

# **Durable Response to Serial Tyrosine Kinase Inhibitors (TKIs) in an Adolescent with Metastatic TFG-ROS1 fusion positive Inflammatory Myofibroblastic Tumor (IMT)**

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## **ABSTRACT**

Here, we present the case of an adolescent with a rare metastatic Inflammatory myofibroblastic tumor (IMT) harboring a TFG-ROS1 fusion initially detected on tumor progression and retrospectively identified in the primary tumor after targeted RNA sequencing. The patient benefitted from sequential TKIs over a 5-year period with response to the third generation ALK/ROS inhibitor, lorlatinib leading to resection of the primary tumor. Detailed molecular analysis can identify targetable oncogenic kinase fusions that alters management in patients with unresectable disease and should be considered in all patients.

## KEY WORDS

Inflammatory myofibroblastic tumor (IMT), tyrosine kinase inhibitors (TKI), crizotinib, lorlatinib, RNA sequencing, ROS1

## ABBREVIATIONS

IMT	Inflammatory myofibroblastic tumor
TKIs	Tyrosine kinase inhibitors
NGS	Next generation sequencing
ATP	Adenosine triphosphate
NSCLC	Non-small cell lung cancer
FISH	Fluorescent in-situ Hybridization
IHC	Immunohistochemistry
EORTC	European Organisation for Research and Treatment of Cancer
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
FFPE	Formalin-fixed paraffin-embedded
EpSSG	European pediatric Soft Tissue Sarcoma Study Group

## 1.0 INTRODUCTION

Inflammatory Myofibroblastic Tumors (IMTs) are rare mesenchymal tumors with a variable natural history that affect patients of all ages including children and adolescents. IMT is often a diagnostic challenge due to a wide and varied morphologic spectrum.<sup>1</sup> Targeted Next Generation Sequencing (NGS) has shown that up to 85% of IMT are driven by kinase fusions, the majority involve *ALK* (~60%) and other gene fusions, *ROS1* (~10%) and *PDGFR $\beta$*  (~3%).<sup>2</sup> *ROS1* and *ALK* receptor tyrosine kinase domains share homology in amino acid sequencing and structurally related adenosine triphosphate (ATP) binding sites.<sup>3</sup> Small molecule TKIs with dual inhibitory activity against *ALK* and *ROS1* have demonstrated efficacy across *ALK* and *ROS1*-rearranged human cancers, most notably in NSCLC.<sup>3</sup>

*ALK* and *ROS1*-rearranged cancers acquire resistance to TKIs leading to disease progression. Second and third generation selective *ALK*/*ROS1* inhibitors are able to overcome this resistance with inhibitors such as Lorlatinib (Pfizer) demonstrating a 42% response rate in previously treated *ALK*- and *ROS1*-rearranged NSCLC and are highly active in the CNS and against TKI-resistant mutants.<sup>4</sup> There is no standard of care for management of patients with unresectable IMT. CREATE, a biomarker driven study conducted by the EORTC is the only prospective phase 2 trial conducted in IMT and demonstrated crizotinib to be highly active in *ALK*-positive tumors, as well as achieving disease control for *ALK*-negative patients.<sup>5</sup> Recent case reports on patients with *ALK*-rearranged IMT have demonstrated partial response to lorlatinib, leading to complete resection for one patient.<sup>6,7</sup> Here, we describe the first case of an adolescent with a metastatic *ROS1* -rearranged IMT treated with multiple *ALK*/*ROS1* inhibitors

including lorlatinib demonstrating a durable response that led to resection of the primary tumor.

## **2.0 CASE PRESENTATION**

A 14-year-old female presented with a 2-month history of neuropathic pain centred on the right scapula, radiating into the axilla and arm with a profound impact on quality of life. An Xray then magnetic resonance imaging (MRI) revealed a heterogeneous 9- cm mass located in the right upper lobe (RUL) of the lung extending to the lung apex, pleura and mediastinum and staging revealed multiple enhancing cortical brain lesions (Figs. 1A and 1B). Open biopsy of the right frontal brain metastasis, and three computed tomography (CT)- guided biopsies of the primary thoracic mass with IHC and FISH for ALK led to the diagnosis of metastatic ALK-negative IMT following several expert pathology opinions (Figs. 2A-C).

