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Untersuchungen zur Pharmakotherapie gastrointestinaler Tumore mit dem Schwerpunkt hepatozelluläres Karzinom

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Meiner Frau Claudia
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Inhaltsverzeichnis

1. Abkürzungsverzeichnis

BCLC	Barcelona Clinic Liver Cancer
Brachy	Brachytherapie
CCC	Cholangiozelluläres Karzinom
CI	Confidence Intervall
CR	Complete Response
CPI	Checkpoint-Inhbitor
DEG	Differentiell exprimierte Gene
EBRT	External Beam Radiation Therapy
FOLFIRI	5-Fluorouracil-Leucovorin-Irinotecan (FOLFIRI)
FOLFOX	5-Fluorouracil-Leucovorin-Oxaliplatin (FOLFOX)
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HCC	Hepatozelluläres Karzinom
HEP	Hepatische Metastasierung
HR	Hazard Ratio
mOS	Medianes Gesamtüberleben
NAFL	Nichtalkoholische Fettleber Erkrankung
NASH	Nichtalkoholische Steatohepatitis
ns	Nicht signifikant
PCT	Procalcitonin
PD	Progressive Disease
PR	Partial Response
SD	Stable Disease
StAbw	Standardabweichung

2. Verzeichnis der in der Habilitationsschrift zusammengefassten Publikationen

Paula Sagmeister, Jimmy Daza, Andrea Ofner, Andreas Ziesch, Liangtao Ye, Najib Ben Khaled, Matthias Ebert, Julia Mayerle, Andreas Teufel, Enrico N De Toni, **Stefan Munker**; Comparative Response of HCC Cells to TKIs: Modified in vitro Testing and Descriptive Expression Analysis; J Hepatocell Carcinoma. 2022 Jul 9;9:595-607. doi: 10.2147/JHC.S356333. Epub 2022 Jul 9. IF 4.1 (2022)

Najib Ben Khaled, Bernhard Mörtl, Dominik Beier, Florian P Reiter, Dorota Pawlowska-Phelan, Andreas Teufel, Daniel Rössler, Daniel F Schwade, Alexander Philipp, Ilja Kubisch, Ursula Ehmer, Andreas Geier, Christian M Lange, Julia Mayerle, Karin Berger-Thürmel, Enrico N De Toni, **Stefan Munker**; Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study; Eur J Cancer. 2023 Oct:192:113248. doi: 10.1016/j.ejca.2023.113248. Epub 2023 Jul 21..; IF 8.4 (2022)

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3. Einleitung

3.1. Klinische Relevanz gastroenterologisch-onkologischer Erkrankungen

Gastroenterologisch-onkologische Erkrankungen machen einen Großteil der soliden Tumore aus und sind zusammengenommen die häufigste weltweite onkologische Todesursache (33%, 3,3 Millionen Tote/Jahr) (1). Zudem nimmt die Inzidenz gastroenterologisch onkologischer Erkrankungen insgesamt zu (insbesondere das Hepatozelluläres Karzinom (HCC) und das Pankreaskarzinom), was eine erhebliche Herausforderung für die Onkologie und die medizinischen Systeme weltweit darstellt (2, 3). Speziell in fortgeschrittenen Stadien bestehen leider weiterhin oft nur begrenzte Behandlungsoptionen. Und trotz der Entwicklung neuer vielversprechender Therapien verbessern sich die Überlebensraten nur langsam(4-7). Dies wird zum Teil mit dem noch ungleichen Zugang zu neuen Therapien erklärt (8), jedoch ist auch die geringfügige Verbesserung systemischer Therapiekonzepte ist als Ursache anzusehen (9, 10).

3.2. Entitätsspezifische Trends

Ursächlich für die Steigerung der weltweiten Inzidenz eines Großteils der gastrointestinal-onkologischen Erkrankungen zeigen sich verschiedene entitätsspezifische Trends und ungeklärte Ursachen (Tabelle1): Die Inzidenz des HCC steigt signifikant aufgrund des Anstiegs von Adipositas und des metabolischen Syndroms (11). Beim kolorektalen Karzinom (KRK) beobachtet man eine steigende Inzidenz in Ländern mit niedrigerem Entwicklungsstand (erklärt durch die steigende Lebenserwartung) und einen Anstieg der Inzidenz bei unter 50-Jährigen in westlichen Ländern, dessen Ursache noch nicht abschließend geklärt ist (12-14), erfreulicherweise jedoch auch eine Abnahme der Inzidenz bei über 50-Jährigen durch die zunehmende Implementierung von Früherkennungsprogrammen (15). Ferner wird ein Anstieg der Pankreas-Karzinom Inzidenz beobachtet (16). Die Inzidenz und Mortalität des Pankreaskarzinoms nehmen weltweit zu. Die Ursachen hierfür sind jedoch noch nicht gut verstanden(17). Ebenso zeigt sich ein signifikanter Anstieg der Ösophagus Karzinom Inzidenz speziell des Adenokarzinom Subtyps, dies wird mit der Zunahme der Adipositas und damit verbundener Reflux und Barrett Erkrankung erklärt (18). Beim Gallengangskarzinom (CCA) steigt die Inzidenz des intrahepatischen Subtyps, hierbei wird die Exposition mit unbekannten Umweltfaktoren als Ursache vermutet, während die Inzidenz des extrahepatischen CCA in

vielen Regionen stabil bis leicht rückläufig ist (19, 20). Es zeigen sich jedoch auch erfreuliche Trends, so sinkt die Inzidenz des Helicobacter Pylori assoziierten Magenkarzinoms aufgrund einer zunehmenden Eradikation von Helicobacter pylori und eines rückläufigen Nikotinkonsums (21). Zudem wird angenommen, dass die Screening-Koloskopie dazu geführt hat, dass die Inzidenz kolorektaler Karzinome (KRK) in der Altersgruppe über 50 Jahren in entwickelten Ländern deutlich gesunken ist (15).

Tumor Entität	Trend der Inzidenz	Ursachen/Zitat
Kolorektales Karzinom	Insgesamt steigende Inzidenz (12-14)	Anstieg in Ländern mit niedrigem Einkommen und in jungen Bevölkerungs-gruppen (12)
Magenkarzinom	Rückläufig(21)	Rückläufiger Nikotinkonsum, H.P. Eradikation(21)
Pankreaskarzinom	Steigend weltweit	Unklar (17)
Ösophaguskarzinom	Weltweit steigend	Mehr Barrett Karzinom Fälle und Adipositas (18)
Hepatozelluläres Karzinom	Weltweit steigende Inzidenz	Adipositas Epidemie und NAFLD führende Ursache des Anstiegs (11)
Gallengangskarzinom	Steigende Inzidenz	Erhöhte Exposition gegen-über Risikofaktoren (19, 20)

Tabelle 1 epidemiologische Trends GI onkologischer Erkrankungen.

Da spezifische Frühsymptome fehlen und strukturierte Vorsorgeprogramme für die meisten GI-Tumoren - mit Ausnahme von KRK (22) und HCC (23) nicht etabliert sind - wird die klinische Versorgung dieser zunehmend häufiger werdenden Patienten erschwert. Ein signifikanter Anteil an GI-Tumoren wird nämlich häufig erst in fortgeschrittenen Stadien diagnostiziert. In der Folge suchen viele Patienten erst mit metastasierten oder lokal weit fortgeschrittenen Tumoren einen Arzt auf. In diesen Fällen sind kurative Therapieansätze meist nicht mehr möglich, sodass hauptsächlich palliative und systemische Maßnahmen erfolgen können.

3.3. Herausforderungen in der Therapie metastasierter GI-Tumore

Die bislang entwickelten empirischen Therapien berücksichtigen die biologische Heterogenität der Tumoren nur unzureichend. Deswegen spricht ein erheblicher Anteil der Patienten nicht adäquat auf die Therapien an und erleidet zudem erhebliche Nebenwirkungen. Es fehlen geeignete Biomarker, die die Therapiewahl leiten könnten (24). Die Folge sind Therapien, die nicht auf das jeweilige Tumorprofil und die Sensitivität des Patienten abgestimmt sind.

Zwar hat sich mit der Einführung von Checkpoint-Inhibitoren die Therapielandschaft für gastrointestinale-onkologische Erkrankungen verbessert. Insbesondere für das hepatozelluläre

Karzinom (HCC), Cholangiokarzinom (CCC) sowie für Magen- und Ösophaguskarzinome, hat sich die Therapiesequenz geändert (25-27). Allerdings weisen auch diese modernen immuntherapeutischen Verfahren weiterhin erhebliche Limitationen auf: Die Mehrheit der Patienten mit den genannten Entitäten (HCC (5)., CCC, Magen-Ca, Ösophagus-Ca) zeigen im Verlauf der Erkrankung entweder ein fehlendes Primäransprechen oder eine rasche Progression. Es besteht daher ein dringender Bedarf an weiteren effektiven Folgetherapien (26, 28) .

Trotz Fortschritten in der Systemtherapie bleibt die Prognose im metastasiertem Stadium gastrointestinaler Tumore insgesamt schlecht. Ein zentraler limitierender Faktor hierbei ist das Fehlen valider prädiktiver Biomarker, die ein Therapieansprechen verlässlich vorhersagen könnten. Während bei manchen GI-Tumoren spezifische Biomarker etabliert sind, fehlen sie bei anderen Entitäten fast vollständig. Beispiele für Biomarker sind KRAS-, NRAS-, und BRAF-Mutationsstatus beim kolorektalen Karzinom oder BRCA1/2-Mutationen beim Pankreaskarzinom. Bei anderen Entitäten, wie dem HCC, ist bislang lediglich ein AFP-Wert von >400 ng/ml als prädiktiver Marker etabliert - jedoch auch hier nur für die Therapie mit Ramucirumab (siehe Tabelle 2). Um die individualisierte Behandlung nachhaltig zu verbessern, besteht somit großer Bedarf an der Entwicklung neuer, verlässlicher Biomarker, die eine bessere Vorhersage des Therapieansprechens ermöglichen.

Tumor Entität	Zugelassene Therapie	Prädiktive Biomarker
Colorectal Cancer (CRC)	FOLFOX FOLFIRI Cetuximab, Panitumumab Bevaciczumab, Ramucirumab, Ziv-Aflibercept Trifluridine/Tipiracil Pembrolizumab, Nivolumab Adagrasib/Sotorasib Encorafenib	Keine Keine KRAS, NRAS, BRAF – Mutation Keine Keine MSI-H, dMMR KRAS G12C V600E
Gastric Cancer	5FU, Capecitabine, Cisplatin Trastuzumab Pembrolizumab	Keine Her2neu PD-L1-IHC
Pancreatic Cancer	FOLFIRINOX NALIRI Gemcitabine-Nab-Paclitaxel PARP Inhibitoren	Keine Keine Keine BRCA1/2 Mutationen
Esophageal Cancer	5FU-Cisplatin ECX/ECF FLOT Trastuzumab Pembrolizumab	Keine Keine Keine Her2neu PD-L1
Hepatocellular Carcinoma (HCC)	Atezolizumab-Bevacizumab	Keine

	Durvalumab-Tremelimumab Sorafenib Regorafenib Lenvatinib Cabozantinib Ramucirumab	Keine Keine Keine Keine Keine AFP>400ng/ml
Gastrointestinal Stromal Tumors (GIST)	Imatinib Sunitinib	KIT PDGFRA
Cholangiocarcinoma	Gemcitabine-Cisplatin FOLFIRI Durvalumab Ivosidenib Futibatinib	Keine Keine Keine IDH1/2 Mutation FGFR2 Fusion

Tabelle 2 Therapielandschaft GI-onkologischer Erkrankungen und zugehörige Biomarker.

3.4. Motivation und Zielsetzung dieser Arbeit

Die Behandlung von gastrointestinalen (GI) Tumorerkrankungen, insbesondere des hepatozellulären Karzinoms (HCC), ist auch mit modernen Therapieansätze nach wie vor eine große klinische Herausforderung. Als Gastroenterologe mit dem Schwerpunkt GI-Onkologie war es mein Ziel, klinisch relevante Fragestellungen retrospektiv und experimentell zu untersuchen, um daraus Erkenntnisse für eine verbesserte Patientenversorgung zu gewinnen.

In der vorliegenden kumulativen Habilitationsschrift werden in mehreren klinisch-retrospektiven Studien die Therapien und die prognostische Relevanz klinischer Parameter gastrointestinaler Tumorerkrankungen, mit besonderem Fokus auf das HCC, umfassend evaluiert. Untersucht wurden unter anderem Prognosefaktoren beim HCC unter systemischer Therapie, der Effekt von Begleittherapien wie Checkpoint-Inhibitoren (CPI) und Strahlentherapie sowie modifizierte in-vitro-Testsysteme zur Generierung neuer Biomarker. Ergänzend erfolgte eine Analyse relevanter therapeutischer Fragestellungen bei weiteren gastrointestinalen Tumorerkrankungen, einschließlich des kolorektalen Karzinoms und hochgradiger neuroendokriner Neoplasien.

Das übergeordnete Ziel dieser Habilitationsschrift ist die klinisch-retrospektive Aufarbeitung relevanter Fragestellungen aus der GI-Onkologie mit Fokus auf dem HCC. Daraus sollen Schlussfolgerungen für verbesserte Behandlungsstrategien und zukünftige klinische Studien abgeleitet werden (siehe **Abbildung 1**).

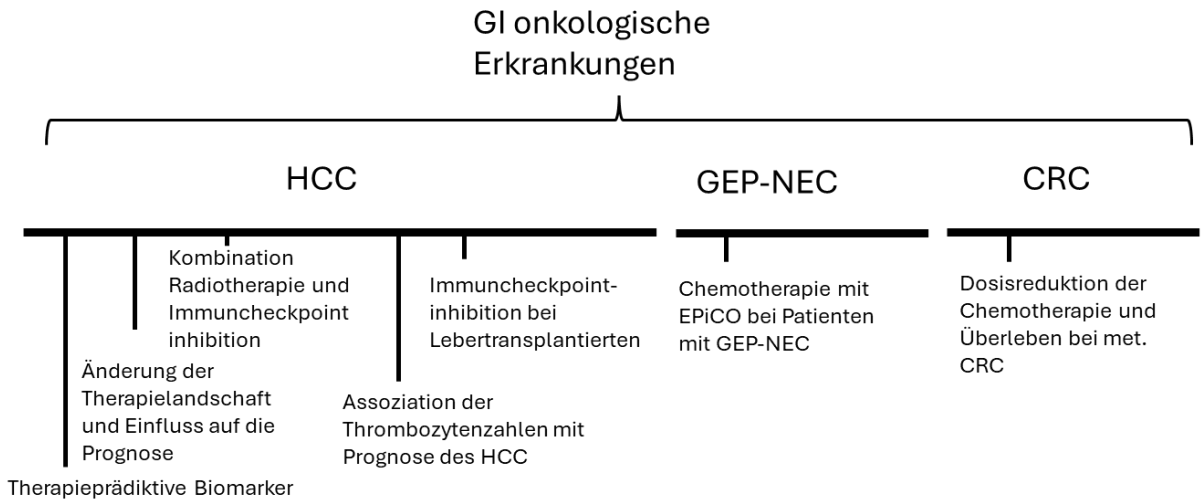


Abbildung 1. Schematischer Überblick über das Habilitationsprojekt.

Hepatozelluläres Karzinom Entwicklung der Prognose, neue Therapieansätze und Biomarker:

1) In späteren Therapielinien besteht beim HCC das Problem, dass mehrere Therapien (Tyrosinkinase-Inhibitoren wie Sorafenib, Regorafenib, Lenvatinib oder Cabozantinib) miteinander konkurrieren. Leider konnten bisher keine Biomarker identifiziert werden, die das Ansprechen zuverlässig vorhersagen (29-31). Im Rahmen dieses Habilitationsprojektes konnte in einer grundlagenwissenschaftlichen Arbeit mit neun HCC-Zelllinien gezeigt werden, dass die verschiedenen Tyrosinkinase-Inhibitoren unterschiedliche Ansprechmuster in den Zelllinien erzeugen. Dieses individuelle Ansprechen könnte eine Rationale für die Entwicklung neuer Biomarker und für eine individuelle Testung liefern könnte (32).
2) Umfangreiche epidemiologische Studien konnten zeigen, dass sich die Prognose von HCC-Patienten im Verlauf der letzten Jahrzehnte deutlich verbessert hat. Einerseits verbesserte sich die Flächenversorgung von HCC-Patienten (4, 7), andererseits wird auch eine höhere Effektivität der neuen Therapien als Ursache vermutet. Im Rahmen des Habilitationsprojektes konnte anhand umfangreicher Versicherungsdaten gezeigt werden, dass sich die Prognose auch in den letzten Jahren weiter verbessert hat und dass diese Verbesserung mit einer Änderung der Therapielandschaft einherging (5).
3) Die Zulassung der CPI-Therapie Atezolizumab in Kombination mit einem Anti-VEGF-Antikörper Bevacizumab in der ersten Therapielinie führte zu einer Neuordnung der Therapiesequenz und ermöglicht erstmals eine sehr gut verträgliche und nebenwirkungsarme Therapie in der ersten Linie. Dennoch weisen nach 18 Monaten bis

zu 80 % der Patienten unter Atezolizumab-Bevacizumab einen Progress auf, sodass Folgetherapien notwendig werden. Aufgrund des milden Nebenwirkungsspektrums wären Eskalationskonzepte, die auf CPI-Therapien basieren, wünschenswert (33). Bei hepatischer Progression werden lokoregionäre Verfahren in Kombination mit CPI-Therapien geprüft (34, 35). Eine vielversprechende Alternative wäre eine Strahlentherapie in Kombination mit einer Immuntherapie, für die es bisher jedoch nur wenige Daten gibt (36). Dies würde auch eine Behandlung extrahepatischer Läsionen ermöglichen. Im Rahmen dieser Habilitationsarbeit wurde mittels einer retrospektiven Analyse die Anwendung einer Radiotherapie in Kombination mit einer CPI-Therapie hinsichtlich ihres Nebenwirkungsspektrums und ihrer Wirksamkeit geprüft. In einer Serie von sechs Patienten, die die Radiotherapie neben der CPI-Therapie zur Symptom- oder Tumorkontrolle erhielten, zeigte sich, dass die Radiotherapie bei einem Teil der Patienten zu einer lokalen Tumorkontrolle führte und dass keine unerwarteten Nebenwirkungen durch die Kombinationstherapie auftraten (37).

4) Alpha-Fetoprotein (AFP) sowie andere seltener gebräuchliche Biomarker wie das Neutrophilen-zu-Lymphozyten Verhältnis, Glypican-3 und PIVKA-II (Des-γ-Carboxyprothrombin) wurden als prognostisch relevante Biomarker für das hepatozelluläre Karzinom (HCC) identifiziert (38-41). Diese Biomarker sind entscheidend für die Beurteilung der Krankheitsschwere und der Prognose. Thrombozyten spielen bisher eine wichtige Rolle bei der Vorhersage von postoperativen Blutungskomplikationen; erniedrigte Werte deuten häufig auf eine portale Hypertonie hin, die mit einem erhöhten Risiko bei chirurgischen Eingriffen verbunden ist (42, 43). In Kontrast zum frühen, operablen HCC (Barcelona Clinic Liver Cancer (BCLC) A), zeigen zunehmende Untersuchungen interessanterweise eine inverse Assoziation der Thrombozytenzahlen mit dem Überleben bei fortgeschrittenem HCC (BCLC B und C) (44-46). Im Rahmen dieser Habilitationsarbeit konnte in einer multizentrischen Analyse gezeigt werden, dass erhöhte Thrombozytenzahlen mit einer schlechteren Prognose bei HCC-Patienten einhergehen (47).

5) Leider ist die Evidenz für Systemtherapien bei Organtransplantierten mit konsekutiver maligner Grunderkrankung limitiert, da Organtransplantationen häufig ein Ausschlusskriterium für klinische Prüfungen darstellen und deswegen nur wenige Erfahrungswerte bestehen (48). CPI-Therapien zeigen eine sehr gute Wirksamkeit bei

Patienten mit Melanomen, Nierenzellkarzinomen, HCC und anderen Tumorentitäten und weisen im Vergleich zu anderen Therapien ein gutes Nebenwirkungsspektrum auf (33). Bei Organtransplantierten bestehen jedoch besondere Bedenken hinsichtlich des Einsatzes von Checkpoint-Inhibition, da theoretisch das Risiko besteht, eine fulminante Abstoßung des Organs herbeizuführen. Im Rahmen der Habilitationsarbeit konnte speziell für Lebertransplantierte mittels einer Fallsammlung ein genaueres Bild des Risikos gezeichnet werden (49).

High-grade Gastroenteropathische Neuroendokrine Tumore (GEP-NET) und Second-Line Therapieoptionen

6) High-Grade-GEP-NET Tumore stellen ein sehr seltenes gastroenterologisch-onkologisches Krankheitsbild dar (53). Man unterscheidet die high-grade-GEP-NET mit einer noch erhaltenen neuroendokrinen Differenzierung und niedrigeren Proliferationsindizes (in der Regel von 20-60 %) von GEP-NEC, die entdifferenziert sind und in der Regel höhere Proliferationsindizes von 60-90 % aufweisen (54). Trotz der distinkten biologischen Unterschiede ist für beide Tumore eine Chemotherapie mit Carboplatin Etoposid in der Erstlinie etabliert (55). In der Zweitlinie zeigt FOLFIRI Wirksamkeit, jedoch ist diese nicht hoch, während die Nebenwirkungen teilweise stark ausgeprägt sind. Gleichzeitig besteht ein großer Bedarf an besseren Therapieoptionen (56). Im Rahmen der Habilitationsarbeit konnte aus retrospektiven Daten die Therapiealternative einer Kombination aus Epirubicin, Cyclophosphamid und Vincristin (EpiCo) hinsichtlich Wirksamkeit und Nebenwirkungsprofil aus retrospektiven Daten untersucht werden (57).

Kolorektales Karzinom Dosisreduktion der Chemotherapie und der Einfluss auf das Überleben

7) Patienten mit metastasiertem kolorektalem Karzinom erhalten in der Regel in der ersten Therapielinie sowie in Folgetherapielinien die Standardchemotherapien 5-Fluorouracil-Leucovorin-Irinotecan (FOLFIRI) oder 5-Fluorouracil-Leucovorin-Oxaliplatin (FOLFOX). Wenn möglich, werden diesen Therapien, Anti-EGFR- oder Anti-VEGF-Antikörper hinzugefügt (50). Bisher wurde angenommen, dass die Notwendigkeit einer Dosisreduktion mit einem schlechteren Performance Status und geringeren biologischen Reserven der Patienten einhergeht (51). Im Rahmen der Habilitationsarbeit konnte in einer retrospektiven Analyse gezeigt werden, dass die

Notwendigkeit einer Dosisreduktion nicht mit einer schlechteren Prognose einhergeht, sondern vielmehr als Titrierung entlang der biologischen Tolerabilität des Patienten betrachtet werden kann (52).

Die vorliegenden Projekte zeigen neue Ansätze in der Therapie gastroenterologisch-onkologischer Erkrankungen mit einem Schwerpunkt auf dem hepatozellulären Karzinom.

4. Ergebnisse

4.1. Vergleichende Analyse des Ansprechens von HCC Zelllinien auf Tyrosinkinase-inhibitoren: Angepasste *in vitro* Testung und deskriptive Expressionsanalyse)

„Comparative Response of HCC Cells to TKIs: Modified in vitro Testing and Descriptive Expression Analysis“ (Munker et al., Journal of Hepatocellular Carcinoma 2022

Es besteht ein ungedeckter Bedarf an prädiktiven Biomarkern, die das Ansprechen auf Tyrosinkinase-Inhibitoren beim HCC vorhersagen können. Dieser Bedarf besteht auch nach der Zulassung der Immuncheckpoint-Inhibitoren in Kombination mit Antiangiogenese-Antikörpern für das HCC, die die Therapiesequenz deutlich geändert haben (58), da nach Wirkverlust der Immuntherapie die Tyrosinkinase-Inhibitoren die einzigen zugelassenen Therapien in zweiter und dritter Linie sind (59).

Eine mögliche Erklärung für das Fehlen prädiktiver Biomarker in der klinischen Routine kann in einem Mangel an Gewebeproben und entsprechenden Untersuchungen in den Zulassungsstudien gesucht werden (60, 61). Ferner erschwert die Testung in wenig repräsentativen in-vitro-Systemen und -Metriken die Etablierung prädiktiver Biomarker. So ist beispielsweise die Behandlungsdauer (*in vitro* 48–72 Stunden) nicht annähernd mit der klinischen Situation vergleichbar (Behandlungen über mehrere Wochen). Darüber hinaus wurde kürzlich gezeigt, dass *in-vitro*-Testungen mit der IC50 Metrik stark durch die individuelle Proliferationsgeschwindigkeit der Zelllinien beeinträchtigt sind. Um diese Beeinträchtigung zu adressieren, entwickelten Hafner et al. die GR-50-Metrik, die eine Beurteilung des Therapieansprechens unabhängig von der Proliferationsgeschwindigkeit ermöglicht (62).

Die Multi-Tyrosinkinase-Inhibitoren (TKI) Regorafenib, Sorafenib, Cabozantinib und Lenvatinib hemmen breit gefächerte Rezeptor-Tyrosinkinasen, blockieren dadurch deren Autophosphorylierung und unterbrechen die nachgeschalteten MAPK/ERK- sowie PI3K/AKT/mTOR-Signalwege.

- Sorafenib wirkt sowohl auf B-/C-RAF als auch auf VEGFR-2/3 und PDGFR-β (63).
- Regorafenib antagonisiert u. a. VEGFR-1-3, TIE2, PDGFR-β, FGFR-1 und KIT (64).
- Lenvatinib blockiert vorzugs-weise VEGFR-1-3, FGFR-1-4, PDGFR-α, KIT und RET (65).
- Cabozantinib zeigt hohe Affinität zu MET, AXL, VEGFR-2, RET, FLT3 und KIT (66).

Im Rahmen der Habilitationsarbeit untersuchten wir neun etablierte HCC-Zelllinien hinsichtlich ihres Ansprechens auf für das HCC zugelassene TKI mit einem verlängerten Behandlungsschema von sechs Tagen (ähnlich der Therapiezyklen beim Patienten) und der neuen GR50 Metrik. Dabei konnten differenzielle Ansprechraten für alle vier Substanzen festgestellt werden. Bei Lenvatinib zeigte sich eine größere Bandbreite des Ansprechens als bei den anderen Therapien (siehe Abbildung 2). Im Gegensatz zu den Publikationen, die mit den gleichen Zelllinien und Substanzen deutlich von den klinischen Steady-State Konzentrationen abweichende Werte zeigten, lagen alle GR50-Werte in etwa im Bereich der aus den Phase I-II Studien bekannten Steady-State-Konzentrationen (Tabelle 3) (67-70).

