

Data S1

DNA extraction, library construction and sequencing. DNA extraction, library construction and sequencing were conducted in a clinical testing laboratory (Nanjing Geneseeq Technology Inc., Nanjing, China) accredited by the Clinical Laboratory Improvement Amendments and the College of American Pathologists. Formalin-fixed paraffin-embedded (FFPE) samples were de-paraffinized with xylene, and genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Inc.; cat. no. 56404), following the manufacturer's instructions. Peripheral blood samples were centrifuged at 1,800 x g for 10 min at room temperature, followed by extraction and purification of cell-free DNA using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Inc.; cat. no. 55114). Genomic DNA of white blood cells in sediments was used as the normal control, and its extraction was carried out using the DNeasy Blood and Tissue Kit (Qiagen, Inc.; cat. no. 69504). Nanodrop2000 (Thermo Fisher Scientific, Inc.) was used to assess the quality of genomic DNA, while the fragment distribution of DNA was analyzed using the High Sensitivity DNA Kit (Agilent Technologies, Inc.; cat. no. 5067-4626) on a Bioanalyzer 2100. The dsDNA HS assay kit was used to quantify DNA on a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Inc.). Library preparations were performed using the KAPA Hyper Prep Kit (KAPA Biosystems; Roche Diagnostics) with optimized protocols. Libraries with different indices were pooled for targeted enrichment with GeneseeqPrime™ targeted NGS panel (437 cancer-related genes) and xGen Lock-down Hybridization and Wash Reagents Kit (Integrated DNA Technologies, Inc.), and then were quantified by qPCR (43.06 nM). The target-enriched library was then sequenced on DNBSEQ-T7 platform (MGI Tech Co., Ltd.) according to the manufacturer's instructions.

Targeted next-generation sequencing (tNGS) technical parameters and validation methods. Using tNGS (Geneseeq Prime™; Nanjing Geneseeq Technology, Inc.) that covered exons, fusion-related introns and microsatellite loci of 437 cancer-related genes with a target region of 1.53 Mb, two rare STRN3-ALK and ALK-MTUS2 fusions, as well as a heterozygous germline frameshift mutation (p.M3011Dfs*6, located in exon 63) in the ATM gene were identified. The sequencing was performed on the DNBSEQ-T7 platform (MGI Tech Co., Ltd.) with 150 bp paired-end reads. The median sequencing depth was 467x for tumor tissue samples, 9,631x for plasma samples and 345x for white blood cell control samples (normal control), with a target region coverage of $\geq 99.7\%$ (Q30, $>95\%$; sequence alignment rate, $>99.9\%$). All samples were derived from the same patient. Bioinformatics analysis was conducted using the GSCAP pipeline (GRCh37/hg19 reference genome). Fusion variants were cross-validated by dual algorithms (FACTERA and Arriba) and orthogonally confirmed by long-range PCR and Sanger sequencing. Germline mutations were double-verified by familial segregation analysis (validated by white blood cell samples) and droplet digital PCR with a detection limit of 0.1% (QX200 system). The entire experimental process included quality control using the NA12878 standard (consistency, $>99\%$) and strictly followed

quality control criteria (tumor cell content, $\geq 80\%$; total DNA, ≥ 50 ng; pre-library total, ≥ 100 ng).

Mutation calling. Sequence reads were processed using bcl2fastq V.2.16.0.10 to generate FASTQ files, followed by quality filtering with Trimmomatic (1). High-quality reads were aligned to the human genome (hg19, GRCh37) using Burrows-Wheeler Aligner (BWA-mem, v0.7.12; <https://github.com/lh3/bwa/tree/master/bwakit>) (2). BAM files were generated and sorted using Picard V.1.119; local realignment around indels and base quality recalibration were performed with the Genome Analysis Toolkit (GATK 3.4.0; <https://software.broadinstitute.org/gatk/>). VarScan2 was used to identify single-nucleotide variations (SNVs) and insertion/deletion mutations. Variant annotation was performed using ANNOVAR, ClinVar, HGMD, LOVD, 1,000 g, ExAC and gnomAD. Variants with a frequency of $\geq 1\%$ in any of these databases, including our in-house database, 1,000 g, ExAC and gnomAD, were removed. SNVs and indels with a variant allele frequency of $>1\%$ for tissues and $>0.1\%$ for circulating tumor DNA (ctDNA) and at least three unique mutant reads were retained to establish a further mutational landscape. Furthermore, matched white blood cell DNA was used as the negative control to remove germline variants and clonal hematopoiesis in tumor and plasma samples. Copy number variants were analyzed using copy number values adjusted by sample ploidy using CNVkit (3) with a cutoff of fold change ≥ 1.6 and ≤ 0.6 for gain and loss, respectively. Gene fusions were identified by DELLY (4) with a minimum split read threshold of 3 for tissues and 1 for ctDNA. Mutations and fusions validated using the Integrative Genomics Viewer (IGV) (5).

