

Genomic Testing in the Emerging Era of Precision Medicine: Lessons Learned from Studies in Larotrectinib

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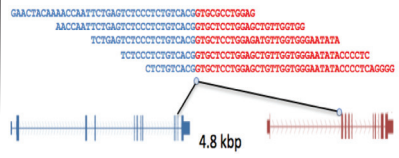
INTRODUCTION

- Identifying the genetic driver of a tumour may allow use of targeted therapies, which typically have fewer side effects than chemotherapies.
- Genomic testing can identify oncogenic alterations, including gene mutations and chromosomal rearrangements, that can be targeted with specific therapies.
- Chromosomal rearrangements may lead to gene fusions, resulting in novel oncogenic fusion proteins.¹
 - Selective inhibitors of oncogenic fusion proteins enable precision medicine-based treatment of gene fusion cancers.¹
 - Molecular testing is essential to identify patients, with rare tumour types, who may benefit from such agents (Figure 1).^{2,3}

Figure 1. Molecular testing for gene fusions^{2,3}

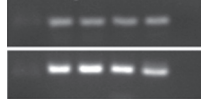
Next-generation sequencing (NGS)

- Tests for different genomic alterations in parallel using DNA or RNA
- RNA is preferred for gene fusions



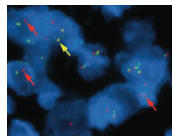
Reverse transcriptase polymerase chain reaction (RT-PCR)

- Detects known fusion transcripts in RNA
- Detects 5'/3' imbalance as a fusion signature, but cannot determine novel partners



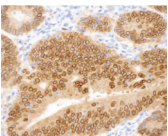
Fluorescence in situ hybridisation (FISH)

- Detects gene rearrangements in DNA that may generate a fusion transcript



Immunohistochemistry (IHC)

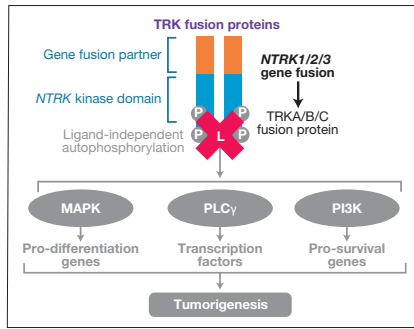
- Detects protein expression, which may result from a fusion event



NTRK GENE FUSIONS

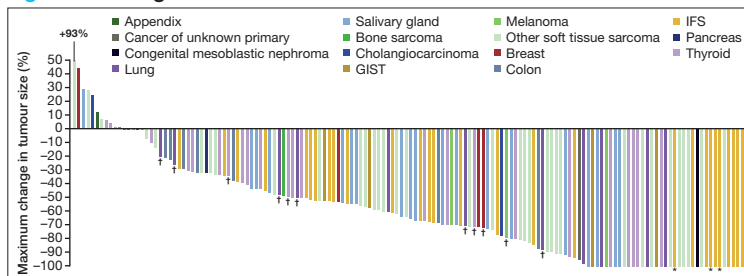
- Tropomyosin receptor kinase (TRK) fusions are oncogenic drivers of various tumours; they arise from rearrangements between neurotrophic tyrosine receptor kinase (*NTRK*) 1, 2, or 3 genes and an unrelated gene (Figure 2).¹
- NTRK* gene fusions identified in >20 paediatric and adult tumour types, and ~1% of all solid tumours.¹
 - Rare in common cancers (e.g., lung, colon) but nearly pathognomonic in certain rare cancers (e.g., infantile fibrosarcoma, secretory carcinoma of the salivary gland, secretory breast carcinoma).

Figure 2. TRK fusions are rare but recurrent oncogenic drivers



- Larotrectinib is a highly selective, CNS-active TRK inhibitor approved in Brazil, Canada, the US and Europe to treat paediatric and adult patients with advanced solid tumours harbouring *NTRK* gene fusions.^{1,4,5}
- In a pooled analysis of 159 patients across three phase I/II trials, objective response rate was 79% and median duration of response was 35.2 months.⁶
- Efficacy was seen regardless of tumour type (Figure 3) and age.⁶
- Larotrectinib was well tolerated. Most adverse events were mild (Grade 1) or moderate (Grade 2) in severity (Table 1).⁶

Figure 3. Change in tumour size on larotrectinib treatment⁶



[†]Patients with a pathological complete response. [‡]Patients with brain metastases. GIST, gastrointestinal stromal tumour; IFS, infantile fibrosarcoma.

