Secondary EML4-ALK-positive lung adenocarcinoma in a patient previously treated for acute lymphoblastic leukemia in childhood: a case report

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Running Title: ALK-positive lung adenocarcinoma in an ALL survivor

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ABSTRACT

It is widely recognized that the risk of secondary neoplasms increases as childhood cancer survivors progress through adulthood. These are mainly hematological malignancies, and recurrent chromosome translocations are commonly detected in such cases. On the other hand, while secondary epithelial malignancies have sometimes been reported, chromosome translocations in these epithelial malignancies have not. A 33-year-old man who had been diagnosed with acute lymphoblastic leukemia and treated with chemotherapy almost 20 years earlier was diagnosed with lung adenocarcinoma. After chromosomal rearrangement of echinoderm microtubule-associated protein-like 4 gene (*EML4*) and the anaplastic lymphoma kinase gene (*ALK*) was detected in this adenocarcinoma, he responded to treatment with crizotinib. It was therefore concluded that this *EML4-ALK*-positive lung adenocarcinoma was a secondary epithelial malignancy.

Mini-abstruct

A 33-year-old man who had been diagnosed with ALL and treated with

chemotherapy in childhood was diagnosed with lung adenocarcinoma. *EML-ALK* was detected in this secondary epithelial malignancy.

Key Words: Cancer survivor, EML4-ALK, secondary lung adenocarcinoma

INTRODUCTION

The survival rate of patients with childhood cancer has increased from 1970 to the present, with 5-year survival rates now approaching 80% (1). Coincident with this improved survival has been the recognition that survivors are at risk for subsequent malignancies (2–6). It has been widely accepted that the anti-cancer drugs used in childhood damage the genomic DNA of some hematopoietic progenitors, resulting in secondary hematological malignancies (7). Indeed, recurrent chromosome translocations were found in many cases of such malignancies (7, 8). On the other hand, no chromosomal translocations have been reported in secondary epithelial malignancies.

Chromosomal rearrangement of the echinoderm microtubule-associated protein-like 4 gene (*EML4*) and of the anaplastic lymphoma kinase gene (*ALK*) leads to an oncogenic fusion gene that was first reported in lung adenocarcinoma (9). This translocation may occur in less than 5% of non-small cell lung cancers (NSCLCs) and has come to be recognized as a new target of chemotherapy (9). To the best of our knowledge, this is the first report of a case of an *EML4-ALK*-positive lung adenocarcinoma that

occurred almost 20 years after treatment for acute lymphoblastic leukemia (ALL).

CASE REPORT

A 33-year-old man who had never smoked was referred to our institution for further examination of a massive right pleural effusion on chest X-ray. Thoracentesis was performed, and pleural fluid cytology was positive for adenocarcinoma. A computed tomography (CT) scan of his chest after the thoracentesis revealed a nodule in the right upper lobe approximately 3 cm in diameter (Fig. 1), and stage IV NSCLC was diagnosed. When the patient was 11 years old, he had been diagnosed with ALL and enrolled in a clinical trial for juvenile high-risk ALL (10). He had been treated with the AL851 regimen, receiving vincristine, prednisolone, daunorubicin, l-asparaginase, methotrexate, 6-mercaptopurine, doxorubicin, cytosine arabinoside, 6-mercaptopurine, and cyclophosphamide. Cranial irradiation was used to prevent central nervous system leukemia; however, thoracic irradiation was not performed (10). Complete remission was achieved.

