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PRECISION ONCOLOGY CONNECT

ACTIONABLE FUSIONS IN SOLID TUMOURS

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DEVELOPED BY PRECISION ONCOLOGY CONNECT

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CLINICAL TAKEAWAYS

1. Several fusions are now actionable in solid tumours

2. Most of them were initially studied in NSCLC (*ALK, ROS1 and RET*), but many are now being developed in a tumour-agnostic manner (*NTRK, RET, FGFR*)

3. The gold standard for detecting them is RNA-based NGS, but IHC, FISH and real-time PCR can also be used

ALK, anaplastic lymphoma kinase; FGFR, fibroblast growth factor receptor; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tropomyosin-receptor kinase; PCR, polymerase chain reaction; RNA, ribonucleic acid; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase

EDUCATIONAL OBJECTIVES

1. Understand **what a gene fusion is** and the different types of genetic variations used in precision oncology

2. Know why gene fusions are oncogenic and **how they can be identified**

3. Have an awareness of the **different types of gene fusions**, their **distribution across tumour types** and **key data related to associated treatments**

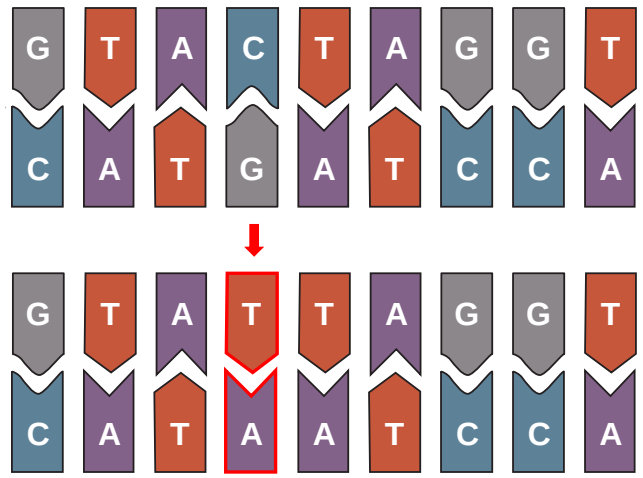
CONTENT

- What is a fusion?
- Why are fusions oncogenic?
- How can we identify fusions?
- When should we search for fusions?
  - The lung carcinoma paradigm
  - The tumour agnostic (r)evolution

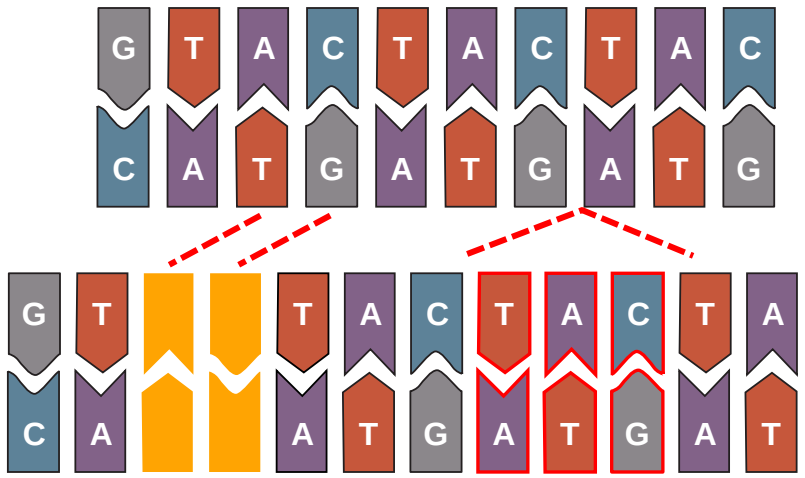
WHAT IS A FUSION?

TYPES OF GENETIC VARIATION

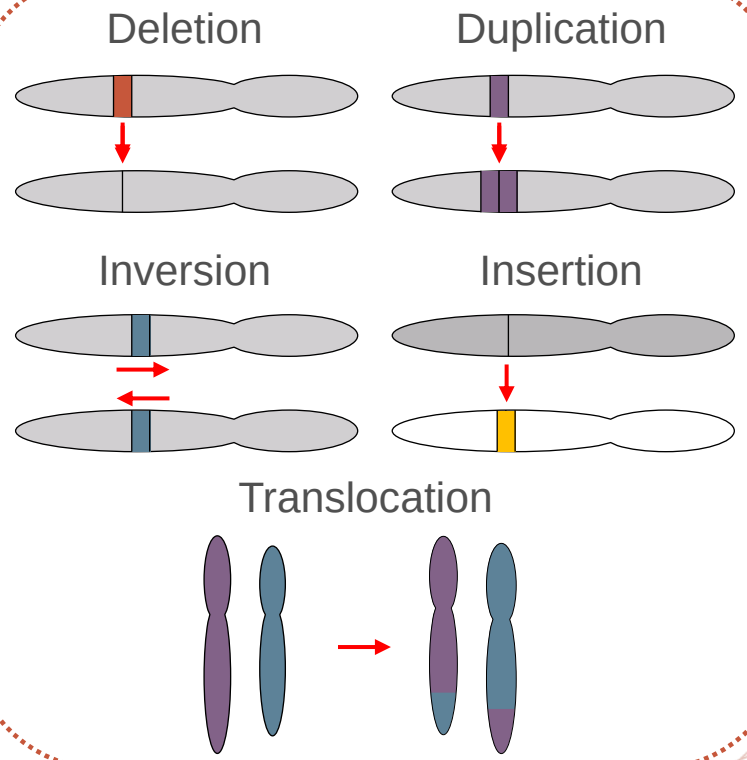
Single nucleotide variants (SNV)



Short insertions or deletions (Indel)



