

# Lung Squamous Cell Carcinoma With Anaplastic Lymphoma Kinase Rearrangement and TP53 Co-mutation Treated Successfully with Ensartinib: A Case Report

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## Research Article

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## Abstract

**Background:** Detection of anaplastic lymphoma kinase (ALK) gene rearrangements is an important step in the selection of effective therapies for patients with advanced lung adenocarcinoma. However, there have been few reports of ALK-positive lung squamous cell carcinoma (LSCC) and, even more rarely, LSCC with ALK rearrangement and TP53 co-mutation. Thus, it remains unclear whether ALK and TP53 co-mutant LSCC responds to ALK inhibitor treatment. Ensartinib is a novel ALK tyrosine kinase inhibitor (TKI).

**Case presentation:** A 73-year-old female nonsmoker was diagnosed with advanced squamous cell carcinoma of the right lung (grade 3, cT4N3M1c, stage IVB). Targeted next-generation sequencing indicated that the cancer cells harbored both the EML4-ALK variant 1 rearrangement (E13;A20) and TP53 exon10 p.L348S (c.1043T>C) mutation, accounted for 70.04%. After only one cycle of ensartinib therapy, the patient exhibited a good partial response in most target lesions, and her performance status improved from 4 to 2.

**Conclusions:** This result strongly suggests that ensartinib, a novel second-generation ALK inhibitor, may be an effective and rapid treatment for patients with this rare subtype of squamous cell carcinoma. Routine molecular analysis of ALK status in patients with LSCC is recommended. Results of the present study may provide some insight into treatment strategies for patients with ALK- and TP53-positive LSCC.

## Background

Lung cancer is the leading cause of cancer-related death worldwide, and approximately 80% of lung cancers are non-small-cell lung cancer (NSCLC)(1). Lung squamous cell carcinoma (LSCC) accounts for approximately 30% of NSCLC cases and is the second most common histological type of lung cancer (2). Despite recent advances in diagnosis, surgery, and chemotherapy strategies, the 5-year survival rate for LSCC remains quite low(3). The development of molecular targeted anticancer agents has resulted in better therapeutic responses in some types of lung cancer, including NSCLC with mutant anaplastic lymphoma kinase (ALK) rearrangements. Clinically, targeted therapies inhibiting the activity of ALK have proven to be efficacious, significantly extending overall survival compared to standard therapies(3). However, ALK-positive NSCLC accounts for only 2–5% of all NSCLC cases, and is almost exclusively detected in patients with lung adenocarcinoma (LUAD). There have been few reports of ALK-positive LSCC and, even more rarely, LSCC with ALK rearrangement and TP53 co-mutation. Thus, ALK testing is not routinely performed in the LSCC population, and the efficacy of such treatment for ALK-rearranged LSCC remains unknown.

Ensartinib is a novel second-generation ALK inhibitor developed by Chinese investigators. In preclinical models, ensartinib demonstrated better anticancer efficacy than crizotinib(4). To our knowledge, only one study to date has reported that LSCC patients with ALK and ROS1 double-rearrangements respond to crizotinib treatment (5). However, it remains unclear whether patients with ALK and TP53 co-mutation LSCC would respond to ALK inhibitor treatment. A previous study reported that TP53 mutations, especially nondisruptive mutations, negatively affected the objective response to crizotinib in patients with ALK-rearranged NSCLC(6). Herein, we describe, to our knowledge, the first case involving a patient with both ALK-and TP53 positive LSCC, and the clinical efficacy of ensartinib therapy.

## Case Presentation

In November 2020, a 73-year-old Chinese woman with no history of smoking was referred to the authors' hospital for a one-month history of severe chest and back pain, cough, dyspnea, asthenia, anorexia, weight loss, and multiple masses observed on chest radiography.