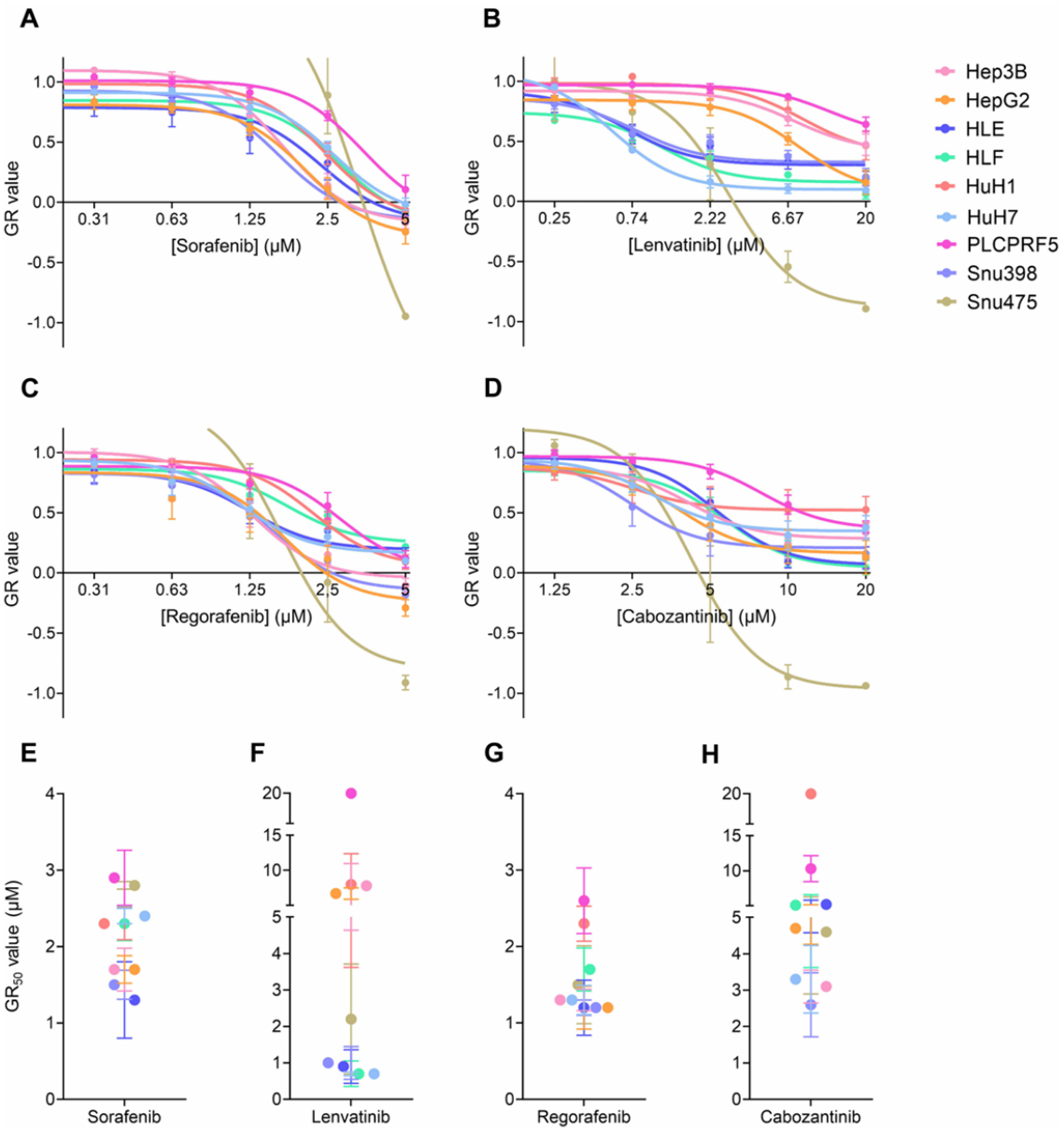


Abbildung 2 Darstellung des Ansprechens von 9 HCC-Zelllinien auf die vier Tyrosinkinaseinhibitoren Sorafenib,

Lenvatinib, Regorafenib und Cabozantinib. (A-D) Viabilität und Dosis-Wirkungskurven jeweils der Substanzen mit sigmoidalem Curve-Fitting (E-H) GR50 Werte der jeweiligen Subsanzen als 1-dimensional scatter plot dargestellt. Statistische Signifikanz bei P<0,05 durch ANOVA bestätigt wurde. (Abk.: GR, Growth-Rate; HCC, hepatocellular carcinoma; µM, Micromolar)

	Sorafenib			**Lenvatinib**			**Regorafenib**			**Cabozantinib**			**RoS**
Cell-Line	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**R**
Hep3B	1.7	−0.17	1	7.8	0.47	0	1.3	−0.11	1	3.1	0.34	1	1
HepG2	1.7	−0.25	1	6.7	0.16	0	1.2	−0.29	1	4.7	0.13	1	1
HLE	1.3	−0.12	1	0.9	0.19	1	1.2	0.12	1	5.2	0.13	0	0
HLF	2.3	−0.08	0	0.7	0.06	1	1.7	0.22	0	5.1	0.05	0	1
HuH1	2.3	−0.06	0	8.0	0.20	0	2.3	0.09	0	20	0.53	0	0
HuH7	2.4	−0.01	0	0.7	0.09	1	1.3	0.10	1	3.3	0.38	1	1
PLC-PRF5	2.9	0.11	0	20	0.64	0	2.6	0.12	0	10.3	0.38	0	0
Snu398	1.5	−0.16	1	1.0	0.47	1	1.2	−0.18	1	2.6	0.16	1	0
Snu475	2.8	−0.95	0	2.2	−0.95	1	1.5	−0.92	0	4.6	−0.98	1	1

Tabelle 3 tabellarische Darstellung Potenz (GR50 in µM), Effizienz (GRmax) und Reaktion (R) für jede Zelllinie und jedes Medikament, einschließlich einer Regorafenib-über-Sorafenib (RoS)-Gruppe Die TKIs sind in weniger empfindlich (0) und empfindlicher (1) unterteilt. Die Reaktionsschwellenwerte wurden basierend auf der GR50-Werteverteilung und der Steady-State-Plasmakonzentration der Medikamente festgelegt, um ein 4:5-Verhältnis der Untergruppen zu erreichen: 2µM für Sorafenib, 1,5µM für Regorafenib, 5µM für Lenvatinib und Cabozantinib. Für die RoS-Gruppe gelten Zelllinien als gleich empfindlich auf Regorafenib und Sorafenib (0) oder zeigen eine signifikante höhere Empfindlichkeit auf Regorafenib (1). Ergebnisse basieren auf dem Mittelwert von mindestens drei Experimenten. P<0,05 für alle Medikamente nach ANOVA.

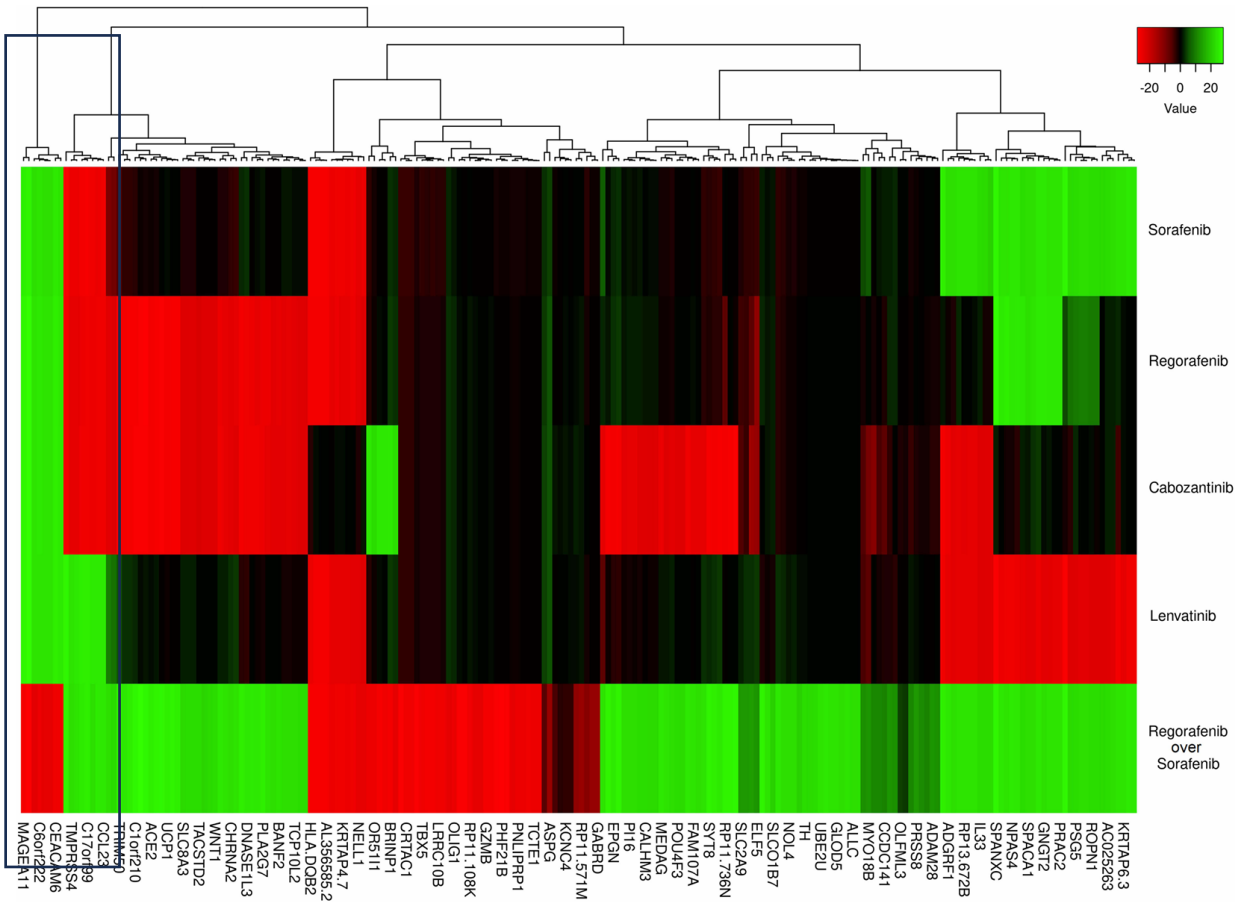


Abbildung 3 Vergleich der differentiellen Genexpressionswerte zu Behandlungsgruppen Diese Heatmap zeigt die differentiellen Expressionswerte für das jeweilige Medikament aufgeschlüsselt. Der „fold-change“ wurde mit dem DESeq2-Algorithmus analysiert. Dunklere Farben repräsentieren FC-Werte zwischen 0 und ± 10, während hellere Farben FC-Werte über ± 10 darstellen. Links enthält das Rechteck zwei Cluster in hellgrün und rot für alle Behandlungsgruppen, was auf zwei erkennbare Genexpressions-Signaturen hindeutet: die linke mit Überexpression in vier Gruppen (Sorafenib [S], Regorafenib [R], Cabozantinib [C] und Lenvatinib [L]) und Unterexpression in jenen, die differenziell besser auf Regorafenib als auf Sorafenib reagierten (RoS), und die rechte mit Genen, die in den ersten drei Gruppen stark unterexprimiert sind ((S), (R) und (C)) und in den letzten beiden überexprimiert (L und RoS).

Die Expressionsanalyse zeigte die differentielle Expression mehrerer Gene (**Abbildung 3**). Dabei wiesen zwei distinkte Cluster eine starke Über- und Unterexpression (FC > ±20) auf, die in allen Zelllinien konstant war und die Zelllinien hinsichtlich der unterschiedlichen Medikamentenreaktionen in zwei Gruppen von Genexpressions-Signaturen teilte. Es zeigten sich 966 differentiell exprimierte Gene (DEG) (FC > |2|, pAdj > 0,001) in mindestens einer Behandlungsgruppe. Es lagen 217 DEG für in der Sorafenib-, 296 in der Lenvatinib-, 201 in der Regorafenib-, 240 in der Cabozantinib- und 210 für in der RoS-Gruppe vor. Es zeigen sich hierbei

zwei distinkte Cluster, die eine starke Über- und Unterexpression (FC > ±20) aufweisen, die in allen Zelllinien konstant ist und die Zelllinien in zwei Gruppen von hinsichtlich der Medikamentenreaktionen unterteilen. Drei Haupt-Signalwege konnten identifiziert werden, die sich auf Medikamentenmetabolismus, Neurotransmitter und Herzfunktion bezogen. Zwei wesentliche Signalwege waren in allen Gruppen angereichert: „CREB-Signalgebung in Neuronen“ und „Regulation von Brustkrebs durch Stathmin1“. Die Filterung nach differentieller Expression zeigte 14 Gene die konsistent signifikant über- oder unterexprimiert (FC > |20|) waren, zusammen mit der Information des Ansprechens konnten hieraus drei definierte Expressionsprofile gebildet werden (Tabelle 4)

Candidates for Gene Expression Signature (FC >|2|, pAdj > 0.001)	Sorafenib (217 DEG)	Regorafenib (201 DEG)	Cabozantinib (240 DEG)	Lenvatinib (296 DEG)	RoS (210 DEG)
Signature Group		**A**		**B**	**C**
GAGE12H	+	+	+	+	–
GJB6	+	+	+	+	–
PTCHD3	+	+	+	+	–
PRH1-PRR4	+	+	+	+	-
C6orf222	+	+	+	+	-
HBB	+	+	+	+	-
C17orf99	-	-	-	+	+
GOLGA6A	-	-	-	+	+
CRYAA	-	-	-	+	+
CCL23	-	-	-	+	+
RP11-347C12.3	-	-	-	+	+
RP11-514O12.4	-	-	-	+	+
FAM180B	-	-	-	+	+
TMPRSS4	-	-	-	+	+

Tabelle 4: Diese Tabelle zeigt die differentiell exprimierten Gene (DEG), die die Filterkriterien erfüllen. Gruppe A (Sorafenib, Regorafenib und Cabozantinib Gruppe) zeigt eine Überexpression mit einem FC > 20 in GAGE12H, GJB6, PTCHD3, PRH1-PRR4, C6orf222 und HBB, sowie eine Unterexpression mit einem FC < -20 in C17orf99, GOLGA6A, CRYAA, CCL23, RP11-347C12.3, RP11-514O12.4, FAM180B und TMPRSS4. Gruppe B (Lenvatinib) zeigt eine Überexpression in allen genannten Genen, und Gruppe C (Regorafenib gegenüber Sorafenib) kehrt das in Gruppe A gesehene Profil vollständig um. (Abkürzungen: FC, Fold-change; DEG, Differenziell exprimierte Gene)

Sollte diese Signatur mit einer größeren Anzahl an Zelllinien *in vitro* und *in vivo* validiert werden, könnte dies die Entwicklung besserer Vorhersagemethoden für HCC-Patienten und zukünftig eine Behandlungsteuerung auf Basis von transkriptionellen Analysen ermöglichen. Auch zeigte die Arbeit, dass veränderte Behandlungsschemata (3 Tage versus 6 Tage) einen hoch signifikanten Einfluss auf die *in-vitro* wirksamen Konzentrationen haben und die gemessenen wirksamen Konzentrationen der Substanzen viel näher den Serumkonzentrationen von Patienten entsprechen. Angesichts der bekannten klinisch unterschiedlichen Ansprechraten und dem gleichzeitig ähnlich differenziellen Ansprechens der Zelllinien auf die verschiedenen Therapeutika könnte dies als eine Rationale für eine individuelle Testung betrachtet werden. Wir planen, in Folgestudien patientennähere *in-vitro*-Systeme wie die Tumoroid-Zellkulturtechnik zu nutzen (71), um die gesamte Bandbreite der Tumorbiologie besser darzustellen und bessere Ansätze für eine individuelle Testung zu etablieren.

4.2. Verändernde Therapielandschaft beim fortgeschrittenen hepatozellulären Karzinom assoziiert mit verbesserter Prognose: eine landesweite, populationsbasierte Studie „Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study." (Munker et al. 2023)

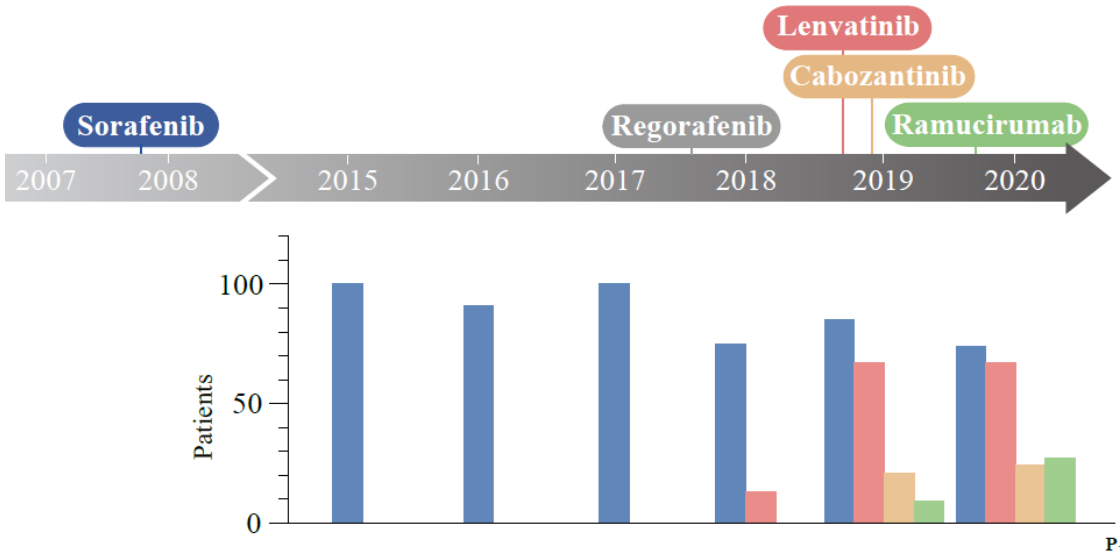
Das HCC ist eine der häufigsten Krebsarten weltweit und weist eine steigende Inzidenz auf (72). Nachdem im Jahr 2007 Sorefenib als erste gut wirksame Systemtherapie für das fortgeschrittene HCC zugelassen werden konnte (60), sind über die letzten zwei Jahrzehnte zahlreiche neue Therapien hinzugekommen. Dazu zählen neben den Tyrosinkinaseinhibitoren Regorafenib (73), Lenvatinib (61) und Cabozantinib (74) auch antivaskuläre Therapien wie Ramucirumab (75) und zuletzt die sehr gut wirksame Immuntherapie Atezolizumab in Kombination mit Bevacizumab (58). Zwar wurde in großen klinischen Studien der Nutzen und die Wirksamkeit der neuen Therapien gezeigt, jedoch ist bislang wenig über deren Einfluss auf die Überlebensraten in einem westlichen Real-World-Kollektiv bekannt. Im Rahmen des Habilitationsprojektes konnte mithilfe der Firma InGef Zugriff auf ein landesweit repräsentatives Kollektiv aus den Versichertendaten von über 4 Millionen in Deutschland Versicherten erhalten werden(76, 77). Aus diesen Daten wurden 2876 HCC-Patienten identifiziert und untersucht, um den Einfluss der Zulassung und des Einsatzes der neuen Therapien auf das Überleben zu charakterisieren (78).

Das repräsentative Kollektiv der 2876 HCC Patienten zeigte eine gute Übereinstimmung hinsichtlich der bekannten Häufigkeiten von Mortalität, Geschlechterverteilung und Therapieeinsatz und der vorbekannte Trend der Abnahme der Hepatitis C assozierten HCC Inzidenz **(Tabelle 5)** (7, 79, 80).

Year	2015 n=855	2016 n=895	2017 n=904	2018 n=941	2019 n=1017	2020 n=974	
Variable							**P-value**
Mean age ± SD (years)	69·3 ± 11·0	70·3 ± 10·1	69·9 ± 10·5	70·7 ± 10·3	70·5 ± 10·8	71·1 ± 10·6	0·0064
Female (%)	224 (26·2)	228 (25·5)	237 (26·2)	244 (25·9)	277 (27·2)	262 (26·9)	0·9609 ns
HCC etiology							
HBV	45 (5·3)	54 (6)	49 (5·4)	51 (5·4)	56 (5·5)	54 (5·5)	0·9713 ns
HBV/HDV	9 (1·1)	7 (0·8)	7 (0·8)	5 (0·5)	6 (0·6)	9 (0·9)	0·5713 ns
HCV	109 (12·7)	95 (10·6)	82 (9·1)	81 (8·6)	83 (8·2)	83 (8·5)	0·0015
Alcoholic liver cirrhosis	183 (21·4)	188 (21)	199 (22)	199 (21·1)	197 (19·4)	200 (20·5)	0·4504 ns
NAFL	143 (16·7)	154 (17·2)	183 (20·2)	168 (17·9)	196 (19·3)	186 (19·1)	0·2311 ns
NASH	23 (2·7)	16 (1·8)	24 (2·7)	32 (3·4)	27 (2·7)	25 (2·6)	0·5535 ns
Autoimmune	7 (0·8)	12 (1·3)	9 (1)	6 (0·6)	8 (0·8)	8 (0·8)	0·4331 ns
Other chronic hepatitis	19 (4·75)	16 (3·9)	15 (3·8)	13 (3·4)	11 (2·7)	12 (3·3)	0·0341
Mild liver disease	608 (71·1)	646 (72·2)	654 (72·3)	687 (73·0)	724 (71·2)	694 (71·3)	0·9988 ns
Moderate to severe liver disease	268 (31·3)	268 (30·0)	283 (31·3)	281 (29·9)	326 (32·1)	307 (31·5)	0·9676 ns
Metastatic disease (%)	288 (33·7)	310 (34·6)	292 (32·3)	316 (33·6)	329 (32·4)	367 (37·7)	0·5293 ns
Systemic therapy (%)[a]	101 (11·8)	93 (10·4)	107 (11·8)	101 (10·7)	159 (15·6)	147 (15·1)	0·0050

Tabelle 5 Merkmale der Patienten nach Jahr. Die einfaktorielle Varianzanalyse (ANOVA) wurde verwendet, um kontinuierliche Variablen zu untersuchen, und der Chi-Quadrat-Test für Häufigkeitsdaten. Statistische Signifikanz wird mit P < 0,05 erreicht. Leichte, moderate und schwere Lebererkrankung wird durch die entsprechenden ICD-10-GM-Codes definiert. Anzahl der Patienten mit mindestens einer Verschreibung von einem der folgenden Medikamente: Sorafenib, Lenvatinib, Regorafenib, Cabozantinib, Ramucirumab, Nivolumab, Pembrolizumab.

In Abbildung 4 sind die Verordnungen neuer Therapeutika neben dem Datum der Zulassung neuer Therapien aufgeschlüsselt. Es lässt sich eine diverse Verordnungspraxis feststellen.



							P-value reference
All patients	855 (100)	895 (100)	904 (100)	941 (100)	1017 (100)	974 (100)	
Sorafenib (%)	100 (11·7)	91 (10·2)	100 (11·1)	75 (8·0)	85 (8·4)	74 (7·6)	**0·0207**
Lenvatinib (%)	0 (0)	0 (0)	<5 (na)	13 (1·4)	67 (6·6)	67 (6·9)	**<0·001**
Regorafenib (%)	<5 (na)	<5 (na)	0 (0)	0 (0)	0 (0)	0 (0)	**na**
Cabozantinib (%)	0 (0)	0 (0)	0 (0)	<5 (na)	21 (2·1)	24 (2·5)	**<0·001**
Ramucirumab (%)	0 (0)	<5 (na)	<5 (na)	<5 (na)	9 (0·9)	27 (2·8)	**<0·001**

Abbildung 4 Die Zulassungen der jeweiligen Systemtherapie für die Indikation HCC sind auf der oberen Zeitlinie aufgeschlüsselt. Das Balkendiagramm unten zeigt die Anzahl der Patienten mit mind. 1x Verordnung des jeweiligen Medikaments in diesem Jahr. Die Farben der Balken entsprechen den Medikamenten aus der Zeitlinie. Der Chi-Quadrat Test wurde verwendet um Häufigkeitsdaten zu untersuchen. P < 0,05 wurde als statistisch signifikant

betrachtet. Aufgrund der Anonymisierung der Ingef Datenbank können Verschreibungshäufigkeiten, die kleiner als 5 sind nicht aufgeschlüsselt werden. (Abkürzung na: nicht anwendbar).

Im nächsten Schritt wurde untersucht inwiefern die Zulassung neuer Therapeutika mit einer Verbesserung des Überlebens koinzidiert. Seit 2008 bestand die Systemtherapie des HCC nur aus Sorafenib (60). Da Regorafenib das 2017 in der EU für das HCC zugelassen wurde von den Ärzten aufgrund des negativen Votums des Instituts für Qualität und Wirtschaftlichkeit im Gesundheitswesen und der deswegen fehlenden Vergütung durch die Kassen nicht von den Ärzten verordnet (81) markierte erst die Zulassung von Lenvatinib in der EU (82) die Trendwende in der Therapielandschaft des HCC's mit darauf folgenden Zulassungen von Cabozantinib, Ramucirumab und Atezolizumab-Bevacizumab. Wir teilten daraufhin die HCC Patienten mit BCLC Stadium C in zwei Gruppen, eine von 01/2015-07/2018 Gruppe A (Zulassung von Lenvatinib in der EU) und eine zweite Gruppe 08/2018-12/2020 Gruppe B (korrespondierende Patientencharakteristika in Tabelle 6).

Year	Period A 2015-07/2018 n=255	Period B 08/2018-2020 n=205	Total 2015-2020 n=460	
Variable				**P-value**
Mean age ± SD (years)	68·6 ± 9·2	70·2 ± 9·6	69·3 ± 9·4	0·0731 ns
Female (%)	46 (18·0)	42 (20·5)	88 (19·1)	0·5516 ns
HCC etiology (%)				
HBV	21 (8·2)	6 (2·9)	27 (5·9)	0·0271
HCV	26 (10·2)	14 (6·8)	40 (8·7)	0·3189 ns
Alcoholic liver cirrhosis	69 (27·1)	51 (24·9)	120 (26·1)	0·7641 ns
NAFL	51 (20·0)	49 (23·9)	100 (21·7)	0·4393 ns
NASH	10 (3·9)	10 (4·9)	20 (4·3)	0·6528 ns
Autoimmune	<5[a]	0 (0)	0 (0)	na
Other chronic hepatitis	11 (4·3)	7 (3·4)	18 (3·9)	0·8098 ns
Moderate to severe liver disease	89 (34·9)	69 (33·7)	158 (34·3)	0·3189 ns
Metastatic disease (%)	129 (50·6)	110 (53·7)	239 (52·0)	0·7641 ns

Tabelle 6 Patientencharakteristika der Patienten aus Gruppe A und Gruppe B Interessanterweise zeigte sich eine Abnahme der HBV assoziierten BCLC C Patienten. Der Chi-Quadrat Test wurde verwendet um Häufigkeitsdaten zu untersuchen. P < 0,05 wurde als statistisch signifikant betrachtet. Aufgrund der Anonymisierung der Ingef Datenbank können Verschreibungshäufigkeiten, die kleiner als 5 sind nicht aufgeschlüsselt werden. (Abkürzung na: nicht anwendbar).

