MOLECULAR TUMOR BOARD WEBINAR SERIES

SAVE THE DATE **WEDNESDAY, MARCH 31ST**

EASTERN TIME (ET): 8:00 A.M.

Andrew Kelly, MD, Ph.D

Scientist - International Medical Affairs,
Foundation Medicine.

Exploring fusion genes in cancer with comprehensive genomic profiling.

Case Presentation:

- Breast carcinoma by Dr.Juha Kononen, M.D., Ph.D. (Docrates Cancer Center, Finland)
- Unknown primary undifferentiated neuroendocrine carcinoma by Dr. Chen Ming-Huang, MD., Ph.D (Taipei Veterans General Hospital, Taiwan)

Registration Link: https://foundationmedicinewebinar.com

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Gene Fusions in Comprehensive Genomic Profiling

Andrew Kelly, MD, PhD
Scientist – International Medical Affairs, Foundation Medicine
March 31, 2021

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M-TH-00000817

Disclosures

- Employee of Foundation Medicine
- Shareholder in Roche

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Webinar Objectives

1. Background on gene fusions in cancer
2. Fusion detection using comprehensive genomic profiling
3. Clinical targetability of gene fusions
4. Resistance to fusion-directed agents

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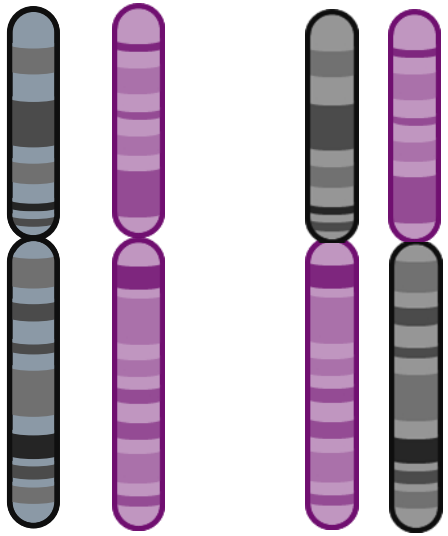
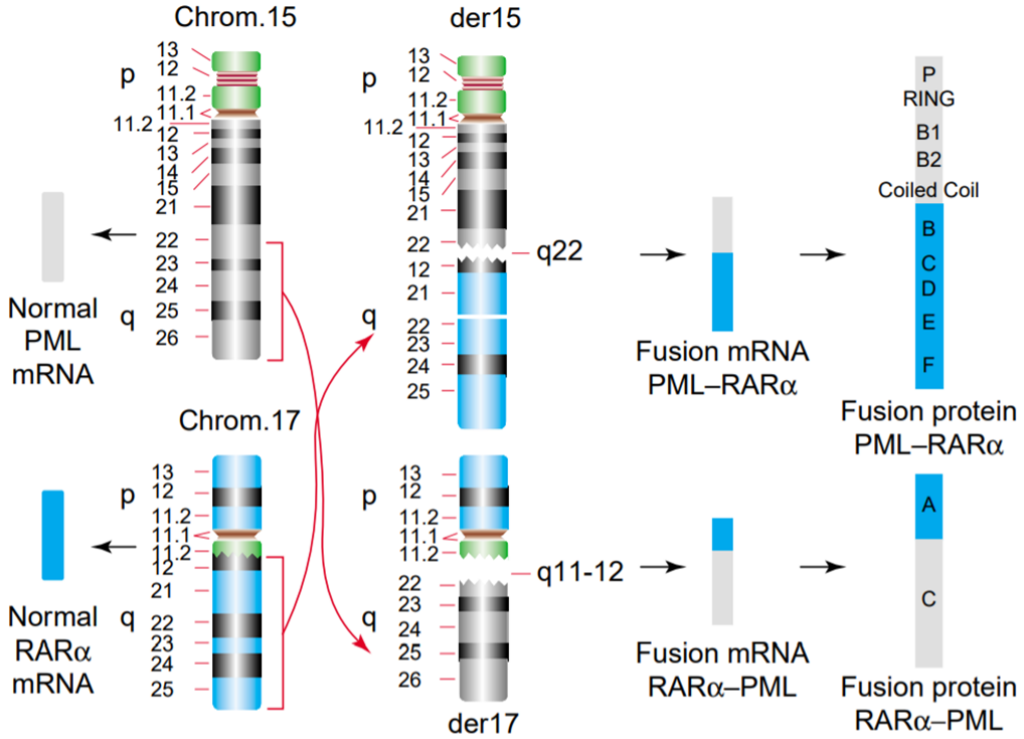
Rearrangements and translocations

Rearrangements or **translocations** = large-scale genetic change where a piece of one chromosome breaks off and attaches to another chromosome. Sometimes pieces from two different chromosomes will trade places with each other. As a result, a translocation can join parts of two different genes, creating a **gene fusion.**





1.Rego EM, Pandolfi PP. Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders? *Trends Mol Med.* 2002;8(8):396-405.

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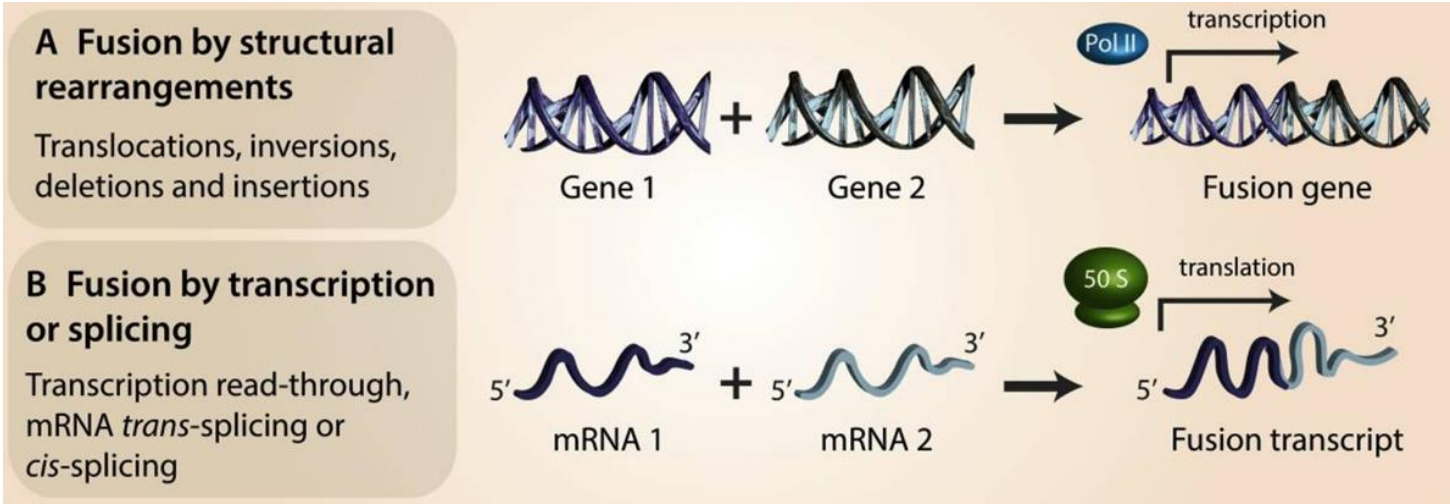
Gene fusions are formed when two previously independent genes become juxtaposed

Mechanisms of gene fusion formation

- **Structural rearrangements of chromosomes**.
  - Translocations
  - Inversions
  - Deletions
  - Insertions

- **Non-structural rearrangement mechanisms**
  - Transcription read-through of neighboring genes
  - Splicing of mRNA molecules
  - Does not involve rearrangement of genomic material



1.Latysheva NS, Babu MM. Discovering and understanding oncogenic gene fusions through data intensive computational approaches. *Nucleic Acids Res.* 2016;44(10):4487-4503.

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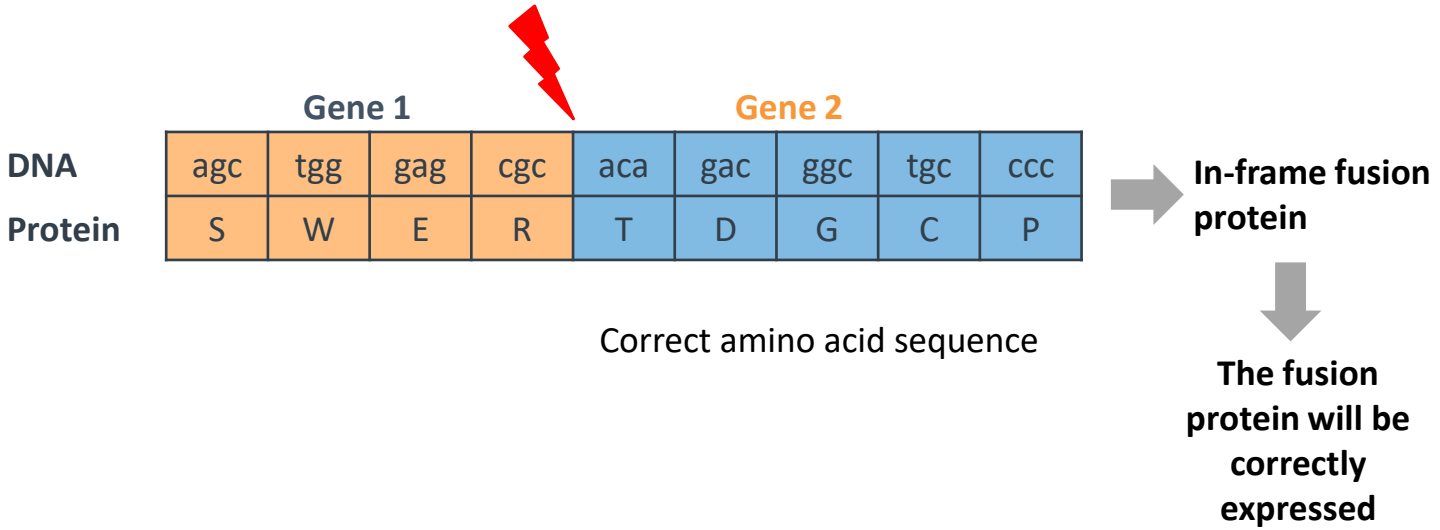
Functional consequences of in-frame versus out-of-frame gene fusions

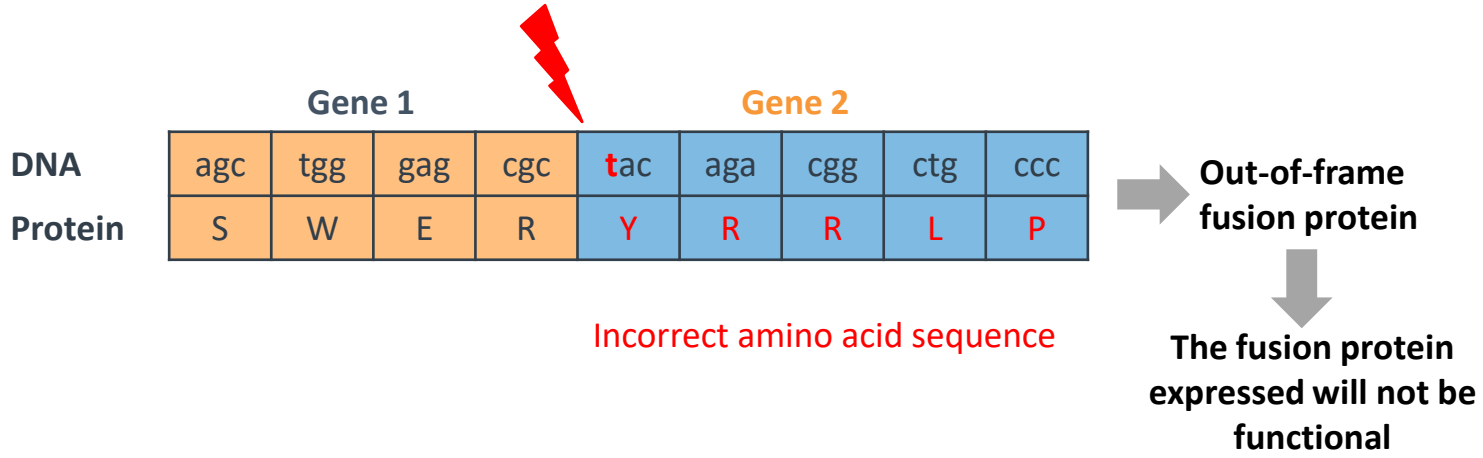
In-frame gene fusion

- Reading frame of the downstream gene maintained: Amino acid sequence **correctly expressed**



Out-of-frame gene fusion

- Introduces a frameshift: Amino acid sequence of the downstream gene will be incorrect and **the fusion protein expressed will not be functional**



1.Voet D, Voet JG. *Biochemistry*. 2nd ed: John Wiley & Sons, Inc.; 1995.

