NTRK fusion: the importance of detection

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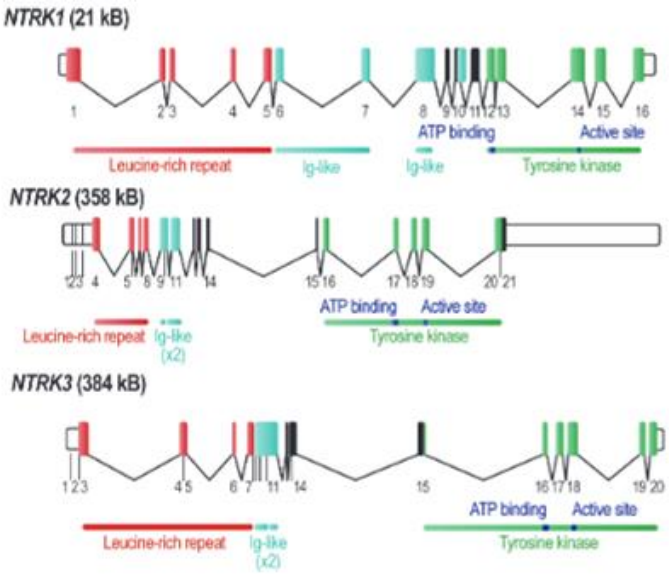
NTRK: structure and function

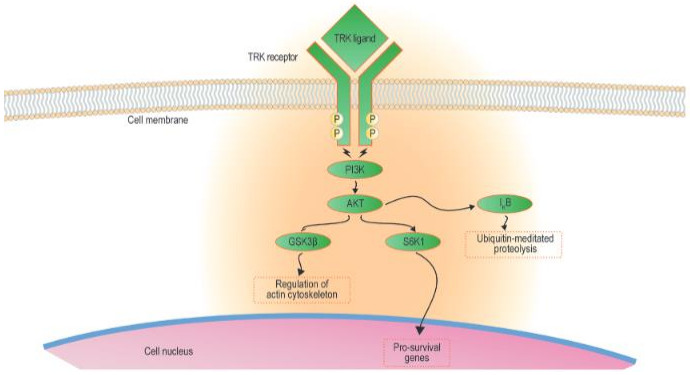
- TRK proteins (tropomyosine kinase A/B/C) are encoded by the genes *NTRK1 (chr1q23.1), NTRK2 (chr9q21.33) and NTRK3 (chr15q25.3).*

- Transmembrane receptors

- Ligands = neurotrophines
  - nerve growth factor (NGF) active TRKA
  - brain-derived neurotrophic growth factor (BDNF) et neurotrophine 4/5 active TRKB
  - neurotrophine 3 active TRKC

- homodimerization and activation of the TK domain:
  - phosphorylation cascade of proteins belonging to signaling pathways regulating functions within the cell.





- Expression limited to the SNC (development and function of the central and peripheral NS)
  - TRKA triggers functions related to pain reception or thermoregulation.
  - TRKB triggers functions related to movement, memory or appetite.
  - TRKC triggers proprioception functions.

Penault-Llorca F, Rudzinski ER, Sepulveda AR. J Clin Pathol 2019;**72**:460–467.

NTRK: gene fusion

- Trk = proto-oncogene (1990)
- 1998 : ETV6-NTRK3 discovery
- Intra- or inter-chromosomal rearrangement
- Juxtaposes region 3 ’of the NTRK gene (TK domain) with region 5’ of a partner gene (dimerization domain)

MOLECULAR AND CELLULAR BIOLOGY, Aug. 1990, p. 4202–4210 Vol. 10, No. 8
0270-7306/90/084202-09$02.00/0

Human *trk* Oncogenes Activated by Point Mutation, In-Frame Deletion, and Duplication of the Tyrosine Kinase Domain

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Received 19 March 1990/Accepted 16 May 1990

Letter | Published: 01 February 1998

A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma

Stevan R. Knezevich, Deborah E. McFadden, Wen Tao, Jerian F. Lim & Poul H.B. Sorensen