Positron emission tomography-computed tomography (PET-CT) revealed 18-fluoro-2-deoxy-d-glucose (FDG) showing abnormally increased radiopharmaceutical uptake at a 4.7- × 4.2 cm mass in the right upper lobe of the lung with a maximum standardized uptake value 11.9, accompanied by multiple metastases in bilateral lungs, bilateral adrenal nodules, liver, bones, bilateral hilar, mediastinal, supraclavicular lymph nodes, and right pleural metastases with pleural effusion (Fig. 1). Pathological examination of the CT-guided core needle biopsy of the liver metastases specimen revealed undifferentiated cancer with a mild tendency of squamous differentiation (hematoxylin and eosin staining [Fig. 2]). Upon immunohistochemical analysis, the tumor cells exhibited strong positive staining for cytokeratin and p63 (Fig. 2), but negative for thyroid transcription factor-1 (TTF-1) (Fig. 2). These findings led to the final diagnosis of advanced LSCC, grade 3, cT4N3M1c, stage IVB.

Targeted next-generation sequencing (NGS) of 16 lung cancer-related genes (Repu Gene Technology Company, China) was performed on DNA isolated from the tumor biopsy specimen, and results indicated that the cancer cells harbored both the EML4-ALK variant 1 rearrangement (E13;A20) and TP53 exon10 p.L348S (c. 1043T > C) mutation accounted for 70.04% (Fig. 3).

At that time, the patient was too ill to undergo conventional chemotherapy due to severely deteriorated liver function and poor physical constitution. According to the Eastern Cooperative Oncology Group criteria, her performance status (PS) was 4. Carbohydrate antigen (CA) 125 and CA19-9 levels were 16,125 U/mL (reference value < 35 U/mL) and 181.7 U/mL (reference value < 40 U/mL), respectively. Considering the molecular results, first-line therapy with the new second-generation ALK inhibitor, ensartinib (225 mg once per day), was administered on December 3, 2020. The accompanying treatment plan consisted of zoledronic acid injections for bone metastasis. On February 24, 2021, CT (performed after 83 days of ensartinib therapy) revealed good partial response in most target lesions according to the Response Evaluation Criteria in Solid Tumors scores (Fig. 4). The levels of CA125 and CA19-9 were reduced to 107 U/mL and 4.1 U/mL, respectively. Moreover, adverse events experienced by the patient were diminished during treatment, and only involved a mild rash. Her physical condition improved significantly, and PS recovered to 2. At the latest follow-up, 3.5 months after commencing ensartinib treatment, there was no evidence of progression or any remarkable toxicity.

## Discussion

In NSCLC, ALK fusions have been reported to occur at a frequency of 2–5%, and mostly occur in younger female patients with LUAD (7). A previous cohort study involving Asian patients reported that ALK gene rearrangement is very rare, with a positive prevalence of 0.7% in those with LSCC (8). ALK and TP53 double mutations and effective treatment have never been reported in LSCC. To the best of our knowledge, this is the first report to describe the successful management of ALK-rearranged and TP53 co-mutation LSCC using the novel ALK inhibitor ensartinib.

As shown in Table 1, only 10 cases of ALK-rearranged LSCC treated with ALK inhibitors, including crizotinib, ceritinib, and alectinib, have been reported in the literature to date (5, 9–17). In these few studies, only one reported co-mutation of ALK and RoS1 double-rearrangement (5). As such, the patient described herein is the second reported case of ALK co-mutant LSCC. NGS was applied to these two cases as a newer technology to replace the previous detection technology and obtain new findings. We believe that technical limitations inherent to fluorescence in situ hybridization, immunohistochemistry, or real-time polymerase chain reaction result in a failure to detect more comprehensive mutations of DNA targets simultaneously. This suggests that the use of NGS techniques will pave the way for greater understanding in this field.

Table 1  
Brief summary of all LSCC cases with ALK inhibitors treatment to date.