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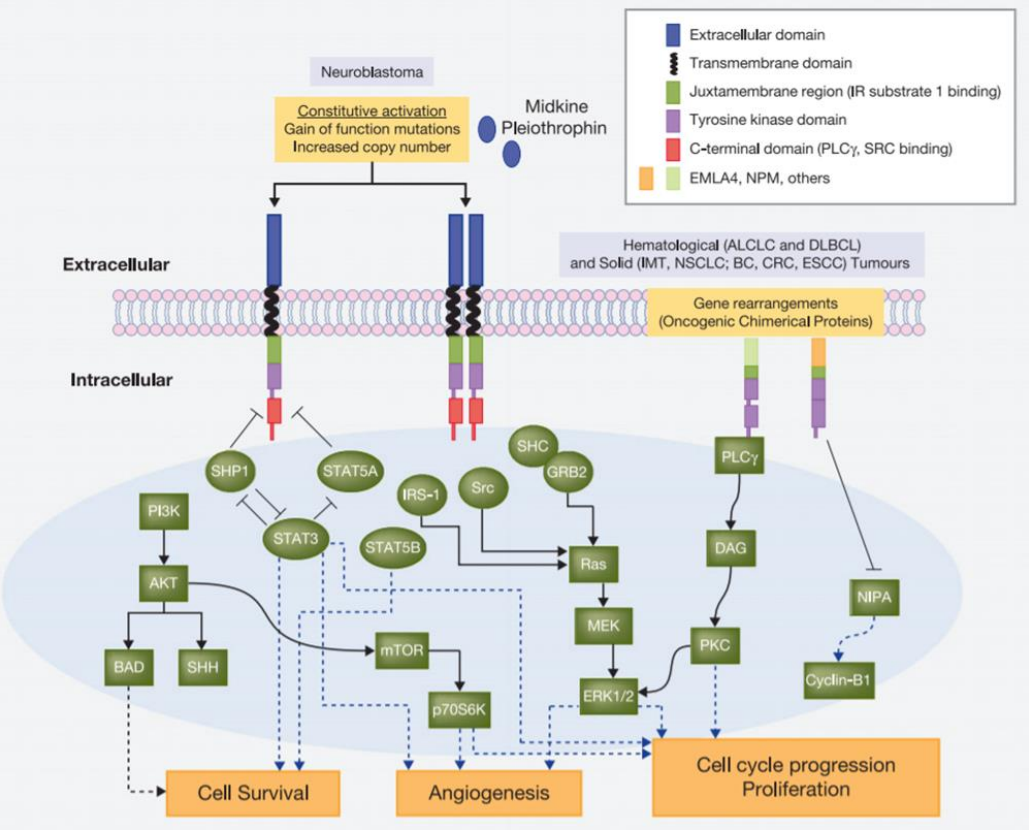
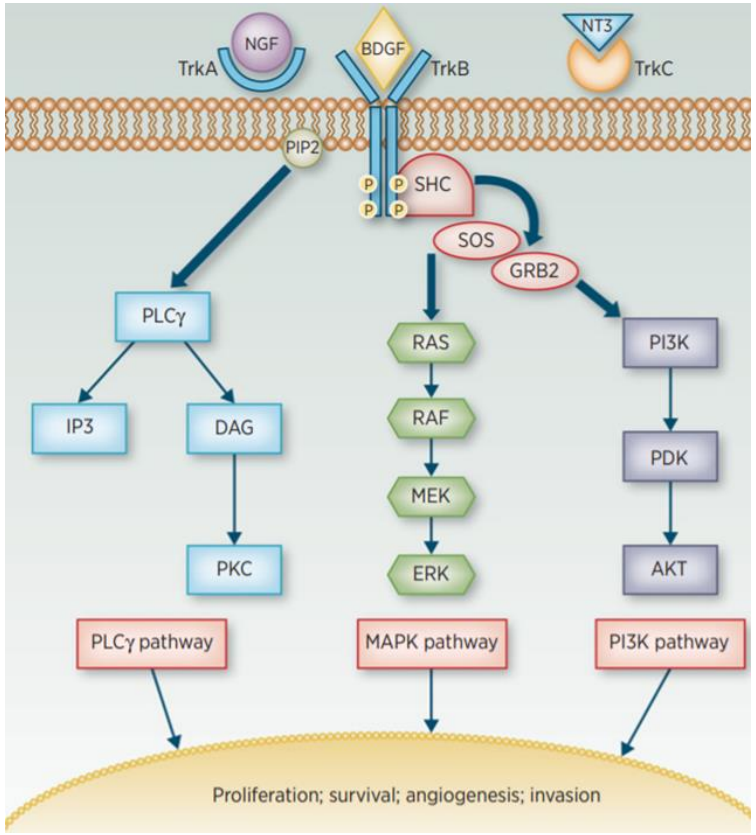
Receptor tyrosine kinases as fusion partners feed into multiple parallel pathways

ALK and downstream signaling



TRK and downstream signaling



1.Grande E, Bolos MV, Arriola E. Targeting oncogenic ALK: a promising strategy for cancer treatment. *Mol Cancer Ther.* 2011;10(4):569-579.
2.Kheder ES, Hong DS. Emerging Targeted Therapy for Tumors with NTRK Fusion Proteins. *Clin Cancer Res.* 2018;24(23):5807-5814.

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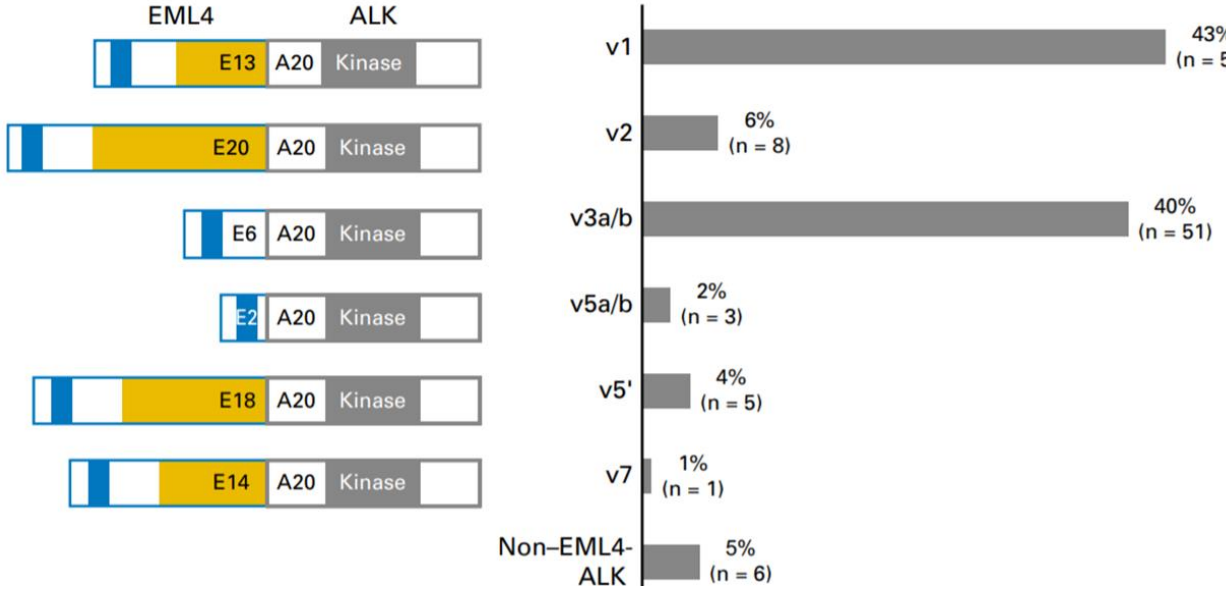
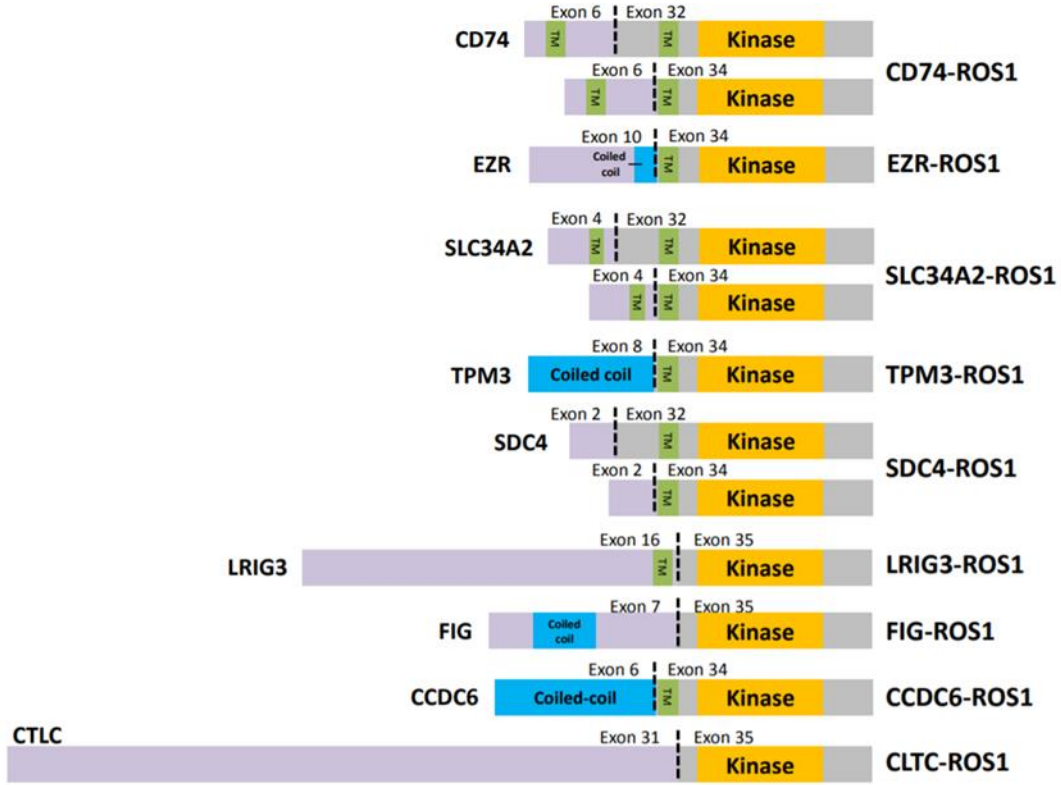
Diverse oncogenic fusions in lung cancer typically join partner genes to kinase domain-containing regions





1. Lin JJ, Zhu VW, Yoda S, et al. Impact of EML4-ALK Variant on Resistance Mechanisms and Clinical Outcomes in ALK-Positive Lung Cancer. *J Clin Oncol.* 2018;36(12):1199-1206.
2.Kohno T, Nakaoku T, Tsuta K, et al. Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. *Transl Lung Cancer Res.* 2015;4(2):156-164.

