Aus dem CharitéCentrum für Innere Medizin und Dermatologie
Medizinische Klinik mit Schwerpunkt Infektiologie und Pneumologie
einschließlich Arbeitsbereich ambulante Pneumologie
Direktor: Prof. Dr. med. Norbert Suttorp

# Habilitationsschrift

## Aktuelle patientenindividuelle Behandlungsansätze beim metastasierten nicht-kleinzelligen Lungenkarzinom

zur Erlangung der Lehrbefähigung
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von

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## Abkürzungen

| | |
|---|---|
| ALK | Anaplastische Lymphomkinase |
| BRAF | Rapidly accelerated fibrosarcoma Isoform B |
| CK | Zytokeratin |
| CML | Chronisch myeloische Leukämie |
| CTLA-4 | Cytotoxic T-lymphocyte-associated protein 4 |
| EGFR | Epidermal growth factor receptor |
| ERK | Mitogen-activated protein kinase |
| HR | Hazard ratio |
| KRAS | Kirsten rat sarcoma virus |
| MEK | Mitogen-activated protein kinase kinase |
| NEC | Neuroendokrines Karzinom |
| NET | Neuroendokriner Tumor |
| NGS | Next-generation sequencing |
| NSCLC | Non-small cell lung cancer |
| | Nicht-kleinzelliges Lungenkarzinom |
| NTRK | Tropomyosinrezeptorkinase A |
| OMD | Oligometastatic disease |
| | Oligometastasierung |
| ORR | Overall response rate |
| OS | Overall survival |
| | Gesamtüberleben |
| PD-L1 | Programmed death ligand 1 |
| PFS | Progression-free survival |
| | Progressionsfreies Überleben |
| PS | Performance status |

| | |
|---|---|
| RAF | proto-oncogene c-RAF |
| RET | Rearranged during transfection |
| ROS1 | C-ros oncogene 1 |
| TKI | Tyrosinkinaseinhibitor |
| TMB | Tumor mutational burden |
| | Tumormutationslast |
| TME | Tumormicroenvironment |
| TNM | T: Tumor; N: Lymph node; M: Metastasis |
| TP53 | Tumor protein P53 |
| TTF-1 | Thyreoidaler Transkriptionsfaktor 1 |
| VEGF | Vascular endothelial growth factor |
| ZNS | Zentrales Nervensystem |

# 1 Einleitung

## 1.1 Epidemiologie und Bedeutung

Krebserkrankungen stellen in Ländern mit mittlerem bis hohem Einkommen mittlerweile die führende Todesursache dar [1]. Ein Drittel aller Fälle entfallen auf Lungenkarzinome, den mit Abstand häufigsten Krebstod [2]. Da die Diagnose meist im fortgeschrittenen Lebensalter gestellt wird [3-5] und, dem jahrzehntelangem Nikotinkonsum geschuldet, häufig multiple Komorbiditäten bestehen [6], stellen Diagnostik und Therapie nicht selten eine Herausforderung dar. Eine metastasierte Tumorerkrankung ohne Aussicht auf eine kurativ-intendierte Behandlung wird je nach Histologie bei 50-75% aller Patienten diagnostiziert [7, 8]. Das Überleben ist im Vergleich zu anderen Tumorentitäten deutlich kürzer und beläuft sich im Stadium IV auf sechs bis zwölf Monate [9-11].

## 1.2 Histologische Diagnostik

80% aller Lungenkarzinome weisen eine nicht-kleinzellige Histologie (non-small cell lung cancer, NSCLC) auf, 20% entfallen auf pulmonal neuroendokrine Tumore (NET) und Karzinome (NEC). Adenokarzinome stellen mit 40% aller Lungenkrebsneudiagnosen den häufigsten Subtyp dar [12, 13]. Die Prognose wird entscheidend vom Grading beeinflusst, wobei dies lediglich bei den neuroendokrinen Tumoren integraler Bestandteil der Diagnosestellung ist [14-16]. Eine genaue histologische Zuordnung erfolgt mittels Immunhistochemie unter Verwendung spezifischer Adeno- (CK7, TTF-1), Plattenepithelkarzinom- (p40, CK5/6) und neuroendokrinen Markern (CD56, Synaptophysin) [17, 18]. Da die Expression des „programmed death ligand 1“-Proteins (PD-L1) auf den Tumorzellen prädiktiv für das Ansprechen auf immunonkologische Behandlungsansätze ist [19], sollte eine immunhistochemische Reflextestung bereits bei Diagnosestellung erfolgen [20, 21].

## 1.3 Molekulare Alterationen beim NSCLC

Bis zu 20% aller Adenokarzinome weisen zielgerichtet behandelbare Treibermutationen auf, die das Tumorgeschehen aktiv und wesentlich beeinflussen. Eine onkogene Abhängigkeit besteht dann, wenn ein Tumor selbst bei großer genomischer Heterogenität durch gezielte Ausschaltung eines einzigen Gens in die Apoptose getrieben werden kann [22], wie es bspw. bei der chronisch-myeloischen Leukämie (CML) mit BCR-ABL Translokation durch eine Behandlung mit Imatinib möglich ist [23]. Entsprechende genetische Alterationen können sich im Bereich membranständiger Rezeptortyrosinkinasen, bzw. der für Zellzyklus und -proliferation zuständigen intrazellulären Signaltransduktionskaskaden befinden [24]. (Punkt-) Mutationen der Kinasedomäne mit nachfolgend ligandenunabhängiger Daueraktivierung kommen beim Lungenkarzinom membranständig im Bereich des epidermalen Wachstumsfaktorrezeptors (EGFR, 10-20%) sowie intrazellulär dem RAS-RAF-MEK-ERK Signalweg mit der $BRAF^{V600E}$-Mutation (1-2%) vor. Mit ca. 30% aller Treibermutationen sind Punktmutationen des KRAS-Gens beim Adenokarzinom mit Abstand am häufigsten, bislang jedoch nicht zielgerichtet behandelbar. Eine dauerhafte Genaktivierung kann weiterhin durch ein Bruchereignis mit anschließender Neuanordnung der DNA, einem sogenannten chromosomalen „rearrangement“ erfolgen. Hierzu zählen Fusionen der anaplastischen Lymphomkinase (ALK, 3-5%), des „c-ros oncogene 1“ (ROS1, 1-2%), des „rearranged during transfection“-Proteins (RET, 1-2%) und der Tropomyosinrezeptorkinase A (NTRK, 0,5-1%) [24]. Patienten mit einem nicht kurativ behandelbaren NSCLC sollten vor Einleitung einer palliativen Erstlinienbehandlung eine entsprechende Paneldiagnostik erhalten, die alle zielgerichtet behandelbaren molekularen Veränderungen abdeckt [20], da nur mittels spezifischer Targettherapie eine Verlängerung des Überlebens möglich ist [25].

## 1.4 Systemtherapien in der palliativen Therapiesituation

### 1.4.1 Platinbasierte Polychemotherapie

Eine platinbasierte Polychemotherapie stellt seit den 1990er Jahren das Rückgrat der Systemtherapie beim metastasierten NSCLC dar, wenngleich damit nur moderate Lebenszeitverlängerungen verglichen mit einem reinen „best supportive care“-Konzept zu erzielen waren [26]. Bis zur Etablierung Histologie-spezifischer Regime zeigte sich kein relevanter Wirk- und Überlebensunterschied zwischen den einzelnen Behandlungsoptionen [27]. Erst die Einführung des Folatantagonisten Pemetrexed [28] sowie antiangiogener Wirkprinzipien (Bevacizumab) führten im Verlauf bei Adenokarzinomhistologie zu einem moderat verbesserten Überleben [29].

### 1.4.2 Zielgerichtete Behandlung des molekular alterierten NSCLC

Die Entdeckung aktivierender EGFR-Mutationen markierte 2004 den Auftakt in der Identifikation onkogen alterierter Lungenkarzinome und verbesserte die Prognose für betroffene Patienten mittels zielgerichteter Behandlungsansätze (Targettherapie) erheblich [30]. Patienten mit EGFR-Mutationen bzw. ALK-/RET-/ROS1-Rearrangement unterscheiden sich phänotypisch zum Teil deutlich vom klassischen Lungenkrebspatienten, da sie meist deutlich jünger sind und selten bzw. nie geraucht haben [31]. Aktuell (Stand März 2021) sind in Deutschland neben EGFR spezifische Behandlungen für ALK, BRAF, NTRK, RET und ROS1 verfügbar. Im Verlauf der Targettherapie kommt es jedoch in der Regel innerhalb von 1-3 Jahren erneut zum Progress. Die Identifikation alternativer Signalwege und spezifischer Resistenzmutationen eröffnet jedoch zumindest in einem Teil der Fälle sequentielle Behandlungsoptionen [32] und ermöglicht so Langzeitüberleben für mehrere Jahre [33].

### 1.4.3 Immuntherapie beim NSCLC

Die dritte Säule der Behandlung stellen immunonkologische Therapieansätze dar. Tumore entziehen sich über eine Aktivierung negativer Checkpoints wie dem „Cytotoxic T-lymphocyte-associated protein 4“ (CTLA-4) und PD-L1 sowie Rekrutierung immunsuppressiver Zellen in der unmittelbaren Tumorumgebung, dem sogenannten Tumormicroenvironment (TME), der Immunantwort. Diese Entdeckung wurde 2018 mit dem Nobelpreis für Medizin geehrt [34, 35]. Seit 2017 steht mit Pembrolizumab eine chemotherapiefreie, hoch effektive Erstlinienbehandlung für Patienten mit NSCLC und einer PD-L1 Expression von ≥50% der Tumorzellen zur Verfügung [36], bei geringerer Expression sind zwischenzeitlich verschiedene Immunchemotherapiekombinationen zugelassen worden.

## 1.5 Oligometastasierung

Während die Behandlung in der metastasierten Situation für die meisten Patienten einen klar palliativen Charakter aufweist, ist in ausgewählten Situationen einer Oligometastasierung („oligometastatic disease“, OMD) ein kuratives Konzept möglich. Die Fähigkeit zur, bzw. Ausprägung der Metastasierung ist unter anderem von der Tumorentität, Lokalisation und Gefäßversorgung abhängig. Der Spektrumtheorie zufolge kann in einem frühen Stadium der Generalisierung eine begrenzten OMD auftreten, solange der Tumor noch keine vollständige Metastasierungsfähigkeit erlangt hat [37-39]. Eine radikale Behandlung in diesem Zwischenstadium kann Langzeitüberleben und ggf. sogar Heilung ermöglichen. Bis zu 7% aller Patienten mit Erstdiagnose im Stadium IV weisen eine begrenzte Metastasierung auf und sind somit potentiell Kandidaten für eine radikale Behandlung [40]. Eine sehr restriktive Definition der OMD mit solitärer extrathorakaler Metastase hat mittlerweile Eingang in die achte Auflage der TNM-Klassifikation gefunden und wird als M1b bezeichnet [10]. Eine lokalablative Behandlung

von Primarius und Metastase sollte bei Patienten mit einer bis drei Metastasen bei Diagnosestellung (synchrone OMD) evaluiert werden [41].

## 1.6 Ausgangspunkte der Arbeit

Während sich die Gesamtprognose des metastasierten NSCLC in den vergangenen Jahren nur moderat verbesserte [42], hat sich die Situation für ausgewählte Patienten durch die Einführung, zum Teil Biomarker-adjustierter patientenindividueller Behandlungen fundamental verändert. Hierunter fallen langanhaltend auf Immuntherapie ansprechende, PD-L1 überexprimierende Tumore, sowie molekular alterierte bzw. im Rahmen einer OMD einem lokal aggressiven Therapiekonzept zugängliche NSCLCs.

Prädiktive und prognostische Biomarker stellen einen Grundpfeiler für die Wahl geeigneter Tumortherapien dar (vgl. 1.2.1 und 1.2.2). Ein diagnostischer Biomarker kann eine Einschätzung hinsichtlich der Prognose der Erkrankung ermöglichen und ist im Idealfall prädiktiv für eine spezifische Therapie. Die Expression des thyreoidalen Transkriptionsfaktors 1 (TTF-1) wird in der Primärdiagnostik zwecks Unterscheidung pulmonaler (TTF-1 positiv) und extrapulmonaler (TTF-1 negativ) Adenokarzinome regelhaft untersucht. Ein Viertel aller pulmonalen Adenokarzinome ist jedoch prinzipiell TTF-1 negativ und weist bei geringerer Anzahl behandelbarer Treibermutationen eine insgesamt deutlich schlechtere Prognose auf [43, 44]. Ein darüber hinaus gehend potentieller prädiktiver Wert ist bei fehlender Berücksichtigung in klinischen Studien bislang nicht etabliert. PD-L1 stellt aktuell den einzigen in der klinischen Routine verwendeten prädiktiven Marker für eine Immuntherapie dar. Jedoch sprechen einige Patienten auch bei hoher Expression aufgrund fehlender tumorinfiltrierender Lymphozyten und immunologisch kaltem TME trotz hoher PD-L1 Expression mitunter nur kurz oder gar nicht auf die Behandlung an [45]. Eine umfassende prätherapeutische

klinisch-pathologische Charakterisierung des Tumors hinsichtlich dem potentiellen Therapieansprechens ist außerhalb von Studien derzeit nicht etabliert und die hoch relevante Frage nach im Rahmen der Routineversorgung bestimmbaren prädiktiven Markern bislang ungelöst. KRAS- und TP53-Mutationen korrelieren mit einer tendenziell höheren PD-L1 Expression [46] sowie einem inflammatorischen TME [47], scheinen ein besseres Therapieansprechen auf Immuntherapie vorherzusagen [48, 49] und könnten daher als zusätzliche Parameter in die Therapieentscheidung bzw. –steuerung bei Patienten mit hoher PD-L1 Expression einfließen. Im Gegensatz zu immunonkologischen Behandlungsansätzen sind TP53-Mutationen beim molekular alterierten Lungenkarzinom aufgrund eines dadurch bedingten intrinsischen Resistenzmechanismus mit reduziertem Ansprechen und Überleben assoziiert [50, 51].

Hinsichtlich der individuellen Wirksamkeit einer Systemtherapie steht der Behandler jedoch vor einem stetig wiederkehrenden Dilemma, da sich in klinische Studien eingeschlossene Patienten teils erheblich von denjenigen aus der klinischen Routine unterscheiden. Eine Vielzahl prädefinierter Ein- und Ausschlusskriterien sollen den Einfluss von Sekundärvariablen auf die Behandlung und das Outcome minimieren, schließen jedoch einen relevanten Anteil potentieller Probanden systematisch aus. Darunter fallen bspw. Patienten mit reduziertem Allgemeinzustand, aktiven Hirnmetastasen, chronischen Organschäden und Infektions- oder Autoimmunerkrankungen. Da häufig eine breite Zulassung neuer Präparate ohne Berücksichtigung der eng gesteckten Ein- und Ausschlusskriterien der jeweiligen Studien erfolgt, stellt sich für den Behandler die Frage einer entsprechenden Effektivität im Individualfall permanent.

Im Gegensatz zu einem klar palliativen Therapieansatz mit Fokus auf eine effektive Systemtherapie stellt die Behandlung von Patienten mit OMD gewissermaßen einen Hybridansatz dar, bei dem durch Integration effektiver Lokalverfahren eine

Langzeitkontrolle der Erkrankung ermöglicht werden soll. Ein in diesem Zusammenhang häufig geäußerter Kritikpunkt ist die mangelnde Evidenz angesichts fehlender qualitativ hochwertiger klinischer Studien. In retrospektive Fallserien eingeschlossene Patienten weisen mit niedrigerem Alter, gutem Allgemeinzustand und zumeist einer N0 oder N1-Situation prognostisch günstige Kriterien auf, deren Einfluss sich angesichts fehlender Kontrollgruppen nicht gut von der applizierten Lokaltherapie trennen lässt [52].

## 1.7 Fragestellungen

Für die vorliegende Arbeit waren die folgenden Fragestellungen von Relevanz:

- Kann TTF-1 neben seinem bekannten prognostischen Wert auch als prädiktiver Biomarker für eine platinbasierte Chemotherapie verwendet werden?
- Gibt es in der Gruppe der PD-L1 Hochexprimierer (≥50%) mit KRAS- und TP53-Mutationen weitere sinnvolle prognostische und ggf. prädiktive Biomarker?
- Welchen Stellenwert hat die Integration des TP53-Mutationsstatus hinsichtlich einer zielgerichteten Behandlung mit Lorlatinib beim ALK- oder ROS1-positiven Lungenkarzinom?
- Inwiefern lassen sich angesichts restriktiver Ein- und Ausschlusskriterien klinischer Studien die entsprechenden Ergebnisse auf die allgemeine Patientenpopulation aus der täglichen klinischen Routine übertragen?
- Welchen Stellenwert hat eine zusätzliche lokalablative Behandlung beim oligometastasierten Lungenkarzinom?

# 2 Eigene Arbeiten

## 2.1 Prädiktive Bedeutung von TTF-1 bei platinbasierter Erstlinienchemotherapie

**Frost N**, Zhamurashvili T, von Laffert M, Klauschen F, Ruwwe-Glösenkamp C, Raspe M, Brunn M, Ochsenreither S, Temmesfeld-Wollbrück B, Suttorp N, Grohé C, Witzenrath M. Pemetrexed-Based Chemotherapy Is Inferior to Pemetrexed-Free Regimens in Thyroid Transcription Factor 1 (TTF-1)-Negative, EGFR/ALK-Negative Lung Adenocarcinoma: A Propensity Score Matched Pairs Analysis.
*Clinical Lung Cancer 2020. Nov;21(6):e607-e621. doi: 10.1016/j.cllc.2020.05.014.*

Eine Pemetrexed-basierte Platindoublette stellt einen Therapiestandard des metastasierten Adenokarzinoms der Lunge dar. Ob der in der Routinediagnostik pulmonaler Adenokarzinome angewendete diagnostische wie prognostischen Biomarker TTF-1 auch eine prädiktive Wertigkeit hinsichtlich der Wahl des Therapieregimes darstellen könnte wurde im Rahmen dieser Arbeit untersucht.

Mittels klinischer Charakterisierung von 741 therapienaiven Patienten, davon 529 mit platinbasierter Erstlinientherapie, wurde ein spezifischer TTF-1 negativer Phänotyp identifiziert, der sich durch das Überwiegen männlicher Patienten in reduziertem Allgemeinzustand, mit höherer Metastasenlast bei Diagnosestellung und insbesondere prädominant adrenaler Metastasierung auszeichnet. Unter Berücksichtigung dieser prognostisch relevanten Unterschiede mittels 1:1 Propensity-Score Matching führten Pemetrexed-freie Regime bei TTF-1 negativen Patienten zu einem verbesserten progressionsfreien (PFS, Hazard ratio (HR) 0.42, $p<0.001$) wie Gesamtüberleben (OS, HR 0.40, $p<0.001$). Im Gegensatz dazu war die Therapiewahl bei TTF-1 positiven Patienten ohne Relevanz für das Outcome.

