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Prädiktive Biomarker und klinische Prognosefaktoren in der Radiochemotherapie von lokal fortgeschrittenen Lungenkarzinomen

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Vorgelegt von
Kathrin Johanna Gennen

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Einleitende Zusammenfassung

der schriftlichen, kumulativen Promotion

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Eidesstattliche Versicherung

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Ich erkläre hiermit an Eides statt,

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in der Radiochemotherapie
von lokal fortgeschrittenen Lungenkarzinomen

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München, 08.07.22 K. Gennen

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Inhaltsverzeichnis

I. Abkürzungsverzeichnis

Deutsches Abkürzungsverzeichnis

ALK:	Anaplastische Lymphomkinase
CT:	Computertomographie
18F-FDG:	Fluor-18-Fluorodesoxyglucose
IMRT:	Intensitätsmodulierte Radiotherapie
MeV:	Megaelektronenvolt
NK-Zellen:	Natürliche Killerzellen
PET:	Positronenemissionstomographie
Ros1:	Protoonkogen-Tyrosin-Protein-Kinase-1
TILS	Tumorinfiltrierende Lymphozyten
TNM:	Tumor, Nodus, Metastasen
UICC:	Union for International Cancer Control
vs.:	Versus

Englisches Abkürzungsverzeichnis

ART:	Adaptive Radiotherapy
BRAF:	V-raf murine sarcoma viral oncogene homolog B1
CD8:	Cluster of Differentiation 8
CHART:	Continuous hyperfractionated accelerated radiotherapy
CTCAE:	Common Terminology Criteria for Adverse Events
CTLA-4:	Cytotoxic T-lymphocyte-associated Protein 4
CTV:	Clinical Target Volume
ECOG:	Eastern Co-operative of Oncology Group
EGFR:	Epidermal Growth Factor Receptor
GTV:	Gross Tumor Volume
IASLC:	International Association for the Study of Lung Cancer
MHC-I:	Major Histocompatibility Complex-I
NSCLC:	Non-small cell lung cancer
OS:	Overall Survival

PD-L1: Programmed-Death ligand 1

PD-1: Programmed cell death protein 1

PORT: Postoperative Radiotherapy

SCLC: Small cell lung cancer

VMAT: Volumetric Modulated Arc Therapy

II. Publikationsliste

Erstautorenschaft:

Gennen K, Käsmann L, Taugner J, Eze C, Karin M, Roengvoraphoj O, Neumann J, Tufman A, Orth M, Reu S, Belka C, Manapov F.

Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy

Radiation Oncology 2020; 15(1):5. doi:10.1186/s13014-019-1453-3

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Koautorenschaft I:

Roengvoraphoj O, Käsmann L, Eze C, Taugner J, Gjika A, Tufman A, Hadi I, Li M, Mille E, Gennen K, Belka C, Manapov F.

Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy

Translational Lung Cancer Research 2020;9(3):541–8. doi:10.21037/tlcr.2020.04.04

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Koautorenschaft II:

Käsmann L, Taugner J, Eze C, Roengvoraphoj O, Dantes M, Gennen K, Karin M, Petrukhnov O, Tufman A, Belka C, Manapov F.

Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Undergoing Multimodal Treatment

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III. Einleitung

III.I. Epidemiologie

Die Inzidenz von Krebserkrankungen in Deutschland liegt derzeit bei etwa 480.000 Neuerkrankungen pro Jahr (1).

Aufgrund der demografischen Altersentwicklung der Industriestaaten ist davon auszugehen, dass die Inzidenzrate weiterhin steigen wird (1,2).

Das Lungenkarzinom befällt in Deutschland als zweithäufigste Tumorentität jährlich circa 35.960 Männer und als dritthäufigste 21.500 Frauen (1,3).

Es stellt zudem die Tumorentität mit der höchsten Mortalitätsrate dar und ist in Deutschland die dritthäufigste Todesursache (1,4,5).

Zu beachten ist zudem eine geschlechterbezogene gegenläufige Entwicklung: Die Anzahl der Neuerkrankungen bei Männern zeigt sich derzeit rückläufig, wohingegen die Lungenkrebsrate bei Frauen stetig zunimmt (6). Dies ist vor allem auf den zunehmenden Anteil der rauchenden Frauen in der deutschen Gesellschaft zurückzuführen (1,7–9).

Diese Epidemiologie spiegelt die klinische und auch wirtschaftliche Relevanz der wissenschaftlichen Auseinandersetzung mit diesem Thema wider.

Das Lungenkarzinom gliedert sich zudem in zwei unterschiedliche Morphologien: Zum einen in das nicht-kleinzellige Karzinom (non-small cell lung cancer, NSCLC), welches mit 85 Prozent den Hauptteil der Lungenkarzinome ausmacht (10), und zum anderen in das kleinzellige Lungenkarzinom (small cell lung cancer, SCLC).

Ein großer Unterschied hinsichtlich der Prognose dieser beiden Entitäten ist zu beachten: Das kleinzellige Lungenkarzinom hat aufgrund einer Tumorverdopplungszeit von 10 bis 50 Tagen (zum Vergleich: nicht-kleinzelliges Lungenkarzinom 180-300 Tage) eine deutlich schlechtere Prognose als das nicht-kleinzellige Lungenkarzinom (10). Die durchschnittliche 5-Jahres-Überlebensrate in einem lokal begrenzten Stadium (ein Hemithorax ohne Fernmetastasen) beträgt 20% bis 30% (11). Bei einer lokal fortgeschrittenen Erkrankung (Fernmetastasen) beträgt die 2-Jahres-Überlebensrate des kleinzelligen Karzinoms 5% bis 10% (11). 5-Jahres-Überlebensraten können hier nur in Einzelfällen verzeichnet werden (11). Eine weitere geschlechterspezifische Differenz betrifft die Tumorentität: Frauen sind hier sechsmal häufiger von einem Adenokarzinom betroffen als Männer (9,12).

Diese kumulative Promotion wird sich im Folgenden auf das nicht-kleinzellige Karzinom, seine

Tumorgenetik und die Etablierung seiner prognostischen Marker und Prognosefaktoren fokussieren.

III.II. Stadieneinteilung

Die Stadieneinteilung des nicht-kleinzelligen Lungenkarzinoms erfolgt zunächst anhand der TNM-Klassifikation (Tumor, Nodus, Metastasen), aus welcher sich im Anschluss das zugehörige Stadium der Union for International Cancer Control (UICC) ableitet (siehe Tabellen 1 und 2) (13).
Die 8. Auflage stellt Stand 2021 die aktuellste Version dar und wird weitestgehend als Grundlage für die Therapieplanung herangezogen (13).

Klassifikation	Stadium
Tumor (T)	**Tis** (Carcinoma in situ) **T1** (Tumordurchmesser bis 3cm) **T2** (Tumor >3cm oder <5cm oder Infiltration des Hauptbronchus, der viszeralen Pleura oder tumorbedingte Atelektase oder Pneumonie **T3** (Tumordurchmesser >5cm oder <7cm oder Infiltration der Thoraxwand, des Nervus phrenicus und des parietalen Perikards oder weiterer Tumorherd im gleichen Lungenlappen wie der Primärtumor) **T4** (Tumordurchmesser >7cm oder Infiltration von großen Gefäßen, Zwerchfell, Mediastinum, Herz, Ösophagus, Wirbelkörper oder Karina oder zusätzlicher Tumorknoten in einem anderen Lungenlappen auf der ipsilateralen Seite)
Lymphknoten (N)	**N0** (keinerlei nachweisbare Lymphknotenmetastasen) **N1** (Metastase ipsilateral oder peribronchial in hilären Lymphknoten) **N2** (Metastase in ipsilateralen Lymphknoten im Mediastinum oder in subkarinalen Lymphknoten) **N3** (Metastasen in kontralateralen Lymphknoten)
Metastasen (M)	**M0** (keinerlei nachweisbare Fernmetastasen) **M1** (Fernmetastasen) **M1a** (separater Tumorherd im kontralateralen Lungenflügel) **M1b** (Pleurabefall) **M1c** (maligner Pleura- oder Perikarderguss, isolierte Fernmetastase mit Sitz in einem extrathorakalen Organ, multiple Fernmetastasen in mehreren Organen)

Tabelle 1: **TNM Klassifikation (8.Auflage) des nicht-kleinzelligen Lungenkarzinoms nach dem International Association for the Study of Lung Cancer (IASLC) Lung Cancer Staging Projekt** (14–16)

UICC Stadium	TNM Kriterium
0	Carcinoma in situ
I	T1 N0 oder T2 N0, geringe Tumorausbreitung
II	T2b N0 oder T1 N1 oder T3 N0, Tumor auf die Lunge begrenzt
IIIa **IIIb**	T1-T2, N2 oder T3, N1 oder T4, N0/N1 T1-T2, N3 oder T3-T4, N2 oder T3-T4, N3
IVa **IVb**	Jedes T, M1a oder M1b Jedes T, M1c

Tabelle 2: **UICC Stadien (8. Auflage) des nicht-kleinzelligen Lungenkarzinoms** (13,17)

III.III. Histologie und Molekulargenetik

Ein weiterer wichtiger Faktor in Bezug auf die Therapieoptionen ist die histologische Einteilung des nicht-kleinzelligen Lungenkarzinoms. Unterschieden werden hierbei das Plattenepithelkarzinom, das Adenokarzinom, das großzellige Karzinom, das Karzinoid sowie das adenosquamöse Karzinom (18).

Mit 30-40 Prozent stellt hierbei das Plattenepithelkarzinom die häufigste Entität dar, gefolgt vom Adenokarzinom mit 30 Prozent (10).

Im Laufe der medizinischen Entwicklung und der damit einhergehenden Fortschritte gewinnt zudem die Molekulargenetik bei der histologischen Einteilung eines Tumors zunehmend an Relevanz.

Aus molekulargenetischer Sicht sind insbesondere der Programmed-Death ligand 1 (PD-L1), der epidermale Wachstumsfaktor-Rezeptor (Epidermal Growth Factor Receptor, EGFR), die Protoonkogen-Tyrosin-Protein-Kinase-1 (Proto-oncogene tyrosine-protein kinase, ROS-1), die anaplastische Lymphomkinase (ALK) und die B-Raf-Kinase (proto-oncogene B-Raf, BRAF) von klinischer Relevanz (19,20).

PD-L1 stellt ein Transmembranprotein dar, welches an den dazugehörigen Rezeptor Programmed cell death protein 1 (PD-1) bindet und damit das Immunsystem zugunsten der

Tumorzellen beeinflusst (21). Über die Induktion eines Interleukin-10 Signalwegs in den Monozyten und eine konsekutive Hemmung der Interleukin-2 Expression sowie der T-Zell Proliferation wird die Immunantwort gehemmt (22). Die Unterdrückung des wachstumshemmenden Einflusses des Immunsystems führt in Folge zur ungehinderten Vergrößerung des Tumors (23).

Ein weiterer immunmodulierender Faktor ist das Cluster of Differentiation Protein 8 (CD8). CD8 ist ein Korezeptor des T-Zellrezeptors und wird auf zytotoxischen und regulatorischen T-Zellen sowie auf natürlichen Killerzellen (NK-Zellen), Thymozyten und dendritischen Zellen exprimiert (18). Das Protein bindet an den Major Histocompatibility Complex-I (MHC-I) und sorgt für eine stabile Bindung eines Antigens an eine T-Zelle (24).

Alle zellkernhaltigen Zellen des menschlichen Körpers sind in der Lage MHC-I-Komplexe zu produzieren: Es werden kurze Peptide synthetisiert, welche im Proteasom der Zelle gebildet und über einen Antigenpeptidtranstporter in das endoplasmatische Retikulum geschleust werden (24). Hier werden die MHC-I-Komplexe dann gebunden (25).

Zytotoxische T-Zellen erkennen nun mit ihrem CD8-Rezeptor, ob es sich um virale oder mutierte Proteine handelt (25). Über Kostimulatoren kann nach Aktivierung der CD8 positiven T-Zelle eine Zerstörung der MHC-I exprimierenden Zelle erfolgen (25). Tumorinfiltrierende Lymphozyten können so durch einen Tumor verursachte mutierte Zellen detektieren und angreifen (26).

Die onkologische Relevanz des CD8-Rezeptors konnte bereits im Hinblick auf die Beeinflussung des Krankheitsverlaufes des Mammakarzinoms nachgewiesen werden (27).

Hinsichtlich der Genese und der Progression des NSCLC ist CD8 jedoch derzeit Gegenstand aktueller Forschung und im Rahmen dessen auch Teil dieser Promotionsarbeit (28).

Trotz der fortschreitenden Entdeckungen der Tumorgenetik ist zu beachten, dass die verschiedenen oben genannten Expressionsmuster aufgrund der aktuellen Forschungslage bisher noch keine Etablierung derselben als Prognosefaktor zulassen (28,29).

III.IV. Immuncheckpoint-Inhibitoren

Die Entdeckung der Tumorgenetik bietet zudem neue spezifische Angriffspunkte in Bezug auf die Therapie des nicht-kleinzelligen Lungenkarzinoms, zum Beispiel in Form von Immuncheckpoint-Inhibitoren (30–32) (siehe Tabelle 3).

Immuncheckpoint-Inhibitor	Angriffspunkt	Zulassung in Deutschland
Durvalumab	Monoklonaler Antikörper gegen PD-L1	2018 (Progressionsfreies Überleben unter Durvalumab 16,8 Monate versus Placebo 5,6 Monate; 2-Jahres-Überlebensrate unter Durvalumab 66.3% vs. 55.6% in der Placebogruppe) (33)
Atezolizumab	Monoklonaler Antikörper gegen PD-L1	2017 (Gesamtüberleben unter Atezolizumab 12.6 Monate vs. Chemotherapie 8.9 Monate) (34)
Pembrolizumab	Monoklonaler Antikörper gegen PD-1	2019 (Progressionsfreies Überleben unter Pembrolizumab 10,3 Monate vs. Chemotherapie 6 Monate) (35,36)
Nivolumab	Monoklonaler Antikörper gegen PD-1	2016 (Progressionsfreies Überleben unter Nivolumab 4.2 Monate vs. Chemotherapie 5.9 Monate) (37)
Ipilimumab	Monoklonaler Antikörper gegen cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4)	2011 (Kombinationstherapie Ipilimumab + Nivolumab wirksamer als Monotherapie mit Nivolumab, Progressionsfreies Überleben unter Ipilimumab + Nivolumab 14.9 Monate vs. Chemotherapie 6.2 Monate) (38)

Tabelle 3: **Beispiele für Immuncheckpoint-Inhibitoren und ihre klinische Relevanz**

Doch die Entwicklung solcher individueller Therapieverfahren geht auch mit klinisch relevanten Nebenwirkungen einher. Diese können sich prinzipiell an allen Organsystem manifestieren. Am häufigsten sind Hautreaktionen (46-72%), Kolitiden (22-48%), Hepatitiden (7-33%) und Endokrinopathien (12-34%) (39).

Seltenere, jedoch schwerwiegende Nebenwirkungen sind Pneumonitiden (3-8%) (39).

Unter dem PD-1 Inhibitor Pembrolizumab beispielsweise zeigt sich eine erhöhte Inzidenz von Pneumonitiden jeden Common Terminology Criteria for Adverse Events (CTCAE) Grades (bei

Monotherapie 5%, bei Kombinationstherapie höher (40)), während unter Durvalumab vermehrt Pneumonitiden Grad 3 und 4 beschrieben wurden (33).
Insgesamt ist die Inzidenz von Pneumonitiden unter PD-1 Inhibitoren, bei denen schwere bis tödliche Nebenwirkungen in 17-21% der Fälle auftreten, höher als bei PD-L1 Inhibitoren (39). Bei PD-L1 Inhibitoren jedoch kann im Falle eines Auftretens einer Pneumonitis ein höherer Schweregrad beobachtet werden (41,42).
Weiterhin wurde in der 2018 im New England Journal of Medicine publizierten Studie von Scott J. Antonia et al. gezeigt, dass unter Durvalumab bei 30.5% der Patienten Nebenwirkungen des CTCAE Grades 3 oder 4 auftraten, in der Placebogruppe hingegen nur bei 26.1% (33).
J.D. Wolchok et al. zeigten in ihrer Studie, dass sogar 96% der Patienten, welche eine Kombinationstherapie bestehend aus einem Checkpointinhibitor (Nivolumab oder Ipilimumab) und einer Chemotherapie erhielten, unerwünschte Nebenwirkungen jeden Grades nach Therapiebeendigung aufwiesen, allen voran Hautreaktionen (34%) und Endokrinopathien (23.8%) (20,39).