Der direkte Vergleich des Überlebens dieser zwei Gruppen zeigte eine signifikante Verbesserung der Prognose auch in der multivariaten Cox-Regression bei der auch Komorbiditäten berücksichtigt wurden (Abbildung 5).

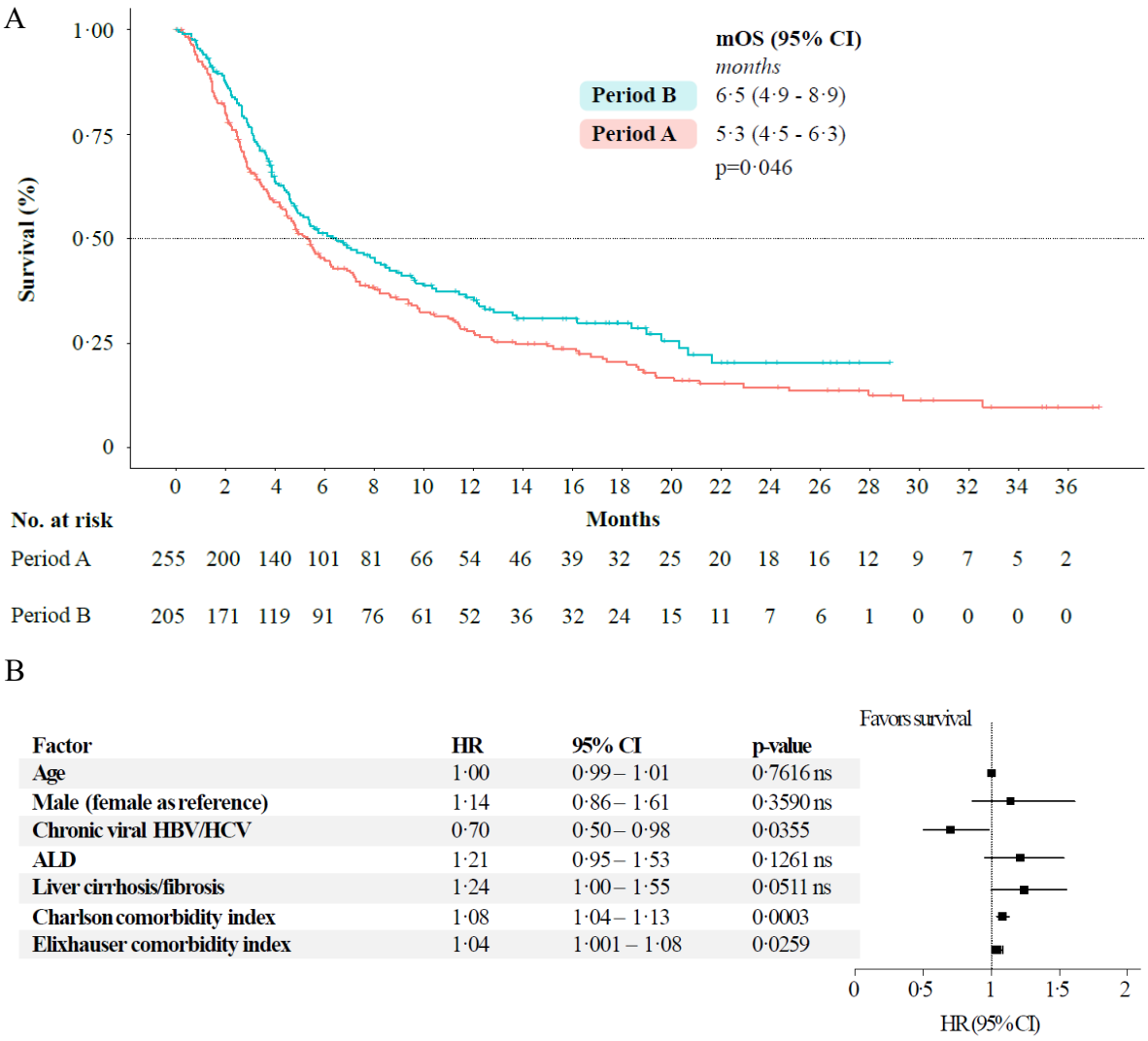


Abbildung 5 Gesamtüberleben von Patienten mit HCC, die in Periode A (2015-07/2018) und Periode B (08/2018-2020) eine Erstlinien Systemtherapie begonnen haben. (A) Kaplan Meier Analyse des Gesamtüberlebens. (B) Multivariate Cox-Regressions Analyse des Überlebens von Periode B im Vergleich zu A in Monaten. Abkürzungen: CI, Konfidenzintervall; HR, Hazard-Ratio; HBV, Heptitis B-Virus; HCC, hepatozelluläres Karzinom; HCV, Hepatitis C Virus; mOS, medianes Gesamtüberleben; NAFL, nichtalkoholische Fettleber; NASH, nicht-alkoholische Steatohepatitis; ns, nicht signifikant; SD, Standardabweichung

Zusammenfassend zeigten die Ergebnisse, dass Patienten in Periode B (August 2018 bis 2020), die nach der Zulassung neuerer Medikamente wie Lenvatinib behandelt wurden, ein besseres medianes Gesamtüberleben aufwiesen als jene in Periode A (2015 bis Juli 2018), was auf die Effektivität der neuen Therapieoptionen hinweist. Die Studie unterstreicht die Bedeutung und

den Nutzen der kontinuierlichen Erweiterung des Behandlungsspektrums mit neuen Therapien zur Verbesserung der Prognose von HCC Patienten.

4.3. Gleichzeitige Strahlen- und Checkpointinhibitor-Therapie bei Patienten mit Hepatozellulärem Karzinom: Eine systematische retrospektive Zentrumsanalyse

„Concomitant Irradiation to Checkpointinhibitor-Therapy of Hepatocellular Carcinoma Patients: A Systematic Retrospective, Single-Center Analysis". (Munker et al., Oncol Res Treat. 2023)

Immuntherapie hat sich als Standarderstlinientherapie für Patienten mit fortgeschrittenem hepatozellulärem Karzinom (aHCC) etabliert. Trotz erhöhter Wirksamkeit bei gutem Nebenwirkungsspektrum kommt es bei einem größeren Anteil der Patienten auch nach einer objektiven Antwort häufig zu einem Krankheitsfortschritt, so dass eine Folgetherapie notwendig wird. Zugelassene Therapien in den Folgelinien umfassen Tyrosinkinase Inhibitoren (59), aufgrund der sehr guten Wirksamkeit und Verträglichkeit der Immuntherapien (33) wäre jedoch auch eine Immuntherapie basierte Folgetherapie oder auf Immuntherapien basierte Eskalationskonzepte wünschenswert.

Strahlentherapie ist eine hochwirksame Behandlungsmodalität für die lokale Tumorkontrolle und stellt eine potenzielle Option für eine Kombinationstherapie mit Immuntherapien dar. Mit High-Dose-Rate-Brachytherapie (HDRBT) und stereotaktischer Körperstrahlentherapie (SBRT) stehen zwei wirksame strahlentherapeutische Modalitäten zur Behandlung des HCC zur Verfügung (83). HDRBT ermöglicht eine minimalinvasive Tumorablation, um lokale Tumorkontrolle zu gewährleisten und systemische Therapien zu unterstützen. Diese Technik ermöglicht es, hohe Strahlendosen direkt im Tumorbereich zu applizieren, während das umliegende gesunde Lebergewebe geschützt wird. Sie überwindet die Einschränkungen thermischer Ablationsmethoden und kann als Überbrückungstherapie vor einer Lebertransplantation dienen, wobei sie in Kombination mit Immunmodulatoren starke lokale und systemische Antitumor-Reaktionen induziert (84). Die externe Strahlentherapie (RT) wird speziell bei HCC-Patienten angewendet, bei denen eine lokoregionale Behandlung nicht möglich ist (85). Fortschritte in der Strahlentechnik wie intensitätsmodulierte und bildgeführte SBRT ermöglichen eine präzise und sichere Dosisverabreichung, die das Leberparenchym schützt und die früher häufige hepatische Dekompensation reduziert (86). SBRT kann auch als

effiziente Überbrückungstherapie zur Tumorkontrolle vor Lebertransplantation eingesetzt werden(83, 87).

Strahlentherapie (RT) kann die Wirksamkeit der Checkpoint-Hemmung durch zusätzliche Immun-Sensibilisierung erhöhen, wie in präklinischen Modellen und klinischen Studien zum malignen Melanom gezeigt wurde (88, 89). Gleichzeitig kann die Strahlentherapie die Expression von MHC Komplexen steigern (90) und es wird vermutet, dass es speziell in Kombination mit einer Immuntherapie zu spezifischen Nebenwirkungen oder Off-Target Effekten kommen könnte (91, 92). Derzeit sind nur wenige Daten verfügbar, die die Verträglichkeit und Wirksamkeit der begleitenden Strahlentherapie bei mit CPI behandelten HCC-Patienten charakterisieren. Zu diesem Zweck haben wir systematisch HCC-Fälle in unserem Zentrum retrospektiv ausgewertet, die im Kontext der Checkpoint-Inhibition eine Strahlentherapie erhalten haben.

Ziel dieser Studie war es, die Nebenwirkungen und die Wirksamkeit der begleitenden Strahlentherapie bei mit Checkpoint-Inhibitoren behandelten Patienten mit aHCC retrospektiv zu analysieren. Das vordefinierte Einschlusskriterium war eine Strahlentherapie nach eingeleiteter Checkpoint-Inhibition und Fortsetzung der ICI-Therapie für mindestens 8 Wochen. Mittels einer Datenbanksuche konnte in den Jahren 2016 bis 2021 sechs konsekutive Patienten gefunden werden, die die vordefinierten Kriterien für eine begleitende ICI- und Strahlentherapie erfüllten. Zu dem Zeitpunkt war der heutige Standard Atezolizumab und Bevacizumab in der Therapie des HCC noch nicht zugelassen und ein Teil der Patienten erhielt die Immuncheckpointtherapie als Teil einer Studienmedikation oder als Salvage Treatment im Rahmen eines Off-Label Einsatzes (1x Patient Ipilimumab-Nivolumab, 3x Nivolumab Monotherapie, 1x Pembrolizumab, Durvalumab+Tremelimumab) . Von diesen 6 Patienten erhielten drei Patienten eine High-dose-Rate-Brachytherapie (15 Gy) zur Behandlung fortschreitender Leberläsionen. Zwei Patienten erhielten eine stereotaktische Körperstrahlentherapie (SBRT) (25-30 Gy) zur Symptomkontrolle, und ein Patient erhielt sowohl Brachytherapie als auch SBRT zur Behandlung von Metastasen. Es wurden keine schwerwiegenden Nebenwirkungen im Zeitraum (<6 Monate) im Anschluss an die begleitende Strahlentherapie gemeldet. Lokale Kontrolle wurde durch standardisierte Messungen der Tumordiameter evaluiert (Abbildung 6).

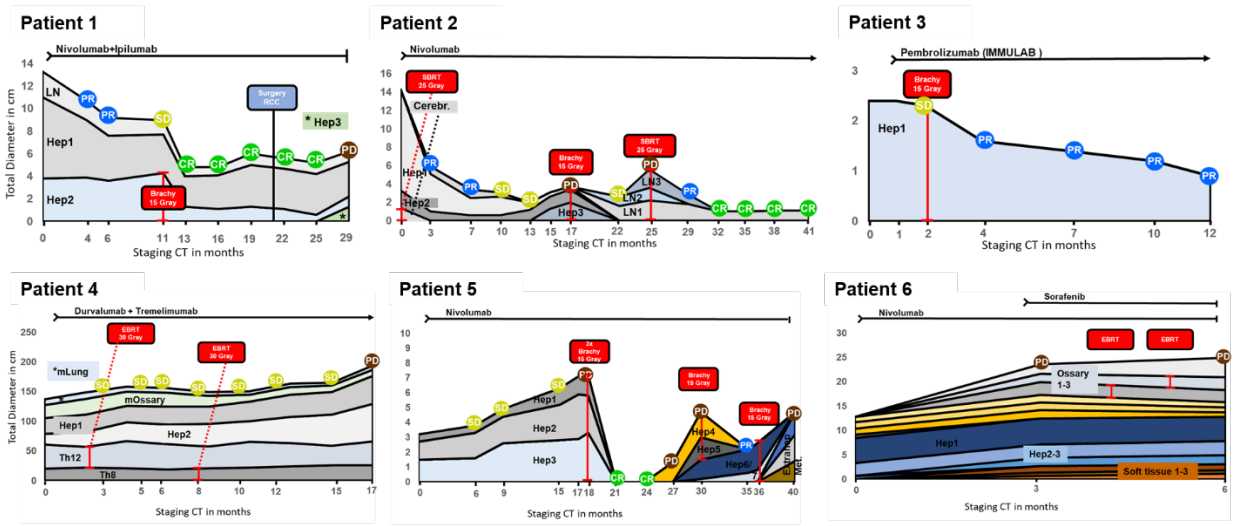


Abbildung 6: Visualisierung des Therapieansprechens von Patient 1-6 mittels eines modifizierten Spider-Plots und der Darstellung der Summe der längsten Durchmesser (nach Recist 1.1). (Abk.: PR, Partial Response; CR, Complete Response; SD, Stable disease; PD, progressive disease, Hep, Hepatic; mLung, Lung Metastasis, Brachy, Brachytherapy, EBRT, external beam radiation therapy)

In 5 von 6 Fällen konnte durch diese therapeutische Kombination eine langfristige Tumorkontrolle erreicht werden (**Tabelle 6**). Speziell die in-field Läsionen sprachen in der Regel komplett auf die Strahlentherapie an. Signifikante Off-target Wirksamkeit im Sinne von abskopalen Effekten konnte nicht beobachtet werden.

Pat. No.	BCLC	Cirrhosis	ICI therapy	RT modality	RT target	Number of fractions × single dose	Indication	Coincidental adverse effect	Best response in-field	Best overall response
1	C	Child A	Nivolumab and ipilimumab	Brachytherapy	Seg VIII and VII	1 × 15 Gy	Local tumor control	No AE	CR	CR
2	C	Child A	Nivolumab	SBRT	Lymph node metastases	5 × 5 Gy (80% isodose)	Local tumor control	No AE	CR	CR
				Brachytherapy	Seg III and V	1 × 15 Gy	Local tumor control	No AE	CR	
				SBRT	Cerebral metastasis	5 × 5 Gy	Local tumor control		CR	
3	B	Child A	Pembrolizumab (Immulab)	Brachytherapy	Seg VI/VIII	1 × 15 Gy	Local tumor control	No AE	PR	PR
4	C	No	Durvalumab and tremelimumab (Himalaya)	3D conformal EBRT	Bone metastasis T11-L1	10 × 3 Gy	Palliative	Hypothyreosis	SD	SD
				3D conformal EBRT	Bone metastasis B6-B10	10 × 3 Gy	Palliative		SD	
5	B	Child A	Nivolumab	Brachytherapy	3 lesions, seg IVb/a and V	1 × 15 Gy	Local tumor control	Nummulary eczema	CR	CR
				Brachytherapy	2 lesions, seg VI/VII	1 × 15 Gy	Local tumor control	Xerosis cutis	CR	
				Brachytherapy	1 lesion, seg V/VIII	1 × 15 Gy	Local tumor control;		CR	

6	C	Child B	Nivolumab	3D conformal EBRT	T12-L1, popliteal fossa right, tibia li, B6-8, pelvis/right os ischii	10 × 3 Gy	Palliative	No AE	SD	PD

Tabelle 6: Patientenmerkmale des jeweiligen Patienten aufgeschlüsselt nach zugehörigem Checkpoint-Inhibitor, radiotherapeutischer Modalität, begleitenden Nebenwirkungen und Behandlungsergebnissen (Abkürzungen: SBRT, stereotaktische Körperstrahlentherapie; EBRT, externe Strahlentherapie; PD, progressive Erkrankung; PR, partielle Remission; CR, komplette Remission).

In den aufgearbeiteten Fällen zeigte sich erfreulicherweise keine mit dem Bestrahlungsfeld assoziierte Immuntoxizität (z.B. Hepatotoxizität bei brachytherapierten Patienten oder Pneumonitis bei pulmonal bestrahlten Patienten). Dies kann als Signal für eine gutes Toxizitätsprofil der Kombination aus Strahlen- und Immuntherapie gewertet werden. Studien, die Radiotherapie in Kombination mit Checkpointinhibition prüfen werden momentan durchgeführt (93). Eine low-dose Radiotherapie in Kombination mit Immuntherapie ist ein vielversprechender konzeptueller Ansatz für ein on Demand Konzept für extrahepatische progrediente Metastasen, die sich unter Immuntherapie progredient zeigen gemäß dem Demand Konzept (94).

Zusammenfassend lässt sich sagen, dass diese Studie zeigt, dass die Kombination aus Strahlentherapie und Immuncheckpoint-Therapie, insbesondere beim HCC, effektiv sein kann und ein günstiges Nebenwirkungsprofil aufweist. Angesichts dieser vielversprechenden Signale sind größere Studien notwendig, um den Nutzen dieser Therapiekombination weiter zu untersuchen und zu bestätigen.

4.4. Prognostische Bedeutung erhöhter Thrombozytenzahlen (>200 x 10^9 per L) in BCLC Stadien B und C des hepatozellulären Karzinoms

“Prognostic Significance of Elevated Platelet Count (>200 x 10^9 per L) in BCLC Stages B and C of Hepatocellular Carcinoma”

Beim HCC gelten niedrige Thrombozytenzahlen traditionell als negativer Prognosefaktor, speziell im frühen operablen Krankheitsstadium (BCLC A)(42, 43). Neuere Daten legen jedoch nahe, dass auch eine erhöhte Thrombozytenzahl mit einer schlechteren Prognose assoziiert sein kann, speziell in fortgeschrittenen Krankheitsstadien (44-46). Ziel dieser multizentrischen retrospektiven Studie war es, den prognostischen Einfluss der Thrombozytenzahl in verschiedenen BCLC-Stadien unter Berücksichtigung der portalen Hypertension zu analysieren.

In die Analyse wurden 1112 Patienten aus München, Mannheim und Wien eingeschlossen, die zwischen 2006 und 2022 mit einem HCC diagnostiziert worden waren. Um den Einfluss der Thrombozytenzahl auf das Überleben in Abhängigkeit vom Tumorstadium zu ermitteln, wurden mittels einer iterativen Bestimmung verschiedene Cut-off-Werte getestet. Der optimale Bereich wurde zwischen 84 und 200 × 10^9/L identifiziert. Patienten außerhalb dieses Bereichs mit niedrigen (<84) und mit hohen (>200) Thrombozytenzahlen zeigten eine signifikant schlechtere Prognose. Um den Einfluss der portalen Hypertension „herauszurechnen“, erfolgte die Einbeziehung des Milzdurchmesser als Surrogat Parameter.

Die multivariate Analyse ergab eine stadienabhängige prognostische Assoziation der Thrombozytenzahl: In BCLC-Stadium A war eine niedrige Thrombozytenzahl mit einem schlechteren Überleben assoziiert, wahrscheinlich als Ausdruck fortgeschrittener portaler Hypertension und einem erhöhten postoperativen Risiko für Komplikationen(42, 43). In den Stadien B und C hingegen wiesen Patienten mit Thrombozytenwerten über 200/nl ein signifikant schlechteres Gesamtüberleben auf (**Abbildung 7**). Diese Assoziation blieb auch nach Adjustierung auf Milzdurchmesser, Makroinvasion, Metastasenstatus und andere klinische Parameter bestehen. Auch erfolgte eine interne Validierung mittels Bootstrapping, die diesen Zusammenhäng ebenso bestätigte.

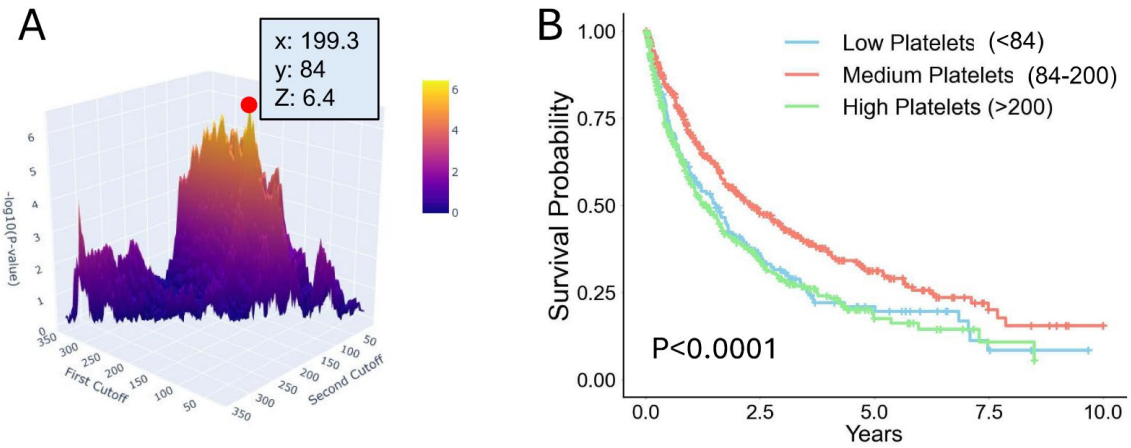


Abbildung 7 (A) Heatmap, die die statistische Signifikanz (z-Achse) verschiedener getesteter Grenzwertpaare anhand des Logrank-Überlebenstests (z-Achse) darstellt. Die beste Diskriminierung im Hinblick auf das Überleben wurde dabei für einen oberen Grenzwert von 200.000 Thrombozyten/µL und einen unteren Grenzwert von 84.000 Thrombozyten/µL ermittelt. (B) Zeigt eine Kaplan-Meier-Überlebensanalyse mit neu definierten Thrombozyten-Grenzwerten, wobei die Überlebenswahrscheinlichkeit der Patienten innerhalb des neuen Thrombozytenbereichs

(84–200 × 10^9/L) mit derjenigen außerhalb dieses Bereichs verglichen wurde (Logrank, p<0,0001). Abkürzung: BCLC, Barcelona Clinic Liver Cancer Stadieneinteilungssystem

Diese Ergebnisse legen nahe, dass die Thrombozytenzahl zusätzlich zu der Bedeutung zur Abschätzung des operativen Risikos auch für Patienten mit BCLC Stadium B oder C ein hochrelevanter prognostischer Marker im klinischen Management sein kann. Eine erneute externe Validierung vorausgesetzt, kann ein Thrombozytenwert >200/nl bei Erstdiagnose eines HCC im Stadium BCLC B oder C kann als Hinweis auf eine schlechtere Prognose gewertet werden und könnte in zukünftige Risikostratifizierungsmodelle und Therapieentscheidungen einbezogen werden. Gleichzeitig bleibt in frühen Stadien eine niedrige Thrombozytenzahl weiterhin Ausdruck portaler Hypertension und eingeschränkter Leberfunktion und negativer Einflussfaktor speziell in Bezug auf das operative Risiko.

Die Studie von Munker et al. liefert eine solide Grundlage für die differenzierte Betrachtung der Thrombozytenzahlen in der Prognoseabschätzung von HCC. Zukünftig könnten Kombinationen mit anderen Markern wie dem Neutrophil-Lymphozyten-Verhältnis (NLR), der Platelet-Lymphocyte-Ratio oder der CD8+ T-Zellen in Scores integriert werden. Auch könnte untersucht werden, ob möglicherweise eine kausale Rolle der Thrombozyten hinsichtlich der Prognoseeinteilung vorliegt. Eine externe Validierung des 200/nl-Cutoffs in unabhängigen Kohorten sowie prospektive Studien zur Integration dieses Parameters in klinische Entscheidungsalgorithmen ist dringend erforderlich.

4.5. Einsatz von Checkpointinhibitoren bei Lebertransplantierten

„Use of checkpoint inhibitors in liver transplant recipients“ (Munker & De Toni, United European Gastroenterol J. 2018)

Für Checkpointinhibitoren (CPI) liegen bei Organtransplantierten bis dato wenig Erfahrungswerte vor und gelten bei Lebertransplantierten aufgrund des Risikos einer akuten Transplantatabstoßung als kontrainidiziert. In der folgender im Rahmen der Habilitation verfasstenretrospektiven Übersichtsarbeit werden 14 Fälle aus der Literatur und von Kongressen beschrieben, in denen CPI nach Lebertransplantation eingesetzt wurden.

Vier Patienten entwickelten akute Transplantatabstoßungen – in drei Fällen mit tödlichem Verlauf. Die mediane Überlebenszeit betrug 1,2 Monate, wobei in Einzelfällen ein langanhaltendes Ansprechen mit bis zu 18 Monaten beobachtet wurde.

PD-1/PD-L1-Inhibitoren erscheinen häufiger mit Abstoßung assoziiert als CTLA-4-Inhibitoren, wobei dies wegen kleiner Fallzahlen nur als Signal gewertet werden sollte. PD-L1-Expression war bei Patienten mit Abstoßung nachweisbar; bei Patienten ohne Abstoßung meist negativ. Dies legt eine mögliche Rolle als prädiktiver Marker nahe. In mehreren Fällen erfolgte eine Reduktion der Immunsuppression auf subtherapeutische Dosen. Präventive Steroidgabe zeigte in Einzelfällen keinen negativen Einfluss auf das Tumoransprechen. Häufig wurde CPI erst bei Therapieversagen anderer Optionen eingesetzt.

Der Einsatz von CPI nach Lebertransplantation ist mit einem relevanten Risiko einer letalen Transplantatabstoßung assoziiert. Dennoch konnten in ausgewählten Fällen signifikante Tumorregressionen ohne Organverlust erreicht werden. Prospektive Studien sind dringend erforderlich, um Nutzen und Risiken dieser im Kontext einer vorliegenden Lebertransplantation als ultima-ratio anzusehenden Therapie besser zu charakterisieren.