The patient received minimal benefit from cytotoxic chemotherapy including high-dose methylprednisolone and mycophenolate mofetil (MMF), methotrexate and vinblastine and ifosfamide. Neuropathic pain and fatigue escalated rendering her housebound. Eighteen months post diagnosis the patient commenced crizotinib (250mg twice daily) through entry to the CREATE study.<sup>5</sup> Immunohistochemistry, FISH and RNA analysis using the Archer CTL fusion panel performed on study was negative for ALK and ROS1.<sup>5</sup> The patient achieved a rapid and excellent clinical response to crizotinib with resolution of all symptoms and near complete resolution of brain metastases over 18 months (Figs. 1C and 1D). The primary tumor however remained stable by RECIST 1.1 criteria and despite ongoing clinical benefit, CT and MRI imaging following 22 cycles demonstrated small volume new pleural disease, consistent with RECIST

progressive disease and the patient was withdrawn from the study, quickly becoming symptomatic once again. Despite multiple biopsies it was not possible to obtain sufficient tumor cells for further genetic analysis.

The patient had a rapid clinical response to the second generation ALK/ ROS1 inhibitor brigatinib (180mg daily) obtained on compassionate access for 9 months before further disease progression (Figs. 1E and 1F, Figure 2D). Biopsy of the primary thoracic tumor at this time detected a TFG-ROS1 fusion through RNA sequencing performed via entry to an ongoing paediatric sequencing study, CRUK-Stratified Medicine Paediatrics study, SMPaeds. Third generation lorlatinib (100mg daily) was initiated through a compassionate access scheme with a good partial response (Figs. 1G and 1H). The patient completed 1-year of lorlatinib and was able to undergo complete surgical resection of residual disease 5-years from diagnosis Figs. 2E and 2F).

## **2.1 METHODS**

### *Molecular analysis*

Patient informed consent for molecular analysis and related research was obtained through the UCL/UCLH biobank for studying health and disease (National Research Ethics Committee reference 15/YH/0311) and CRUK Stratified Medicine Paediatrics (ISRCTN21731605). A biopsy from the primary thoracic tumor at diagnosis and relapse were evaluated by NGS (see Molecular methods, Appendix 1). Tumor content was 30% and 90%, respectively. Of significance, the primary tumor sample had appreciable reactive changes and crush artefact hindering accurate estimation of tumor content.

Multiple DNA sequencing approaches in the DNA derived from FFPE from the primary and relapse biopsies and blood for germline analysis failed to identify any clinically significant variants associated with the IMT phenotype (Figure 3). RNA analysis, using the TruSight RNA Pan-Cancer Panel and RNA-Sequencing Alignment App v2.0.1. (Illumina Inc., San Diego, CA, USA) identified a TFG-ROS1 fusion in the relapse biopsy that was retrospectively confirmed in the primary biopsy (Figure 4).

## **2.2 DISCUSSION**

This patient required repeat biopsies and expert pathology review to confirm a morphological diagnosis of IMT, with IHC and FISH negative for ALK. Targeted RNA sequencing at disease progression identified a TFG-ROS1 fusion that was discordant with the previous negatively reported FISH for ROS1. Challenges in accurate diagnosis of IMT are well described with a European pediatric Soft Tissue Sarcoma Study Group (EpSSG) prospective trial demonstrating 20 patients (25%) with an initial diagnosis of IMT having their diagnosis amended after central review.<sup>8</sup> In addition, molecular diagnostics are not routinely available or standardised for this rare malignancy. Chang *et al* demonstrated in a cohort of 33 patients with thoracic IMT that by using an array of molecular techniques an oncogenic tyrosine kinase fusion protein could be identified in every patient, including 6 *ROS1*-rearranged IMTs, 1 of which did not stain for ROS1 by IHC.<sup>9</sup> Similarly, they report an overall sensitivity for FISH at 86%, with 4/30 thoracic IMT cases (13%) negative by FISH that had a fusion confirmed with targeted RNA sequencing, including one case of TFG-ROS1.<sup>9</sup>

Here, we demonstrated the utility of targeted RNA sequencing to detect therapeutically actionable kinase fusions in a patient with unresectable IMT. It was challenging to identify the kinase fusion in the primary sample, that was initially detected in the relapse sample. This is most likely due, at least in part to lower tumor content in the primary sample; tumor heterogeneity, the lability of RNA in archival FFPE, low expression levels of fusion transcripts and limitations in testing sensitivity and PCR amplification bias may also be contributing factors. These potential limitations in diagnostic testing lend support to repeat molecular re-evaluation at progression for any fusion negative IMT's.