Hematoxylin-eosin staining. The samples were obtained from lung biopsy tissues and processed by paraffin embedding. The tissues were fixed with 10% neutral formalin at room temperature for 24 h. The sections were cut at a thickness of 4-5 μm . For antigen retrieval, the heating temperature was set at 95-100°C, followed by washing with phosphate-buffered saline (PBS). Then, the sections were rehydrated through a series of graded alcohols (100, 95, 85 and 70%) for 5 min each, and finally washed with distilled water. For hematoxylin staining, the sections were stained at room temperature for 5 min, differentiated with 1% hydrochloric acid alcohol for several seconds and then blued in running tap water for 10 min. Eosin staining was performed at room temperature for 1 min. Subsequently, the sections were dehydrated through a series of graded alcohols (70, 85, 95 and 100%), cleared with xylene and mounted with neutral gum. The stained sections were examined using a light microscope.

Immunohistochemical analysis. The immunohistochemical analysis was performed using fully automated immunohistochemistry instruments from Dako (Agilent Technologies, Inc.) and Roche Diagnostics. The samples were permeabilized in 0.1% Triton X-100 (dissolved in PBS) at room temperature for 10 min. To block endogenous peroxidase/phosphatase activity the samples were incubated with 3% hydrogen peroxide at room temperature for 10 min. For non-specific protein blocking, 5% bovine serum albumin (BioFroxx; neoFroxx) was employed at

room temperature for 30 min. The primary antibodies did not require dilution. Cytokeratin 7 (cat. no. M7018), programmed death-ligand 1 (22C3; cat. no. M3653) and p40 (cat. no. DAK-P40) (all Dako; Agilent Technologies, Inc.) were detected using the DAKO LinK48 platform. Thyroid transcription factor 1 (cat. no. SP141), Napsin A (cat. no. MRQ-60), p53 (cat. no. 800-2912), Ki67 (cat. no. 790-4286) and anaplastic lymphoma kinase (D5F3; cat. no. 790-4794) (all Roche Diagnostics) were detected using the VENTANA BenchMark ULTRA platform. All primary antibody incubations were performed at room temperature for 2 h. The samples were then incubated with the HRP conjugated the anti-mouse secondary antibody (Dako; Agilent Technologies, Inc.; cat. no. K800221-2CN) diluted at 1:20 and the anti-rabbit secondary antibody (Dako; Agilent Technologies, Inc.; cat. no. GV809) diluted at 1:20 at room temperature for 30 min. For the chromogenic detection, the sample was incubated with DAB+ solution (Dako; cat. no. K346811-2) at room temperature for 5 min and then terminated

with distilled water. The stained sections were examined using a light microscope.

References

1. Bolger AM, Lohse M and Usadel B: Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics* 30: 2114-2120, 2014.
2. Li H and Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754-1760, 2009.
3. Talevich E, Shain AH, Botton T and Bastian BC: CNVkit: Genome-wide copy number detection and visualization from targeted DNA sequencing. *PLoS Comput Biol* 12: e1004873, 2016.
4. Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V and Korbel JO: DELLY: Structural variant discovery by integrated paired-end and split-read analysis. *Bioinformatics* 28: i333-i339, 2012.
5. Thorvaldsdóttir H, Robinson JT and Mesirov JP: Integrative genomics viewer (IGV): High-performance genomics data visualization and exploration. *Brief Bioinform* 14: 178-192, 2013.