Authors	Year	Age	Sex	Detection method	ECOG	Co-mutant	TKI	Therapy line	Efficacy	PFS(m)
Srivastava et al.(9)	2013	80	F	FISH	unclear	No	crizotinib	first	PD	1.0
Wang et al.(10)	2014	55	F	FISH	unclear	No	crizotinib	second	PR	6.3*
Mikes et al.(11)	2015	36	M	FISH, RT-PCR	unclear	No	crizotinib	first	PR	4.7*
Zhang et al.(13)	2015	55	F	IHC	2	No	crizotinib	second	PR	6.0
Vergne et al.(14)	2015	58	F	IHC, FISH	unclear	No	crizotinib	third	PR	7.0
Tamiya et al.(12)	2015	78	M	IHC, FISH	unclear	No	alectinib	first	PD	1.4
Wang et al.(15)	2016	37	F	IHC	unclear	No	crizotinib	second	PR	9.0*
Mamesaya et al.(17)	2017	52	F	IHC, FISH	unclear	No	alectinib	second	PR	11.0*
Bolzacchini et al.(16)	2017	51	M	unclear	unclear	No	crizotinib/ ceritinib	second /third	PR /PD	10 /4
Li et al.(5)	2017	45	F	NGS	0	ROS1	crizotinib	first	PR	3
This case	2020	73	F	NGS	4	TP53	ensartinib	first	PR	3.5*
* Progression free at the last follow-up, ALK anaplastic lymphoma kinase, ECOG Eastern Cooperative Oncology Group, F female, FISH fluorescence in situ hybridization, IHC immunohistochemistry, M male, m month, RT-PCR real time polymerase chain reaction, PD progressive disease, PFS progression-free survival, PR partial response, TKI Tyrosine kinase inhibitors.										

The proportion of coexisting mutations of EML4-ALK variant 1 (E13:A20) and TP53 in ALK-rearranged LUAD is approximately 5.5%, with no relevant data in squamous cell carcinoma (18). Moreover, the impact of concomitant TP53 mutations in ALK-rearranged NSCLC remains unclear. Although TP53 mutations have been reported to be associated with inferior response to EGFR-TKIs and poor outcome in EGFR-mutated NSCLC patients (19), the association between TP53 mutations and the effect of crizotinib treatment in ALK-rearranged NSCLC patients remains controversial(6, 18). To date, only one previous study reported that TP53 co-mutation negatively affected the objective response to crizotinib in patients with ALK-rearranged NSCLC(6). However, the multiple masses in this patient rapidly decreased in size after treatment with ALK-TKI, which suggests that further studies aiming to elucidate the exact effect of TP53 co-mutation on ALK-rearranged LSCC are warranted.

Regardless, the short progression-free survival (PFS) of the retrospective 10 ALK-rearranged cases showed that ALK inhibitors were less effective in LSCC than in LUAD. Furthermore, worse prognosis has emerged among elderly patients. In the cohort cases, two patients > 70 years of age underwent PD with a one-month PFS in the first-line treatment of ALK inhibitors, whether crizotinib or alectinib. However, the 73-year-old patient described in the present report presented with

PS 4 and improved quickly with only mild side effects after treatment with ensartinib, strongly suggesting that this new ALK inhibitor is promising.

## Conclusions

In summary, we described a previously unreported patient with LSCC harboring EML4-ALK variant 1 rearrangement and a high abundance of TP53 co-mutation. This proves that ensartinib, a novel second-generation ALK inhibitor, can effectively and rapidly treat patients with this rare subtype of squamous cell carcinoma. Routine molecular analysis of ALK status in patients with LSCC is recommended. The results of our study may provide some insight into treatment for patients with ALK- and TP53-positive LSCC.

## Abbreviations

ALK Anaplastic lymphoma kinase LSCC Lung squamous cell carcinoma TKI Tyrosine kinase inhibitor NSCLC Non-small-cell lung cancer PET-CT Positron emission tomography-computed tomography FDG 18-fluoro-2-deoxy-d-glucose TTF-1 Thyroid transcription factor-1 NGS Next-Generation Sequencing PFS Progression-free survival

## Declarations

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### Author Contributions

Hu X and Fei YC were major contributors to the drafting of the manuscript; Chen KX and Lv DL treated the patient; Lv DL revised the manuscript and interpreted the molecular biology findings; all authors approved the manuscript submitted for publication.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Statement of Ethics