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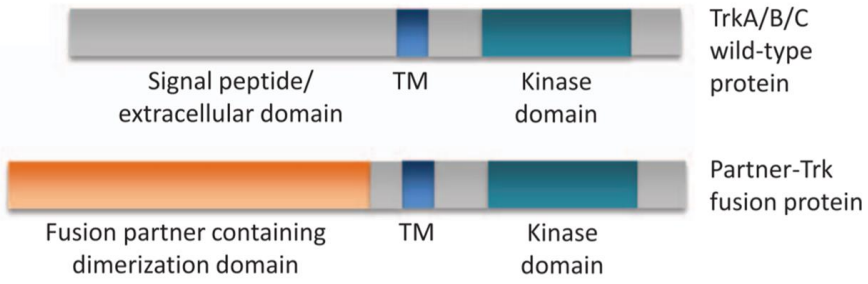
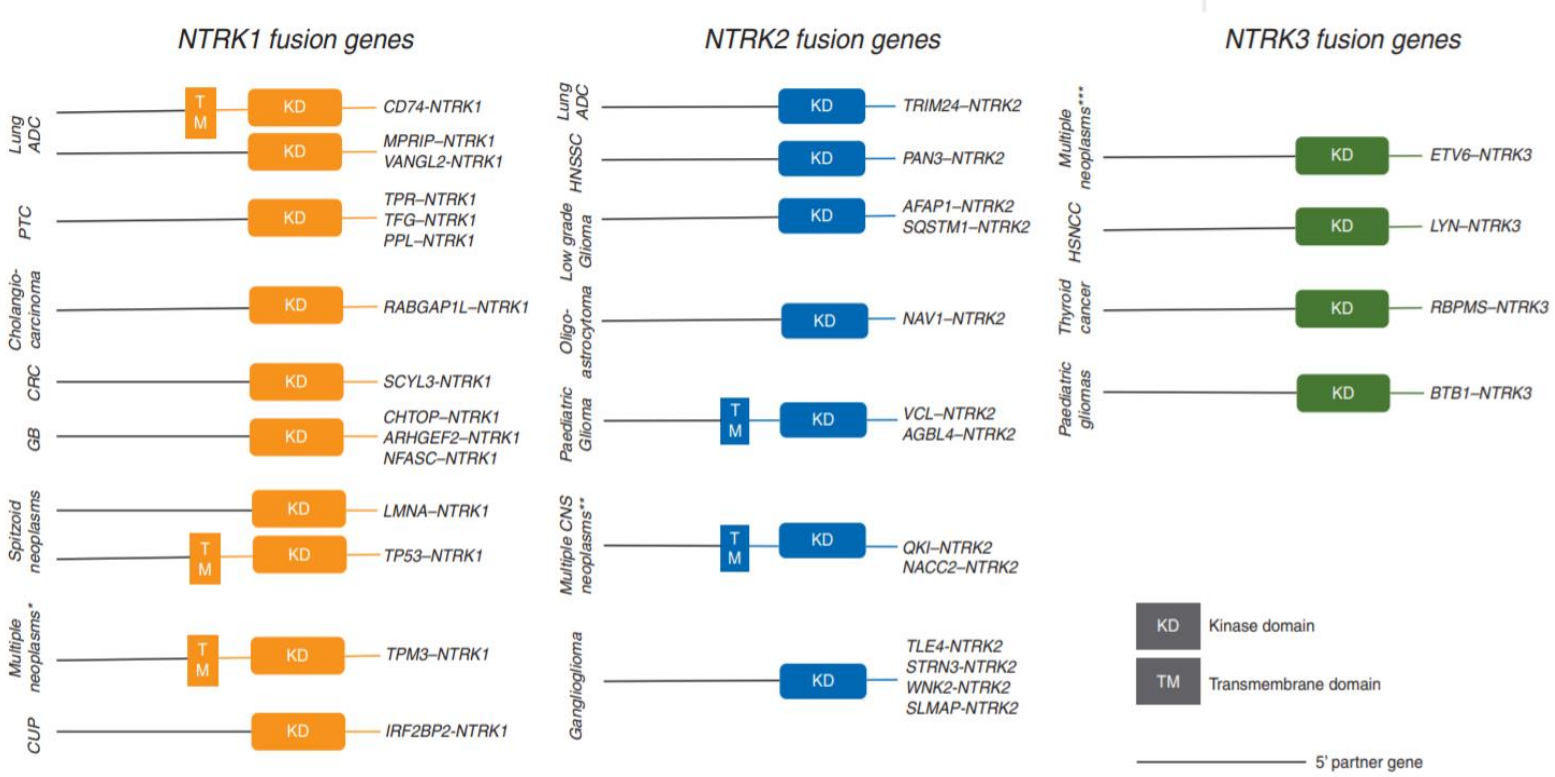
TRK gene fusions are typically retain the TRK kinase domain



Diversity of NTRK fusion partners across cancer types



NT: neurotrophin; NTRK: neurotrophic tropomyosin receptor kinase; TM: transmembrane domain; TRK: tropomyosin receptor kinase.

1.Farago AF, Le LP, Zheng Z, et al. Durable Clinical Response to Entrectinib in NTRK1-Rearranged Non-Small Cell Lung Cancer. *J Thorac Oncol.* 2015;10(12):1670-1674.
2.Marchio 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;30(9):1417-1427.

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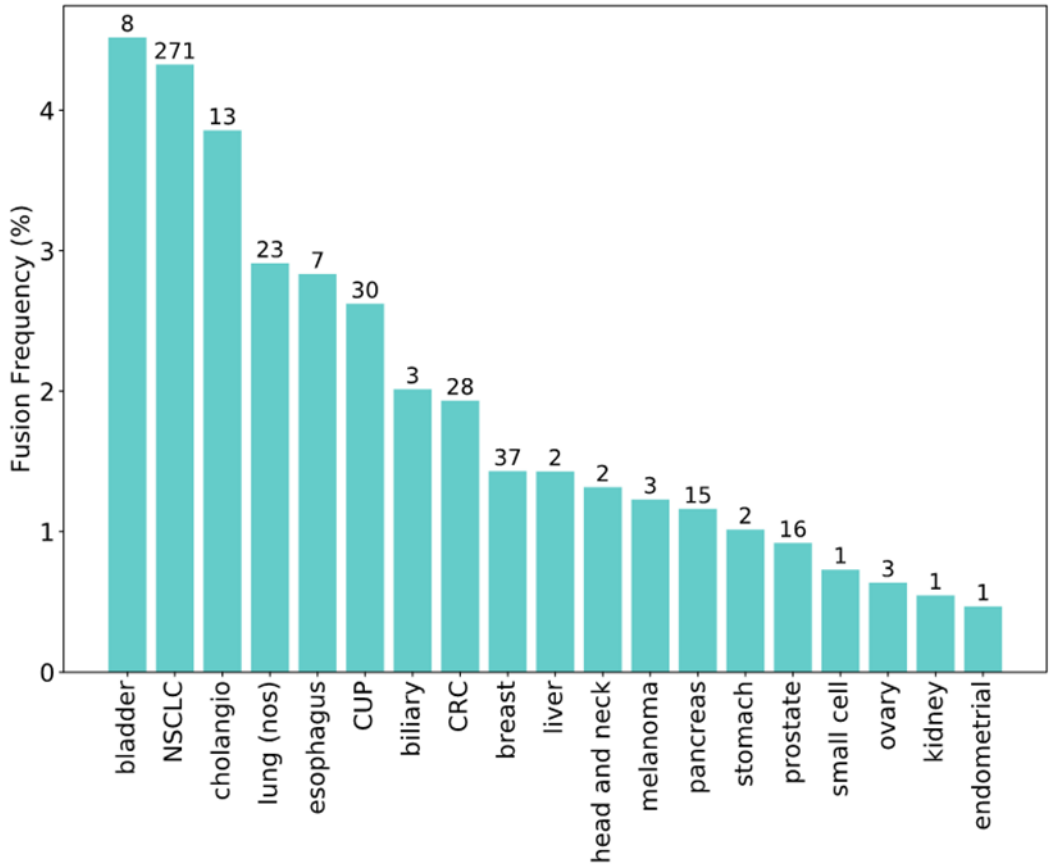
Fusion genes are rare, but detected in a wide variety of solid and hematologic malignancies

Tumour Type	*NTRK* gene fusions involved	Frequency
Breast secretory carcinoma	*NTRK3*	96%
Infantile fibrosarcoma	*NTRK3*	95.5%
MASC ~90%	*NTRK3*	89.1%
Congenital mesoblastic nephroma	*NTRK3*	72.0%
Spitz tumours and spitzoid melanoma	*NTRK1*	16.4%
Papillary thyroid carcinoma	*NTRK1,3*	8.8%
Intrahepatic cholangiocarcinoma	*NTRK1*	3.6%
Astrocytoma	*NTRK2*	3.1%
High-grade glioma	*NTRK1,2,3*	2.1%
Uterine sarcoma	*NTRK1,3*	2.1%
GIST	*NTRK3*	1.9%
Lung cancer	*NTRK1,2*	1.7%
Thyroid carcinoma	*NTRK1,3*	1.2%
Glioblastoma	*NTRK1,2*	1.2%
Sarcoma	*NTRK1*	1.0%
Ph-like ALL	*NTRK3*	0.7%
Colorectal cancer	*NTRK1,3*	0.61%
Melanoma	*NTRK3*	0.3%
Head and neck cancer	*NTRK2,3*	0.24%
Invasive breast cancer	*NTRK3*	<0.1%



https://oncologypro.esmo.org/oncology-in-practice/anti-cancer-agents-and-biological-therapy/targeting-ntrk-gene-fusions/overview-of-cancers-with-ntrk-gene-fusion/ntrk-gene-fusions-as-oncogenic-drivers/epidemiology-of-cancers-with-ntrk-gene-fusion

1. Chen Y, Chi P. Basket trial of TRK inhibitors demonstrates efficacy in TRK fusion-positive cancers. *J Hematol Oncol.* 2018;11(1):78.
2. Lee JK, Lieber D, Madison R, et al. Pan-tumor analyses of kinase fusions detected in circulating tumor DNA (ctDNA) and concordance with paired tissue. *Journal of Clinical Oncology.* 2020;38(15_suppl):3517-3517.