Figure S1. Brain MRI documented the changes in the patient during alectinib treatment. (A) Brain MRI shows an enhanced nodule in the right frontal lobe at baseline. (B) Brain MRI shows the disappearance of a right frontal lobe brain metastasis after 3 months of alectinib treatment. No recurrence was observed following (C) 6 and (D) 11 months of continued alectinib treatment. (E) Brain MRI shows an enhanced nodule in the left cerebellar hemisphere. (F) Brain MRI shows the disappearance of a left cerebellar hemisphere brain metastasis after 3 months of alectinib treatment. No recurrence was observed following (G) 6 and (H) 11 months of continued alectinib treatment. MRI, magnetic resonance imaging.

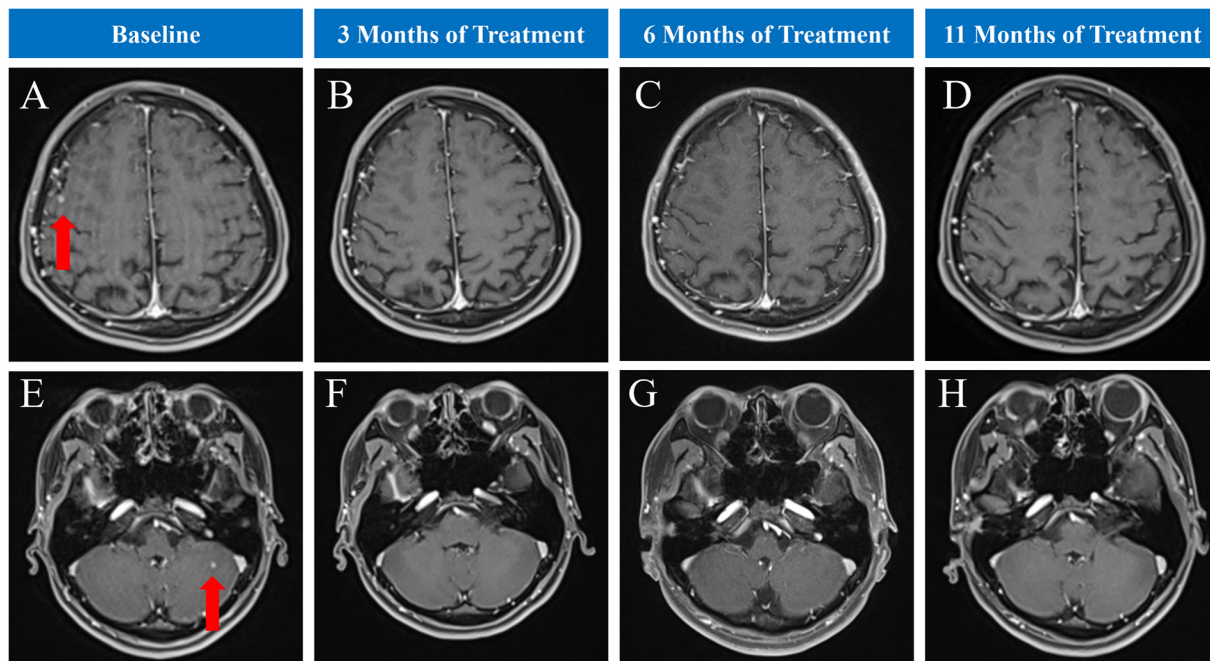


Figure S2. Baseline PET/CT shows a lung mass with bone metastasis and widespread lymph node metastasis throughout the body. (A) PET/CT demonstrates a mass in the basal segment of the right lower lobe, measuring $\sim 4.3 \times 4.0$ cm in its largest cross-sectional area, with significantly elevated radiotracer uptake ($SUV_{max}=11.1$). (B) PET/CT reveals a right hilar lymph node with a maximum diameter of 3.1 cm, exhibiting a marked increase in radiotracer uptake ($SUV_{max}=11.7$). (C) PET/CT reveals multiple enlarged lymph nodes in the right supraclavicular area and within the mediastinum, specifically in zones 1R, 2R, 4 and 7, with a mild increase in glucose metabolism ($SUV_{max}=2.7$). (D) PET/CT reveals a slightly hyperdense nodule on the left side of the sacrum, with a maximum cross-sectional size of $\sim 2.3 \times 2.0$ cm and increased radiotracer uptake ($SUV_{max}=9.8$). SUV, standardized uptake value; PET/CT, positron emission tomography/computed tomography.