Considering his medical history, he was found to have a secondary lung

adenocarcinoma at an advanced stage. Mutation analysis of mononuclear cells in his pleural fluid indicated that his tumor carried the wild-type epidermal growth factor receptor gene (*EGFR*). After pleurodesis, treatment with carboplatin and paclitaxel with bevacizumab was started. During this first-line treatment, it was suspected that his lung adenocarcinoma could be an *EML4-ALK*-positive tumor, given the fact that he was young and his tumor was negative for *EGFR* mutations. Thus, the multiplex reverse-transcription polymerase chain reaction (RT-PCR) was performed to test for *EML4-ALK* fusion complementary DNA (cDNA) in his tumor cells (Forward primer; 5′-GTGCAGTGTTTAGCATTCTTGGGG-3′ and reverse primer; 5′-TCTTGCCAGCAAAGCAGTAGTTGG-3′) (11). As shown in Fig. 2, RT-PCR showed that his tumor did indeed carry *EML4-ALK*. Nucleotide sequencing of this PCR product further confirmed the presence of *EML4-ALK* variant 1 cDNA in which exon 13 of *EML4* was fused in-frame to exon 20 of *ALK* (Fig. 3). These data suggest that the patient had a secondary *EML4-ALK*-positive NSCLC.

After four cycles of carboplatin and paclitaxel with bevacizumab and two cycles of bevacizumab maintenance treatment, his tumor progressed. He

was then treated with crizotinib, an ALK-specific inhibitor. The tumor initially responded to crizotinib, but became refractory after several months of treatment. After that, several chemotherapy regimens, including pemetrexed, cisplatin plus docetaxel, gemcitabine, and TS-1, were administered; however, all were ineffective. He died almost 2 years after the diagnosis of lung cancer.

DISCUSSION

This patient had been diagnosed with and treated for ALL almost 20 years earlier and subsequently diagnosed with lung adenocarcinoma. It is widely recognized that the risk of secondary malignancies increases as childhood-cancer survivors progress through adulthood (1–6). There is no widely accepted definition of secondary malignant neoplasm; however, considering the definition of the Childhood Cancer Survivor Study (CCSS) (1), we concluded that his lung adenocarcinoma was a secondary malignant neoplasm. In the CCSS report, the most frequent secondary epithelial malignancies occurring ≥5 years after childhood cancer were breast and thyroid cancer. However, bronchial and lung cancers were also epithelial

malignancies whose subsequent risks were elevated. The 30-year cumulative incidence for selected subsequent cancers was 0.1% for lung cancer, and the standardized incidence ratio (SIR) of second bronchial and lung cancers was 3.4 (95% confidence interval [CI]=1.9–6.1) (1).

Recurrent chromosome translocations were found in most cases of secondary hematological malignancies after childhood ALL treated with Berlin-Frankfurt-Munster (BFM)-based regimens (8). Several anti-cancer drugs are considered to be potent clastogenic agents. Thus, secondary hematological malignancies may result from illegitimate recombination of chromosomal fragments caused by such anti-cancer agents (7). In this regard, BFM-based treatment might play a direct role in chromosome translocations detected in secondary malignancies. On the other hand, oncogenic driver mutations, such as *EGFR* mutant or *K-ras* mutant, have not been reported in relation to secondary lung cancer so far. This case also did not harbor any *EGFR* mutations. In this regard, oncogenic point mutations in lung cancer may have only a weak association with carcinogenicity of anti-cancer drugs.

It was previously accepted that recurrent chromosome translocations play a major role in the molecular pathogenesis of hematological

malignancies, but not solid tumors. However, the discovery of *EML4-ALK* generated through inv(2)(p21p23) has changed this notion (9). This fusion gene encodes an oncogenic EML4-ALK fusion-type tyrosine kinase. Wild-type ALK is thought to undergo transient homodimerization in response to binding to its specific ligands, resulting in its activation. On the other hand, EML4-ALK is constitutively oligomerized via the coiled-coil domain within EML4, leading to persistent mitogenic signals that eventually lead to malignant transformation (9).

Brenner et al. described the risk of second malignancies associated with CT (12). Indeed, Mathews et al. identified CT scans as a risk factor for second malignancies in a large cohort trial (13). However, the risk of lung cancer was not increased in their report, and the present patient received only one or two CT scans during his leukemia treatment and never underwent CT prior to that. Thus, we consider that the effect of irradiation was very limited in this secondary lung adenocarcinoma.