III.V. Allgemeinzustand

Neben den therapiebedingten biologischen Faktoren spielen auch patientenorientierte Faktoren eine entscheidende Rolle in der Therapie und Prognose des nicht-kleinzelligen Lungenkarzinoms (43).
Klinisch objektiviert wird der Allgemeinzustand des Patienten in Form des 1949 entwickelten Karnofsky Index und des Index der Eastern Co-operative of Oncology Group (ECOG) (siehe Tabelle 4) (43).

Karnosfky Index		ECOG	
100	Keine Einschränkungen des Allgemeinzustandes	Normale Aktivität möglich	**0**
90	Minimale Einschränkungen		
80	Geringe Einschränkungen	Einschränkungen bei	**1**
70	Arbeitsunfähigkeit	körperlicher Anstrengung	
60	Leistungsfähigkeit eingeschränkt	Geh- aber nicht arbeitsfähig	**2**
50	Benötigung von pflegerischer Hilfe, aber nicht dauernd bettlägerig		
40	Bettlägerig, dauerhaft Pflege nötig	Nur eingeschränkte	**3**
30	Krankenhauspflege notwendig	Selbstversorgung möglich	
20	Schwerst krank, Krankenhauspflege und supportive Maßnahmen nötig	Schwerst pflegebedürftig	**4**
10	Moribund		
0	Exitus letalis	Exitus letalis	**5**

Tabelle 4: **Gegenüberstellung des ECOG und des Karnofsky Index** (10)

III.VI. Radiologische / Nuklearmedizinische Diagnostik

Hauptbestandteil der radiologischen Diagnostik des Lungenkarzinoms ist die computertomographische (CT) Thoraxübersichtsaufnahme, die sich bei suspektem Befund im Röntgen Thorax anschließt (11).

Mit Hilfe der Computertomographie beispielsweise kann die TNM-Situation radiologisch beurteilt werden, was im Hinblick auf die Therapieplanung essenziell ist (11). Beurteilt werden hier vor allem die Beziehung zwischen Tumor und Thoraxwand (Sensitivität und Spezifität zwischen 40% und 90% (44)) sowie maligne Pleura- beziehungsweise Perikardergüsse (Sensitivität annähernd 100%, Spezifität 40% (44)).

Hinsichtlich der nuklearmedizinischen Diagnostik und mit besonderem Augenmerk auf die erste Koautorenschaft wird im Folgenden das Positronenemissionstomographie (PET) mit Fluor-18-Fluorodesoxyglukose (FDG, 18F-FDG-PET/CT) als Radionuklid hervorgehoben.

Dieses nuklearmedizinische Schnittbildverfahren quantifiziert mittels Radionuklidaufnahme verschiedene biochemische Stoffwechselvorgänge und physiologische Funktionen (11). Die Strahlenexposition kommt in etwa der einer Röntgenübersichtsaufnahme gleich (45). Das

Glukose-Uptake wird als Standardized Uptake Value (SUV) bezeichnet und kennzeichnet solche Gewebe, die einen erhöhten Verbrauch an Glukose aufweisen (beispielsweise Tumore, Entzündungen, solide Metastasen und Lymphknotenmetastasen) (46).

Die ersten Ergebnisse zur PET-Untersuchung wurden bereits 1975 publiziert (47). Reine PET-Geräte wurden allerdings durch die geringe Ortsauflösung von 4-6 Millimetern (10) zugunsten der PET-CT Scans (höhere Ortsauflösung von bis zu 0,35 Millimetern (10)) im klinischen Alltag verdrängt (48).

Mittels oben genannter Verfahren können die UICC-Stadien des nicht-kleinzelligen Lungenkarzinoms präzise eingeteilt und basierend darauf eine anschließende Therapie geplant werden.

III.VII. Therapie

Die Therapie des nicht-kleinzelligen Lungenkarzinoms richtet sich unter anderem nach dem Tumorstadium (10,11,49).

In den operablen Stadien I, II und IIIa ist eine Lobektomie mit systematischer Dissektion der zugehörigen Lymphknoten als Therapie der Wahl in kurativer Absicht anzustreben (11).

John L. Mikell et al. bewiesen in ihrer PORT (Postoperative Radiotherapy)-Studie, dass eine postoperative Radiotherapie das mediane Gesamtüberleben von Patienten mit NSCLC verbessert (42 Monate vs. 38 Monate) (50). Die 5-Jahres-Überlebensrate von Patienten mit PORT betrug 39.8%, die 5-Jahres-Überlebensrate von Patienten ohne PORT betrug 34.7% (50).

Eine adjuvante Chemotherapie kann unter Berücksichtigung des Alters, der Komorbiditäten und der perioperativen Komplikationen im Anschluss erfolgen (10).

Mittel der Wahl sind hier platinhaltige Chemotherapeutika in Kombination mit Vinorelbin oder Taxanen (11). Beim Adenokarzinom findet vor allem eine Chemotherapie mit Pemetrexed Anwendung (11).

Ein Überlebensvorteil durch eine alleinige adjuvante Radiatio in diesen Stadien als alleinige Alternative zur Chemotherapie mit Dosen von 30-60 Gray verteilt auf 10 bis 30 Fraktionen konnte nicht belegt werden (49). Eine Kombination aus Radio- und Chemotherapie sollte hier favorisiert werden (49).

Bei Patienten in Stadium I oder II, welche aufgrund von Komorbiditäten nicht operiert werden können oder eine Operation ablehnen, konnte die CHART (continuous hyperfractionated

accelerated radiotherapy)-Studie den Nutzen der Strahlentherapie belegen (51).

Ab Stadium III sollte eine multimodale Therapie aus Bestrahlung und Chemotherapie erfolgen (11). Eine Operation kommt hier aufgrund der fortgeschrittenen Tumorausbreitung nicht mehr infrage (10). Mittel der Wahl hierbei ist Cisplatin, welches bevorzugt in 4 Zyklen verabreicht wird, in Kombination mit weiteren Chemotherapeutika, die entsprechend ihres Wirkungs- und Nebenwirkungsprofils ausgesucht werden (11). Hinsichtlich der Bestrahlung sollte eine Fraktionierung mit 1,8 bis 2 Gray täglich bei einer Gesamtdosis von 60 bis 70 Gray gewählt werden (11).

Eine kombinierte Radiochemotherapie ist hinsichtlich des Überlebens vorteilhafter als die alleinige Radiotherapie (49).

Als Erhaltungstherapie nach Radiochemotherapie eignet sich ab Stadium III Durvalumab bei Patienten mit einer PD-L1 Expression von mehr als 1% (11). Studien belegen hier eine signifikante Verlängerung des progressionsfreien Überlebens, des Gesamtüberlebens und der Überlebensrate nach 2 Jahren (33,52).

In Stadium IV mit Oligometastasierung kann ein kurativer Ansatz angestrebt werden, solange eine lokale Tumorausbreitung im Stadium TIIIa vorliegt (11).

Das Stadium IVb hingegen verlangt in der Regel einen palliativen Ansatz (11). Eingesetzt werden hier meist platinhaltige Chemotherapeutika (11). Je nach PD-L1-, EGFR-, ROS-1-, ALK- und BRAF-Mutationsstatus kann die Therapie immunmodulierend oder zytostatisch erweitert werden (11). Standardtherapie ist eine kombinierte Chemotherapie, beispielsweise mit Cisplatin, Pemetrexed und Pembrolizumab (49). Primäres Ziel in diesem Stadium ist die Reduktion der krankheits- und therapieassoziierten Komplikationen und damit die Erhaltung der Lebensqualität des Patienten (53–55).

Wie oben bereits erwähnt spielt die Strahlentherapie in der Therapie des nicht-kleinzelligen Lungenkarzinoms eine tragende Rolle. Eine australische Studie von Delaney et al., die sich mit der Anwendung von Bestrahlungen beim nicht-kleinzelligen Lungenkarzinom beschäftigte, hat gezeigt, dass über 70% der NSCLC Patienten im Verlauf auf eine Bestrahlung angewiesen sind (56).

Moderne Systeme mit bildgeführten Radiotherapien (Image Guided Radiotherapy und Gating, also das Bestrahlen in bestimmten Atemphasen) ermöglichen eine genaue Reproduzierbarkeit der Lagerung (10).

Das Zielvolumen des Bestrahlungsplanes setzt sich zusammen aus dem Gross Tumor Volume (GTV), also der makroskopischen Tumorausdehnung, und dem Clinical Target Volume (CTV), welches einem Sicherheitsrandsaum um das GTV entspricht (10). So wird eine lokale Dosissteigerung unter möglichst geringem Einbezug des umliegenden Gewebes ermöglicht (57).

Die kurative Radiotherapie wird mit Photonen unter der Verwendung konformaler Mehrfeldertechniken nach dreidimensionaler, intensitätsmodulierter beziehungsweise volumenintensitätsmodulierter Bestrahlung durchgeführt (58).

Besonderes Augenmerk gilt außerdem der Entwicklung neuer Bestrahlungstechniken.

Ein wichtiger Bestandteil derselben ist vor allem die Intensitätsmodulierte Radiotherapie (IMRT), bei der höhere Einzeldosen auf das makroskopische Tumorvolumen abgegeben werden können, während gleichzeitig die mikroskopische Tumorausdehnung mit geringeren Einzeldosen versehen werden kann (59). So kann eine größtmögliche Schonung der umliegenden Risikostrukturen, wie zum Beispiel des Ösophagus, gewährleistet werden (59,60).

Ebenso findet die Volumetric Modulated Arc Therapy (VMAT) in der klinischen Praxis Anwendung, welche eine Weiterentwicklung des IMRT darstellt (10,60). Hierbei rotiert die Strahlenquelle um den Patienten und moduliert so das Strahlenfeld unter kontinuierlicher Anpassung des Kollimators (10). Neben dem Strahlenfeld können so auch die Rotationsgeschwindigkeit, die Dosisleistung und der Kollimatorwinkel variiert werden (10). Zudem erfolgte die Entwicklung der Adaptive Radiotherapy (ART), welche eine adaptive Technik der Radiotherapie darstellt (10). Im Laufe der Therapie wird hier das Behandlungsvolumen an die aktuelle Tumorkonfiguration angepasst (10,61).

III.VIII. Zielsetzung

Ziel der ersten Studie mit dem Titel “Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy” ist die Untersuchung der verschiedenen Expressionen des programmed death-ligand 1 (PD-L1) auf Tumorzellen und des cluster of differentiation 8 (CD8) auf tumorinfiltrierenden T-Lymphozyten im Hinblick auf die mögliche Etablierung solcher Expressionsmuster als Marker für die individuelle onkologische Prognose

(62).

In dieser Promotion gilt es jedoch nicht nur die Tumorgenetik und -biologie im Hinblick auf die Prognose des NSCLC zu beleuchten, sondern auch die klinischen und bildmorphologischen Aspekte in der Diagnostik und Nachsorge. Zu diesem Zwecke wurde die erste Koautorenschaft „Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy" verfasst (63). Als letzter Punkt befasst sich die zweite Koautorenschaft „Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Under Multimodal Treatment" mit der Bedeutung des klinischen Allgemeinzustandes des Patienten und dessen Dynamik im Verlauf der Radiochemotherapie für die Prognose des nicht-kleinzelligen Lungenkarzinoms (64). Denn obwohl der Karnofsky Index bereits 1949 etabliert wurde (43), hat er unverändert heutzutage eine hohe Bedeutung im klinischen Alltag, was in Anbetracht der Geschwindigkeit und des ständigen Wandels des medizinischen Fortschrittes bemerkenswert ist.

IV. Publikationen der kumulativen Promotion

IV.I. Erstautorenschaft

Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy (62)

IV.I.I. Zielsetzung und Zusammenfassung

Die Veröffentlichung "Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TILs density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy" beschäftigt sich insbesondere mit der Tumorbiologie von nicht-kleinzelligen Lungenkarzinomen.

Ziel dieser Arbeit war die Etablierung von PD-L1 und CD8 als mögliche Prognosemarker beim nicht-kleinzelligen Lungenkarzinom (62).

Hierzu wurden retrospektiv 31 inoperable lokal fortgeschrittene nicht-kleinzellige Lungenkarzinompatienten mit ihren klinischen Daten (Alter, Geschlecht, Raucherstatus und

andere) und Initialbiopsien erfasst (62).

Aus den Initialbiopsien wurde der prognostische Einfluss der PD-L1 Expression auf den Tumorzellen (Cut-Offs: 0%, klassifiziert als negativ vs. ≥1%, klassifiziert als positiv) und der Dichte der CD8 positiven tumorinfiltrierenden Lymphozyten (Cut-Offs: 0-40%, klassifiziert als negativ vs. 41-100%, klassifiziert als positiv) auf das Gesamtüberleben, das progressionsfreie Überleben und die lokale Kontrolle untersucht (62). Es wurden insgesamt vier Tumormikromilieus unterschieden: Typ I (PD-L1 Negativität auf Tumorzellen und fehlende Invasion CD8 positiver tumorinfiltrierender Lymphozyten (TILS), implizierte Immunignoranz), Typ II (PD-L1 Positivität mit Invasion CD8 positiver TILS, implizierte adaptive Immunresistenz), Typ III (PD-L1 Negativität mit Invasion CD8 positiver TILS, mögliche Immuntoleranz) und Typ IV (PD-L1 Positivität ohne Invasion CD8 positiver TILS, implizierte intrinsische Induktion) (62). Ebenso wurde analysiert, ob und inwiefern die unterschiedlichen Expressionen von PD-L1 und CD8 miteinander agieren und einen Einfluss auf den Therapieerfolg beziehungsweise das Gesamtüberleben der Patienten haben (62).

Grafik 1 zeigt exemplarisch die oben genannten Initialbiopsien im Lichtmikroskop in 10-facher Vergrößerung (62). Gezeigt sind hier Adenokarzinome mit positivem (a, 80%) und negativem (b, 0%) PD-L1 Befund auf Tumorzellen sowie mit positiver (c, 70%) und negativer (d, 2%) CD8 Expression auf tumorinfiltrierenden Lymphozyten (62).

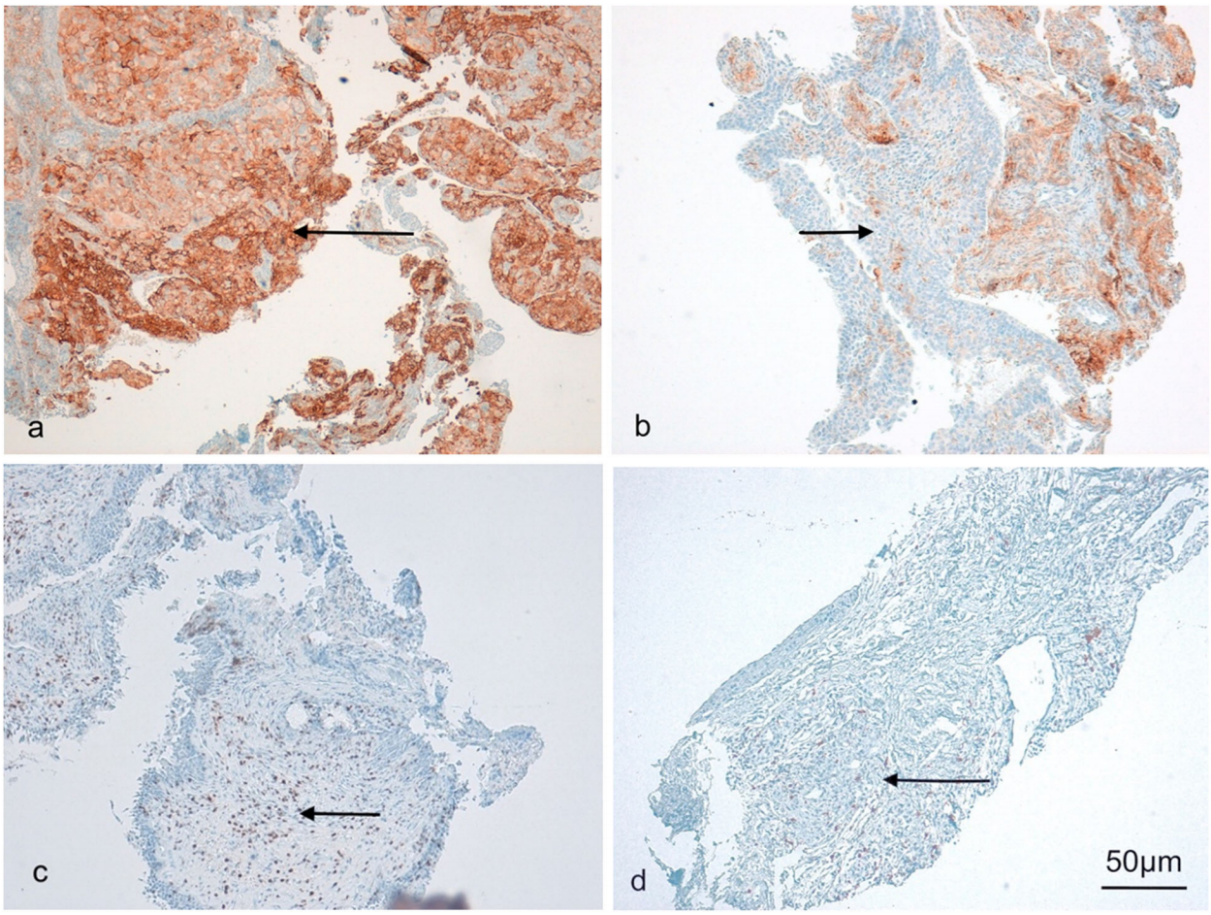

Abb. 1: **Initialbiopsien mit PD-L1 (a, b) und CD8 (c, d) Immunhistochemie in 10-facher Vergrößerung, die Pfeile zeigen positive (a, c) oder negative (b, d) Färbung an** (62)

Das mediane Überleben der Kohorte betrug 14 Monate (62).