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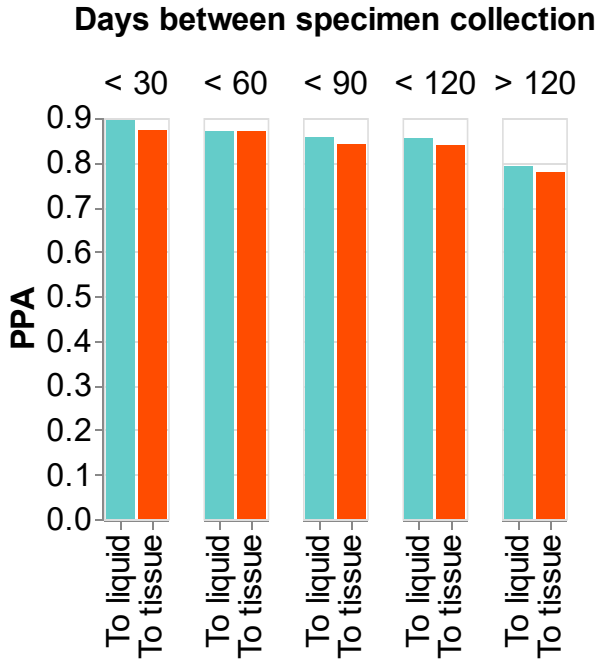
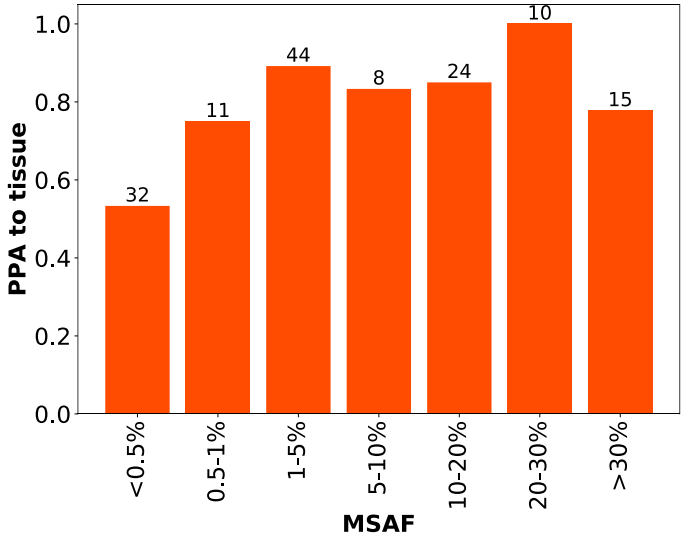
Detection of gene fusions with comprehensive genomic profiling

Principle: Bait for specific cancer genes, paired-end next-generation sequencing, map reads to reference. Evidence for fusions in observing reads mapping to two partners.

RNA sequencing allows for detection of novel fusions and is highly concordant with orthogonal methods.

Cell free DNA sequencing can detect recurring gene fusions with sensitivity depending on tumor fraction





1. Lee JK, Lieber D, Madison R, et al. Pan-tumor analyses of kinase fusions detected in circulating tumor DNA (ctDNA) and concordance with paired tissue. *Journal of Clinical Oncology.* 2020;38(15_suppl):3517-3517.

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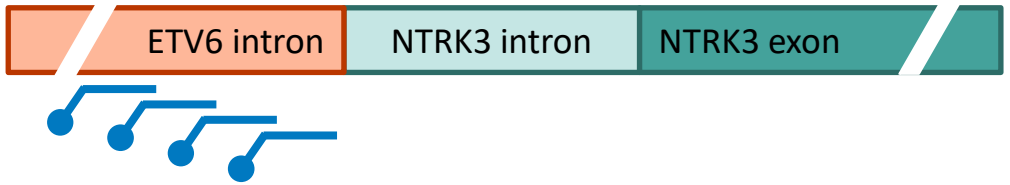
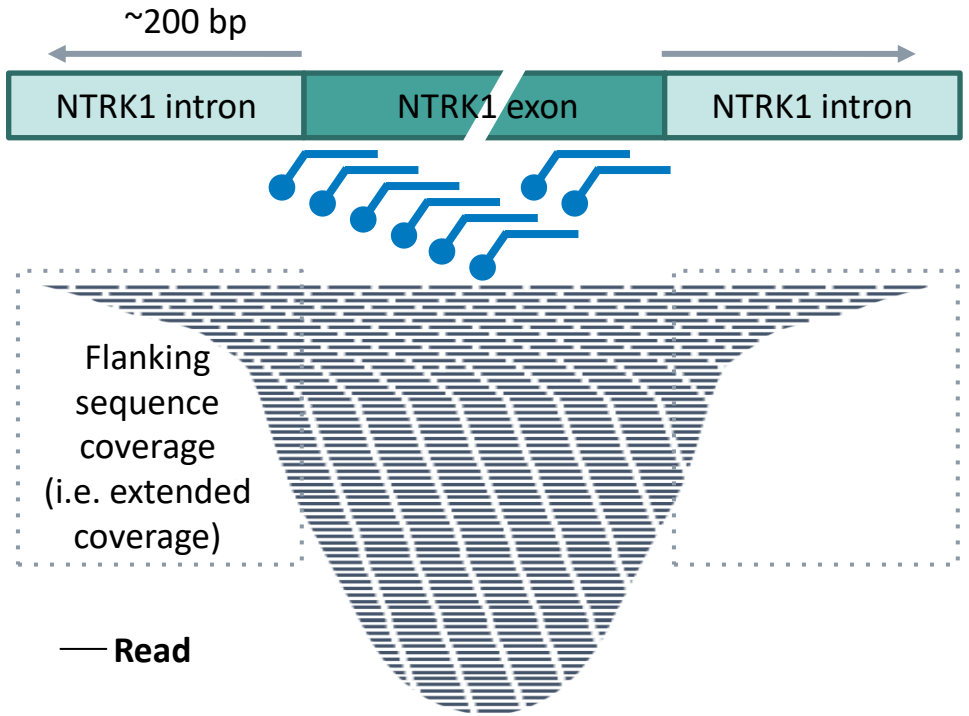
Benefits of hybrid capture in gene fusion detection

- Partner gene concept - F1CDx does not have intronic coverage of NTRK3, but still detect fusion events via partner gene if this is on the baitset (e.g. ETV6)



Bait on ETV6 pulling DNA fragment containing rearrangement partner gene NTRK3

- Flanking sequence coverage[1] - hybrid capture allows extended coverage into the exon-intron junction. Not intronic coverage but the exonic coverage will extend into the intron (~ 200 bp) which may detect fusions in the intron in NTRK1/2/3.



1.Henry IM, Nagalakshmi U, Lieberman MC, et al. Efficient Genome-Wide Detection and Cataloging of EMS-Induced Mutations ng Exome Capture and Next-Generation Sequencing. *Plant Cell.* 2014;26(4):1382-1397.

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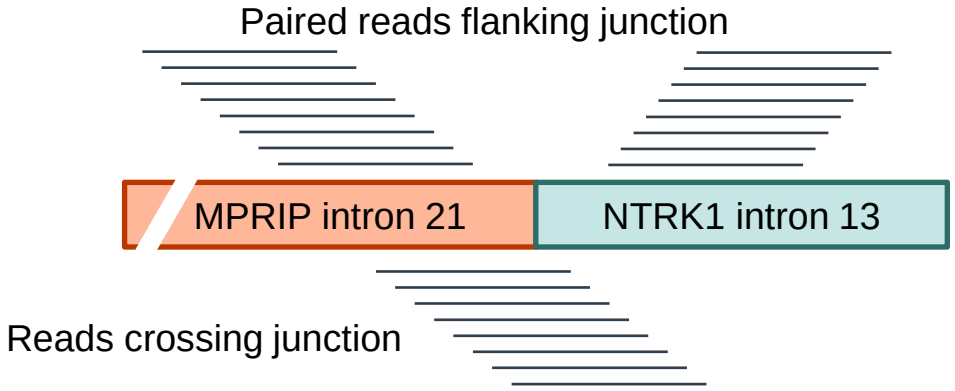
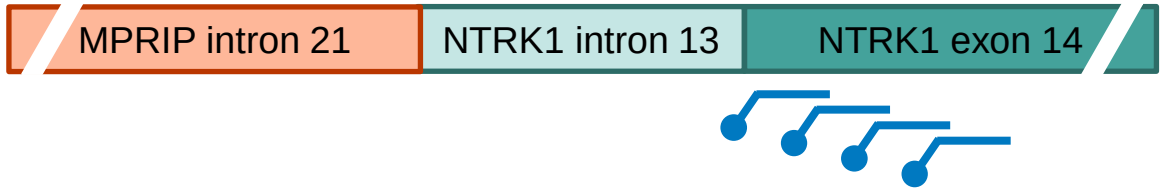
Benefits of hybrid capture in gene fusion detection

- Hybrid capture allows flanking sequence coverage which allows extended coverage into the exon-intron junction
- Vaishnavi et al[1] reported MPRIP-NTRK1 fusion (M21; N14) detected using FoundationOne where the rearrangement was between MPRIP intron 21 and NTRK1 intron 13
  - Detected despite the fact NTRK1 intron 13 was not covered by the FoundationOne baitset





Bait on NTRK1 exon 14 pulling DNA fragment containing rearrangement between NTRK1 intron 13 and partner gene MPRIP intron 21

1.Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med.* 2013;19(11):1469-1472.

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Recent tyrosine kinase inhibitors with activity against gene fusion driven cancer

Table 1. TRK inhibitors

	Larotrectinib	Entrectinib	Selitrectinib	Repotrectinib
Generation				
First	✓	✓		
Second			✓	✓
Inhibits				
TRKA/B/C	✓	✓	✓	✓
ROS1		✓		✓
ALK		✓		✓
Resistance				
Inhibits most *NTRK* mutations			✓	✓

The features of four TRK tyrosine kinase inhibitors (larotrectinib, entrectinib, selitrectinib and repotrectinib) are summarised by tyrosine kinase inhibitor generation, major kinase targets and activity against resistance.

1.Drilon A. TRK inhibitors in TRK fusion-positive cancers. *Ann Oncol.* 2019;30 Suppl 8:viii23-viii30.