Nature Genetics **18**, 184–187 (1998) | Download Citation
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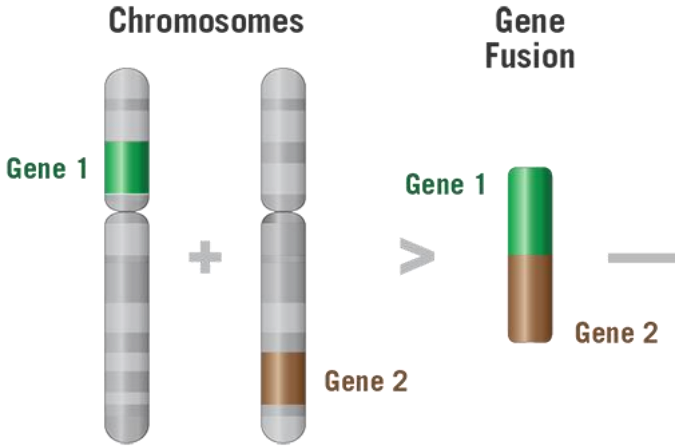
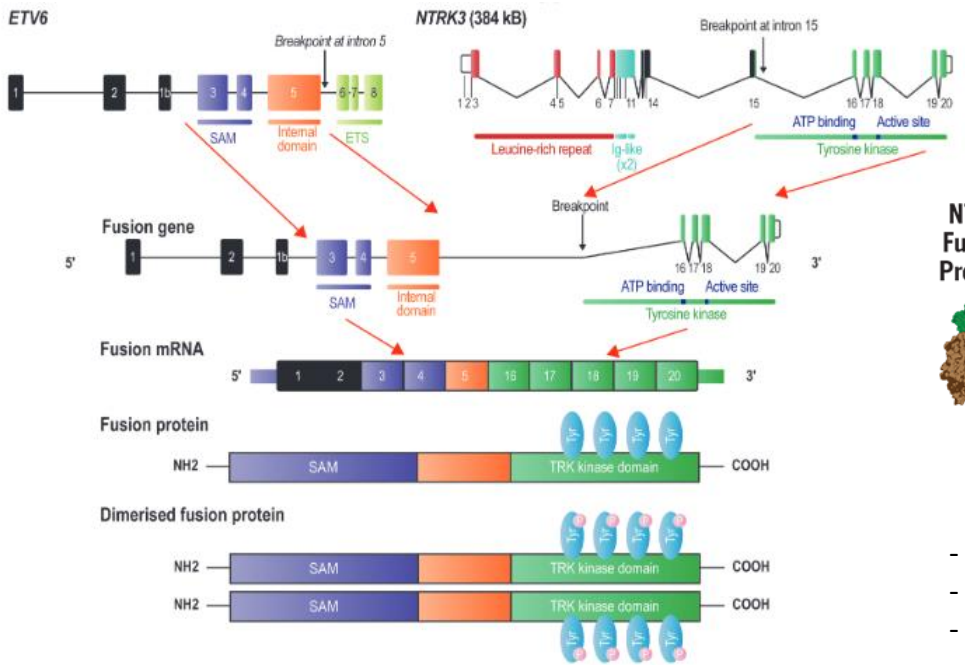
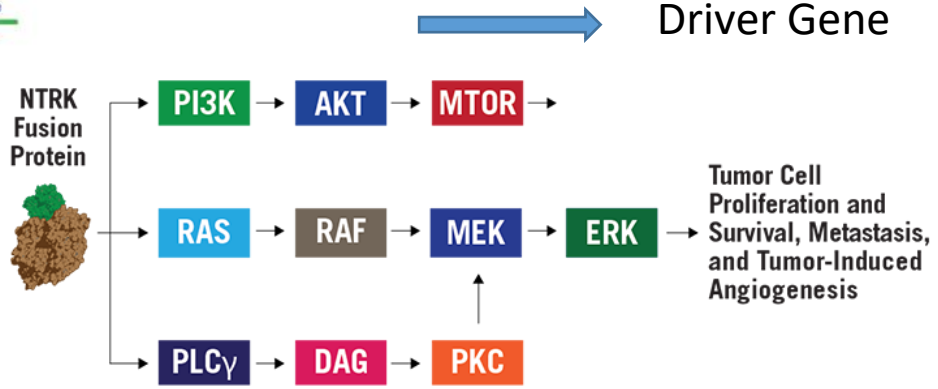




Encodes for a constitutive express fusion protein



- increased cell proliferation
- decrease in apoptosis.
- uncontrolled proliferation of cells that cause a tumor to form.

Penault-Llorca F, Rudzinski ER, Sepulveda AR. J Clin Pathol 2019;**72**:460–467.