## 2.2 Langzeitansprechen des PD-L1 positiven (≥50%) Lungenkarzinoms unter palliativer Erstlinienbehandlung bei KRASG12C/TP53-Komutationen

**Frost N**, Kollmeier J, Vollbrecht C, Grah C, Matthes B, Pultermann D, von Laffert M, Lüders H, Olive E, Raspe M, Mairinger T, Ochsenreither S, Blum T, Hummel M, Suttorp N, Witzenrath M, Grohé C. KRAS$^{G12C}$/TP53 co-mutations identify long-term responders to first line palliative treatment with pembrolizumab monotherapy in PD-L1 high (≥50%) lung adenocarcinoma.

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Angesichts ungenügender Trennschärfe auch einer hohen PD-L1-Expression (≥50%) hinsichtlich des zu erwartenden Therapieansprechens auf Immuntherapie werden dringend zusätzliche prädiktive Biomarker benötigt, die langanhaltend ansprechende Patienten von denjenigen trennen, für die kein relevanter Nutzen zur erwarten ist.

Für diese Arbeit wurde die im Rahmen der Routinepaneldiagnostik bestimmte molekulare Tumorcharakterisierung mit dem klinischen Ansprechen von 119 primär mit Pembrolizumab behandelten, PD-L1 hochexprimierenden Patienten korreliert. In der untersuchten Kohorte zeigte sich mit 52% eine deutlich erhöhte Rate an KRAS-Mutationen, wovon die Hälfte auf den Subtyp G12C entfiel. Die insgesamt höheren Ansprechraten bei KRAS-Positivität waren auf KRAS$^{G12C}$-mutierte Patienten zurückzuführen, und hier wiederum hauptsächlich durch die Gruppe KRAS$^{G12C}$/TP53-Komutierter bedingt. Alle Patienten in der letztgenannten Konstellation sprachen langanhaltend an (ORR 100%, PFS 33.3 Monate). ORR und PFS der anderen KRAS-Subtypen waren mit denjenigen der Wildtyppatienten vergleichbar und lagen deutlich niedriger als bei Vorliegen einer G12C-Konstellation. Die Kombination anderer KRAS-

Subtypen mit einer TP53-Mutation war sogar mit einem tendenziell schlechteren Überleben assoziiert.

Original Article

# KRAS$^{G12C}$/TP53 co-mutations identify long-term responders to first line palliative treatment with pembrolizumab monotherapy in PD-L1 high (≥50%) lung adenocarcinoma

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**Background:** Pembrolizumab is a standard of care as first line palliative therapy in PD-L1 overexpressing (≥50%) non-small cell lung cancer (NSCLC). This study aimed at the identification of KRAS and TP53-defined mutational subgroups in the PD-L1 high population to distinguish long-term responders from those with limited benefit.

**Methods:** In this retrospective, observational study, patients from 4 certified lung cancer centers in Berlin, Germany, having received pembrolizumab monotherapy as first line palliative treatment for lung adenocarcinoma (LuAD) from 2017–2018, with PD-L1 expression status and targeted NGS data available, were evaluated.

**Results:** A total of 119 patients were included. Rates for KRAS, TP53 and combined mutations were 52.1%, 47.1% and 21.9%, respectively, with no association given between KRAS and TP53 mutations (P=0.24). By trend, PD-L1 expression was higher in KRAS-positive patients (75% *vs.* 65%, P=0.13). Objective response rate (ORR), median progression-free survival (PFS) and overall survival (OS) in the KRAS$^{G12C}$ group (n=32, 51.6%) were 63.3%, 19.8 months (mo.) and not estimable (NE), respectively. Results in KRAS$^{other}$ and wild type patients were similar and by far lower (42.7%, P=0.06; 6.2 mo., P<0.001; 23.4 mo., P=0.08). TP53 mutations alone had no impact on response and survival. However, KRAS$^{G12C}$/TP53 co-mutations (n=12) defined a subset of long-term responders (ORR 100.0%, PFS 33.3 mo., OS NE). In contrast, patients with KRAS$^{other}$/TP53 mutations showed a dismal prognosis (ORR 27.3%, P=0.002; PFS 3.9 mo., P=0.001, OS 9.7 mo., P=0.02).

**Conclusions:** A comprehensive assessment of KRAS subtypes and TP53 mutations allows a highly relevant prognostic differentiation of patients with metastatic, PD-L1 high LuAD treated upfront with pembrolizumab.

**Keywords:** Non-small cell lung cancer (NSCLC); checkpoint inhibitors; KRAS mutations; TP53 mutations

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## Introduction

Pembrolizumab monotherapy is a highly effective standard-of-care in metastatic, programmed death ligand 1 positive (PD-L1 ≥50%) non-small cell lung cancer (NSCLC) (1,2). However, predictive biomarkers distinguishing long-term responders to immune checkpoint inhibitors (ICI) from those experiencing no or only a limited benefit are still an unmet medical need.

Assuming a positive correlation of tumor neoantigens and the respective immune host response, assessment of tumor mutational burden (TMB) may serve as a predictor to ICI treatment (3-6), but several constraints have prevented an extensive integration into daily clinical practice yet. Compared to next-generation sequencing (NGS)-based gene panel tests, TMB testing is substantially more tissue-, time- and cost-consuming and harmonization of methods and cut-offs used is lacking (5,7-10). Finally, prospective clinical trials using upfront immuno-oncologic approaches in metastatic NSCLC have not unanimously demonstrated a predictive value for TMB (11,12).

KRAS mutations account for approximately 30% of driver mutations in lung adenocarcinoma (LuAD) (13,14), but are just rarely identified in squamous carcinoma (15). No specific therapies have been established yet and prognosis, in general, is poor (16). They are clearly tobacco-related and associated to a higher PD-L1 expression (17) as well as TMB (18). As lung cancer is characterized by a high average number of somatic mutations in general (19), co-occurring mutations like TP53 became the focus of attention. In contrast to TMB, both are routinely investigated in NGS assays and, besides distinguishing distinct molecular subgroups, might identify responders to ICI (20,21). Hence, our retrospective study aimed at the identification of KRAS- and TP53-defined prognostic subsets of PD-L1 positive (≥50%) LuAD treated with pembrolizumab monotherapy as first line palliative treatment. We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-958).

## Methods

### *Study population*

For this retrospective study all patients from four certified lung cancer centers in Berlin, Germany, with relapsed or metastatic LuAD, without any actionable target mutation (ALK or ROS1 rearrangements, BRAF$^{V600E}$ or EGFR mutations), with available results for PD-L1 testing and NGS panel diagnostics and having received first line palliative treatment with pembrolizumab in the period between January 2017 and December 2018 were included. The contributing centers were: Department of Infectious Diseases and Pulmonary Medicine at the Charité – Universitätsmedizin Berlin; Department of Pulmonary Medicine at the Evangelische Lungenklinik Berlin-Buch; Department of Pulmonary Medicine at the HELIOS Klinikum Emil-von-Behring, Lungenklinik Heckeshorn and the Department of Pulmonary Medicine at the Gemeinschaftskrankenhaus Havelhöhe.

### *Data collection and endpoints*

Patients' baseline demographics [age, sex, performance status (PS), smoking behavior], tumor-specific data [date of diagnosis, histology, PD-L1 expression, molecular profiling (NGS), initial staging (cTNM), treatments], radiologic evaluation and outcome were collected using the respective hospital's tumor registry, site-specific clinical databases and individual charts. Follow-up data, when not documented in the respective clinical database, were obtained from the patients or their primary care physicians to minimize missing data.

Response was assessed according to national guidelines (22) using "Response Evaluation Criteria in Solid Tumors" (RECIST) version 1.1 (23). PFS was defined as the time in months from the date of first dose pembrolizumab to the first documented progression (RECIST-defined or death), OS as the time in months from the first dose pembrolizumab to death from any cause.

### *PD-L1 testing and targeted NGS used to characterize KRAS and TP53 mutations*

PD-L1 expression was determined as the percentage of tumor cells with positive membranous staining using the E1L3N (n=80; Cell Signaling, Cambridge, UK) or QR1 antibody (n=39; Quartett Immunodiagnostics, Berlin, Germany). Scoring was determined counting ≥100 tumor cells by experienced thoracic pathologists. Multiplex PCR-based, targeted NGS assays used were the Ion AmpliSeq™ Colon and Lung Cancer Panel covering 22 genes (93 patients; Thermo Fisher Scientific, Waltham, USA) and the panel from the German Network Genomic Medicine, Cologne, Germany, covering 14 genes (26 patients) (24). Mutation status was assessed for TP53 and KRAS hotspot regions with focus on non-synonymous variants known or predicted to be pathogenic or non-functional.

### *Statistical analysis*

Demographics and disease data were described and compared using the Pearson $Chi^2$-test, Fisher's exact test or Mann-Whitney-U-test. The Kaplan-Meier method was used to estimate median PFS, time to treatment failure (TTF) and OS. P values comparing survival curves were calculated with log-rank tests. Hazard ratios were calculated using Cox proportional hazard regression. Analyses were performed using IBM SPSS statistics version 24 (IBM, Armonk, NY, USA). A P value <0.05 (two-tailed) was defined as statistically significant.

### *Ethics statement*

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of Charité Universitätsmedizin Berlin (approval number EA2/223/18) and individual consent for this retrospective analysis was waived (patient's written informed consent was obtained within the treatment contract as ICI were administered as standard of care).

## Results

### *Baseline characteristics*

A total of 153 patients had received pembrolizumab as first line palliative treatment from January 2017 until December 2018. One hundred and nineteen patients with available results for PD-L1 testing and targeted NGS assays and with LuAD or related histologies were included in this study. Median age at the beginning of ICI treatment was 68 years (range, 40–86) with a predominance of male patients (n=68, 57.8%). PS was 0-1 in 92 patients (77.3%), and 2 and 3 in 23 (19.4%) and 4 patients (3.4%), respectively. Ninety-eight patients were active or former smokers (91.6%), 9 patients had a history of never-smoking (8.4%). LuAD was the predominant histology in 95 patients (79.8%), adenosquamous carcinoma (ASqC), large cell carcinoma (LCC) and a not-otherwise specified (NOS) pattern were identified in 11 (9.3%), 1 (0.8) and 12 patients (10.1%), respectively. Median PD-L1 expression in the entire cohort was 75% (95% CI, 65–75). Stage at primary diagnosis was III in 19 patients (16.0%) and IV in 100 patients (84.0%). Ten patients underwent a primary therapy with curative intent (8.4%) and received pembrolizumab after disease relapse. Rates for adrenal (ADR), brain (BRA), liver (HEP) and bone metastases (OSS) at the beginning of pembrolizumab were 16.8%, 20.2%, 10.1% and 27.7%, respectively. The main characteristics are reported in *Table 1*.

Frequency of KRAS mutations ($KRAS^{mut}$) was 52.1%, of whom 51.6% were $KRAS^{G12C}$ (*Figure 1A*). Non-synonymous TP53 mutations ($TP53^{mut}$) occurred in 47.1% of the patients, 58.9% displayed missense mutations (*Figure 1B*). No association between $KRAS^{mut}$ and $TP53^{mut}$ was observed (P=0.24). Rates of wild type patients, $KRAS^{mut}$ or $TP53^{mut}$ alone, and KRAS/TP53 co-mutations were 22.7%, 30.3%, 25.2% and 21.8%, respectively (*Figure 1C*). By trend, PD-L1 expression was higher in $KRAS^{mut}$ tumors (75 *vs.* 65%, P=0.13). Whereas no differences were observed among KRAS subgroups, $KRAS^{G12C}/TP53^{mut}$ tumors more frequently had a PD-L1 expression within the highest percentile (≥90%: 41.7% *vs.* 20.0%, P=0.14). Expression levels were similar among TP53 subsets. Apart from a trend to a higher rate of current/former smokers in $KRAS^{mut}$ patients (96.5% *vs.* 86.0%, P=0.08), clinical baseline characteristics were similar across all molecularly defined groups.

### *Treatment characteristics and RECIST-evaluation*

All treatment characteristics are listed in *Tables 2,3*. Median follow-up was 26.4 months for the entire cohort. The median number of cycles administered, duration of therapy and rate of patients still on treatment were 10, 8.2 months and 19.3%, respectively. RECIST-based evaluation was available for 105 patients (88.2%), showing an objective

**Table 1** Patients' baseline demographics for all patients, KRAS mutations, KRAS subgroups, TP53 mutations and KRAS/TP53 co–mutations

| Variable | All patients (n=119) | KRAS$^{mut}$ (n=62) | KRAS$^{wt}$ (n=57) | P value | KRAS$^{G12C}$ (n=32) | KRAS$^{other}$ (n=30) | P value | TP53$^{mut}$ (n=56) | TP53$^{wt}$ (n=63) | P value | KRAS$^{G12C}$/ TP53$^{mut}$ (n=12) | KRAS$^{other}$/ TP53$^{mut}$ (n=14) | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age, y (median, range) | 68 [40–86] | 66 [45–85] | 69 [40–86] | 0.53 | 65 [53–84] | 67 [45–85] | 0.75 | 66 [40–86] | 68 [48–86] | 0.25 | 62 [53–77] | 66 [45–81] | 0.90 |
| Sex, n (%) | | | | 0.20 | | | 0.22 | | | 0.14 | | | 1.0 |
| Female | 51 (42.9) | 30 (48.4) | 21 (36.8) | | 14 (43.8) | 12 (40.0) | | 28 (50.0) | 23 (36.5) | | 6 (50.0) | 7 (50.0) | |
| Male | 68 (57.8) | 32 (51.6) | 36 (63.2) | | 18 (56.3) | 18 (60.0) | | 28 (50.0) | 40 (63.5) | | 6 (50.0) | 7 (50.0) | |
| ECOG–PS, n (%, 0–1 *vs.* ≥2) | | | | 0.18 | | | 0.19 | | | 0.90 | | | 0.37 |
| 0–1 | 92 (77.3) | 51 (82.3) | 41 (71.9) | | 24 (75.0) | 27 (90.0) | | 43 (76.8) | 49 (77.8) | | 8 (66.7) | 12 (85.7) | |
| 2 | 23 (19.3) | 9 (14.5) | 14 (24.6) | | 6 (18.8) | 3 (10.0) | | 13 (23.2) | 10 (15.9) | | 4 (33.3) | 2 (14.3) | |
| 3 | 4 (3.4) | 2 (3.2) | 2 (3.5) | | 2 (6.3) | 0 | | 0 | 4 (6.3) | | 0 | 0 | |
| Smoking history, n (%) | | | | 0.08 | | | 1.0 | | | 1.0 | | | 1.0 |
| Current or former smoker | 98 (91.6) | 55 (96.5) | 43 (86.0) | | 28 (96.6) | 27 (96.4) | | 49 (92.5) | 49 (90.7) | | 12 (100.0) | 14 (100.0) | |
| Never smoker | 9 (8.4) | 2 (3.5) | 7 (14.0) | | 1 (3.4) | 1 (3.3) | | 4 (7.5) | 5 (9.3) | | 0 | 0 | |
| Missing data | 12 (–) | 5 (–) | 7 (–) | | 1 (–) | 2 (–) | | 3 (–) | 9 (–) | | 0 | 0 | |
| Histology, n (%, LuAD *vs.* other) | | | | 0.49 | | | 1.0 | | | 0.89 | | | 0.64 |
| Adenocarcinoma (LuAD) | 95 (79.8) | 51 (82.3) | 44 (77.2) | | 26 (81.3) | 25 (83.3) | | 45 (80.4) | 50 (79.4) | | 9 (75.0) | 12 (85.7) | |
| Other | 24 (20.2) | 11 (17.7) | 13 (22.8) | | 6 (18.8) | 5 (16.7) | | 11 (19.6) | 13 (20.6) | | 3 (25.0) | 2 (14.3) | |
| Adenosquamous carcinoma (ASQ) | 11 (9.3) | 5 (8.0) | 6 (10.6) | | 3 (9.4) | 2 (6.7) | | 5 (8.9) | 6 (9.6) | | 1 (8.3) | 0 | |
| Large cell carcinoma (LCC) | 1 (0.8) | 1 (1.6) | 0(0) | | 0 | 1 (3.3) | | 1 (1.8) | 0 | | 1 (8.3) | 1 (7.1) | |
| Not otherwise specified (NOS) | 12 (10.1) | 5 (8.1) | 7 (12.3) | | 3 (9.4) | 2 (6.7) | | 5 (8.9) | 7 (11.1) | | 1 (8.3) | 1 (7.1) | |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Variable | All patients (n=119) | KRAS$^{mut}$ (n=62) | KRAS$^{wt}$ (n=57) | P value | KRAS$^{G12C}$ (n=32) | KRAS$^{other}$ (n=30) | P value | TP53$^{mut}$ (n=56) | TP53$^{wt}$ (n=63) | P value | KRAS$^{G12C}$/ TP53$^{mut}$ (n=12) | KRAS$^{other}$/ TP53$^{mut}$ (n=14) | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PD–L1 expression (%TC), median (95% CI) | 75 [65–75] | 75 [65–83] | 65 [65–75] | 0.13 | 75 [55–85] | 75 [70–85] | 0.38 | 73 [65–75] | 75 [65–80] | 0.72 | 75 [60–95] | 70 [63–78] | 0.67 |
| 50–59%, n (%) | 34 (28.6) | 16 (25.8) | 18 (31.6) | | 11 (34.4) | 5 (16.7) | | 14 (25.0) | 20 (31.7) | | 3 (25.0) | 2 (14.3) | |
| 60–69%, n (%) | 20 (16.8) | 8 (12.9) | 12 (21.1) | | 3 (9.4) | 5 (16.7) | | 13 (23.2) | 7 (11.1) | | 2 (16.7) | 5 (35.7) | |
| 70–79%, n (%) | 23 (19.3) | 12 (19.4) | 11 (19.3) | | 6 (18.8) | 6 (20.0) | | 11 (19.6) | 12 (19.0) | | 2 (16.7) | 3 (21.4) | |
| 80–89%, n (%) | 17 (14.3) | 11 (17.7) | 6 (10.5) | | 4 (12.5) | 7 (23.3) | | 3 (5.4) | 14 (22.2) | | 0 | 1 (7.1) | |
| 90–100%, n (%) | 25 (21.0) | 15 (24.2) | 10 (17.5) | | 8 (25.0) | 7 (23.3) | | 15 (26.8) | 10 (15.9) | | 5 (41.7) | 3 (21.4) | |
| Stage at primary diagnosis | | | | 0.45 | | | 1.0 | | | 0.98 | | | 0.20 |
| III | 19 (16.0) | 8 (12.9) | 11 (19.3) | | 4 (12.5) | 4 (13.3) | | 9 (16.1) | 10 (15.9) | | 2 (16.7) | 0 | |
| IV | 100 (84.0) | 54 (87.1) | 46 (80.7) | | 28 (87.5) | 26 (86.7) | | 47 (83.9) | 53 (84.1) | | 10 (83.3) | 14 (100.0) | |
| Prior treatment with curative intent, n (%) | 10 (8.4) | 4 (6.5) | 6 (10.5) | 0.52 | 1 (3.1) | 3 (10.0) | 0.35 | 5 (8.9) | 5 (7.9) | 0.85 | 0 | 1 (7.1) | 1.0 |
| Metastatic sites at the begin of IO, n (%) | | | | | | | | | | | | | |
| ADR | 20 (16.8) | 8 (12.9) | 12 (21.1) | 0.33 | 4 (12.5) | 4 (13.3) | 1.0 | 11 (19.6) | 9 (14.3) | 0.47 | 1 (8.3) | 2 (14.3) | 1.0 |
| BRA | 24 (20.2) | 13 (21.0) | 11 (19.3) | 0.82 | 7 (21.9) | 6 (20.0) | 1.0 | 15 (26.8) | 9 (14.3) | 0.11 | 5 (41.7) | 5 (35.7) | 1.0 |
| HEP | 12 (10.1) | 4 (6.5) | 8 (14.0) | 0.23 | 1 (3.1) | 3 (10.0) | 0.35 | 7 (12.5) | 5 (7.9) | 0.55 | 1 (8.3) | 1 (7.1) | 1.0 |
| OSS | 33 (27.7) | 17 (27.4) | 16 (28.1) | 0.94 | 9 (28.1) | 8 (26.7) | 1.0 | 15 (26.8) | 18 (28.6) | 0.84 | 4 (33.3) | 3 (21.4) | 0.67 |

*, P<0.05; CI, confidence interval; ECOG–PS, Eastern Co–operative Oncology Group Performance Status; %TC, percentage of positive tumor cells; ADR, adrenal metastases; BRA, brain metastases; HEP, liver metastases; OSS, bone metastases; KRAS$^{mut}$, KRAS mutation; KRAS$^{wt}$, KRAS wildtype; KRAS$^{other}$, KRAS mutation other than KRAS$^{G12C}$; TP53$^{mut}$, TP53 mutation; TP53$^{wt}$, TP53 wildtype.