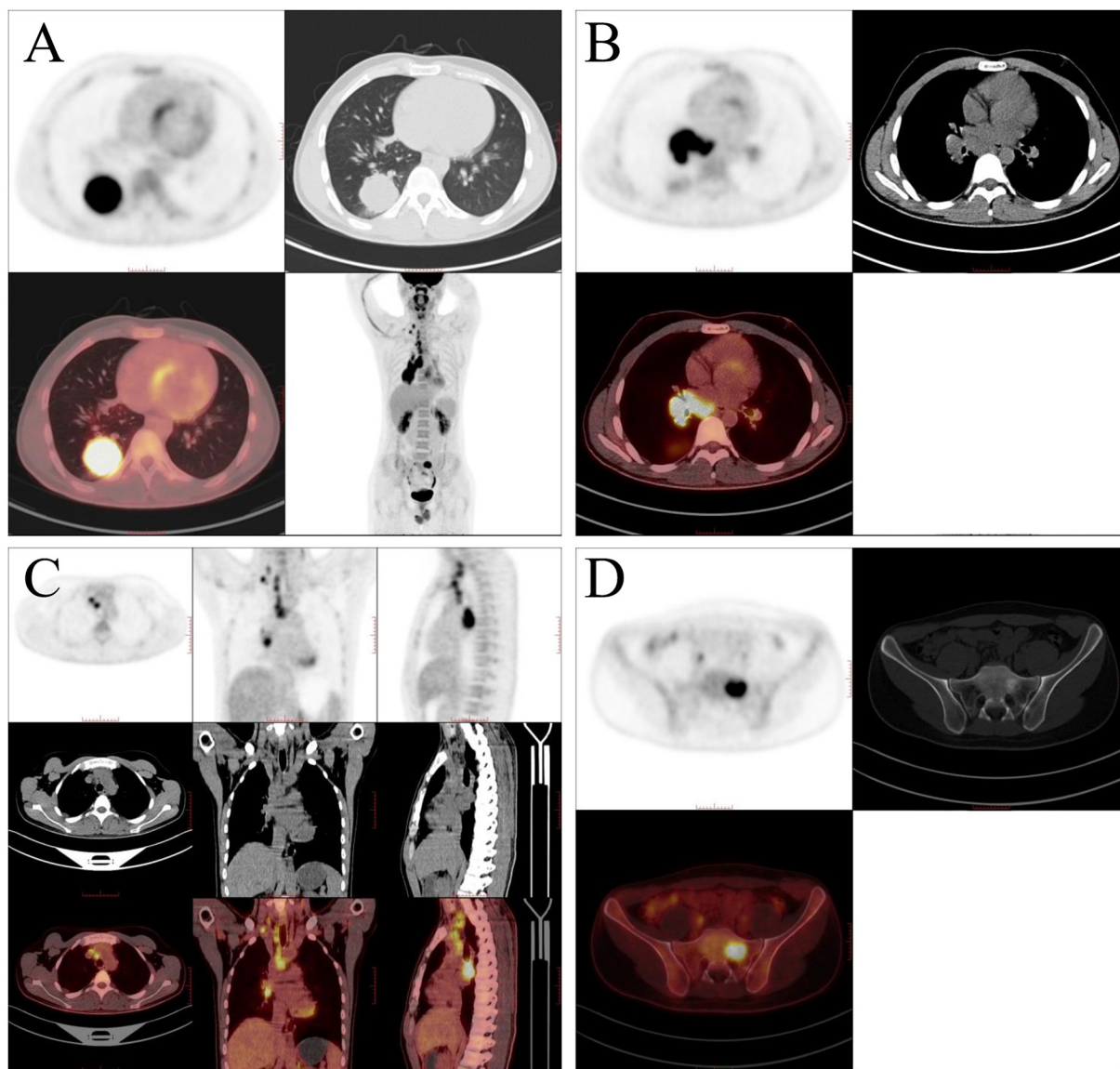
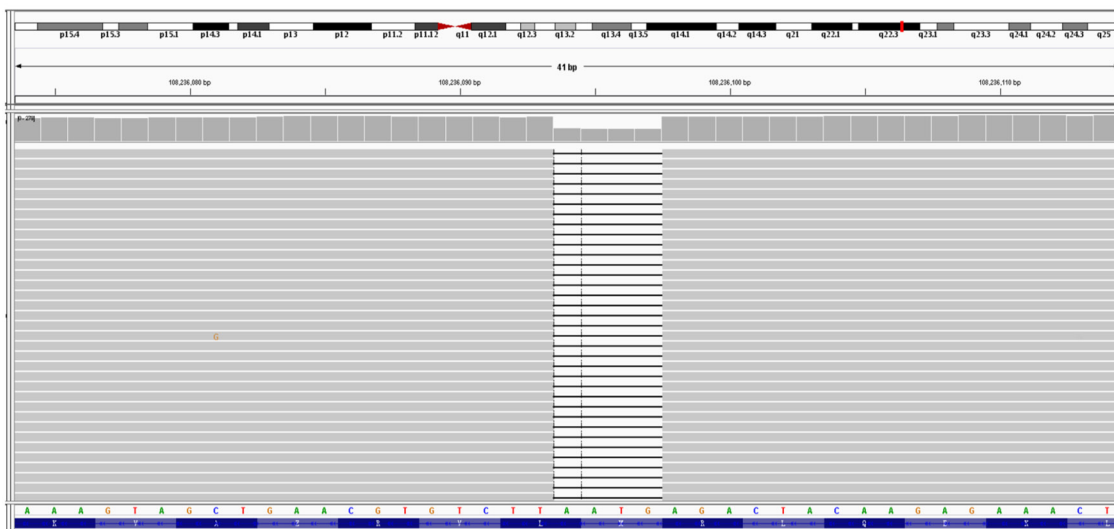