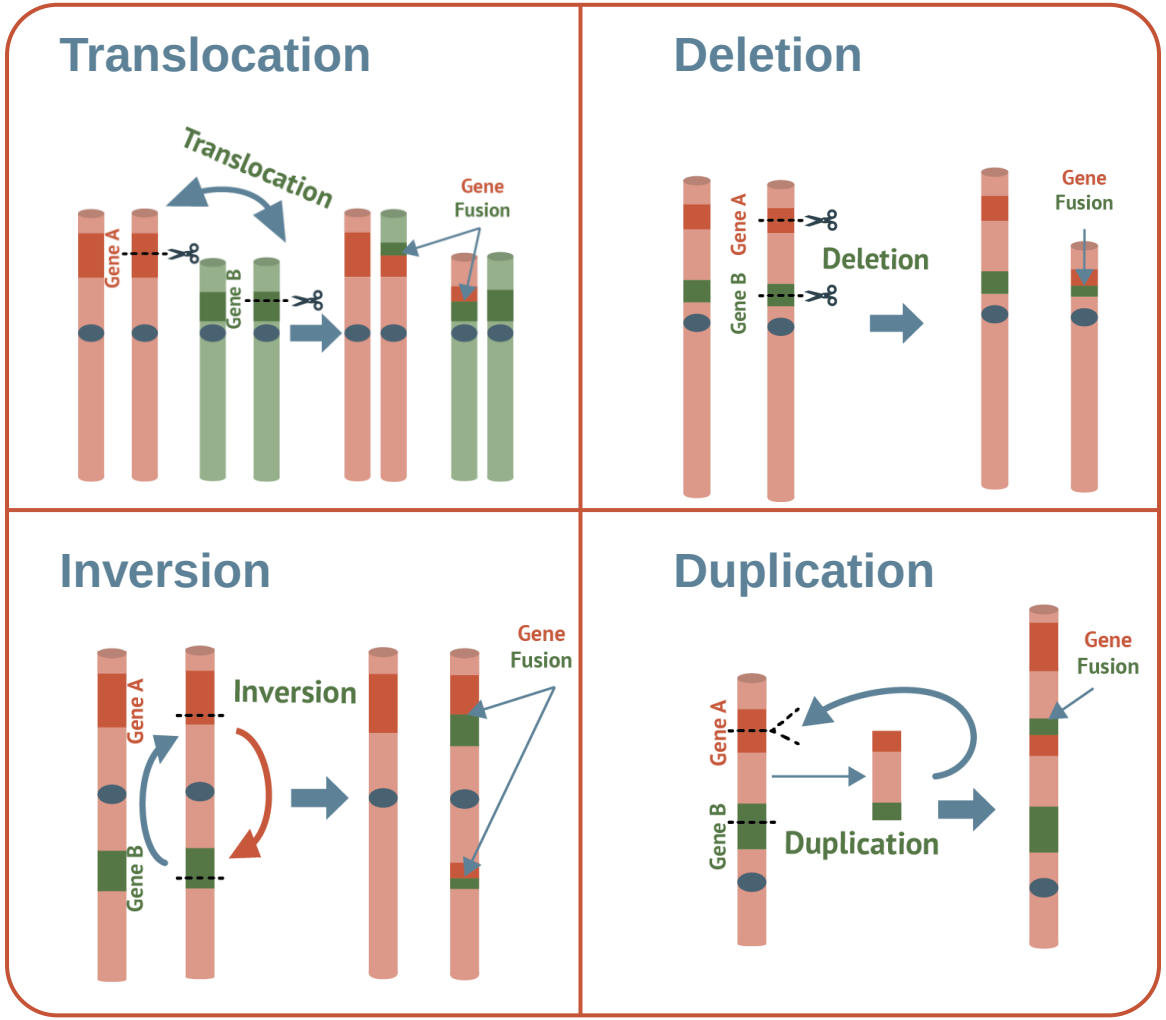
Large structural variants (SV)



Nesta AV, et al. Trends Genet. 2021;37:717-729

WHAT IS A FUSION?

DEFINITION

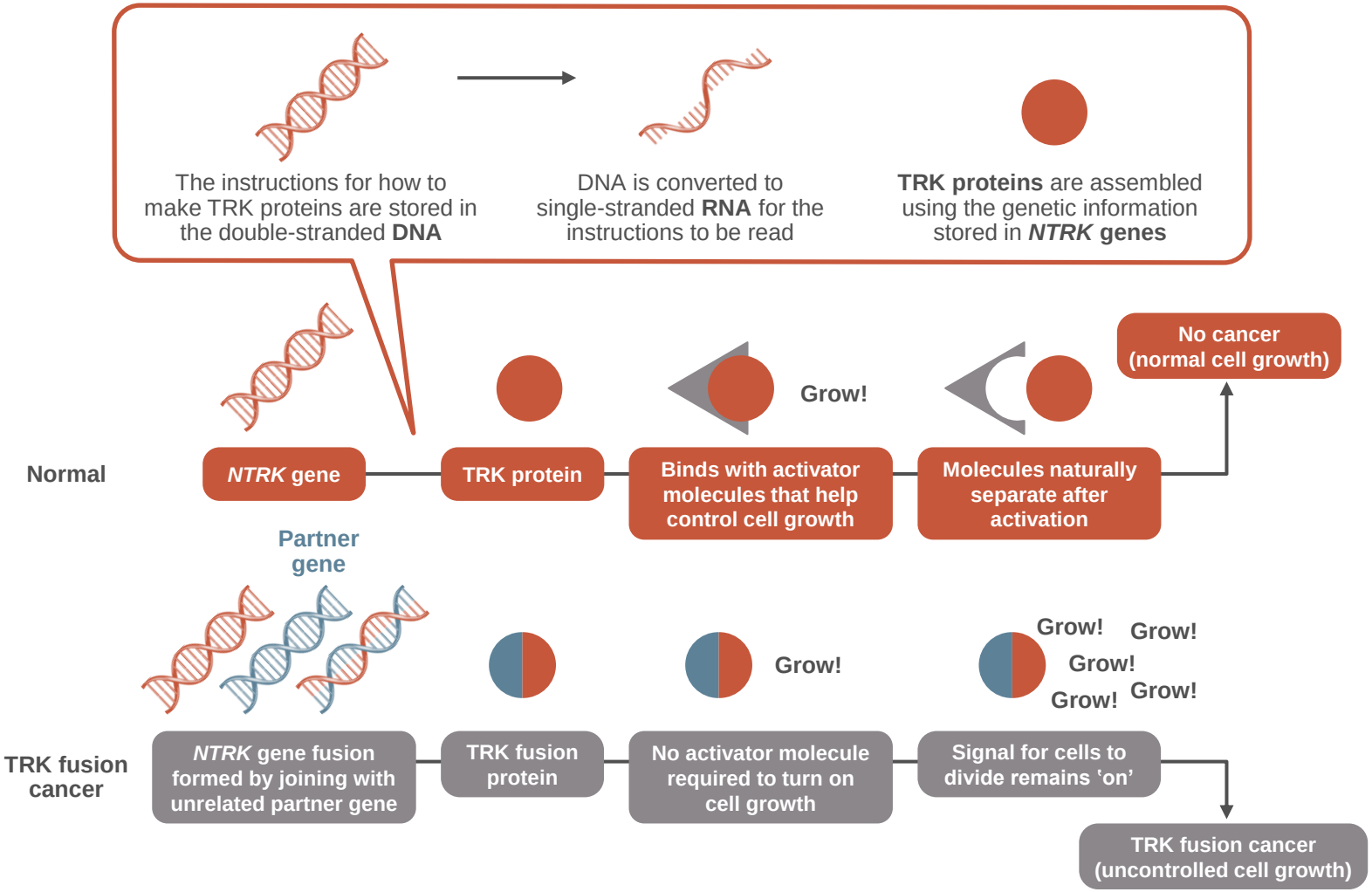


- A novel gene product that is created from two previously separate and independent genes. Gene fusions may arise from genomic rearrangements such as:
- **Chromosomal translocations:** the joining of DNA that previously resided within different chromosomal locations
- **Interstitial deletions:** deletions that occur because of two breakpoints and the rejoining of the terminal end to the main chromosome
- **Inversions:** a region of chromosomal DNA that is reversed
- **Tandem duplications:** replication of the portion of the genomic sequence immediately adjacent to the duplication

DNA, deoxyribonucleic acid

Chakravarty D, et al. J Clin Oncol. 2022;40:1231-1258

WHY ARE FUSIONS ONCOGENIC?