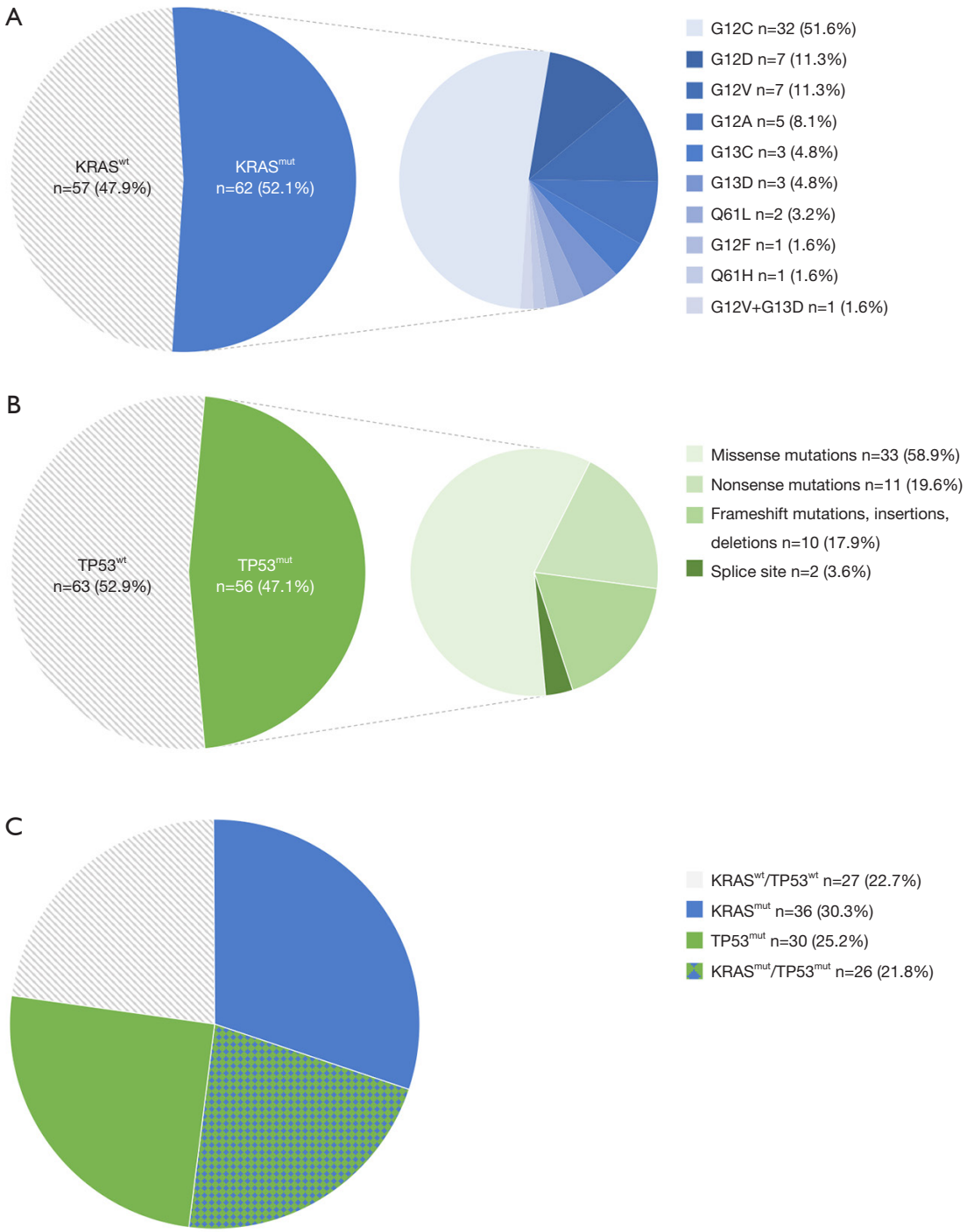


**Figure 1** Distribution of KRAS mutations (A), TP53 mutations (B) and mutational pattern according to both mutations (C). KRASmut, KRAS mutation; KRASwt, KRAS wild type; TP53mut, TP53 mutation; TP53wt, TP 53 wild type.



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**Table 2** Treatment characteristics and response according to RECIST 1.1 for all patients (left column), KRAS–mutations (second column from left side), KRAS subgroups (third column from left side) and TP53 mutations (right column)

| Variable | All patients (n=119) | $KRAS^{mut}$ (n=62) | $KRAS^{wt}$ (n=57) | P value | $KRAS^{G12C}$ (n=32) | $KRAS^{other}$ (n=30) | P value | $TP53^{mut}$ (n=56) | $TP53^{wt}$ (n=63) | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Cycles administered, n (range) | 10 [1–58] | 11 [1–45] | 8 [1–58] | 0.19 | 20 [1–38] | 9 [1–45] | 0.05* | 11 [1–43] | 9 [1–58] | 0.95 |
| Follow–Up, months (median, 95% CI) | 26.4 (24.3–28.5) | 28.9 (26.1–31.6) | 23.0 (19.9–26.1) | 0.05* | 26.9 (23.6–30.1) | 30.7 (27.3–34.2) | 0.18 | 23.7 (9.8–27.5) | 28.0 (23.8–32.1) | 0.07 |
| Duration of treatment, months (median, 95% CI) | 8.2 (5.5–11.0) | 11.2 (6.2–16.2) | 6.2 (2.1–10.3) | 0.20 | 20.0 (12.3–27.6) | 7.6 (4.7–10.5) | 0.03* | 7.2 (4.8–9.6) | 10.0 (3.4–16.7) | 0.51 |
| Therapy ongoing, n (%) | 21 (17.6) | 11 (17.7) | 10 (17.5) | 0.98 | 9 (28.1) | 2 (6.7) | 0.03* | 14 (25.0) | 7 (11.1) | 0.06 |
| RECIST–evaluation available, n (%) | 105 (88.2) | 55 (88.7) | 50 (87.7) | 1.0 | 30 (93.8) | 25 (83.3) | 0.20 | 50 (89.3) | 55 (87.3) | 0.78 |
| ORR, % (95% CI) | 48.6 [39–58] | 50.9 [36–64] | 46.0 [32–60] | 0.62 | 63.3 [47–80] | 36.0 [20–56] | 0.05* | 52.0 [38–66] | 45.5 [33–58] | 0.51 |
| DCR, % (95% CI) | 79.0 [71–86] | 83.6 [73–93] | 74.0 [62–86] | 0.23 | 86.7 [73–97] | 80.0 [64–92] | 0.51 | 76.0 [64–88] | 81.8 [71–91] | 0.47 |

**Table 3** Treatment characteristics and response according to RECIST 1.1 depending on the KRAS/TP53 co–mutational status and for $KRAS^{G12C}$/TP53, respectively

| Variable | $KRAS^{wt}$/ $TP53^{wt}$ (n=27) | $KRAS^{mut}$/ $TP53^{wt}$ (n=36) | $KRAS^{wt}$/ $TP53^{mut}$ (n=30) | $KRAS^{mut}$/ $TP53^{mut}$ (n=26) | P value | $KRAS^{G12C}$/ $TP53^{mut}$ (n=12) | $KRAS^{G12C}$/ $TP53^{wt}$ (n=20) | $KRAS^{other}$/ $TP53^{mut}$ (n=14) | $KRAS^{other}$/ $TP53^{wt}$ (n=16) | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Cycles administered, n (range) | 11 [1–45] | 15 [1–45] | 12 [1–43] | 10 [1–38] | 0.48 | 28 [2–37] | 13 [1–38] | 7 [1–38] | 16 [2–45] | 0.03* |
| Follow-up, months (median, 95% CI) | 25.6 (20.9–30.4) | 29.2 (24.7–33.7) | 21.3 (17.5–25.2) | 28.9 (19.3–38.4) | 0.02* | 26.9 (19.0–34.7) | 28.0 (23.7–32.2) | 29.3 (20.5–38.1) | 30.7 (24.3–37.1) | 0.33 |
| Duration of treatment, months (median, 95% CI) | 3.2 (1.2–5.3) | 12.4 (9.7–15.0) | 7.2 (4.6–9.7) | 6.8 (3.1–10.5) | 0.41 | 22.0 (16.7–26.4) | 12.4 (0.8–24.0) | 4.1 (0.1–11.8) | 12.3 (9.0–15.7) | 0.01* |
| Therapy ongoing, n (%) | 11 (17.7) | 4 (11.1) | 7 (23.3) | 7 (26.9) | 0.26 | 6 (50.0) | 3 (15.0) | 1 (7.1) | 1 (6.3) | 0.01* |
| RECIST–evaluation available, n (%) | 22 (81.5) | 33 (91.7) | 28 (93.8) | 22 (84.6) | 0.45 | 11 (91.7) | 19 (95.0) | 11 (78.6) | 14 (87.5) | 0.51 |
| ORR, % (95% CI) | 50.0 [32–73] | 42.4 [24–61] | 42.9 [25–61] | 63.6 [41–82] | 0.42 | 100.0 [100–100] | 42.1 [21–63] | 27.3 [9–55] | 42.9 [14–71] | 0.003* |
| DCR, % (95% CI) | 77.3 [59–96] | 84.8 [70–97] | 71.4 [54–86] | 81.8 [64–96] | 0.62 | 100.0 [100–100] | 78.9 [58–95] | 63.6 [36–91] | 92.9 [79–100] | 0.09 |

*, P<0.05; CI, confidence interval; RECIST, Response Evaluation Criteria in Solid Tumors; ORR, objective response rate; DCR, disease control rate; KRAS+, KRAS mutation; KRAS–, KRAS wildtype; $KRAS^{other}$, KRAS mutation other than $KRAS^{G12C}$; TP53+, TP53 mutation; TP53–, TP53 wildtype.

response rate (ORR) and disease control rate (DCR) of 48.6% and 79.0%, respectively. Treatment characteristics and responses were comparable for KRAS$^{mut}$ and TP53$^{mut}$ as well as wild type patients (*Table 2*). However, patients with KRAS$^{G12C}$ as compared to KRAS$^{other}$ were significantly longer on therapy (20.0 *vs.* 7.6 months, P=0.03) and ORR was markedly higher (63.3% *vs.* 36.0%, p=0.05). Patients with KRAS$^{G12C}$/TP53$^{mut}$ (n=12) had the longest duration of therapy (22.0 months) and all patients showed a response (ORR 100.0%, *Table 3*).

### *Survival analyses*

Median PFS was 8.8 months (92 events, 77.3% of patients, 95% CI, 4.6–12.9). KRAS$^{mut}$ patients displayed an improved PFS (13.3 *vs.* 6.2 months; HR, 0.66, 95% CI, 0.43–1.0, P=0.05, *Figure 2A*), whereas TP53 status had no impact (8.0 *vs.* 9.7 months; HR 0.97, 95% CI, 0.64–1.46, P=0.88, *Figure 2B*). The substantial increase in KRAS$^{mut}$ was strongly driven by KRAS$^{G12C}$ [19.8 *vs.* 5.8 months (KRAS$^{other}$); HR, 0.37, 95% CI, 0.20–0.68, P=0.001, *Figure 2C*], whereas results for KRAS$^{other}$ and wild type patients (KRAS$^{wt}$) were nearly identical. KRAS$^{G12C}$/TP53$^{mut}$ patients experienced the by far longest PFS (33.3 months; 95% CI, not estimable (NE), 1- and 2-year PFS 83% and 67%) as compared to KRAS$^{G12C}$/TP53$^{wt}$ (15.6 months; 95% CI, 10.8–20.4, HR, 0.48, 95% CI, 0.17–1.35, P=0.16), KRAS$^{other}$/TP53$^{wt}$ (13.1 months; 95% CI, 10.3–15.9; HR 0.23, 95% CI, 0.08–0.72, P=0.01) and KRAS$^{other}$/TP53$^{mut}$, the latter group displaying the worst PFS (2.8 months; 95% CI, 0.0–6.2; HR, 0.18, 95% CI, 0.06–0.53, P=0.002, *Figure 2D*). Patients displaying a PD-L1 expression <70% had a 1.7-fold decreased PFS (HR, 1.72, 95% CI, 1.14–2.60, P=0.01). In multivariate analysis, smoking history and KRAS subtypes were identified as independent predictors for PFS (*Table 4*).

Patients treated beyond RECIST-defined progression (n=19, 22.9%) due to a sustained clinical benefit displayed a time-to-treatment-failure (TTF) of 14.0 months. The probability for a treatment beyond progression was higher in KRAS$^{mut}$ patients (33.3% *vs.* 13.6%, P=0.04). However, TTF was not different according to KRAS mutational status (KRAS$^{mut}$ *vs.* KRAS$^{wt}$, 9.0 *vs.* 6.2 months, P=0.27) and within KRAS subgroups, respectively.

Median OS reached 23.6 months (61 events, 51.3% of patients, 95% CI, 15.0–32.2) and was neither influenced by KRAS (HR, 0.92, 95% CI, 0.55–1.52, P=0.74, *Figure 3A*) nor TP53 mutational status (HR, 0.85, 95% CI, 0.51–1.41, 0.85, P=0.52, *Figure 3B*). Patients with KRAS$^{G12C}$ experienced a longer OS by trend (HR, 0.50, 95% CI, 0.25–1.01, P=0.06, *Figure 3C*). Again, survival was strongly influenced by KRAS$^{G12C}$/TP53$^{mut}$ (median OS not yet reached; 1- and 2-year OS 92% and 79%), as compared to KRAS$^{G12C}$/TP53$^{wt}$ (17.9 months; 95% CI, 12.0–23.8; 1- and 2-year OS 79% and 41%, HR, 0.24, 95% CI, 0.05–1.07, P=0.06) and KRAS$^{other}$/TP53$^{wt}$ (22.0 months; 95% CI, 13.6–30.6, 1- and 2-year OS 81% and 44%, HR, 0.23, 95% CI, 0.05–1.05, P=0.06). KRAS$^{other}$/TP53$^{mut}$ patients experienced the shortest OS (9.7 months; 95% CI, 2.4–17.0; 1- and 2-year OS 48% and 30%, HR, 0.17, 95% CI, 0.04–0.76, P=0.02, *Figure 3D*). A PD-L1 expression level of <70% was associated with a reduced OS (HR, 1.93, 95% CI, 1.16–3.20, P=0.01). In multivariate analysis, the initial PS and molecular status independently predicted OS, with the best HR for KRAS$^{G12C}$/TP53$^{mut}$ (0.20, P=0.03, *Table 3*).

## Discussion

This investigation identified patients with KRAS$^{G12C}$/TP53$^{mut}$ LuAD as long-term responders benefitting most from upfront pembrolizumab. All patients in this molecularly defined subgroup responded to ICI treatment. Our study cohort was markedly enriched by KRAS mutations, present in >50% of the patients (13), subgroups showed the normal distribution pattern of KRAS$^{mut}$ LuAD. KRAS$^{mut}$ patients had a higher PD-L1 expression, probably resulting from KRAS-induced stabilization of PD-L1 (25). A better response to ICI in KRAS$^{mut}$ patients may be attributable to a "KRAS phenotype", clinico-pathologically characterized by its tobacco-association, PD-L1 positivity and an inflamed tumor microenvironment (26). However, results from prospective clinical trials and real-world data are conflicting. A meta-analysis including 509 patients from 3 second and further line studies with ICI demonstrated an OS benefit in KRAS mutations as compared to wild type patients (HR, 0.64, 95% CI, 0.43–0.96, P=0.03) (27). In contrast, real-world data with nivolumab from the Italian expanded access program analyzing 530 patients in the second and further line setting (PFS 4 *vs.* 3 months, P=0.56; OS 11.2 *vs.* 10 months, P=0.86) (28) and a French investigation with 282 patients having received ICI in all lines of therapy showed no survival differences (HR for PFS and OS 0.93) (29). Altogether, patient populations were very heterogeneous; only one study included first line patients and this to a very small degree (8.5%).