4.6. EpiCO (Epirubicin, Cyclophosphamid und Vincristin) als Behandlung für extrapulmonale hochgradige neuroendokrine Neoplasien

„EpiCO (epirubicin, cyclophosphamide and vincristine) as treatment for extrapulmonary high-grade neuroendocrine neoplasms". (Munker et al., Zeitschrift für Gastroenterologie. 2020)

Hochgradige neuroendokrine Neoplasien (NEN) sind eine seltene Tumorentität, für die (aufgrund der geringen Fallzahl) bis vor kurzem wenige randomisierte kontrollierte Studien existierten. Die Therapieempfehlungen basierten z.T. auf Extrapolationen vom pulmonalen Analogon, dem kleinzelligen Lungenkarzinom (SCLC), und wurden mittels retrospektiver Fallserien validiert. Die multizentrische Nordic-NEC-Studie an gastroenteropathischen (GEP) und "cancer of unknown primary" (CUP) NENs mit hohem Grading zeigten ein signifikantes Ansprechen auf Etoposid- und platinbasierte Kombinationstherapien, die nun als Standard-Erstlinientherapie gelten (55). Gemischtzellige hochgradige NEN/MiNEN, früher als MANEC bekannt, weisen ebenfalls eine schlechte Prognose auf und werden ähnlich den GEP/CUP-Neuroendokrinen Karzinomen behandelt. Das Carboplatin/Etoposide-Protokoll erreicht in 50–60 % der Fälle ein Ansprechen, allerdings sind die Remissionen oft kurzlebig (55). Daten aus der PRODIGE 41-BEVANEC Studie randomisierte 133 Patienten in einen FOLFIRI Arm mit oder ohne Bevacizumab, dieser zeigte (unabhängig von Bevacizumab) ein verbessertes Medianes Überleben von 5,3 Monaten (historischer Komparator), so dass seitdem FOLFIRI zunehmend als Zweitlinientherapien betrachtet wird (95). Die Prognose ist jedoch trotz dieser Zweitlinien

Option weiterhin sehr schlecht und im Falle von Unverträglichkeiten z.B. auf Irinotecan bestehen keine guten Therapieoptionen, für die eine gute Effektivität bekannt wäre.

Das als CAV-Regime abgekürzte Therapieschema mit Doxorubicin, Cyclophosphamid und Vincristin, war früher Standardchemotherapie bei SCLC. Bei initial akzeptabler Effektivität beim SCLC, wurde es im Verlauf aufgrund einer besseren Verträglichkeit von Epirubicin abgelöst, woraus das EpiCO-Regime entstand (96). In Analogie zum SCLC wurden ausgewählte Patienten mit hochgradigen NEN am Universitätsklinikum Regensburg in einer Zweitlinientherapie mit EpiCO behandelt. In dieser retrospektiven Studie berichten wir über die Verläufe von fünf konsekutiven Patienten aus den Jahren 2004-2017 mit metastasierten hochgradigen GEP/CUP NEN/MiNEN, die eine Chemotherapie nach diesem Schema erhielten. Vier der fünf Patienten waren auswertbar: ein Patient zeigte ein partielles Ansprechen, zwei Patienten eine Krankheitsstabilisierung, während ein Patient eine Krankheitsprogression erlitt. Die beobachtete Krankheitskontrollrate (drei von vier) sowie das Zeitintervall bis zur Progression waren vergleichbar oder länger als unter der vorangegangenen Carboplatin Etoposid-Erstlinientherapie. Die Studie unterstreicht die potenzielle Rolle von EpiCO als therapeutische Option in der Zweitlinie, insbesondere bei Kontraindikationen gegen Irinotecan-haltige Schemata (z. B. FOLFIRI).

Diese Ergebnisse deuten darauf hin, dass anthrazyklinhaltige Regime wie EpiCO eine potenziell wirksame Zweitlinienoption für Patienten mit hochgradigen GEP-NEN darstellen, insbesondere bei Kontraindikationen gegenüber Irinotecan. Eine weiterführende Evaluation in prospektiven Studien mit größeren Kohorten ist zur Validierung dieser Ergebnisse erforderlich.

4.7. Chemotherapie bei metastasiertem Darmkrebs: Kein Effekt auf das Überleben bei Dosisreduktion aufgrund von Nebenwirkungen.

“Chemotherapy for metastatic colon cancer: No effect on survival when the dose is reduced due to side effects” (Munker S. et al., BMC Cancer 2018)

Darmkrebs ist die dritthäufigste Krebsart weltweit und eine Hauptursache für Morbidität und Mortalität. Bei Diagnosestellung weisen 20% der Patienten eine metastasierte Erkrankung auf, ohne kurative Behandlungsoptionen. In den letzten Jahrzehnten haben verbesserte Therapieregime bei fortgeschrittenem kolorektalem Krebs zu einer signifikanten Steigerung der Wirksamkeit und einer Senkung der Mortalität geführt. Das Rückgrat dieser Chemotherapien

stellt eine Kombination aus 5FU mit entweder Irinotecan (FOLFIRI) oder Oxaliplatin (FOLFOX) dar (50).

Ursprünglich wurden diese Regime an jüngeren, weniger komorbiden Studienpopulationen getestet, spiegeln aber nicht die Realität älterer, multimorbider Patienten wider, die Nebenwirkungen schlechter vertragen. In der Praxis wird oft die Chemotherapiedosis reduziert, um Nebenwirkungen zu mindern, was jedoch die tumorspezifische Überlebensrate beeinträchtigen könnte (51). Um dies auf den Grund zu gehen analysierten wir retrospektiv eine Patientenkohorte mit fortgeschrittenem kolorektalem Krebs der Uniklinik Regensburg, die zwischen den Jahren 2004 und 2012 an unserer Klinik therapiert wurden um die Auswirkungen einer reduzierten Dosierung auf das Gesamtüberleben zu bewerten.

Von 109 Patienten erhielten 67 % (73/109) beide Regime (FOLFOX und FOLFIRI) nacheinander. Die Mehrheit, 78 % (57/73), begann mit FOLFOX und wechselte im Verlauf der Erkrankung zu FOLFIRI. 21 % (23/109) der Patienten erhielten ausschließlich FOLFOX, während FOLFIRI-Chemotherapieregime bei 12 % (13/109) der Patienten ausschließlich verwendet wurden. 43 % (47/109) erhielten zusätzlich eine Behandlung mit einem Biologikum. Wenn die Patienten Chemotherapie assoziierte Nebenwirkungen zeigten, wurde die Dosierung nach Ermessen des Arztes in den meisten Fällen auf 80 % der Protokolldosis reduziert. Daher legten wir eine Reduzierung auf 80 % der Protokolldosis als Grenzwert fest, ab dem die Chemotherapie als reduziert galt. Bei 42 % (46/110) unserer Patienten erfolgte im Verlauf der Chemotherapie eine Dosisreduktion auf 80 % oder weniger. Diese 46 Patienten erhielten durchschnittlich 14 Chemotherapiezyklen in unserer Klinik (Durchschnitt: 13,7 Zyklen; +−12,3 Zyklen). Patienten, die die Chemotherapie mit voller Dosis fortsetzten, erhielten nur 10,5 Zyklen (Standardabweichung 10,8, $p=0,149$). Die Patienten mit reduzierter Chemotherapie erhielten mehr Zyklen als diejenigen mit voller Dosis. Es wurde die durchschnittliche kumulative Dosis und Dosisintensität für 5-Fluorouracil, Irinotecan und Oxaliplatin für die Gruppen mit voller Dosierung und Dosisreduktion berechnet. Wie erwartet waren die relativen Dosisintensitäten signifikant unterschiedlich. Der t-Test zeigte jedoch keine signifikanten Unterschiede bei den kumulativen Dosierungen.

Es zeigte sich kein signifikanter Unterschied im Gesamtüberleben zwischen Patienten, die eine Chemotherapie in voller Dosierung erhielten, und jenen, bei denen eine Dosisreduktion auf

≤ 80 % aufgrund von Nebenwirkungen vorgenommen wurde (Abbildung 7). Die mediane Überlebenszeit lag bei 13,0 Monaten in der Voll-Dosis-Gruppe und bei 14,9 Monaten in der Gruppe mit einer Dosisreduktion (p = 0,430). Auch in der multivariablen Cox-Regression, adjustiert auf relevante klinische Einflussfaktoren wie TNM-Stadium, Komorbiditäten und Therapiesequenz, war die Dosisreduktion nicht mit einer signifikant erhöhten Mortalität assoziiert (HR = 0,861; 95 %-KI: 0,492–1,506; p = 0,600). Diese Ergebnisse sprechen dafür, dass eine moderate Dosisreduktion im palliativen Setting, sofern diese indiziert ist, das Überleben von Patienten mit metastasiertem Kolonkarzinom nicht negativ beeinflusst.

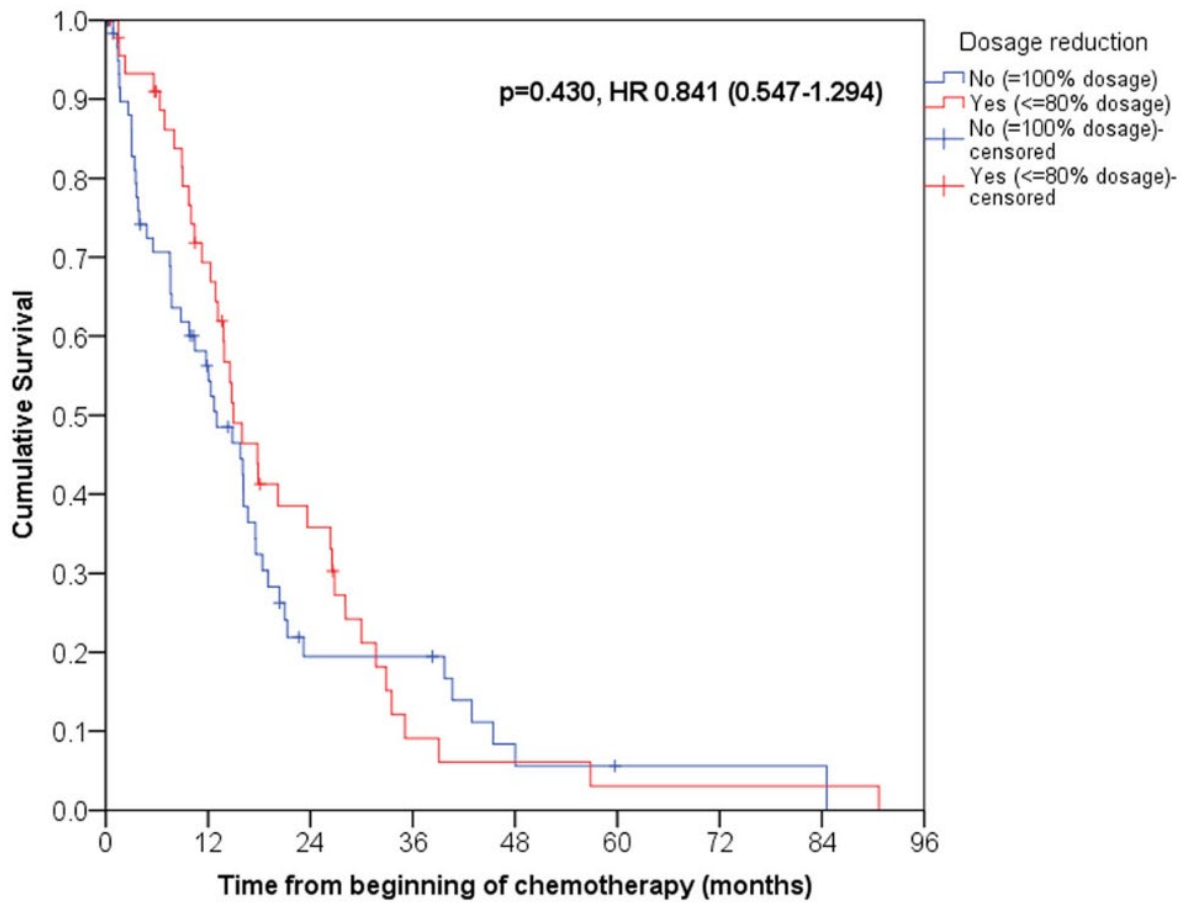


Abbildung 7: Kaplan-Meier Überlebens Analyse von Patienten, die die volle Chemotherapie Dosis erhielten oder die reduzierte Dosis. Die Log-Rank Analyse zeigte keinen Unterschied hinsichtlich der Überlebenswahrscheinlichkeit (p=0.43).

Die Ergebnisse dieser Studie stellen die verbreitete Annahme infrage, dass die Notwendigkeit einer Dosisreduktion mit einer schlechteren onkologischen Prognose einhergeht (52). Vielmehr unterstützen diese Ergebnisse sofern eine Dosisreduktionen indiziert erscheint, diese nicht aufgrund einer potenziell besseren Wirksamkeit der höheren Dosis herauszuschieben, insbesondere für ältere oder multimorbide Patientinnen mit erhöhter Vulnerabilität gegenüber

Toxizitäten. Wahrscheinlich reflektieren die gleichwertigen Überlebensdaten in der Dosisreduktionsgruppe eine bessere Anpassung an individuelle pharmakokinetische Gegebenheiten sowie eine höhere Therapietreue durch bessere Verträglichkeit. Diese Erkenntnisse unterstreichen die Notwendigkeit prospektiver Studien, die toleranzadaptierte Dosierungsstrategien untersuchen (auch Dosissteigerungen). Langfristig könnten derartige Konzepte zu einer personalisierten und patientenzentrierten Therapieentscheidung beim metastasierten Kolonkarzinom beitragen.

5. Zusammenfassung und Ausblick

Diese Habilitationsschrift befasst sich mit der praxisrelevanten Fragestellung der Analyse prognostischer Variablen von Pharmakotherapien bei GI-onkologischen Erkrankungen, mit dem besonderem Fokus auf das hepatozelluläre Karzinom. GI-onkologische Erkrankungen belasten die Gesundheitssysteme weltweit erheblich und erfordern komplexe, interdisziplinäre Behandlungsansätze, in die Fachbereiche wie Gastroenterologie, Onkologie, Chirurgie und Radiologie einbezogen werden. Neue Therapieoptionen und die Erweiterung der zugelassenen Systemtherapien eröffnen Patientinnen und Patienten mit fortgeschrittenen Tumorerkrankungen zunehmend Perspektiven, stellen aber auch die klinische Forschung und Versorgung vor neue Herausforderungen.

Ein zentrales Ergebnis der Arbeit ist die klare Bestätigung, dass sich durch Fortschritte in der Therapielandschaft - insbesondere durch die Einführung neuer Tyrosinkinaseinhibitoren und Immuntherapien - das Überleben und die Lebensqualität von Patientinnen und Patienten mit HCC signifikant verbessern lassen. Die Arbeit belegt zudem die Notwendigkeit, etablierte Therapieschemata regelmäßig zu hinterfragen und an den individuellen Krankheitsverlauf sowie das Nebenwirkungsprofil der Patientinnen und Patienten anzupassen. Dies gilt sowohl für systemische Therapien als auch für die Kombination lokaler und systemischer Ansätze, wie beispielsweise die Kombination von Strahlentherapie und Checkpoint-Inhibitoren.

Ein weiterer bedeutender Ausblick ist, dass sich aus der Beobachtung, dass Dosismodifikationen, im Rahmen der Chemotherapie, nicht zwangsläufig mit einer Verschlechterung der Prognose einhergehen muss. Vielmehr spricht vieles dafür, dass eine individualisierte Dosistitration die Lebensqualität erhalten kann, ohne die Effektivität der

Therapie wesentlich zu beeinträchtigen. Dies könnte die Grundlage für neue, flexible Therapieregime in der klinischen Praxis schaffen.

Auch zukünftig wird es eine vordringliche Aufgabe sein, die translationale Brücke zwischen präklinischer Forschung und klinischer Anwendung weiter auszubauen. Insbesondere die Entwicklung neuer prädiktiver Testsysteme und patientenbasierter Tumormodelle birgt das Potenzial, gezielt neue prädiktive Marker zu identifizieren und die Therapiesequenz noch besser auf die individuellen Charakteristika der Tumorerkrankung abzustimmen.

Insgesamt zeigen die Ergebnisse dieser Habilitationsschrift zeigen insgesamt, dass durch kontinuierliche interdisziplinäre Forschung und die konsequente Integration neuer Erkenntnisse in die klinische Praxis nachhaltige Verbesserungen in der Behandlung gastrointestinaler Tumoren und insbesondere des HCC möglich sind. Die zunehmend personalisierte Onkologie, die auf validen Biomarkern und innovativen Testsystemen basiert, bildet die Grundlage für zukünftige Entwicklungen in Forschung und Patientenversorgung.

6. Literaturverzeichnis

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7. Der Habilitation zugrunde liegende Publikationen im Original

Sagmeister P, Daza J, Ofner A, Ziesch A, Ye L, Ben Khaled N, Ebert M, Mayerle J, Teufel A, De Toni EN, **Munker S.**; Comparative Response of HCC Cells to TKIs: Modified in vitro Testing and Descriptive Expression Analysis. J Hepatocell Carcinoma. 2022 Jul 9;9:595–607. doi: 10.2147/JHC.S356333. PMID: 35845819. PMCID: PMC9278726. IF 6.46 (2022)

Ben Khaled N, Mörtl B, Beier D, Reiter FP, Pawlowska-Phelan D, Teufel A, Rössler D, Schwade DF, Philipp A, Kubisch I, Ehmer U, Geier A, Lange CM, Mayerle J, Berger-Thürmel K, De Toni EN, **Munker S.**; Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study. Eur J Cancer. 2023 Jul 21;192:113248. doi: 10.1016/j.ejca.2023.113248. PMID: 37672814. IF 10.7 (2023)

Munker S, Roessler D, Öcal O, Ben-Khaled N, Bernhart K, Ye L, Piseddu I, Vielhauer J, Reiter FP, Rodriguez I, Ricke J, Teufel A, De Toni E, Seidensticker M, Niyazi M, Corradini S. Concomitant irradiation to checkpoint inhibitor therapy of hepatocellular carcinoma patients: a systematic retrospective, single-center analysis. Oncol Res Treat. 2023;46(11):466–475. doi: 10.1159/000533983. Epub 2023 Oct 12. PMID: 37827135. PMCID: PMC10664332. IF 2.4 (2023)

Munker S#, Rodriguez I#, Bernhart K, Ben Khaled N, Findik M, Siegmund LK, Ye L, Reiter FP, Roessler D, Nasseh D, Balcar L, Pomej K, Scheiner B, Weiss C, Pinter M, Seidensticker M, Mayerle J, Philipp AB, De Toni EN.Prognostic significance of elevated platelet count (>200 × 10^9 per L) in BCLC stages B and C of hepatocellular carcinoma: a retrospective multicenter analysis. J Hepatocell Carcinoma. 2025 May 5;12:855–864. doi: 10.2147/JHC.S511263. PMID: 40352960. PMCID: PMC12063624. IF 6.46 (2024 est.) ; (# these authors contributed equally)

Munker S, De Toni EN.; Use of checkpoint inhibitors in liver transplant recipients. United European Gastroenterol J. 2018 Aug;6(7):970–973. doi: 10.1177/2050640618774631. Epub 2018 Apr 30. PMID: 30228883. PMCID: PMC6137599. IF 3.85 (2018)

Munker S, Vogelhuber M, Bornschein J, Stroszczynski C, Evert M, Schlitt H, Herr W, Teufel A.; EpiCO (epirubicin, cyclophosphamide and vincristine) as treatment for extrapulmonary high-grade neuroendocrine neoplasms. Z Gastroenterol. 2020 Feb;58(2):133–136. doi: 10.1055/a-1042-6504. Epub 2020 Jan 2. PMID: 31896137. IF 1.94 (2020)

Munker S, Gerken M, Fest P, Ott C, Schnoy E, Fichtner-Feigl S, Wiggermann P, Vogelhuber M, Herr W, Stroszczynski C, Schlitt HJ, Evert M, Reng M, Klinkhammer-Schalke M, Teufel A.; Chemotherapy for metastatic colon cancer: no effect on survival when the dose is reduced due to side effects. BMC Cancer. 2018 Apr 23;18(1):455. doi: 10.1186/s12885-018-4380-z. PMID: 29685155. PMCID: PMC5913883. IF 3.03 (2018)

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9. Versicherung an Eides Statt

Hiermit erkläre ich Dr. med. Stefan Munker an Eides Statt, dass ich die schriftliche Habilitationsleistung selbständig verfasst und die Herkunft des verwendeten oder zitierten Materials ordnungsgemäß kenntlich gemacht habe.

Des Weiteren erkläre ich, dass ich an keiner anderen Hochschule habilitiert, ein Habilitationsgesuch eingereicht habe oder ein Habilitationsverfahren ohne Erfolg beendet habe.

Ich erkläre weiter, dass mir kein akademischer Grad entzogen wurde und auch kein entsprechendes Verfahren gegen mich anhängig ist.

Dr. med. Stefan Munker

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ORIGINAL RESEARCH

Comparative Response of HCC Cells to TKIs: Modified in vitro Testing and Descriptive Expression Analysis

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Introduction: Although the treatment paradigm for hepatocellular carcinoma (HCC) has recently shifted in favour of checkpoint inhibitor (CPI)-based treatment options, the tyrosine kinase inhibitors (TKI) currently approved for the treatment of HCC are expected to remain the cornerstone of HCC treatment alone or in combination with CPIs. Despite considerable research efforts, no biomarker capable of predicting the response to specific TKIs has been validated. Thus, personalized approaches to HCC may aid in determining optimal treatment lines for 2nd and 3rd lines. To identify new biomarkers, we examined differential sensitivity and investigated potential transcriptomic predictors.

Methods: To this aim, the sensitivity of nine HCC cell lines to sorafenib, lenvatinib, regorafenib, and cabozantinib was evaluated by a prolonged treatment scheme to determine their respective growth rate inhibition concentrations (GR_{50}). Subgroups discriminated by GR_{50} values underwent differential expression and gene set enrichment analysis (GSEA).

Results: The nine cell lines showed broadly different sensitivities to different TKIs. GR_{50} values of sorafenib and regorafenib clustered closer in all cell lines, whereas treatments with lenvatinib and cabozantinib showed diversified GR_{50} values. GSEA showed the activation of specific pathways in sensitive vs non-sensitive cell lines. A signature consisting of 14 biomarkers (GAGE12H, GJB6, PTCHD3, PRH1-PRR4, C6orf222, HBB, C17orf99, GOLGA6A, CRYAA, CCL23, RP11-347C12.3, RP11-514O12.4, FAM180B, and TMPRSS4) discriminates the cell lines' response into three distinct treatment profiles: 1) equally sensible to sorafenib, regorafenib and cabozantinib, 2) sensible to lenvatinib, and 3) more sensible to regorafenib than sorafenib.

Conclusion: We observed diverse responses to either of the four TKIs. Subgroup analysis of TKI effectiveness showed distinct transcriptomic profiles and signaling pathways associated with responsiveness. This prompts more extensive studies to explore and validate pharmacogenomic and transcriptomic strategies for a personalized treatment approach, particularly after the failure of CPI treatment.

Keywords: biomarkers, sorafenib, lenvatinib, regorafenib, cabozantinib

Introduction

Hepatocellular carcinoma is the sixth most common and third most deadly malignant disease.[1] The tyrosine kinase inhibitors sorafenib,[2] lenvatinib,[3] regorafenib,[4] and cabozantinib[5] are approved for the treatment of advanced HCC for first or second-line treatment. But with the advent of immune oncology (I/O)-based therapies in the first line, the previously established empirical order of TKI treatment has changed. However, even before CPIs, there was an unmet need for adequate predictors of response for either of the four TKIs. Although TKIs impinge on several specific

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intracellular signal transduction mechanisms, their clinical use in monotherapy or in combination with CPIs is based on empirical clinical evidence.[6,7] Previous attempts to establish clinically validated biomarkers of response for these agents have not been implemented in clinical routine so far, leaving an unmet need for biomarkers capable of guiding the choice of TKI.[8–11]

The TKIs' mechanism of action consists of the inhibition of various receptor tyrosine kinases by disrupting their autophosphorylation, as well as inhibiting Raf serine/threonine kinases, eventually leading to a disruption of the PI3K/AKT/mTOR- and MAPK/ERK-pathways, which are believed to play a significant role in the genesis of hepatocellular carcinoma.[12–14] The four TKIs' differences in mechanisms of action and toxicity can be explained through a varying potency towards one target point, as well as slightly different points of target.[15–18] However, despite the well-characterized mechanism of action, there are little to no clinical, phenotypical, or genetic features to guide us to the right therapy.

The lack of predictive biomarkers in the clinical routine may be attributed to the lack of histological specimen acquisition in the initial pharmaceutical studies. Another explanation may be that candidate biomarkers were generated from an analysis made in artificial in vitro systems, and the lack of microenvironment hinders transfer to the in vivo situation. For example, the treatment duration (in vitro 48–72 h) is not remotely close to the clinical situation (weeks to months). In addition, it has recently been shown that pharmacogenomic examinations are strongly confounded by the cell lines' individual proliferative speed. To increase biological relevance, Hafner et al developed the GR_{50} metric, a new approach to assess cellular drug efficacy.[19,20] Another rationale for using the GR50 metric instead of ED50 or AUC, is that it also corrects for cell-line specific differences in read-out measurements by adjusting for read-outs of initial cell populations.[21]

In this study, we used the GR_{50} metric and a prolonged treatment scheme to examine the response of nine hepatoma cell lines to the four viable tyrosine kinase inhibitors to compare responsiveness to either drug and narrow down potential markers that could be further examined in subsequent studies as predictors of response for one of the four TKIs.

Materials and Methods

Cell Lines, Cell Culture and Chemicals

Nine human hepatoma cell lines with different clinical and histological features were used for this research project (Table 1).

Table 1 Clinical and Histological Features of Nine Hepatoma Cell Lines

	Ethnicity	Sex	Age	HBV	AFP	Histological Type	Source	Ref
Hep3B	African	M	8	(+)	(+)	HCC	ATCC	[40]
HepG2	Caucasian	M	15	(-)	(+)	Hepatoblastoma	ATCC, JCRB	[40]
HLE	Asian	M	68	(-)	(-)	HCC	JCRB	[41]
HLF	Asian	M	68	(-)	(-)	HCC	JCRB	[41]
HuH1	Asian	M	53	(+)	(+)	HCC	JCRB	[42]
HuH7	Asian	M	57	(-)	(+)	HCC	RIKEN, JCRB	[43]
PLC-PRF5	African	M	24	(+)	(+)	HCC	ATCC, JCRB	[44]
Snu398	Asian	M	42	(+)	(-)	HCC	ATCC	[45]
Snu475	Asian	M	43	(+)	(-)	HCC	ATCC	[45]

Notes: Modified and recreated from Hirschfield et al,[46] Qiu et al,[8] and Caruso et al.[9]
Abbreviations: AFP, alpha-fetoprotein; ATCC, American Type Culture Collection; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; JCRB, Japanese Collection of Research Bioresources; M, male; RIKEN, short for Rikagaku Kenkyujo – a Japanese research institute.