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

### Consent for publication

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

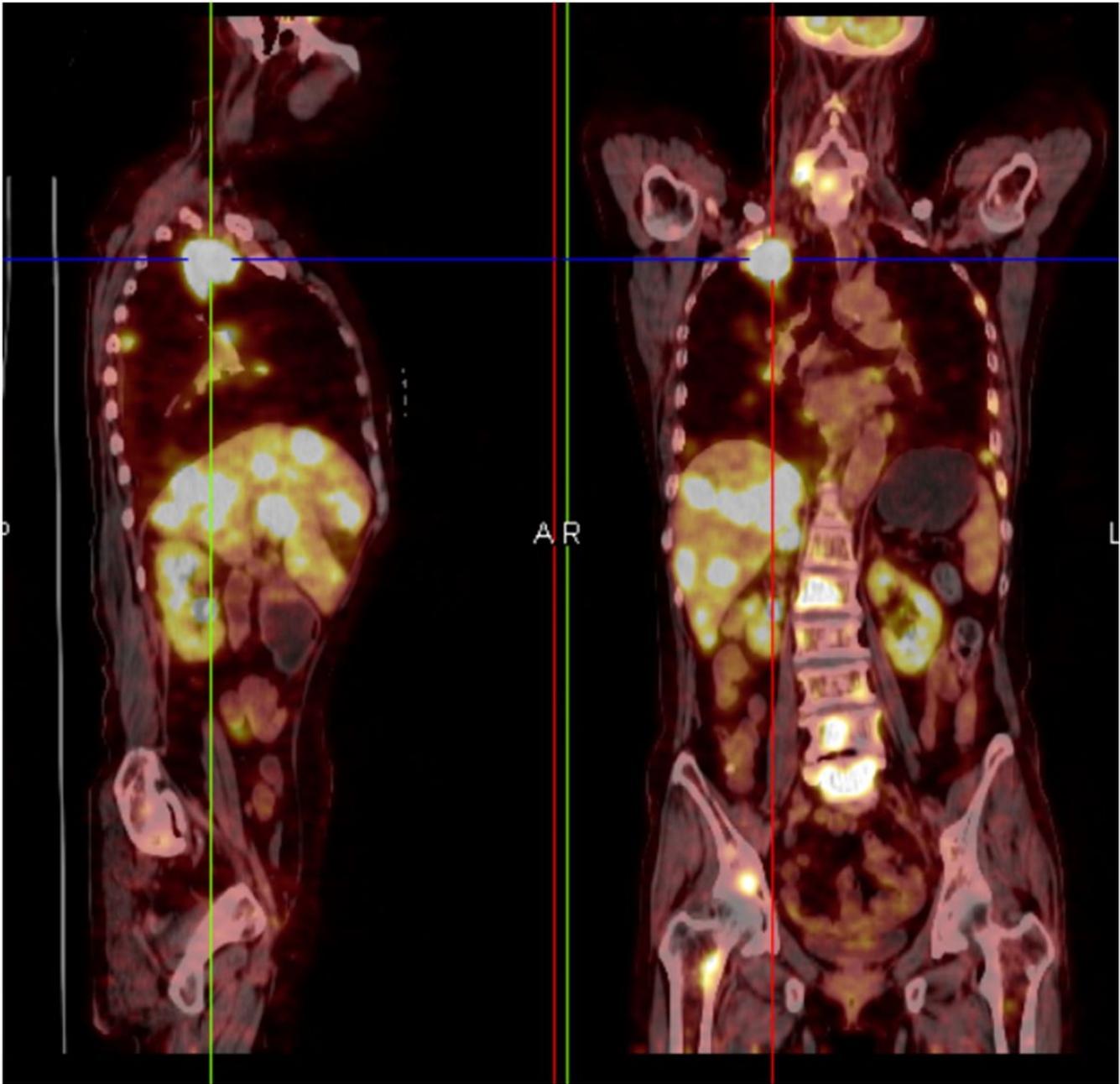
### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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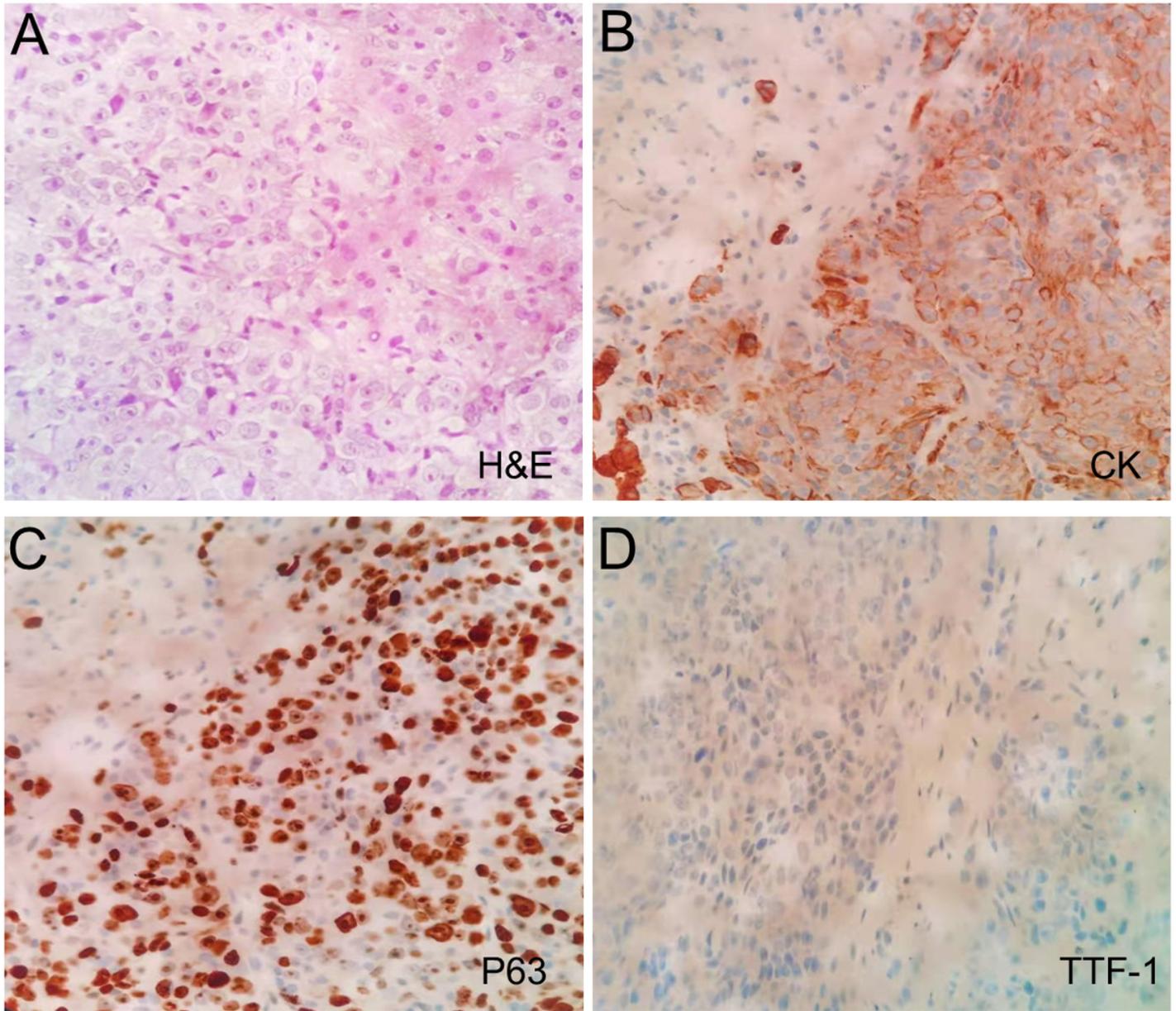
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## Figures