Our results suggest that looking on the KRAS mutational status as positive or negative alone may be inadequate, as

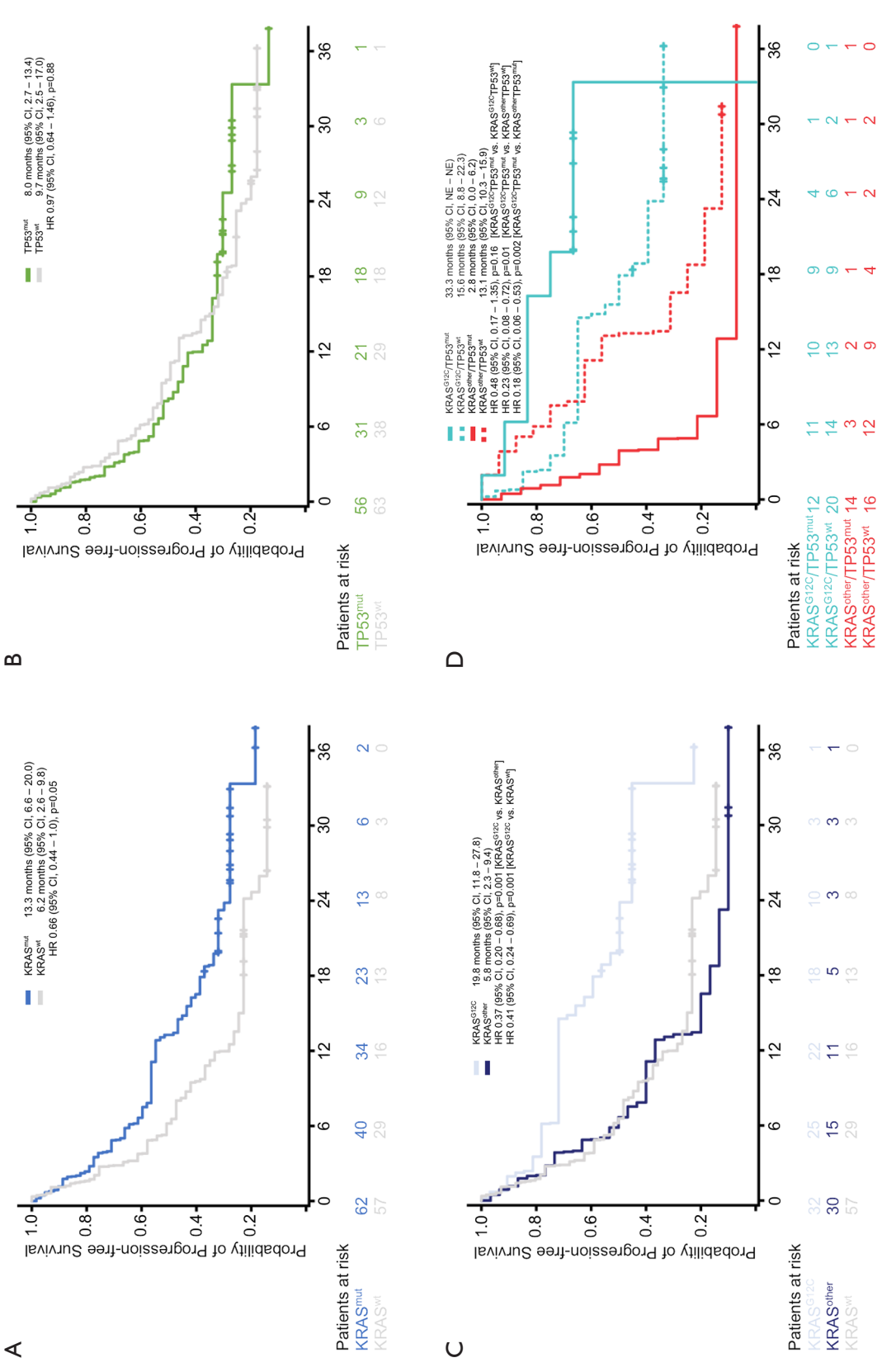


**Figure 2** Kaplan-Meier curves for progression-free survival (PFS) depending on KRAS mutational status (A), TP53 mutational status (B), KRAS subgroups (KRASG12C *vs.* KRASother (C) and KRAS/TP53 co-mutations (KRASG12C/TP53mut *vs.* KRASG12C/TP53wt *vs.* KRASother/TP53mut *vs.* KRASother/TP53wt (D).

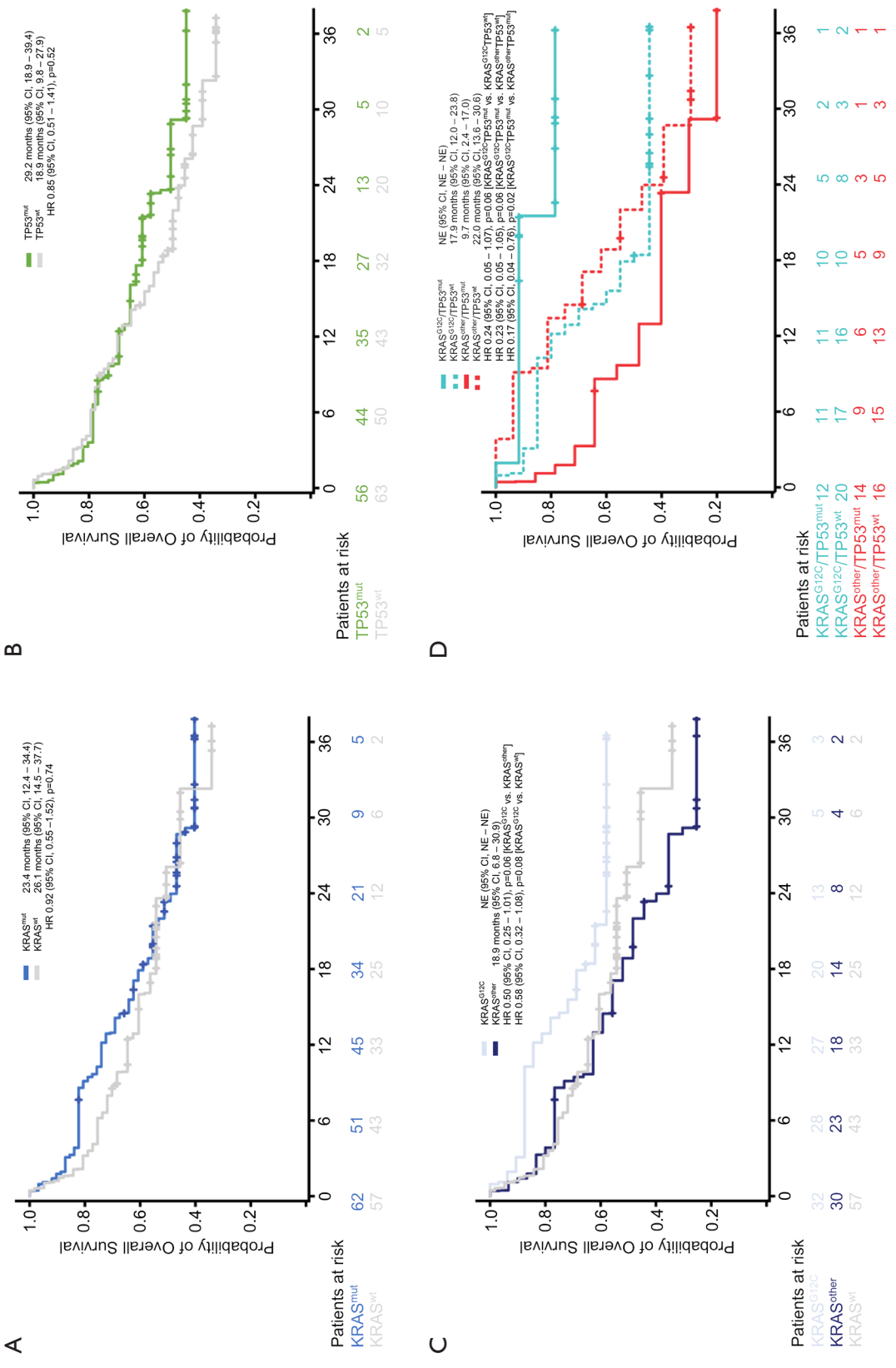


**Figure 3** Kaplan-Meier curves for overall survival (OS) depending on KRAS mutational status (A), TP53 mutational status (B), KRAS subgroups (KRAS$^{G12C}$ *vs.* KRAS$^{other}$ mutations (C) and KRAS/TP53 co-mutations (KRAS$^{G12C}$/TP53$^{mut}$ *vs.* KRAS$^{G12C}$/TP53$^{wt}$ *vs.* KRAS$^{other}$/TP53mut *vs.* KRAS$^{other}$/TP53$^{wt}$ (D).

**Table 4** Univariate and multivariate Cox proportional hazard regression analysis for PFS and OS

| Variable | Univariate analysis (PFS) | | | Multivariate analysis (PFS) | | | Univariate analysis (OS) | | | Multivariate analysis (OS) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | P value | HR | 95% CI | P value | HR | 95% CI | P value | HR | 95% CI | P value |
| Age | | | | | | | | | | | | |
| <70 *vs.* ≥70 years | 1.09 | 0.72–1.64 | 0.70 | | | | 0.79 | 0.48–1.31 | 0.37 | | | |
| Sex | | | | | | | | | | | | |
| Female *vs.* male | 1.08 | 0.71–1.64 | 0.72 | | | | 0.94 | 0.56–1.56 | 0.80 | | | |
| ECOG–PS | | | | | | | | | | | | |
| 0–1 *vs.* ≥2 | 0.71 | 0.43–1.17 | 0.18 | | | | 0.43 | 0.25–0.74 | 0.003* | 0.40 | 0.23–0.71 | 0.002* |
| Smoking history | | | | | | | | | | | | |
| Current or former *vs.* never smoker | 0.36 | 0.18–0.72 | 0.004* | 0.43[a] | 0.21–0.89 | 0.02* | 0.45 | 0.19–1.06 | 0.07 | | | |
| | | | | 0.49[b] | 0.24–1.01 | 0.05* | | | | | | |
| | | | | 0.43[c] | 0.21–0.89 | 0.02* | | | | | | |
| Histology | | | | | | | | | | | | |
| Adenocarcinoma (LuAD) *vs.* other | 1.14 | 0.68–1.91 | 0.62 | | | | 0.82 | 0.46–1.46 | 0.50 | | | |
| PD–L1 expression (%TC) | | | | | | | | | | | | |
| <70 *vs.* ≥70% | 1.72 | 1.14–2.60 | 0.01* | 1.41[a] | 0.90–2.21 | 0.13 | 1.93 | 1.16–3.20 | 0.01* | 1.65 | 0.98–2.76 | 0.06 |
| | | | | 1.51[b] | 0.97–2.35 | 0.07 | | | | | | |
| | | | | 1.40[c] | 0.90–2.19 | 0.13 | | | | | | |
| Molecular alteration | | | | | | | | | | | | |
| KRAS (pos. *vs.* neg.) | 0.66 | 0.44–1.00 | 0.05* | 0.75 | 0.48–1.18 | 0.13 | 0.92 | 0.55–1.52 | 0.73 | | | |
| $KRAS^{G12C}$ (pos. *vs.* $KRAS^{other}$/$KRAS^{wt}$) | 0.41 | 0.24–0.69 | 0.001* | 0.42 | 0.24–0.73 | 0.002* | 0.58 | 0.32–1.08 | 0.08 | | | |
| $KRAS^{G12C}$/$TP53^{mut}$ (pos. *vs.* else) | 0.30 | 0.12–0.74 | 0.009* | 0.32 | 0.13–0.80 | 0.02* | 0.23 | 0.06–0.93 | 0.04* | 0.20 | 0.05–0.82 | 0.03* |
| TP53 (pos. *vs.* neg.) | 0.97 | 0.64–1.46 | 0.88 | | | | 0.85 | 0.51–1.41 | 0.52 | | | |
| Stage at primary diagnosis | | | | | | | | | | | | |
| III *vs.* IV | 1.09 | 0.63–1.90 | 0.76 | | | | 0.78 | 0.37–1.65 | 0.78 | | | |
| Metastatic sites at the begin of IO, n (%) | | | | | | | | | | | | |
| ADR (Y *vs.* N) | 1.26 | 0.74–2.14 | 0.39 | | | | 1.34 | 0.71–2.51 | 0.37 | | | |
| BRA (Y *vs.* N) | 1.00 | 0.60–1.66 | 1.00 | | | | 1.10 | 0.60–2.04 | 0.76 | | | |
| HEP (Y *vs.* N) | 0.75 | 0.36–1.55 | 0.44 | | | | 0.82 | 0.33–2.06 | 0.67 | | | |
| OSS (Y *vs.* N) | 0.88 | 0.55–1.40 | 0.58 | | | | 0.97 | 0.56–1.71 | 0.92 | | | |

*, P<0.05; CI, confidence interval; ECOG-PS, Eastern Co-operative Oncology Group Performance Status; %TC, percentage of positive tumor cells; ADR, adrenal metastases; BRA, brain metastases; HEP, liver metastases; OSS, bone metastases; [a], HR for KRAS (pos. *vs.* neg.); [b], HR for $KRAS^{G12C}$ (pos. *vs.* $KRAS^{else}$/$KRAS^{wt}$); [c], HR for $KRAS^{G12C}$/$TP53^{mut}$ (pos. *vs.* else).

substantial differences between KRAS$^{G12C}$ and KRAS$^{other}$ are given for response and survival. Smoking behavior is correlated to a distinct spectrum of KRAS mutations with KRAS$^{G12D}$ more frequently observed in never smokers and KRAS$^{G12C}$ being the predominant mutation in smokers (30). The lower probability for a high TMB in KRAS$^{G12D}$ mutations might provide a molecular rationale for different responses to IO, whereas KRAS$^{G12C}$ mutations display higher shares of PD-L1 positivity (≥50%) as well as high TMB (31). A prognostic value of KRAS$^{G12C}$ remained to be demonstrated, as KRAS subtyping, if determined, showed no survival difference in the second- and further line setting (29,32). An exploratory analysis from the Keynote-042 study recently suggested a moderate benefit in ORR (67% *vs.* 57%), PFS (15 *vs.* 12 months) and OS (not reached *vs.* 28 months) in favor of KRAS$^{G12C}$ *vs.* KRAS$^{other}$, but the subgroup of patients with PD-L1 ≥50% has not been reported separately (33).

Analogous to KRAS$^{mut}$, TP53$^{mut}$ are associated with an enhanced PD-L1 expression (34,35). These cancers are molecularly characterized by neoantigen accumulation-induced tumor immunogenicity, resulting from a loss of function of this transcriptional key player in cell homeostasis. In PD-L1 non-selected metastatic NSCLC, TP53$^{mut}$ consequently increased response to ICI and improved OS (HR, 0.48, 95% CI, 0.25–0.95, P=0.04) (36). In contrast, no relationship between TP53 and response or outcome was obvious in our study, although OS was numerically also in favor of TP53$^{mut}$. Interestingly, a large and sustained clinical benefit was observed in KRAS$^{G12C}$/TP53$^{mut}$, associated to a higher share of highest PD-L1 expression levels (≥90%: 41.7% *vs.* 20.0% in KRAS$^{other}$). We identified a PD-L1 expression ≥70% as threshold for an improved survival, but observed an even more pronounced benefit in patients with a PD-L1 expression ≥90% (ORR, PFS and OS 68.0%, 13.1 months and NE *vs.* 42.5%, 6.2 and 18.9 months in PD-L1 <90%), thereby confirming recently published findings (37).

The favorable outcome observed in these co-mutated subgroups might thus result from synergistic and complementary effects on PD-L1 expression, TMB and cell cycle repair mechanisms mediated independently by KRAS$^{mut}$ and TP53$^{mut}$ and leading to an inflamed tumor microenvironment with adaptive immune resistance and high immunogenicity (35). In an exploratory analysis from the Keynote-001 trial, all patients with KRAS$^{mut}$/TP53$^{mut}$ were also PD-L1 high (≥50%) and experienced a durable clinical benefit (35). Similar results have been reported from real life cohorts (38,39). However, as KRAS subgroups have not been investigated separately, it remains unclear, whether a "KRAS-TP53-synergy" is independent from the specific KRAS$^{mut}$ or rather might be strongly relying on KRAS$^{G12C}$/TP53$^{mut}$.

To the best of our knowledge, our investigation is the first one demonstrating a strong prognostic value for KRAS$^{G12C}$/TP53$^{mut}$ in the PD-L1 high population. Its strength is a clear focus on a well-defined, uniform patient population in contrast to studies including patients irrespective from PD-L1 strata and line of therapy. The thereby resulting heterogeneity may not only make comparisons impossible, but might also dilute an impact of KRAS and TP53 mutations, as these molecularly defined cohorts might perform differently according to the PD-L1 expression levels.

Recently and after years of discouraging research, promising results have been published for the first small molecules directly targeting specific KRAS mutations. Sotorasib and MRTX849 selectively inhibit KRAS-dependent signaling by modifying mutant cysteine 12 in GDP-bound KRAS$^{G12C}$ (40,41) and are currently investigated in clinical trials. Comparing different modes of action, with ICI on the one hand and specific tyrosine kinase inhibitors on the other, it is tempting to speculate, which therapeutic option for patients with KRAS$^{G12C}$/TP53$^{mut}$ might perform best.

This study has several limitations. Due to its retrospective design, a certain selection bias in favor of patients displaying a better PS cannot be excluded. As only patients with available PD-L1 expression and parallel NGS testing were included, those with a clinically unfavorable prognosis due to a reduced PS in whom molecular testing may have been omitted were not analyzed. Second, the use of different diagnostic antibodies (22C3 in the KEYNOTE trials, E1L3N and QR1 in our investigation) as well as the examination by different pathologists might have biased results for PD-L1 staining. However, a growing body of evidence supports the comparability of different standardized assays and laboratory-developed tests (42,43). All participating centers were certified by the quality management initiative of the German Society of Pathology (QuIP®) after having successfully passed round-robin tests for PD-L1 testing, therefore results can be regarded as comparable. Third, TMB was not evaluated. Thus, molecular groups may be unbalanced and outcome may be biased by a higher neoantigen load in KRAS$^{G12C}$/TP53$^{mut}$ patients (35,44). Forth, we did not account for

additional, presumably negative predictive and prognostic KRAS-associated co-mutations like STK11 or KEAP1, as they were not included into the routine NGS assay (20). Lower frequencies of e.g., STK11 mutations leading to immunologically cold cancers might have contributed to the improved outcome in $KRAS^{G12C}$ patients. However, recently published data in this setting are inconclusive. Whereas no differences among KRAS subgroups were observed in the LC-SCRUM-Japan study, STK11 co-mutations occurred less frequently in $KRAS^{G12D}$ but were equally present in $KRAS^{G12A, C, V \text{ or } Q61X}$ in a large US cohort (31,44). Noteworthy, a favorable survival in $KRAS^{mut}$/$TP53^{mut}$ patients may be even preserved in the presence of STK11 mutations (38). Fifth, as patients were treated within the valid standard of care outside a clinical trial, imaging intervals varied, thereby potentially biasing PFS. Additionally, RECIST assessments were not confirmed independently. Finally, given the inclusion of patients with pembrolizumab monotherapy only without a control group, this study was not designed to evaluate a predictive value of either $KRAS^{G12C}$ alone or in combination with $TP53^{mut}$. However, one should keep in mind that $KRAS^{mut}$ have consistently been associated with a worse outcome in the era of chemotherapy and no survival differences were identified according to the applied regimens. Thus, no predictive value for standard chemotherapy has been established (16,45,46).

## Conclusions

A comprehensive KRAS subtyping and TP53 assessment may allow a prognostic highly relevant differentiation of patients with metastatic, PD-L1 high LuAD, treated upfront with pembrolizumab. The advantage of the proposed approach is its availability for the majority of patients with LuAD, as NGS panel testing has become the method of choice to screen for actionable genetic alterations. In contrast to large panels or whole exome sequencing needed for TMB, a small gene panel might be sufficient to provide the necessary prognostic information. Whether the constellation of PD-L1 ≥50% and $KRAS^{G12C}$/$TP53^{mut}$ favors upfront ICI monotherapy *vs.* an ICI-chemotherapy combination should be addressed in further, prospective studies.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of Charité Universitätsmedizin Berlin (approval number EA2/223/18) and individual consent for this retrospective analysis was waived (patient's written informed consent was obtained within the treatment contract as ICI were administered as standard of care).

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## 2.3 Prädiktive Wertigkeit von TP53-Mutationen und intrakranielle Wirksamkeit einer zielgerichteten Behandlung des ALK- oder ROS1-positiven NSCLC mit Lorlatinib

**Frost N**, Christopoulos P, Kauffmann-Guerrero D, Stratmann J, Riedel R, Schaefer M, Alt J, Gütz S, Christoph DC, Laack E, Faehling M, Fischer R, Fenchel K, Haen S, Heukamp L, Schulz C, Griesinger F. Lorlatinib in pretreated ALK- or ROS1-positive lung cancer and impact of TP53 comutations: results from the German early access program. *Therapeutic Advances in Medical Oncology 2021.* doi: 10.1177/ 1758835920980558.