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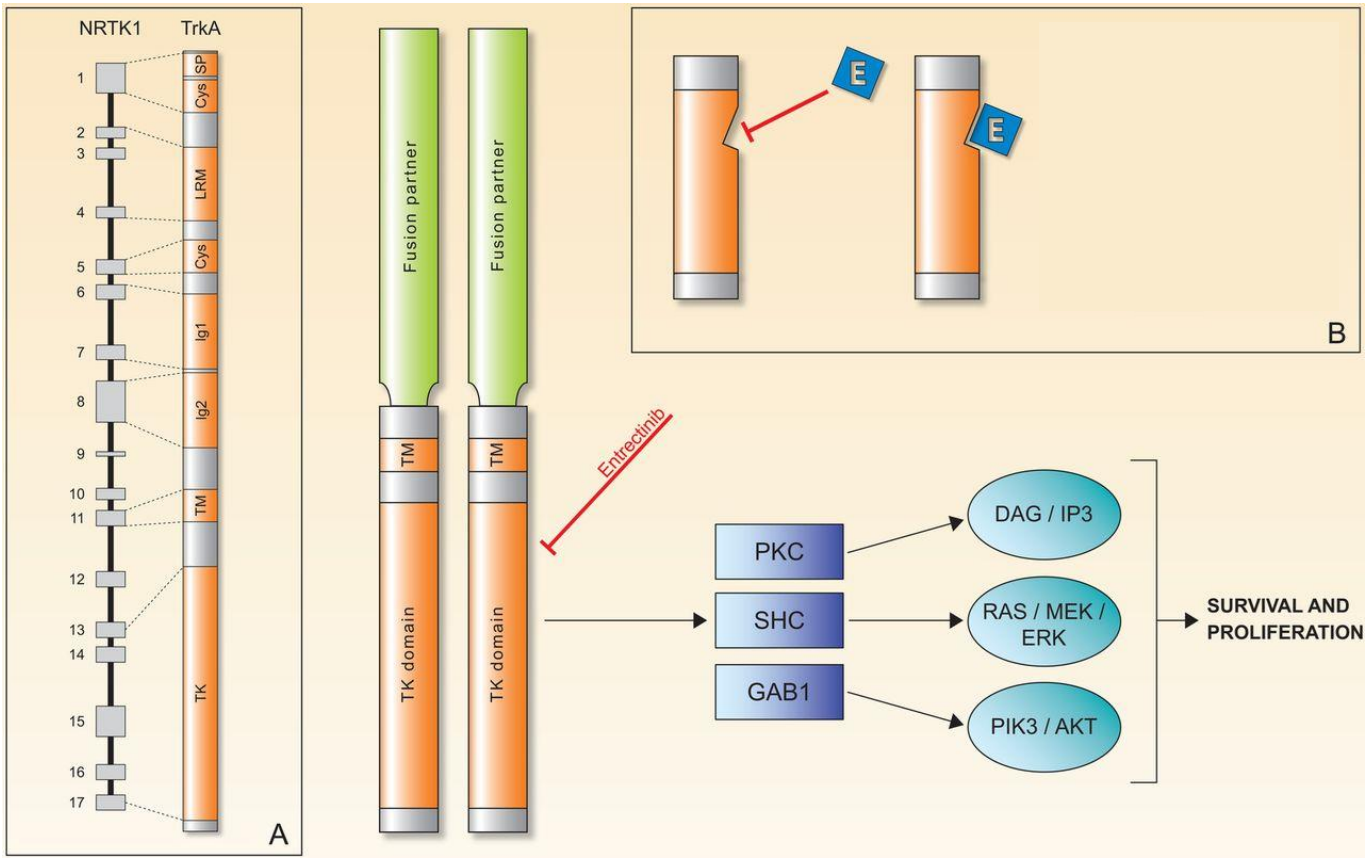
Entrectinib inhibits the constitutive activation of TRK signaling

Oligomerization of the chimeric protein is the main proposed mechanisms for increased tumor cell survival and proliferation via the known pathways of TRK receptors.



Ig1 and Ig2, first and second immunoglobulin-like motifs, respectively; LRM, leucine-rich motifs; SP, signal peptide; TK, tyrosine kinase; TM, transmembrane.

1.Amatu A, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open.* 2016;1(2):e000023.

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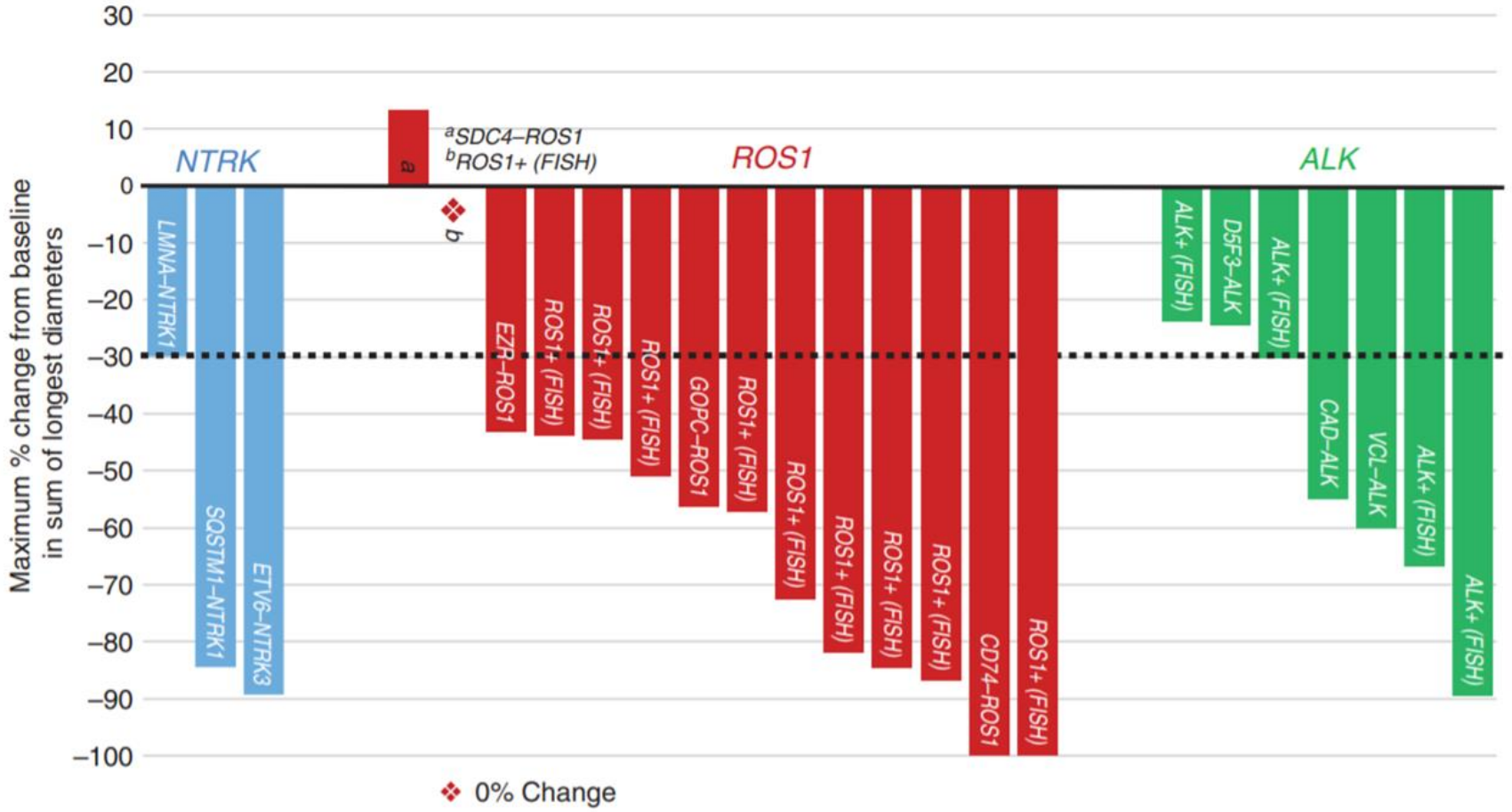
Entrectinib: Activity in multiple solid tumors with NTRK, ROS1, or ALK positivity



1.Drilon A, Siena S, Ou SI, et al. Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1). *Cancer Discov.* 2017;7(4):400-409.

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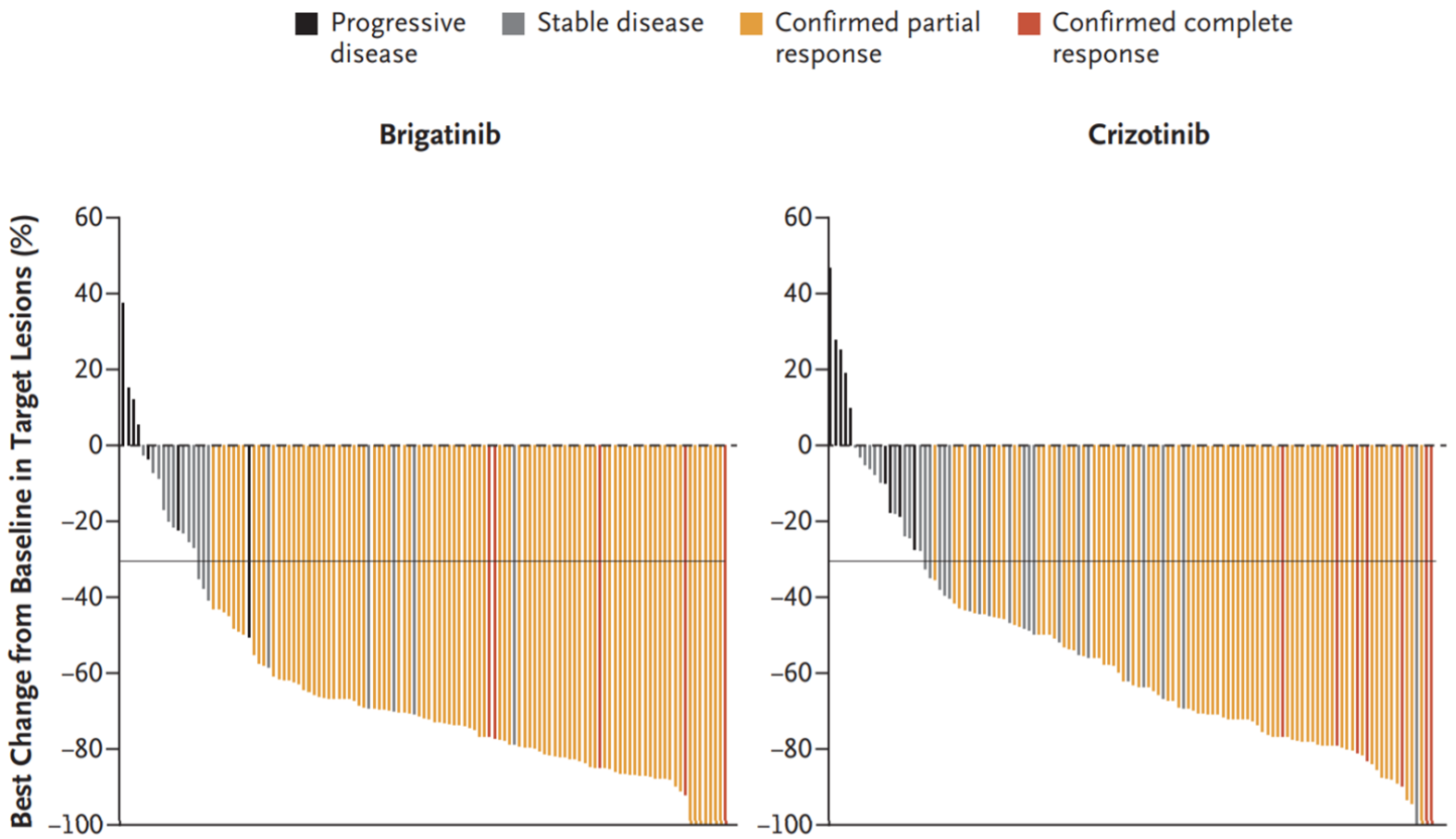
Brigatinib: Improved progression-free survival compared with crizotinib in ALK positive NSCLC



1.Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus Crizotinib in ALK-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2018;379(21):2027-2039.

