Supplementary Materials

Method S1. Diagnosis of NSCLC and detection of EML4-ALK fusion variants and TP53 mutations by next-generation sequencing

The histological diagnosis of NSCLC and the quantification of tumour cell content were performed by experienced pulmonary pathologists on formalin-fixed and paraffin-embedded (FFPE) small biopsies according to the WHO and IASLC/ATS/ERS criteria [1]. Newly diagnosed cases were screened for the presence of an ALK alteration by fluorescence in situ hybridisation (FISH) and reverse-transcription polymerase-chain reaction (RT-PCR) until 2015, or by immunohistochemistry (IHC) and next-generation sequencing (NGS) afterwards, with FISH restricted to discordant results and technical failures.

For the NGS detection of ALK fusions and TP53 mutations, areas with at least 15% tumour cellularity were marked on a hematoxylin and eosin-stained slide and macrodissected manually from consequent 8 μm thick tissue sections, followed by sample deparaffinization, digestion with Proteinase K overnight and nucleic acid extraction with the automated system Promega Maxwell 16 LEV RNA FFPE Purification Kit (Promega, Madison, WI, USA). Samples with at least 1 ng/μL DNA and/or RNA (QuBit 2.0 DNA and RNA high sensitivity kit, ThermoFisher Scientific, Waltham, MA, USA) were split into two and used for DNA and/or RNA library preparation directly and/or after treatment with DNaseI at room temperature for 15 min, respectively. For library preparation, the multiplex PCR-based Ion Torrent AmpliSeq™ technology (ThermoFisher) was used together with the RNA Lung Cancer Fusion Panel and a proprietary Lung Cancer Panel (covering the entire exons 4, 5, 6, 7, 8, 9, and 10 of TP53 including 15 flanking base pairs for each exon), as described previously [2]. Only samples achieving library concentrations > 100 pM were considered suitable for further processing. Raw sequencing data were processed using the Torrent Suite Software (version 4.2.1) and aligned against the human genome (version hg19) using the Torrent Mapping Alignment Program algorithm. ALK fusion transcripts were detected using the fusion workflow integrated in the Ion Reporter Software (versions 4.2 and 5.2) as described previously [2].

Method S2. Detection of the V1 and V3 EML4-ALK fusion variants by RT-PCR

For the RT-PCR based detection of V1 and V3 ALK fusion variants, tumour RNA was reverse transcribed using the SuperScript III One Step RT-PCR System (Invitrogen, ThermoFisher) and amplified with primers specific for the EML4 exons 6 and 13 and the ALK exon 20 (sequences given in the Online Supplements). Direct sequencing of the PCR amplicons was performed for both strands on a 3500 Genetic Analyzer using the BigDye Terminator v1.1 Cycle Sequencing Kit (both from Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Since RT-PCR represented only a temporary
solution in our laboratory and was succeeded by NGS in 2015 for the typing of ALK fusion variants, we chose not to invest additional resources in the establishment of RT-PCR assays specific for less frequent variants like V2.

**Method S3. ALK IHC and FISH**

For the ALK IHC a sensitive and specific antibody (D5F3 clone, Roche, Mannheim, Germany) [3] was used according to current guidelines [4]. ALK FISH analyses were performed on whole block slides of FFPE NSCLC samples using a break-apart probe (ZytoLight SPEC ALK probe, ZytoVision GmbH, Bremerhaven, Germany). At least 100 cells were analysed per case, and cases were considered to be positive when 15% or more of 50 tumour cells showed classic split signals [4].

**Method S4. ctDNA analysis**

For ctDNA analysis, plasma was isolated from blood samples of ALK+ NSCLC patients centrifuged within 30 minutes of collection and processed with the AVENIO ctDNA Analysis Kit (covering the entire TP53 exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11) according to the manufacturer’s instructions (Roche Diagnostics, Mannheim, Germany). Briefly, DNA was isolated from 2 mL of plasma using the AVENIO ctDNA Isolation Kit (Roche) and quantified with the Qubit dsDNA High Sensitivity Kit (ThermoFisher). Targeted sequencing libraries were prepared from 39.5 ng DNA in median (range 15–50 ng) using the AVENIO ctDNA Library Preparation Kit with the AVENIO Targeted Panel (both from Roche) for hybridisation-based enrichment of a 17-gene panel. All protocols were conducted according to the manufacturer’s recommendations. Equal amounts of 16 libraries were pooled and sequenced on an Illumina NextSeq 550 using the High Output Kit V2 (300 cycles) according to the manufacturer’s protocol (Illumina, San Diego, CA, USA) with a median unique target sequence coverage of 7,600x (range 2,013x–12,467x). Automated raw data processing and data analysis was performed with the AVENIO ctDNA analysis software (Roche).
Table S1. TP53 mutations of the study patients.