Disease in this patient was controlled using multigenerational TKIs, but with each TKI, resistance eventually developed. ALK-negative IMT may be less responsive to crizotinib and acquire mutations within the *ROS1* kinase domain more frequently than ALK-positive IMT.<sup>4,5</sup> Despite candidate gene analysis, the resistance mechanism was not identified in this study. Escalation in potency of sequential generation TKI's to the highly effective and CNS penetrant lorlatinib established an objective response that deemed the tumor resectable.

### **2.3 CONCLUSION**

This case study demonstrates the efficacy of serial TKIs in a patient with a very rare tumor, *ROS1*-rearranged IMT. In patients with ALK-negative IMTs, detailed molecular analysis at diagnosis and on a repeat biopsy at progression should be considered to detect rare gene rearrangements and optimise ongoing therapy.

### **CONFLICT OF INTEREST**

Declarations of interest: none.

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## **AUTHOR STATEMENT FILE**

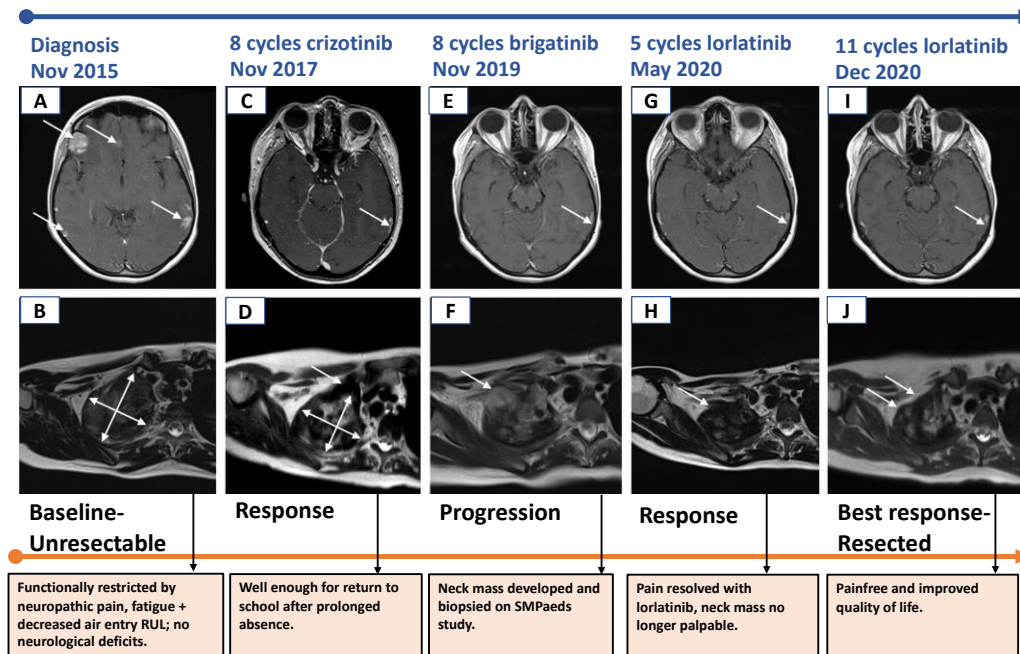
**Katrina Ingley (KMI):** Visualisation; Data curation; Project administration; Original draft. **Debbie Hughes (DH):** Methodology; Formal analysis; Validation; Writing- review & editing. **Michael Hubank (MH):** Methodology, Formal analysis; Validation. **Daniel Lindsay (DL):** Investigation, Writing- review and editing. **Andrew Plumb (AP):** Investigation, Writing- review and editing. **Rachel Cox (RC):** Investigation. **Louis Chesler (LC):** Resources, Writing- review and editing. **Sandra Strauss (SJS):** Conceptualisation; Visualisation; Supervision; Writing- review & editing.