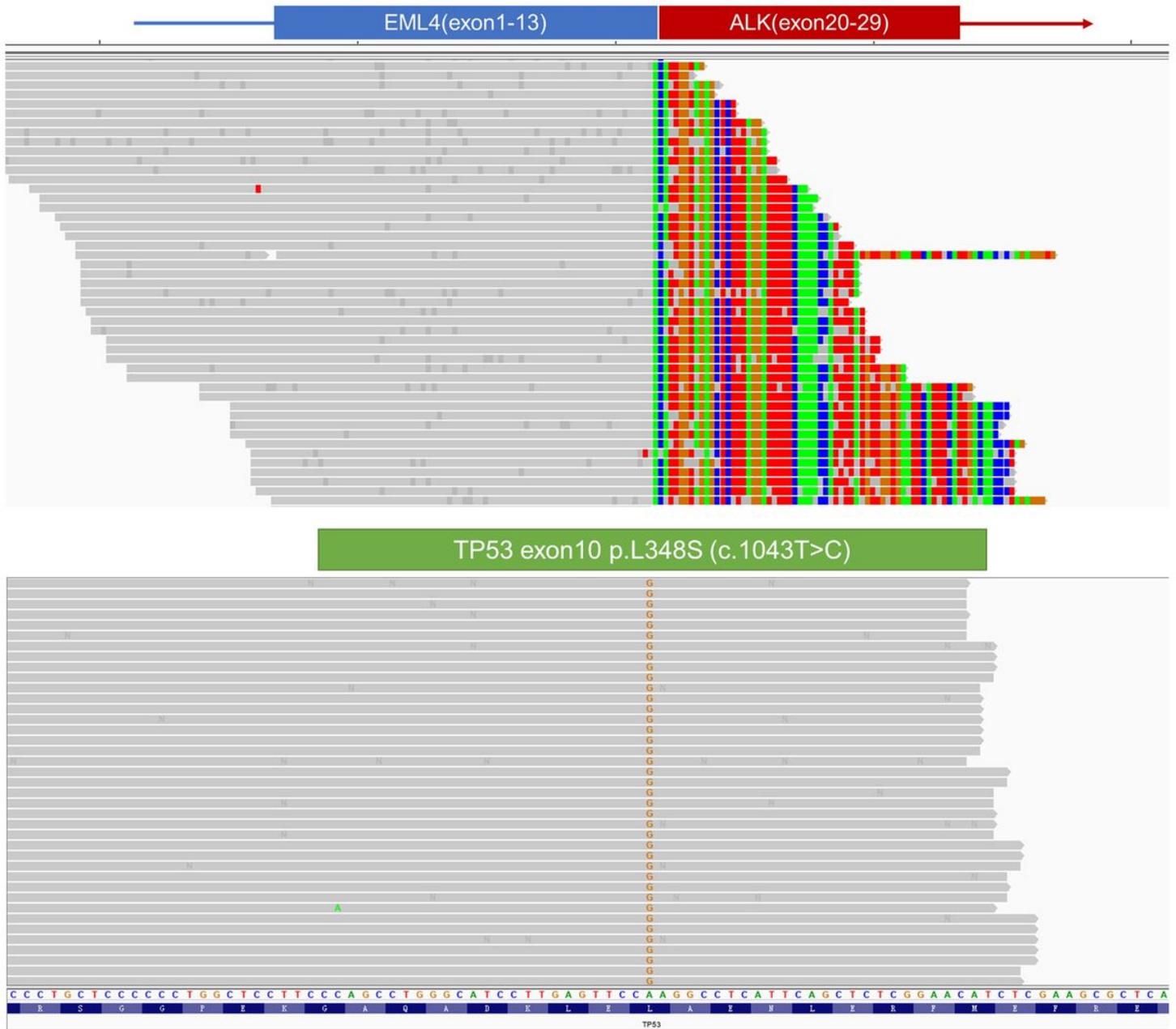
**Figure 1**

Positron emission tomography/computed tomography scan revealing primary and extensive metastatic lesions of lung cancer.