The occurrence of secondary *EML4-ALK*-positive lung cancer in the present case could have been the result of the patient's initial treatment for childhood ALL. Considering this case, known or unknown oncogenic

recurrent chromosome translocations or DNA rearrangement might be present in other secondary epithelial malignancies, which should be carefully investigated by physicians who treat patients with all types of secondary malignancies.

Conflict of Interest statement

The authors confirm that this report has not been published or presented elsewhere, either in whole or in part, and that it is not under consideration by another journal. All authors have approved the content and agree with its submission. No financial support was received for this publication, and none of the authors has any conflicts of interest to declare.

References

1. Friedman DL, Whitton J, Leisenring W, et al. Subsequent neoplasms in 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *J Natl Cancer Inst* 2010; 102:1083-95.
2. Armstrong GT, Liu W, Leisenring W, et al. Occurrence of multiple subsequent neoplasms in long-term survivors of childhood cancer: a report from the childhood cancer survivor study. *J Clin Oncol* 2011; 29:3056-64.
3. Reulen RC, Frobisher C, Winter DL, et al. British Childhood Cancer Survivor Study Steering Group. Long-term risks of subsequent primary neoplasms among survivors of childhood cancer. *JAMA* 2011; 305:2311-9.
4. Davies SM. Subsequent malignant neoplasms in survivors of childhood cancer: Childhood Cancer Survivor Study (CCSS) studies. *Pediatr Blood Cancer* 2007; 48:727-30.
5. Bassal M, Mertens AC, Taylor L, et al. Risk of selected subsequent carcinomas in survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2006; 24:476-83.

6. Mertens AC, Yasui Y, Neglia JP, et al. Late mortality experience in five-year survivors of childhood and adolescent cancer: the Childhood Cancer Survivor Study. *J Clin Oncol* 2001; 19:3163-72.

7. Pui CH, Ribeiro RC, Hancock ML, et al. Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. *N Engl J Med* 1991; 325:1682-7.

8. Schmiegelow K, Al-Modhwahi I, Andersen MK, et al. Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. *Blood* 2009; 113:6077-84.

9. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming *EML4–ALK* fusion gene in non-small-cell lung cancer. *Nature* 2007; 448:561-6.

10. Matsuzaki A, Ishii E, Okamura J, et al. Treatment of high-risk acute lymphoblastic leukemia in children using the AL851 and ALHR88 protocols: a report from the Kyushu-Yamaguchi Children's Cancer Study Group in Japan. *Med Pediatr Oncol* 1996; 26:10-9.

11. Soda M, Isobe K, Inoue A, et al. A prospective PCR-based screening for the *EML4-ALK* oncogene in non-small cell lung cancer. *Clin Cancer Res* 2012; 18:5682-9.

12. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. *N Engl J Med*. 2007,357:2277-2284.

13. Mathews JD, Forsythe AV, Brady Z, et al. Cancer risk in 680,000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. *BMJ*. 2013,346:f2360.

Figure legends

Figure 1. Chest computed tomography scan after thoracentesis. A nodule with a diameter of approximately 3 cm is detected in the right upper lobe.

Figure 2. Gel electrophoresis of RT-PCR products: Multiplex RT-PCR was conducted on pleural effusion premixed with RLT buffer (Qiagen, Hilden, Germany) (Effusion 1), a frozen aliquot of the original effusion (Effusion 2), and a frozen aliquot of whole peripheral blood. The same experiment was also conducted with no template or an *EML4-ALK*-negative specimen (Negative control). For the internal control of RT-PCR, the human ribonuclease P (RNase P) cDNA was also amplified. Size standards (50 bp DNA ladder, Life Technologies Corporation, Carlsbad, CA).