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## FIGURES and LEGENDS

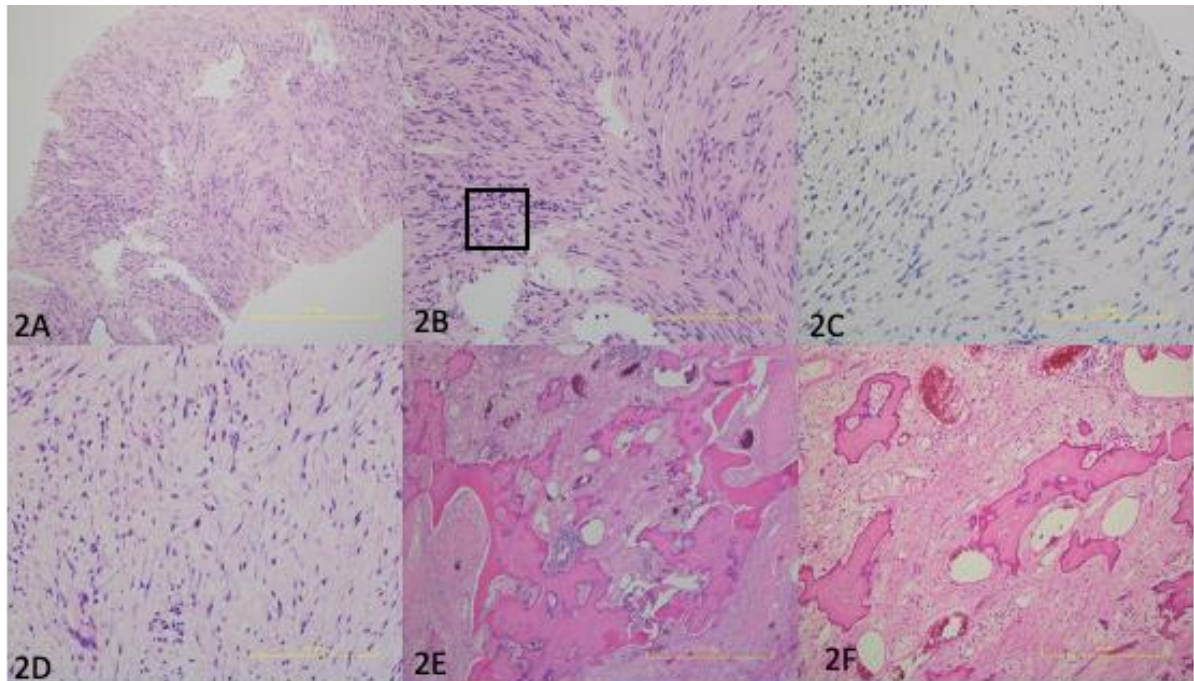


**Figure 1. a-j**

### **Timeline depicting the clinical course and radiological response to treatment.**

Baseline axial T1 weighted MRI of the brain following intravenous gadolinium (A) and axial T2 weighted MRI of the thorax show multiple brain metastases [arrows in (A)] and a bulky mass in the RUL of the lung [measured in (B)]. Following 8 cycles of crizotinib and excision biopsy of the right frontal brain metastasis, only a small rim of dural enhancement remains in the brain [arrow in (C)], and the RUL mass has reduced in size [measured in (D)], with re-aeration of the anterior RUL [arrow in (D)]. Although the brain imaging remained stable [arrowed in (E)], intrathoracic recurrence despite treatment with brigatinib was diagnosed based on the enlarging RUL mass which was beginning to invade into the right chest wall and brachial plexus [arrowed in (F)]. Response is maintained in the brain, shown in (G), with intrathoracic imaging (H) after 5 cycles of lorlatinib showing good partial response in the RUL mass and restoration of the extrapleural fat planes, with retraction of the mass away from the right brachial plexus. After a total of 11 cycles of lorlatinib,

intracranial response was maintained with only a trace of residual dural enhancement [arrowed in (I)]. Further response in the chest is shown in (J), with a clear rim of fat between the RUL tumor and the subclavian vessels and brachial plexus, deeming the disease resectable.