NTRK gene fusion & cancer

Oncogenic *NTRK* gene fusion frequencies reported across multiple tumor types[1-11]

Glioblastoma multiforme[1]
- *NTRK1 fusion frequency:* ***1%***

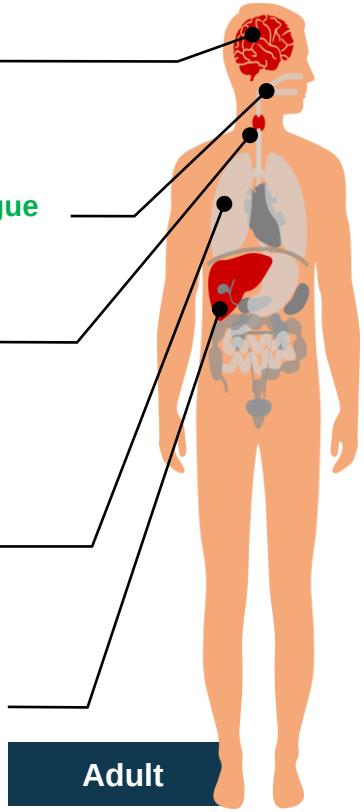
Salivary (MASC = Mammary analogue secretory cancer)[2]
- *NTRK3 fusion frequency:* ***100%***

Papillary thyroid cancer[3,4]
- *NTRK1 fusion frequency:* ***11.8%***
- *NTRK3 fusion frequency (post-Chernobyl):* ***14.5%***

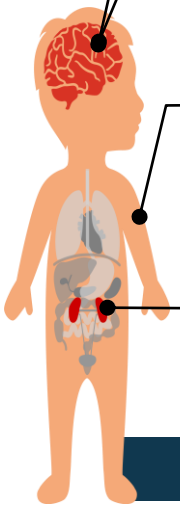
Secretory breast cancer[5]
- *NTRK3 fusion frequency:* ***92%***

Intrahepatic cholangiocarcinoma[6]
- *NTRK1 fusion frequency:* ***3.6%***

Lung cancer / CCRm
- *NTRK fusion frequency:* ***≈ 1%***



Adult



Pediatric/Young adult

Pediatric high-grade glioma[7]
- *NTRK1/2/3 fusion frequency:* ***10%***

Pilocytic astrocytoma[8]
- *NTRK3 fusion frequency:* ***3.1%***

Infantile fibrosarcoma[9,10]
- *NTRK3 fusion frequency:* ***91–100%***

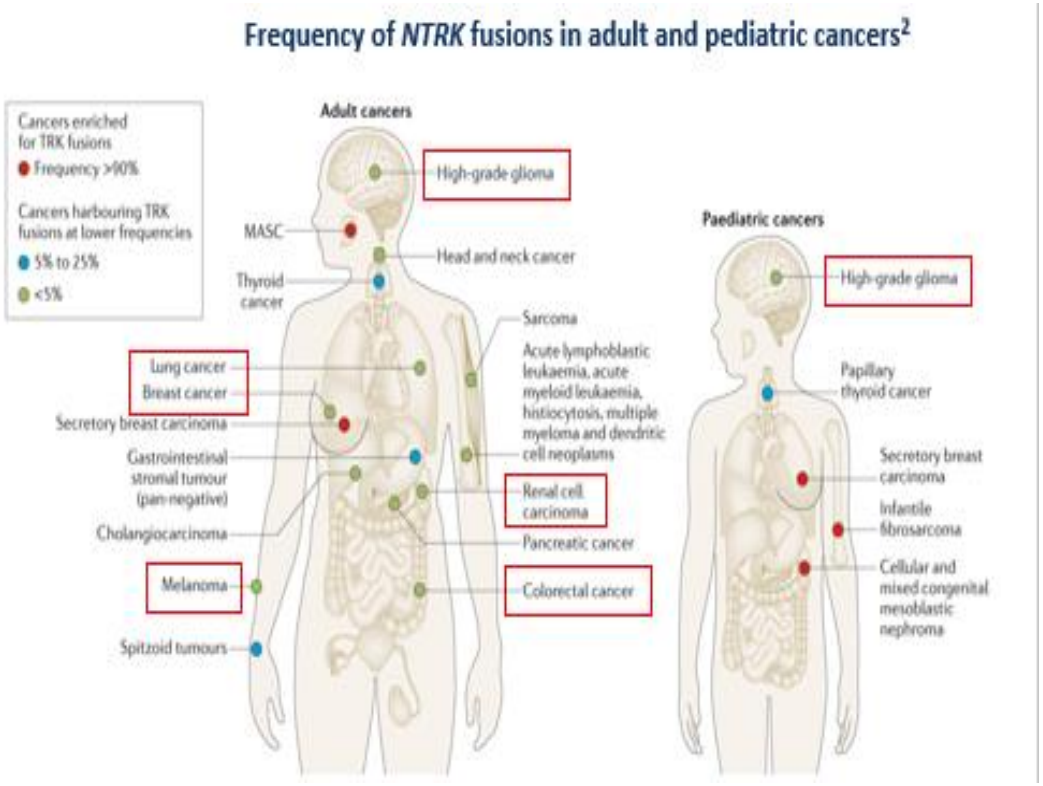
Congenital mesoblastic nephroma[10,11]
- *NTRK3 fusion frequency:* ***83–91%***