Figure 3. The nucleotide sequence of the EML4-ALK variant1 cDNA isolated from this patient is shown. Genome nucleotides corresponding to EML4 or to ALK are shown in blue and red, respectively. An arrowhead indicates the fusion point.

Figure 1.

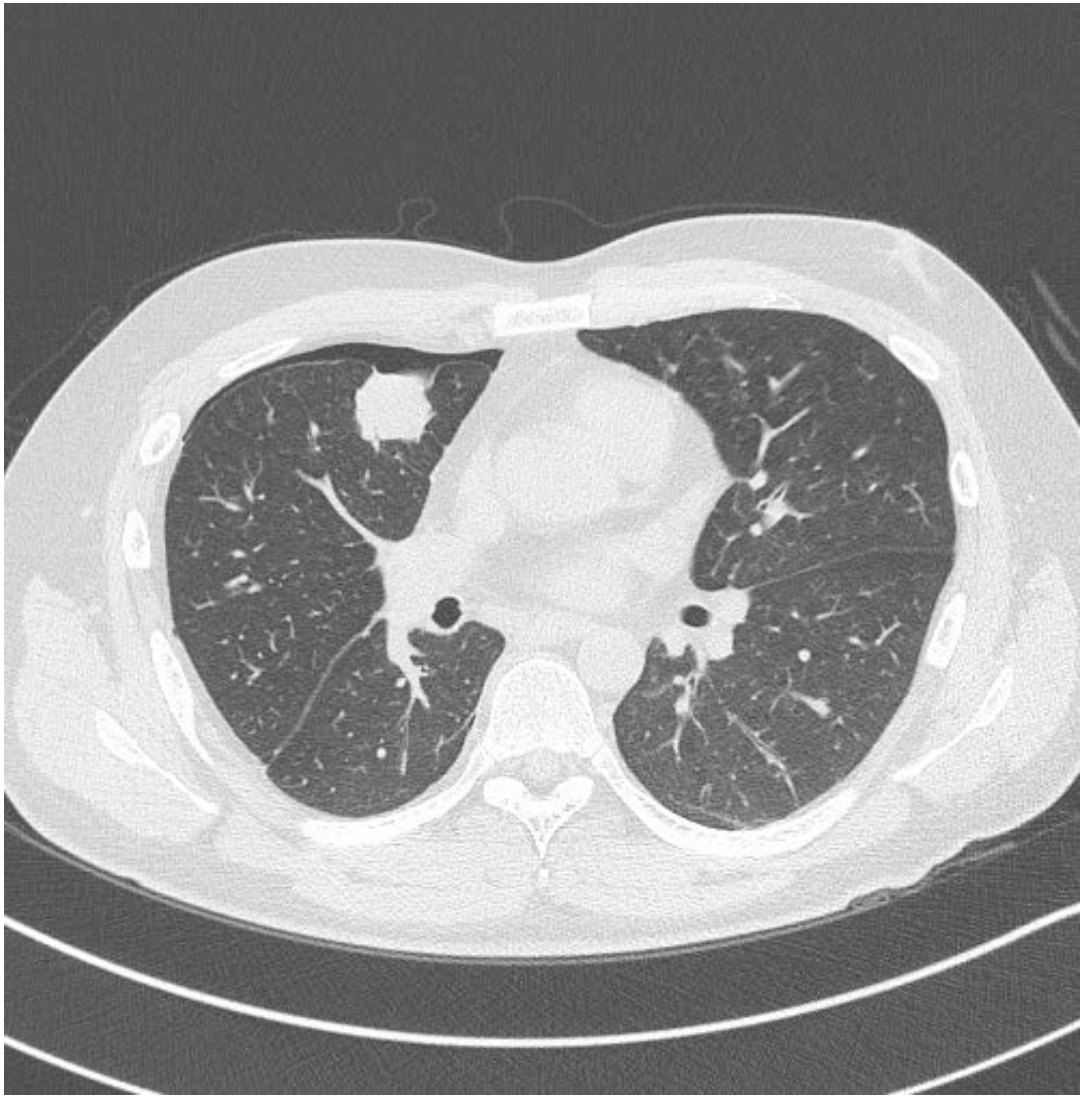


Figure 2.