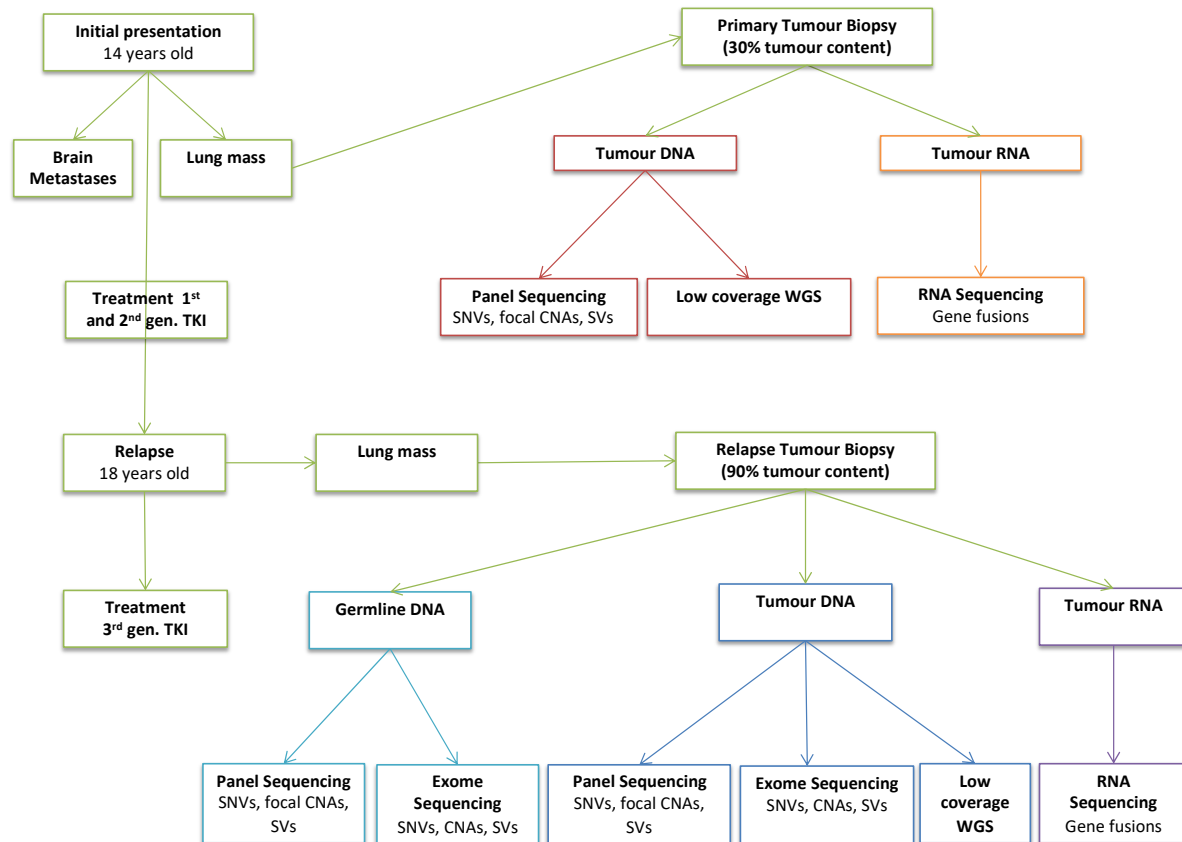


**Figure 2. a-f**

**Pathology and response to treatment.**

2A. VATS biopsy, RUL, February 2016 (H&E x10). A tumor with a vaguely fasciculated architecture composed of elongated spindle cells with mildly atypical spindle shaped nuclei and amphophilic cytoplasm imparting a myofibroblastic morphology. The tumor cells are embedded in a collagenous stroma. There is an associated infiltrate of chronic inflammatory cells composed predominantly of lymphocytes with occasional plasma cells. Note how the tumor cells have a loose fascicular growth with spindle cells featuring elongated cytoplasmic processes, a phenotypic feature associated with *ROS1* rearranged IMT.<sup>10</sup> Very occasional mitotic figures were present (<1/10 high