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Acquired mechanisms of resistance to TRK inhibitors

Resistance to larotrectinib and entrectinib through **NTRK gene mutations**

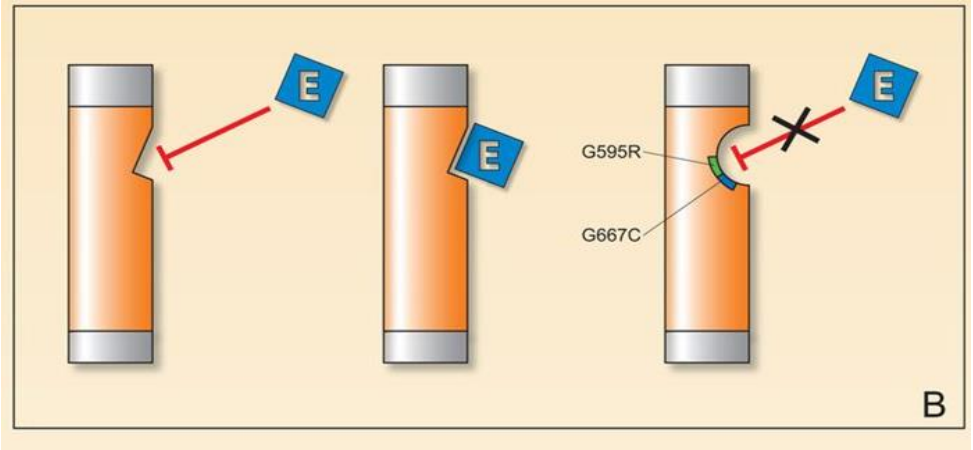
- Amino acid substitutions in solvent-front, gatekeeper residues of the NTRK genes (NTRK1 p. G667C, NTRK3 p. G696A) and xDFG motif substitutions.

Off target resistance → other oncogenic pathway alterations

- BRAF V600E
- KRAS G12D
- MET amplification

Additional TRK inhibitors are being developed to overcome these mechanisms of resistance

- BAY 2731954 (LOXO-195)
- Repotrectinib



Mechanism of entrectinib (E) action and the known mechanisms of acquired resistance (point mutation G595R and G567C) to cysteine clusters;

1.Amatu A, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open. 2016;1(2):e000023.

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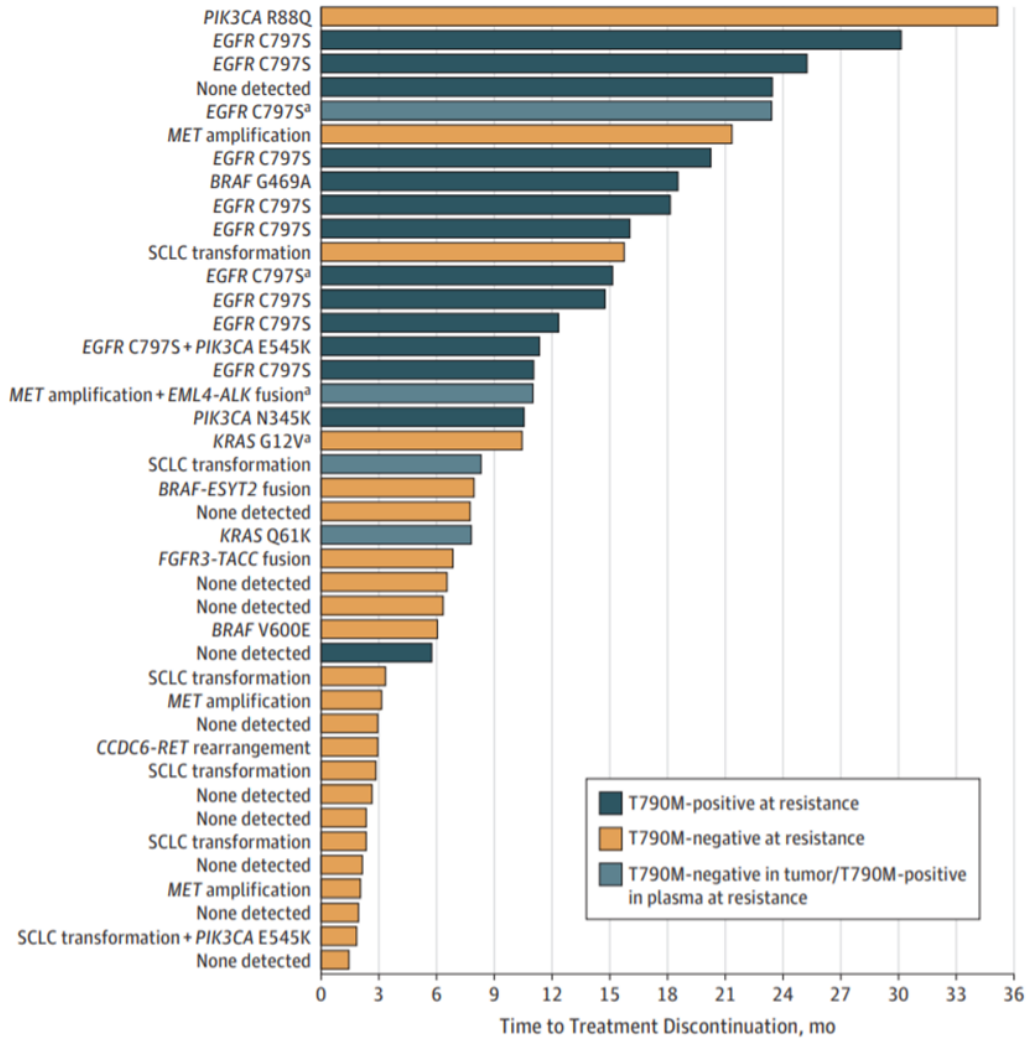
Gene fusions in resistance to EGFR-directed therapy

Patients with non-small cell lung cancer treated with osimertinib can develop targetable gene fusions that mediate resistance

- EML4-ALK
- CCDC6-RET
- FGFR3-TACC
- BRAF-ESYT2

Often clonal elimination of initially targeted EGFR mutation



1.Oxnard GR, Hu Y, Mileham KF, et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients With EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. *JAMA Oncol.* 2018;4(11):1527-1534.

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Conclusions

- Gene fusions are a unique class of genomic driver events resulting from large-scale alterations in chromosome structure
- Comprehensive genomic profiling can identify clinically actionable gene fusions to direct therapy
- Tyrosine kinase inhibitors have impressive activity in fusion-driven cancer
- Resistance mechanisms include acquired mutations and bypass pathway activation
- Fusions can act as mechanisms of acquired resistance to other targeted agents

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Breast carcinoma

Dr.Juha Kononen, M.D., Ph.D.

Chief Clinical Director, Personalized Oncology Specialist
Docrates Cancer Center, Finland

DOCRATES CANCER CENTER

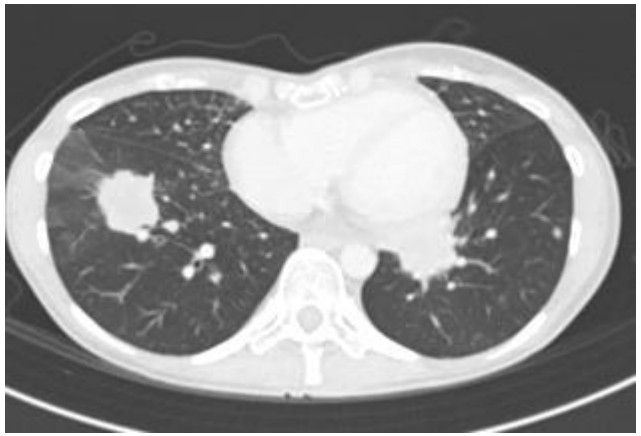
Breast cancer

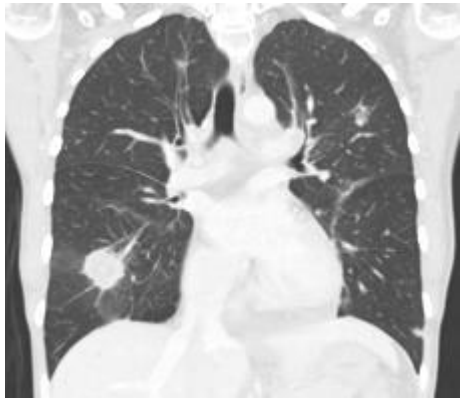
Case History

- 52 year old female with lump in breast 2019
- 5/2019 mastectomy; PAD grade III pT2 ductal cancer, ER 70%, PR 0%. Ki-67 90%, N2 (5/13), ECE+
- Adjuvant treatment with 3 x docetaxel + 3 x CEF + radiotherapy + tamoxifen
- 2/2020 lesions detected in lung. Biopsy attempted but failed. Assumed metastatic breast cancer.
- Docetaxel x 3 -> PD, bone metastases and ascites
- Paclitaxel + carboplatin x 3 -> PR
- Attempted maintenance with palbociclib +letrozol (with LHRH agonist) - > rapid progression
- 9/2020 multiple brain metastases -> WBRT
- 10/2020 liver metastases

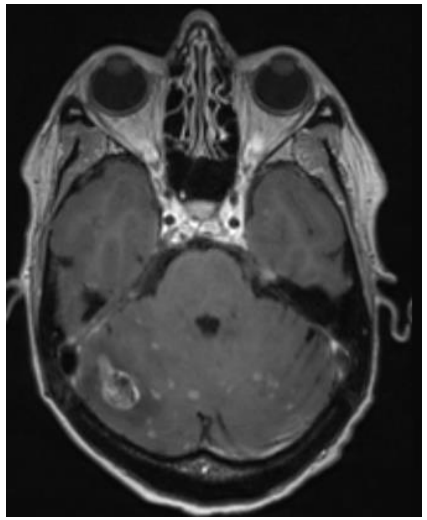
Breast cancer

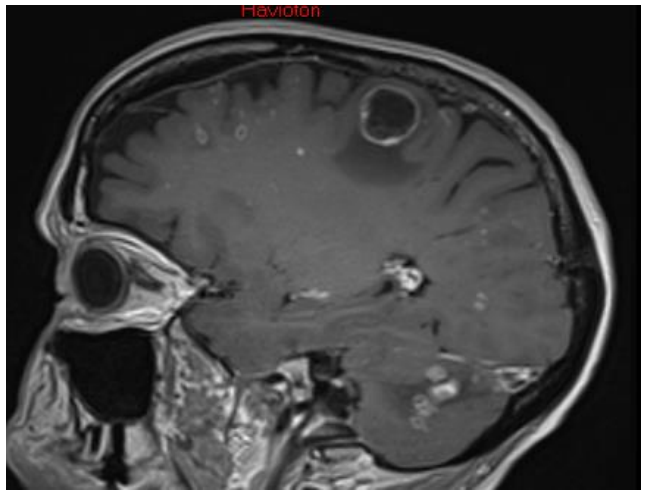
PAD from liver metastasis: morphology in accordance with metastasis from ductal adenocarcinoma.
ER 70%, PR 0%, GATA+

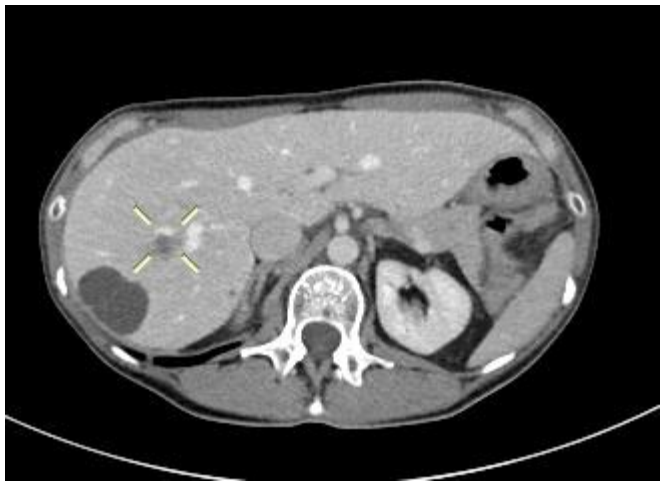




Lung metastases
Liver metastases
Multiple brain metastases







Polling question 1

What to do?