TP53-mutierte Tumore zeichnen sich durch eine hohe Rate an Tumorneoantigenen aus. Was einerseits ein gutes Ansprechen auf Immuntherapie erwarten lässt ist andererseits beim treibermutierten Lungenkarzinom womöglich eher nachteilig, da trotz spezifisch adressierbarer molekularer Alteration aufgrund der Komutation(en) keine onkogene Abhängigkeit mehr besteht.

Die Bedeutung von TP53-Komutationen wurde im Rahmen einer Auswertung des deutschen Härtefallprogramms für den Drittgenerations-ALK- und ROS1-Inhibitor Lorlatinib genauer untersucht. Insgesamt wurden von 2016 bis 2019 37 ALK- und 15 ROS1-positive Patienten nach Versagen zugelassener Behandlungsoptionen bzw. Vorliegen spezifischer Resistenzmutationen oder einer Meningeosis carcinomatosa eingeschlossen. Die Patienten waren größtenteils ausgeprägt vorbehandelt und hatten im Median vier Therapielinien, davon drei zielgerichtete Behandlungen, erhalten. Klinischerseits zeichnete sich die Kohorte durch einen klassischen treibermutierten Phänotyp mit Überwiegen des weiblichen Geschlechts (54%), jüngerem Lebensalter (Median 57 Jahre), einer hohen Rate an Niemalsrauchern (62%) und prädominanter Adenokarzinomhistologie (98%) aus. Der TP53-Status war bei 75% der Patienten vor

Behandlungsbeginn mit Lorlatinib erhoben worden. Mutationen waren mit einem dreifach kürzeren PFS unter Lorlatinib assoziiert (3.7 versus 10.8 Monate, HR 3.3, p=0.003). Darüber hinaus waren sowohl das Gesamtüberleben seit Einschluss in das Härtefallprogramm (OS1, 24.7 versus 10.9 Monate, HR 1.7, p=0.24) als auch seit Stellung der Erstdiagnose (OS2, 42.2 versus 88.9 Monate, HR 3.0, p=0.02) im Falle einer TP53-Mutation deutlich verkürzt.

Aktive Hirnmetastasen bestanden bei Einschluss bei 69%, eine Meningeosis carcinomatosa bei 17% der Patienten. Bei einer Ansprechrate von 54% in der Gesamtkohorte zeigte sich ein nochmals verbessertes Ansprechen von 63%, bzw. 78% im Falle von Hirnmetastasen bzw. einer Leptomeningeosis.

# Lorlatinib in pretreated ALK- or ROS1-positive lung cancer and impact of TP53 co-mutations: results from the German early access program

**Nikolaj Frost, Petros Christopoulos, Diego Kauffmann-Guerrero, Jan Stratmann, Richard Riedel, Monica Schaefer, Jürgen Alt, Sylvia Gütz, Daniel C. Christoph, Eckart Laack, Martin Faehling, Richard Fischer, Klaus Fenchel, Sebastian Haen, Lukas Heukamp, Christian Schulz and Frank Griesinger**

***Abstract***

**Introduction:** We report on the results of the German early access program (EAP) with the third-generation ALK- and ROS1-inhibitor lorlatinib.

**Patients and Methods:** Patients with documented treatment failure of all approved ALK/ROS1-specific therapies or with resistance mutations not covered by approved inhibitors or leptomeningeal carcinomatosis were enrolled and analyzed.

**Results:** In total, 52 patients were included [median age 57 years (range 32–81), 54% female, 62% never smokers, 98% adenocarcinoma]; 71% and 29% were ALK- and ROS1-positive, respectively. G1202R and G2032R resistance mutations prior to treatment with lorlatinib were observed in 10 of 26 evaluable patients (39%), 11 of 39 patients showed TP53 mutations (28%). Thirty-six patients (69%) had active brain metastases (BM) and nine (17%) leptomeningeal carcinomatosis when entering the EAP. Median number of prior specific TKIs was 3 (range 1–4). Median duration of treatment, progression-free survival (PFS), response rate and time to treatment failure were 10.4 months, 8.0 months, 54% and 13.0 months. Calculated 12-, 18- and 24-months survival rates were 65, 54 and 47%, overall survival since primary diagnosis (OS2) reached 79.6 months. TP53 mutations were associated with a substantially reduced PFS (3.7 *versus* 10.8 month, HR 3.3, $p = 0.003$) and were also identified as a strong prognostic biomarker (HR for OS2 3.0 $p = 0.02$). Neither prior treatments with second-generation TKIs nor BM had a significant influence on PFS and OS.

**Conclusions:** Our data from real-life practice demonstrate the efficacy of lorlatinib in mostly heavily pretreated patients, providing a clinically meaningful option for patients with resistance mutations not covered by other targeted therapies and those with BM or leptomeningeal carcinomatosis.

***Keywords:*** ALK, brain metastases, early access program, lorlatinib, NSCLC, ROS1, TP53

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## Introduction

Rearrangements of anaplastic lymphoma kinase (ALK) or c-ros oncogene 1 (ROS1) are identified in 3–5 and 1–2% of patients with lung adenocarcinoma.[1,2] A common pattern of clinical characteristics distinguishes these patients from the general population of non-small-cell lung cancer (NSCLC), including a younger age, a history of never or light smoking (<10 pack years), and a higher prevalence of brain metastases (BM).[3–5] Compared with ALK-positive patients, ROS1-positive patients exhibit fewer extrathoracic and

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BM at first diagnosis as well as during the course of disease.[6] Median progression-free survival (PFS) using the first-generation tyrosine kinase inhibitor (TKI) crizotinib ranges between 7.7 months in ALK-positive and 19.1 months in ROS1 patients, respectively.[7–9] Due to a low intracranial penetration rate, the brain represents the most prominent site of progression on crizotinib treatment.[5,10–12] Consequently, first-line treatment with more potent second-generation TKIs has become the standard of care at least for ALK-positive patients.[13] However, the majority of patients inevitably relapses also using these newer drugs, revealing the medical need for sequential treatment options.[13,14] Acquired mutations in the ALK or ROS1 kinase domain represent a major molecular mechanism of resistance. Lorlatinib is a potent and selective third-generation, ATP-competitive oral ALK and ROS1 kinase inhibitor, especially designed to penetrate the blood–brain barrier and to overcome known ALK and ROS1 resistance mutations. Clinical trials including ALK- and ROS1-positive patients demonstrated the efficacy of lorlatinib in subsequent lines of therapy with high intracranial response rates (RRs).[15,16] We here report on the results of the German early access program (EAP) of lorlatinib, providing data on mostly heavily pretreated patients with ALK- and ROS1-alterated NSCLC from the daily routine.

## Patients and methods

Patients with documented treatment failure of all approved ALK/ROS1-specific therapies and ⩾2 other approved systemic therapies for metastatic NSCLC could be enrolled into the EAP from April 2017 until May 2019. Patients with documented resistance mutations not covered by other inhibitors (e.g. G1202R for ALK and G2032R for ROS1) or leptomeningeal carcinomatosis (LMC) could receive lorlatinib even without having been treated with all approved lines of therapy. Resistance testing was not mandatory prior to enrolment. LMC was defined as the combination of multifocal neurologic signs, typical radiomorphologic findings in brain/spine magnetic resonance imaging (MRI) (e.g. diffuse leptomeningeal contrast enhancement), cytologic identification of malignant cells within the cerebrospinal fluid (CSF) and/or a CSF composition compatible with LMC (e.g. high protein concentration, low glucose concentration, lymphocytic pleocytosis). Detailed inclusion and exclusion criteria were as follows.

### *Inclusion criteria*

1. Diagnosis:

Evidence of histologically or cytologically confirmed diagnosis of metastatic NSCLC (Stage IV, AJCC v7.0) carrying an ALK or ROS1 rearrangement as determined by locally approved tests.

2. Disease Status Requirements:

Documented treatment failure (i.e. disease progression, symptom deterioration or intolerance to therapy) of all locally approved ALK/ROS1 inhibitor therapies and any other alternative approved systemic treatments for metastatic NSCLC had to be documented as a prerequisite.

For patients with ALK-positive NSCLC:

- All approved ALK inhibitors*
- Plus two other approved chemotherapies or immuno-oncologic (IO) therapies

For patients with ROS1-positive NSCLC:

- Minimum crizotinib* plus two other approved chemotherapies or IO therapies

*with the exception of documented resistance mutations not covered by other inhibitors (e.g. ALK G1202R resistance mutation).

3. Adequate Bone Marrow Function, including:
   - Absolute Neutrophil Count (ANC) $\geqslant 1.5 \times 10^9$/L;
   - Platelets $\geqslant 100 \times 10^9$/L;
   - Hemoglobin ⩾9 g/dL.
4. Adequate Pancreatic Function, including:
   - Serum total amylase $\leqslant 1.5 \times$ ULN
   - Serum lipase $\leqslant 1.5 \times$ ULN
5. Adequate Renal Function, including:
   - Serum creatinine $\leqslant 1.5 \times$ ULN or estimated creatinine clearance ⩾60 mL/min as calculated using the method standard for the institution.
6. Adequate Liver Function, including:
   - Total serum bilirubin $\leqslant 1.5 \times$ ULN;
   - Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) $2.5 \times$ ULN; $\leqslant 5.0 \times$ ULN in the case of liver metastases.
7. Acute effects of any prior therapy resolved to baseline severity or to Common Terminology Criteria for Adverse Events (CTCAE)

Grade 1, except for adverse events (AEs) not constituting any safety risk for the patient as judged by the investigator.
8. A negative serum pregnancy test for females of childbearing potential at screening.
9. Evidence of a personally signed and dated informed consent document indicating that the patient has been informed of all pertinent aspects of the "Härtefallprogramm".

*Exclusion criteria*

1. Major surgery (within 4 weeks), minor surgery (within 2 weeks), chemotherapy (within 4 weeks), radiotherapy (RT) (within 2 weeks; 48 h for palliative RT), any investigational agents (within 4 weeks), or other anti-cancer therapy (within 2 weeks; but five half-lives if known for approved TKI).
2. Clinically significant cardiovascular disease (that is, active or $<3$ months prior to enrolment): cerebral vascular accident/stroke, myocardial infarction, unstable angina, congestive heart failure (New York Heart Association Classification Class $\geq$ II), second-degree or third-degree atrioventricular (AV) block (unless paced) or any AV block with PR $>220$ msec. Ongoing cardiac dysrhythmias of NCI CTCAE Grade $\geq 2$, uncontrolled atrial fibrillation of any grade, bradycardia defined as $<50$ bpm (unless patient is otherwise healthy such as long-distance runners, etc.), machine-read ECG with QTc $>470$ msec, or congenital long QT syndrome.
3. Predisposing characteristics for acute pancreatitis (e.g. uncontrolled hyperglycemia, current gallstone disease, alcoholism) in the last month.
4. History of extensive, disseminated, bilateral or presence of Grade 3 or 4 interstitial fibrosis or interstitial lung disease including a history of pneumonitis, hypersensitivity pneumonitis, interstitial pneumonia, interstitial lung disease, obliterative bronchiolitis and pulmonary fibrosis. Patients with history of prior radiation pneumonitis are not excluded.
5. Recent (i.e. within previous 6 months) or active suicidal ideation or behavior.
6. Concomitant use of strong or moderate CYP3A4 inhibitors, strong CYP3A4 inducers, drugs that are CYP3A4 substrates with narrow therapeutic indices:
   - 6.1. Current use or anticipated need for food or drugs that are known strong or moderate CYP3A4 inhibitors, including their administration within 10 days prior to the first dose of lorlatinib [i.e. strong CYP3A4 inhibitors: grapefruit juice or grapefruit/grapefruit related citrus fruits (e.g. Seville oranges, pomelos), ketoconazole, miconazole, itraconazole, voriconazole, posaconazole, clarithromycin, telithromycin, indinavir, saquinavir, ritonavir, nelfinavir, amprenavir, fosamprenavir nefazodone, lopinavir, troleandomycin, mibefradil, and conivaptan; Moderate CYP3A4 inhibitors: erythromycin, verapamil, atazanavir, delavirdine, fluconazole, darunavir, diltiazem, aprepitant, imatinib, tofisopam, ciprofloxacin, cimetidine].
   - 6.2. Current use or anticipated need for drugs that are known strong CYP3A4 inducers, including their administration within 12 days prior to the first dose of lorlatinib (i.e. phenobarbital, rifampin, phenytoin, carbamazepine, rifabutin, rifapentin, clevidipine, St. John's Wort).
   - 6.3. Concurrent use of drugs that are CYP3A4 substrates with narrow therapeutic indices, such as astemizole, terfenadine, cisapride, pimozide, quinidine, tacrolimus, cyclosporine, sirolimus, (alfentanil and fentanyl, including transdermal patch) or ergot alkaloids (ergotamine, dihydroergotamine) is not permitted or caution is warranted. CYP2C9 or P-gp substrates with narrow therapeutic indices, sensitive CYP2B6 substrates, or strong CYP2C8 or CYP2C19 inhibitors.
7. Concurrent use of drugs that are CYP2C9 substrates with narrow therapeutic indices, such as warfarin, phenytoin or a sensitive substrate such as celecoxib is not permitted or caution is warranted.
8. Concurrent use of drugs that are sensitive CYP2B6 substrates, such as bupropion, efavirenz is not permitted or caution is warranted.
9. Current use or anticipated need for drugs that are known strong CYP2C19 inhibitors, including their administration within 12 days prior to study entry (i.e. fluconazole, fluvoxamine, ticlopidine).

10. Current use or anticipated need for drugs that are known strong CYP2C8 inhibitors, including their administration within 12 days prior to study entry (i.e. gemfibrozil).
11. Current use or anticipated need for drugs that are known P-gp substrates with a narrow therapeutic index, including their administration within 12 days prior to study entry (i.e. digoxin).
12. Breastfeeding female patients (including patients who intend to interrupt breastfeeding).
13. Known hypersensitivity to lorlatinib or any of its excipients.

Patients' baseline demographics, tumor-specific data and outcome were collected. As RECIST-based radiologic evaluation is not routinely performed in the real-world setting, estimation of tumor response and definition of disease progression were based on the clinician's estimates, which have nevertheless been shown to correlate reasonably well with RECIST assessments in several recent studies.[17,18] On-treatment imaging was performed according to national guidelines[19] and the respective local standard of care, respectively, using computed tomography scans (thorax, abdomen) and brain MRIs. Positron emission tomography scans were not routinely performed.

Survival endpoints were real-world PFS, time to treatment failure (TTF) and overall survival (OS). PFS was defined as the time in months from the first dose of lorlatinib to the first documented progression, either as radiologically confirmed progression or death, PFS2 as the time in months from the first dose of any subsequent therapy following lorlatinib until death from any cause, TTF as the time in months from the first dose of lorlatinib until loss of clinical efficacy as defined by the treating physician, OS1 as the time in months from the first dose of lorlatinib and OS2 since the date of primary diagnosis until death from any cause.

AEs were graded according to the CTCAE version 5.0. Approval was obtained from the Charité Universitätsmedizin Berlin ethics committee (approval number EA2/159/19).

## Statistical analysis

Demographics and disease data were described and compared using the Pearson Chi$^2$-test, Fisher's exact test or Mann–Whitney $U$ test, according to the level of measurement. The Kaplan–Meier method was used to estimate median PFS, TTF and OS. $p$-values comparing survival curves were calculated with log-rank tests. Hazard ratios were calculated using univariate Cox-regression analysis. All analyses were performed using IBM SPSS statistics version 24 (IBM, Armonk, NY, USA). A $p$-value $< 0.05$ (two-tailed) was defined as statistically significant.

## Results

### Baseline characteristics

In total, 52 patients from 29 institutions were included in the analysis. Some 37 patients were ALK-positive (71.2%), 15 had a ROS1 rearrangement (28.8%). The median number of prior therapies was five (range 2–9) and three (1–5) in ALK and ROS1-positive patients, respectively. ALK-positive patients were pretreated with three (1–4) specific TKIs, and rates for crizotinib, ceritinib, alectinib and brigatinib were 94.6, 89.2, 91.9 and 35.1%, respectively. All ROS1 patients had received crizotinib and 26.7% an additional treatment with ceritinib. In ALK-positive patients, the most recent treatment regimens patients progressed on before lorlatinib were alectinib (17 patients, 45.9%), chemo-/immunotherapy (eight patients, 21.6%), ceritinib (five patients, 13.5%), brigatinib (four patients, 10.8%) and crizotinib (three patients, 8.1%). The most recent treatments in ROS1-positive patients were crizotinib (nine patients, 60.0%) and ceritinib (three patients, 20.0%). One patient each (6.7%) was treated with chemo-/immunotherapy and cabozantinib, respectively. Thirty-six patients (69.2%) had active BM at the time of enrolment and nine (25%) exhibited signs of LMC. The presence of BM was associated with a PS of $\geqslant 2$ ($p = 0.04$). All baseline characteristics are listed in Table 1.