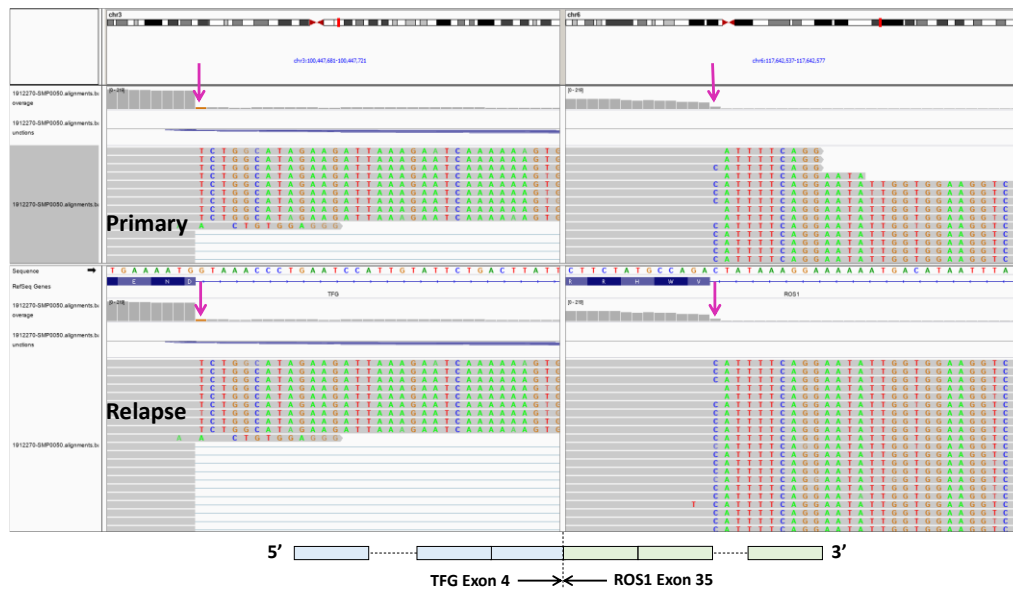
power fields (HPF)) and there was no necrosis. 2B. VATS biopsy, RUL, February 2016 (H&E x20). Higher power image of the lung tumor, further demonstrating the features described in 2A. Note there are scattered larger cells with a rounded morphology and conspicuous eosinophilic nucleoli (within box). 2C. Primary tumor from lung x20 (ALK IHC). Immunohistochemistry for ALK1 (clone 5A4) is negative in the tumor cells. The spindle cells demonstrated low proliferative activity. 2D. Needle biopsy from right supraclavicular fossa mass, October 2019, (H&E x20). Pathology confirms tumor with similar morphological features as described in 2A and B. Note the degree of cytological atypia- greater variation in nuclear size, hyperchromasia and mitotic activity compared to February 2016. This tumor also showed areas of coagulative necrosis (not pictured). 2E. (H&E x4) and 2F. (H&E x10): Resection from RUL/ chest wall December 2020, post lorlatinib treatment. The tumor has shown an excellent response to therapy, with diffuse areas of metaplastic ossification, calcification and fibrosis. Only approximately 5% residual viable tumor was identified on the resection specimen.



**Figure 3. Clinical course and genomic profiling.**

Summary of all analyses performed on the primary, relapsed tissue samples and germline DNA to find the causative variant. DNA sequencing employed a clinically validated custom panel targeting 92 genes associated with paediatric cancers (473kb) and in the relapse sample, an exome panel targeting 19,396 genes (39Mb). DNA analysis was designed to detect single nucleotide, copy number and structural variants. Panel sequencing of germline DNA identified a SNV, *BCOR* c.-34T>C, at a variant allele frequency (VAF) of 56%, confirmed in both the primary (VAF 35%) and the relapse (VAF 21%) tumor but deemed to be of no clinical significance as it is unlikely to affect splicing, has not been reported previously and is not associated with the IMT phenotype. No clinically actionable, somatic variants, defined as those that alter gene function, confer drug resistance or influence disease prognosis or

diagnosis, were identified in the panel data or genes commonly associated with resistance to TKI inhibition including PDGFRA, RET, ROS1 in the WES data.