Es konnte gezeigt werden, dass eine PD-L1 Expression auf den Tumorzellen mit einem verbesserten Gesamt- beziehungsweise progressionsfreien Überleben sowie einer besseren lokalen Kontrolle einhergeht (62). Dem begleitend ließ sich ein längeres Gesamtüberleben und eine höhere lokale Kontrolle mit einer niedrigen CD8+ tumorinfiltrierenden Lymphozytendichte feststellen (62).

Des Weiteren ließ sich ein Zusammenspiel dieser beiden Marker aufzeigen: Patienten mit negativem PD-L1 Status und einer niedrigen CD8 Dichte (Typ I) zeigten die längsten Überlebenszeiten (medianes overall survival (OS): 57±37 Monate), wohingegen Patienten mit positivem PD-L1 Status und niedriger CD8 Dichte (Typ IV) eine kürzere Überlebenszeit (medianes OS: 10±5 Monate, p=0.05) aufwiesen (62).

Zusammenfassend lässt sich daher sagen, dass die Kombination der Expression von CD8 und PD-L1 als prognostische Marker in der Therapie des lokal fortgeschrittenen nicht-kleinzelligen

Lungenkarzinoms eine große Rolle spielen (62).

Durch eine gezielte Differenzierung von Tumorimmunogenität und Tumormikromilieu können Hochrisikopatienten identifiziert, die Therapie personalisiert und möglicherweise Unter- beziehungsweise Übertherapien vermieden werden (62). Therapie-Eskalationen können somit ebenso geplant werden wie Therapie-Deeskalationen bei Patienten, die von einer nebenwirkungsreichen Immuntherapie nicht profitieren würden (62).

Eine Etablierung von PD-L1 und CD8 als Prognosemarker kann somit zur Individualisierung der Therapieregime beitragen (62).

IV.I.II. Englische Zusammenfassung der Erstautorenschaft

31 inoperable locally advanced NSCLC patients were retrospectively recorded with their clinical data (age, sex, smoker status and others) and initial biopsies (62).

From the initial biopsies, the prognostic impact of PD-L1 expression on tumor cells (Cut-Offs: 0%, classified as negative vs. ≥1%, classified as positive) and the density of CD8 positive tumor-infiltrating lymphocytes (TILS) (Cut-Offs: 0-40%, classified as negative vs. 41-100%, classified as positive) on overall survival, progression-free survival and local control was examined (62).

A total of four tumor microenvironments were distinguished: type I (PD-L1 negativity on tumor cells and lack of invasion of CD8 positive TILS, indicating immune ignorance), type II (PD-L1 positivity with invasion of CD8 positive TILS, implying adaptive immune resistance), type III (PD-L1 negativity with invasion of CD8 positive TILS, suggesting immune tolerance) and type IV (PD-L1 positivity without invasion of CD8 positive TILS, indicating intrinsic induction) (62).

The expression of PD-L1 and CD8 interaction was analyzed (62). Further analysis occurred to see how this influenced therapeutic success or overall survival of the patients (62).

Figure 1 shows representative images under a microscope with a 10x enlargement of adenocarcinomas with positive (a, 80%) and negative (b, 0%) PD-L1 staining on tumor cells and adenocarcinomas with positive (c, 70%) and negative (d, 2%) CD8 staining on tumor infiltrating lymphocytes (62).

PD-L1 expression on the tumor cells correlated with improved overall, progression-free survival and better local control (62). This was accompanied by better overall survival and local control with a low CD8+ TILS density (62).

Furthermore, an interaction of these two biomarkers could be shown: Patients with negative PD-L1 status and low CD8 density (Type I) showed the longest survival times (median OS: 57±37 months), patients with positive PD-L1 status and low CD8 density (Type IV) showed a shorter survival time (median OS: 10±5 months, p=0.05) (62).

In summary, CD8 and PD-L1 play an important role as prognostic markers in the therapy of locally advanced NSCLC (62).

A targeted differentiation between high-risk patients with mutation status and patients without mutation but have a suspicious tumor microenvironment can avoid unnecessary under- or overtherapy (62). Therapy escalations can thus be planned as well as therapy de-escalations for patients who do not benefit from immunotherapy with the many side effects (62).

Establishing PD-L1 and CD8 as prognosis markers can thus contribute to the individualization of therapy regimes (62).

IV.II. Koautorenschaft I

Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy (63)

IV.II.I. Zielsetzung und Zusammenfassung

Zweck dieser Publikation war es, einen Überlebensscore für NSCLC Patienten basierend auf 18F-FDG-PET/CT Daten zu entwickeln.

Insgesamt wurden 99 UICC Stadium IIIa oder IIIb Patienten untersucht, welche eine Stadienuntersuchung mittels 18F-FDG-PET/CT vor Beginn der Radiochemotherapie erhielten (63). Das SUVmax des Primarius sowie der Bereich zwischen den am weitesten entfernt gelegenen PET-positiven Lymphknoten (SUV ≥2,5) in zwei Richtungen (vertikal, A-Linie und horizontal, B-Linie) wurde im Hinblick auf eine mögliche Korrelation mit dem Therapieergebnis untersucht (63). Auf Basis dessen wurde dann ein Score etabliert und die Patienten in drei Risikogruppen eingeteilt: niedrig (SUVmax des Primarius ≤8 und B-Linie ≤3,7cm), mittel (SUVmax des Primarius ≥8 oder B-Linie ≥3,7cm) und hoch (SUVmax des Primarius ≤8 und B-Linie ≤3,7cm) (63). 28% der Patienten waren der niedrigen Risikogruppe zugeordnet, 46% der

mittleren und 26% der hohen (63).

Das mediane ereignisfreie Überleben betrug in der niedrigen, mittleren und hohen Risikogruppe 16, 13 und 10 Monate (p=0,002, log-rank Test) (63). Das mediane Gesamtüberleben betrug 40 Monate in der niedrigen, 23 Monate in der mittleren und 14 Monate in der hohen Risikogruppe (p=0,0001, log-rank Test) (63).

Betont werden muss hier allerdings, dass der Überlebensscore insbesondere für Patienten mit einem Adenokarzinom der Lunge eine große Relevanz hat: Das zwei-Jahres-Überleben von Patienten mit Adenokarzinom, welche der Gruppe mit niedrigem Risiko angehörten, betrug 70%; wohingegen Patienten mit Adenokarzinom der Hochrisikogruppe ein zwei-Jahres-Überleben von nur 8% zeigten (63).

Es konnte gezeigt werden, dass der entwickelte PET-CT Score als unabhängiges Prognoseinstrument beim nicht-kleinzelligen Lungenkarzinom Anwendung finden kann (63).

IV.II.II. Englische Zusammenfassung der Koautorenschaft I

The purpose of this publication was to investigate whether and to what extent a self-developed PET-CT staging score in lung cancer patients allows a prediction of the prognosis. A total of 99 UICC stage IIIa or IIIb patients were examined who received a staging score by means of 18F-FDG-PET/CT before starting chemoradiotherapy (63). The SUVmax of the primarius as well as the area between the most distant PET-positive lymph nodes (SUV ≥2.5) in two directions (vertical, A-line and horizontal, B-line) were examined with regard to a possible correlation regarding therapy outcome (63). A score was then established, and the patients were divided into three subgroups: low (SUVmax of primary ≤8 and B-line ≤3.7cm), medium (SUVmax of primary ≥8 or B-line ≥3.7cm) and high (SUVmax of primary ≤8 and B-line ≤3.7cm)-risk (63). 28% of patients were assigned to the low-risk group, 46% to the medium- and 26% to the high-risk group (63).

The median event-free survival in the low-, medium- and high-risk group was 16, 13 and 10 months (p=0.002, log-rank test) (63). Median overall survival was 40 months in the low-, 23 months in the medium- and 14 months in the high-risk group (p=0.0001, log-rank test) (63). In addition, it must be emphasized that the score is of great relevance especially for patients with an adenocarcinoma: The two-year survival of patients with adenocarcinoma who belonged to the low-risk group was 70%; whereas adenocarcinoma patients in the high-risk

group showed a two-year survival of only 8% (63).
Thus, it could be shown that the developed PET-CT score can be used as an independent prognostic tool in non-small cell lung cancer (63).

IV.III. Koautorenschaft II

Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Under Multimodal Treatment (64)

IV.III.I. Zielsetzung und Zusammenfassung

Ziel dieser Publikation war es zu ermitteln, ob und inwiefern der klinische Allgemeinzustand des Patienten mit dem Gesamtüberleben und dem progressionsfreien Überleben korreliert. Insgesamt wurden in dieser Arbeit 99 Patienten mit nicht-kleinzelligem Lungenkarzinom im UICC Stadium III im Hinblick auf ihren Allgemeinzustand (ECOG Status) vor, während und nach der Radiochemotherapie untersucht (64).
Vor Therapiebeginn wurden der Tabakkonsum und mögliche Vorerkrankungen anamnestisch erfragt (64). Des Weiteren wurde standardisiert ein Lungenfunktionstest, eine Bildgebung der Lunge in Form einer CT oder PET-CT und eine Blutabnahme zwecks Prüfung der Leber- und Nierenfunktion und der Blutzellenzahl durchgeführt (64).
Im ersten Jahr nach Therapieabschluss fand eine follow-up Untersuchung alle 3 Monate, dann alle 6 Monate statt (64).
Die Nachsorgeuntersuchungen in den ersten zwei Jahren nach Therapieabschluss enthielten bei Tumorprogression und/oder neuen Metastasen eine Bildgebung mittels CT oder PET-CT, Laborkontrollen, Lungenfunktionsprüfungen und eine klinische Untersuchung (64).
Das mediane Überleben der in die Studie eingeschlossenen Patienten betrug 20.8 Monate (64). Patienten mit einem ECOG 0 vor Therapiebeginn zeigten ein Gesamtüberleben von 26.4 Monaten sowie eine 1-Jahres-Überlebensrate von 85% und eine 2-Jahres-Überlebensrate von 53% (64). Im Vergleich dazu zeigten Patienten mit ECOG 1 ein Gesamtüberleben von 18.9 Monaten sowie eine 1-Jahres-Überlebensrate von 69% und eine 2-Jahres-Überlebensrate von 37% (64). Dies entspricht deutlich schlechteren Werten hinsichtlich des Überlebens als bei Patienten mit niedrigerem ECOG (64).
Weiterhin konnte festgestellt werden, dass eine Verschlechterung des ECOG unter der

Therapie einen negativen prognostischen Faktor für das Gesamtüberleben darstellt (64). Der ECOG-Status der Nachsorgeuntersuchungen korrelierte statistisch signifikant mit dem Überleben ($p<0.001$) (64).

Zusammenfassend lässt sich also sagen, dass Patienten zwar von multimodalen Therapieansätzen in der Radioonkologie profitieren, jedoch der klinische Allgemeinzustand unbedingt Berücksichtigung in der Therapie finden sollte, da dieser entscheidend für das Überleben und die Prognose ist (64).

IV.III.II. Englische Zusammenfassung der Koautorenschaft II

A total of 99 patients with NSCLC stage III were examined with regard to their ECOG status before, during and after chemoradiotherapy (64). The aim was to find out if and how the performance status of the patient correlates with overall survival and progression free survival.

Before the start of the therapy the patient's history of tobacco consumption and previous illnesses were investigated (64). In addition, a standardized lung function test, lung imaging in form of a CT or PET-CT, a blood test to check liver and kidney function and an examination of the blood cell count were performed (64).

The follow-up examinations in the first two years after completion of therapy included imaging by CT or PET-CT, blood samples, lung function tests and a clinical examination in the event of tumor progression and/or new metastases (64). In the first year after the end of therapy, a follow-up took place every 3 months, then every 6 months (64).

The median survival of the patients included in the study was 20.8 months (64).

Patients with an ECOG 0 before the start of therapy showed an overall survival of 26.4 months and a one- and two-year survival rate of 85% and 53% respectively (64). In comparison, patients with ECOG 1 showed an overall survival of 18.9 months and a one- and two-year survival rate of 69% and 37% ($p=0.1$), which is significantly worse than patients with a lower ECOG status (64). It was also found that a deterioration of the ECOG under therapy is a negative prognostic factor for overall survival (64).

The patient’s ECOG performance status evaluated in the follow-up examination also correlated statistically significantly with the overall survival rate ($p<0.001$) (64).

In summary, it can be said that although patients benefit from multimodal therapeutic

approaches in radiooncology it is essential to take their clinical status into account, as this is crucial for the overall survival and the prognosis of the patient (64).

IV.IV. Eigenanteil an den vorgelegten Publikationen

In Zusammenarbeit mit dem Forschungsgruppenleiter Herrn Dr. Farkhad Manapov, dem leitenden Pathologen Herrn Prof. Dr. Jens Neumann und mir als Doktorandin erfolgte zunächst die Entwicklung und Ausarbeitung der grundlegenden Forschungsidee und die Selektion der zu untersuchenden Biomarker.

Es erfolgte bei der Erstautorenschaft zunächst die eigenständige Erhebung und im Anschluss die Auswertung der klinischen Daten der Patienten.

Daraufhin erfolgte die selbstständige Literaturrecherche nach vergleichbaren wissenschaftlichen Arbeiten.

Die Festlegung benötigter Cut-Offs und Grenzwerte sowie eine mikroskopische Analyse der Biopsien und der Tumormarker führte ich in Rücksprache mit Herrn Dr. Manapov und Herrn Prof. Dr. Neumann selbstständig durch.

Ich selbst entwickelte die Etablierung des CD8 Scores unter abschließender Sichtung von Herrn Prof. Dr. Neumann.

Anschließend erfolgten durch mich die die statistische Analyse und die Interpretation der Daten, welche daraufhin durch die Koautoren, insbesondere durch Herrn Dr. Käsmann, geprüft wurden. Aufgrund der Kontrolle der Ergebnisse durch Herrn Dr. Käsmann wurde hier die Erstautorenschaft geteilt.

Zuletzt wurde eigenverantwortlich eine wissenschaftliche Veröffentlichung verfasst und in Rücksprache mit den Koautoren überarbeitet.

Hinsichtlich des Eigenanteils der Koautorenschaften erfolgte durch mich die Selektion passender Patienten, die Analyse ihrer 18F-FDG-PET/CT Scans, die Sammlung der entsprechenden klinischen Daten sowie die Durchführung der Literaturrecherche und die Erarbeitung des Fließtextes für die Veröffentlichung.

V. Diskussion

Das lokal fortgeschrittene nicht-kleinzellige Lungenkarzinom stellt eine sehr heterogene Patientengruppe dar. Trotz verbesserter Behandlungsoptionen wie beispielsweise

zielgerichtete Therapie und Immuntherapie, Verbesserung der Bildgebung (18F-FDG-PET/CT) und der Einführung multimodaler Konzepte unterscheidet sich die Prognose dieser Patienten erheblich (3).

Ziel dieser Promotion ist die Identifikation von Prognosefaktoren und prädiktiven Biomarkern zur Ermöglichung einer Abschätzung der Überlebenszeit und einer Therapieindividualisierung (11).

Insbesondere die Interaktion zwischen Tumorzellen und den residenten Immunzellen wie T- und B-Lymphozyten sowie Makrophagen spielt eine zunehmend relevante Rolle in der Onkologie und wird in der Therapieplanung des lokal fortgeschrittenen nicht-kleinzelligen Lungenkarzinoms berücksichtigt (32,33). PD-L1, ein Transmembranprotein, welches von Tumorzellen exprimiert wird, interagiert mit dem zugehörigen PD-1 Rezeptor der T-Lymphozyten und Makrophagen (23). Über die Induktion eines Interleukin-10 Signalwegs in den Monozyten und einer konsekutiven Hemmung der Interleukin-2 Expression sowie der T-Zell Proliferation wird die Immunantwort gehemmt (30). Somit kann eine Immunevasion seitens des Tumors mit begleitender ungehinderter Vergrößerung erfolgen (23).