1. Kim J, et al. *PLoS ONE* 2014 ; 9 : e91940.
2. Bishop JA, et al. *Hum Pathol*. 2013;44:1982-1988.
3. Bongarzone I, et al. *Clin Canc Res*. 1998;4:223-228
4. Leeman-Neil RJ, et al. *Cancer*. 2014;120:799-807.
5. Tognon C, et al. *Cancer Cell*. 2002;2:367-376.
6. Ross JS, et al. *Oncologist* 2014; 19: 235–442.
7. Wu G, et al. *Nat Genet* 2014;46:444–450.
8. Jones DT, et al. *Nat Genet* 2013;45:927–932.
9. Bourgeois JM, et al. *Am J Surg Pathol*. 2000;24:937-946.
10. Rubin BP, et al. *Am J Pathol*. 1998;153:1451-1458.
11. Argani P, et al. *Mod Pathol*. 2000;13:29-36.

High frequency in rare tumors

→ To be searched systematically at diagnosis

NTRK gene fusion & cancer



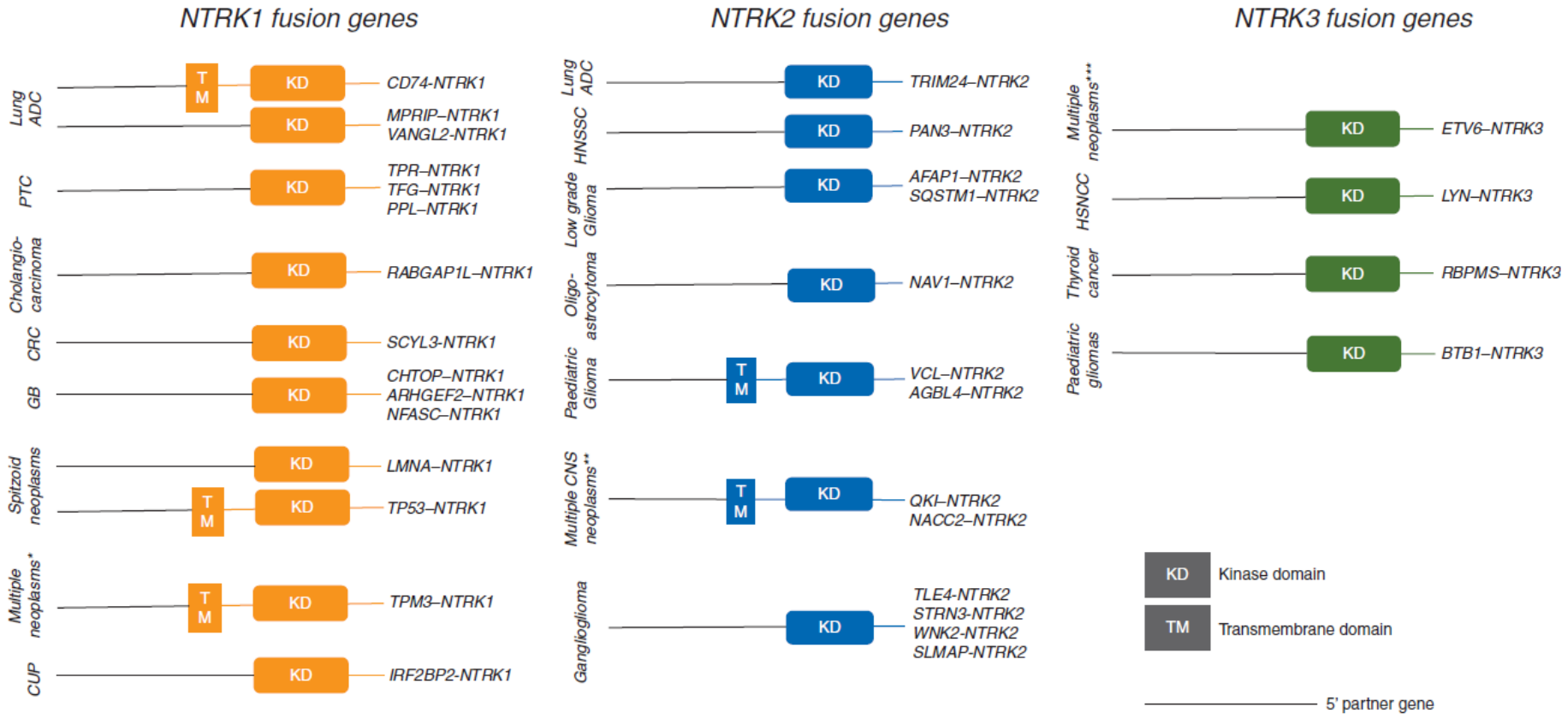
NTRK fusion-positive cancers and TRK inhibitor therapy: DOI:10.1038/s41571-018-0113-0