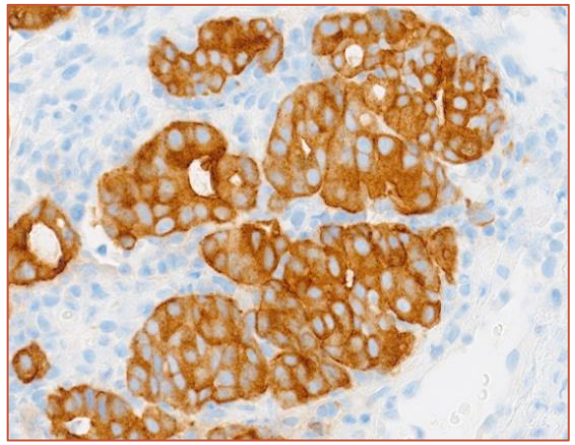
DNA, deoxyribonucleic acid; NTRK, neurotrophic tyrosine receptor kinase; RNA, ribonucleic acid; TRK, tropomyosin receptor kinase

Rudzinski ER, et al. Future Oncol. 2022;18:4141-4151

HOW CAN WE IDENTIFY FUSIONS?

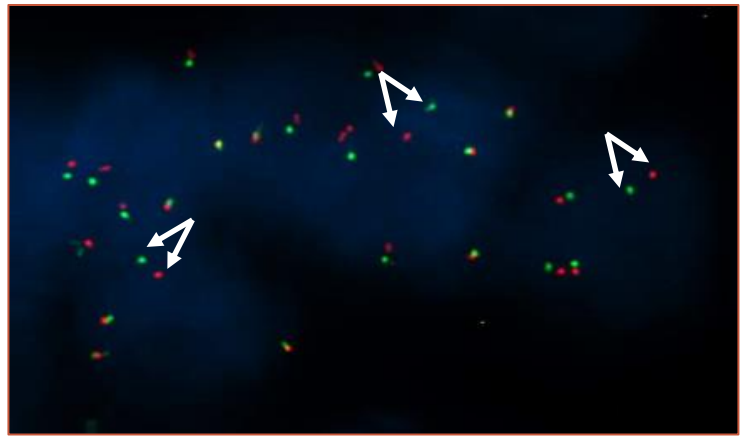
Immunohistochemistry

Cytoplasmic/membranous

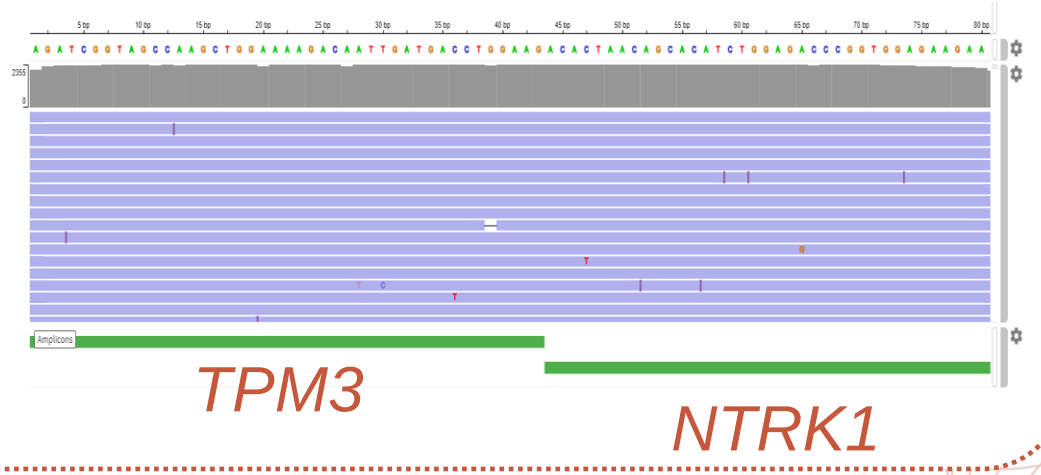


Fluorescence *in situ* hybridisation

Break-apart pattern



Next generation sequencing



NTRK1, neurotrophic tyrosine receptor kinase 1; TPM3, tropomyosin 3

Images provided by Dr Fernando Lopez-Rios

IMMUNOHISTOCHEMISTRY (IHC)

Immunohistochemistry	
Gene fusions detected	*ALK, ROS1, NTRK*
Advantages	Low input material Short turnaround time Usually, high sensitivity Low cost
Challenges	Specificity (*ROS1* and *NTRK* positivity need to be confirmed by a genomic method)

ALK, anaplastic lymphoma kinase; NTRK, neurotrophic tyrosine receptor kinase; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase

FLUORESCENCE IN SITU HYBRIDISATION (FISH)

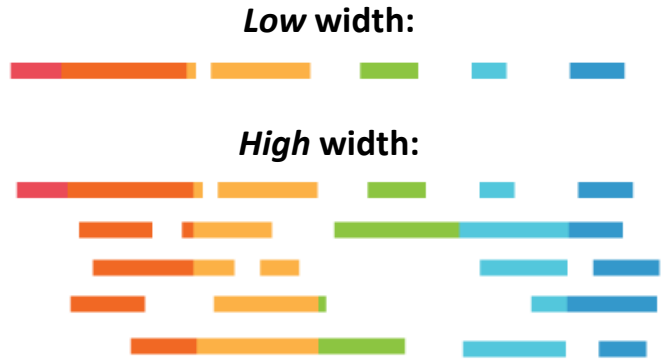
Flourescence in-situ hybridisation	
Methods	Break-apart probes
Advantages	Low input material Short turnaround time Usually, high specificity & sensitivity Low cost
Challenges	Interpretation

REAL-TIME PCR

Real-time PCR	
Methods	RNA real-time PCR
Advantages	Low input material Short turnaround time Usually, high specificity & sensitivity Low cost
Challenges	RNA failure rate Design of the assay • Risk of false negatives • Width – could miss certain alterations depending on selected design

Persevere if RNA fails

- ✓ Re-test (different block/specimen)
- ✓ Re-biopsy
- ✓ Use another assay



RNA, ribonucleic acid; PCR, polymerase chain reaction

NEXT-GENERATION SEQUENCING (NGS)