CD8, ein Oberflächenrezeptor der T-Lymphozyten, ist ebenso Bestandteil des Tumormikromilieus (30). Über dessen Bindung an MHC I Komplexe auf der Tumorzelloberfläche erkennen zytotoxische T-Zellen nun, ob es sich um virale oder mutierte Proteine handelt (18,24). Nach Aktivierung der CD8 positiven T-Zelle erfolgt eine Zerstörung der MHC-I exprimierenden Tumorzelle (26). Basierend auf der Veröffentlichung von Teng et al. lassen sich vier verschiedene Arten des tumorumgebenden Mikromilieus entsprechend der PD-L1 Expression und des Vorhandenseins oder des Fehlens von tumorinfiltrierenden Lymphozyten unterscheiden (65). Dazu gehörten Typ I (PD-L1 negativ ohne TILs, kennzeichnet Immunignoranz), Typ II (PD-L1 positiv mit TILs, impliziert eine adaptive Immunresistenz), Typ III (PD-L1 negativ mit TILs, mögliche andere Suppressoren bei der Förderung der Immuntoleranz) und Typ IV (PD-L1 positiv ohne TILs, intrinsische Induktion) (62).

Frühere Veröffentlichungen, wie die Studie von Vrankar et al. oder die Metaanalyse von Brody et al., legen nahe, dass die PD-L1-Expression auf Tumorzellen ein potenzieller Biomarker hinsichtlich der Prognose in der Behandlung des NSCLC (einschließlich Operation, Strahlentherapie und Checkpoint-Inhibition) ist (66–68). Ebenso zeigt eine post-hoc-Analyse der PACIFIC-Daten, dass die Prognose der Patienten mit nicht-kleinzelligem Lungenkarzinom

von der anfänglichen PD-L1-Expression abhängen kann (33,66,68).

Die wissenschaftliche Datenlage hinsichtlich des prädiktiven Wertes der PD-L1-Ausprägung auf Tumorzellen in Kombination mit CD8 positiven tumorinfiltrierenden Lymphozyten (TILS) ist bei Patienten mit lokal fortgeschrittenem NSCLC allerdings sehr begrenzt (66,69).

Das Hauptergebnis der im Rahmen dieser Promotion veröffentlichten Erstautorenschaft (*"Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy"*) bestätigt die Aussage, dass die anfängliche Expression von PD-L1 auf Tumorzellen als prognostischer Faktor für das lokal fortgeschrittene NSCLC infrage kommt (62). Die längsten Überlebenszeiten zeigten hier Patienten mit einem Tumormikromilieu Typ I, also mit einer Immunignoranz (PD-L1 negativ ohne TILS) (62). Im Gegensatz dazu wiesen Patienten mit einem Tumormikromilieu Typ IV, also mit einer erfolgreichen Immuneversion durch den Tumor (PD-L1 positiv ohne TILS), die kürzesten Überlebenszeiten auf (62). Die kürzeste Überlebenszeit der Patienten mit Tumormikromilieu Typ IV ist dadurch zu erklären, dass die Patienten der Kohorte dieser Veröffentlichung keine Immuncheckpointinhibitoren gegen die PD-L1 Expression, sondern nur eine Radiochemotherapie erhielten, da die Immuncheckpointinhibitoren zur Therapie des nicht-kleinzelligen Lungenkarzinoms in der Zeit der Datenerfassung noch nicht zugelassen waren.

Ebenso zeigte sich ein Trend für ein verbessertes Gesamtüberleben und eine bessere lokale Kontrolle bei Patienten mit initial niedriger CD8 positiven TIL Dichte (≤40%) (62).

Des Weiteren konnte eine negative Korrelation zwischen der TIL Dichte und der PD-L1 Expression und dem Karnofsky Index aufgezeigt werden: Eine hohe TIL Dichte und eine Expression von PD-L1 auf Tumorzellen weisen dementsprechend auf einen schlechteren Allgemeinzustand der Patienten hin (62).

Neben den biologischen Tumoreigenschaften haben aber auch klinische Prognosefaktoren wie der Allgemeinzustand des Patienten und die Zielvolumendefinition durch das PET-CT einen Einfluss auf die Prognose des NSCLC (63).

Die Zielvolumendefinition durch das 18F-FDG-PET/CT wurde als Prognosefaktor für das NSCLC bereits erfolgreich etabliert (70). In der PET-Plan Studie von Nestle et al. konnte nachgewiesen werden, dass eine bildgebungsbasierte Zielvolumendefinition durch das 18F-FDG-PET/CT einen positiven Einfluss auf das progressionsfreie Überleben hat und die lokale Kontrolle von

Patienten mit lokal fortgeschrittenem NSCLC erhöht (70).

Aufbauend auf diesen Ergebnissen wurde in unserer Studie (*„Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy",* 1. Koautorenschaft) ein Überlebensscore basierend auf 18F-FDG-PET/CT Parametern entwickelt (63). Dieser Score umfasst zum einen das SUVmax (Glukoseaufnahme) als metabolischer Parameter zur Charakterisierung der Tumorvitalität und des Tumorwachstums sowie zum anderen die B-Linie (horizontale Verteilung zwischen PET-positiven Lymphknoten) als ein wichtiges Merkmal der metabolisch aktiven beteiligte Lymphknotenkompartimente und des Tumorvolumens (63). Beide Parameter zeigten einen signifikanten Einfluss auf das Überleben der Studienteilnehmer: Bei Patienten mit SUVmax ≥8 betrug das mediane Gesamtüberleben nur 19 Monate, bei Patienten mit einem SUVmax <8 hingegen 40 Monate (63). Das mediane ereignisfreie Überleben betrug bei Patienten mit hohem SUVmax (≥8) 11,6 Monate und bei Patienten mit niedrigem SUVmax (<8) 16 Monate (63). In ähnlicher Weise verhielt es sich mit der B-Linie: Patienten mit einer B-Linie ≥3,7 cm zeigten eine signifikant schlechtere Überlebensrate im Vergleich zum Rest der behandelten Kohorte (63).

Des Weiteren können mit Hilfe dieses Überlebensscores Patienten mit lokal fortgeschrittenem Lungenkarzinom in 3 Risikogruppen unterteilt werden: niedriges Risiko (SUVmax <8, B-Linie <3,7cm), mittleres und hohes Risiko (SUVmax >8, B-Linie ≥3,7cm) (63). Insbesondere das Gesamtüberleben, das heißt die geschätzten 3-Jahres-Überlebensraten, waren zwischen den Untergruppen signifikant unterschiedlich: von 52% bei den Niedrigrisikopatienten bis nur 4% bei den Hochrisikopatienten (63).

Basierend auf unserem Überlebensscore zeigten Patienten mit einem niedrigen Risiko eine gute Prognose mit einer medianen Überlebenszeit von 40 Monaten (63). Demgegenüber zeigten Hochrisikopatienten trotz eines guten anfänglichen Allgemeinzustands (ECOG 0 oder 1) und einer abgeschlossenen definitiven Radiochemotherapie eine schlechte Prognose (63).

In der dritten Veröffentlichung dieser Promotion (*"Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Under Multimodal Treatment"*, 2. Koautorenschaft) erfolgte nun die Untersuchung eines möglichen Zusammenhanges zwischen dem klinischen Allgemeinzustand der Patienten und dem posttherapeutischen

Gesamtüberleben (64).

Antonia et al. belegten erstmals, dass eine simultane platinbasierte Radiochemotherapie mit anschließender Erhaltungstherapie mit dem PD-L1 Inhibitor Durvalumab zu einer signifikanten Verbesserung des Gesamt- und progressionsfreien Überlebens führt (33). Basierend auf den Ergebnissen dieser Phase-3 Studie ist eine Erhaltungstherapie bei inoperablen Patienten mit PD-L1 positiven nicht-kleinzelligen Lungenkarzinomen in Stadium III mit einer platinbasierten definitiven Radiochemotherapie allgemeiner Behandlungsstandard (31,33).

In der klinischen Praxis sind jedoch nicht alle Patienten für eine intensive multimodale Therapie geeignet. Diesbezüglich stellt der Allgemeinzustand des Patienten einen wichtigen Entscheidungsfaktor in der Therapieplanung dar (64). Dieser kann standardisiert durch den Karnofsky Index oder ECOG Status bestimmt und objektiviert werden (43). Im Vergleich zu Patienten mit einem besseren Allgemeinzustand, also Patienten ohne körperliche Einschränkungen, ist ein schlechter Allgemeinzustand (beispielsweise pflegebedürftiger Patient ohne die Fähigkeit der Selbstversorgung) mit einem erhöhten Risiko für behandlungsbedingte Toxizität und schlechten onkologischen Ergebnissen verbunden (43). In unserer Studie zeigten Patienten mit einem Stadium III NSCLC und einem initialen ECOG 0 (aktivitätsuneingeschränkter Patient) ein verbessertes medianes Überleben von 26,4 Monaten im Vergleich zu Patienten mit ECOG 1 (Patient mit leichten Einschränkungen) mit 18,9 Monaten (64). Eine Verschlechterung des ECOG Status nach multimodaler Behandlung hatte einen negativen prognostischen Einfluss auf das Gesamtüberleben der Patienten (64). Basierend auf einem Ansprechen auf die Behandlung erfuhren einige Patienten eine signifikante Verbesserung ihres anfänglichen Allgemeinzustands (64). Das Hauptergebnis unserer Studie war, dass der ECOG Status bei der ersten Nachuntersuchung nach abgeschlossener Radiochemotherapie signifikant mit der medianen Überlebensdauer korreliert: Die Ein-Jahres-Überlebensrate bei Patienten mit einem ECOG 0 nach abgeschlossener Therapie betrug 88%, bei Patienten mit einem ECOG 1 82% und bei Patienten mit einem ECOG 2 (Selbstversorgung des Patienten nur noch eingeschränkt möglich) nur 50% (64). Ein niedriger ECOG Status nach multimodaler Behandlung scheint also ein starker negativer Prognosefaktor für das Überleben bei Patienten mit lokal fortgeschrittenem nicht-kleinzelligem Lungenkarzinom zu sein (64).

VI. Ausblick

Es zeigt sich also, dass die in dieser Promotion aufgegriffenen Aspekte der Prognoseforschung, nämlich zum einen die Charakterisierung des Tumormikromilieus und zum anderen der klinischen Prognosefaktoren wie die Zielvolumendefinition in der Bildgebung und der Allgemeinzustand des Patienten, einen zunehmenden Einfluss in der Therapieplanung und -individualisierung gewonnen haben.

Die initiale PD-L1-Expression auf Tumorzellen kann ein prognostischer Faktor für die lokale Kontrolle, das progressionsfreie Überleben und das Gesamtüberleben sein und korreliert mit der CD8 positiven TIL Dichte bei inoperablem lokal fortgeschrittenem NSCLC (62). Die Beurteilung der PD-L1-Expression in Kombination mit der CD8 positiven TIL Dichte, anstatt der PD-L1-Expression allein, scheint bei NSCLC Patienten, die mit einer gleichzeitigen Radiochemotherapie behandelt werden, von hoher prognostischer Relevanz zu sein (62). Ebenso ist der von uns zur Abschätzung der Patientenprognose entwickelte PET-CT-Score für Patienten mit NSCLC Stadium III unter Radiochemotherapie von klinischer Relevanz (63). Es konnte gezeigt werden, dass dieser Score ein unabhängiger prognostischer Faktor ist und zusammen mit patienten- und behandlungsbezogenen Faktoren zur weiteren Optimierung der multimodalen Therapie in Betracht gezogen werden kann (63).

Ebenso hat der ECOG Status der Patienten vor der multimodalen Behandlung, nach Abschluss der Radiochemotherapie sowie dessen Veränderung während der Behandlung einen starken prognostischen Einfluss auf das Gesamtüberleben und das ereignisfreie Überleben der Patienten (64).

Dies unterstreicht die Notwendigkeit von Prognosescores im Allgemeinen. Die Heterogenität der Patientenkohorte kann so detaillierter differenziert und eine Therapieindividualisierung vorgenommen werden. In Anbetracht der in dieser Promotion dargestellten Ergebnisse sind weitere prospektive Studien gerechtfertigt, um die veröffentlichten Scores zu verifizieren und damit das heterogene Patientenspektrum des nicht-kleinzelligen Lungenkarzinoms detaillierter abbilden zu können.

VII. Originalarbeiten der Promotion

VII.I. Veröffentlichung 1 (Erstautorenschaft)

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Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy

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Abstract

Background/aim: mmune checkpoint inhibition (CPI) has an increasing impact in the multimodal treatment of locally advanced non-small cell lung cancer (LA-NSCLC). Increasing evidence suggests treatment outcome depending on tumor cell PD-L1 expression. The purpose of this retrospective study was to investigate the prognostic value of PD-L1 expression on tumor cells in combination with CD8+ tumor stroma-infiltrating lymphocyte (TIL) density in inoperable LA-NSCLC treated with concurrent chemoradiotherapy (CRT).

Patients and method: We retrospectively assessed clinical characteristics and initial tumor biopsy samples of 31 inoperable LA-NSCLC patients treated with concurrent CRT. Prognostic impact of tumor cell PD-L1 expression (0% versus ≥1%) and CD8+ TIL density (0–40% vs. 41–100%) for local control, progression-free (PFS) and overall survival (OS) as well as correlations with clinicopathological features were evaluated.

Results: Median OS was 14 months (range: 3–167 months). The OS rates at 1- and 2 years were 68 and 20%. Local control of the entire cohort at 1 and 2 years were 74 and 61%. Median PFS, 1-year and 2-year PFS were 13 ± 1.4 months, 58 and 19%. PD-L1 expression < 1% on tumor cells was associated with improved OS, PFS and local control in patients treated with concurrent CRT. Univariate analysis showed a trend towards improved OS and local control in patients with low CD8+ TIL density. Evaluation of Tumor Immunity in the MicroEnvironment (TIME) appears to be an independent prognostic factor for local control, PFS and OS. The longest and shortest OS were achieved in patients with type I ($PD\text{-}L1^{neg}/CD8^{low}$) and type IV ($PD\text{-}L1^{pos}/CD8^{low}$) tumors (median OS: 57 ± 37 vs. 10 ± 5 months, $p = 0.05$), respectively.

Conclusion: Assessment of PD-L1 expression on tumor cells in combination with CD8+ TIL density can be a predictive biomarker in patients with inoperable LA-NSCLC treated with concurrent CRT.

Keywords: TILs, PDL1, Chemoradiotherapy, Prognostic factors, Checkpoint inhibition

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide [1–3]. Locally-advanced non-small cell lung cancer (LA-NSCLC) represents as a heterogeneous disease including large tumor volume, extensive lymph node involvement, tumor-related atelectasis and infiltration of the thoracic wall, mediastinum and spine [4–6]. The majority of LA-NSCLC patients are inoperable and multimodal approaches are considered a cornerstone of treatment [7–10]. Historically, administering platinum-based chemotherapy concurrently to thoracic irradiation resulted in modest improvements of local control, metastasis-free and overall survival (OS) compared to radiotherapy alone [11, 12]. In the last years, the role of immune checkpoint inhibition (CPI) in the multimodal treatment of LA-NSCLC has evolved [9, 12]. In 2015, the first programmed cell death protein 1 (PD-1) inhibitor (nivolumab) was approved by the Food and Drug Administration (FDA) for advanced or metastatic NSCLC in the second-line setting following progression during or after platinum-based chemotherapy [13, 14]. Subsequently in 2016, the FDA approved monotherapy with the PD-1 inhibitor pembrolizumab in the first-line setting for patients with metastatic NSCLC with programmed cell death 1 ligand 1 (PD-L1) Tumor Proportion Score (TPS) ≥ 50% and expanded the indication in April 2019 based on the results of the KEYNOTE-042 trial for the first-line treatment of patients with stage III patients who are not candidates for surgical resection or definitive CRT or metastatic NSCLC with TPS ≥ 1% determined by an FDA-approved test. Patients' tumors had no Epidermal Growth Factor Receptor (EGFR) or Anaplastic lymphoma kinase (ALK) genomic aberrations [15, 16].