### Response to lorlatinib

After a median follow-up time of 16.1 months [95% confidence interval (CI), 12.2–18.1], the median duration of treatment was 10.4 months (95% CI, 6.5–12.8), 25 patients (48.1%) still received lorlatinib. In 46 patients with documented responses (88.5%, no information: $n = 4$; response not evaluable: $n = 2$), RR was 54.3% with two complete responses (CR; 4.3%) and 23 partial responses (PR; 50.0%). The

**Table 1.** Patients' baseline demographics in the entire cohort (left column) and in ALK and ROS1-patients separately (middle and right column).

| | **All patients (*n* = 52)** | **ALK-positive (*n* = 37)** | **ROS1-positive (*n* = 15)** |
|---|---|---|---|
| Age, years (range) | 57 (32–81) | 58 (32–70) | 56 (36–81) |
| Sex, *n* (%) | | | |
| Female | 28 (53.8) | 18 (48.6) | 10 (66.7) |
| Male | 24 (46.2) | 19 (51.4) | 5 (33.3) |
| Smoking history, *n* (%) | | | |
| Current smoker | 4 (7.7) | 3 (8.1) | 1 (6.7) |
| Former smoker | 14 (26.9) | 10 (27.0) | 4 (27.6) |
| Never smoker | 32 (61.5) | 22 (59.4) | 10 (66.7) |
| Missing data | 2 (3.8) | 2 (5.4) | |
| Performance Status at primary diagnosis, *n* (%) | | | |
| 0 | 31 (59.6) | 24 (64.9) | 7 (46.7) |
| 1 | 16 (30.8) | 10 (27.0) | 6 (40.0) |
| 2 | 1 (1.9) | 1 (2.7) | |
| missing data | 4 (7.7) | 2 (5.4) | 2 (13.3) |
| Histology, *n* (%) | | | |
| Adenocarcinoma | 51 (98.1) | 37 (100.0) | 14 (93.3) |
| Adenosquamous carcinoma | 1 (1.9) | | 1 (6.7) |
| Brain metastases at primary diagnosis, *n* (%) | | | |
| Yes | 13 (25.0) | 11 (29.7) | 2 (13.3) |
| No | 38 (73.1) | 25 (67.7) | 13 (86.7) |
| Missing data | 1 (1.9) | 1 (2.7) | |
| Stage at primary diagnosis | | | |
| III | 6 (11.5) | 4 (10.8) | 2 (13.3) |
| IV | 46 (88.5) | 33 (89.2) | 13 (86.7) |
| Prior systemic therapies, *n* (range) | 4 (1–9) | 5 (2–9) | 3 (1–5) |
| Prior targeted therapies, *n* (range) | 3 (1–4) | 3 (1–4) | 1 (1–2) |
| Crizotinib | 50 (96.2) | 35 (94.6) | 15 (100.0) |
| Ceritinib | 37 (71.2) | 33 (89.2) | 4 (26.7) |
| Alectinib | 34 (65.4) | 34 (91.9) | |
| Brigatinib | 13 (25.0) | 13 (35.1) | |

*(Continued)*

**Table 1.** (Continued)

| | All patients ($n=52$) | ALK-positive ($n=37$) | ROS1-positive ($n=15$) |
|---|---|---|---|
| Performance Status at enrolment, *n* (%) | | | |
| 0 | 12 (23.1) | 8 (21.6) | 4 (26.7) |
| 1 | 27 (51.9) | 19 (51.4) | 8 (53.3) |
| 2 | 9 (17.3) | 7 (18.9) | 2 (13.3) |
| 3 | 2 (3.8) | 2 (5.4) | |
| 4 | 2 (3.8) | 1 (2.7) | 1 (6.7) |
| Brain metastases at enrolment, *n* (%) | | | |
| Yes | 36 (69.2) | 26 (70.3) | 10 (66.7) |
| No | 16 (30.8) | 11 (29.7) | 5 (33.3) |
| Leptomeningeal disease at enrolment, *n* (%) | | | |
| Yes | 9 (25.0) | 6 (23.1) | 3 (30.0) |
| No | 27 (75.0) | 20 (76.9) | 7 (70.0) |

disease-control rate was 82.6%. RR according to the rearrangement were 42.4% for ALK-positive patients ($n=14$) and 84.6% for ROS1 ($n=11$, $p=0.02$). RR in ALK-positive patients with two ($n=4$), three ($n=15$) or four ($n=16$) previous TKIs were 25.0, 40.0 and 43.8%, respectively. In patients with prior alectinib ($n=34$), RR was 38.3% as compared with 33.3% without ($n=3$). In ROS1-positive patients having received crizotinib only ($n=10$) and two previous TKIs ($n=5$), respectively, RR were 80.0 and 60.0%. Comparing patients with BM with those without, RR were 62.5% and 35.7% ($p=0.09$). Seven out of nine patients with LMC showed a PR (77.8%).

### *Survival analysis, efficacy in brain metastases and subsequent therapies*

At the time of data cut-off (30 April 2020), 34 PFS events were recorded (65.4%), median PFS was 8.0 months (95% CI, 4.0–12.0, Figure 1A). 59.4% of PFS events occurred extracranially ($n=19$), an isolated brain progression was documented in 15.6% ($n=5$), one patient had a simultaneous extra- and intracranial progression [site of progression unknown in seven cases (21.9%)]. Ten out of 15 patients with baseline BM progressed intracranially (75%) as compared with no patient without ($p=0.04$). The presence of baseline BM had no influence on PFS (HR 0.97, 95% CI, 0.47–1.99, $p=0.93$).

Treatment with lorlatinib was continued in 14 patients (41.2%) beyond documented progression, resulting in a TTF of 13.0 months (95% CI, 8.8–17.3). Out of 34 patients with progressive disease (PD), 12 received at least one subsequent therapy (23.1%), containing chemotherapy, immunochemotherapy or immunotherapy alone in seven, one and two patients, respectively. Seven patients were re-exposed to a specific TKI. PFS2 for subsequent treatments was 7.1 months for all patients (95% CI, 1.5–12.8) and 2.2 months for TKIs (95% CI, 1.9–2.5) *versus* not reached in patients receiving chemotherapy ($p=0.11$). Swimmer plots visualizing the treatment with lorlatinib and subsequent therapies for each individual patient are depicted in Figure 2.

Within the indicated follow-up time 22 deaths occurred (42.3%). The estimated median OS1 reached 24.7 months [95% CI, not evaluable (NE)–NE], calculated 12-, 18- and 24-months survival rates were 64.9, 54.0 and 47.3%, respectively (Figure 1B). OS2 reached 79.6 months (95% CI, 75.1–102.1).

### *Molecular characterization and identification of potential resistance mechanisms*

Next-generation sequencing (NGS)-based analyses of potential tyrosine kinase resistance mutations prior to therapy with lorlatinib were performed in

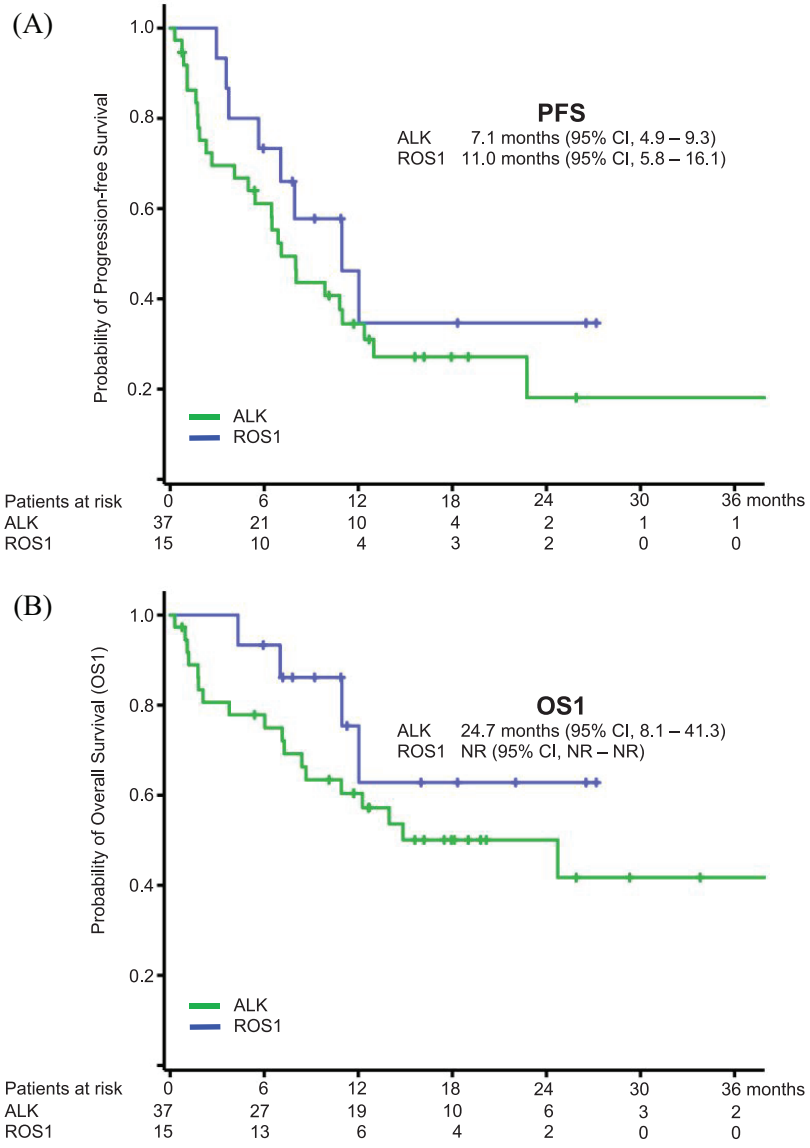


**Figure 1.** Kaplan–Meier curves for progression-free (PFS, Figure 1A) and overall survival since treatment with lorlatinib (OS1, Figure 1B) for ALK and ROS1-positive patients.

26 patients (50.0%, Table 2). Substantially more ALK-positive patients underwent a re-assessment (either tissue-based or liquid biopsy) than those with ROS1 (62.2 *versus* 20.0%, $p=0.01$). Specific mutations were identified in 15 patients (57.7%), six of whom displayed compound mutations with ⩾2 mutations. G1202R and G2032R-mutations represented the most frequent resistance pattern, detected in eight ALK (61.5%) and two ROS1-positive patients (100.0%). PFS and TTF for patients with G1202R/G2032R/without detectable mutations *versus* those with compound mutations were 6.9 (95% CI, 3.4–10.3) and 12.8 months (95% CI, 6.9–18.8) *versus* 1.9 (95% CI, 0.0–4.8) and 3.2 months (95% CI, 0.0–12.5), respectively.

NGS-based analyses of TP53-mutations at any timepoint prior to lorlatinib were conducted in 41 patients (78.8%). Pathogenic mutations were detected in 11 patients (26.8%), occurring more frequently in ROS1- (6/10 patients, 60%) than ALK-positive patients (5/31 patients, 16.1%, $p=0.01$). TP53 mutations were associated with a threefold decrease in PFS of 3.7 (95% CI, 2.5–5.0) *versus* 10.8 months (95% CI, 6.2–15.5, HR, 3.3, 95% CI, 1.5–7.5, $p=0.003$, Figure 3A). By trend, survival differences were also observed for OS1 (10.9 *versus* 24.7 months, $p=0.24$, Figure 3B). In general, TP53-mutations carried out a strongly negative prognosis throughout the entire course of disease with an OS2 of 42.2

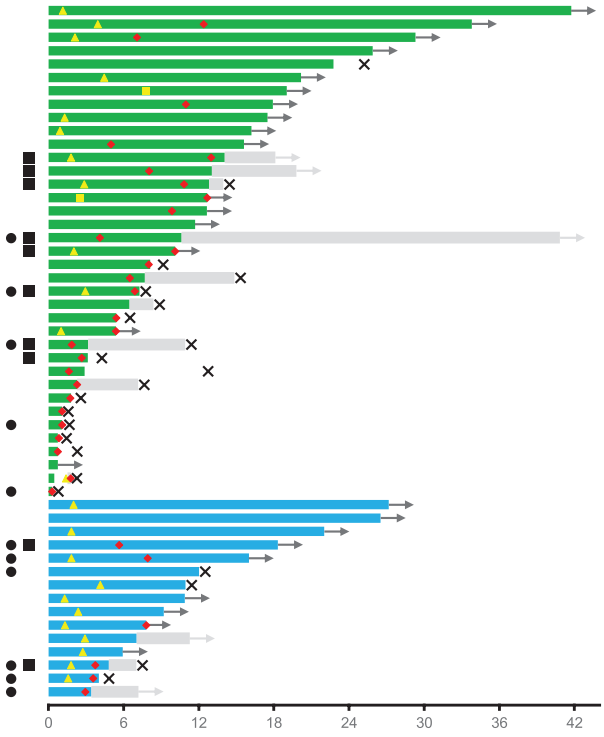


**Figure 2.** Swimmer plot for each individual patient indicating the duration of treatment with lorlatinib and subsequent therapies (time in months). Green bars (■) indicate ALK-positive patients on lorlatinib, blue bars (■) ROS1-positive patients on lorlatinib. Black arrows (→) indicate ongoing treatment with lorlatinib. Yellow triangles (▲) define the date of partial remission, yellow squares (■) the date of complete remission. Red hashes (◆) indicate the date of progressive disease. Gray bars (■) indicate subsequent therapies, gray arrows (→) ongoing subsequent treatment(s). Black crosses (✕) define the date of death. Black circles (●) indicate TP53 mutations, black squares (■) G1202R or G2032 gatekeeper mutations.

**Table 2.** Tyrosine kinase resistance mutations and TP53 mutations in the entire cohort (left column) and in ALK and ROS1-patients (middle and right column).

| | All patients ($n = 52$) | ALK-positive ($n = 37$) | ROS1-positive ($n = 15$) |
|---|---|---|---|
| Assessment of specific tyrosine kinase mutations (NGS), *n* (%) | 26 (50.0) | 23 (62.2) | 3 (20.0) |
| Tyrosine kinase mutation* | 15 (57.7) | 13 (56.5) | 2 (66.6) |
| V1149A | | 1 | |
| C1156Y | | 2 | |
| I1171N | | 1 | |
| F1174V | | 1 | |
| L1196M | | 4 | |
| G1202R | | 8 | |
| D1203N | | 1 | |
| G1269A | | 2 | |
| G2032R | | | 2 |

*(Continued)*

**Table 2.** (Continued)

| | All patients ($n=52$) | ALK-positive ($n=37$) | ROS1-positive ($n=15$) |
|---|---|---|---|
| No tyrosine kinase mutation | 11 (42.3) | 10 (43.5) | 1 (33.3) |
| Assessment of TP53 mutations, *n* (%) | 41 (78.8) | 31 (83.8) | 10 (66.7) |
| TP53 mutation | 11 (26.8) | 5 (16.1) | 6 (60.0) |
| No TP53 mutation | 28 (68.3) | 25 (80.7) | 3 (30.0) |
| TP53 not evaluable | 2 (4.9) | 1 (3.2) | 1 (10.0) |

*compound mutations ($n=6$): L1196M-based: +I1171N, +F1174V, +D1203N; G1202R-based: +V1149A+L1196M, +C1156Y, +G1269A.
NGS, next-generation sequencing.

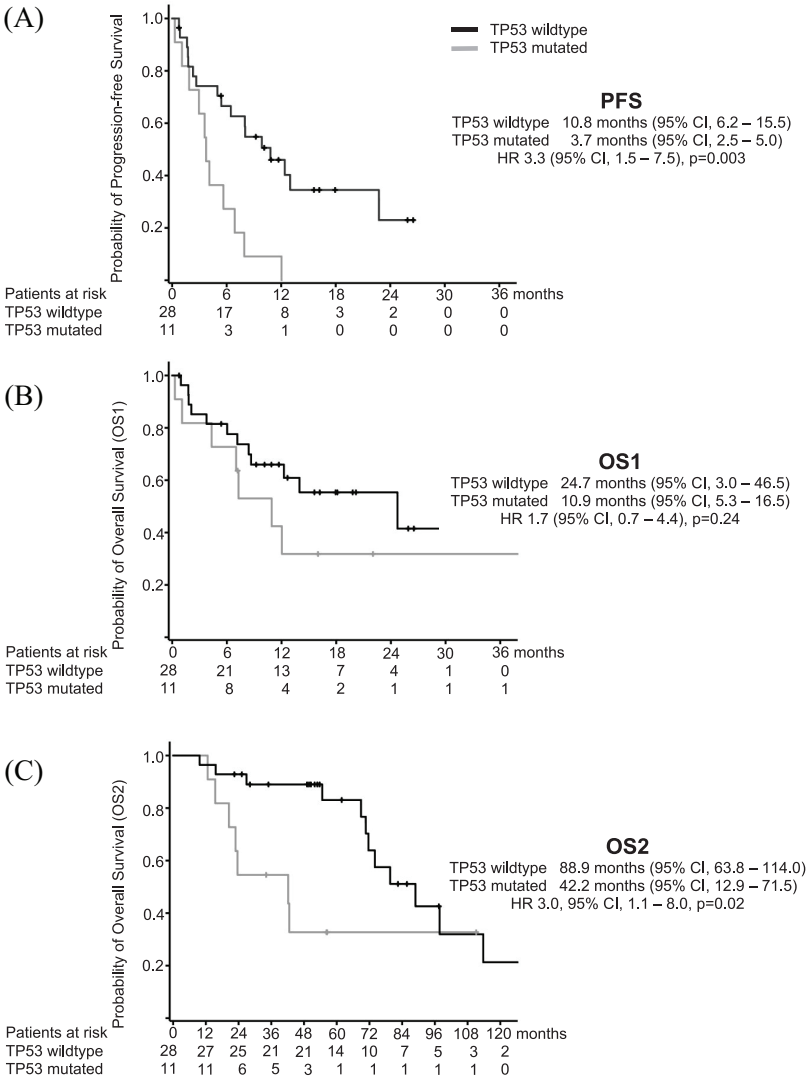


**Figure 3.** Kaplan–Meier curves for progression-free survival (PFS, Figure3A), overall survival since treatment with lorlatinib (OS1, Figure 3B) and overall survival since primary diagnosis (OS2, Figure 3C), each depending on the TP53 mutational status.

**Table 3.** Summary of adverse events.

| Event | Any grade | G1 or 2 | G3 | G4 | Grade not reported |
|---|---|---|---|---|---|
| Patients with adverse events (AE), *n* | 37 | 37 | 8 | 2 | 4 |
| AE reported, *n* | 91 | 59 | 9 | 2 | 21 |
| AE leading to discontinuation, *n* | 5 | 1 | 1 | 1 | 2 |
| Gastrointestinal disorders | 34 | 29 | 4 | 0 | 1 |
| Diarrhea | 3 | 3 | 0 | 0 | 0 |
| Dysgeusia | 2 | 1 | 0 | 0 | 1 |
| Hypercholesterolemia | 17 | 15 | 2 | 0 | 0 |
| Hypertriglyceridemia | 6 | 5 | 1 | 0 | 0 |
| Lipase/amylase increased | 4 | 3 | 1 | 0 | 0 |
| Mucositis | 1 | 1 | 0 | 0 | 0 |
| Nausea | 1 | 1 | 0 | 0 | 0 |
| General disorders | 21 | 13 | 2 | 0 | 6 |
| Edema | 11 | 8 | 2 | 0 | 1 |
| Fatigue | 2 | 2 | 0 | 0 | 0 |
| Pruritus | 1 | 0 | 0 | 0 | 1 |
| Rash | 4 | 1 | 0 | 0 | 3 |
| Sweating | 1 | 0 | 0 | 0 | 1 |
| Weight gain | 1 | 1 | 0 | 0 | 0 |
| Neurologic disorders | 16 | 7 | 1 | 0 | 8 |
| Central nervous system effects* | 13 | 5 | 1 | 0 | 7 |
| Peripheral neuropathy | 3 | 2 | 0 | 0 | 1 |
| Respiratory disorders | 11 | 5 | 2 | 1 | 3 |
| Dyspnea | 7 | 5 | 0 | 0 | 2 |
| Pleural effusion | 1 | 0 | 1 | 0 | 0 |
| Pneumonia | 1 | 0 | 0 | 0 | 1 |
| Pneumonitis | 2 | 0 | 1 | 1 | 0 |
| Other disorders | 9 | 5 | 0 | 1 | 3 |
| Creatinine increased | 1 | 0 | 0 | 0 | 1 |
| Hypertension | 1 | 1 | 0 | 0 | 0 |
| Hypothyroidism | 2 | 2 | 0 | 0 | 0 |
| Myalgia | 2 | 1 | 0 | 0 | 1 |
| Thromboembolism | 2 | 1 | 0 | 1 | 0 |
| Tongue swelling | 1 | 0 | 0 | 0 | 1 |

*Psychiatric disorders: *n* = 4 (aggressiveness, hallucinations, persecution mania, panic attack); visual defects: *n* = 3; dizziness: *n* = 3; slow speech, headache and daze: *n* = 1 each.

(95% CI, 12.9–71.5) *versus* 88.9 months (95% CI, 63.8–114.0, HR 3.0, 95% CI, 1.1–8.0, $p = 0.02$, Figure 3C).