A) Start eribulin

B) Re-challenge with paclitaxel + carboplatin and do molecular profiling from primary tumor and metastatic lesion to look for additional treatment options

C) Start capecitabine

D) Refer patient to palliative care

FoundationONE CDx's Comprehensive Genomic Profiling (CGP) result from breast biopsy in May 2019

Genomic Signatures

Microsatellite status - MS-Stable

Tumor Mutational Burden - 3 Muts/Mb

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

ALK STRN-ALK fusion

MSH6 duplication exons 4-9

TP53 R306*

4 Disease relevant genes with no reportable alterations: ***BRCA1, BRCA2, ERBB2, PIK3CA***

6 Therapies approved in the EU

0 Therapies with Lack of Response

8 Clinical Trials

FoundationONE CDx's Comprehensive Genomic Profiling (CGP) result from liver biopsy in Oct 2020

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Genomic Signatures

Microsatellite status - MS-Stable
Tumor Mutational Burden - 3 Muts/Mb

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

ALK STRN-ALK fusion
CCND2 amplification
BRAF amplification
FGF23 amplification
FGF6 amplification
KDM5A amplification
KEL amplification
LYN amplification - equivocal†
MSH6 duplication exons 4-9
TP53 R306*

4 Disease relevant genes with no reportable alterations: ***BRCA1, BRCA2, ERBB2, PIK3CA***

† See About the Test in appendix for details.

Poll question 2

Which of the below statement is incorrect?

A) Receptor tyrosine kinases can be a fusion partner

B) TRK gene fusions retain the TRK kinase domain

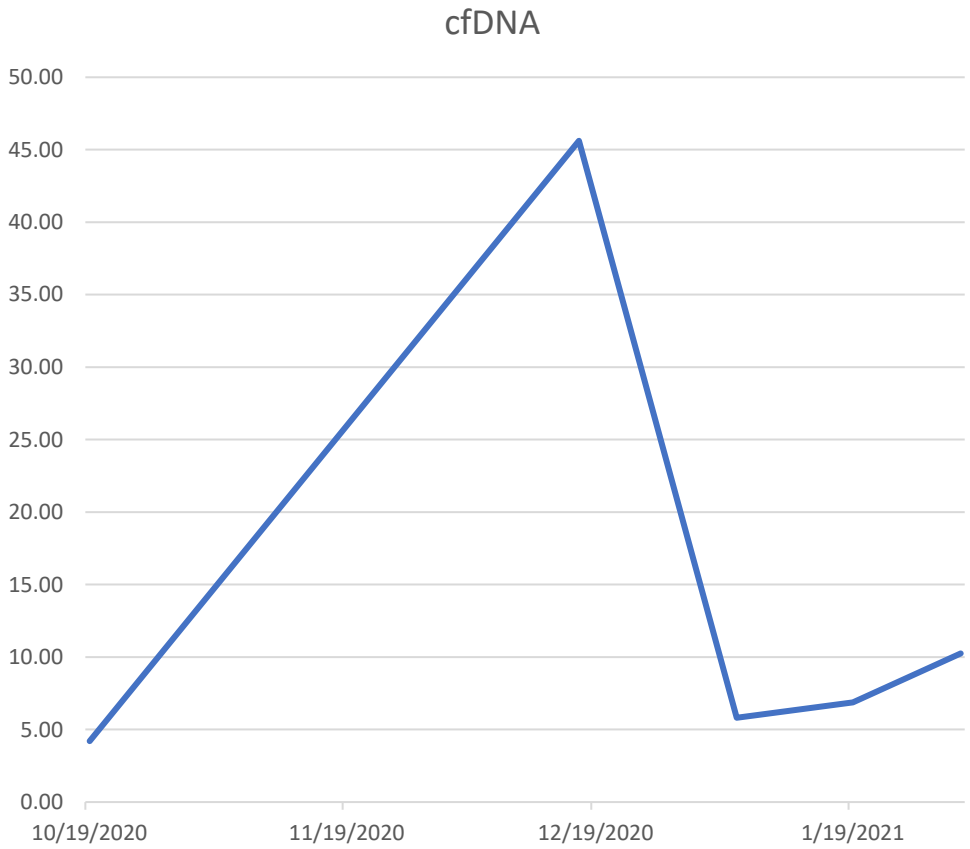
C) Functional fusion protein can be expressed from an out-of-frame gene fusion

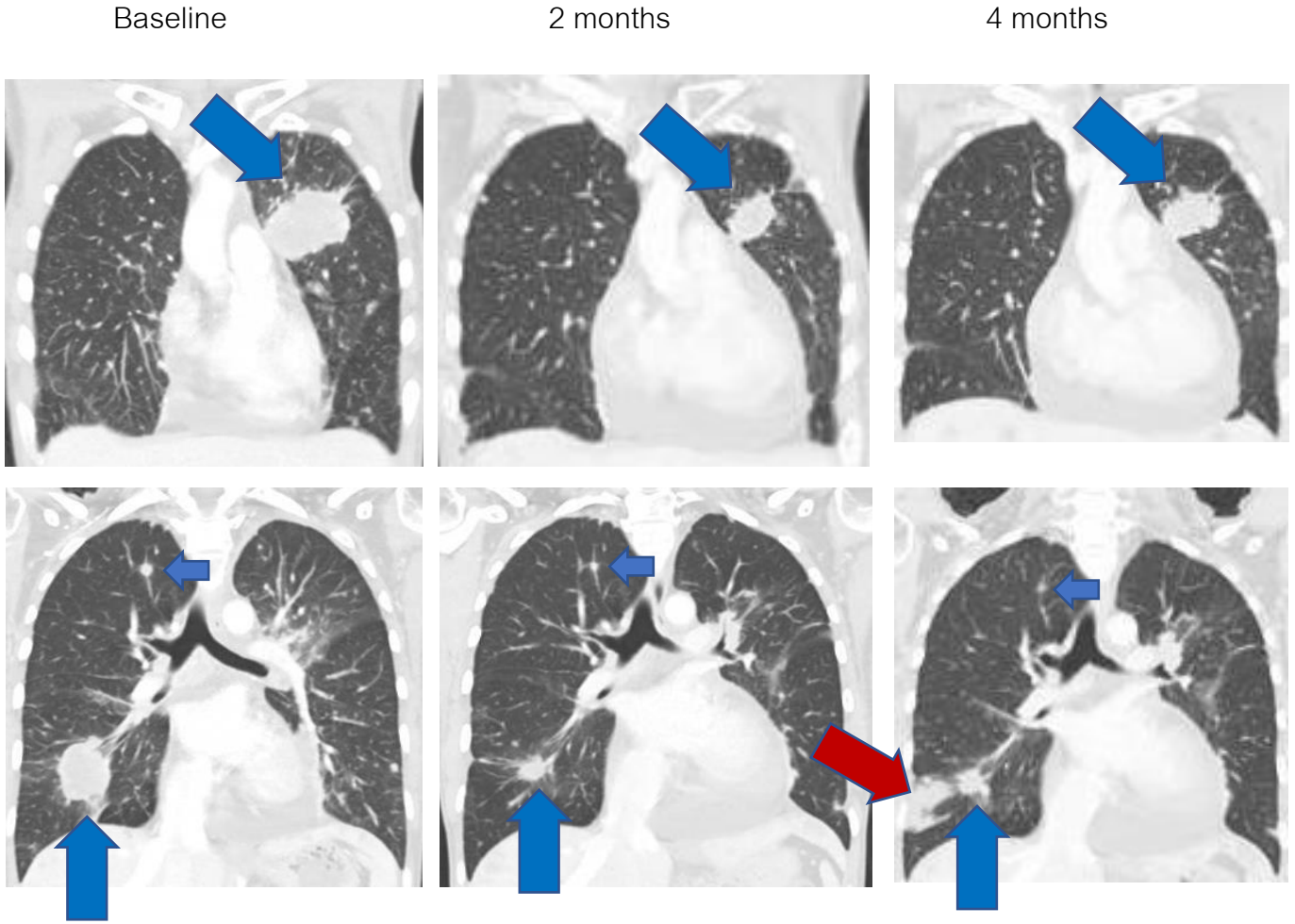
D) Gene fusions can be formed by splicing of mRNA molecules

Clinical history

- Disease progression after paclitaxel + carboplatin rechallenge
- Decision to start ALK-inhibitor
- Alectinib chosen considering CNS metastases
- Initially, good symptom relief and radiological and biochemical response
- Unfortunately, progressive lesions appear quickly

Response to alectinib





Rapid but short-lived response to targeted treatment

Pancreas neuroendocrine carcinoma

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Taipei Veterans General Hospital, Taiwan

FOUNDATION MEDICINE®

Pancreas neuroendocrine carcinoma

Case History

1. 56 y/o male

   Pancreatic head NEC, Grade 3 (ki67:70%), cT4N1M1 with liver metastases [Diagnosed @2019/4]

1. Initial presentation: Jaundice

   s/p ERCP and metallic stent insertion on 2019/3/25

   s/p Chemotherapy with atezolizumab+ etoposide +cisplatin x 4 cycles with PD (2019/5-2019/8)

   s/p FOLFIRINOX (fluorouracil, leucovorin, irinotecan, and oxaliplatin) x 4 cycles with PD (2019/8-2019/10)

   s/p dacarbazine and fluorouracil x 3 cycles with PD (2019/11-2020/1)