The addition of pembrolizumab to chemotherapy resulted in significantly higher rates of response and longer PFS than chemotherapy alone in a phase 2 cohort of the KEYNOTE-021 trial [17] and the FDA granted accelerated approval in May, 2017. CPI and chemotherapy combination therapy was also tested in the first-line setting in the KEYNOTE-189 and KEYNOTE-407 trials for metastatic nonsquamous NSCLC without sensitizing EGFR or ALK mutations and squamous NSCLC, respectively [18, 19]. Both studies reporting significantly improved OS and progression-free survival (PFS) than chemotherapy alone. Furthermore, the IMpower150 trial demonstrated superior PFS and OS for carboplatin/paclitaxel, bevacizumab and the PD-L1 blocking antibody atezolizumab vs. carboplatin/paclitaxel and bevacizumab in metastatic nonsquamous NSCLC, regardless of PD-L1 status and EGFR or ALK genetic alteration status [20]. Both combinations have been approved by the FDA.

Vis-à-vis stage III NSCLC, as a result of the PACIFIC trial, maintenance treatment with PD-L1 inhibitor durvalumab after successful completion of platinum-based concurrent chemoradiotherapy (CRT) has demonstrated significantly improved PFS and OS and became a new standard of care in inoperable stage III NSCLC [8, 9]. Currently, predictors for response to CPI are unclear and potential biomarkers are under investigation including PD-L1 expression of tumor cells, tumor-infiltrating lymphocytes (TIL), T-effector-interferon-γ-associated gene expression and tumor mutational burden (TMB) [21–23]. High mutation load has been shown to correlate with an immunogenic tumor microenvironment with increased expression of tumor-specific neoantigens that can be targeted by activated immune cells e.g. cytotoxic CD8+ TILs [24, 25].

Considering the importance of PD-L1 expression on tumor cells and CD8 TIL density in defining the tumor immune microenvironment, we aimed to study PD-L1 expression alone and in combination with CD8 TIL density with relation to clinicopathologic characteristics and survival in patients treated with concurrent CRT.

Methods

Patients and samples

This study included 31 patients who received concurrent CRT for locally advanced or metastatic NSCLC. From their medical records, we retrieved patients' clinical data, such as sex, age, histologic type and grading, pack years and TNM stage (using the 8th UICC TNM Staging System of lung cancer). Evaluation of EGFR/ALK genomic aberrations was performed in nonsquamous metastatic patients and was negative. All patients were closely followed-up according to an in-house protocol - every 3 months in the first 2 years, every 6 months up to 5 years and afterwards once per year. Expert pathologists (J.N. and S.R.) re-reviewed hematoxylin-eosin–stained slides from all cases, and corresponding formalin-fixed, paraffin-embedded specimens and performed the immunohistochemical staining.

Immunohistochemistry

All immunohistochemical stainings were done on 5 μm whole standard tissue sections of formaldehyde-fixed paraffin-embedded tissue (FFPE) tumor samples (see Fig. 1). For the detection of PD-L1 prediluted PD-L1 rabbit monoclonal antibody (SP263; Ventana Medical Systems, Oro Valley, Arizona) was used as the primary antibody. Immunohistochemical staining for CD8 was carried out with an anti- CD8α mouse monoclonal antibody (C8/144B, Cell Marque, Rocklin, California, dilution 1:50) as the primary antibody. Both stainings were performed on a Ventana Benchmark Ultra autostainer using the UltraView diaminobenzidine kit (Ventana Medical Systems, Oro Valley, AZ).

Assessment of PD-L1 expression

PD-L1 expression on tumor cells was measured quantitatively using an established immunohistochemistry assay (Ventana SP 263) which had been used recently published randomized phase III studies [8, 9, 26]. All of

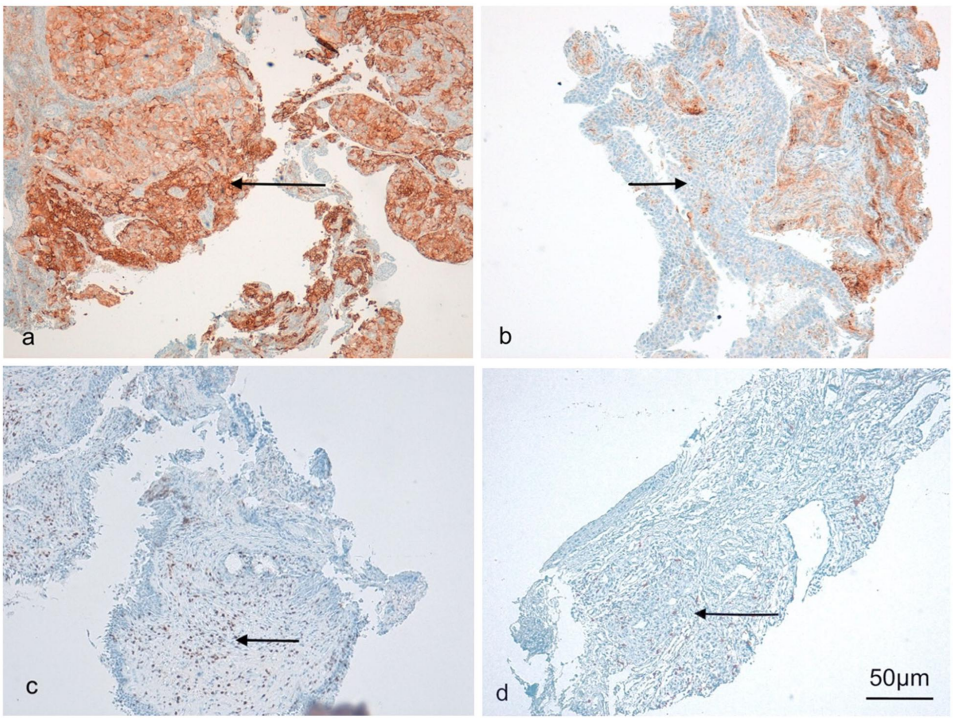

Fig. 1 PD-L1 expression on tumor cells and CD8 expression on tumor infiltrating lymphocytes**.** Representative images under a microscope with a 10x enlargement of adenocarcinomas with positive (a, 80%) and negative (b, 0%) PD-L1 staining on tumor cells and adenocarcinomas with positive (c, 70%) and negative (d, 2%) CD8 staining on tumor infiltrating lymphocytes. The arrows indicate positive (**a**,**c**) or negative (**b**,**d**) staining

the stained sections were scored in five randomly selected areas containing tumor cells, which showed membranous and cytoplasmic staining. The percentage of positive tumor cells was graded on a scale of 0–2: 0 (< 1%), 1 (1–5%); 2 (> 5%). The intensity of staining was scored as follows: 0 (no staining), 1 (weak staining), 2 (moderate or strong staining). The H-score, ranging from 0 to 12, was calculated by multiplying the percentage of positive tumor cells by the intensity of staining on the tissue sections. The H-scores were categorized as follows: 0: negative (−), 1–4: weak positive (+), 5–8: moderately positive (+ +), 9–12: strong positive (+ + +).

Assessment of CD8+ TIL density

Assessment of CD8+ TIL density was performed according to established breast cancer protocols [23]. In literature, common cut-off points ranged between 2.5 and 40% in order to differentiate between high and low CD8+ TIL density. In our study we divided the patient cohort in two subgroups (low and high density of CD8+ TILs: 0–40% vs. 41–100%).

Assessment of tumor immunity in the MicroEnvironment (TIME)

Based on previous studies, four different types of tumour immune microenvironment have been identified according to PD-L1 expression of tumor cells and presence or absence of TILs in the tumor microenvironment [27, 28]. These included type I (PD-L1 – with no TILs indicating immune ignorance), type II (PD-L1 + with TILs implying adaptive immune resistance), type III (PD-L1 – with TILs suggesting the role of other suppressor(s) in promoting immune tolerance) and type IV (PD-L1 + with no TILs indicating intrinsic induction). All patients were stratified according to TIME classification and TIME subgroups were evaluated for prognostic outcome, local control, PFS and OS.

Table 1 patient characteristics

	Number of patients (%)
Age	
≤ 65 years	16 (52)
> 65 years	15 (48)
Gender	
Female	26 (84)
Male	5 (16)
Karnofsky performance status	
> 80%	11 (35)
≤ 80%	20 (65)
UICC stage	
III	28 (90)
IV	3 (10)
T category	
1–2	6 (19)
3–4	25 (81)
N category	
0–1	3 (10)
2–3	28 (90)
Histology	
Squamous cell carcinoma	16 (52)
Non-squamous cell carcinoma	15 (48)
Tobacco consumption (PY)	
0	8 (26)
20–40	8 (26)
> 40	15 (48)
Grading	
Moderately differentiated	2 (6)
Poorly differentiated	27 (87)
anaplastic	2 (6)
TIME	
I	10 (32)
II	5 (16)
III	5 (16)
IV	7 (23)

Statistical analysis

Each clinicopathologic characteristic was evaluated using Pearson's chi-squared test or Fisher's exact test (categorical variables). OS was measured from the date of the initial diagnosis until the date of death. The Kaplan-Meier method and log-rank test were applied to assess OS. In multivariate analysis, the Cox regression proportional hazard model was used to assess the clinicopathologic characteristics significantly related to OS with HRs and 95% CIs. A two-sided p value of ≤0.05 was considered statistically significant. All statistical analyses were performed using SPSS 25 software (IBM, Armonk, NY).

Results

The clinicopathologic characteristic of all patients are shown in Table 1. Median age was 65 years (range: 51–76 years). Histopathological biopsy was taken before treatment by all patients and reviewed by pathology specialists. Sixteen (52%) patients were diagnosed with squamous cell carcinoma, 9 (29%) patients with adenocarcinoma and 6 (19%) with a non-specified non-small cell lung cancer. Twenty-eight (90.3%) patients had stage III NSCLC according to the 8th UICC TNM Staging System of lung cancer and 3 (9.7%) patients were diagnosed with stage IV NSCLC due to pleural involvement or malignant pleural effusion. All 3 stage IV patients were without sensitizing EGFR or ALK mutations. At diagnosis, 23 (74.2%) patients were heavy smokers (median pack years (PY):40) and 8 (25.8%) patients never smokers.

All patients were treated with definitive concurrent CRT. Twenty-five (81%) patients received platinum-based chemotherapy. A taxane-based combination was applied in 16 (52%) patients. Median biologically equivalent dose (EQD2) to the primary tumor and involved nodes was 65Gy (range: 50-70Gy). Follow-up was conducted as per in-house protocol every 3 months in the first 2 years, every 6 months up to 5 years and afterwards once per year.

The median overall survival in the entire patient collective was 14 months (range: 3–167 months). The 1-year and 2-year OS rates were 67.7 and 19.4%, respectively. The 1 and 2-year actuarial local control rates were 74 and 61%, respectively. Median PFS, 1-year and 2-year PFS were 13 ± 1.4 months, 58 and 19%, respectively.

Correlations of PD-L1 expression and clinicopathologic characteristics

Correlations of PD-L1 expression and clinicopathologic characteristics are shown in Table 2. PD-L1 inversely correlates with Karnofsky performance status ($p = 0.023$) and positively with CD8+ TIL density ($p = 0.020$).

Correlations of CD8+ TIL density and clinicopathologic characteristics

Correlations of CD8+ TIL density and clinicopathologic characteristics are shown in Table 3. CD8+ TIL density inversely correlates with Karnofsky performance status ($p = 0.038$) and positively with PD-L1 expression ($p = 0.020$).

Prognostic impact of PD-L1 expression for local control, PFS and OS

Univariate and multivariate analysis for OS, PFS and local control concerning PD-L1 expression are shown in Tables 4,

Table 2 Correlations of PD-L1 expression and clinicopathologic characteristics

	Positive, n (%)	Negative, n (%)	*p*-value
Age			
≤ 65 years	8 (50)	8 (50)	
> 65 years	8 (57)	6 (43)	0.834
Gender			
Female	13 (52)	12 (48)	
Male	4 (80)	1 (20)	0.513
Karnofsky performance status			
90–100%	8 (80)	2 (20)	
70–80%	8 (40)	12 (60)	0.023
UICC stage			
III	15 (56)	12 (44)	
IV	1 (33)	2 (67)	0.447
T category			
1–2	4 (67)	2 (33)	
3–4	12 (50)	12 (50)	0.073
N category			
0–1	2 (67)	1 (33)	
2–3	14 (52)	13 (48)	0.402
Histology			
Squamous cell carcinoma	9 (60)	6 (40)	
Non- Squamous cell carcinoma	7 (47)	8 (53)	0.864
Tobacco consumption (PY)			
0	5 (63)	3 (38)	
20–40	3 (38)	5 (63)	
> 40	8 (57)	6 (43)	0.105
Grading			
Moderately differentiated	1 (50)	1 (50)	
Poorly differentiated	14 (54)	12 (46)	
anaplastic	1 (50)	1 (50)	0.223
CD8+ TILs density			
≤ 40%	5 (50)	5 (50)	
> 40%	10 (59)	7 (41)	0.020

5 and 6. Univariate analysis for OS showed significance (p = 0.048). However, multivariate analysis with cox regression failed (p = 0.648). In univariate analysis for PFS and local control, PD-L1 expression was associated with improved PFS (p = 0.006) and improved local control rate (p = 0.017).

Prognostic impact of CD8+ TIL density for local control, PFS and OS

Univariate and multivariate analysis for OS, PFS and local control concerning CD8+ TIL density are shown in Tables 4, 5 and 6. Univariate analysis showed a trend for improved OS and better local control in patients with low CD8+ TIL density (p = 0.055; p = 0.092).

Prognostic impact of tumor immunity in the MicroEnvironment (TIME)

According to the Tumor Immunity in the Micro-Environment (TIME) classification [27, 28], TIME subgroups were evaluated for prognostic outcome for OS, PFS and local control. The longest and shortest OS were achieved in patients with type I (PD-L1^{neg}/CD8^{low}) and type IV (PD-L1^{pos}/CD8^{low}) (median OS: 57 ± 37 vs. 10 ± 5 months, p = 0.05). In univariate and multivariate analysis for OS, TIME subgroups had significant differences (p = 0.05; p = 0.048) as well as in univariate analysis for PFS and local control (p = 0.05; p = 0.035).

Table 3 Correlations of CD8+ TILs density and clinicopathologic characteristics

	high, n (%)	low, n (%)	p-value
Age			
≤ 65 years	12 (80)	3 (20)	
> 65 years	6 (46)	7 (54)	0.403
Gender			
Female	14 (61)	9 (39)	
Male	4 (80)	1 (20)	0.384
Karnofsky performance status			
> 80%	9 (90)	1 (10)	
≤ 80%	9 (50)	9 (50)	0.038
UICC stage			
III	17 (65)	9 (35)	
IV	1 (50)	1 (50)	0.409
T category			
1–2	3 (60)	2 (40)	
3–4	15 (65)	8 (35)	0.751
N category			
0–1	2 (67)	1 (33)	
2–3	16 (64)	9 (36)	0.899
Histology			
Squamous cell carcinoma	8 (62)	5 (39)	
Non- Squamous cell carcinoma	10 (67)	5 (33)	0.681
Tobacco consumption (PY)			
0	6 (75)	2 (25)	
20–40	5 (71)	2 (29)	
> 40	7 (54)	6 (46)	0.11
Grading			
Moderately differentiated	0 (0)	2 (100)	
Poorly differentiated	16 (67)	8 (33)	
anaplastic	2 (100)	0 (0)	0.067
PD-L1 expression			
0%	7 (58)	5 (42)	
≥ 1%	10 (67)	5 (33)	0.02

Discussion

LA-NSCLC represents a heterogeneous disease which can include large tumor volumes, extensive lymph node involvement and infiltration of the thoracic wall, mediastinum and spine [4–6]. An interdisciplinary strategy is required to define optimal multimodal approaches based on disease stage, patients' general condition and treatment options according to the latest evidence [29]. The majority of these patients are inoperable due to comorbidities and lymph node involvement. In this situation, multimodal treatment including concurrent application of chemo- and radiotherapy is associated with a moderate toxicity profile and improved patient outcome compared to sequential CRT or radiotherapy alone [7].

Based on the results of the PACIFIC trial, consolidation PD-L1 inhibition with durvalumab is currently considered as standard of care for stage III NSCLC patients without progressive disease following platinum-based concurrent CRT [8, 9]. In stage IV disease, patients with initial TPS ≥ 50% can be offered pembrolizumab monotherapy. Stage IV patient with tumor cell PD-L1 expression < 1% and good PS can receive a combination of platinum-based chemotherapy with PD-1 or PD-L1 inhibition [17–19].

Previous studies suggest that PD-L1 expression can be a potential biomarker for efficacy of NSCLC treatment including surgery, radiotherapy and checkpoint inhibition [19, 21, 30–32]. Retrospective post-hoc analysis of PACIFIC data suggests that outcome of patients appears to depend on initial PD-L1 expression [9, 33].