### *Adverse events*

AEs were reported in 37 patients (71.2%); the median number per patient was two (range 1–8; 14 patients without reported AEs, information missing $n = 1$). All AEs are listed in Table 3. The most frequent clustered AEs concerned gastrointestinal disturbances (34 patients), followed by neurological and respiratory disorders (16 and 11 patients, see footnote below Table 3 for a detailed description of all neuro-psychiatric disorders). The most common isolated AEs were hypercholesterolemia ($n = 17$) and peripheral edema ($n = 11$). The majority of AEs were mild or moderate grade (G1 or G2, 59 events, 37 patients). G3 and four events occurred in eight (nine events) and two patients (two events), respectively, concerning laboratory abnormalities in lipid metabolism and pancreatic enzymes as well as edema, central nervous system effects and pneumonitis. Grading was not reported for 21 events. Therapy with lorlatinib was discontinued due to AEs in five patients, suffering from dyspnea (G3), anasarca (G3), an increase in serum creatinine [grade not reported (NR)] and pneumonitis (G4). Psychiatric disorders leading to treatment discontinuation concerned hallucinations and persecution mania (grade NR) in two ALK-positive patients with BM and LMC.

## Discussion

Our data from real-world treatment demonstrates the efficacy of lorlatinib in mostly heavily pretreated patients with either ALK- or ROS1-rearrangements. RR was 42.4% for ALK-positive patients ($n = 14$), comparable to a phase II study[16] and the results from the expanded access program in Asian countries and the US.[20] In contrast to these studies, RR was not influenced by the number of prior TKIs and was also substantially higher for ROS1-positive patients (84.6%) as compared with the respective phase I/II study.[15]

The brain represents a frequent site of progression in ALK- and ROS1-positive NSCLC, affecting nearly 60% of patients after 3 years.[21] In the crizotinib-refractory setting, intracranial RR for alectinib, brigatinib and ceritinib ranged between 35 and 73%.[22] According to the presence *versus* absence of BM at baseline, lorlatinib showed rates of brain progression after 12 months of 22 *versus* 9% after crizotinib, and 23 *versus* 12% after ⩾1 s generation ALK-TKI.[23] Although BM have not been evaluated separately in our investigation, the reported RRs were higher in patients with BM (62.5% *versus* 35.7%) suggesting a high intracranial efficacy. In contrast to the published literature, the brain also represented the most frequent site of progression in patients suffering from BM when entering the EAP (75.0%), whereas no patient without BM progressed intracranially. Due to these uneven results, a clear brain-protecting effect of lorlatinib could not be identified from our dataset. LMC represents a difficult to treat manifestation of BM, mostly refractory to standard treatment and associated with a dismal prognosis. In this setting, case series suggest a high efficacy using effective targeted therapy even after treatment failure of preceding ALK TKIs.[24] The prevalence of LMC in NSCLC in general is estimated to range between 3% and 5%[25] and substantially increases in molecularly altered subgroups, benefiting from a longer survival due to targeted therapies. The 25% of patients with LMC in our study may represent an enriched share of patients even for this special population,[20] showing a promising RR of 77.8%.

The median PFS reached 8.0 months and was 7.1 and 11.0 months for ALK and ROS1 patients, consistent with phase I/II studies reporting a PFS of 7.3 and 8.5 months in the ALK and ROS1 subsets, respectively.[15,16] Treatment beyond progression was continued in almost half of the patients with PD (14 patients, 41.2%) and resulted in a meaningful increase in TTF of 13.0 months. Treatment continuation beyond PD given an ongoing clinical response nowadays represents an established option for patients with molecularly altered NSCLC and has been proven beneficial. Hence, retrospective data suggest that these patients might represent a subset with a more favorable prognosis in general.[26]

In the era of molecularly driven therapies, offering sequential targeted treatment options in ALK and ROS1-positive NSCLC, re-characterization of the tumor *via* repeated biopsies has become an established procedure and was carried out in 50% of patients in the routine setting. G1202R-solvent-front mutations are associated with clinical resistance to first and second-generation ALK TKIs but may be sensitive to lorlatinib.[27] G2032R-mutations represent a similar situation for ROS1-patients. The available molecular data

from the present investigation confirm the clinical efficacy of lorlatinib for both solvent-front mutations, whereas PFS and TTF were clearly reduced with the evidence of compound mutations. Furthermore, TP53 mutations were associated with a markedly reduced PFS of 3.7 *versus* 10.8 months. As differences in OS since primary diagnosis depending on the TP53 status were even more pronounced (42.2 *versus* 88.9 months), these alterations might represent an intrinsic mode of TKI resistance. In this line, several investigations have identified TP53 mutations, either present at diagnosis or acquired at disease progression, as a predictive as well as prognostic biomarker in ALK-positive NSCLC.[28,29] To our knowledge, our study is the first report transferring the validity of these results also on a treatment with lorlatinib, indicating that TP53 mutations confer a negative impact on PFS and OS irrespective of the TKI.

Whether re-exposure to a frontline TKI after progression on a subsequent TKI may be beneficial remains an unresolved question. In our study, patients receiving another TKI after lorlatinib had a shorter PFS2 than those treated with chemotherapy. However, selection bias may represent a significant confounder, as patients with a subsequent TKI may have been judged not fit enough for chemotherapy and data on post-lorlatinib PS were not reported. A significant fraction of the so far identified lorlatinib-resistant compound mutations is not sensitive to earlier generation TKIs.[30–32] Therefore, a re-exposure without a carefully performed molecular analysis suggesting sensitivity to first- or second-generation TKIs has to be regarded cautiously. Thus, patients may benefit more from chemotherapy.

The reported rates of AEs in our study were substantially smaller than those in the respective clinical trials, probably attributable to a less rigorous reporting in the daily routine. However, as patients were not treated according to a dedicated study protocol, dose modifications may have been used preemptively to avoid (more severe) AEs. It is noteworthy, that two of five patients with AE-related treatment cessation were discontinued due to psychiatric disorders, effects that have not been reported for other ALK or ROS1-TKIs. Especially BM (e.g. next to the limbic system) and LMC might predispose to psychiatric AEs. In this connection, prior brain radiotherapy and steroids administered to most patients with symptomatic BM have been described as potential trigger factors.[33] Albeit the biologic connection with the administration of lorlatinib is uncertain, it would be highly desirable to identify patients at risk in advance, as these AEs have a substantial impact on the patients' quality of life and might require discontinuation of a highly effective treatment.

Due to its retrospective design, this study has some limitations. ALK fusion variants, proven to impact response and survival on TKIs, were not assessed routinely and thus were not evaluable.[34,35] On the other hand, TP53 status and molecular resistance mechanisms at the time of disease progression were assessed at all participating centers in laboratories and by NGS platforms certified by the quality management initiative of the German Society of Pathology (QuIP®), therefore the results can be considered homogenous and reliable. Patients were treated within the valid standard of care outside a clinical trial with varying imaging intervals, potentially biasing PFS, and imaging was not routinely performed using RECIST assessments. In this line intra- and extracranial responses were not assessed separately and are not reported from our cohort. Nonetheless and with special regard to these limitations, a growing body of evidence suggests that radiologic outcome generated from a real-life cohort may be comparable to a RECIST-defined study cohort.[17,18] An important limitation results from the special patient characteristics of nearly every EAP. Data from real-world practice demonstrate a substantial loss of patients in the transition from one line of therapy to the other.[36] The recently reported FLAURA trial comparing osimertinib with erlotinib in EGFR-mutant NSCLC indicated that 22% of patients with PD did not receive any second-line treatment and 46–49% had no third-line therapy.[37] Thus, patients included into an EAP represent a clearly positively selected population and immortal-time bias should be taken into consideration with regard to OS. Results must be regarded with caution and may not be translated to a general population.

## Conclusion

Our data from a real-world setting confirm the effectiveness of lorlatinib in heavily pretreated ALK and ROS1-positive patients, in whom all treatment options are exhausted. Control of BM is promising, especially as those patients are at a high risk to develop BM during the course of their disease. Integration of the TP53 status into molecular testing strategies may provide a helpful tool in the management of disease, as patients

displaying mutated TP53 have a more aggressive course of disease and may derive a clinical benefit from a closer monitoring.

## Conflict of interest statement

**Dr. Frost** reports personal fees and other from AstraZeneca, personal fees and other from BMS, personal fees and other from AbbVie, personal fees and other from Boehringer Ingelheim, personal fees from Pfizer, personal fees from Roche, personal fees from MSD, personal fees from Takeda, outside the submitted work; **Dr. Christopoulos** reports grants and personal fees from Novartis, grants and personal fees from Roche, grants and personal fees from AstraZeneca, personal fees from Pfizer, grants and personal fees from Takeda, personal fees from Chugai, personal fees from Boehringer, outside the submitted work; **Dr. Kauffmann-Guerrero** reports personal fees from Pfizer, personal fees from Takeda, outside the submitted work; **Dr. Stratmann** reports personal fees from Bristol-Myers Squibb, personal fees from Novartis, personal fees from Roche Pharma, outside the submitted work; **Dr. Riedel** has nothing to disclose. **Dr. Schäfer** has nothing to disclose. **Dr. Alt** has nothing to disclose. **Dr. Guetz** has nothing to disclose. **Dr. Christoph** reports personal fees and non-financial support from Pfizer, during the conduct of the study; personal fees and non-financial support from Amgen, personal fees and non-financial support from AstraZeneca, personal fees and non-financial support from Bayer, personal fees and non-financial support from Boehringer Ingelheim, personal fees and non-financial support from Bristol-Myers Squibb, personal fees and non-financial support from Chugai, personal fees and non-financial support from Merck, Sharp & Dohme, personal fees and non-financial support from Novartis, personal fees and non-financial support from Roche, personal fees and non-financial support from Takeda, outside the submitted work; **Dr. Laack** has nothing to disclose. **Dr. Faehling** has nothing to disclose. **Dr. Fischer** has nothing to disclose. **Dr. Fenchel** has nothing to disclose. **Dr. Haen** has nothing to disclose. **Dr. Heukamp** reports personal fees from Roche, personal fees from NEO NewOncology, personal fees from Pfizer, personal fees from Bayer, personal fees from BMS, personal fees from Astra Zeneca, outside the submitted work; **Dr. Schulz** reports personal fees from Pfizer, personal fees and non-financial support from Roche, outside the submitted work; **Dr. Griesinger** reports grants, personal fees and non-financial support from AstraZeneca, grants, personal fees and non-financial support from Boehringer Ingelheim, grants, personal fees and non-financial support from BMS, grants, personal fees and non-financial support from Eli Lilly, grants, personal fees and non-financial support from MSD, grants, personal fees and non-financial support from Novartis, grants, personal fees and non-financial support from Pfizer, grants, personal fees and non-financial support from Roche, grants, personal fees and non-financial support from Takeda, personal fees from AbbVie, personal fees from Tesaro/GSK, grants and personal fees from Amgen, personal fees from Blueprint Medicines, outside the submitted work.

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## 2.4 Pembrolizumab als palliative Erstlinienbehandlung des PD-L1 hochexprimierenden (≥50%) NSCLC in der klinischen Routine

**Frost N**, Kollmeier J, Misch D, Vollbrecht C, Grah C, Matthes B, Pultermann D, Olive E, Raspe M, Ochsenreither S, von Laffert M, Suttorp N, Witzenrath M, Grohé C. Pembrolizumab as first-line palliative therapy in PD-L1 overexpressing (≥50%) NSCLC: Real-world results with special focus on PS ≥2, brain metastases and steroids. *Clinical Lung Cancer 2021.* doi: 10.1016/j.cllc.2021.02.001.

Die Zulassung von Pembrolizumab als palliative Erstlinienbehandlung des PD-L1 hochexprimierenden (≥50%) NSCLC erfolgte ohne Einschränkung auf bestimmte Patientenkohorten, obwohl ein relevanter Anteil an Patienten aus der täglichen Routine mit symptomatischen Hirnmetastasen, reduziertem Allgemeinzustand oder Steroiddauermedikation nicht in die KEYNOTE-024-Studie eingeschlossen werden konnte. Was aus Sponsorensicht nachvollziehbar erscheint, um den Einfluss prognostisch ungünstiger Sekundärvariablen auf den Behandlungserfolg zu minimieren, lässt die Frage offen, ob und in welchem Ausmaß diese Patientengruppen überhaupt von einer Behandlung mit Pembrolizumab profitieren.

In der untersuchten Kohorte aus 153 im ersten Zulassungsjahr (2017) therapierten Patienten wies jeder Zweite ein Ausschlusskriterium für eine Studienbehandlung auf. Bei Patienten ohne Ausschlusskriterien war die Effektivität in der klinischen Routine mit den Ergebnissen der KEYNOTE-024-Studie vergleichbar. Zudem zeigte sich ein teils langanhaltendes Therapieansprechen bei asymptomatischen Hirnmetastasen oder einem zu Behandlungsbeginn tumorbedingt reduzierten Allgemeinzustand, der sich dank einer hoch effektiven Krebstherapie im Verlauf besserte. Im Gegensatz dazu hatten Patienten mit auf Komorbidität zurückzuführendem schlechten Allgemeinzustand,

symptomatischer ZNS-Metastasierung oder mit Steroiden bei Behandlungsbeginn keinen wesentlichen Nutzen von Pembrolizumab. Letztere stellen womöglich jedoch eher ein Surrogat für Komorbidität und einen aggressiven Tumor als eine eigenständige Variable dar, da eine erst im Verlauf, meist zur Behandlung von Immuntoxizitäten, begonnene Steroidtherapie zu keinem schlechteren Outcome führte.

## 2.5 Kurativ-intendierte Lokaltherapie bei Patienten mit synchron oligometastasiertem Lungenkarzinom

Frost N, Tessmer A, Schmittel A, van Laak V, Raspe M, Ruwwe-Glösenkamp C, Brunn M, Senger C, Böhmer D, Ochsenreither S, Temmesfeld-Wollbrück B, Furth C, Schmidt B, Neudecker J, Rückert JC, Suttorp N, Witzenrath M, Grohé C. Local ablative treatment for synchronous single organ oligometastatic lung cancer – A propensity score analysis of 180 patients.

*Lung Cancer 2018 Nov;125:164-173. doi: 10.1016/j.lungcan.2018.09.021*.

In der oligometastasierten Situation besteht ein potentiell kurativer Behandlungsansatz durch die Hinzunahme ablativer Verfahren für Primarius und Metastase. Der zusätzliche Nutzen der Lokaltherapie war jedoch bislang aufgrund fehlender klinischer Evidenz durch Untersuchungen mit entsprechenden Kontrollgruppen nicht hinreichend quantifiziert.

Für diese Studie wurde daher für 90 lokal kurativ behandelte Patienten anhand von 12 patienten- bzw. tumorspezifischen Charakteristika mittels 1:1 Propensity Score Matching eine vergleichbare Kontrollgruppe modelliert, die im Rahmen des „standard of care“ eine System- sowie ggf. zusätzliche Lokaltherapie in palliativer Intention erhalten hatte. Eine synchrone Oligometastasierung wurde als 1-4 Fernmetastasen in einem Organ definiert, die in einem Zeitraum von maximal 90 Tagen nach Stellung der Primärdiagnose auftraten. Die Mehrheit der Patienten wies eine ZNS-Metastasierung auf (57%). Eine zusätzliche kurative Lokaltherapie reduzierte die Wahrscheinlichkeit für einen Tumorprogress signifikant (PFS, HR 0.30, $p<0.001$) und war ebenfalls mit einem deutlich verlängerten Gesamtüberleben assoziiert (OS, HR 0.42, $p<0.001$). Im Falle eines Rezidivs nach kurativer Behandlung war bei einem Drittel der Patienten aufgrund einer

weiterhin bestehenden Oligometastasierung eine erneute kurative Behandlung möglich. Bei einem auch in der Kontrollgruppe mit 22.5 Monaten deutlich verlängerten OS scheinen Patienten mit synchroner Oligometastasierung eine insgesamt günstigere Prognose aufzuweisen.

# 3 Diskussion

Die Therapie des metastasierten Lungenkarzinoms hat sich in den letzten Jahren zunehmend diversifiziert, weg von der Platindoublette für alle und hin zu patientenindividuellen Behandlungsansätzen. Fortschritte in der Diagnostik gingen Hand in Hand mit der Entwicklung neuer zielgerichteter Substanzen und mit der Immunonkologie wurde ein gänzlich neues Feld beschritten. Häufig fehlt es jedoch an validen, in der klinischen Alltagsroutine einsetzbaren praktikablen Biomarkern, anhand derer das potentielle Therapieansprechen abgeschätzt werden kann.

Der diagnostische Biomarker TTF-1 unterscheidet zwei prognostisch unterschiedliche Phänotypen pulmonaler Adenokarzinome [53, 54]. TTF-1 positive Karzinome stammen von Typ II-Pneumozyten und Clarazellen ab und sind meist hochdifferenziert [55], während sich TTF-1 negative Tumore aus dysplastischen Mukosazellen der zentralen Lungenabschnitte heraus entwickeln [56]. Letztere teilen mit Plattenepithelkarzinomen neben der anatomischen Lokalisation auch eine starke Tabakassoziation sowie höhere Raten prognostisch ungünstiger Nebennierenmetastasen [57]. Das in der vorliegenden Arbeit aufgezeigte schlechtere Überleben unter Pemetrexed-haltiger Chemotherapie [58] legt somit auch ein biologisches Verhalten nahe, das dem pulmonaler Plattenepithelkarzinome ähnelt. Analog hierzu profitieren Patienten mit TTF-1 negativem Adenokarzinom auch nicht von einer antiangiogenen Behandlung mit dem VEGF-Inhibitor Bevacizumab, der lediglich bei Adenokarzinom- nicht aber plattenepithelialer Histologie wirksam ist [59]. In klinischen Studien ist TTF-1 als Stratifizierungsmarker bislang nicht untersucht worden. Aufgrund der beschriebenen negativen prognostischen Bedeutung wird möglicherweise ein deutlich geringerer Anteil TTF-1 negativer Patienten in klinischen Studien behandelt, da diese die engen Ein- und Ausschlusskriterien häufiger nicht erfüllen. Obwohl die vorliegende Arbeit lediglich den prädiktiven Wert von TTF-1 im Rahmen platinbasierter Erstlinientherapie untersuchte können die Ergebnisse

auch im aktuellen Therapiealgorithmus von Nutzen sein, da Patienten mit einer PD-L1 Expression <50% derzeitig eine kombinierte Immunchemotherapie erhalten. Hier besteht für den Behandler die Wahl zwischen Pemetrexed-basierten [60, 61] und Pemetrexed-freien Regimen [62-64].