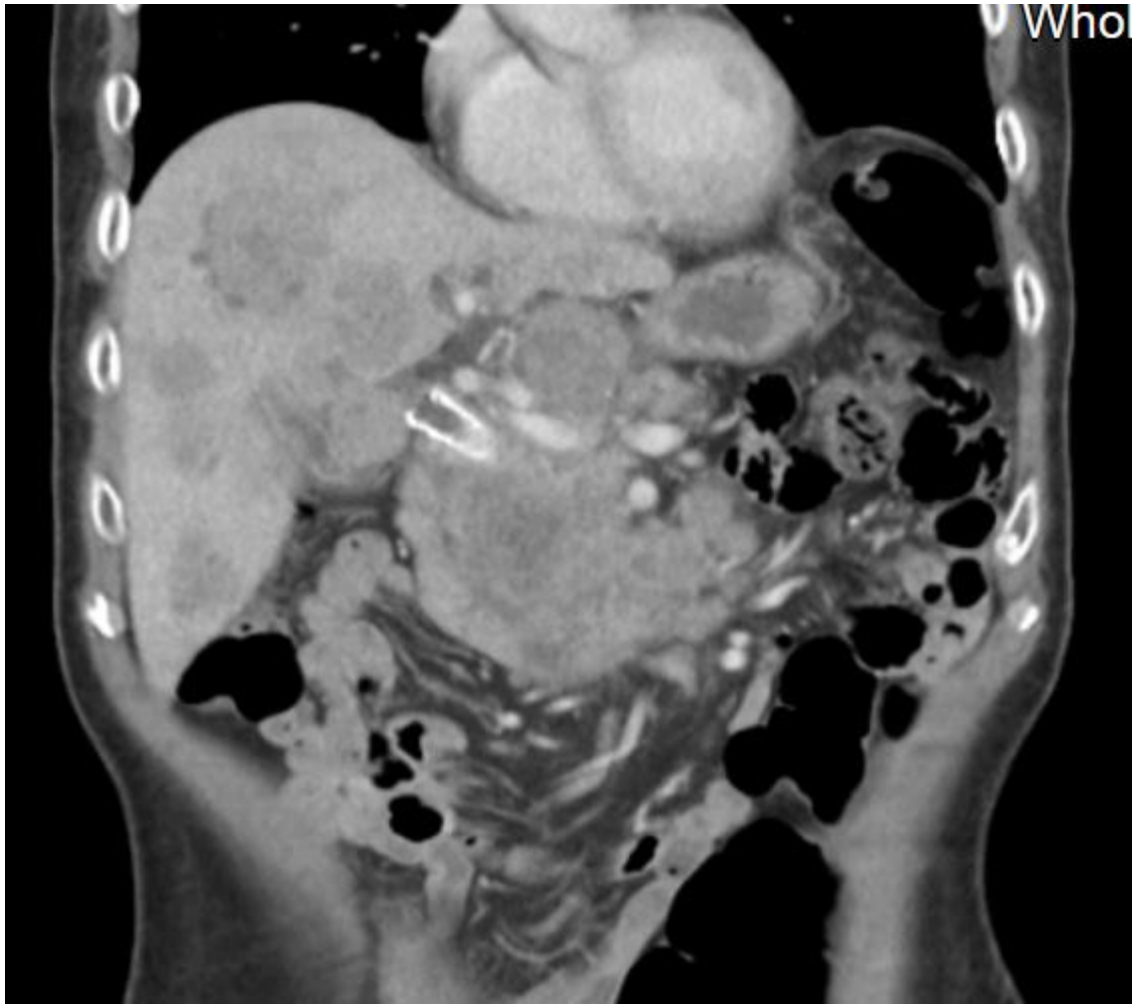
   s/p gemcitabine + nab-paclitaxel x 8 cycles with PD (Best response: SD) (2020/2-2020/05)

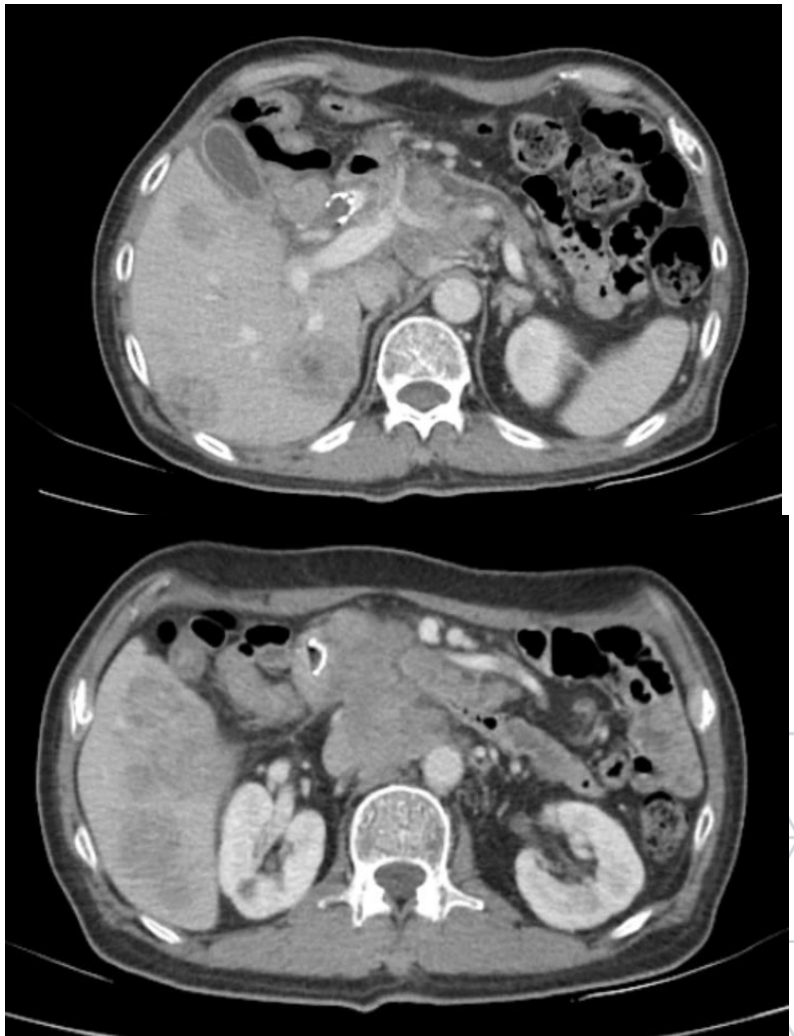
   s/p Ipilimumab + Nivolumab x3 cycles with PD (2020/6-2020/7) with complicated type 1 DM

3. Due to no further treatment choices, the patient received FMI **Liquid** CDx.

Pancreatic neuroendocrine carcinoma

Image CT scan (2020/9): pancreas head tumor (4.2 x 2.8 cm), multiple liver, multiple LN and lung metas





Pancreatic neuroendocrine carcinoma

Pathology

Liver biopsy (2019/4):

1. Sections show liver tissue infiltrated with poorly differentiated carcinoma cells in nested and cord-like patterns.
2. The tumor cells are positive for **CK and INSM1** negative for CD56, CK7, trypsin and synaptophycin. **Ki-67 index is 70%.**

Conclusion: The above histologic and immunophenotypical features are suggestive of a poorly differentiated neuroendocrine carcinoma

Comprehensive Genomic Profiling report

FOUNDATIONONE®LIQUID CDx

TUMOR TYPE
Unknown primary undifferentiated neuroendocrine carcinoma
COUNTRY CODE
TW

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT

DISEASE Unknown primary undifferentiated neuroendocrine carcinoma

Genomic alterations	VAF%
ETV6-NTRK3 fusion	24.3%
KRAS K117N	0.23%
RB1 R320*	50.9%
TP53 R213*	65.8%

Biomarker Findings

Blood Tumor Mutational Burden - 10 Muts/Mb
Microsatellite status - Cannot Be Determined
Tumor Fraction - 62%

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

NTRK3 **ETV6-NTRK3 fusion**
KRAS **K117N**
RB1 **R320***
TP53 **R213***

Poll question 1

NTRK fusions were observed in 0.3% of whole population (0.31% of adult tumors and 0.34% of pediatric tumors). What level of patient in whole population do you think will be identified as NTRK+ using FMI liquid CDx?

A. 0.3% in the whole population
B. 0.1~0.3 % in the whole population
C. < 0.1% in the whole population
D. NTRK fusion could not be detected by liquid biopsy

Poll question 2

Which of the below statement is correct?

A. Gene fusions are common genomic alterations

B. Off target resistance to NTRK inhibitors is mediated via convergent MAPK pathway activation

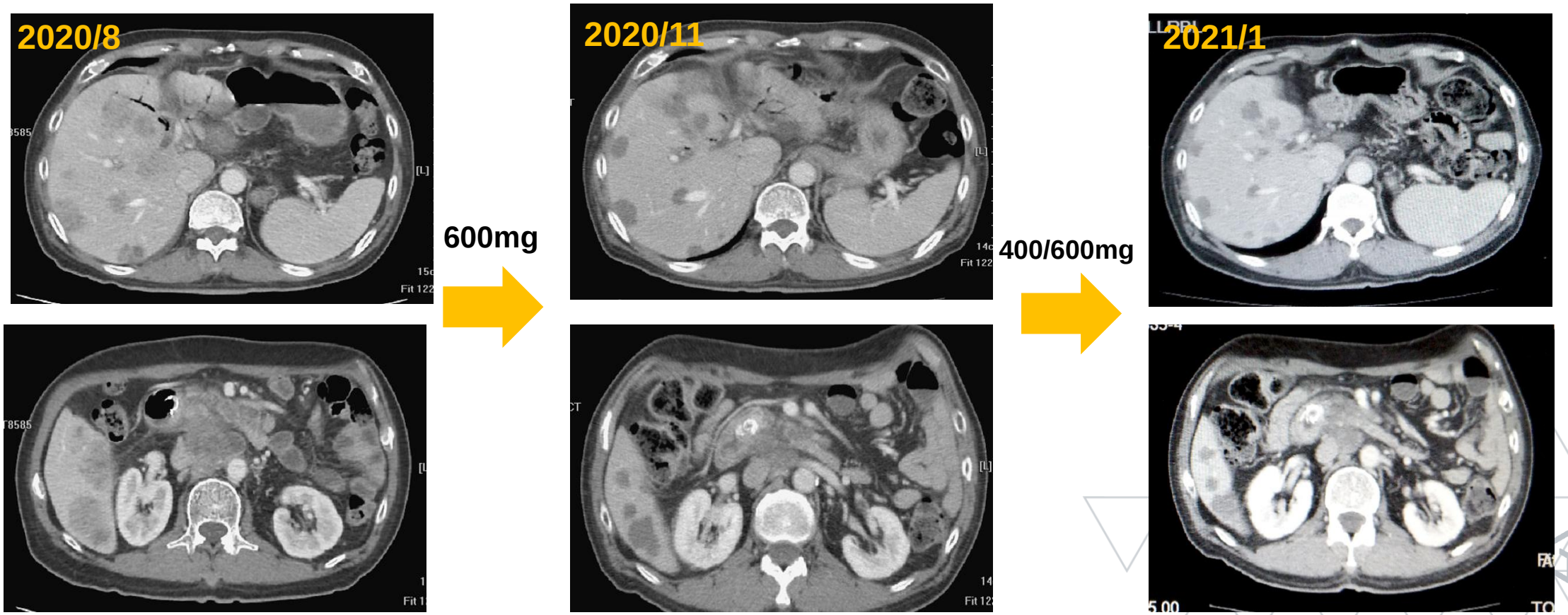
C. Fusion genes can only be detected in solid tumors

D. Tyrosine kinase inhibitors don’t exert activity in fusion-driven cancers

Pancreatic neuroendocrine carcinoma

Patient's outcome

- Patient received **Entrectinib** since September 2020 (600mg self-pay)
- **Tx-related AE: grade 1 fatigue, BW increase 3 kg/month**
- **Disease status: PR**



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