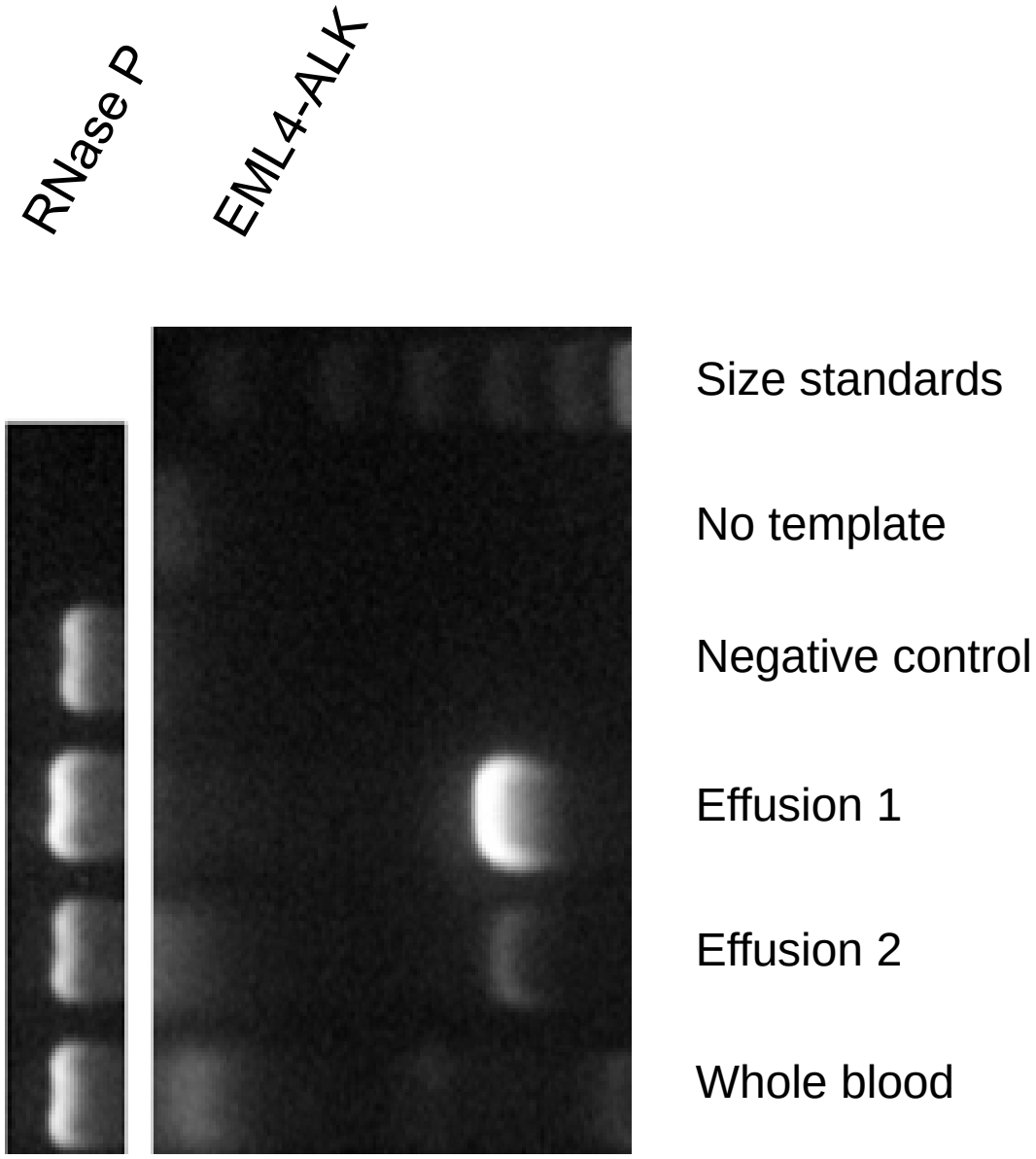
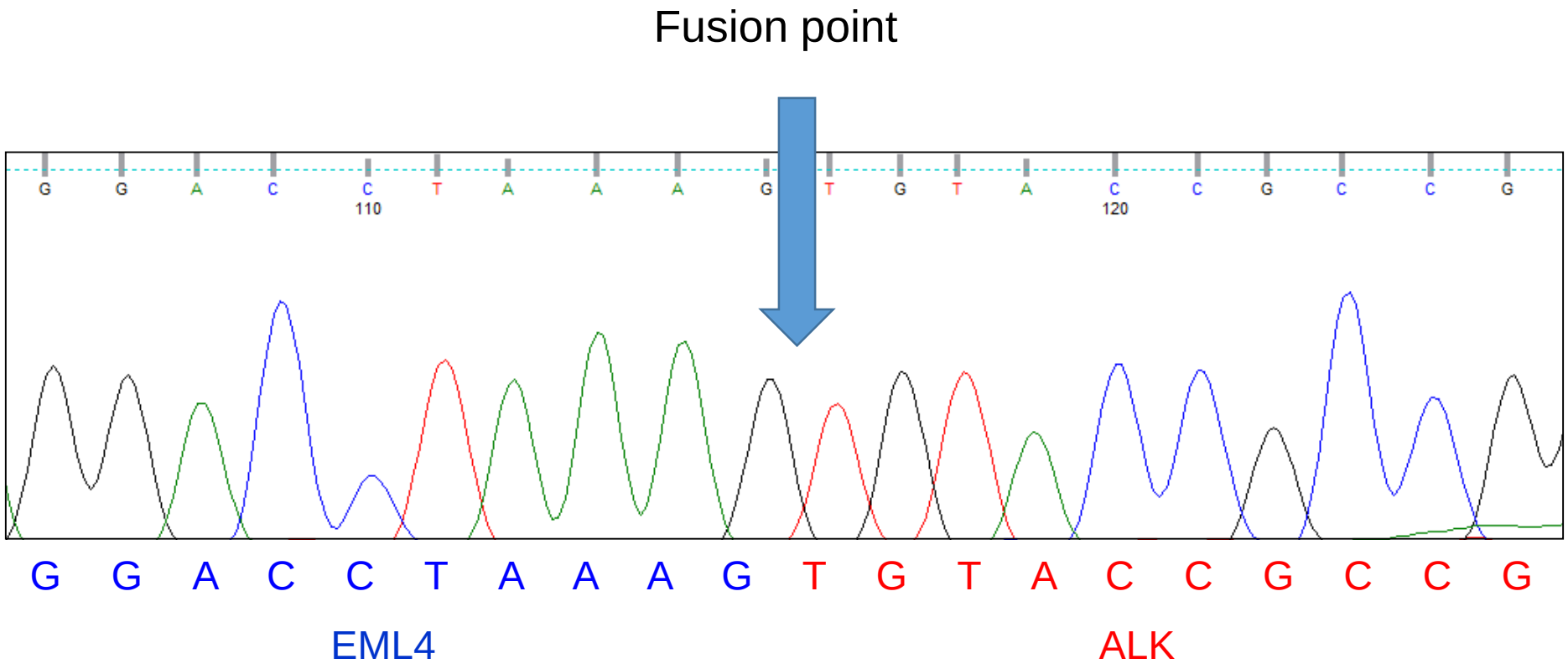

RNase P
EML4-ALK
Size standards
No template
Negative control
Effusion 1
Effusion 2
Whole blood

Figure 3.


Fusion point
G G A C C T A A A G T G T A C C G C C G
110
120
G G A C C T A A A G T G T A C C G C C G
EML4
ALK