Bei einer PD-L1 Expression ≥50% hingegen kann eine Monotherapie mit Pembrolizumab angewendet werden [20, 65, 66], die in der KEYNOTE-024 Studie verglichen mit dem bisherigen Standard einer Platindoublette zu einem deutlich verlängerten PFS (HR 0.50; 95% CI, 0.37 – 0.68; p<0.001) wie OS (HR, 0.63; 95% CI, 0.47 – 0.86; p=0.002) führte [36, 67]. Trotz des teils langanhaltenden Ansprechens über mehrere Jahre profitiert ein relevanter Anteil an Patienten trotz hoher PD-L1 Expression nicht oder nur kurz von der Therapie. Da die Anzahl somatischer Mutationen im Tumorgenom mit dem Ansprechen auf eine Immuntherapie korreliert und somit prädiktiv ist [46, 68, 69], kann deren Quantifizierung alternativ als Tumormutationslast („tumor mutational burden“, TMB) bestimmt werden. Mehrere methodenbedingte Einschränkungen haben jedoch bislang die routinemäßige Verwendung verhindert. So ist die TMB-Bestimmung im Vergleich zu einer Panel-basierten Diagnostik („next generation sequencing“, NGS) zeitaufwändiger und teurer, es wird mehr Gewebe benötigt und letztlich sind die angewendeten Verfahren und Grenzwerte für das Reporting bislang nicht standardisiert worden [70-74]. Es erscheint daher sinnvoll, mögliche zusätzliche prädiktive Faktoren aus dem bereits aktuell im Rahmen der Routinediagnostik erfolgenden NGS abzuleiten. KRAS-Mutationen könnten aufgrund ihrer Assoziation mit höherer PD-L1 Expression, starkem Nikotinkonsum und einem mit Immunzellen angereicherten TME geeignete Kandidaten sein [47, 75]. Allerdings ist die klinische Datenlage diesbezüglich heterogen. Während eine Metaanalyse aus Zweit- und Nachfolgelinienstunden einen günstigeren Verlauf unter Immuntherapie bei KRAS-Mutationen nahelegte [76], zeigte sich sowohl im italienischen Härtefallprogamm mit Nivolumab wie einer französischen Kohorte kein Unterschied [49, 77]. Allerdings zeichneten sich alle Untersuchungen durch eine

ausgeprägte Heterogenität hinsichtlich PD-L1 Expression und Therapielinie aus und nur ein geringer Anteil an Patienten hatte eine Erstlinienbehandlung erhalten (8.5%). Weiterhin ist die Gruppe der KRAS-Mutationen klinisch wie biologisch keineswegs homogen konfiguriert. So treten G12C-Mutationen hauptsächlich bei Rauchern und G12D-Mutationen eher bei Nichtrauchern auf [78]. Die daraus resultierende unterschiedliche Tumormutationslast könnte sich somit auch in ein divergentes Ansprechen auf Immuntherapie übersetzen [79]. In einer explorativen Analyse der KEYNOTE-042 Studie waren G12C-Mutationen tatsächlich auch mit einer tendenziell höheren Ansprechrate sowie längerem PFS und OS assoziiert. Allerdings konnten Patienten mit jedweder PD-L1 Expression in die Studie eingeschlossen werden und es liegt bislang keine gesonderte Auswertung der PD-L1 Hochexprimierer vor [48]. Analog zu KRAS zeichnen sich TP53-mutierte Tumore durch höhere PD-L1 Expression wie Tumorneoantigene aus [80, 81] und zeigen ein besseres Ansprechen und Überleben auf Immuntherapie [82]. In der vorliegenden Arbeit profitierten insbesondere Patienten mit $KRAS^{G12C}$/TP53-Komutationen und sprachen langanhaltend auf eine Behandlung mit Pembrolizumab an [83]. Pathophysiologisch könnten dem komplementäre, bzw. synergistische Effekte auf dysfunktionale Reperaturmechanismen im Zellzyklus mit konsekutiv erhöhter Tumormutationslast sowie auf die PD-L1 Expression zugrundeliegen. Molekular derartig konfigurierte Tumore sind stark immunogen, zeichen sich durch ein entzündliches TME aus [81] und weisen somit alle Merkmale eines gut auf Immuntherapie ansprechenden Tumors auf [45]. Eine a priori-Aussage könnte daher mittels genauer Analyse des KRAS- und TP53-Mutationsstatus bei Patienten mit metastasiertem, PD-L1 hochexprimierendem Adenokarzinom möglich sein. Da jeder Patient in der nicht-kurativen Behandlungssituation eine NGS-Paneldiagnostik erhalten sollte, liegen die notwendigen Informationen bei Behandlungsbeginn zumeist vor. Der große Vorteil gegenüber einer TMB-Testung besteht zudem in der Fokussierung auf ein relativ umschriebenes Genpanel, welches gleichwohl ausreichend zur Beantwortung der relevanten Fragestellungen sein sollte. Inwiefern der KRAS/TP53-Status über seine

prognostische Wertigkeit hinaus auch einen prädiktiven Nutzen hat ist bislang nicht geklärt. Aus Zeiten der klassischen Platindoublette sind jedoch keine Unterschiede bzgl. Mutationsstatus und Outcome berichtet worden [84-86], so dass den Mutationen seit Einführung immunonkologischer Behandlungsansätze durchaus ein prädiktiver Wert zugeschrieben werden kann.

Während prädiktive Biomarker für eine Immuntherapie somit derzeitig noch eingehend erforscht und evaluiert werden (müssen), ist deren Stellenwert beim onkogen alterierten Lungenkarzinom unstrittig [25]. Aufgrund von im Bereich der Kinasedomäne oder alternativer Signalwege im Verlauf auftretender Resistenzen kommt es unter zielgerichteter Behandlung jedoch unweigerlich zum Progress [87]. Weiterhin stellt die Entwicklung (symptomatischer) Hirnmetastasen aufgrund des mittlerweile deutlich verlängerten Überlebens eine Herausforderung dar [31, 88, 89], da nicht alle TKIs gleich gut liquorgängig sind [90, 91]. Die ungünstigste Konstellation einer leptomeningealen Tumoraussaat mit ausgeprägten klinischen Symptomen und schlechtem Outcome tritt beim molekular alterierten Lungenkarzinom ebenfalls häufiger auf [92]. Neue Substanzen sollten daher einerseits bekannte Resistenzmechanismen abdecken und andererseits eine gute intrakranielle Penetration aufweisen. Beim Tumorprogress werden zunehmend gewebe- oder blutbasierte („liquid biopsy“) Re-Biopsien durchgeführt, um über eine erneute molekulare Charakterisierung des Tumors die ideale Nachfolgetherapie auszuwählen. So ist bspw. beim EGFR-mutierten Lungenkarzinom eine Behandlung mit dem Drittgenerations-TKI Osimertinib nur nach Nachweis einer unter Erst- oder Zweitgenerations-TKI aufgetretenen T790M-Mutation möglich [93]. Im Rahmen dieser zumeist als NGS durchgeführten (seriellen) Testung werden TP53-Mutationen regelhaft mitbestimmt. In der vorliegenden Arbeit waren diese mit deutlich reduziertem PFS und OS assoziiert [94] und scheinen einen intrinsischen, TKI-unabhängigen Resistenzmechanismus darzustellen [50, 51, 95], der sich zudem unabhänig von der vorliegenden Treibermutation manifestiert [96]. Neben einem TP53-

assoziierten Verlust prognostisch relevanter antiproliferativer und antiapoptotischer Wirkungen scheint eine verminderte Wirksamkeit zielgerichteter Therapien zu bestehen [97]. TP53-Mutationen stellen somit je nach Tumorbiologie mit stark immunogenen, KRAS-positiven und Nikotin-assoziierten Entitäten einerseits und onkogen abhängigen Erkrankungen andererseits ein zweischneidiges Schwert dar: Wo mit immunonkologischer Behandlung ein guter Therapieerfolg möglich ist, resultiert eine deutlich geringere Effektivität beim molekular alterierten Lungenkarzinom.

Die Übertragbarkeit von Studienergebnissen auf die Behandlungsrealität im klinischen Alltag stellt eine relevante, sich stets auf Neue stellende Frage dar. Der Wunsch nach übersichtlichen Studienprotokollen für eine „Allcomer“-Population steht hier seit vielen Jahren im Widerspruch zu einer komplexen Studienlandschaft mit stetig zunehmender Anzahl an Ein- und Ausschlusskriterien sowie Screening- und Studienprozeduren [98]. Klinisch relevant aber deutlich unterrepräsentiert sind beispielsweise Patienten in reduziertem Allgemeinzustand, mit aktiven Hirnmetastasen sowie unter dauerhafter Steroidbehandlung. Obwohl ein Performance-Status ≥2 bei etwa jedem dritten Patienten vorliegt [99-101], ist diese Gruppe in Studien kaum vertreten. Immuntherapie scheint der bislang gewonnenen Evidenz zufolge hier auch bei PD-L1 Positivität ≥50% deutlich weniger effektiv zu sein [102, 103]. Allerdings wird auch hier eine oberflächliche Betrachtung der komplexen Situation nicht gerecht, da eine Vielzahl an einerseits Tumor- und andererseits Komorbiditäts-bedingten Faktoren für den reduzierten Allgemeinzustand ursächlich sein kann [104]. Dementsprechend war der Therapieerfolg in der vorliegenden Arbeit stark von der Ursache des PS ≥2 abhängig. Lediglich Patienten mit tumorassoziiert reduziertem PS profitierten von einer effektiven Behandlung, die sich im Verlauf in einem gebesserten Allgemeinzustand niederschlug [105]. Hirnmetastasen liegen bei Erstdiagnose bei 20-25% der Patienten vor [106, 107], machen in Studien mit Immuntherapie allerdings nur einen Bruchteil davon aus [108]. Und während die meisten Studienprotokolle nur vorbehandelte stabile Metastasen

erlauben, erhält im klinischen Alltag nur ein geringer Anteil an Patienten eine Vorbehandlung [109], so dass die Beurteilung der intrakraniellen Wirksamkeit einer Immuntherapie aus Studien nicht klar abgeleitet werden kann. Dies ist jedoch für den Alltag von immenser Relevanz, da eine vorgeschaltete Strahlentherapie nicht immer möglich, bzw. ggf. auch nicht notwendig ist. Die Ergebnisse einer Phase 2-Studie mit Pembrolizumab bei unbehandelten oder progredienten Hirnmetastasen legen eine Wirksamkeit nur für PD-L1 positive Patienten (≥1%) nahe [110]. In der Berliner Pembrolizumabkhohorte hatte ⅓ der Patienten mit asymptomatischen Hirnmetastasen keinerlei Lokaltherapie für den Kopf erhalten, so dass bei sorgfältiger Vorauswahl ein abwartendes Verhalten hinsichtlich Strahlentherapie gerechtfertigt erscheint [105]. Schließlich erhalten bis zu 2% der Erwachsenen eine dauerhafte Behandlung mit Steroiden [111, 112]. Während diese Patienten aus klinischen Studien ausgeschlossen waren, legen dem klinischen Behandlungsalltag entnommene Beobachtungen einen Zusammenhang zwischen Gabezeitpunkt und Ansprechen auf Immuntherapie nahe. Insbesondere scheint eine Medikation zu Behandlungsbeginn nachteilig zu sein [113]. Inwiefern Steroide tatsächlich einen unabhängigen Einflussfaktor darstellen oder nicht vielmehr Ausdruck von Komorbidität und aktiver Tumorerkrankung sind ist bislang nicht geklärt.

Eine synchrone Oligometastasierung mit potentiell kurativem Behandlungsansatz ist mit 7-25% bei Diagnosestellung nicht selten [40, 114, 115]. Dennoch beruht die Behandlungsevidenz weitgehend auf retrospektiven Fallserien und unterliegt womöglich einem Selektionsbias, so dass die berichteten Überlebensdaten womöglich eher einer Positivselektion denn der spezifischen Behandlung als solcher zuzuschreiben sind [116]. Das im Verhältnis zum erwartbaren Überleben im Stadium IV deutlich verbesserte Outcome der Kontrollgruppe der aus drei Berliner Lungenkrebszentren zusammengetragenen Patienten mit OMD läge diesen Schluss nahe [117]. Allerdings hatte ein relevanter Anteil eine palliative Strahlentherapie erhalten, die bei gegebenem

Dosis-Effektivitäts-Zusammenhang ebenfalls zu einer Verbesserung des Überlebens geführt haben mag [118]. Der zusätzliche Nutzen einer Lokaltherapie bei OMD ist zwischenzeitlich auch durch Studien untermauert worden. So verlängerte sich das PFS bei Patienten mit synchroner bzw. unter Erstlinientherapie induzierter OMD und lokaler Konsolidierung mittels Stereotaxie von 3.9 auf 11.9 Monate [119]. Zudem profitierten auch Patienten der Kontrollgruppe, die beim Progress eine zusätzliche effektive Lokaltherapie erhielten [120]. Bislang fokussierte sich eine OMD-Behandlung hauptsächlich auf Patienten mit NSCLC. Da sich der Nutzen einer hochdosierten lokalen Strahlentherapie jedoch auch beim SCLC belegen lässt, sollten diese Patienten ebenfalls für entsprechende Behandlungsverfahren evaluiert werden [121]. Bis zu welchem Ausmaß einer Metastasierung Patienten überhaupt von einem zusätzlichen Lokalverfahren profitieren ist weiterhin ungeklärt. Eine tatsächlich kurativ-intendierte Behandlung erscheint bei gutem Allgemeinzustand für thorakal nodal-negative Adenokarzinome mit solitärer Hirnmetastase möglich [122]. Eine mittlerweile solide Datengrundlage legt darüber hinaus eine teils deutlich verlängerte Palliation für entsprechend behandelte Patienten nahe. Historisch betrachtet wurden zunächst die meisten Patienten operiert [40]. Zwischenzeitlich hat sich die zumindest theoretisch beliebig oft wiederholbare stereotaktisch-geführte Strahlentherapie als Alternative etabliert [123]. Ein direkter Vergleich zwischen Chirurgie und Strahlentherapie zur Beantwortung der Frage der besten Behandlungsoption steht noch aus. Der Stellenwert einer Systemtherapie im Kontext der OMD ist aktuell noch nicht hinreichend beleuchtet worden. Bislang erfolgt die Behandlung analog der üblichen Empfehlungen im Stadium IV, die Frage der optimalen Therapiemodalität und –sequenz ist jedoch noch offen. Für eine zusätzliche Immuntherapie unabhängig vom PD-L1 Status konnte im indirekten Vergleich mit historischen Daten zwar ein verbessertes PFS erzielt werden. Dieses unterscheidet sich jedoch nicht wesentlich vom PFS der vorliegenden Arbeit, in der aufgrund des Erfassungszeitraums kein Patient eine Immuntherapie erhalten hatte [124].

# 4 Zusammenfassung und Ausblick

Therapeutische und diagnostische Fortschritte der letzten Jahre haben in Kombination mit einer patientenindividuellen, Biomarker-stratifizierten Therapie das Überleben von Patienten mit metastasiertem Lungenkarzinom zum Teil erheblich verbessert. Hierzu zählen zielgerichtete Behandlungen des onkogen alterierten Lungenkarzinoms sowie eine Immuntherapie mit Pembrolizumab bei PD-L1 hochexprimierenden Tumoren (≥50%). Mitunter mangelt es jedoch weiterhin an im klinischen Alltag verwendbaren prädiktiven Biomarkern, um im Vorfeld der Behandlung den zu erwartenden Nutzen besser einschätzen zu können.

Für den diagnostischen Biomarker TTF-1 wurde in Abhängigkeit der verwendeten platinbasierten Erstlinienchemotherapie eine zusätzliche prädiktive Wertigkeit bei TTF-1 negativen Adenokarzinomen erarbeitet. Diese biologisch wie klinisch pulmonalen Plattenepithelkarzinome ähnelnden Tumore sind mit Pemetrexed-freien Behandlungsregimen deutlich effektiver zu behandeln, was im aktuellen Therapiealgorithmus mit indizierter Immunchemotherapie für alle Patienten mit einer PD-L1 Expression <50% von anhaltend hoher Relevanz ist.

Bei erwartbar gutem Ansprechen auf eine Monotherapie mit Pembrolizumab bei einer PD-L1 Expression ≥50% zeigt die klinische Realität mitunter einen leider teils nur kurz anhaltenden Effekt. Eine genauere Einschätzung des potentiellen Therapieerfolgs ist möglicherweise durch die Hinzunahme des KRAS- und TP53-Mutationsstatus möglich. Beide Mutationen zeichnen sich nicht nur durch eine insgesamt höhere PD-L1 Expression aus, sondern korrelieren auch mit einer höheren Tumormutationslast und einem immunologisch aktiven Tumormicroenvironment. Ein langanhaltender Therapieeffekt bei Patienten mit $KRAS^{G12C}$/TP53-Komutationen kontrastiert mit teils erheblich reduziertem Überleben im Falle anderer molekularer Konstellationen. Eine prognostische Einschätzung scheint somit durch ein fokussiertes Genpanel möglich zu

sein, welches bereits jetzt im Rahmen der im Stadium IV etablierten Paneldiagnostik weitgehend flächendeckend zur Verfügung steht.

Was ein gutes Ansprechen auf Immuntherapie erwarten lässt ist beim onkogen alterierten Lungenkarzinom nachteilig. Hier lösen TP53-Komutationen die onkogene Abhängigkeit zum Teil auf und reduzieren die Wirksamkeit einer zielgerichteten Behandlung. So waren progressionsfreies und Gesamtüberleben in einer Auswertung des deutschen Härtefallprogramms mit dem Drittgenerations-ALK/ROS1-Inhibitor Lorlatinib deutlich reduziert. Dies gilt jedoch unabhängig von der verwendeten Substanz, da TP53-Mutationen beim zielgerichtet behandelbaren Lungenkarzinom wahrscheinlich einen generellen, unabhängigen Resistenzmechanismus darstellen.

Inwiefern sich angesichts rigider Ein- und Ausschlusskriterien für klinische Studien die abgeleiteten Ergebnisse auf alle Patienten übertragen lassen ist eine Frage von regelmäßiger klinischer Relevanz. Während eine Erstlinienbehandlung mit Pembrolizumab bei tumorbedingt reduziertem Allgemeinzustand und bei asymptomatischer ZNS-Metastasierung durchaus effektiv sein kann, bestimmt das Ausmaß an Komorbiditäten die Gesamtprognose, die bei den Betroffenen auch durch eine weniger toxische Immuntherapie nicht wesentlich verbessert werden kann. Das unter Steroiden zu Behandlungsbeginn beobachtete deutlich verkürzte Überleben reflektiert eine aggressive Tumorerkrankung und ausgeprägte Komorbidität und keinen Kausalzusammenhang.

Patienten mit synchroner oligometastasierter Erkrankung können potentiell kurativ behandelt werden. Die Hinzunahme zusätzlicher lokalablativer Behandlungsverfahren verbessert das Überleben dieser insgesamt prognostisch günstigeren Kohorte nochmals erheblich. Der Stellenwert einer Biomarker-stratifizierten Systemtherapie bei der OMD, bzw. das optimale Verhältnis von Lokal- und Systemtherapie sind noch nicht hinreichend geklärt.

Eine integrale und flächendeckende molekulare Tumorcharakterisierung könnte künftig in Kombination mit den entsprechenden klinischen Daten weitere aufschlussreiche Hinweise zur effektiven Behandlung des metastasierten Lungenkarzinoms ergeben. Weiterhin ist die Sammlung klinischer Routinedaten für in Studien nicht oder unzureichend repräsentierte Patientengruppen von besonderer Bedeutung, für die ansonsten in Ermangelung entsprechender Evidenz keine belastbare Aussage zum Therapieerfolg möglich ist. Zusätzlich sollten jedoch alle Anstrengungen unternommen werden, diese Patienten tatsächlich auch im Rahmen klinischer Prüfungen behandeln zu können.

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# Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

…….  …….

Datum  Dr. Nikolaj Frost