The principal finding of our study confirms the statement that initial tumor cell PD-L1 expression can be a prognostic factor for inoperable LA-NSCLC treated with concurrent CRT alone. In the study by Vrankar et al., the prognostic relevance of PD-L1 expression was evaluated in 102 patients with stage III NSCLC treated with concurrent chemoradiotherapy [30]. PD-L1 expression ≥5% on tumor cells resulted in significantly unfavorable PFS and OS. However, several limitations of this study need to be taken into account: only a very small patient number ($n = 7$) was considered PD-L1 positive. In addition, negative and unknown states of PD-L1 expression were evaluated together. In our study, 52% of all patients were considered PD-L1 positive according to the cut-off value in the PACIFIC trial.

Data of the predictive value of PD-L1 expression on tumor cells in combination with CD8+ tumor-infiltrating lymphocyte (TIL) density in patients with locally advanced NSCLC is limited [34, 35]. Tokito et al. found CD8+ TIL density is an independent prognostic factor for OS [34]. Interestingly, PD-L1 expression (≥5%) on tumor cells has shown no prognostic role in this study in contrast to previous reports [19, 32, 36]. Indeed, patients with low or no PD-L1 expression on tumor cells could respond to PD-1/PD-L1 inhibition as well and show a durable response [22, 37]. In addition, PD-L1 expression can vary between tumor cells, surrounding non-malignant tissue and peripheral immune cells [38–40]. Treatment modality appears to have an impact on PD-L1 expression [41, 42]. Fujimoto et al. evaluated PD-L1 expression on tumor cells before and after CRT and found that alteration of PD-L1 expression was associated with survival in patients with LA-NSCLC [42].

As a result, the interaction of tumor and immune cells in the treatment and immune response is still poorly understood. Based on preclinical and clinical data, the

Table 4 univariate and multivariate survival analysis

	Survival		*p*-value	
	at 12 months (%)	at 24 months (%)	univariate Analysis	multivariate Analysis
Age				
≤ 65 years	56	19		
> 65 years	80	27	0.676	
Gender				
Female	80	20		
Male	65	23	0.629	
Karnofsky performance status				
> 80%	75	30		
≤ 80%	55	10	0.041	0.077
UICC stage				
III	64	21		
IV	100	33	0.537	
T category				
1–2	67	0		
3–4	68	28	0.395	
N category				
0–1	33	0		
2–3	71	25	0.299	
Histology				
Squamous cell carcinoma	69	25		
Non- Squamous cell carcinoma	67	20	0.935	
Tobacco consumption (PY)				
0	75	25		
20–40	50	12,50		
> 40	73	27	0.758	
Grading				
Moderately differentiated	50	50		
Poorly differentiated	67	19		
anaplastic	100	50	0.758	
PD-L1 expression				
0%	86	29		
≥ 1%	50	19	0.048	0.648
CD8+ TILs density				
≤ 40%	70	40		
> 40%	61	17	0.055	
TIME type				
I	100	60		
II	50	20		
III	71	14		
IV	40	20	0.05	0.048

Table 5 univariate and multivariate analysis of local control

	Local control		p-value	
	at 12 months (%)	at 24 months (%)	univariate Analysis	multivariate Analysis
Age				
65 years	58	58		
> 65 years	83	63	0.380	
Gender				
Female	68	57		
Male	80	80	0.941	
Karnofsky performance status				
> 80%	73	67		
≤ 80%	66	33	0.233	
UICC stage				
III	66	62		
IV	100	67	0.862	
T category				
1–2	66	62		
3–4	100	67	0.970	
N category				
0–1	33	33		
2–3	75	64	0.154	
Histology				
Squamous cell carcinoma	70	60		
Non- Squamous cell carcinoma	70	62	0.766	
Tobacco consumption (PY)				
0	83	83		
20–40	51	34		
> 40	72	60	0.417	
Grading				
Moderately differentiated	100	100		
Poorly differentiated	70	59		
anaplastic	50	50	0.487	
PD-L1 expression				
0%	92	79		
≥ 1%	44	44	0.017	0.045
CD8+ TILs density				
≤ 40%	86	75		
> 40%	62	62	0.092	
TIME type				
I	100	80		
II	41	41		
III	83	83		
IV	67	67	0.05	0.694

Table 6 univariate and multivariate analysis of progression free survival (PFS)

	PFS		P-value	
	at 12 months (%)	at 24 months (%)	univariate Analysis	multivariate Analysis
Age				
≤ 65 years	50	19		
> 65 years	67	20	0.925	
Gender				
Female	80	20		
Male	54	19	0.868	
Karnofsky performance status				
> 80%	65	25		
≤ 80%	46	9	0.134	
UICC stage				
III	54	18		
IV	100	33	0.458	
T category				
1–2	67	0		
3–4	56	20	0.292	
N category				
0–1	33	0		
2–3	61	20	0.235	
Histology				
Squamous cell carcinoma	56	19		
Non- Squamous cell carcinoma	60	20	0.855	
Tobacco consumption (PY)				
0	63	25		
20–40	38	13		
> 40	67	20	0.633	
Grading				
Moderately differentiated	50	50		
Poorly differentiated	59	15		
anaplastic	50	50	0.831	
PD-L1 expression				
0%	86	29		
≥ 1%	31	13	0.006	0.061
CD8+ TILs density				
≤ 40%	70	30		
> 40%	50	17	0.201	
TIME type				
I	100	60		
II	30	20		
III	71	14		
IV	40	0	0.035	0.144

involvement of CD8+ TILs plays a crucial role in tumor-associated immune response [43]. The CD8+ TIL density in the tumor microenvironment has been suggested to predict the oncologic outcome in different cancer types such as colorectal cancer, malignant melanoma and anal cancer [28, 44, 45]. Based on previous studies, four different types of tumor immune microenvironment have been identified according to PD-L1 expression of tumor cells and presence or absence of TILs in the tumor microenvironment. These included type I (PD-L1^{neg} with no TILs indicating immune ignorance), type II (PD-L1^{pos} with TILs implying adaptive immune resistance, type III (PD-L1^{neg} with TILs suggesting a role of other suppressor(s) in promoting immune tolerance) and type IV (PD-L1^{pos} with no TILs indicating intrinsic induction). In our study, the longest OS was achieved in patients with type I (PD-L1^{neg}/CD8^{low}) in contrast to previous studies investigating the prognostic value of PD-L1 expression combined with CD8+ TIL density. In the studies of Tokito et al. and El-Guindy et al., patients with PD-L1^{neg}/CD8^{high} had the longest OS and according to Yang et al. the patient subgroup with PD-L1^{pos}/CD8^{high} showed the longest OS [34, 35, 46]].

The shortest OS in our study was seen in patients with type IV (PD-L1pos/CD8low) and well in accordance with the published literature [33, 34, 45]. This finding could be explained by the lack of immune-mediated tumor response. Tumor cells can decrease their immunogenicity through interaction of PD-L1 with PD-1 on T cells. As a result, the tumor can evade the immune surveillance. In addition, a lack of CD8+ TILs can account for most non-responders to PD-1/PD-L1 inhibition [28].

Several limitations of this study need to be considered when interpreting the results. Firstly, the retrospective nature of this study and the possibility of unknown biases. Secondly, the relatively small number of patients included in the analysis and lastly, all patients were treated at a single center. However, we are convinced that our findings supporting the assessment of CD8+ TIL density combined with PD-L1 expression, instead of PD-L1 expression alone is of important clinical relevance and requires special consideration in future trials.

Conclusion

Initial PD-L1 expression on tumor cells can be a prognostic factor for local control, PFS and OS and **correlates** with CD8+ TILs density in inoperable LA-NSCLC. Assessment of PD-L1 expression in combination with CD8+ TILs density, instead of PD-L1 expression alone, appears to be of strong prognostic relevance in patients treated with concurrent CRT. Future prospective studies are warranted to verify our findings.

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The piece has not been previously published and is not under consideration elsewhere. The persons listed as authors have given their approval for the submission.

Authors' contributions
LK, KG, JN, CE, OR, MO, MK, AT, SR, CB and FM analysed and interpreted the data, performed the statistical analysis and wrote the manuscript. LK, KG, JT and FM helped with the statistical analysis and editing the manuscript. All authors helped in drafting the manuscript. All authors read and gave their stamp of approval for the submission of the final version of the manuscript.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All patients gave express written informed consent. This retrospective analysis is in compliance with the principles of the Declaration of Helsinki and its subsequent amendments. This work was approved by the Ethics Committee of the Ludwig Maximilian University of Munich.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

VII.II. Veröffentlichung 2 (Koauotorenschaft I)

Original Article

Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy

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Contributions: (I) Conception and design: O Roengvoraphoj, L Käsmann, A Tufman, C Belka, F Manapov; (II) Administrative support: O Roengvoraphoj, L Käsmann, A Tufman, M Li, E Mille, C Belka, F Manapov; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: O Roengvoraphoj, L Käsmann, A Tufman, C Belka, F Manapov; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: 18F-FDG-positron emission tomography (PET)/computed tomography (CT) is a standard for initial staging in patients with locally advanced stage III non-small cell lung cancer (NSCLC). We evaluated a PET/CT staging score to characterize disease extension and patient outcome in this disease.

Methods: Ninety-nine consecutive patients with NSCLC stage IIIA–B (UICC 7th edition), who underwent 18F-FDG-PET/CT before the start of chemoradiotherapy (CRT) were analyzed. Maximum standardized uptake value of primary tumor (SUVmax_PT) and range between two most distant PET-positive (SUV ≥2.5) lymph nodes in two directions were analyzed for their correlation with patient outcome. The vertical distance was defined as A- and the horizontal as a B-line.

Results: According to the results of univariate analysis, score included the SUVmax_PT and horizontal B-line, patients were divided into three risk subgroups: low, intermediate and high-risk subgroups. Subgroups were defined as SUVmax_PT <8 and B-line <3.7 cm, SUVmax_PT >8 or B-line >3.7 cm and SUVmax_PT >8 plus B-line >3.7 cm, respectively. Twenty-eight (28%), 45 (46%) and 26 (26%) patients were assigned to the low, intermediate and high-risk subgroup, respectively. Median event-free survival (EFS) in low, intermediate and high-risk subgroups was 16 (95% CI: 7–25), 13 (95% CI: 12–15) and 10 (95% CI: 7–13) months (P=0.002, log-rank test). Median OS in the low, intermediate and high-risk subgroups was 40 (95% CI: 11–69), 23 (95% CI: 15–31) and 14 (95% CI: 13–14) months (P=0.0001, log-rank test). In the multivariate analysis, SUV, B-line and PET/CT score were significantly associated with EFS [harard ratio (HR) 2.12 (95% CI: 1.27–3.55) and intermediate risk HR 2.01 (95% CI: 1.13–3.59), P=0.003] and OS [high-risk HR 2.79 (95% CI: 1.16–4.55) and intermediate risk HR 2.30 (95% CI: 1.58–4.94), P=0.001].

Conclusions: A PET/CT score was developed for inoperable stage III NSCLC patients treated with CRT and was an independent predictor of patient outcome in the single-center cohort.

Keywords: Chemoradiotherapy (CRT); combined modality therapy; positron emission tomography computed tomography (PET-CT); survival rate

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Introduction

For patients with inoperable stage III non-small cell lung cancer (NSCLC), concurrent chemoradiotherapy (CRT) followed by consolidation therapy with PD-L1 inhibition is the current standard of care (1). Exact T- and N-stage definition is very important for the planning of multimodal treatment and patient prognosis. Also an assessment tool for functional impairment with Eastern cooperative oncology group (ECOG) is a metric to characterize patient prognosis (2). Previous prognostic scores have been proposed (3,4) and one of such scores was developed by our group from patient- and tumor-related factors (age, pack years, tumor-associated atelectasis and histology) and predicted prognosis in inoperable stage III NSCLC patients (5).

Whole body 18F-FDG-positron emission tomography (PET)/computed tomography (CT) is an established and validated diagnostic tool for initial staging in stage III NSCLC. FDG uptake is an indicator of tumor vitality that is usually measured using standardized uptake values (SUV), metabolic tumor volume (MTV) and total lesion glycolysis (TLG). FDG uptake intensity correlated with overall survival in different studies (6,7). Our previous data showed that an initial primary tumor (PT) MTV and its changes in the course and after completion of CRT has a prognostic impact in inoperable stage III NSCLC (8). Moreover, in a meta-analysis by Na *et al.*, high pre-treatment SUVmax_PT was associated with poor outcome (9). Other studies have also shown a correlation between tumor histology and the SUV values in locally-advanced NSCLC (10,11).

The aim of present single-center analysis was to evaluate the impact of pre-treatment SUVmax_PT and range between two most distant PET-positive (SUV ≥2.5) lymph nodes in two directions (vertical and horizontal) on patient survival in a real-life inoperable stage III cohort. Hence, a new PET/CT score that combines these parameters was proposed and validated. To the best of our knowledge, no previous studies have combined multiple imaging parameters.

Methods

Patients

A total of 99 consecutive patients with locally advanced NSCLC stage IIIA–B (UICC 7th edition) between 2011 and 2016, treated with curative intent CRT were enrolled. All patients received initial staging including bronchoscopy with biopsy, contrast-enhanced CT or MRI of the brain. Histology yielded 49 adenocarcinomas (49.5%) and 50 non-adenocarcinomas (50.5%). Patients with distance metastasis on PET/CT and poor performance status (ECOG >1) were excluded from this analysis. This study was approved by the institutional review board.

CRT

All patients received platinum-based CRT (sequentially or concurrently). Various chemotherapy regimens were allowed. The majority of the cohort was treated with a combination of cisplatin 20 mg/m^2 body surface area on days 1–4 and 50 mg/m^2 oral vinorelbine on days 1, 8, 15 for 2 cycles.

A planning PET/CT was performed in the treatment position for improved target volume definition (tumor and affected lymph node regions). The description of target volume definition was previously published (5,12). Forty patients (40.4%) were treated with three-dimensional conformal radiotherapy, whereas 59 patients (59.6%) with intensity-modulated radiotherapy (IMRT). Treatment was delivered by a multi-energy linear accelerator. Image-guidance was performed with cone-beam CT several times a week.

18F-FDG-PET/CT

Emission scans were initiated 60–90 minutes after intravenous administration of 20 mg furosemide, 10–20 mg butylscopolamine and 18F-FDG. The examination was performed in the treatment position (patient's arms overhead, wingstep) on carbon fiber couch at the same institution. After PET data collection, whole body CT scans were performed after intravenous injection of 100–120 mL iodized contrast. CT scans were also used to correct PET attenuation. The weight adapted to the body weight maximum SUV (SUVmax) of PT was determined. The SUV measurements were created using automated software in a 3D volume tool. (Hybrid Viewer 3D, Hermes Medical

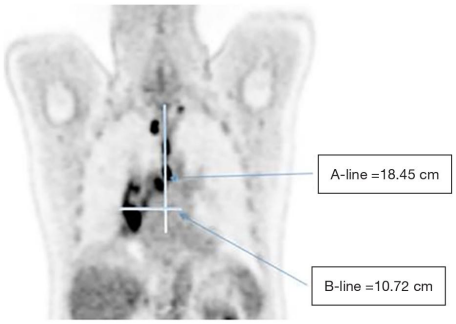

Figure 1 Definition of A- and B-line.

Table 1 Patient and treatment characteristics

Parameters	N (%)
Sex	
Male	62 (62.6)
Female	37 (37.4)
Age	
Mean [range]	67 [43–88]
Histology	
Adenocarcinoma	49 (49.5)
Adenocarcinoma with EGFR mutation	3 (3.0)
Adenocarcinoma with wild-type EGFR	30 (30.3)
Squamous cell carcinoma	42 (42.4)
Others	8 (8.1)
Endobronchial ultrasound	
Yes	79 (79.8)
No	20 (20.2)
CRT	
Concurrent	77 (77.8)
Platinum-based chemotherapy	
Yes	67 (67.7)
No	32 (32.3)
Radiotherapy	
Three-dimensional conformal radiation therapy	40 (40.4)
Intensity-modulated radiation therapy	59 (59.6)

CRT, chemoradiotherapy.

Solutions, Stockholm, Sweden).

Lymph nodes with SUVmax ≥2.5 were indicated for malignant potential (13,14) and selected for the measurement of the two widest involved lymph nodes in selected CT layers with optimal imaging of lymph nodes in both directions. A-line was defined as the vertical distance of the 2 most distant involved pet-positive lymph nodes with SUVmax ≥2.5. B-line consisted of the horizontal distance of the 2 most distant affected lymph nodes (*Figure 1*). The measurement was performed by two experienced radiation oncologists and nuclear medicine specialists.