Figure S3. Heterozygous germline frameshift mutation in the ATM gene (p.M3011Dfs*6, in exon 63) through targeted next-generation sequencing. AF, allele frequency; ATM, ataxia-telangiectasia mutated.

Germline ATM c.9031_9034del(p.M3011Dfs*6)

Tumor sample
AF: 44.16%



Normal control
AF: 47.04%

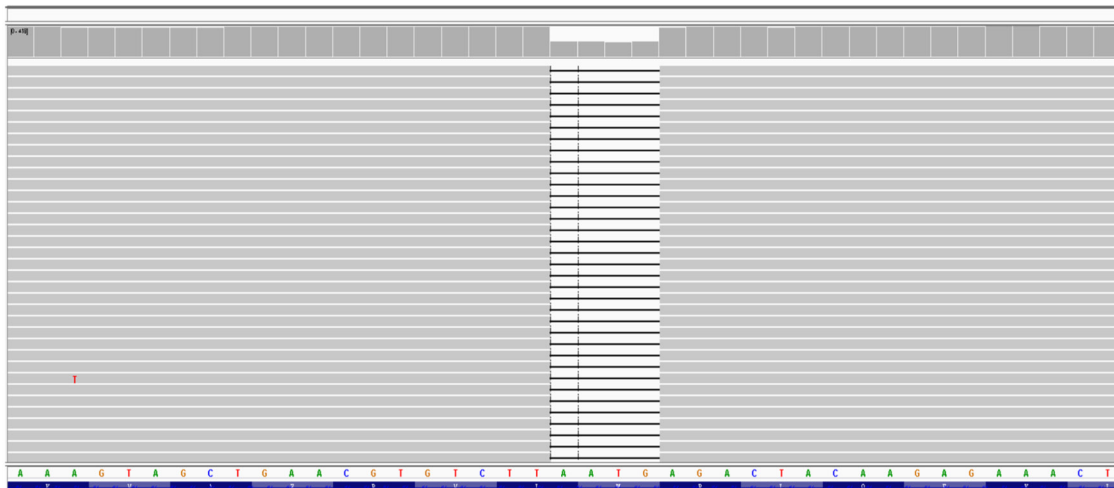


Figure S5. Tumour tissue immunohistochemistry. (A) Ki67 exhibited ~15% positive expression (magnification, x200). (B) Programmed death-ligand 1 (22C3) had a tumour proportion score of ~20% (magnification, x200). (C) p53 showed ~80% positive expression (magnification, x200). (D) Cytokeratin 7 exhibited diffuse positive staining (magnification, x200). (E) Thyroid transcription factor 1 showed diffuse positive staining (magnification, x200). (F) Napsin A showed diffuse positive staining (magnification, x200). (G) p40 was negatively expressed (magnification, x200).