- Lower frequencies (<1%) for the most common types of cancer: Lung, mRCC, melanoma, high-grade glioma, etc.

→ In which patient should I look for NTRK?

the marker is present only in 0.1% to 3% of tumors

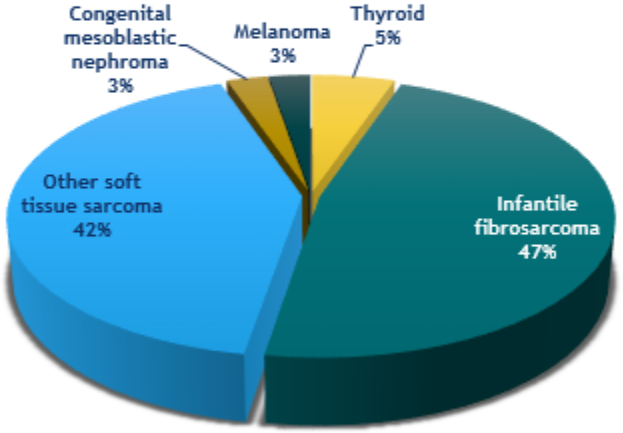
NTRK gene fusion & cancer



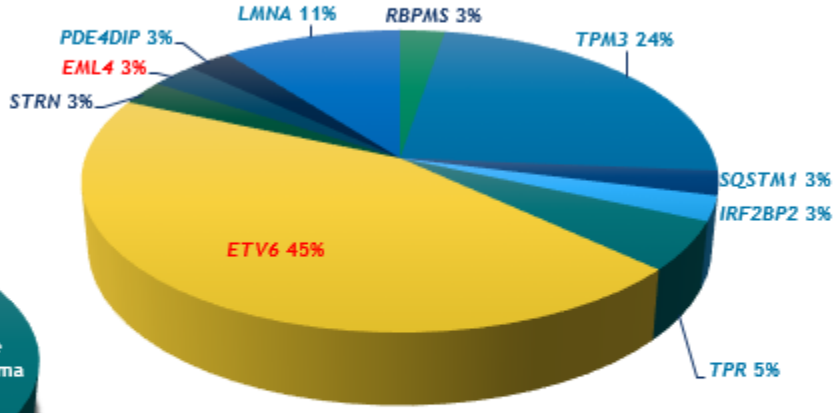
- NTRK1 and NTRK3 >> NTRK2
- 60 partner genes have been described
- Depends on tumor type
- Pediatric / adult tumor

- NTRK1 et NTRK3 >> NTRK2
- 60 gènes partenaires ont été décrit
- Dépend du type tumoral
- Tumeur pédiatrique / adulte

Pediatric population by tumor type and *NTRK* fusion (N=38)