Statistics

Statistical analysis was performed using software of SPSS statistics 25 (IBM, New York, USA). The median follow-up achieved 17.2 (range: 2.2–92.1) months. Kaplan-Meier analyses were used to compare survival curves for the subgroups according to the SUVmax, vertical A- and horizontal B-lines. A receiver-operating characteristic (ROC) curve analysis was performed to define the best cut-off of SUVmax as well as vertical A- and horizontal B-lines in terms of overall survival. For the multivariate analysis, Cox regression model was used. All variables with P<0.1 (log-rank test) from previous analysis (age, pack years, tumor-associated atelectasis and histology) with this end point on univariate analysis were included in a multivariate cox regression analysis.

Results

Patient and treatment characteristics

A summary of patient and treatment characteristics is provided in *Table 1*. Of the 99 patients treated, 62 (62.6%) were men and 37 (37.4%) were women. The median age was 67 (range: 43–88) years. The median radiation dose was 60 (range: 45–70) Gy. The median interval between PET/CT and start of CRT was 12 (range: 0–67) days.

There was a significant positive relationship between: PT SUV and gross tumor volume, P<0.01; N stage and the sum of vertical A- and horizontal B-line, P<0.01; N stage and the horizontal B-line, P<0.01. In these patients no correlation could be found between SUVmax, vertical A- or horizontal B-line and histology.

SUVmax_PT

The mean SUVmax_PT was 11.6 (range: 0–41). The optimal cut-off value of pre-treatment SUVmax_PT was 8 based on the ROC analysis. The sensitivity, specificity and AUC using this cut-off values achieved 72%, 64% and 0.74 respectively. Among patients with SUVmax_PT ≥8, median OS was 19 months (95% CI: 15–23.2) *vs.* 40 (95% CI: 9.8–70.2) in patients < SUVmax 8 (P<0.0001, log-rank test).

The median event-free survival (EFS) was 11.6 months (95% CI: 8.6–14.5) in the SUVmax ≥8 group and 16 months (95% CI: 13.9–17.8) in patients < SUVmax 8 (P<0.058, log-rank test).

Vertical (A-) and horizontal (B-) lines (involved lymph node distribution)

Vertikal A-line had no impact on overall survival at different cut-offs. Horizontal B-line cut-off ≥3.7 cm was significantly associated with worse survival compared to the rest of the treated cohort. The sensitivity, specificity and AUC using this cut-off values were 52%, 85% and 0.63 respectively. The median OS was 30 when horizontal B-line <3.7 cm *vs.* 16.3 months when horizontal B-line ≥3.7 cm (P<0.01, log-rank test).

Table 2 Definition of new 18F-FDG-PET/CT staging score for inoperable stage III NSCLC patients treated with CRT

Parameters	Risk group	Point(s)
SUVmax ≥8; B-line ≥3.7 cm	Low risk	0
	Intermediate risk	1
	High-risk	2

PET, positron emission tomography; CT, computed tomography; NSCLC, non-small cell lung cancer; CRT, chemoradiotherapy; SUV, standardized uptake value.

Evaluation of 18F-FDG PET-CT score

The score was developed on the basis of PET parameters correlated with OS on univariate analysis and included the SUVmax_PT and horizontal B-line. The patients were divided into 3 subgroups (*Table 2*). Low risk subgroup (0 points) included patients with SUVmax_PT <8 and horizontal B-line <3.7 (n=28, 28%). Forty-five patients (46%) had 1 point (intermediate risk) and 26 patients (26%) had 2 points (high-risk).

Median OS in the low, intermediate and high-risk subgroups was 40 (95% CI: 11–69), 23 (95% CI: 15–31) and 14 (95% CI: 13–14) months, respectively (P=0.0001, log-rank test) (*Figure 2*). The 2-year OS rates were 70% for low, 47% and 15% for intermediate and high-risk subgroups. The estimated 3-year OS rates were 52%, 24%, and 4% in the low, intermediate and high-risk patients, respectively.

EFS was also significantly superior in the low (16 months) compared to intermediate (13 months) and high-risk patients (10 months) (P=0.002, log-rank test) (*Figure 3*). A

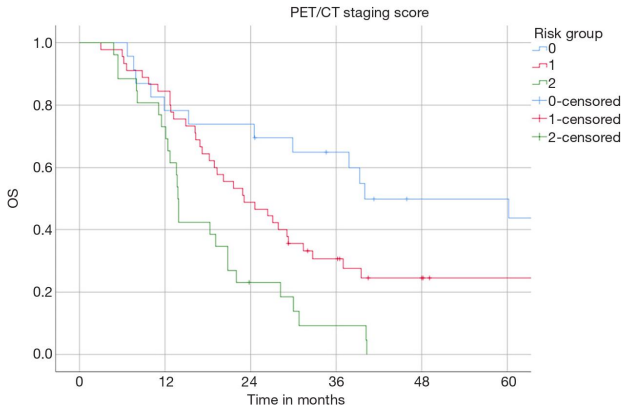

Figure 2 OS according to PET/CT staging score (P<0.001, log-rank test). PET, positron emission tomography; CT, computed tomography.

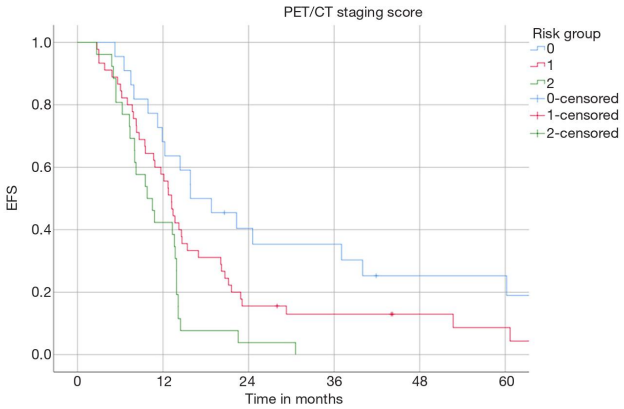

Figure 3 EFS according to PET/CT staging score (P=0.002, log-rank test). EFS, event-free survival; PET, positron emission tomography; CT, computed tomography.

2-year EFS rate in the low risk was 32% *vs.* 0% in high-risk patients.

On multivariate Cox regression analysis, SUVmax_PT <8 and horizontal B-line <3.7 cm were significantly associated with OS [P=0.001, HR 3.06 (1.61–5.81) and P=0.002, HR 2.38 (1.36–4.16), respectively] and EFS [P=0.03, HR 1.91 (1.06–3.43) and P=0.04, HR 1.99 (1.02–3.87), respectively]. In addition, PET staging score was also an important variable and was a strong predictor of superior OS and EFS (P=0.001 and P=0.003, respectively) (*Table 3*).

18F-FDG-PET/CT score and histology

After stratification for histopathological tumor features, patients were divided into subgroups according to the PET-CT score. In the non-adenocarcinoma cohort, the analysis showed that patients with low risk had significantly longer overall survival than intermediate and high-risk (39 *vs.* 18 months, respectively, P=0.045). However, the subgroups showed only a trend for improved EFS (P=0.09). In contrast, OS and EFS differences among patients with adenocarcinoma were highly significant (P<0.0001 and P<0.001, respectively). The estimated median OS was not reached in the low-risk patients with adenocarcinoma. Median OS for the adenocarcinoma high-risk patients was only 12 (range: 10–14) months.

Discussion

Inoperable stage III NSCLC represents a heterogeneous disease based on total tumor volume and extension. These factors strongly influence multimodal treatment strategies and patient prognosis (15,16). Tumor extension has also a significant impact on the definition of treated (irradiated) volume and corresponding treatment toxicity (17,18).

An important role of 18F-FDG-PET/CT for initial staging and response characterization in locally-advanced NSCLC has been extensively described. Based on our data, initial PT-MV was defined as an important survival predictor (17). Patients with higher PT-MV (>63 cm³) before the start of multimodal treatment had significantly worse long-term outcome. Regarding the tumor response assessment after CRT, PT-MV reduction of at least 80% was necessary to significantly improve patient outcome (8). Furthermore, the last publication from the ACRIN 6668/RTOG 0235 trial showed that TLG at 175 and MTV at 35 cm³ were cutoff values for the definition of patient prognosis after completion of multimodal treatment (19). Furthermore, SUVmax_PT was also reported as a predictor of patient outcome in stage III NSCLC treated with CRT. Different SUVmax_PT cut-off values have been investigated (20). Kumasaka *et al.* showed that SUVmax_PT above the median of 9.7 significantly affects patient overall survival (HR 2.24, 95% CI: 1.29–3.88, P<0.01) (21).

Table 3 Univariate und multivariate analysis

Parameters	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Overall survival				
Age >60 years	3.13 (13.16–25.44)	0.02	1.40 (0.76–2.55)	0.277
Sex (male)	2.40 (12.18–21.62)	0.007	1.55 (0.88–2.75)	0.132
Pack years ≥20	2.25 (14.69–23.51)	0.015	2.52 (1.19–5.32)	0.015
Tumor-associated atelectasis	4.59 (0–17.79)	0.004	3.42 (1.62–7.25)	0.001
Histology (non-adenocarcinoma)	1.83 (14.62–21.78)	0.011	0.94 (0.55–1.63)	0.835
SUVmax ≥8	2.10 (14.98–23.22)	<0.0001	3.06 (1.61–5.81)	0.001
A-line ≥3.8 cm	2.48 (15.95–25.65)	0.232		
B-line ≥3.7 cm	2.85 (10.72–21.88)	0.001	2.38 (1.36–4.16)	0.002
PET-score		0.001		0.001
2 points	4.20 (14.95–31.25)		2.79 (1.16–4.55)	
1 point	0.19 (13.43–14.18)		2.30 (1.58–4.94)	
EFS				
Age >60 years	0.94 (11.37–15.03)	0.80		
Sex (male)	1.44 (9.93–15.57)	0.51		
Pack years ≥20	0.58 (11.08–14.42)	0.65		
Tumor-associated atelectasis	3.21 (0–12.57)	0.05	2.08 (0.98–4.41)	0.06
Histology (non-adenocarcinoma)	1.22 (9.30–14.09)	0.06	1.00 (0.62–1.62)	0.99
SUVmax ≥8	1.63 (0.98–2.70)	0.060	1.84 (1.06–3.22)	0.03
A-line ≥3.8 cm	1.60 (1.03–2.49)	0.039	1.36 (0.74–2.48)	0.32
B-line ≥3.7 cm	2.21 (1.40–3.49)	0.001	1.89 (1.00–3.58)	0.05
PET-score		0.002		0.003
2 points	1.65 (6.55–13.03)		2.12 (1.27–3.55)	
1 point	0.86 (11.51–14.89)		2.01 (1.13–3.59)	

PET, positron emission tomography; EFS, event-free survival; SUV, standardized uptake value.

Analysis of 139 stage III NSCLC patients revealed an SUVmax_PT of 8.47 as a prognostic factor (22). The mean survival times of patients with SUVmax_PT >8.47 was only 15.9 *vs.* 32 months for the rest of the treated cohort.

On the basis of previous data and the present results, a PET/CT score was developed for patients with good performance status treated with CRT. This score includes SUVmax_PT as a metabolic parameter characterizing tumor vitality and growth as well as B-line (horizontal distribution) as an important characteristic of the metabolically active involved lymph node compartments. Both factors included in the present score were shown to have a significant impact on patient survival in the uni- and multivariate analyses.

According to the cumulative point number (0 to 2), the score was able to identify three different risk subgroups. Relevant survival differences could be demonstrated between patients with low, intermediate and high-risk, respectively. Especially long-term patient outcome, i.e., estimated 3-year OS rates, was significantly different between subgroups: from 52 in low to only 4% in the high-risk patients. Also, an estimated 2-year EFS rate of 32% in the low-risk versus 0% in high-risk patients. According to the presented score,

low risk patients had the most favorable prognosis with a median survival of 40 months. In contrast high-risk patients demonstrated a dismal outcome in spite of good initial performance status and completed CRT. High-risk patients might mostly benefit from the individualization of the current multimodal approach. In this setting, intensification of combined treatment with chemo- and/or immunotherapy induction as well as the role of concurrent and sequential immune checkpoint inhibition require new studies.

The subgroup analysis demonstrated the special value of the PET-CT score for patients with adenocarcinoma. According to our score, the 2-year OS rate in low risk patients was 70% *vs.* only 8% in high-risk patients.. As a result, this PET-CT score reliably identifies adenocarcinoma patients with a very poor outcome after CRT.

Important limitations of the present analysis are its retrospective and single-center design. Therefore, an independent validation in an external cohort is ongoing. In addition, PET emission scans were initiated 60 to 90 minutes after tracer injection. It is well known that SUV values increase over time after injection, so the involved nodes may be influenced depending on the time of scanning. False positive lymph nodes may explain the fact that in the subgroup analysis, the scoring system performed worse in SCC patients.

Conclusions

A PET/CT score for inoperable stage III NSCLC treated with CRT has been developed to estimate patient prognosis. The score includes two parameters: SUVmax_PT and horizontal B-line, characterizing PT vitality and growth as well as metabolically active involved lymph node compartments. This score was shown to be an independent prognostic factor in a single-center cohort and might be considered together with patient and treatment related factors for further optimization of multimodal therapy.

Acknowledgments

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr.2020.04.04). The authors have no conflicts of interest to declare.

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. This analysis is in compliance with the principles of the Declaration of Helsinki and its subsequent amendments. The study was approved by the Ethics Committee of the Ludwig Maximilian University of Munich (No. 17-230).

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VII.III. Veröffentlichung 3 (Koautorenschaft)

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Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Undergoing Multimodal Treatment

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Abstract. *Background/Aim: Patient performance scores are used widely in clinical practice to assess a patient's general condition. The aim of this study was to evaluate the prognostic role of Eastern Cooperative Oncology Group performance score (ECOG PS) before, after and its changes during chemoradiotherapy in patients with stage III non-small cell lung cancer (NSCLC). Patients and Methods: Records of 99 patients with stage III NSCLC were evaluated. ECOG PS before, during and after chemoradiotherapy was analyzed for prognostic impact on overall (OS) and event-free (EFS) survival. Results: Median OS considering the entire cohort was 20.8 months (range=15.3-26.2 months). Median OS, and 1- and 2-year survival rates were 26.4 months, 85% and 53% in patients with ECOG PS 0 versus 18.9 months, 69% and 37% in patients with ECOG PS 1 (p=0.1, log-rank test), respectively. After the first follow-up, 35% of patients presented worsening ECOG PS, while in 65% it was stable or improved. Median EFS according to ECOG PS 0, 1, 2 and 3 was 9.6, 9.0, 7.9 and 3.5 months, respectively, at the first follow-up (p=0.018, log-rank test). Deterioration of ECOG PS after chemoradiotherapy resulted in reduced OS in the subgroups with initial ECOG PS 0 and 1 (p=0.005 and p=0.001, log-rank test). Conclusion: ECOG PS and its changes have a strong impact on patient outcome. Deterioration of performance status was a strong negative prognostic factor for EFS and OS.*

Lung cancer remains the leading cause of cancer-related mortality worldwide (1-4). Over 80% of all lung cancers are characterized as non-small cell lung cancer (NSCLC), mainly squamous cell carcinoma, adenocarcinoma and large-cell carcinoma (3-5). Stage III NSCLC represents a locally advanced stage with heterogenous characteristics such as extensive lymph node (N3) involvement, large tumour volumes or infiltration of surrounding structures *e.g.* mediastinum, heart or spinal column (3, 4, 6).

Karnofsky's performance status (KPS) or the Eastern Cooperative Oncology Group Performance Status Scale (ECOG PS) are widely used methods of assessing the functional status of cancer patients (7-11). Success of the individualized multimodal treatment highly depends on general and functional patient performance. A multimodal approach including chemo-, immunotherapy and locoregional thoracic irradiation is considered a standard of care in the treatment of inoperable stage III NSCLC. Patients with a good performance status (ECOG PS 0 or 1) should receive definitive concurrent chemoradiotherapy (CRT) followed by consolidation programmed cell death 1 ligand 1 (PD-L1) inhibition (3, 4, 12, 13). However, not all patients will be able to tolerate intensified multimodal approaches and understanding the role of patient performance during the course of treatment is necessary for personalized decision making. The aim of this retrospective study was to evaluate the prognostic role of ECOG PS before, during and after CRT in stage III NSCLC.

The data were partly presented at the ESTRO and ELCC congress 2019.

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Key Words: NSCLC, chemoradiotherapy, survival, prognostic factor, ECOG.