Next-generation sequencing	
Methods	The study of thousands of genomic alterations
Advantages	Comprehensive Usually high specificity & sensitivity
Challenges	Longer turnaround time High cost Reduced sensitivity of DNA-only NGS for fusion testing RNA failure rate of RNA-only NGS

Chip-use optimisation

Persevere if RNA fails

- ✓ Re-test (different block/specimen)
- ✓ Re-biopsy
- ✓ Use single analyte assays

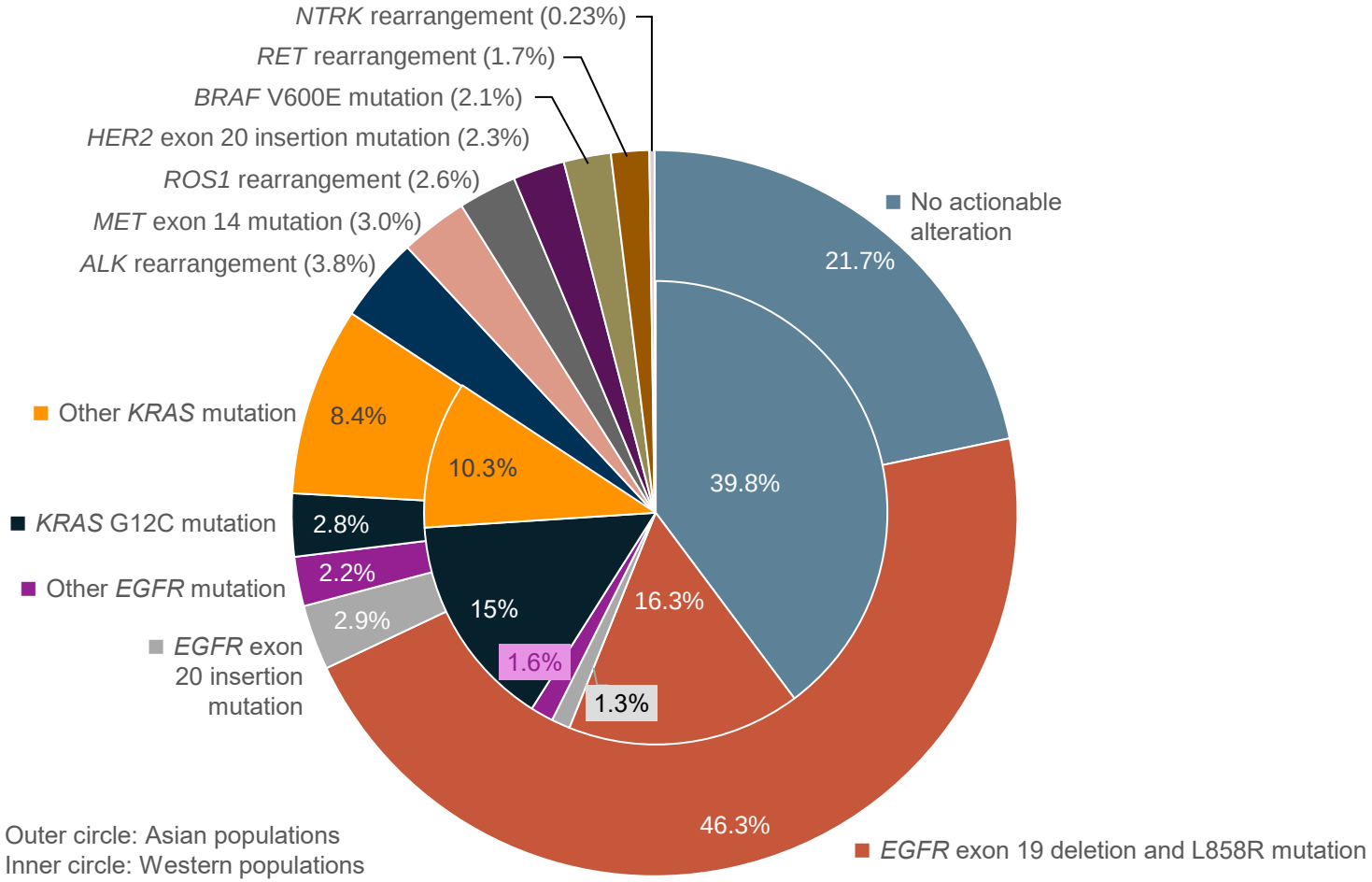
Persevere if DNA-only NGS is negative

- ✓ Use an RNA-based method

DNA, deoxyribonucleic acid; NGS, next-generation sequencing; RNA, ribonucleic acid

WHEN SHOULD WE SEARCH FOR FUSIONS?

GENE FUSIONS HAVE A PIVOTAL ROLE IN LUNG CARCINOMA



BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten ras oncogene; MET, MET proto-oncogene; NTRK, neurotrophic tyrosine receptor kinase; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase

Tan AC and Tan DSW. J Clin Oncol. 2022;40:611-625

DISTRIBUTION OF KINASE FUSIONS ACROSS PRIMARY SITES

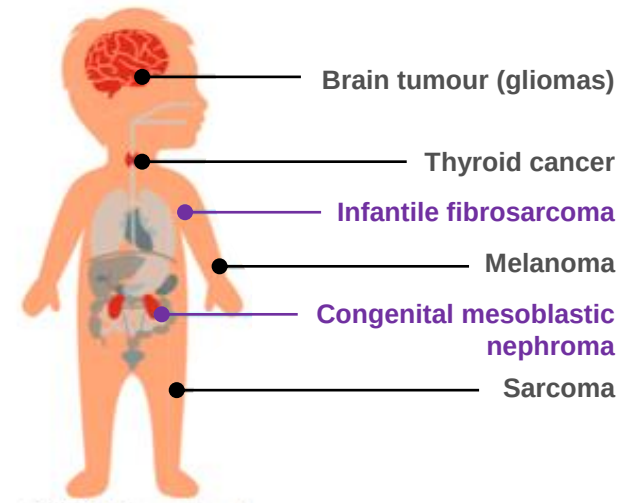
THE TUMOUR AGNOSTIC (R)EVOLUTION

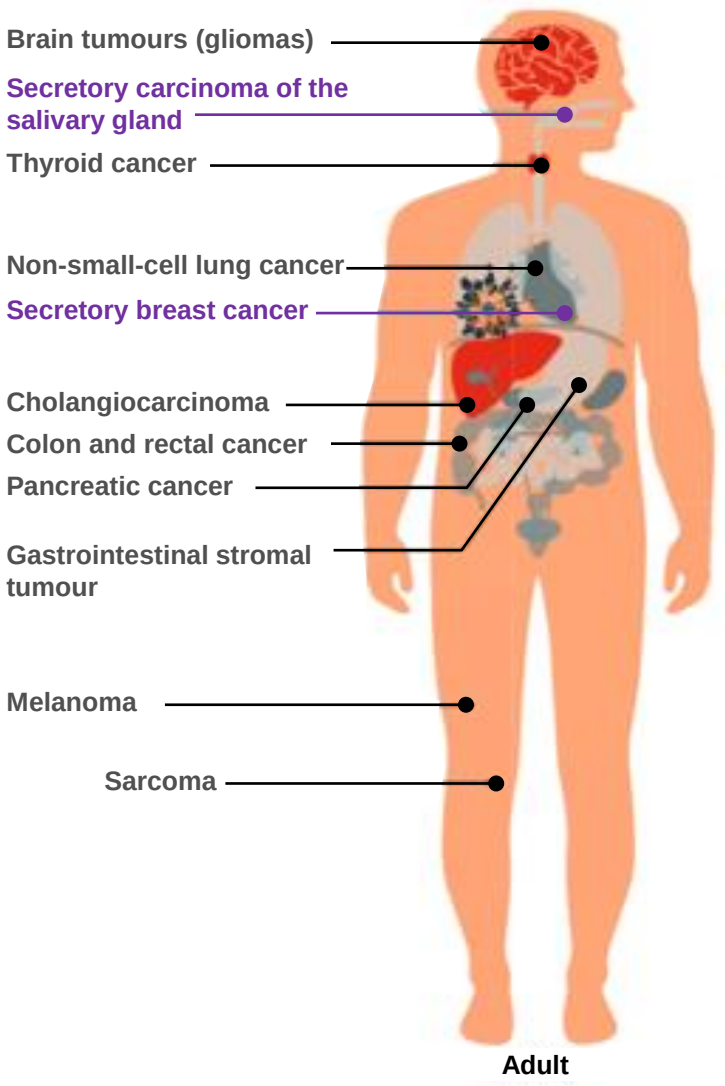
	ALK	*ROS1*	*RET*	*NTRK1/2/3*	*FGFR1/2/3*	*BRAF/CRAF*
Brain		X		X	X	X
Parotid gland				X		
Oesophagus	X					
Head and neck				X	X	
Thyroid	X		X	X	X	X
Lung	X	X	X	X	X	X
Breast	X	X	X	X	X	
Bones/soft tissue	X	X		X		
Skin	X	X	X	X		X
Stomach		X			X	X
Liver/gall bladder	X	X		X	X	
Pancreas						X
Kidney	X			X	X	
Colon/rectum	X	X	X	X		X
Ovary		X				
Bladder	X				X	
Prostate					X	X

ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; CRAF, c-RAF proto-oncogene; FGFR1/2/3, fibroblast growth factor receptor 1/2/3; NTRK1/2/3, neurotrophic tyrosine receptor kinase 1/2/3; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase

Schram AM, et al. Nat Rev Clin Oncol. 2017;14:735-748

TYPES OF NTRK FUSION-POSITIVE CANCERS IN CHILDREN AND ADULTS





Rare tumours with high TRK fusion protein frequency

More common tumours with low TRK fusion protein frequency

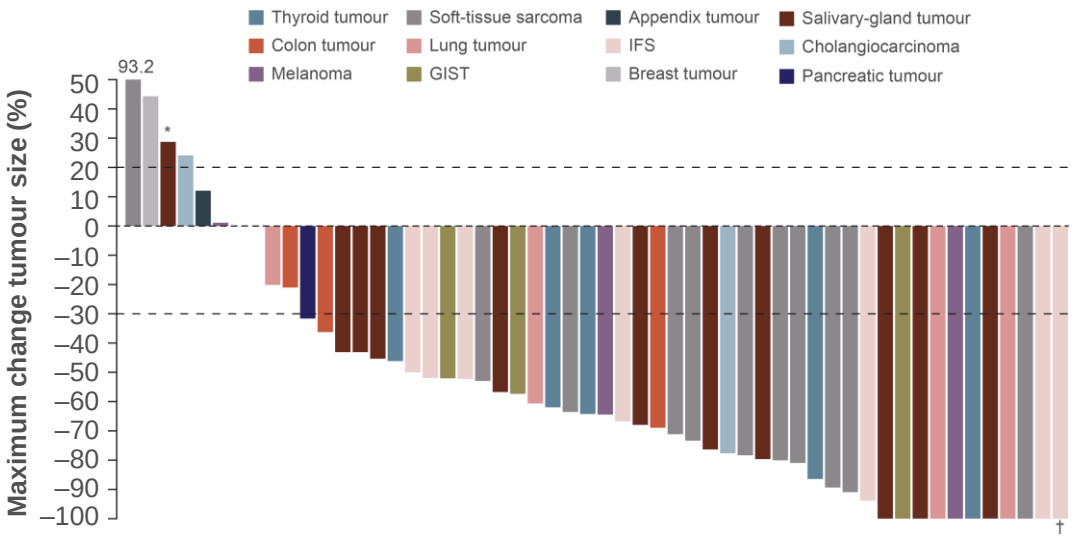
NTRK, neurotrophic tyrosine receptor kinase; TRK, tropomyosin receptor kinase

Rudzinski ER, et al. Future Oncol. 2022;18:4141-4151

INITIAL EFFICACY RESULTS OF APPROVED TRK INHIBITORS: RESPONSES BY TUMOUR TYPE

Larotrectinib[1]

Data cut-off: 17 July 2017

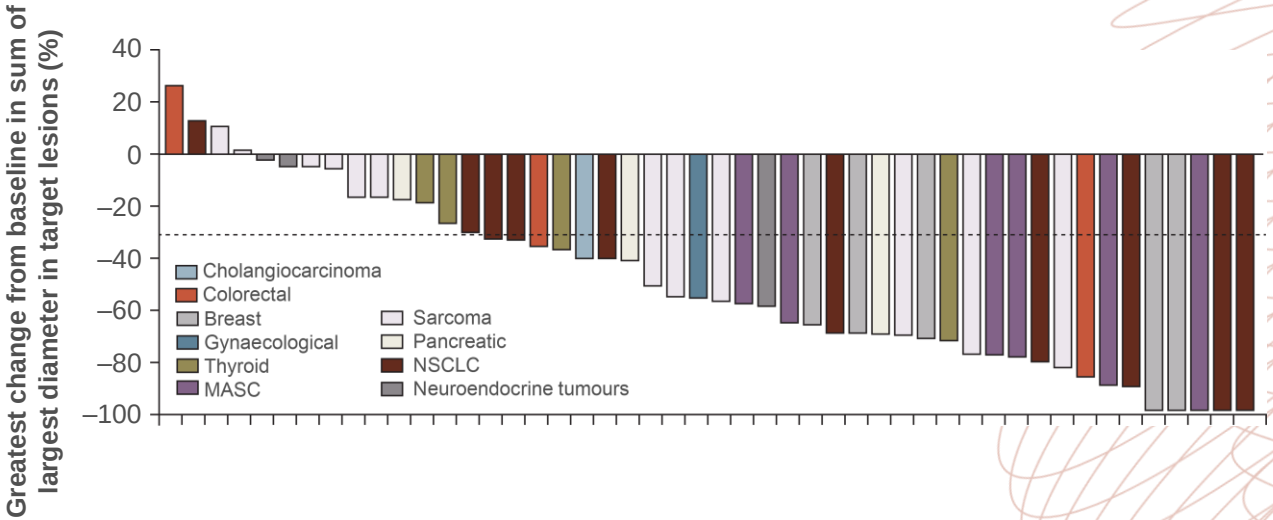


Objective responses with larotrectinib are seen in multiple tumour types and in most of the patients:

80%, 95% CI: 67-90

Entrectinib[2]

Data cut-off: 31 May 2018



Objective responses with entrectinib are seen in multiple tumour types and in most of the patients:

57%, 95% CI: 43.2-70.8

One patient (dagger) had a pathological complete response

CI, confidence interval; GIST, gastrointestinal stromal tumour; IFS, infantile fibrosarcoma; MASC, mammary analogue secretory carcinoma; NSCLC, non-small-cell lung cancer; TRK, tropomyosin receptor kinase

1. Drilon A, et al. N Engl J Med. 2018;378:731-739; 2. Doebele RC, et al. Lancet Oncol. 2020;21:271-282

TYPES OF *RET*-REARRANGED TUMOURS

FOCUS ON GASTRO-INTESTINAL AND NEUROENDOCRINE?

ARROW: pralsetinib in patients with *RET*-fusion positive tumours[1]

Tumour type, n (%)	*RET* fusion-positive solid tumours	
	Efficacy-evaluable population (n=23)	Safety population (n=29)
Pancreatic	4 (17)	5 (17)
Cholangiocarcinoma	3 (13)	4 (14)
Neuroendocrine	3 (13)	3 (10)
Sarcoma	3 (13)	3 (10)
Head and neck	2 (9)	2 (7)
Colorectal	2 (9)	5 (17)
SCLC	2 (9)	2 (7)
Unknown primary	1 (4)	1 (3)
Gastric	1 (4)	1 (3)
Ovarian	1 (4)	1 (3)
Thymic	1 (4)	1 (3)
CNS	0	1 (3)

LIBRETTO-001: selpercatinib in patients with *RET*-fusion positive tumours[a2]

Primary tumour diagnosis, n (%)	*RET* fusion tumour-agnostic population (n=45)
Pancreatic	12 (27)
Colon	10 (22)
Salivary	4 (9)
Sarcoma	3 (7)
Unknown primary	3 (7)
Breast	2 (4)
Carcinoma of the skin	2 (4)
Cholangiocarcinoma	2 (4)
Xanthogranuloma	2 (4)
Carcinoid	1 (2)
Ovarian	1 (2)
Pulmonary carcinosarcoma	1 (2)
Rectal neuroendocrine	1 (2)
Small intestine	1 (2)

[a] excluding lung and thyroid

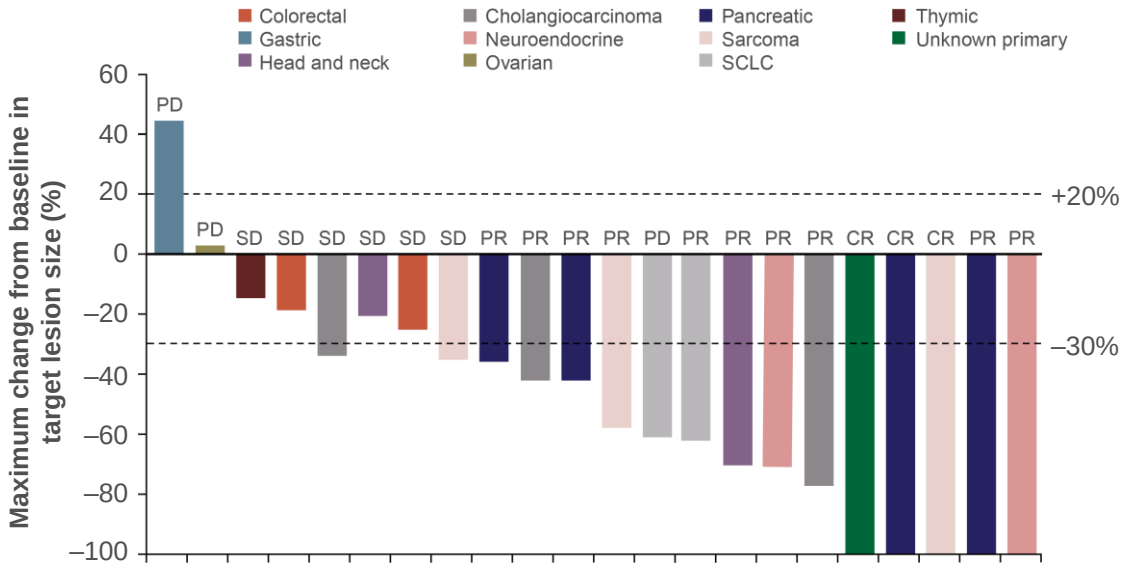
CNS, central nervous system; RET, RET proto-oncogene; SCLC, small-cell lung cancer

1. Subbiah V, et al. Nat Med. 2022;28:1640-1645; Subbiah V, et al. Lancet Oncol. 2022;23:1261-1273

INITIAL EFFICACY RESULTS OF *RET* INHIBITORS: RESPONSES BY TUMOUR TYPE

ARROW: pralsetinib[1]

Data cut-off: 18 Oct 2021

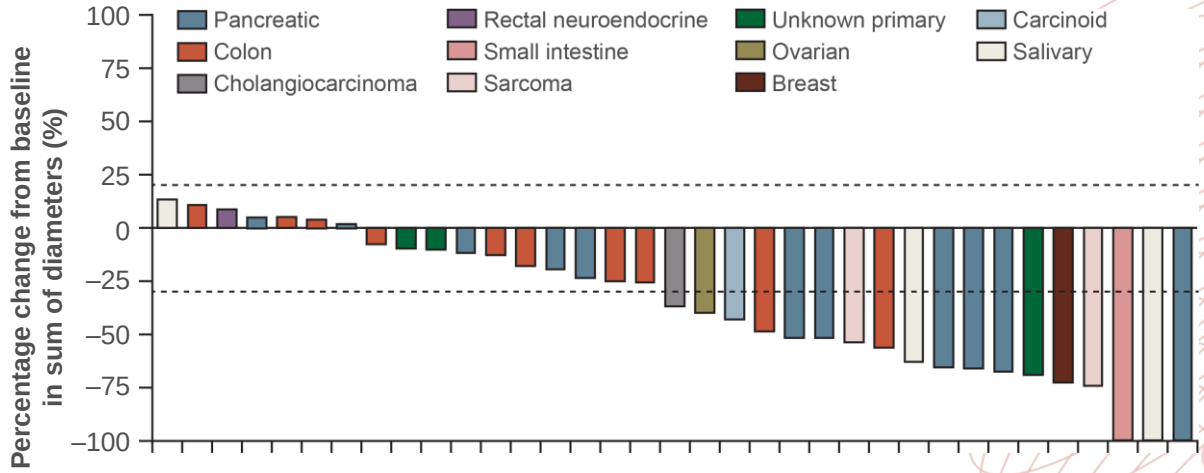


Objective responses with pralsetinib are seen in multiple tumour types and in most of the patients:

57%, 95% CI: 35-77

LIBRETTO-001: selpercatinib[2]

Data cut-off: 24 Sep 2021



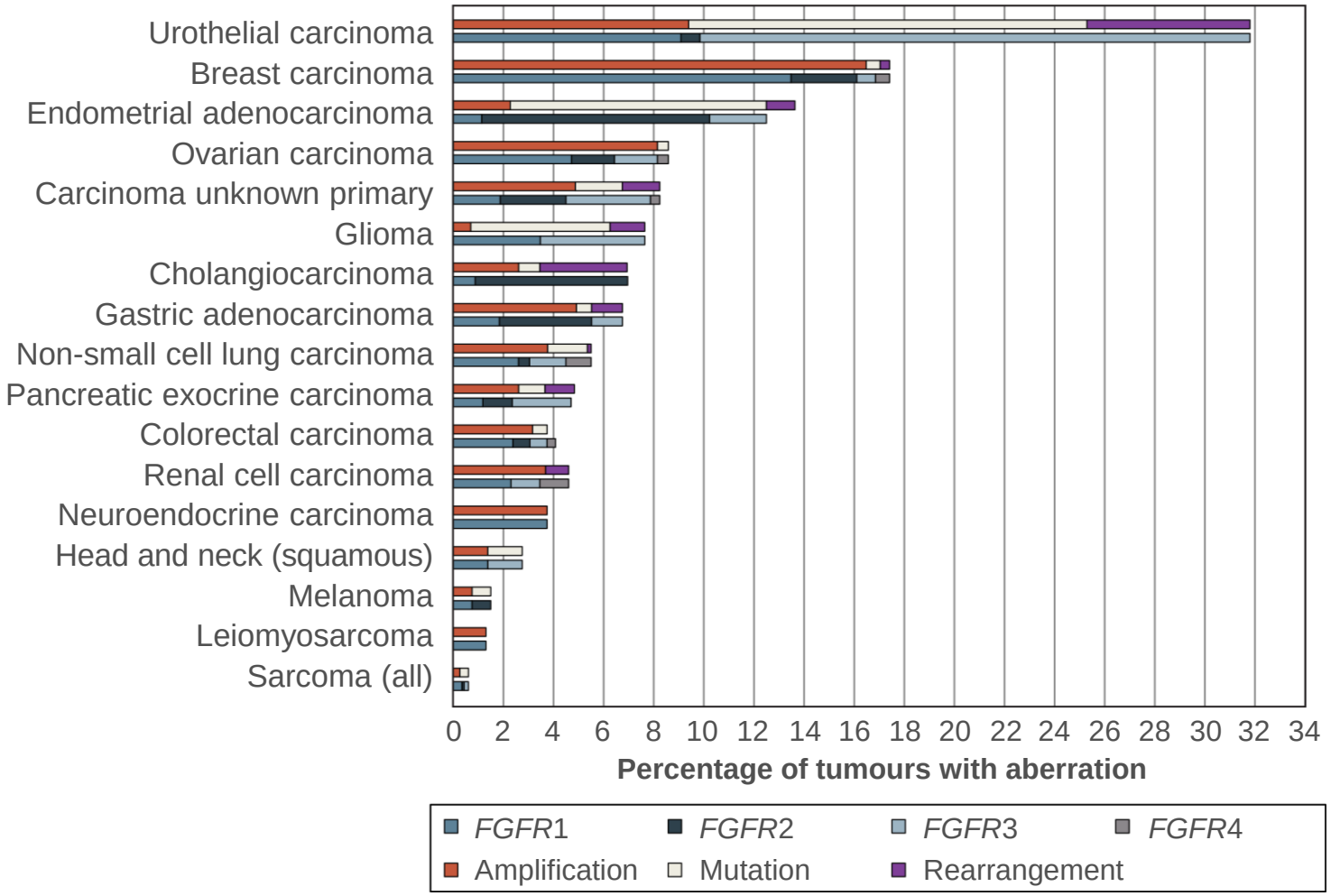
Objective responses with selpercatinib are seen in multiple tumour types and in most of the patients:

43.9%, 95% CI: 28.5-60.3

CI, confidence interval; CR, complete response; PD, progressive disease; PR, partial response; RET, RET proto-oncogene; SCLC, small-cell lung cancer; SD, stable disease