| ALK fusion variant | TP53 status | TP53 mutation | Material of 1st detection | TP53 exon affected | Effect |
|--------------------|-------------|---------------|----------------------------|-------------------|--------|
| V1 (E13;A20)       | TP53mut<sup>conv</sup> | p.?: c.994-2A>G; AF 0.24% (ctDNA); CV: 5446 | ctDNA | loss of exon 10 | Loss-of-function<sup>1,2</sup> |
| V1 (E13;A20)       | TP53mut<sup>conv</sup> | p.H214R: c.641A>G; AF: 0.07% (ctDNA); 12103 | ctDNA | 6 | Loss-of-function<sup>2</sup> |
| V2 (E20;A20)       | TP53mut<sup>conv</sup> | p.K139E: c.415A>G; AF: 0.25% (ctDNA); 7158 | ctDNA | 5 | Loss-of-function<sup>2</sup> |
| V3 (E6;A20)        | TP53mut<sup>conv</sup> | p.E285K: c.853G>A; AF: 11%; CV: 2506 | FFPE | 8 | Likely loss-of-function<sup>1</sup> |
| V3 (E6;A20)        | TP53mut<sup>conv</sup> | p.Y234fs*8:c.699_700insCATCCAC; AF:26%; CV:2918 | FFPE | 7 | Loss-of-function<sup>2</sup> |
| V3 (E6;A20)        | TP53mut<sup>conv</sup> | p.M237I: c.711G>A; AF: 0.18% (ctDNA); CV: 8496 | ctDNA | 7 | Loss-of-function<sup>2</sup> |
| V3 (E6;A20)        | TP53mut<sup>conv</sup> | p.R158S: c.472C>A; AF: 0.09% (ctDNA); CV: 5487 | ctDNA | 5 | Loss-of-function<sup>1</sup> |
| V3 (E6;A20)        | TP53mut<sup>conv</sup> | p.A276D: c.827C>A; AF: 0.64% (ctDNA); CV: 5657 | ctDNA | 8 | Loss-of-function<sup>1</sup> |
| K9;A20             | TP53mut<sup>conv</sup> | p.I195Y: c.584T>C; AF 0.16% (ctDNA), CV: 6290 p.V216L: c.646G>T; AF 0.15% (ctDNA), CV: 7542 | ctDNA | 6 | Loss-of-function<sup>2</sup> |
| E9;A20             | TP53mut<sup>basi</sup> | p.5241C: c.722C>G; AF 0.17% (ctDNA); CV: 5147 | FFPE | 7 | Loss-of-function<sup>2</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.L194P:c.581T>C; AF 14%, CV: 277 | FFPE | 6 | Loss-of-function<sup>2</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.R342P: c.1025G>C; AF 30%, CV: 900 | FFPE | 10 | Loss-of-function<sup>1</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.spl?: c.375+1G>A; AF 14%, CV: 2639 | FFPE | likely loss of exon 5 | Likely loss-of-function<sup>2,3</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.L330fs*15: c.988delC; AF 35%, CV: 1996 | FFPE | 9 | Loss-of-function<sup>1</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.V272M: c.814G>A; AF: 23%, CV: 13056 | FFPE | 8 | Loss-of-function<sup>1</sup> |
| V1 (E13;A20)       | TP53mut<sup>basi</sup> | p.S183*: c.548C>G; AF: 24%, CV: 883 | FFPE | 5 | Loss-of-function<sup>1</sup> |
| V2 (E20;A20) | TP53mutbas | p.R273C: c.817C>T; AF: 23%, CV: 6755 | FFPE | 8 | Loss-of-function¹ |
|-------------|-----------|-----------------------------------|------|----|------------------|
| V2 (E20;A20) | TP53mutbas | p.P152S: c.454C>T; AF 26%, CV: 971 | FFPE | 5 | Loss-of-function¹ |
| V2 (E20;A20) | TP53mutbas | p.H193D: c.577C>G; AF 54%, CV: 530 | FFPE | 6 | Loss-of-function¹ |
| V3 (E6;A20) | TP53mutbas | p.R249W: c.745A>T; AF 45%, CV: 1120 | FFPE | 7 | Loss-of-function¹ |
| V3 (E6;A20) | TP53mutbas | p.M66fs*84: c.195_196insCAGA; AF:33%, CV: 1735 | FFPE | 4 | Likely loss-of-function⁴ |
| V3 (E6;A20) | TP53mutbas | p.E286delinsDRRTEE: c.857_858insCCGGCGCACAGAGGA; AF: 20%, CV: 1914 | FFPE | 8 | Likely loss-of-function¹ |
| V3 (E6;A20) | TP53mutbas | p.R213Q: c.638G>A; AF: 11%, CV:2215 | FFPE | 6 | Loss-of-function¹² |
| V3 (E6;A20) | TP53mutbas | p.Q331*: c.991C>T; AF: 10%, CV: 1591 | FFPE | 9 | Loss-of-function¹² |
| V3 (E6;A20) | TP53mutbas | p.C135F: c.404G>T; AF:12%, CV: 1566 | FFPE | 5 | Loss-of-function¹²⁴ |
| V3 (E6;A20) | TP53mutbas | p.spl?: c.920-2A>G; AF:13%, CV: 1676 | FFPE | 10 | Likely loss-of-function²³ |
| V3 (E6;A20) | TP53mutbas | p.Y220C: c.659A>G, AF: 0.19% (ctDNA), CV: 2146 | ctDNA ⁵ | 6 | Loss-of-function¹ |
| V3 (E6;A20) | TP53mutbas | p.R249W: c.745A>T; AF: 17%, CV: 4162 | FFPE | 7 | Loss-of-function¹ |
| V3 (E6;A20) | TP53mutbas | p.Q192*: c.574G>T; AF:13%, CV: 308 | FFPE | 6 | Loss-of-function¹ |
| V9 (E18;A20) | TP53mutbas | p.Y220C: c.659A>G; AF: 15%, CV: 607 | FFPE | 6 | Loss-of-function² |
| V9 (E18;A20) | TP53mutbas | p.Leu167fs*41: c.508_508A>delinsGG; AF: 24%, CV: 1445 | FFPE | 5 | Loss-of-function² |
| unknown | TP53mutbas | p.R249W: c.745A>T; AF: 17%, CV: 4162 | FFPE | 7 | Loss-of-function¹ |
| unknown | TP53mutbas | p.A138V: c.413G>T; AF: 20%, CV: 2889 | FFPE | 5 | Loss-of-function² |
| unknown | TP53mutbas | p.R110L: c.329G>T; AF13%, CV: 352 | FFPE | 4 | Likely loss-of-function¹ |