HuH1, HuH7, HepG2, PLC-PRF5, HLE, and HLF were cultivated in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, USA), Hep3B in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F12, Sigma-Aldrich, USA), supplemented with 200mM L-glutamine (Sigma-Aldrich, USA), and Snu398 and Snu475 in RPMI-1640 medium (Sigma-Aldrich, USA). The disparity of media for the various cell lines is due to our intent to continue using the same medium compound for one cell that has been used in the original publications and in our laboratory in the past, in order to protect the cells from sudden unfamiliarity. All the media were supplemented with 10% Filtrated Bovine Serum (FBS, PAN-Biotech GmbH, Germany) and 1% Penicillin–Streptomycin (P/S, Sigma-Aldrich, USA). Cells were constantly maintained at 37°C and 5% CO_2. Genetic fingerprinting was performed on all cell lines by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures to confirm authenticity. Furthermore, all cell lines have been tested negative for mycoplasms by polymerase chain reaction. Sorafenib tosylate, lenvatinib (E7080), regorafenib (BAY 73–4506), and cabozantinib (XY184 free base) were acquired from AdooQ Bioscience, USA, dissolved in 100% dimethyl sulfoxide (DMSO, Carl Roth GmbH + Co. KG, Germany) at a stock concentration of 100µM and stored at −80°C until used.

Cell Viability Assay

After seeding 800–3000 cells from each cell line in 96-well-plates and leaving the cells to attach for approximately 24 hours, the cells were then treated with seven different concentrations of each drug, ranging from 0.03µM to 20µM in a 1:2–3 serial dilution. The individual concentration ranges for each drug were determined in preliminary experiments that have been conducted beforehand. A DMSO medium solution was used as a control group. After six days, the cell count was estimated using SYBR green assay (Lonza, USA), and fluorescence was measured using the Cytofluor Series 4000 (Applied Biosystems, Germany), as previously described.[22] All experiments were performed in triplicates with at least three individual biological replicates per drug.

To minimize the effect of proliferation, we decided on using the growth rate method established by Hafner et al. To calculate the normalized GR(c)-value at concentration c, the following equation is being used:

$$GR(c) = 2^{\frac{log_2(x(c)/x_0)}{log_2(x_{ctrl}/x_0)}} - 1$$

with x(c) being the cells treated with one drug at concentration c at day 7, x_{ctrl} being the DMSO treated control wells from the same plate at day 7, and x_0 being the untreated cells from the time of treatment at day 1. Typically, the GR value lies between −1 and +1, whereas a positive value (0 to +1) correlates to partial growth inhibition, GR equals 0 stands for complete cytostasis, ie, no increase or decrease in cell number, and negative values (0 to −1) stand for cytotoxicity, that is cell death. Afterwards, the GR values were fitted to a sigmoid curve, and the GR_{50} and GR_{max} values were calculated. The GR_{max} value, which is a metric for drug efficacy, represents the GR value for one drug at the highest concentration of this drug. The GR_{50} value is a metric for drug potency and represents the concentration at which GR(c) = 0.5.[19]

Processing of Gene Expression Data

To determine the driving genes affecting the response to our medication groups (sorafenib, lenvatinib, regorafenib and cabozantinib) and of an additional "regorafenib over sorafenib" (RoS) subset, differential expression analysis was performed using the "DESeq2" package in R Version 4.1.1 and RStudio Desktop 2021.09 release,[23] evaluating our treatment responses in a binary "sensitive vs resistant" notation against the established cell lines RNA-seq gene expression profiles obtained from the Liver Cancer Model Repository (LIMORE, https://www.picb.ac.cn/limore/), a cell models compilatory project with 81 human liver cancer cell models.[8] The resulting information was filtered to contain only genes with ±2-fold changes (FC), and a cut-off value of 0.001 was set as statistically significant using the Bonferroni-Holm adjusted *p*-value.

Gene Clustering and Pathway Enrichment Analysis

To visualize the DESeq2 data, a heatmap focusing on the gene groups of interest was created using the Heatmapper webtool (http://www.heatmapper.ca).[23,24] Clustering was performed using the Complete Linkage method, and the Manhattan distance method was selected due to the high dimensionality of the data. Furthermore, KEGG pathways enrichment analysis was performed on the resulting differential expression data using a cut-off value of 0.05 in the ShinyGO webtool version 0.64,[25] and further pathway analysis and filtering of our data with the same cut-off value was performed using the commercial tool QIAGEN's Ingenuity Pathways Analysis (IPA).[26] A selection of the most statistically significant over- and under-expressed genes was performed by filtering the genes with a fold change ±2 and an adjusted *p*-value under 0.001.

Graphics

Graphics and figures were generated using GraphPad Prism 8 (GraphPad Software Inc., USA), Heatmapper (University of Alberta, Canada), or QIAGEN's Ingenuity Pathway Analysis (QIAGEN Inc., USA).

Statistical Analysis

Data were analyzed using IBM SPSS statistics software version 27 (SPSS Inc., IBM, USA) or GraphPad Prism 8 (GraphPad Software Inc., USA). Results are shown as mean ± standard deviation for at least three individual experiments. One-way analysis of variance (ANOVA) or the more robust Welch-ANOVA, in case equal variances using Levene's test could not be shown, followed by a Tukey- or Games Howell post hoc analysis, was conducted. If $p < 0.05$ between two cell lines, these two cell lines showed a statistically significant difference in response to the drug that has been examined.

Additionally, the correlation coefficients, which show the strength of correlation between our GR_{50} values, our GR_{max} values, Qiu et al's IC_{50} values,[8] and Caruso et al's GI_{50} values,[9] for every drug, as well as overall, were calculated. According to the linearity of the correlation between two factors, Pearson's correlation or the more robust Spearman's Rho correlation were calculated.

Results

Differential Responsiveness to Clinically Viable TKIs Sorafenib, Lenvatinib, Regorafenib, and Cabozantinib

The effect of four tyrosine kinase inhibitors, sorafenib, lenvatinib, regorafenib, and cabozantinib, on cell viability was assessed on nine hepatoma cell lines, exhibiting different clinical and molecular features (Table 1). We used a six-day treatment protocol to mimic the clinical application of TKIs. Each TKI induces a decrease in cell viability in a dose-dependent manner in all cell lines (Figure 1A–D). In addition, we observed a diversified response to the four TKIs (Figure 1E–H).

Upon sorafenib treatment (Figure 1A), HLE, Snu398, Hep3B, and HepG2 are more sensitive to GR_{50} concentrations below 2μM and HLF, HuH1, HuH7, Snu475, and PLC-PRF5 are less sensitive to GR_{50} concentrations above 2μM. The difference in sensitivity between the most (1.3μM) and the least sensitive cell line (2.9μM) only differs by a factor of 2.2. Almost all cell lines display a slight cytotoxic effect at the highest concentration of sorafenib (Table 2).

Lenvatinib displays a heterogeneous pattern of response (Figure 1B), dividing all cell lines but Sun475 into two groups. The first group, containing HuH7, Snu398, HLE, and HLF, shows GR_{50} values between 0.7μM and 1.0μM. The second group, containing HepG2, Hep3B, PLC-PRF5, and HuH1, displays GR_{50} values between 6.7μM and >20μM. Hence, the GR_{50} value of the most sensitive cell lines (HuH7 and HLF) differs from the most resistant cell line (PLC-PRF5) by more than 28.5 times.

Regorafenib's range of efficacy is similar to sorafenib's (Figure 1A and C). However, some cell lines, such as Hep3B, HepG2, HLF, HuH7, and Snu475, show a difference in potency when treated with sorafenib and

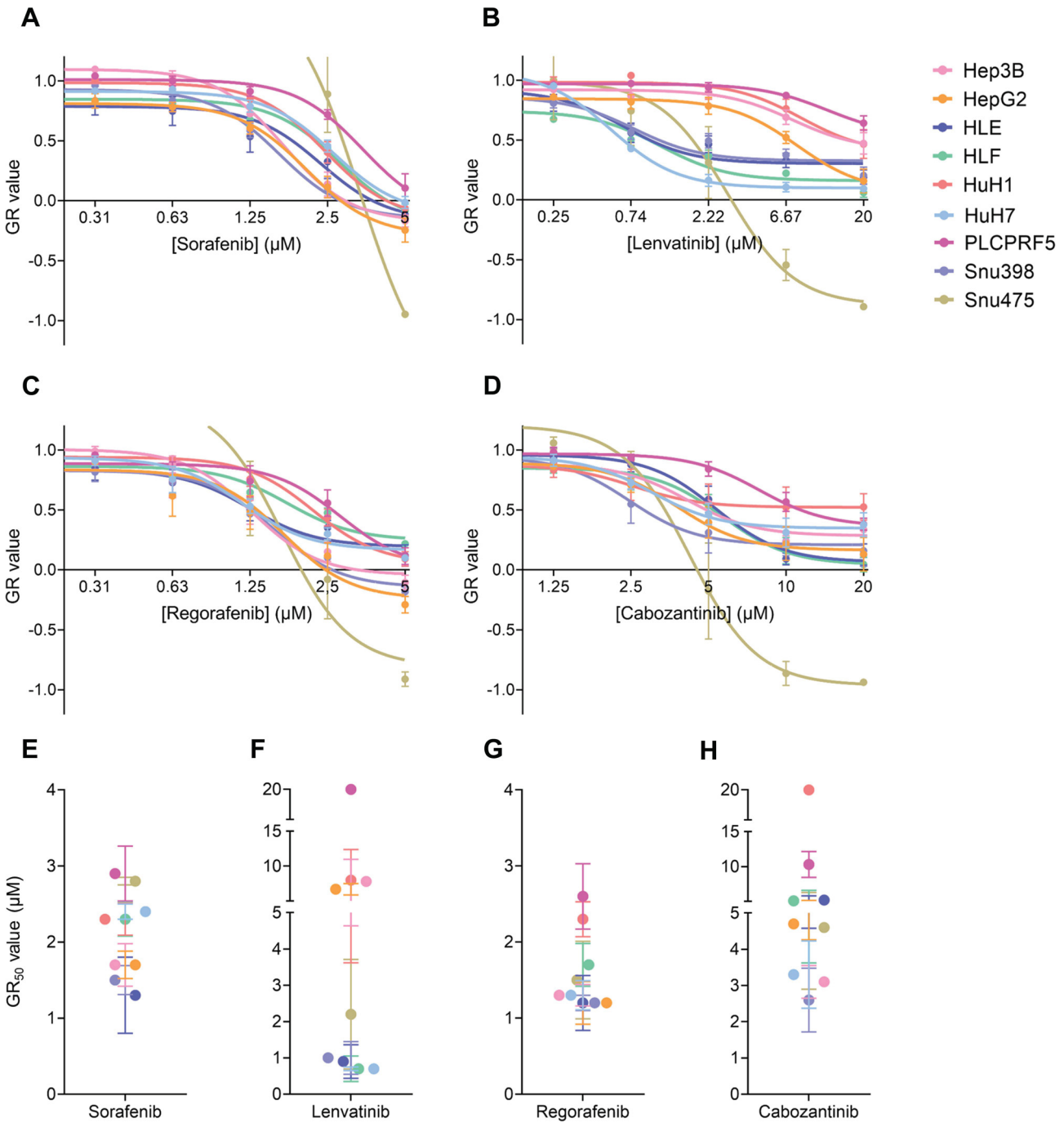


Figure 1 The four tyrosine kinase inhibitors, sorafenib, lenvatinib, regorafenib, and cabozantinib, cause a reduction in cell viability in all nine cell lines. (**A**) Effect of sorafenib on all cell lines with a concentration range of 0.31μM to 5μM in a 1:2 serial dilution. (**B**) Effect of lenvatinib on all cell lines with a concentration range of 0.25μM to 20μM in a 1:3 serial dilution. (**C**) Effect of regorafenib on all cell lines with a concentration range of 0.31μM to 5μM in a 1:2 serial dilution. (**D**) Effect of cabozantinib on all cell lines with a concentration range of 1.25μM to 20μM in a 1:2 serial dilution. Potency (GR_{50} values) for (**E**) sorafenib, (**F**) lenvatinib, (**G**) regorafenib, and (**H**) cabozantinib. Results are presented as the mean and standard deviation for at least three experiments. $P<0.05$ for all drugs by analysis of variance (ANOVA). Due to the increased proliferation of Snu475 under sorafenib and regorafenib at the lower concentrations and to better fit the representation in this graph, we do not show Snu475 at said lower concentrations. The complete dose–response curves of Snu475 can be seen in Supplementary Figure 1.

regorafenib, with all of them being more sensitive to the second-line treatment option regorafenib (Figure 1A and C, Table 2).

Lastly, cabozantinib shows a little more variety of its effect on different cell lines (Figure 1D). The most sensitive (Snu398, $GR_{50,\ C\text{-}Snu398}$= 2.6μM) and the least sensitive cell line (HuH1, $GR_{50\ C\text{-}HuH1}$ >20μM) differ by a factor greater than 7.6, respectively.

Table 2 GR Values for Potency and Efficacy

	Sorafenib			Lenvatinib			Regorafenib			Cabozantinib			RoS
Cell-Line	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**GR_{50}**	**GR_{max}**	**R**	**R**
Hep3B	1.7	−0.17	1	7.8	0.47	0	1.3	−0.11	1	3.1	0.34	1	1
HepG2	1.7	−0.25	1	6.7	0.16	0	1.2	−0.29	1	4.7	0.13	1	1
HLE	1.3	−0.12	1	0.9	0.19	1	1.2	0.12	1	5.2	0.13	0	0
HLF	2.3	−0.08	0	0.7	0.06	1	1.7	0.22	0	5.1	0.05	0	1
HuH1	2.3	−0.06	0	8.0	0.20	0	2.3	0.09	0	20	0.53	0	0
HuH7	2.4	−0.01	0	0.7	0.09	1	1.3	0.10	1	3.3	0.38	1	1
PLC-PRF5	2.9	0.11	0	20	0.64	0	2.6	0.12	0	10.3	0.38	0	0
Snu398	1.5	−0.16	1	1.0	0.47	1	1.2	−0.18	1	2.6	0.16	1	0
Snu475	2.8	−0.95	0	2.2	−0.95	1	1.5	−0.92	0	4.6	−0.98	1	1

Notes: This table shows the potency (GR_{50} in μM), efficacy (GR_{max}), and response (R) for each cell line and drug, as well as the response for one regorafenib over sorafenib (RoS) group. The response of the four TKIs is subgrouped into less sensitive (0) and more sensitive (1). The cut-off points for response chosen for each drug were determined by looking at the spread of GR_{50} values of the different cell lines, factoring in the steady-state plasma concentration of each drug, as well as trying to achieve a 4:5 ratio for the two subgroups: 2μM for sorafenib, 1.5μM for regorafenib, 5μM for lenvatinib and cabozantinib. For the RoS-group, the cell lines were divided into being equally responsive to regorafenib and sorafenib (0) and showing a statistically significant difference in response to those two drugs, with all of them being more sensitive to regorafenib (1). Results are presented as the mean of at least three experiments. $P<0.05$ for all drugs by analysis of variance (ANOVA). GRmax values can only be compared if the highest concentrations are equal. Therefore, they can be used for interpretation when comparing the efficacy of different cell lines to one drug, but not when comparing how one cell line reacts to different drugs.

Snu475 appeared to be the overall least responsive cell line. Snu475 proliferates approximately 12 times slower than the next slowest proliferating cell line, assuming exponential growth. Snu475 is the only cell line, that shows a complete cytotoxic response to all four drugs, with GR_{max} values almost reaching −1 ($GR_{max,S\text{-}Snu475} = -0.95$, $GR_{max,L\text{-}Snu475} = -0.95$, $GR_{max,R\text{-}Snu475} = -0.92$ and $GR_{max,C\text{-}Snu475} = -0.98$, respectively) (Table 2).

Differential Expression Analysis of the Four Treatment Groups and a Fifth Category

The bioinformatics evaluation following the responsiveness analysis allowed the addition of a fifth category ("regorafenib over sorafenib") to determine the genes involved in the differential potency response of a specific subset of cell lines to the second-line treatment regorafenib in comparison to the first-line treatment sorafenib. Expression data is filtered for significance as previously described in the methods. Our differential gene expression data are translated into an expression heatmap (Figure 2). On the far left of Figure 2, we can see two distinct clusters that show a highly over- and under-expression (FC > ±20) consistent in all our cell lines (Figure 2, blue rectangle), showing two groups of identifiable gene expression signatures concerning the distinct drug response groups.

Gene Sets Enrichment Analysis

Out of the 19.761 human genes evaluated on the RNA-seq data, a total of 966 fulfilled our differentially expressed genes (DEG) filtering criteria (*FC >|2|, pAdj > 0.001*) in at least one of the treatment groups. Specifically, there were 217 DEG for sorafenib, 296 for lenvatinib, 201 for regorafenib, 240 for cabozantinib, and 210 for the RoS group (ie those related to the differential response between sorafenib and regorafenib).

These gene groups were analyzed for pathway enrichment using ShinyGO (to evaluate KEGG) and QIAGEN's IPA. The KEGG pathway enrichment analysis (Table 3) showed three pathways – two of them associated with drug metabolism in the sorafenib responding group, one – related to neurotransmitters – in the lenvatinib group, seven pathways – most of them related to cardiac function – in the regorafenib group, three receptor-related pathways in the

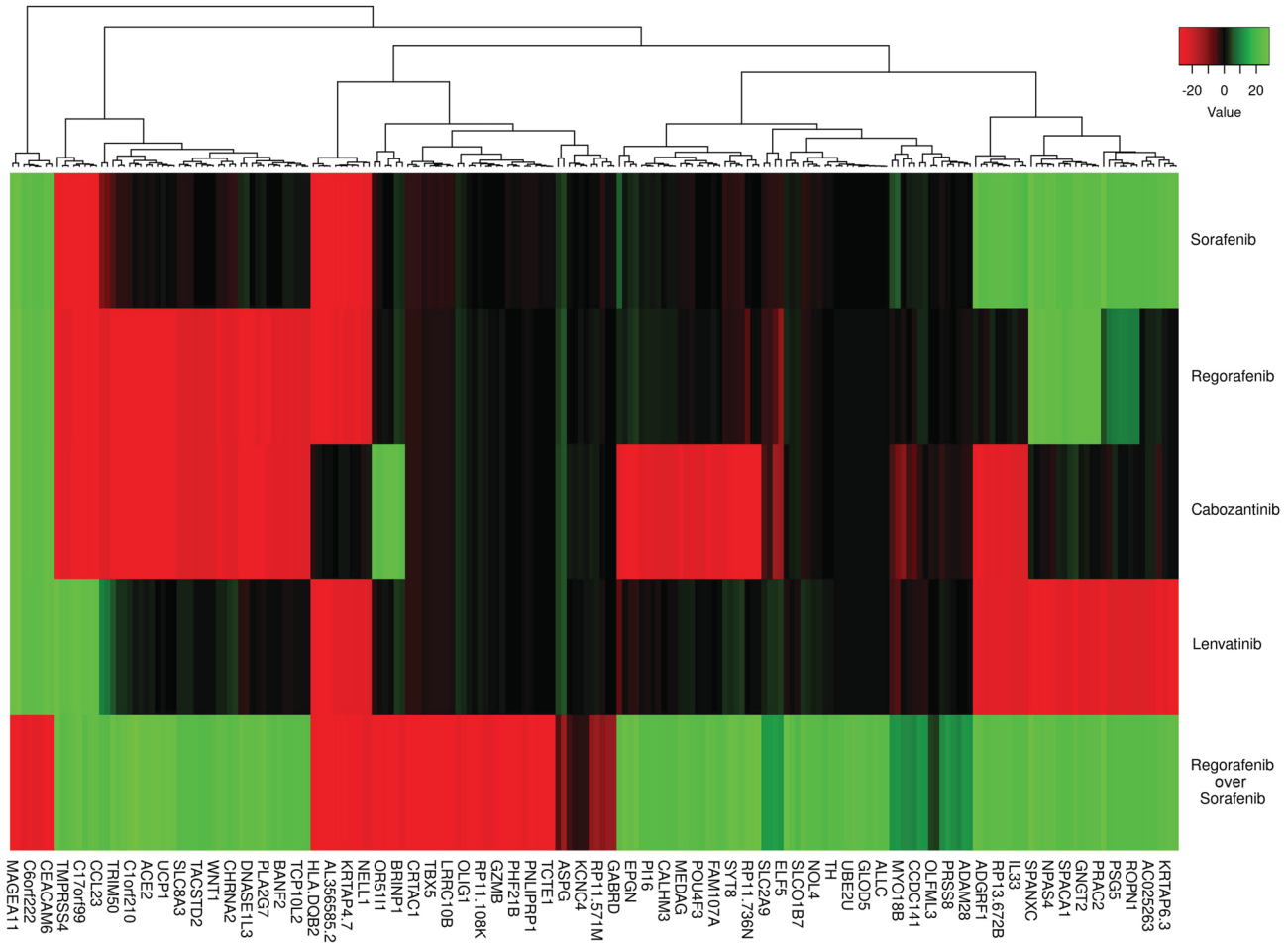


Figure 2 Gene differential expression values compared to treatment groups. This heatmap shows the differential expression values for each drug. The scale is measured in expression fold changes (FC) in relation to the standardized expression profiles of the original cell lines after being analyzed with the DESeq2 algorithm. The dark colors represent FC values between 0 and ± 10, and the lighter colors present FC values over ± 10. At the left, the blue rectangle includes two clusters tinted in light green and red in all the treatment groups, suggesting two identifiable gene expression signatures: the left one, with overexpression in four groups (sorafenib [S], regorafenib [R], cabozantinib [C] and lenvatinib [L]) and underexpression in those that responded differentially better to regorafenib than sorafenib (RoS), and the right one, with genes highly underexpressed in the first three groups ((S), (R), and (C) and overexpressed in the last two (L and RoS).

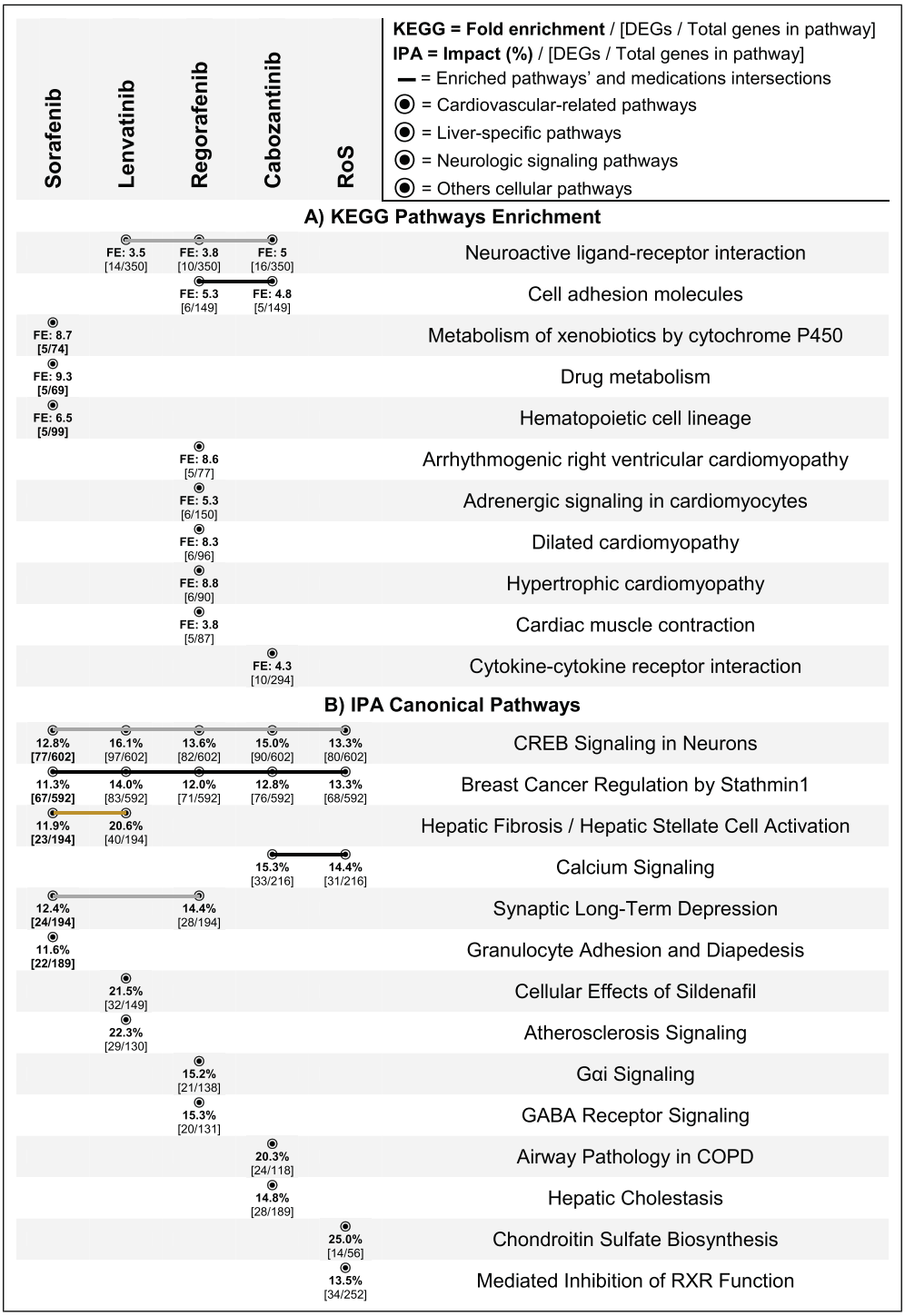
cabozantinib group, and no enriched pathways in the RoS group. Noticeably, "Neuroactive ligand-receptor interaction" was shared among lenvatinib, regorafenib, cabozantinib, and "Cell adhesion molecules" among regorafenib and cabozantinib.

Furthermore, among the top five canonical pathways detected using the IPA analysis (Table 3), two were enriched in all the treatment groups: "CREB Signalling in Neurons" and "Breast Cancer Regulation by Stathmin1", and three were partially shared: "Hepatic Fibrosis/Hepatic Stellate Cell Activation" between lenvatinib and sorafenib, "Synaptic Long-Term Depression" between sorafenib and regorafenib, and "Calcium Signaling" between cabozantinib and RoS.

Resulting Gene Expression Signature

After filtering the DEG consistently significant in the five treatment groups, 14 genes (GAGE12H, GJB6, PTCHD3, PRH1-PRR4, C6orf222, HBB, C17orf99, GOLGA6A, CRYAA, CCL23, RP11-347C12.3, RP11-514O12.4, FAM180B, and TMPRSS4) fulfilled our criteria. Moreover, they all presented a highly significant level of over or under expression (FC > |20|). When organized as in the heatmap clusters, three different expression profiles were formed: one comprehending those that respond to sorafenib, regorafenib, and cabozantinib; one comprehending those that respond to lenvatinib; and one with those that respond better to regorafenib compared to sorafenib (Table 4).

Table 3 KEGG and IPA enriched pathways by treatment group.