Tumor types



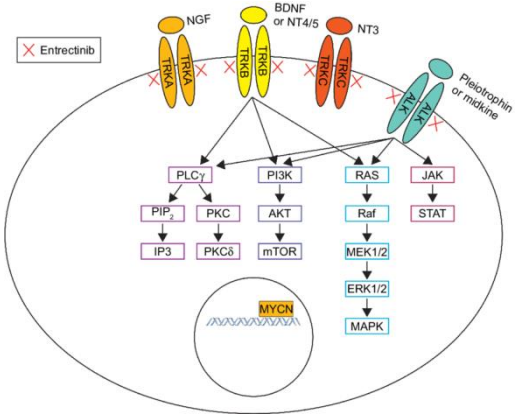
NTRK fusion partners

NTRK gene	
NTRK1	47%
NTRK2	5%
NTRK3	47%

NTRK & target therapy

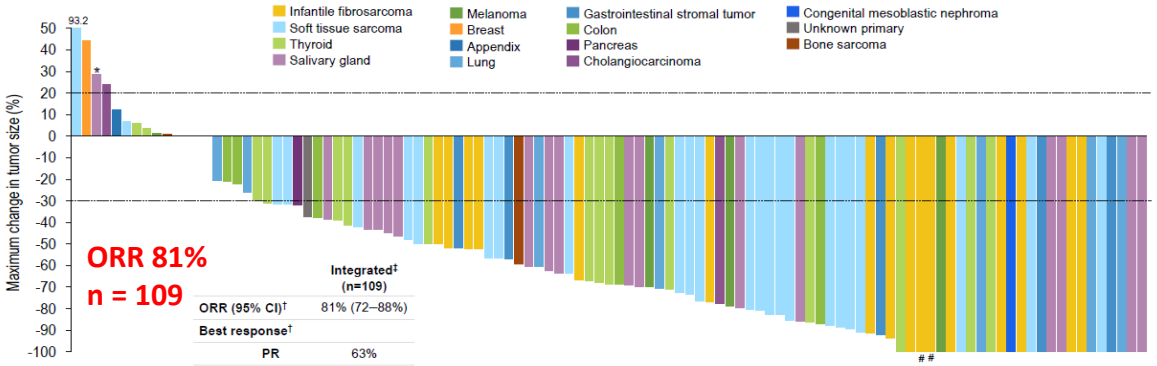
- NTRK inhibitors actson the kinase domain, which allows transactivation, to block the activation of the downstream pathways and therefore the proliferation of tumor cells.
  - Larotrectinib (Bayer)
  - Entrectinib (Roche)

VITRAKVI (larotrectinib)
TEST. TRK. TREAT.



- Regardless of histology or tumor type

Integrated dataset: Larotrectinib is efficacious regardless of tumor type



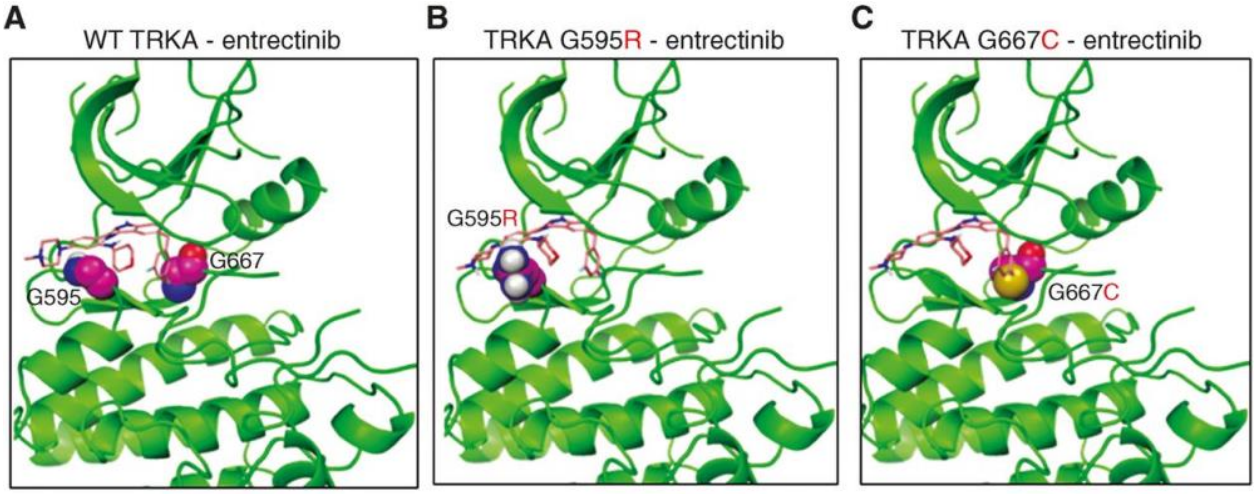
‡Includes 9 unconfirmed PRs pending confirmation; does not include 13 patients continuing on study and awaiting initial response assessment
*Patient had TRKC solvent front resistance mutation (G623R) at baseline due to prior therapy; #Surgical CR; †RECIST 1.1
Note: Two patients not shown here. These patients discontinued treatment prior to any post-baseline tumor measurements.
CR, complete response; ORR, objective response rate; PR, partial response

MUNICH 2018 ESMO congress

Investigator response assessments, as of 30 July 2018

NTRK & target therapy

And already described resistance mutations !!