AF: allelic frequency; CV: coverage; TP53mut<sup>conv</sup>: cases with wild-type TP53 at baseline and detection of TP53 mutations at disease progression; TP53mut<sup>bas</sup>: cases with detection of TP53 mutations at baseline.

¹ according to the OncoKB database, http://oncokb.org/#/gene/TP53, accessed on 04.04.2018 [5].
² according to the COSMIC database v84, released 13 Feb 2018, https://cancer.sanger.ac.uk/cosmic/.
according to the ClinVar database, https://www.ncbi.nlm.nih.gov/clinvar/, accessed on 04.04.2018.

according to the The Jackson Laboratory database, https://ckb.jax.org/gene/show?geneld=7157, accessed on 04.04.2018.

for this single case, the TP53 mutation at baseline was detectable with ctDNA analysis only, while the FFPE tumour sample was negative.
Figure S1. Progression-free survival of patients with metastatic ALK+ NSCLC under treatment with chemotherapy according to TP53 status at baseline and under therapy. The median progression-free survival (PFS) under chemotherapy treatment was 5 months for patients with TP53 mutations at baseline (TP53mutbas) vs. 7 months for patients with initially wild-type status and detection of TP53 mutations in a subsequent biopsy (TP53mutconv) vs. 8 months for patients without subsequent detection of TP53 mutations (TP53wtprogr, logrank p = 0.60). The treatment details are given in Table 1; ns: not statistically significant.

PFS under chemotherapy

|                      | No. at risk | at risk | 12 | 18 | 24 | 30 |
|----------------------|-------------|---------|----|----|----|----|
| TP53wtprogr/ALK+     | 21          | 21      | 11 | 4  | 3  | 1  | 0  |
| TP53mutconv/ALK+     | 12          | 12      | 6  | 2  | 1  | 1  | 0  |
| TP53mutbas/ALK+      | 13          | 13      | 4  | 3  | 2  | 1  | 0  |
Figure S2. Overall survival of study patients from initial diagnosis. The median overall survival (OS) from initial diagnosis was 44 months for patients with TP53 mutations at baseline (TP53mutbas) vs. 45 months for patients with initially wild-type status and detection of TP53 mutations in a subsequent biopsy (TP53mutconv) vs. not reached for patients without subsequent detection of TP53 mutations (TP53wtprogr, logrank p=0.0012). As shown in Table 1, 10/30 TP53wtprogr and 2/23 TP53mutbas patients had secondary development of metastatic disease after relapse of initially nonmetastatic ALK+ NSCLC, causing the curves in this plot to differ slightly from these of Figure 1; ns: not statistically significant.

**OS from initial diagnosis**

![Graph showing OS from initial diagnosis](image)

| No. at risk | TP53wtprogr | TP53mutconv | TP53mutbas |
|------------|------------|------------|------------|
|            | 30         | 9          | 23         |
| 1          | 29         | 9          | 17         |
| 2          | 25         | 7          | 9          |
| 3          | 20         | 4          | 3          |
| 4          | 15         | 3          | 3          |
| 5          | 13         | 3          | 1          |
| 6          | 11         | 1          | 1          |
| 7          | 8          | 0          | 0          |
| 8          | 5          |             |            |
| 9          | 3          |             |            |
| 10         | 0          |             |            |

$p=0.0012$ for TP53mutbas vs. TP53wtprogr

$p=0.0038$ for TP53mutconv vs. TP53wtprogr

ns (p=0.39) for TP53mutbas vs. TP53mutconv
References

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