Notes: This table shows the enriched pathways for every treatment group as detected by **A**) KEGG pathways enrichment and **B**) QIAGEN'S IPA analysis. To establish each pathway's relevance, Fold Enrichment (FE; the percentage of DEGs belonging to a pathway divided by the corresponding percentage in the background) is used on KEGG, and Impact (the number of DEGs divided by the total number of pathway genes) on IPA pathways. The intersections of pathways between treatments are shown with a line, and the main type of enriched pathways is expressed by colors: red denotes cardiovascular-related pathways, gray the neurological, brown the liver-specific, and black other pathways.

Table 4 Gene Expression Signature Candidate

Candidates for Gene Expression Signature (FC >|2|, pAdj > 0.001)	Sorafenib (217 DEG)	Regorafenib (201 DEG)	Cabozantinib (240 DEG)	Lenvatinib (296 DEG)	RoS (210 DEG)
Signature Group		**A**		**B**	**C**
GAGE12H	+	+	+	+	–
GJB6	+	+	+	+	–
PTCHD3	+	+	+	+	–
PRH1-PRR4	+	+	+	+	-
C6orf222	+	+	+	+	-
HBB	+	+	+	+	-
C17orf99	-	-	-	+	+
GOLGA6A	-	-	-	+	+
CRYAA	-	-	-	+	+
CCL23	-	-	-	+	+
RP11-347C12.3	-	-	-	+	+
RP11-514O12.4	-	-	-	+	+
FAM180B	-	-	-	+	+
TMPRSS4	-	-	-	+	+

Notes: This table shows the DEG that fulfills the filtering criteria. Group A (sorafenib, regorafenib, and cabozantinib group) present an overexpression with an FC > 20 in GAGE12H, GJB6, PTCHD3, PRH1-PRR4, C6orf222, and HBB, and an underexpression with an FC < −20 in C17orf99, GOLGA6A, CRYAA, CCL23, RP11-347C12.3, RP11-514O12.4, FAM180B, and TMPRSS4. Group B (lenvatinib) presents an overexpression in all the named genes, and Group C (regorafenib over sorafenib) completely inverts the profile seen in group A.

Discussion

The current gold standard for treating HCC in an advanced stadium are I/O-based therapies, such as atezolizumab plus bevacizumab or durvalumab plus tremelimumab, which are presently investigated in the ongoing randomized Phase III HIMALAYA study and show promising efficacy in the preliminary analysis.[27,28] However, if the tumor is non-responsive to the I/O-treatment or contraindications prohibiting I/O-based therapies are present, inclusion in second-line studies or the usage of TKIs may be considered as the next choice in the treatment algorithm for advanced HCCs.[6] If a patient must be treated with a TKI, there is no established biomarker to guide treatment. Therefore, the use and the application sequence of these agents in different lines of treatment are solely determined by empirical evidence. To narrow potential gene sets and explore the feasibility of biomarker-guided approaches, we investigated the differential responsiveness of nine hepatoma cell lines to the four currently approved TKIs, followed by a differential expression and gene set enrichment analysis.

Cell line-specific heterogeneity of proliferative rates influences the comparison of in vitro efficacy measurements. It is assumed that only 40% of statistically significant associations between IC_{50} values and genomic alterations in large-scale screening experiments are not confounded.[20] The recently developed GR_{50} metric adjusts for proliferation and integrates cytostatic and cytotoxic effects.[19]

In the last years, extensive experiments have been conducted examining the response of various hepatoma cell lines to the four tyrosine kinase inhibitors among an array of other substances.[8,9] Given that we used the GR_{50} metric, a prolonged treatment protocol, and a different assay, a comparison of these two papers to our experiments can only be made to a certain extent.

In our experiments, we used the GR method with the GR_{50} value as a marker for potency, as previously described. Qiu et al used the half-maximal inhibitory concentration (IC_{50}) as a value for potency, which shows the drug

concentration having caused 50% inhibition on cell viability and Caruso et al decided to portray the potency of their substances in the form of the GI_{50} value, which is the renamed version of the IC_{50} value correcting for the cell count at time zero.[29] In their work, some inhibitory concentrations of regorafenib, lenvatinib, and cabozantinib were outside the metrics range (Supplementary Table 1). While we can see an apparent decrease in cell viability in our experiments, both Qiu et al and Caruso et al show that while the four TKIs lead to a decrease in cell viability on all cell lines, their effect on hepatoma cell lines in vitro is among the smallest out of all the agents that were evaluated in their experiments. In addition, comparing the efficacy measurements in all three datasets, that is, Caruso et al's, Qiu et al's and ours, for the four TKIs, a difference in responses can be seen, with our experiments showing the most potent effect in cell decrease for all four TKIs (Supplementary Tables 1 and 2). Duration of treatment also affects the validity and clinical relevance of in vitro studies.[30] In a clinical setting, the four TKIs are usually administered over several weeks to months. Therefore, we decided on using a drug assay with a six-day treatment duration instead of a shorter drug assay to mimic this clinically proven treatment schedule better. Both Qiu et al and Caruso et al decided on a shorter duration of drug exposition (48–72 hours) compared to our experiment (144 h). In contrast, we observed significantly decreased GR_{50} values compared with the other two studies (Supplementary Table 1) and attribute it to the prolonged treatment. Since cytostasis is the primary mode of action of the four TKIs, a transcriptomic lag or cellular accumulation of the cytostatic factors may explain the observed differences with the prolonged treatment scheme. Additionally, we can also assume that the different metrics for efficacy, the varying execution of the drug assays, and the mode of action of the TKIs play a role in the different results of these three studies.[8,9]

A diversified response can be observed for all four TKIs. In particular, lenvatinib and cabozantinib, but also sorafenib and regorafenib, showed a pronounced spread of GR_{50} values. Thus, the diverse pharmacological response reflects the divergent biology of these cell lines and gives justification to pursue biomarker-guided approaches such as transcriptomic-driven approaches or individualized testing strategies. In this regard, Broutier et al recently showed the feasibility of ex vivo culture of individual tumoroid HCC and cholangiocarcinoma cell lines and the possibility of ex vivo drug testing.[31]

Interestingly, the response in most cell lines to sorafenib and regorafenib correlates strongly, which is not surprising considering their chemical structure only differs by one additional fluorine atom,[16] they display almost identical target points,[32] and regorafenib is only approved for therapy in patients with HCC in the second line after they have previously been treated with sorafenib.[33]

Moreover, the differential responsiveness of each drug allowed us to compare their related canonical pathways and the different gene expression profiles. Among the shared sets, KEGG enrichment analysis identified the "neuroactive ligand-receptor interaction pathways" in the lenvatinib, regorafenib, and cabozantinib responders, and "Cell adhesion molecules" in regorafenib and cabozantinib responders. This further validates the enrichment of these pathways in sorafenib-resistant cell lines as reported by Gan et al, including focal adhesion, neuroactive ligand/receptor, and cytokine/cytokine receptor.[34] The rest of the pathways were enriched only in treatment-specific responses, being the sorafenib responders related to drug metabolism and regorafenib responders to cardiac-related pathways. Notably, no specific KEGG enriched set was identified in the regorafenib over sorafenib group and none of our TKIs' key target pathways in KEGG (eg "Pathways in cancer", "VEGF signaling pathway", "MAPK signaling pathway", among others) was enriched.

On the other hand, Qiagen's IPA analysis showed two pathways enriched in all five groups: "CREB Signalling in Neurons" and "Breast Cancer Regulation by Stathmin 1". As recently reviewed by Zhang et al, the cAMP-PKA-CREB pathway has a versatile role in multiple targets, and it can promote or inhibit HCC in a context-dependent matter, suggesting that CREB's homeostasis is vital for HCC progression.[35] Stathmin 1′s importance for hepatoma seems to be on the rise, since it has recently been proposed as a tumorigenic and progression regulator due to its interaction with YAP1,[36] and, when overexpressed, as a novel treatment target for HCC.[37] Furthermore, sorafenib and regorafenib shared the "Synaptic Long-Term Depression" pathway, which is not surprising given that it encompasses the activation of glutamate and other neurotransmitter receptors,[38] as seen in the KEGG neuroactive ligand/receptor enriched set.

Additionally, we found the "Hepatic Fibrosis/Hepatic Stellate Cell Activation" in both the sorafenib and lenvatinib responders and "Calcium Signalling" in the cabozantinib and "regorafenib over sorafenib" groups.

As discussed in a recent review by Qian et al, the creation of an HCC gene expression signature is challenging because it usually arises from the cirrhotic liver, and the comparison of tumoral samples against non-tumorous tissue is complicated as the control tissue itself may have significant changes in gene expression.[39] Nonetheless, the determination of a common DEG signature capable of predicting multiple cell lines' treatment response into three distinct TKI treatment profiles has been achieved: A) those equally sensible to sorafenib, regorafenib, and cabozantinib, B) those more sensible to lenvatinib, and C) those more sensible to regorafenib than sorafenib. This signature comprised the 14 genes that were statistically significant in each treatment, and additionally, they had a Fold-change of ±20, turning them into easily discernible molecules. Consequently, despite not being able to discern the best responders to cabozantinib, it may be used to decide whether the group sorafenib/cabozantinib, lenvatinib, or regorafenib should be used for a patient's best possible outcome according to their tumor DEG. However, due to the limited number of cell lines investigated, these data must be validated in more extensive cell-line or tumoroid-line collections before being tested in a clinical approach. Another limitation of our study, is that we have conducted our experiments on cell lines that were generated from untreated HCCs and did not undertake any pre-treatments. As the currently preferred first-line therapy for advanced HCCs are CPI therapies, TKIs are mainly used upon progression to the preferred first line. Upon progression to CPI therapies, it is likely that the tumor biology in untreated tumors differs significantly from post-CPI tumors, for example, due to the process of acquiring resistance. Therefore, inference of our results to the post-CPI situation is limited. We are planning on using in vitro systems such as the tumoroid cell culture method in follow-up studies, to better reflect the whole spectrum of tumor biology and the clinical context of the HCC tumor entity.[31]

Upon evaluation of multiple hepatoma cell lines in vitro and in silico, a common gene expression signature was characterized that, if validated in vitro and in vivo, could accurately predict a patient's HCC response to the different available TKIs, and it could help us define the best treatment for the individual patient according to transcriptomic analysis in our near future.

Conclusion

We observed heterogeneous responsiveness to the four currently approved TKIs. This generates a rationale for evaluating genomic and transcriptomic biomarkers and individualized approaches, such as patient-derived tumoroid culture and testing. In addition, interobserver heterogeneity highlights the need for additional investigations evaluating the applicability of modified in vitro strategies (ie, prolonged testing) and new efficacy metrics. The transcriptomic-analysis-generated genes candidating as biomarkers showed distinct signaling pathways associated with sensitivity to either TKI and found a candidate gene signature capable of discriminating response to the TKIs.

Disclosure

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Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study

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List of Abbreviations: AFP, alpha-fetoprotein; ATC, Anatomical Therapeutic Chemical; BCLC, Barcelona Clinic Liver Cancer; CCI, Charlson comorbidity index; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EMA, European Medicines Agency; HR, hazard ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; ICD-10-GM, International Classification of Diseases version 10 German; MTT, molecular targeted therapies; mOS, median overall survival; NAFLD, nonalcoholic fatty liver disease; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; ns, not significant; PHT, portal hypertension; SD, standard deviation; SHI, statutory health insurance; TKI, tyrosine kinase inhibitor.

Ethics approval: The study did not require Institutional Review Board approval since only anonymized data was analyzed. This study was conducted in accordance with both the Declarations of Helsinki.

Informed Consent Statement: Informed consent was unnecessary since only anonymized data was analyzed.

Data Availability Statement: Data were obtained from the Ingef database. Due to German data protection laws (Bundesdatenschutzgesetz), the data used in this study cannot be made available directly to other parties. Scientists can apply for data access at Ingef (info@ingef.de).

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Abstract

Background & Aims: The treatment of hepatocellular carcinoma (HCC) is undergoing a historic transformation with the approval of several new systemic therapies in the last years. The aim of this study was to examine the impact of this changing landscape on survival and costs in a Western nationwide, real-world cohort.

Methods: A nationwide representative claims database (InGef database) was screened for HCC cases between 2015 and 2020. Survival in an era with only sorafenib (period A, 01/2015-08/2018) and after approval of lenvatinib and other systemic treatments (period B, 08/2018-12/2020) was analyzed. Health care costs were assessed.

Results: We identified a total of 2876 individuals with HCC in the study period. The proportion of patients receiving systemic therapy increased significantly over time from 11.8% in 2015 to 15.1% in 2020 (p=0.005). Median overall survival in period B was 6.5 months (95% confidence interval (CI): 4.9 – 8.9) and in period A 5.3 months (95% CI: 4.5-6.3) (p=0.046). In period B, median overall survival with lenvatinib was 9.7 months (95%

CI: 6.3 - 18.4) versus 4.8 months with sorafenib (95% CI: 4.0 – 7.1, p=0.008). Costs for prescription drugs per patient increased from €6,150 in 2015 to €9,049 in 2020 (p<0.0001), and costs for outpatient care per patient from €1,646 to €2,149 (p=0.0240).

Conclusion: The approval of new systemic therapies resulted in a survival benefit in patients with HCC. The magnitude of the effect is modest and associated with a moderate increase in health costs.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer type worldwide, with an increasing incidence and a significant socioeconomic burden (1-4). Despite well-defined risk factors and surveillance programs in patients with chronic liver disease (5), most patients with HCC are diagnosed with cancer at an advanced stage, where curative treatment is not feasible (6). In 2007, sorafenib, a tyrosine kinase inhibitor (TKI), was the first agent to show significant clinical benefit in patients with advanced HCC (7, 8). Real-world analyses revealed an improved survival in patients with advanced HCC in the years after sorafenib's approval (9, 10).

Eleven years after the introduction of sorafenib, the treatment landscape of HCC has changed and continues to change dramatically. In 2018, lenvatinib, another TKI, was approved as an alternative treatment option for treatment-naïve patients with advanced HCC based on the non-inferiority of overall survival compared to sorafenib (11). Two more TKIs, regorafenib and cabozantinib, and the vascular endothelial growth factor receptor two inhibitor ramucirumab were approved in the second-line after sorafenib in the following years based on the results of the respective pivotal trials (12-15). In 2020, the highly effective combination regimen of atezolizumab and bevacizumab received approval as first-line treatment for advanced HCC (16).

The benefit of adopting new effective systemic therapy options into the treatment of patients with HCC has been demonstrated in large clinical trials (11-15). HCC patients in clinical practice differ substantially from clinical trial populations and little is known about the translation of this trial data into the real world. A study in an Asian HCC cohort from three institutions published recently showed improvements in survival after the introduction of multiple TKIs (17). It remains to be shown that the new treatment landscape led to an improved survival in a Western population.

This nationwide retrospective study exploits a comprehensive sample of 2876 patients with HCC from a database representative for the German population to investigate survival outcomes and treatment costs associated with the higher availability of HCC systemic therapies.

2. Methods

2.1 Study design

We performed a retrospective study of patients with HCC using the InGef database (Institute for Applied Health Research Berlin GmbH [Institut für angewandte Gesundheitsforschung Berlin]) (18). This German nationwide healthcare claims database includes anonymized, longitudinal data of around 8.8 million people with statutory health insurance (SHI) covering all federal states of Germany. The database was extensively externally validated (18, 19) and offers an ideal platform to study healthcare developments (20-22). A sample of around 4.8 million people was used for this study and represents the German population in terms of gender and age. The database shows good overall accordance with regards to mortality, morbidity, and drug usage (18, 19). The database comprises information on demographics, inpatient and outpatient services (e.g., diagnoses, operations, procedures, provider specialty), prescriptions, and other claims (e.g., drugs, aids, remedies), as well as health care costs. Diagnoses are coded according to the International Classification of Diseases, version 10, German modification (ICD-10-GM). Drug prescriptions are classified using the Anatomical Therapeutic Chemical (ATC) codes defined by the AOK Research Institute (23). The study did not require Institutional Review Board approval since only anonymized data was analyzed. Due to German data privacy regulations, the time period covered by the patient population is six years and no exact numbers can be provided for items with patient numbers below five.

2.2 Patient population

We identified all patients with a diagnosis of HCC using the ICD-10-GM code C22.0 (liver cell carcinoma, hepatocellular carcinoma) between January 01, 2015, and December 31, 2020, within a sample of four million individuals (approximately 5% of the German population) in the claims database (see study flow chart, supplementary fig. S1). All patients prescribed systemic therapies approved within the study period were selected using the respective ATC codes (supplementary table 1). Within the study period, the following five molecular targeted therapies (MTT) were approved by the European Medicines Agency (EMA) for the systemic treatment of HCC in chronological order (24): (1) Sorafenib was approved for the treatment of HCC in 11/2007 independent of the line of therapy. (2) Regorafenib was approved after previous treatment with sorafenib in 08/2017, (3) lenvatinib was approved for first-line therapy in 08/2018, (4) cabozantinib was approved after previous treatment with sorafenib in 11/2018, and (5) ramucirumab was approved after previous treatment with sorafenib in patients with an alpha-fetoprotein (AFP) of ≥ 400 ng/ml in 09/2019. Within the approval, sorafenib and lenvatinib could be started in the first-line setting. After first-line treatment, sorafenib, cabozantinib, regorafenib, or ramucirumab (if AFP ≥ 400 ng/ml) could be used. After first-line therapy with lenvatinib, sorafenib, and, subsequently, cabozantinib, regorafenib, or ramucirumab (if AFP ≥ 400 ng/ml) could be administered. The German HCC guideline valid in the study period recommended the use of systemic therapy in patients with metastasized or locally untreatable HCC with preserved liver function defined by Child-Pugh Score A, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and a life expectancy of > three months (25). During the study period, the checkpoint inhibitors pembrolizumab and nivolumab were approved as monotherapy in the United States for patients with advanced HCC (26), which could serve

as a basis for their off-lable use in later lines of therapy in Germany. Therefore, we also included nivolumab and pembrolizumab among the investigated systemic therapies.

To investigate the effect of increased treatment options on survival, we identified patients who received approved first-line systemic therapies and grouped the patients according to time of treatment initiation. Since the manufacturer withdrew regorafenib from the German market before its approval for treatment of HCC, we defined the cut-off date of the two groups as the approval of lenvatinib: Period A (01/01/2015 – 31/07/2018), period B (01/08/2018 – 31/12/2020). Period A was subdivided into period A1 (01/01/2015 – 31/12/2016) and period A2 (01/01/2017 – 31/07/2018), to investigate wheter a gradual improvement in survival could be seen before introduction of new therapeutic options. To examine the impact of first-line choice on survival in this patient cohort, we also compared patients who received sorafenib or lenvatinib in the first-line. The date of the first systemic therapy prescription was defined as the index date. All study subjects were followed until death, end of the study period, or disenrollment from SHI.

2.3 Endpoints and covariates

Overall survival (OS), defined as the time from treatment initiation to death from any cause, was assessed and compared for each group. The cut-off date for follow-up was the end date of each study period. To investigate the impact of underlying HCC etiology, we categorized patients based on their background liver disease, including chronic hepatitis B and hepatitis C, alcoholic and nonalcoholic fatty liver disease (NAFLD) (ICD-10-GM codes in supplementary table 2). Furthermore, we assessed other patients' comorbidities and calculated the Charlson comorbidity index (CCI) and the Elixhauser comorbidity index using the respective ICD-10-GM codes (supplementary table 3, 4), as described before (27, 28). Other outcomes of interest included health care costs (without inflation adjustement), as measured by mean annual costs and mean annual costs per patient for inpatient and outpatient health care, prescription drugs, and aids and remedies.

2.4 Statistics

Demographics and clinical information were compared using one-way ANOVA or t-test for continuous variables and the chi-square or Fishers exact test for frequency data, as indicated. The Kaplan-Meier method was used to estimate median survival and 95% confidence intervals (CI). Kaplan-Meier curves were compared using the log-rank test. Independent predictors of survival were investigated using Cox's proportional hazards regression. Uni- and multivariate regression models were used to estimate the hazard ratios for variates of interest. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute GmbH, Heidelberg, Germany), R Software, Version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria), GraphPad Prism 9 Software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Cohort characteristics

We identified a total of 2876 individuals with a diagnosis of HCC in the study period between 2015 and 2020 in the database (supplementary fig. S1). 2317 cases were new, which is in line with previous reports (29, 30). The mean age was 69.3 (standard deviation (SD) ± 11.0) at the start of the observation period and increased to 71.1 (SD ± 10.6) by its end (p=0.0064) (table 1). As expected, the female proportion of patients was lower with around 26% and remained unchanged throughout the study (p=0.96). Alcoholic liver cirrhosis was the leading HCC etiology in the cohort, followed by nonalcoholic fatty liver (NAFL) plus nonalcoholic steatohepatitis (NASH), and chronic viral hepatitis. We observed a statistically significant decline in chronic hepatitis C virus infection (p=0.0015), as described before (31), and a nonsignificant trend for an increase in NAFL (p=0.2311). Around 70% of individuals suffered from mild liver disease, defined as liver cirrhosis without portal hypertension (PHT) or chronic hepatitis by the respective ICD-10-GM codes (supplementary table 2), while around 30% had moderate (cirrhosis and PHT) or severe (cirrhosis, PHT, history of variceal bleed) liver disease. The proportion of patients with mild or moderate to severe liver disease did not change over time (table 1). The most common comorbidities among HCC patients included cardiovascular (e.g., hypertension, cardiac arrhythmias), metabolic (diabetes, obesity), renal (renal insufficiency, disturbances of electrolytes and fluids), and psychiatric disorders (alcohol abuse, depression). No significant changes in the spectrum of comorbidities were observed during the study period, except for a slight increase in patients with depression (p=0.03) (supplementary fig. 2).

The proportion of HCC patients with metastatic disease was around one-third and showed no significant changes (p=0.5293) (table 1). The proportion of patients receiving systemic therapies increased significantly over time from 11.8% (n=101) in 2015 to 15.1% (n=147) in 2020 (p=0.005) (table 1). Following the respective approvals of the MTTs in Europe, a significant increase in their prescriptions was observed (Fig. 1). Sorafenib remained the most commonly prescribed MTT during the study period (n=417), followed by lenvatinib (n=110), cabozantinib (n=46), and ramucirumab (n=36). The checkpoint inhibitors nivolumab and pembrolizumab were approved in the United Status during the study period and prescribed to only a small number of patients in our cohort (nivolumab n=21, pembrolizumab n=20) (supplementary table 5).

3.2 Association between survival and higher availability of MTT

In the next step, we aimed to investigate if the increased use of different MTTs was associated with improved survival of patients with HCC in a nationwide cohort representative of the German population. We identified 460 patients with HCC that started on first-line systemic therapy in the study period (supplementary fig. S1). These patients were grouped according to the time of treatment initiation, defining the approval of lenvatinib as the cutoff date of the two subgroups. We chose the approval date of lenvatinib as the cutoff for the two subgroups because (1) no prescriptions could be identified in our database for the previously approved regorafenib and (2) beginning in late 2018, the range of HCC drugs prescribed broadened significantly beyond sorafenib only (Fig. 1). The

following time periods were defined accordingly: period A (01/2015 - 07/2018, n=255), period B (08/2018 - 12/2020, n=205). Baseline characteristics among patients in both periods were similar with respect to age, gender, liver disease, and proportion of patients with metastatic HCC (Fig. 2A). The proportion of patients with chronic hepatitis B virus infection was significantly lower in period B (p=0.0271). Median overall survival (mOS) in the whole cohort was 5.5 months (95% CI: 4.8 – 6.9). In period B, a superior mOS of 6.5 months (95% CI: 4.9 – 8.9) was identified as compared to period A with an mOS of 5.3 months (95% CI: 4.5 - 6.3) (p=0.046) (Fig. 2B). We performed bi- and multivariate Cox's regression analysis to adjust for confounders and identify prognostic factors for patients treated with first-line systemic therapy in both periods. In bivariate analysis, survival was significantly correlated with patients' Charlson comorbidity index (hazard ratio (HR) 1.08, 95% CI 1.04 – 1.13, p=0.0003) and Elixhauser comorbidity score (HR 1.04, 95% CI 1.001 – 1.08, p=0.0259) (supplementary figure S3). Patients with chronic viral hepatitis had a prolongued survival (HR 0.70, 95% CI 0.50 – 0.98, p=0.0355). Multivariate Cox's regression analysis revealed that treatment initiation in period B was an independent, statistically significant factor for a longer survival (period B vs. period A, HR 0.77, 95% CI: 0.61 – 0.96, p=0.0196) (Fig. 3). Furthermore, a higher Charlson comorbidity index persisted in the multivariate analysis as an independent negative prognostic marker (HR 1.07, 95% CI 1.02 – 1.12, p=0.0028). Patients with viral HCC etiology had longer survival (HR 0.73, 95% CI 0.53 – 0.99, p=0.0436). Survival was independent of age (HR 1.00, 95% CI 0.99 – 1.02, p=0.6227), gender (HR 1.15, 95% CI 0.87 – 1.52, p=0.3416), and non-viral etiology (HR 1.29, 95% CI 0.95 – 1.74, p=0.1033).

3.3 Subgroup analyses

By comparing the two observation periods A and B, we revealed an association between a higher availability of systemic therapies and survival in patients with HCC. Next, we aimed to investigate whether the improved survival could also be seen in previous periods independent of higher drug availability. We divided period A into period A1 (01/2015 – 12/2016) and A2 (01/2017 – 07/2018). In this cohort, 255 patients with HCC started first-line systemic therapy, 145 patients in period A1, and 110 in period A2. Baseline characteristics were equally distributed (Fig. 4A). Median overall survival (mOS) in the whole cohort was 4.9 months (95% CI: 4.3 – 6.2). Median survival for period A2 of 5.3 months (95% CI: 4.4 – 7.3) was not statistically different from period A1 (4.5 months, 95% CI: 4.4 – 7.3, p=0.707) (Fig. 4B).

Lastly, we compared the outcomes of patients treated with sorafenib or lenvatinib as first-line therapy after the approval date of lenvatinib (08.2018 – 12.2020). 102 patients were treated with sorafenib and 103 with lenvatinib in the observation period. There were no statistically significant differences in baseline characteristics between both groups (Fig. 5A). Lenvatinib showed a longer overall survival as compared to sorafenib (Fig. 5B). Median OS with lenvatinib was 9.7 months (95% CI: 6.3 - 18.4) versus 4.8 months with sorafenib (95% CI: 4.0 – 7.1, p=0.008). Multivariate Cox's regression analysis confirmed that treatment with sorafenib was an independent, statistically significant factor for a shorter survival

(sorafenib vs. lenvatinib, HR 1.63, 95% CI: 1.15 – 2.31, p=0.001) (Fig. 6). OS was independent of age (HR 1.00, 95% CI 0.98– 1.02, p=0.7924), gender (HR 1.14, 95% CI 0.75 – 1.74, p=0.5442), and HCC etiology (viral: HR 0.92, 95% CI 0.54 – 1.57, p=0.7554; non-viral: HR 1.59, 95% CI 0.97 – 2.60, p=0.0661).