D

Gene	Codon	Mutation at resistance	Tissue of detection	Associated with resistance to
NTRK1	595	p.G595R	Colon	Entrectinib
NTRK1	667	p.G667C	Colon	Entrectinib

Russo et al. Cancer discovery, January 2016

NTRK fusion: detection strategies

- **IHC**
- test for the presence of the protein, which is not normally present in the tissue, except for nerve tissue.
- use of an antibody directed against the TRK proteins sequence common to the three TRKs (A, B or C).
- Advantage: prescreening, fast and inexpensive. without it being possible to conclude with certainty that this protein was produced by a rearrangement.
- Disadvantage: does not apply to tumors of the nervous tissue. no information on the breakpoint or the fusion partner involved.

- **FISH**
  - DNA probes that hybridize to one of the NTRK genes, on either side of the breakpoint.
  - Advantage: gene-specific probes.
  - Disadvantage: false negatives possible if the gap between the two probes is small. Furthermore, DNA rearrangements are not always translated into protein. However, in the absence of fusion protein, the drug will have no effect.
  - partner gene / breakpoint not known.

NTRK fusion: detection strategies

- **NGS (RT-PCR on RNA)**
  - Advantage: panel of several transcripts can be analyzed
  - Disadvantage: it is necessary to know all the partner genes among all the partner genes for all the NTRK genes. Does not make possible to identify novel fusions.

Table 1. Summary of main features, strengths and weaknesses of all available techniques to detect *NTRK* rearrangements

Method	Sensitivity	Specificity	Detection of all fusion genes	Detection of partner	Detection of expression	Screening
IHC	High[a]	High[b]	Yes	No	Yes	Yes
FISH[c]	High	High	One per probe	No	No	No
RNA seq NGS	High	High	Yes	Yes	Yes	Yes
DNA seq[c]	Moderate	High	Yes	Yes	No	Yes

[a]False negatives reported mainly in *NTRK3* fusions.
[b]In the absence of smooth muscle/neuronal differentiation.
[c]Detected rearrangements by DNA-based assays may not result in fusions, correlation with surgical pathology and predicted transcript (for sequencing) is needed.

NTRK fusion: detection strategies

Panel Oncomine™ Focus Assay RNA & Oncomine™ Comprehensive Assay v3 RNA

RNA Assay Type	Isoform Count	Pool 1	Pool 2	Panel RNA Focus
Fusions	amplicons, 762 isoformes, 51 gènes drivers	363*	394*	272 amplicons , 194 isoformes, 23 gènes drivers
Gene Expression	99	74**	23**	
Expression Controls	6	HMBS, ITGB7, MYC	LRP1, MRPL13, TBP	5 (LMNA, TBP, MYC, ITGB7, HMBS)

	Panel OCA v3			Panel FOCUS	
Driver Gene	Partners in Pool 1	Partners in Pool 2	Total Isoforms	Partners	Total Isoforms
NTRK1	DYNC2H1, EPS15, LMNA, TPM3	ARHGEF2, BCAN, CD74, CEL, CHTOP, EPHB2, EPS15, IRF2BP2, **LMNA**, MPRIP, MRPL24, NFASC, PPL, RABGAP1L, RNF213, SQSTM1, SSBP2, TFG, TP53, **TPM3**, TPR	48	BCAN,CD74,CEL,IRF2BP2, **LMNA**,MPRIP, NFASC, DYNC2H1,RNF213,SQSTM1, SSBP2,TFG,**TPM3**,TPR	19
NTRK2		AFAP1, AGBL4, DAB2IP, NACC2, NAV1, QKI, SLMAP, SQSTM1, TRIM24, VCL	11	AFAP1,AGBL4,NACC2,QKI,SQSTM1,TRIM24,VCL	7
NTRK3	HOMER1	AKAP13, BTBD1, COX5A, **EML4, ETV6,** FAT1, LYN, RBPMS	12	BTBD1,COX5A,**ETV6**	6

NTRK fusion: detection strategies

- What detection strategy should be adopted?
  - for the most common tumors: perform an IHC test and confirm it with an NGS technique
  - for rare tumors: perform a FISH or RT-PCR test.