Table 1. Adverse events in the safety dataset (N=260)⁶

	Treatment-emergent AEs, (%)				Treatment-related AEs, (%)		
	Grade 1 or 2	Grade 3	Grade 4	Any grade	Grade 3	Grade 4	Any grade
Fatigue	30	2	0	33	<1	0	17
ALT increased	25	3	<1	28	3	<1	22
Cough	27	<1	0	28	0	0	1
Constipation	27	<1	0	27	0	0	11
Anaemia	17	10	0	27	2	0	10
AST increased	24	2	<1	27	<1	0	20
Dizziness	25	<1	0	25	<1	0	18
Nausea	24	<1	0	25	<1	0	13
Vomiting	24	<1	0	25	0	0	9
Diarrhoea	23	1	0	24	0	0	6
Pyrexia	19	<1	<1	20	0	0	2
Dyspnoea	13	2	0	16	0	0	<1
Myalgia	15	1	0	16	<1	0	8
Oedema peripheral	15	<1	0	16	0	0	6
Headache	15	<1	0	15	<1	0	5
Neutrophil count decreased	7	5	<1	12	2	<1	7

Grade 1/2 Grade 3 Grade 4
 Mild/moderate Severe Life threatening

Adverse events that occurred at any grade in ≥15% of patients, or at grade 3 or 4 in ≥5% of patients, regardless of attribution, are listed. AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

CONSIDERATIONS OF GENOMIC TESTING

Benefits and drawbacks of different testing methods^{2,3}

	NGS	FISH	RT-PCR	Pan-TRK IHC
BENEFITS	<ul style="list-style-type: none"> Possible to detect novel fusion partners, and fusions expressed in RNA Ability to test multiple actionable targets in parallel Relevance of NGS increases as number of actionable targets grows Potential for high sensitivity and specificity 	<ul style="list-style-type: none"> Location of target within cell is visible High sensitivity and specificity Several fluorophores can be used at once to detect multiple targets in one sample 	<ul style="list-style-type: none"> High sensitivity and specificity Low cost 	<ul style="list-style-type: none"> Low cost Identifies subcellular location of protein Decentralised; available in most laboratories Established reimbursement codes Turnaround time: 1–2 days
DRAWBACKS	<ul style="list-style-type: none"> Turnaround time: ~1–3 weeks Technically complex and costly Requires highly centralised testing model and specialty infrastructure Reimbursement currently restricted Sensitivity and specificity vary widely 	<ul style="list-style-type: none"> Requires fluorescence microscopy Separate tests required for each <i>NTRK</i> gene Cannot show that functional protein has been generated 	<ul style="list-style-type: none"> Target sequences must be known; cannot detect novel fusion partners Separate tests required for each <i>NTRK</i> gene 	<ul style="list-style-type: none"> Cannot differentiate between fusion and wild-type TRK Scoring algorithms not standardised Confirmatory testing required

- Genomic testing requires accredited laboratories using approved platforms. In larotrectinib clinical trials, *NTRK* gene fusions were identified by NGS in most patients. Patients' tumours were tested in Clinical Laboratory Improvement Amendments-certified laboratories, or equivalent for ex-US sites.⁷

Examples of companies providing commercial NGS services	Archer	www.archerdx.com
	Caris Life Science	www.carislifesciences.com
Foundation Medicine, Inc.	www.foundationmedicine.com	
illumina	www.illumina.com	
Paradigm Diagnostics	www.paradigmidx.com	
PathGroup	www.pathgroup.com	
Tempus	www.tempus.com	
ThermoFisher	www.thermofisher.com	

- Panel of mutations tested for vary between companies and depend on which panel is ordered. NGS costs may be covered by private insurance or Medicare, and financial assistance programs may also be available.^{8,9}

SUMMARY

- Genomic testing is essential and highly recommended to identify patients who may benefit from treatment with targeted therapies.
- Selective inhibitors of oncogenic fusion proteins can enable precision medicine-based treatment of patients with gene fusion cancers.
- Larotrectinib is effective and well tolerated in patients with TRK fusion cancer, regardless of age or tumour type. Rapid and reliable molecular testing is required to identify patients for larotrectinib therapy.
- The identification of *NTRK* gene fusions as actionable drug targets, highlights the importance of *NTRK* gene fusion testing across solid tumours.

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