3.3 Costs

In the previous sections, we showed that the approval of new systemic therapies increased their use in clinical practice and was associated with a statistically significant prolongation of survival in patients with HCC. Cancer care represents a substantial economic burden within healthcare systems, and new treatment modalities can raise healthcare expenditures (32). We aimed to analyze the costs caused by the treatment of the HCC patients in our cohort from an insurance perspective. Cumulative costs for HCC cancer care amounted to €113.6 million in the observation period (supplementary table 6) with a mean of €18.9 million per year. Inpatient care accounted for 62.1% of total costs per patient, prescriptions drugs for 28.5%, outpatient care for 8.5%, and aids and remedies for 3.7% (supplementary fig. S4). Mean total costs per patient were €21,681 at the beginning of the observation period in 2015 and increased significantly to €24,533 by its end in 2020 (p=0.0002) (Fig. 7). We observed a statistically significant increase in mean costs per patient for prescriptions drugs (p<0.0001), outpatient care (p=0.0240), and aids and remedies (p<0.0001), while mean costs per patient for inpatient care remained stable (p=0.0748) (supplementary table 7). Because the COVID-19 pandemic may have impacted cost trends for patients with HCC, we repeated the analysis for the 2014 - 2019 period before the pandemic. A statistically significant increase in mean costs per patient (p=0.0175) and costs for prescription drugs (p=0.01) could be observed (supplementary table 8, supplementary fig. S5). In general, mean costs per patient were characterized by high standard deviations reflecting the significant variations in resource usage among patients with HCC.

4. Discussion

This study based on a representative sample of 2876 patients with HCC demonstrates a statistically significant improvement in survival in a nationwide cohort in 2015 to 2020, coinciding with an increased approval of systemic treatment options. At the same time, we could observe a moderate increase in costs for prescription drugs and outpatient care per patient. Some population-based studies by our and other groups have described trends in HCC mortality over the years but with older time frames not encompassing the new systemic therapies (3, 33, 34). In contrast to these studies, our work analyzes outcomes and health care costs in the era of novel treatment options.

In this work, we divided a large cohort of patients with HCC based on the date of first systemic therapy prescription into (1) patients treated in a period with multiple available molecular targeted therapies (MTT) (08/2018 – 2020) and (2) patients treated in a period with only sorafenib available (2015 – 07/2018). By comparing the survival of both cohorts, we demonstrated that higher availability of MTTs was associated with longer patient survival. The prolongation of survival observed is unlikely to be attributable to a lead-time

bias, as the proportion of patients with metastatic disease was unchanged over time. In addition, we consider it unlikely that this result was due to a general improvement in managing patients with HCC since only one version of the German HCC guideline was valid during our 6-year study period (25). Nevertheless, we divided the first observation period with only sorafenib available into two subgroups (A1: 2015 – 2016, A2: 2017 – 07/2018) to assess a gradual improvement of HCC care. We did not observe any changes in patient survival within these two subgroups. Therefore, we conclude that the improvement in survival is likely due to more systemic therapy options.

The trend towards improvements in the survival of patients with advanced HCC could already be observed based on large phase III trials in which sorafenib was used as a comparator. In the pivotal SHARP trial, median survival with sorafenib was months (7). The REFLECT study published ten years later reported a median survival of 12.4 months for sorafenib (11). Recently, the IMbrave150 and HIMALAYA trials described a survival of 13.4 and 13.8 months with sorafenib, respectively (35, 36). In addition to these large randomized, prospective trials, several retrospective single- or multicenter studies with varying numbers of patients have also analyzed the outcomes of patients with advanced HCC receiving MTT. Kobayashi et al. recently underlined the importance of an expanding armamentarium of MTT in HCC patients from three Japanese institutions, reporting increases in the proportion of patients receiving MTTs and improvements in survival from 10.4 months to 15.2 months (17). Leyh et al. (37), Kuo et al. (38), and Lee et al. (39) confirmed the real-world efficacy of lenvatinib and sorafenib in the era of multiple treatment options for HCC systemic therapy in smaller cohorts using single- or multicenter data. Overall survival in these studies ranged from 8.4 months to 13.5 months. In contrast to these prospective and retrospective studies, our nationwide analysis is not biased by (1) strict eligibility criteria within randomized clinical trials and (2) recruitment of patients from a limited number of selected study sites.

The population-level benefit detected in our study lagged far behind benefits seen in the randomized trials or retrospective single-/multicenter studies cited before. Median survival was 6.5 months in the era of multiple MTTs, which was only around one month longer than in the period before. Despite the statistically significant improvement in survival, the magnitude of the effect is limited, which may also be explained by older patients with more comorbidities compared to the pivotal trials. There is an urgent need to improve the antitumor efficacy of HCC systemic therapies. Around two years ago, atezolizumab and bevacizumab became the first approved immunotherapeutic regimen for first-line therapy of patients with unresectable HCC (16, 24). Atezolizumab and bevacizumab markedly enhanced survival compared to sorafenib, with a median OS of 19.2 months versus 13.4 months (35). Double checkpoint blockade with durvalumab and tremelimumab recently emerged as another effective treatment option for first-line treatment of patients with HCC (36). We expect that the advent of immunotherapy will further improve the outcomes of patients with HCC at the population level. Up-to-date HCC guidelines will be crucial in ensuring the rapid translation of new treatments into clinical practice. Similar to previous reports (40-43), the proportion of patients with metastatic disease among incident cases

was around one-third (data not shown), which is in line with a previous population-based study from our group (3). This highlights the need to increase efforts in HCC surveillance implementation by abdominal ultrasound to detect cancers earlier, where curative therapy can be achieved.

(16, 36)

Choice of first-line regimen and survival

Lenvatinib has become an alternative to sorafenib based on a non-inferiority trial (11). Lenvatinib performed better in the secondary endpoints progression-free survival and response rate. These findings might affect treatment choice in individual patients. A fierce debate is on which agent may perform best under real-life conditions. We compared how the choice of first-line agent affected survival, specifically first-line lenvatininb versus sorafenib. A controversial finding of this study constitutes the statistically significant increased survival with lenvatinib compared to sorafenib. Real-world studies and meta-analyses that compared lenvatinib and sorafenib report conflicting survival data, with some favoring lenvatinib (44, 45) and others not (37, 46, 47). Several factors limit this particular analysis in our work. First, the sample size for this subgroup analysis is comparatively small, which reduces statistical power. Second, due to the lack of information on laboratory liver function tests and presence of macrovascular invasion, we cannot account for differences in patient's baseline characteristics, which might have influenced treatment allocation.

Limitations

This study has several limitations, mainly from its retrospective nature using a claims database. First, laboratory values to determine liver function scores, such as Child-Pugh Score or Albumin-Bilirubin Grade, were not available within the database. These variables could represent a bias in patient selection for a specific treatment option. However, we could describe liver function in our cohort to a certain extent by categorizing our patients into mild, moderate, or severe liver disease based on the respective ICD-10-GM codes. Furthermore, we could account for the influence of comorbidities on survival by including the established Charlson comorbidity index and Elixhauser score in Cox regression analysis (27, 28). Second, imaging data to determine Barcelona Clinic Liver Cancer (BCLC) classification and to assess treatment response were missing (48, 49). Nevertheless, we could describe the percentage of patients with metastatic disease using the respective ICD-10-GM code. In addition, response rates with MTT are low, and it is unclear if response can be used as a surrogate parameter for survival in this setting (50). Third, information on MTT dosage was unavailable, and it is unclear to what extent patients took the prescribed

drugs. However, this is unlikely to have influenced the results of this study since the treatment protocols used in clinical practice are based on the respective pivotal trials and approvals, which remained unchanged during the study period. Fourth, secondary outcomes in this work, such as the proportion of patients with certain comorbidities, could be limited by differences in coding quality, leading to underreporting of particular conditions.

In compensation for the limitations described above, our work provides insights from a nationwide, population-based database with a high degree of national coverage. The database represents the German population, in contrast to other highly selective cohorts, such as prospective, randomized trials, or single-/multicenter cohorts from high-volume tertiary institutions. Furthermore, available cost data allow important insights into the interplay of changing treatments, outcomes, and healthcare costs. Finally, since this study includes data until the end of 2020, it gives an up-to-date picture of clinical practice in German HCC care.

5. Conclusions

Our study reveals that higher availability of systemic therapies is associated with a statistically significant longer survival in patients with HCC in a large, nationwide cohort. The magnitude of the effect is limited and accompanied by a moderate increase in treatment costs. Novel checkpoint inhibitor-based regimens and better implementation of HCC surveillance may help to improve the poor diagnosis of patients with HCC in the future.

Figure legends

Table 1. Patients' characteristics by year. One-way ANOVA was used to examine continuous variables and chi-square test for frequency data. $P < 0.05$ was considered statistically significant. Mild, moderat and severe liver disease is defined by the respective ICD-10-GM codes (see supplementary table 3). aNumber of patients with at least one prescription of any of the following medications: sorafenib, lenvatinib, regorafenib, cabozantinib, ramucirumab, nivolumab, pembrolizumab.

Fig. 1. HCC systemic therapy prescriptions by year. The European Medicines Agency approval dates are displayed on the timeline by the respective drug names in boxes. The bar chart below shows the number of patients with at least one prescription for each drug in the respective year. The colors correspond to the colors of the drugs in the timeline. Chi-square test was used to examine frequency data. $P < 0.05$ was considered statistically significant. Due to data protection laws, no exact numbers can be provided for patient numbers below five, and the respective items were excluded from calculations. Abbreviations: na, not applicable.

Fig. 2. Overall survival of patients with HCC starting first-line systemic therapy in period A (2015 – 07/2018) versus period B (08/2018 – 2020). (A) Baseline characteristics. (B) Kaplan-Meier analysis of overall survival. aDue to data protection laws, no exact numbers can be provided for patient numbers below five and the respective items were excluded

from calculations. Abbreviations: CI, confidence interval; HR, hazard ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; mOS, median overall survival; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; No., number; ns, not significant; SD, standard deviation.

Fig. 3. Multivariate analysis of survival of period B versus period A in months. Abbreviations: CI, confidence interval; HR, hazard ratio; ns, not significant.

Fig. 4. Overall survival of patients with HCC starting first line systemic therapy in period A1 (2015 – 2016) versus period A2 (2017 – 07/2018). (A) Baseline characteristics. (B) Kaplan-Meier analysis of overall survival. aDue to data protection laws, no exact numbers can be provided for patient numbers below five and the respective items were excluded from calculations. Abbreviations: CI, confidence interval; HR, hazard ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; mOS, median overall survival; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; No., number; ns, not significant; SD, standard deviation.

Fig. 5. Overall survival of patients with HCC starting first line systemic therapy with sorafenib or lenvatinib after approval of lenvatinib (08/2018 - 2020). (A) Baseline characteristics. (B) Kaplan-Meier analysis of overall survival. aDue to data protection laws, no exact numbers can be provided for patient numbers below five and the respective items were excluded from calculations. Abbreviations: CI, confidence interval; HR, hazard ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; mOS, median overall survival; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; No., number; ns, not significant; SD, standard deviation.

Fig. 6. Multivariate analysis of survival of sorafenib versus lenvatinib in months. Abbreviations: CI, confidence interval; HR, hazard ratio; ns, not significant.

Fig. 7. Mean health care costs per patient per year in the study period 2015 - 2020. Data are presented as costs per patient per year in Euro.

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Concomitant Irradiation to Checkpoint Inhibitor Therapy of Hepatocellular Carcinoma Patients: A Systematic Retrospective, Single-Center Analysis

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Keywords

Immunotherapy · Hepatocellular carcinoma · Checkpoint inhibitor · Radiotherapy · Locoregional therapy

Abstract

Introduction: Immunotherapy has been established as the standard treatment option for patients with advanced hepatocellular carcinoma (aHCC). Despite the increased efficacy, disease progression occurs in a relevant proportion of patients even after an objective response. Combination concepts with locoregional therapy are currently under investigation for hepatic disease but are also in discussion for the control of distant metastasis. Radiotherapy is a highly effective treatment modality for local tumor control. It is also thought to increase the efficacy of checkpoint inhibition and sensitize distant lesions to the effects of immunotherapy, but may potentially increase adverse effects. In our center, few patients with aHCC treated with immune checkpoint inhibitors (ICIs) received concomitant radiotherapy for symptom or disease control. The aim of this study was to retrospectively analyze adverse effects and efficacy of concomitant radiotherapy in patients with aHCC treated with checkpoint inhibition. ***Methods:*** To this aim, patients who received a combination of ICI and radiotherapy in our institution were retrospectively considered for analysis. The predefined inclusion criterion was radiotherapy after initiated checkpoint inhibition and continuation of ICI therapy for at least 8 weeks. Adverse effects and efficacy measurements were performed according to local standards. ***Results:*** The database search of 2016–2021 revealed six consecutive patients fulfilling the predefined criteria for concomitant ICI and radiotherapy. Three patients received

The study was conducted at the gastrointestinal cancer unit of the University Hospital LMU Munich, Germany.

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high-dose-rate brachytherapy (15 Gy) to treat progredient hepatic lesions. Two patients received stereotactic body radiotherapy (SBRT) (25–30 Gy) for symptom control, and 1 patient received brachytherapy and SBRT to treat metastases. No severe adverse events were reported in the period (<6 months) after concomitant radiotherapy. In 5 out of 6 cases, long-term tumor control could be achieved by this therapeutic combination. ***Conclusion:*** A good efficacy of concomitant radiotherapy and checkpoint inhibition has been achieved with no safety concerns. Further investigations should evaluate the safety, appropriate clinical context, and efficacy of this promising approach.

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Introduction

The treatment landscape for hepatocellular carcinoma (HCC) is observing an enormous paradigm shift toward immune checkpoint inhibitor (ICI) treatments, which have emerged as a promising therapeutic option [1]. ICI, in combination with anti-VEGF antibody therapy, became standard of care in the first-line treatment of advanced hepatocellular carcinoma (aHCC) [2]. In the case of a systemic progress, escalation strategies are currently developed by combining, for example, ICI with tyrosine kinase inhibitors in a second-line setting [3]. In the case of progressive disease limited to the liver, an additive locoregional approach might be feasible and is currently tested in the DEMAND trial [4]. However, in case of limited progression of extrahepatic disease, new multimodal treatment concepts are urgently needed [5]. Radiotherapy (RT) may increase efficacy of checkpoint inhibition through additional immune sensitization, as shown in preclinical models [6] and clinical studies of malignant melanoma [7, 8]. With high-dose-rate brachytherapy (HDRBT) and stereotactic body radiation therapy (SBRT), two effective radiotherapeutic modalities are available for the treatment of HCC.

HDRBT is used for minimally invasive tumor ablation, to achieve local tumor control and support systemic therapies. It can also be used as a bridging therapy prior to liver transplantation [9]. HDRBT enables the administration of high-dose radiation to the tumor region while maintaining a low radiation dose to the remaining healthy hepatic tissue. It has also enabled us to overcome the limitations associated with thermal ablation techniques. Such limitations included tumor size, proximity to adjacent thermosensitive structures, and vascular cooling effects [10]. Besides local tumor ablation, using the destructive alpha radiation-based brachytherapy induces a lasting local immune response and a systemic anti-tumor response [11]. When used with immunomodulators, this anti-tumor response is further potentiated [12].

External beam RT is a well-characterized therapeutic modality for HCC and remains an essential treatment option, especially in patients with hepatic lesions not amenable to locoregional treatment due to size, anatomical reasons, or other accompanying circumstances such as ascites or an impaired coagulation status [13]. In the past, irradiation of hepatic lesions could lead to hepatic decompensation in up to 5% of the cases [14, 15] because radiation has the potential to harm surrounding healthy liver parenchyma. Therefore, its utilization as a treatment option has historically been limited [16, 17]. However, new technological improvements such as intensity-modulated and image-guided SBRT allow administering the dose safely in carefully selected patients, thereby protecting the proper functioning of liver parenchyma [18, 19]. Previous studies have supported the utilization of SBRT as a therapeutic approach for treating liver cancer with the aim of achieving a favorable toxicity profile [20, 21]. Moreover, SBRT can be used in combination with locoregionary therapies as a bridging therapy before transplantation to efficiently control the spread of the tumor in pre-transplant conditions [9, 22].

RT may increase efficacy of checkpoint inhibition through additional immune sensitization, as shown in preclinical models [6] and clinical studies of malignant melanoma [7, 8]. The combination enhances the expression of MHC-I and CD8 cells ultimately leading to the major improvements in the cytotoxic activity and overall efficacy of ICI [23]. RT may be an effective additive immune sensitizing therapy, but in the context of checkpoint inhibition, it is quite plausible that radiation may trigger immunogenic off-target effects [24, 25]. Only few data are currently available, characterizing tolerance and efficacy of concomitant RT in ICI-treated HCC patients. To this aim, we systematically evaluated HCC cases in our center that received RT in the context of checkpoint inhibition.

Methods

Study Design

A retrospective analysis was performed based on clinical data obtained from our gastrointestinal cancer unit at the University Hospital LMU Munich, Germany. All consecutive patients between 2016 and 2021 with the diagnosis C22.0 were retrospectively screened for receiving RT at our outpatient clinic at the University Medical Center Großhadern LMU. The only inclusion criterion was that RT was begun after initiated checkpoint inhibition and continuation of ICI therapy for at least 2 months after RT, including efficacy measurements. Due to the low number, the individual patient cases are presented each as separate case vignettes. Data collection and retrospective analysis of patient information were anonymized in accordance with the Declaration of Helsinki. Ethics approval from the Local Institutional Review Board was obtained (18–604).

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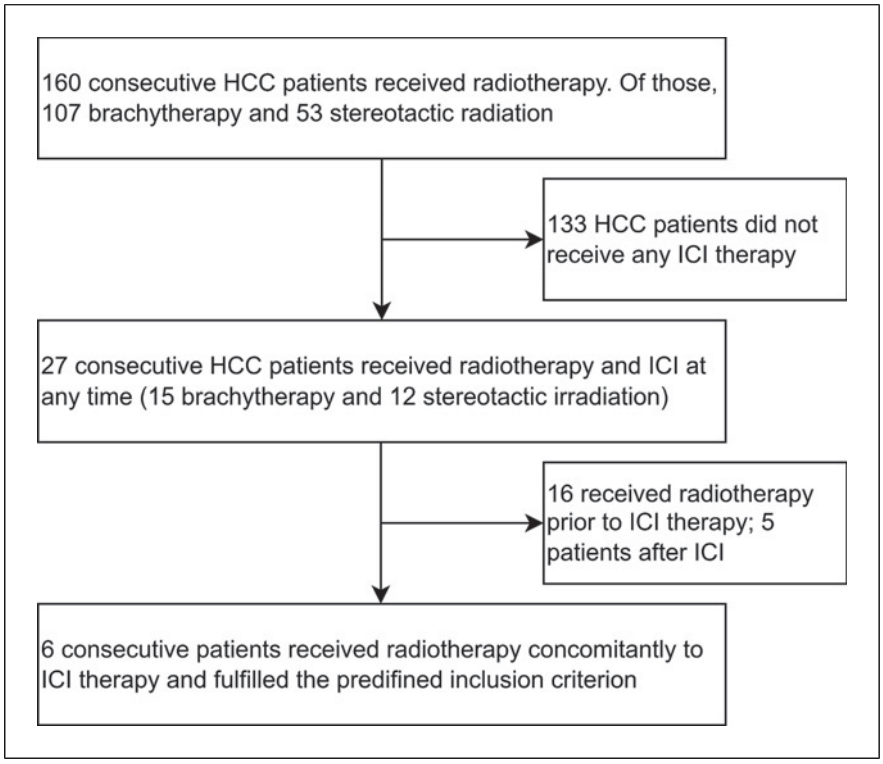

Fig. 1. Flowchart depicting results of the database search.

Treatment Schedule

One patient with HCC received nivolumab and ipilimumab according to the established protocol (1 mg/kg nivolumab followed by ipilimumab 3 mg/kg on the same day every 3 weeks for 4 doses, followed by 240 mg nivolumab monotherapy maintenance 2-weekly) [26]. Three patients received full-dose (240 mg) nivolumab monotherapy 2-weekly [27]. Another patient received pembrolizumab in combination with local treatment as part of a study treatment [28]. One patient received durvalumab and tremelimumab treatments (single dose treme 300 mg, durva 1,500 mg every 4 weeks) as part of a study treatment.

Regarding external beam RT, different techniques were applied. For bone metastases, a 3D conformal technique was used, while for other metastases SBRT techniques were used. Therefore, various fractionation schemes were used (10 × 3 Gy, 5 × 4 Gy, 5 × 5 Gy).

The brachytherapy procedure was conducted under conscious sedation under sterile conditions. Under CT fluoroscopy, the HCC was punctured with a 17G needle which was exchanged over a stiff wire to a 6-French sheath that can hold a brachytherapy catheter. The number of catheters was dependent on size and number of target lesions. A tumor surrounding prescription dose of 15 Gy was aimed at as a single fraction using high-dose brachytherapy with an iridium-192 afterloading unit. Organ at risk constraints were as follows: bowel/colon and stomach D1 ccm: 12 Gy, D0.1 ccm: 15 Gy; esophagus D1 ccm: 12 Gy, D0.01 ccm: 15 Gy; spinal cord D0.01 ccm: 10 Gy; skin D0.01 ccm: 10 Gy. One-third of the uninvolved liver was irradiated with less than 5 Gy [9].

Assessments

Routine efficacy assessments were conducted by computed tomography of the chest, abdomen, and pelvis or magnetic resonance imaging of the abdomen in every 3 months. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 and modified RECIST for hepatocellular cancer (mRECIST) retrospectively by two experienced hepatobiliary radiologists.

Results

Database Search

A database search showed a total of 27 consecutive HCC patients receiving RT and checkpoint inhibition at any time. Of those, only six patients fulfilled the predefined inclusion criterion with concomitant RT. Another 16 patients received ICI before and 5 patients after RT (Fig. 1).

Patient characteristics of the six patients receiving concomitant RT and checkpoint inhibition are depicted in Table 1. The age range was from 42 to 80 years old.

Case Vignettes

Patient 1 was diagnosed with poorly differentiated HCC (verified by histology) and renal carcinoma with suspected lymph node metastasis of the renal cell carcinoma (RCC). Treatment with nivolumab and ipilimumab for metastasized RCC was initiated (Fig. 2a). One of two HCC lesions responded to the initiated treatment with shrinkage, while the second lesion showed isolated tumor growth. The progressive lesion was treated with brachytherapy. Afterward, the HCC and the lymph node metastasis showed sustained

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Table 1. Patient characteristics with the corresponding checkpoint inhibitor, radiotherapeutic modality, coincidental adverse effects, and treatment outcomes

Pat. No.	BCLC	Cirrhosis	ICI therapy	RT modality	RT target	Number of fractions × single dose	Indication	Coincidental adverse effect	Best response in-field	Best overall response	Time to best response after ICI	Time to best response after RTX	Duration of the best response
1	C	Child A	Nivolumab and ipilimumab	Brachytherapy	Seg VIII and VII	1 × 15 Gy	Local tumor control	No AE	CR	CR	12	3	15
2	C	Child A	Nivolumab	SBRT	Lymph node metastases	5 × 5 Gy (80% isodose)	Local tumor control	No AE	CR	CR	35	8	12
				Brachytherapy	Seg III and V	1 × 15 Gy	Local tumor control	No AE	CR				
				SBRT	Cerebral metastasis	5 × 5 Gy	Local tumor control		CR				
3	B	Child A	Pembrolizumab (Immulab)	Brachytherapy	Seg VI/VIII	1 × 15 Gy	Local tumor control	No AE	PR	PR	3	2	11
4	C	No	Durvalumab and tremelimumab (Himalaya)	3D conformal EBRT	Bone metastasis T11-L1	10 × 3 Gy	Palliative	Hypothyreosis	SD	SD	4	3	14
				3D conformal EBRT	Bone metastasis B6-B10	10 × 3 Gy	Palliative		SD				
5	B	Child A	Nivolumab	Brachytherapy	3 lesions, seg IVb/a and V	1 × 15 Gy	Local tumor control	Nummulary eczema	CR	CR	37	3	20
				Brachytherapy	2 lesions, seg VI/VII	1 × 15 Gy	Local tumor control	Xerosis cutis	CR				
				Brachytherapy	1 lesion, seg V/VIII	1 × 15 Gy	Local tumor control; tumor-control		CR				
6	C	Child B	Nivolumab	3D conformal EBRT	T12-L1, popliteal fossa right, tibia li, B6-8, pelvis/right os ischii	10 × 3 Gy	Palliative	No AE	SD	PD	?	?	?

SBRT, stereotactic body radiation therapy; EBRT, external beam radiation therapy; PD, progressive disease; PR, partial response; CR, complete response.

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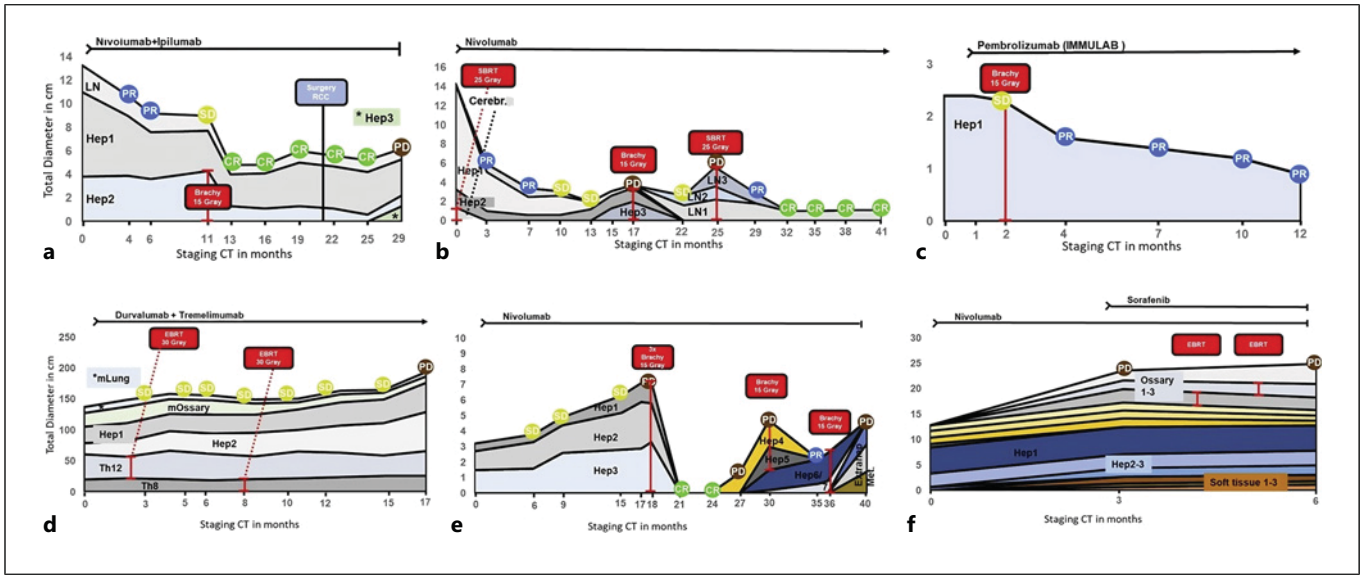

Fig. 2. a–f Schematic representation of the 6 patient courses. The longest tumor axis was used for diameter measurements. Hep, hepatic metastasis; LN, lymph node metastasis; SBRT, stereotactic intensity-modulated radiotherapy; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

complete remission. Due to good tumor control and sustained good performance status, nephrectomy of the RCC was performed (histologically confirmed RCC R0 resection), whereas histology of the lymph nodes showed HCC metastasis with a good regression status. Due to the good efficacy of ICI therapy, it was further continued. Eight months later, a new hepatic tumor nodule developed, and ICI therapy was discontinued.