- At CHU Amiens: Oncomine™ Focus Assay and Comprehensive
  - Transcript produced immediately: Thyroid, Salivary glands
  - Frequent tumor (lung, colon, ...): NGS DNA panel, if Not mutated => discussion to have Oncomine™ Focus Assay RNA
  - Rare tumor: discussion in molecular tumor board, comprehensive panel

NTRK fusion: example case

- November 2015: discovery of left parotid swelling with complete left peripheral facial paralysis.

- February 2016: Surgery
  - poorly differentiated invasive adenocarcinoma pT4N0M0
  - adjuvant radio-chemotherapy with 3 cures of CISPLATIN
- Oct 2016: Secondary pulmonary location = Chemotherapy with Taxol + Carboplatin
- April 2017: secondary choroidal lesion treated by radiotherapy
- May 2017: paclitaxel alone

- Nov 2017: two cerebral infra-centimeter lesions, infra and supra-tentorial, asymptomatic, treated by RT
- Dec 2017: included in acesé Nivolumab (at IGR) (4 courses)

- March 2018: pleural evolution + hepatic and ovarian localizations
  - DOXORUBICIN + ENDOXAN then CARBOPLATIN GEMZAR
- August 2018: evolution on scanner = resumption of the Taxol hebdo
- January 2019: Capecitabine - increase in serum marker CA15-3
- April 2019: Folfox-Avastin - General condition deterioration, weight loss

NTRK fusion: example case

- Molecular Tumor Board: Large Panel proposal (OCA)
  - Direct debit received on 05/31 - Result June 18, 2019: transcript NTRK3 (15) -ETV6 (5)
  - **Larotrectinib in ATU at the end of June**

Analysis Results

MyVariants | Download | Visualize | Selected Variants | Send to Report Role | Switch To | Generate Report

Analysis Name: p35_2019_X4_191511030ARN_RNA_v3_c2040_2... **Fusion Sample QC**: PASS,[TotalMappedFusionPanelReads>500000;... **Fusion Overall Call**: POSITIVE ,[3pGene=NTRK3,IsoformsDetected=E... **Total Mapped Fusion Panel Reads**: 1614878
Total Unmapped Reads: 28691 **Pool-1 Mapped Fusion Reads**: 111074 **Pool-2 Mapped Fusion Reads**: 1503804 **Pool-1,2 Mapped Fusion Reads**: 0

To learn more about reviewing your results, visit the help guide.

Fusions | Search | Go | Preferences | FILTER OPTIONS

Classification	Locus	Type	Filter	Genes (Exons)	Read Counts	Oncomine Variant Class	Oncomine Gene Class	Detection	3'/5' Imbalan...	Ratio To Wild Type	Norm Co
Unclassified	chr11:118960975	EXPR_CON	PASS	HMBS	3607			Present			
Unclassified	chr8:128751265	EXPR_CON	PASS	MYC	51954			Present			
Unclassified	chr8:121455461	EXPR_CON	PASS	MRPL13	126543			Present			
Unclassified	chr12:53585787	EXPR_CON	PASS	ITGB7	10806			Present			
Unclassified	chr12:57591167	EXPR_CON	PASS	LRP1	206469			Present			
Unclassified	chr6:170871321	EXPR_CON	PASS	TBP	434250			Present			
Unclassified	chr12:12022903 - chr15:88483984	FUSION	PASS	ETV6(5) - NTRK3(15)	437486	Fusion	Gain-of-function	Present			
Unclassified	chr2:29443584	GENE_EXPF	PASS	ALK	2102			Present			
Unclassified	chr2:29446432	GENE_EXPF	PASS	ALK	348			Present			
Unclassified	chr2:29455224	GENE_EXPF	FAIL	ALK	0			Absent, READ_COUNT<=			

1 | 30 items per page | 1 - 10 of 10 items

Panel FOCUS : 325 521 reads / 147 547 reads ETV6(5)-NTRK3(15)
Panel OCA : 1 614 878 reads / 437 485 reads ETV6(5)-NTRK3(15)

- September 2019 (3 months of larotrectinib): good general condition, + 6 kg, No respiratory signs even during exercise when the patient was dependent on oxygen, Regular drop in markers, especially CEA

- Rediscussion of the diag: MASC = Mammary analog secretory cancer

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