acid
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridization
GIST	Gastrointestinal stromal tumour
IDH1	Isocitrate dehydrogenase 1
IHC	Immunohistochemistry
MAPK	Mitogen-activated protein kinase
MASC	Mammary analogue secretory carcinoma
MSI	Microsatellite instability
NDA	New drug application
NGF	Nerve growth factor
NGS	Next-generation sequencing
NT-3	Neurotrophin 3
NT-4/5	neurotrophin 4/5
NTRK	Neurotrophic tyrosine receptor kinase
PKC	Protein kinase C
PI3K	Phosphoinositide 3-kinase
RNA	Ribonucleic acid
RT-PCR	Reverse-transcriptase polymerase chain reaction
TK	Tyrosine kinase
TRK	Tropomyosin receptor kinase

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