**Figure 4. TFG Exon 4 is fused to ROS1 Exon 35 in the primary and relapse biopsies.**

Primary and relapsed RNA samples showed a TFG:ROS1 fusion (Breakpoint chr3:100,447,701:chr6:117,642,557) as identified by arrows. In all instances paired end sequencing (2x75bp) generated a minimum of 3 million unique aligned reads per sample, in line with supplier recommendations. Analysis software failed to identify the fusion in the primary biopsy for both TruSight RNA Pan-Cancer and Tru-Sight Tumor 170 panel data; fusion calls are calculated as a weighted average of individual features including percentage of fusion supporting reads, read counts across fusion breakpoint, alignment qualities and additional quality metrics fusion. The fusion did not meet the confidence score criteria but is visible when inspecting the data in the genome browser Integrative Genome Viewer at the breakpoint. All RNA sequencing was performed to diagnostic/ISO standards.

## **APPENDIX 1: MOLECULAR METHODS**

### Fluorescent in situ hybridisation (FISH):

The *ALK* dual colour break-apart rearrangement probe (Abbott Molecular, USA) specific for the *ALK* locus on chromosome band 2p23 was undertaken by FISH on 4µm formalin-fixed paraffin-embedded (FFPE) tissue sections to detect an *ALK* rearrangement.<sup>1</sup> This testing was performed and interpreted at the South West Genomic Laboratory Hub at Bristol Genetics Laboratory, UK. As part of the CREATE study, *ROS1* expression was tested for using the D4D6 antibody (Cell Signaling Technology) and FISH performed with the KREATECH *ROS1* (6q22) probe.<sup>2</sup> *ROS1* fusion was investigated by the Archer FusionPlex CTL Panel (Archer).<sup>2</sup>

### DNA sequencing:

Genomic sequencing was performed on FFPE. DNA library preparation was performed using the KAPA HyperPlus Kit and SeqCap EZ adapters (Roche, NimbleGen, Madison WI, USA) that included dual-SPRI size selection of the libraries (250-450 bp). 1 µg of the pooled library DNA was hybridised to a custom panel of 92 genes (473kb) (NimbleGen SeqCap EZ library, Roche, Madison, WI, USA).<sup>3</sup> Additionally, 500ng of the relapse biopsy library was hybridised to IDT xGen Exome Research Panel v1.0 targeting 19,396 genes (39Mb) (Integrated DNA technologies, Coralville, Iowa, USA) for WES.<sup>4</sup> DNA analysis was performed using Molecular Diagnostics Information Management System v4.0, based on genome build hg19, that follows Genome Analysis Toolkit (GATK) best practice guidelines and uses open access tools for data generated on Illumina platforms.

Single Nucleotide Variants (SNVs), Copy Number Alterations (CNAs) and Structural Variants (SVs) were identified and visually examined in the genome browser, Integrative Genome Viewer (IGV).<sup>5</sup> Target genes commonly associated with resistance to TKI inhibition in the WES data included *PDGFRA*, *RET*, *ROS1*.

### Germline DNA sequencing:

Germline DNA analysis was performed using the same custom and exome methods as above.

### TruSight RNA Pan-Cancer Panel, Illumina:

RNA was analysed using the Illumina TruSight RNA Pan-Cancer Panel (Illumina, San Diego, CA, USA), which comprehensively detects gene fusions and gene expression changes with a focus on 1,385 genes cited in public databases and implicated in cancer.<sup>6</sup> Bioinformatic analysis was performed using the RNA-Sequencing Alignment App v2.0.1 (BaseSpace Sequencing Hub) that used STAR for alignment and Manta for gene fusion calling.<sup>7</sup> RNA results were confirmed using TruSight Tumor 170 panel (Illumina,



San Diego, CA, USA) targeting 170 genes.<sup>8</sup> In all instances paired end sequencing (2x75bp) generated a minimum of 3 million unique aligned reads per sample, in line with supplier recommendations.

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