Patients and Methods

Medical records of 99 patients consecutively treated with curative-intent multimodal treatment between December 2010 and December 2016 for stage IIIA/B NSCLC according to the seventh edition of the Union for International Cancer Control (UICC) classification were included (14). Pre-treatment evaluation included: patient history *i.e.* tobacco consumption, comorbidities, pulmonary function testing, radiographic imaging including computed tomography (CT) for all patients and positron-emission tomography (PET)-CT in 94%, routine blood work to assess kidney, liver function and blood cell count.

Tumor histology was obtained *via* transbronchial biopsy in 80 patients, *via* CT-guided-biopsy in nine patients and with mediastinoscopy in 10. Therapeutic approach was discussed in Multidisciplinary Tumor Boards with. Informed consent was given by all patients for evaluation of the acquired data for research purposes. There was Ethical Committee approval for analysis and publishing of the patients' data (approval number: 17-230).

Treatment. Treatment was planned and delivered at one European tertiary cancer center. Three-dimensional (3D) conformal radiotherapy was delivered to the primary tumor and involved lymph nodes to a median total dose of 66 Gy (range=45-70 Gy). Elective nodal irradiation included directly adjacent nodal stations and was delivered to a total dose of 45-54 Gy to 85% of patients. Radiotherapy was delivered on a linear accelerator with megavoltage capability (6-15 MV) using 3D-CRT in 60% of patients and Intensity-modulated radiotherapy in 40% of patients. Image guidance was performed with cone-beam CT two or three times a week.

Patient follow-up. Local and locoregional progression and new distant metastases were documented with CT, PET-CT and magnetic resonance imaging scans. For the first 2 years after therapy, all patients underwent CT or PET-CT scans, routine blood work, lung-function testing and clinical examination every 3 months, and afterwards twice a year. Event-free survival was calculated from the first day of radiation therapy.

Statistical analysis. All statistics were performed with IBM SPSS version 25 (IBM, Armonk, NY, USA). Survival curves were calculated with the Kaplan–Meier method and log-rank test (univariate analysis). Factors showing a significantly negative association with patient prognosis ($p<0.05$) were included in multivariate analysis using Cox regression.

Results

A summary of patient and tumor characteristics is shown in Table I. The median survival was 20.8 months (range=15.3-26.2 months) in the entire patient cohort. Squamous cell carcinoma was diagnosed in 42% of patients, adenocarcinoma in 50% and not otherwise specified in 8% at initial diagnosis. The majority of patients were male (63%) and the median age at diagnosis was 67.4 years (range=43-88 years). Overall, 56% of all patients had NSCLC stage IIIB according to the UICC (seventh edition). Patients were mostly diagnosed with T-stage 3 (30%) or 4 (40%) and N-stage 2 (36%) or 3 (45%). The majority of all patients (78%) received concurrent CRT. The predominant concurrent chemotherapy regimen consisted of cisplatin given intravenously at a dose of 20 mg/m^2 on days 1-4 and oral vinorelbine (Navelbine) 50 mg/m^2 on days 1, 8, and 15, every 4 weeks for two courses (46% of patients).

Patients with an initial ECOG PS 0 had a median OS of 26.4 months and an 1- and 2-year survival rate of 85% and 53% compared to patients with an ECOG PS 1 with a median OS of 18.9 months an 1- and 2-year survival rate of 69% and 37% ($p=0.1$, log-rank test) (see Table II). At the first follow-up after multimodal treatment, 34% of all patients had ECOG PS 0, 46% ECOG PS 1, 18% ECOG PS 2 and 2% ECOG PS 3. Median OS, 1- and 2-year survival rates were: 40.3 months, 88% and 64% in patients with ECOG PS 0 at the first follow-up; 19.3 months, 82% and 40% for ECOG PS 1; 11.9 months, 50% and 28% for ECOG PS 2; and 7.6 months, 0% and 0% for ECOG PS 3 ($p<0.001$, log-rank test), respectively. Decline of ECOG PS after multimodal treatment had a negative prognostic impact on OS in patients with initial ECOG PS 0 [median OS 19.1 *vs.* 31.4 months ($p=0.005$, log-rank test)] and 1 [median OS 22.9 *vs.* 11.1 months ($p=0.001$, log-rank test)]. In the multivariate analysis, male gender (hazard ratio=1.964; 95% confidence interval=1.201-3.211; $p=0.007$) and ECOG PS after treatment (hazard ratio=1.67, 95% confidence interval=1.082-2.577; $p=0.021$) achieved significance. Median EFS according to ECOG PS 0, 1, 2 and 3 was 9.6, 9.0, 7.9 and 3.5 months at the first follow-up ($p=0.018$, log-rank test). Deterioration of ECOG PS after CRT resulted in reduced EFS (median time 9.4 *vs.* 7.7 months, $p=0.049$, log-rank test). No factor achieved significance in the multivariate analysis for EFS.

Discussion

Management of inoperable stage III NSCLC is very heterogeneous and may include different treatment modalities such as chemotherapy, locoregional thoracic irradiation, concurrent CRT, immunotherapy, targeted therapy and best supportive care depending on the performance status patient's (3, 4). As a result of the PACIFIC trial, concurrent platinum-based CRT followed by consolidation PD-L1 inhibition for over 1 year represents the actual standard of care for patients with inoperable stage III with good initial performance status (13).

In the real-life setting, not all patients will be able to tolerate and successfully complete such an intensified multimodal approach. In this situation, clinicians need to assess the suitability for the defined treatment approach continuously. Since their development 50 years ago, the KPS and ECOG PS have been established as standard simple assessment tools to determine the patient functional status

Patients and Methods

Medical records of 99 patients consecutively treated with curative-intent multimodal treatment between December 2010 and December 2016 for stage IIIA/B NSCLC according to the seventh edition of the Union for International Cancer Control (UICC) classification were included (14). Pre-treatment evaluation included: patient history *i.e.* tobacco consumption, comorbidities, pulmonary function testing, radiographic imaging including computed tomography (CT) for all patients and positron-emission tomography (PET)-CT in 94%, routine blood work to assess kidney, liver function and blood cell count.

Tumor histology was obtained *via* transbronchial biopsy in 80 patients, *via* CT-guided-biopsy in nine patients and with mediastinoscopy in 10. Therapeutic approach was discussed in Multidisciplinary Tumor Boards with. Informed consent was given by all patients for evaluation of the acquired data for research purposes. There was Ethical Committee approval for analysis and publishing of the patients' data (approval number: 17-230).

Treatment. Treatment was planned and delivered at one European tertiary cancer center. Three-dimensional (3D) conformal radiotherapy was delivered to the primary tumor and involved lymph nodes to a median total dose of 66 Gy (range=45-70 Gy). Elective nodal irradiation included directly adjacent nodal stations and was delivered to a total dose of 45-54 Gy to 85% of patients. Radiotherapy was delivered on a linear accelerator with megavoltage capability (6-15 MV) using 3D-CRT in 60% of patients and Intensity-modulated radiotherapy in 40% of patients. Image guidance was performed with cone-beam CT two or three times a week.

Patient follow-up. Local and locoregional progression and new distant metastases were documented with CT, PET-CT and magnetic resonance imaging scans. For the first 2 years after therapy, all patients underwent CT or PET-CT scans, routine blood work, lung-function testing and clinical examination every 3 months, and afterwards twice a year. Event-free survival was calculated from the first day of radiation therapy.

Statistical analysis. All statistics were performed with IBM SPSS version 25 (IBM, Armonk, NY, USA). Survival curves were calculated with the Kaplan–Meier method and log-rank test (univariate analysis). Factors showing a significantly negative association with patient prognosis ($p<0.05$) were included in multivariate analysis using Cox regression.

Results

A summary of patient and tumor characteristics is shown in Table I. The median survival was 20.8 months (range=15.3-26.2 months) in the entire patient cohort. Squamous cell carcinoma was diagnosed in 42% of patients, adenocarcinoma in 50% and not otherwise specified in 8% at initial diagnosis. The majority of patients were male (63%) and the median age at diagnosis was 67.4 years (range=43-88 years). Overall, 56% of all patients had NSCLC stage IIIB according to the UICC (seventh edition). Patients were mostly diagnosed with T-stage 3 (30%) or 4 (40%) and N-stage 2 (36%) or 3 (45%). The majority of all patients (78%) received concurrent CRT. The predominant concurrent chemotherapy regimen consisted of cisplatin given intravenously at a dose of 20 mg/m^2 on days 1-4 and oral vinorelbine (Navelbine) 50 mg/m^2 on days 1, 8, and 15, every 4 weeks for two courses (46% of patients).

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performance status is of critical importance because decision-making needs to be based on its correct assessment, including the eligibility for and planning of clinical trials and allocation of healthcare resources such as palliative care. In our study, ECOG PS was scored by experienced radiation oncologists. However, we were unable to evaluate interobserver variability. Therefore, future studies need to prospectively confirm our findings and assess interobserver variations.

Conclusion

In inoperable stage III NSCLC, despite the prognostic value of the ECOG PS before multimodal treatment, ECOG PS after completion of CRT as well as its change during treatment application have a strong prognostic impact on patient OS and EFS.

Conflicts of Interest

The Authors have declared that there are no conflicts of interest with regard to this work.

Authors' Contributions

L.K., J.T., C.E., O.R., M.D., K.G., M.K., O.P., A.T., C.B. and F.M. contributed to the design and implementation of the research, L.K., J.T., C.E. and O.R. to the analysis of the results and L.K., J.T., C.E., O.R., and F.M. to the writing of the article.

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IX. Gesamtpublikationsverzeichnis

Gennen K, Käsmann L, Taugner J, Eze C, Karin M, Roengvoraphoj O, Neumann J, Tufman A, Orth M, Reu S, Belka C, Manapov F.

Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy

Radiation Oncology 2020; 15(1):5. doi:10.1186/s13014-019-1453-3

Käsmann L, Abdo R, Eze C, Dantes M, Taugner J, Gennen K, Roengvoraphoj O, Rades D, Belka C, Manapov F.

External Validation of a Survival Score for Limited-Stage Small Cell Lung Cancer Patients Treated with Chemoradiotherapy

Lung Cancer 2020; 198(1):201–6. doi:10.1007/s00408-019-00312-6

Taugner J, Käsmann L, Eze C, Dantes M, Roengvoraphoj O, Gennen K, Karin M, Petruknov O, Tufman A, Belka C, Manapov F.

Survival score to characterize prognosis in inoperable stage III NSCLC after chemoradiotherapy

Translational Lung Cancer Research 2019;8(5):593–604. doi:10.21037/tlcr.2019.09.

Käsmann L, Taugner J, Eze C, Roengvoraphoj O, Dantes M, Gennen K, Karin M, Petrukhnov O, Tufman A, Belka C, Manapov F.

Performance Status and Its Changes Predict Outcome for Patients With Inoperable Stage III NSCLC Undergoing Multimodal Treatment

Anticancer research. 2019;39(9):5077–81. doi:10.21873/anticanres.13701

Roengvoraphoj O, Käsmann L, Eze C, Taugner J, Gjika A, Tufman A, Hadi I, Li M, Mille E, Gennen K, Belka C, Manapov F.

Maximum standardized uptake value of primary tumor (SUVmax_PT) and horizontal range between two most distant PET-positive lymph nodes predict patient outcome in inoperable stage III NSCLC patients after chemoradiotherapy

Translational Lung Cancer Research 2020;9(3):541–8. doi:10.21037/tlcr.2020.04.04

Taugner J, Eze C, Käsmann L, Roengvoraphoj O, Gennen K, Karin M, Petrukhnov O, Tufman A, Belka C, Manapov F.

Pattern-of-failure and salvage treatment analysis after chemoradiotherapy for inoperable stage III non-small cell lung cancer

Radiation Oncology 2020;15(1):148. doi:10.1186/s13014-020-01590-8

2019, Abstract für die deutsche Gesellschaft für Radioonkologie (DEGRO), Koautorenschaft

Lehrformate der Radioonkologie – ein interuniversitärer Vergleich

2019, Abstract für die DEGRO, Koautorenschaft

Ernährungstherapie während der Strahlentherapie / Radiochemotherapie

2020, Abstract für den deutschen Krebskongress in Berlin, Koautorenschaft

Undergraduate medical education in radiation oncology: A student's perspective

X. Wissenschaftliche Vorträge im Rahmen der Promotion

Posterpräsentation auf der 24. Jahrestagung der deutschen Gesellschaft für Radioonkologie in Leipzig, 2018

Patterns of Care for Patients with Small Cell Lung Cancer: A Survey of German Radiation Oncology Institutions on Recommendations for Prophylactic Cranial Irradiation

Posterpräsentation auf der 24. Jahrestagung der deutschen Gesellschaft für Radioonkologie in Leipzig, 2018

Prophylactic Cranial Irradiation in SCLC: A Survey of German Radiation Oncology Institutions on Recommendations for Brain Imaging

Vortrag auf der 24. Jahrestagung der deutschen Gesellschaft für Radioonkologie in Leipzig, 2018

Natürliche Strahlenexposition und Krebsentstehung

Posterpräsentation auf der ELCC (European Lung Cancer Congress) in Genf, 2019

Prognostic value of CD8-postive tumor stroma-infiltrating lymphocytes and PD-L1 positive tumor cells at initial biopsy in patients with locally advanced NSCLC treated with chemoradiotherapy

Posterpräsentation auf der ESTRO (Congress of the European Society for Radiotherapy and Oncology) in Mailand, 2019

Prognostic value of CD8-postive tumor stroma-infiltrating lymphocytes and PD-L1 positive tumor cells at initial biopsy in patients with locally advanced NSCLC treated with chemoradiotherapy

Postervortrag auf der 25. Jahrestagung der deutschen Gesellschaft für Radioonkologie in Münster, 2019

Prognostic value of CD8-postive tumor stroma-infiltrating lymphocytes and PD-L1 positive tumor cells at initial biopsy in patients with locally advanced NSCLC treated with chemoradiotherapy

Best of Five Vortrag auf der 25. Jahrestagung der deutschen Gesellschaft für Radioonkologie in Münster, 2019

Strahlentherapie und Ernährung

XI. Lebenslauf

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Name	Kathrin Johanna Gennen
Geburtsdatum	16.09.1996
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Nationalität	Deutsch

Schulische und akademische Ausbildung

2006-2012	Gymnasium Erzbischöfliche Liebfrauenschule Köln
2012-2013	Private High School Forsyth Country Day School (North Carolina, USA)
2013-2015	Friedrich-Wilhelm-Gymnasium Köln Abschluss: Abitur (1,2)
2015-2017	Absolvierung der Vorklinik an der Rheinischen Friedrich-Wilhelms-Universität Bonn, Erwerb des Physikums in Regelstudienzeit mit der Note „gut“
2017-2021	Studentin der LMU München (Studium in Regelstudienzeit), Erwerb des Dritten Abschnitts der Ärztlichen Prüfung am 11.11.2021 mit der Note „sehr gut“

Famulaturen

19.02.2018-28.03.2018	Hausarztpraxis Dr. Gennen/Dr. Neufang, Lehrpraxis der Universität zu Köln
28.07.2018-26.08.2018	Strahlentherapie der LMU München

08.03.2019-07.04.2019	Radiologie und Nuklearmedizin, Klinikum Dritter Orden München Nymphenburg
24.08.2019-22.09.2019	Kinderchirurgie, Klinikum Dritter Orden München Nymphenburg

Praktisches Jahr

Erstes Tertial (16.11.2020-07.03.2021)	München, Krankenhaus Neuwittelsbach, Pflichtbereich Innere Medizin (Akademisches Lehrkrankenhaus der LMU München)
Zweites Tertial (08.03.2021-27.06.2021)	München, Krankenhaus Martha Maria, Wahlbereich Hals-Nasen-Ohrenheilkunde und Kopf-Hals-Chirurgie (Akademisches Lehrkrankenhaus der LMU München)
Drittes Tertial (28.06.2021-17.10.2021)	Erding, Klinikum Erding, Pflichtbereich Chirurgie mit Fokus auf plastischer Chirurgie und geschlechtsangleichende Operationen (Akademisches Lehrkrankenhaus der TU München)

Akademische Auszeichnungen und Stipendien

Sommersemester 2018	Erwerb des TEAM-G Zertifikates (Trauma Evaluation and Management Germany)
2018	Kongressstipendium der deutschen Gesellschaft für Radioonkologie e.V. (DEGRO)
Seit 2018	Beiratsmitglied des Vorstandes des Club 100 (Studentische Arbeitsgruppe der deutschen Gesellschaft für Radioonkologie e.V.)

2019	Kongressstipendium der deutschen Gesellschaft für Radioonkologie e.V. (DEGRO)

Interessen und Freizeit

Sport	Tennis (10 Jahre Leistungssportlerin des TC LESE GW Köln, dort langjährige Mannschaftsführerin der 3. Damen), Golf, Fitness, Joggen, Ski
Kultur	Lesen, Reisen