1. Subbiah V, et al. Nat Med. 2022;28:1640-1645; 2. Subbiah V, et al. Lancet Oncol. 2022;23:1261-1273

FGFR FUSIONS ARE COMMON IN A WIDE VARIETY OF CANCERS

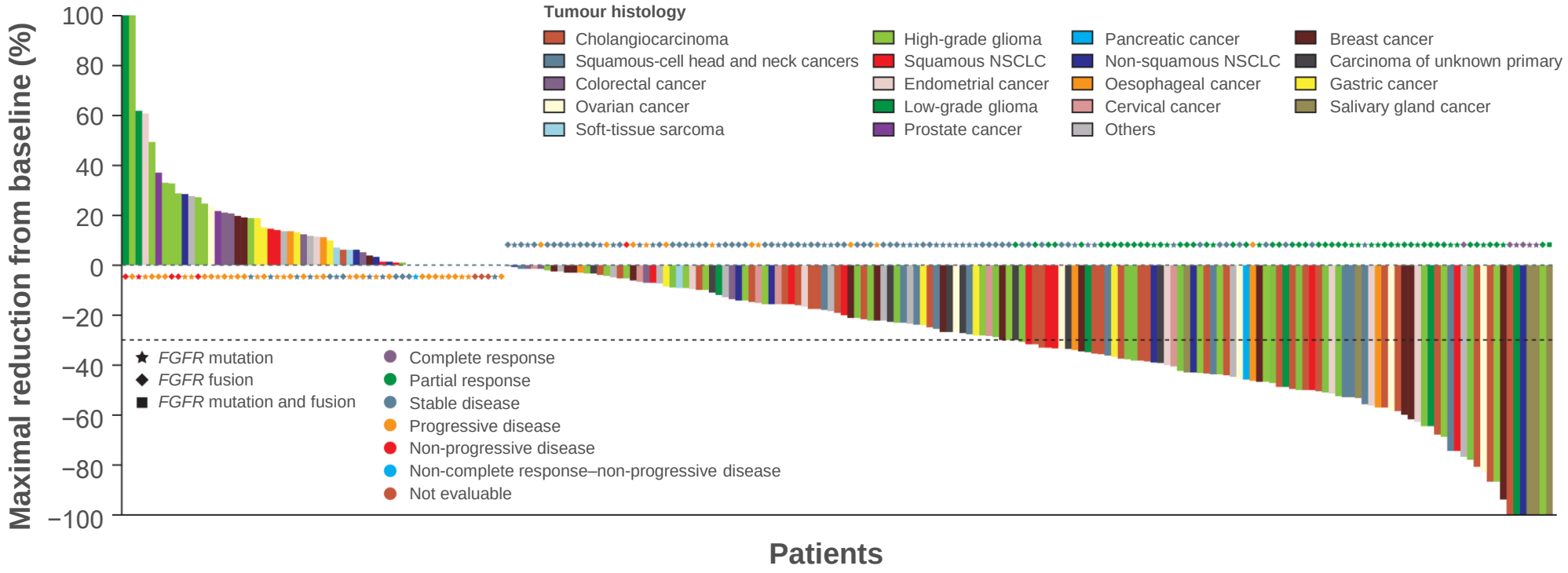


- 4,853 solid tumours analysed on physician request by NGS
- *FGFR* aberrations were found in 7.1% of cancers [66% gene amplifications, 26% mutations, 8% rearrangements]
- *FGFR*1 was affected in 3.5% of 4,853 patients; *FGFR*2 in 1.5%; *FGFR*3 in 2.0%; and *FGFR*4 in 0.5%

FGFR(1/2/3/4), fibroblast growth factor receptor (1/2/3/4); NGS, next-generation sequencing

Helsten T, et al. Clin Cancer Res. 2016;22:259-267

RAGNAR TRIAL – ERDAFITINIB IN *FGFR*-ALTERED ADVANCED SOLID TUMOURS

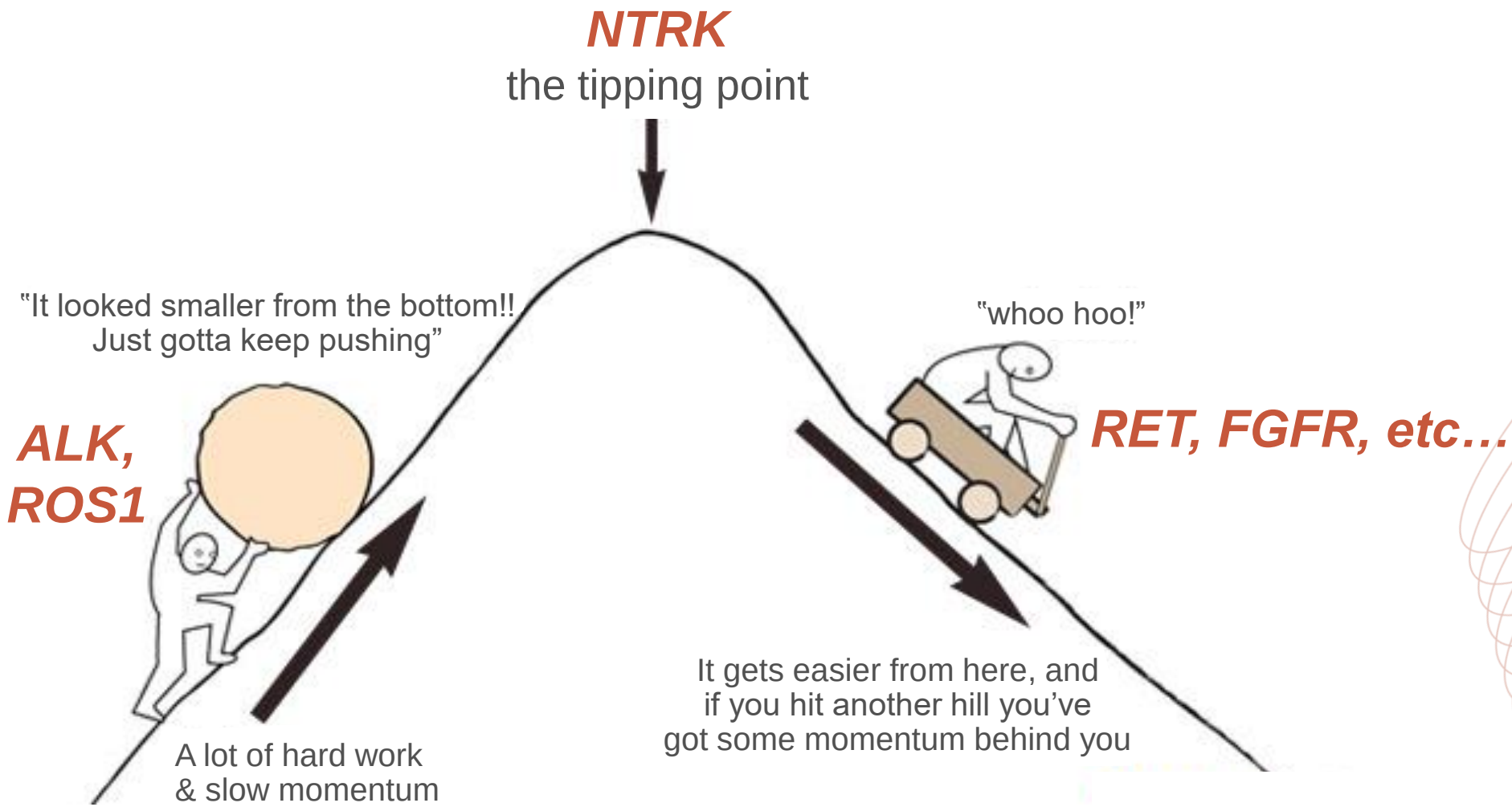


FGFR, fibroblast growth factor receptor; NSCLC, non-small cell lung cancer

Pant S, et al. Lancet Oncol. 2023;24:925-935

THE TIPPING POINT FOR NGS

THE NEED TO SEARCH FOR ACTIONABLE FUSIONS



ALK, anaplastic lymphoma kinase; FGFR, fibroblast growth factor receptor; NGS, next-generation sequencing; NTRK, neurotrophic tyrosine receptor kinase; RET, RET proto-oncogene; ROS1, ROS proto-oncogene 1 receptor tyrosine kinase

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