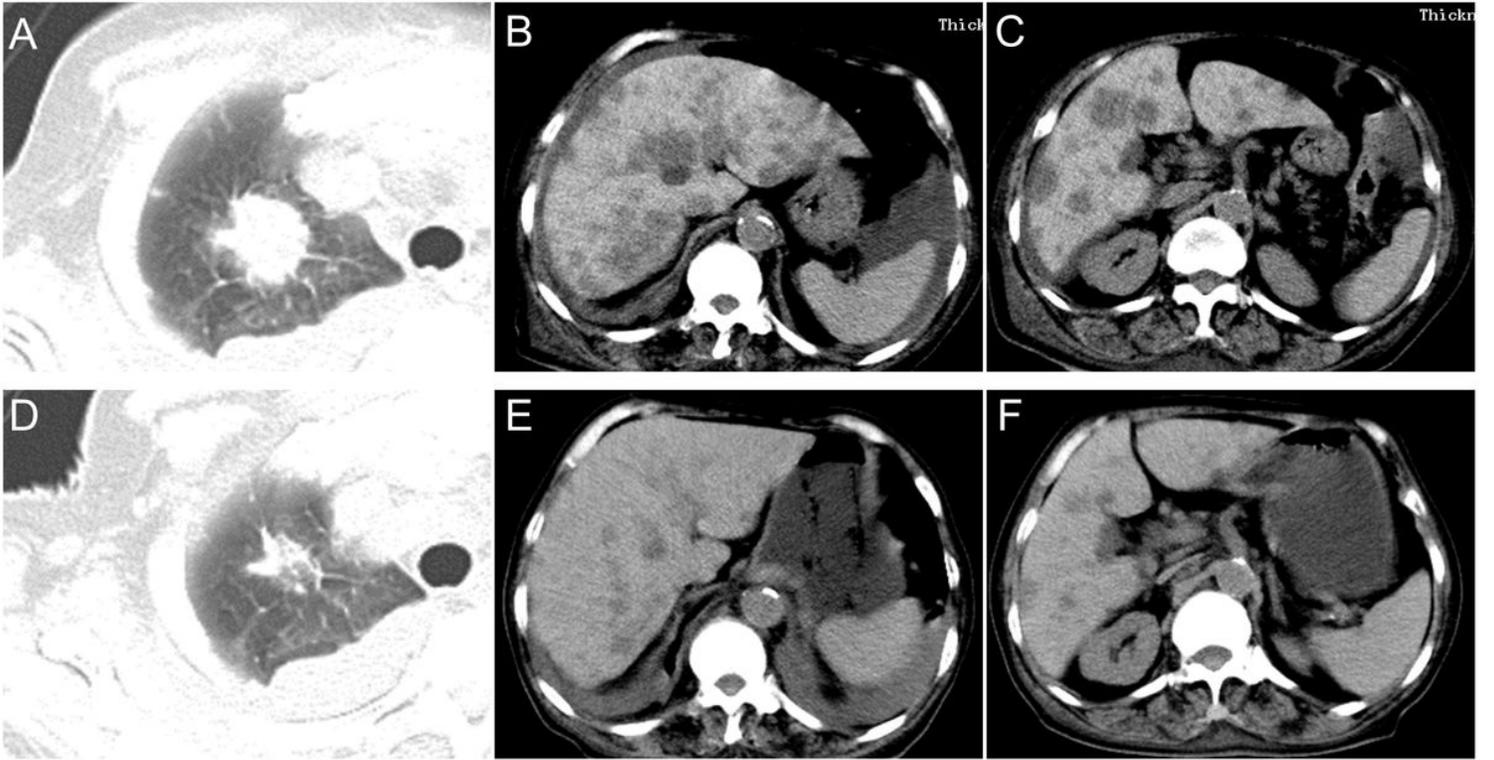
**Figure 2**

Histopathological and immunohistological findings from tumor tissue samples. (A) Hematoxylin and eosin stain revealing poorly differentiated squamous cell carcinoma (original magnification  $\times 100$ ). (B, C) Immunostaining for cytokeratin and P63. The tumor was strongly positive (original magnification  $\times 100$ ). (D) No expression of thyroid transcription factor-1 was apparent (original magnification  $\times 100$ ).



**Figure 3**

Next-generation sequencing findings from the metastatic liver tumor tissue samples.



**Figure 4**

Several target lesions before (A-C) and after (D-F) one cycle of ensartinib.