Patient 2 was diagnosed with metastatic HCC in 2017 with cerebral metastasis. Upon initiation with sorafenib, he developed an intracerebral hemorrhage originating from a cerebral metastasis. The bleeding lesion was evacuated surgically. Treatment was switched to nivolumab, and the patient received postoperative SBRT of the surgical cavity (Fig. 2b). The hepatic tumor had an excellent response. Seventeen months later, two new hepatic nodules developed and local brachytherapy was administered, and reasonable local tumor control was achieved. Eight months later, progressive retroperitoneal lymph node metastasis was evident, which was treated by stereotactic irradiation. Sustained, complete response was achieved, and so far, no treatment-related adverse effects have been reported. The patient is still doing well after 41 months of treatment.

Patient 3 had a singular HCC lesion and received pembrolizumab as a study treatment additive to locoregional treatment (Fig. 2c). Brachytherapy was performed. The staging CT showed tumor devascularization indicating good local tumor control and no adverse effects were reported.

Patient 4 was diagnosed with HCC with bone, pulmonary, and hepatic metastases. He received durvalumab and tremelimumab (Fig. 2d). Due to symptomatic vertebral metastasis, he received palliative RT for symptom control. Other than hypothyreosis, no additional adverse effects were evident. However, he had an intrahepatic progress after 17 months, and the study medication was discontinued.

Patient 5 presented with intrahepatic disseminated HCC. Initial therapies consisted of selective internal RT, transarterial chemo embolization, and radiofrequency thermal ablation. A therapy with sorafenib was initiated upon intrahepatic progression and evidence of peritoneal carcinosis. Due to the progression of the hepatic lesion, the patient was included in a trial and entered the placebo arm. Due to hepatic progression, therapy with nivolumab was initiated (Fig. 2e). Progressive lesions were treated with brachytherapy and local control was achieved. Except for nummulary eczema and xerosis cutis, no adverse events occurred. After 40 months, extrahepatic metastasis was diagnosed, and nivolumab treatment was discontinued.

Patient 6 was diagnosed with HCC in 2012. He received orthotopic liver transplantation in February 2014 and had an intrahepatic HCC recurrence in January 2015. He received local ablative treatments. When extrahepatic disease (bone, lymph nodes) was evident, a treatment with nivolumab was started (Fig. 2f). Due to symptomatic vertebral (B12-L2 and C6-B3) metastases, a palliative RT treatment for symptom control was performed and sorafenib was added. As he progressed, systemic treatment with nivolumab was discontinued.

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Table 2. Ongoing clinical trials using checkpoint inhibitors with RT for HCC (www.ClinicalTrials.gov)

Clinical trial identification	Study start date	Phase	Type of radiative intervention	Type of CPI	Est. enrollment	Primary endpoint
NCT04167293	November 16, 2019	II/III	SBRT	Sintilimab	116	6-month PFS
NCT03482102	May 14, 2018	II	RT (not specified)	Durvalumab and tremelimumab	70	Best ORR
NCT03817736	March 1, 2019	II	TACE+ SBRT	Not specified	33	No. of patients eligible to curative surgical intervention
NCT03316872	February 15, 2018	II	SBRT	Pembrolizumab	30	ORR
NCT04611165	November 15, 2019	II	EBRT	Nivolumab	50	PFS
NCT04547452	July 1, 2020	II	SBRT	Sintilimab	84	PFS
NCT02608385	December 2015	I	SBRT	Pembrolizumab	130	Recommended SBRT dose
NCT04547452	July 1, 2020	II	SBRT	Sintilimab	84	PFS
NCT04193696	January 10, 2020	II	RT	Anti-PD-1 agent	39	ORR
NCT03817736 (START-FIT)	March 1, 2019	II	TACE + SBRT	Not specified	33	No. of patients eligible to curative surgical intervention
NCT05286320	March 28, 2022	Ib/II	SBRT	Lenvatinib/ pembrolizumab	27	Safety rate ORR PFS OS
NCT03753659 (IMMULAB)	May 9, 2019		RFA MWA Brachytherapy TACE	Pembrolizumab	30	ORR OS TTR Incidence and severity of adverse events

RT, radiotherapy; RE, radioembolization; SBRT, stereotactic body radiation therapy; PR, pathological response; TACE, transcatheter arterial chemoembolization; RR, response rate; ORR, overall response rate; MW, microwave ablation; MTD, maximum tolerated dose; TTR, time to response; PFS, progression-free survival; AEs, adverse events; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; EBRT, external beam radiotherapy; PDL-1, programmed cell death ligand 1.

Discussion

Current studies have demonstrated the role of RT in potentiating and modulating the tumor immunity. With emerging clinical evidence supporting the use of RT in combination with immunotherapy, there is a need to discuss and analyze their effects from the standpoint of toxicity and safety. Here, we present a systematic retrospective database analysis highlighting six consecutive cases of HCC patients receiving concomitant checkpoint inhibition and RT. Immunotherapy in combination with antiangiogenic therapy is considered the standard first-line treatment choice for treating HCC patients [29]. Locoregional therapies hold numerous potentials to act synergistically or in combination with immunotherapy to produce more efficient results in HCC patients. Although many clinical trials are currently investigating the synergistic combination of RT and ICI, few results have been published. A summary of ongoing clinical trials using this combination is outlined in Table 2.

To our knowledge, this is the first study describing the clinical courses of concomitant checkpoint inhibition and radiation therapy in patients with HCC. The combination therapy did not raise safety concerns, i.e., an increased rate of adverse effects of the respective irradiated tissue, and gave a good efficacy signal for patients with limited disease progression.

Brachytherapy irradiation has a favorable dose deposition with little radiation scattering and minor off-target parenchymal tissue damage. Nevertheless, a small area of irradiation of surrounding hepatic parenchyma occurs. This may provoke local autoimmune phenomena and ultimately induction of checkpoint hepatitis may be plausible. In HCC patients, the liver function is additionally limited due to frequently underlying cirrhosis or fibrosis; therefore, triggering autoimmune hepatitis in the context of liver disease may more frequently lead to worsening of liver function up to liver failure. In 4 patients receiving seven local RT treatments, no autoimmune hepatitis occurred, indicating only a limited immune sensitization due to RT of liver tissue in the context of checkpoint inhibition.

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Stereotactic irradiation or intensity-modulated RT of other tissues may similarly provoke autoimmune processes. Few studies have investigated the adverse effects of combination concepts of irradiation and checkpoint inhibition. Welsh et al.'s [7] prospective clinical trial focused on non-melanoma carcinoma patients receiving a combination scheme of concurrent ipilimumab and stereotactic ablative RT with 50 or 60 Gy. In light of the various pretreated cancer entities, an acceptable overall disease control rate (26%) could be achieved. Interestingly, the study found that low-dose (5–10 Gy) irradiated lesions responded in 31% versus 5% of nonirradiated lesions. No grade 4–5 toxicities and only a few grade 3 toxicities were evident. The number of patients with liver diseases was not stated. However, only 1 HCC patient was included.

Various recent trials have also demonstrated the high safety and tolerance levels with little or no toxicity reactions in cancer patients that were treated with this multimodal combination. In a pooled analysis of patient data from prospective trials in the US Food and Drug Administration databases, Anscher et al. [30] found that patients with cancer receiving RT within 90 days prior to the start of ICI treatment did not have an increased risk for severe adverse events compared to those who did not receive RT. Similar results were also found in a multicenter safety analysis by Bang et al. [19] corroborating the safety of the combination. There were no significant differences in adverse events between those receiving RT during/after checkpoint inhibitors and before checkpoint inhibitors ($p = 0.053$), and between those receiving RT within 14 days or outside 14 days of checkpoint blockade ($p = 0.06$). Immune-related adverse events occurred in 39% of patients receiving RT with ICI, compared to 23% of patients who used RT only. All of these adverse events were mild, easily manageable, and were independent of the irradiated site. In a phase I trial recruiting 22 melanoma patients, Victor et al. reported no grade 4 toxicities when treated with ipilimumab in combination with RT (6 Gy × 2–3 or 8 Gy × 2–3 to one site). Grade 3 toxicities were reported in the form of anemia (4/22) and colitis (1/22) [6]. In another study, multiple regimens of RT (8.0–12.5 Gy, administered to 1–2 sites) and ipilimumab were used. There was only one case of grade 4 colitis, one grade 3 hypophysitis, skin rashes (4/22), and no other significant adverse results greater than grade 3 [31]. Tang et al. [32] also conducted a phase I trial in which doses of ipilimumab were administered in combination with SBRT (50 Gy in four fractions or 60 Gy in ten fractions) either sequentially or simultaneously. There were no grade 4 or 5 toxicities found in patients, while 12 patients out of 35 showed grade 3 toxicities.

The scarcity of severe toxicities in these studies mirrors our observations. In our study, 3 patients received in total five radiation treatments. Despite irradiation of abdominal masses, cerebral mass, or bone masses with off-target volumes in either abdominal organs or the lung, no severe colitis, pneumonitis, or other severe adverse effect was triggered. The use of dual ICI with brachytherapy also seems to be well-tolerated (patients 1 and 4).

The manifestation of brain metastasis in HCC patients generally causes severe complications and is associated with poor prognosis and a survival rate of less than 4 weeks. Our patient 2, which has been previously reported by Ye et al. [33] was a proof of concept of the efficacy of sorafenib and nivolumab in combination with RT in aHCC with brain metastasis. After treatment, a profound durable response in terms of shrinkage of metastatic lesions and a major decline in the number of alphafetoprotein was observed in the patient. The patient remained in remission for >5 years until today. Our results therefore favor the utilization of combination therapy for potentially controlling the presence of brain metastasis in aHCC.

An appropriate escalation concept should address the subset of patients with limited systemic progression upon checkpoint inhibition [25]. Our analysis showed that the 2 patients with extensive HCC tumor load (patients 4 and 6) did not show radiologic response after RT treatment, whereas the other 4 patients, who had only few progressive metastases, could be well addressed with local RT, and subsequently showed a good local control.

This analysis has several limitations. First, the numbers of patients are very limited. The HCC patients were heterogeneous, as they had disease limited to the liver or extrahepatic disease. In addition, different RT techniques were used. These data leads to the hypothesis that concomitant radiation therapy may be applied in HCC patients with oligoprogression and that this combination therapy should be evaluated in a prospective clinical study setting.

Further research is needed to validate the best-suited RT regimens and the optimal dose given in conjunction with ICI. It is also of utmost importance to evaluate late toxicity and immune-related responses. Further understanding of the patient factors associated with toxicities is needed for risk stratification, to minimize their effects, and to tailor personalized treatments.

Conclusion

The results of our case series indicate a good efficacy of concomitant RT and checkpoint inhibition with a favorable safety profile. Further investigations should evaluate the safety, the appropriate clinical context, and efficacy of this combination therapy.

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Statement of Ethics

Data collection and retrospective analysis of patient information were anonymized in accordance with the Declaration of Helsinki. This study protocol was reviewed and approved by the Ethics Committee of the Ludwig-Maximilians-University (LMU) Munich, Germany, approval number (18-604). As this was a retrospective study, the need for informed consent was waived by the Ethics Committee of the University Hospital LMU Munich.

Conflict of Interest Statement

M.S. has received travel support from Ipsen, EISAI, and Roche and research funding by Ipsen. D.R. had received advisory fees from Bayer, speaker fees from Ipsen, and travel support from Ipsen and Bayer. F.P.R. has received honoraria for lectures and travel support from the Falk Foundation, Gilead, Ipsen, and Novartis. A.T. received advisory fees and honoraria from Roche. A.T. received grants from the Sino-German Center for Research Promotion (Grant No. GZ-1546 and C-0012), the State Ministry of Baden-Wuerttemberg for Sciences, Research and Arts supporting the Clinical Cooperation Unit Healthy Metabolism at the Center for Preventive Medicine and Digital Health (grant identifier: CCU Healthy Metabolism), the Foundation for Biomedical Alcohol Research (grant identifier: N/A), and the Baden-Wuerttemberg center for digital early disease detection and prevention (grant identifier: BW-ZDFP). E.D.T. has served as a paid consultant for AstraZeneca, Bayer, BMS, EISAI, Eli Lilly & Co., MSD, Mallinckrodt, Omega, Pfizer, IPSEN, Terumo, and Roche. He has received reimbursement of meeting attendance fees and travel expenses from ArQule, AstraZeneca, BMS, Bayer, Celsion, and Roche, and lecture honoraria from BMS and Falk. He has received third-party funding for scientific research from ArQule, AstraZeneca, BMS, Bayer, Eli Lilly, IPSEN, and Roche. M.S., research grants: SIRTEX Medical and Bayer Healthcare; lecture honoraria: Bayer Healthcare, Cook, Balt, Boston Scientific, LIAM, and SIRTEX Medical; and advisory board: Bayer Healthcare, Siemens Healthineers, and AstraZeneca. S.C. has received research grants from Elekta, ViewRay, and Brainlab and speaker fees/travel support from Elekta, ViewRay, C-RAD, Roche, and Brainlab.

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Author Contributions

Conception and design of the study: Stefan Munker, Osman Öcal, Jens Ricke, Max Seidensticker, and Maximilian Niyazi. Acquisition, analysis, and interpretation of data: Daniel Rössler, Najib Ben-Khaled, Liangtao Ye, Ignazio Piseddu, and Jakob Vielhauer. Drafting of the manuscript: Kathrin Bernhart, Isaac Rodriguez, Florian P. Reiter, and Stefanie Corradini. Critical revision of the manuscript: Liangtao Ye, Ignazio Piseddu, Jakob Vielhauer, Jens Ricke, Andreas Teufel, Enrico N. De Toni, and Maximilian Niyazi. Final approval of the manuscript: Stefan Munker, Daniel Rössler, Osman Öcal, Jakob Vielhauer, Florian P. Reiter, Andreas Teufel, Enrico N. De Toni, Max Seidensticker, Maximilian Niyazi, and Stefanie Corradini. Guarantor of the study: Stefan Munker, Enrico N. De Toni, Max Seidensticker, and Maximilian Niyazi.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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ORIGINAL RESEARCH

Prognostic Significance of Elevated Platelet Count (>200 x 10^9 per L) in BCLC Stages B and C of Hepatocellular Carcinoma: A Retrospective Multicenter Analysis

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Introduction: In hepatocellular carcinoma (HCC) comorbidities related to decreased liver function or to portal hypertension often limit treatment options. Traditionally, low platelet count has been considered a negative prognostic factor in HCC, especially in early stages. However, recent evidence suggests that elevated platelet count may also predict worse outcomes in advanced stages, suggesting a stage-dependent prognostic impact.

Aim: This study evaluated the prognostic role of platelet counts across BCLC stages, adjusted for portal hypertension, to improve individualized patient management.

Methods: In this retrospective, multicenter study, platelet count of 1112 patients with HCC in different tumor stages was analyzed. Various platelet count cutoffs (X to Y × 10^9/L) were tested to identify the optimal prognostic threshold. To isolate the effect of platelet levels from portal hypertension, spleen diameter was incorporated as an adjustment variable in multivariate analyses, with variceal status considered when available (in about two thirds of patients). Using an optimized cut-off, survival analysis was performed using univariate and multivariate Cox proportional hazards models. Bootstrapping was performed for internal validation.

Results: Platelet count outside 84–200 × 10^9/L was associated with poorer survival (HR = 0.66, 95% CI = 0.57–0.78, p < 0.0001). Bootstrapping showed robustness of the final model. Subgroup analysis revealed worse survival in BCLC stages B and C but not stage A for elevated platelet counts (>200 × 10^9/L) in multivariate analysis (including spleen diameter).

Conclusion: Platelet counts showed a stage-dependent prognostic impact in HCC. A platelet count above a cutoff of 200/µL at diagnosis was associated with poorer prognosis. Using this cutoff may improve survival prediction in BCLC B and C patients with potential usage for risk stratification and guidance of treatment decisions. Further external validation is required to confirm these findings and evaluate their clinical applicability.

Keywords: hepatocellular carcinoma, HCC, platelets, survival, cutoff, biomarker, BCLC

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Introduction

Hepatocellular carcinoma (HCC) is a common and deadly cancer, frequently arising in patients with chronic liver disease or cirrhosis.[1,2] Treatment is often complicated by portal hypertension-related comorbidities, contributing to end-stage liver disease symptoms such as ascites, kidney failure, variceal bleeding, and thrombocytopenia.[3,4] Platelet count, a critical marker of significant portal hypertension, is effective in predicting postoperative outcomes in BCLC Stage A patients and guiding clinical decisions.[5,6] Despite increasing data on the relevance of platelet count across other BCLC stages its role has to be further refined.

Early studies have shown that in BCLC Stages B and C, the prognostic role of platelet counts exhibits an opposite trend compared to BCLC Stage A.[7,8] Recent evidence complementing these findings by Scheiner et al, showed in a large patient cohort that increased mean platelet volume and low platelet count correlate with improved survival in HCC patients.[9] Similarly, Huo et al reported that elevated platelet counts across all BCLC stages (0-D) are associated with advanced tumor characteristics and predict worse overall survival in HCC patients[10] and Chen et al demonstrated a U-shaped curve associating platelet count with survival in BCLC Stage B patients.[11] These studies highlight the complex role of platelet counts in HCC progression across different BCLC stages. However, the precise role of platelet counts remains unclear and a clinically relevant cutoff for BCLC B and C patients still needs to be established.

Non-invasive methods, such as CT-derived liver and spleen volumes, are increasingly being used for the evaluation of portal hypertension in HCC.[12] Whereas liver stiffness measurements may not reliably assess clinically significant portal hypertension in HCC patients.[13] These imaging approaches underscore the utility of certain Baveno criteria elements, particularly spleen diameter, in the non-invasive assessment of portal hypertension in HCC patients.

Delineating the relationship between platelet count, portal hypertension, and additional underlying factors is important for prognostic assessment, improvement of treatment strategies and new therapeutic approaches in HCC. Our study focuses on evaluating platelet count comprehensively across BCLC stage to validate previous findings and to find better clinically meaningful cutoffs for platelet count at diagnosis of HCC.

Methods

Study Design

Epidemiological data, survival, and recurrence rates were analyzed in a cohort of patients diagnosed with HCC between 2006 and 2022. Consecutive Patients were included with following criteria:[1] age ≥18 years, and[2] a radiological or histological diagnosis of HCC. The study included patients from the University Medical Center Munich, University Clinic Mannheim, and University Clinic Vienna. Data collection and retrospective analysis were conducted in compliance with the Declaration of Helsinki and approved by the local ethics committee (LMU Ethics Committee: 18–604). Patient consent was not required due to the study's retrospective design. To ensure data confidentiality, all patient information was anonymized throughout the study.

Background and Data Collection

We conducted a retrospective analysis of 1112 patients with HCC from three centers: Munich (n = 754), Vienna (n = 250), and Mannheim (n = 108). In total 632 deceased during the study period while 480 were censored. Diagnosis was made either histologically (postoperatively or by puncture) or by imaging and typical contrast enhancement on the basis of progressed liver disease. Most current lab values available at time or shortly before initial treatment (<3 months) were used for the analysis. Macrovascular invasion was diagnosed based on contrast-enhanced imaging (CT and/or MRI) following the standard diagnostic criteria for HCC. Baseline characteristics assessed included age, sex, height, weight, performance status (according to the Eastern Cooperative Oncology Group), presence of cirrhosis, Child-Pugh score, etiology of liver disease, presence of ascites, portal hypertension, spleen diameter, BCLC Stage and laboratory values. Data were extracted, recorded, and entered into a central database by trained personnel. Information on patient survival and disease recurrence was obtained from clinical reports, death certificates issued by local public health departments, and registration offices. Data processing and storage adhered to the Bavarian Law of Cancer Registries.

Exploratory Analysis of Platelet Range for HCC Patients

To find optimal cut-off values we tested the Log rank test with different cut-offs and chose the cut-offs with the lowest p-value as previously described.[14,15] Patients for which lab values were not available according to the requirement (<3 months prior to first treatment) were not considered for subsequent analysis. To determine the optimal platelet range for patients with hepatocellular carcinoma, we considered the lowest (11 × 10^9/L) and highest (1073 × 10^9/L) platelet counts in our cohort. We iteratively tested various platelet ranges from X to Y x 10^9/L including combinations such as 10–12, 12–14, 100–150, 400–700, until all possible range sizes were covered. Patients were categorized as either inside or outside each range. An exhaustive analysis was conducted by plotting survival curves for all platelet ranges and assessing the separation of these curves. For each range, we fitted a survival model, visualized survival curves, and used the Log rank test to evaluate differences in survival probabilities. The p-value from the Log rank test was computed for each range. The range associated with the best survival outcomes allowed the classification of patients into three groups: "normal range", "low platelet counts", and "high platelet counts", which differed from conventional platelet ranges.

Statistical Analysis

Continuous data were summarized using means, medians, minimum and maximum values, and standard deviations, while categorical data were presented as absolute frequencies and relative percentages. Comparisons of patient characteristics were made using analysis of variance (ANOVA) or the Kruskal–Wallis test for continuous data and Chi-square tests for categorical variables. Survival analysis was conducted from the date of diagnosis until death or the last patient contact. Follow-up and survival times were censored as of December 31, 2022. Univariable analysis involved fitting a series of Cox proportional hazards models to assess the relationship between individual variables and survival. Significant variables were identified, and a multivariable Cox regression model was developed to determine independent predictors of survival. Rows with missing values were excluded to ensure robust analysis. Stepwise forward selection was used to refine the model, starting from a null model and progressively adding variables. The final model summarized significant predictors of survival, with a significance level set at 0.05. All analyses were performed using RStudio 2023.09.1+494 "Desert Sunflower" release.

Results

In this retrospective cohort study, we analyzed a total of 1112 patients diagnosed with HCC from all BCLC stages across three European centers. Baseline characteristics are provided in Table 1.

To identify clinically relevant platelet cutoffs, platelet count prognostic potential was examined across BCLC stages. Based on previous observations, we hypothesized that two cutoffs better discriminate survival of HCC patients. To explore the prognostic potential of cutoff pairs, we iteratively tested cutoff pairs that stratified risk of patients in low, medium, and high platelet count groups. A cutoff pair dividing the patients into low (<84/nl), medium (84–200) and high platelet counts (>200/nl) showed the strongest predictive potential (Figure 1A). This cutoff pair showed a significantly better model fit compared to any single cutoff (spp. Table 1). Patients with platelet counts within 84 to 200 x 10^9/L at diagnosis were significantly associated with better survival, whereas platelet counts outside this range were linked to poorer survival (Figure 1B).

As shown in Table 2, low platelet counts were significantly associated with a higher prevalence of cirrhosis (96.1% vs 84.8% and 56.3%, $p < 0.001$), more advanced liver disease (reflected by higher Child-Pugh scores), as well as higher incidences of metastasis, macroscopic portal vein infiltration, ascites (50.2%), and portal hypertension (90.3%) ($p < 0.001$). On the other hand, platelet numbers above 200/nl were significantly associated with BCLC C stages.

According to the new platelet cutoffs we performed a multivariate Cox Regression analysis across BCLC stages and for each BCLC stage separately (Table 3 and Figure 2). Spleen diameter was included in the model to adjust for portal hypertension and to adjust for potential confounders. We further included macroscopic portal hypertension or metastasis. The multivariate analysis showed that in BCLC A patients lower platelet counts (<84/nl) were associated to poorer survival whereas in BCLC B and C patients primarily elevated platelet counts (>200/μL) were associated to poorer survival. Due to the incomplete assessment of varice status (available in 868 of the 1112 patients) it was not included in

Table 1 Baseline Characteristics of the HCC Patients Across BCLC Stages

Variable	BCLC A	BCLC B	BCLC C	BCLC D	p-value
n	**364**	**334**	**355**	**59**	
Age (mean (SD))	65.99 (10.33)	67.65 (10.18)	65.69 (10.74)	62.12 (8.65)	0.001
Female (%)	89 (24.5)	54 (16.2)	56 (15.8)	6 (10.2)	0.003
Height (mean (SD))	1.73 (0.09)	1.73 (0.07)	1.74 (0.09)	1.76 (0.07)	0.044
Weight (mean (SD))	79.21 (16.69)	81.71 (15.52)	80.21 (14.60)	85.36 (16.09)	0.028
BMI (mean (SD))	26.41 (4.50)	27.19 (4.50)	26.39 (4.10)	27.78 (4.44)	0.021
ECOG (%)					<0.001
0	271 (74.7)	186 (55.7)	160 (45.2)	26 (44.1)	
1	74 (20.4)	134 (40.1)	159 (44.9)	23 (39.0)	
2	16 (4.4)	14 (4.2)	33 (9.3)	9 (15.3)	
3	2 (0.6)	0 (0.0)	2 (0.6)	1 (1.7)	
Cirrhosis = 1 (%)	261 (71.7)	274 (82.0)	268 (75.5)	57 (96.6)	<0.001
Childscore (%)					<0.001
1 – Child-Pugh A	287 (78.8)	233 (69.8)	233 (65.6)	2 (3.4)	
2 – Child-Pugh B	67 (18.4)	92 (27.5)	105 (29.6)	3 (5.1)	
3 – Child-Pugh C	10 (2.7)	9 (2.7)	17 (4.8)	54 (91.5)	
Etiology (%)					<0.001
Alcohol	100 (27.5)	132 (39.5)	106 (29.9)	29 (49.2)	
Viral	106 (29.1)	78 (23.4)	102 (28.7)	16 (27.1)	
Other (NASH included)	61 (16.8)	41 (12.3)	39 (11.0)	10 (16.9)	
Unknown	97 (26.6)	83 (24.9)	108 (30.4)	4 (6.8)	
Ascites = 1 (%)	84 (23.1)	94 (28.1)	135 (38.0)	48 (81.4)	<0.001
Portal Vein thrombosis = 1 (%)	19 (5.5)	55 (19.0)	77 (36.7)	9 (52.9)	<0.001
Portal hypertension = 1 (%)	198 (54.4)	222 (66.5)	214 (60.6)	47 (79.7)	<0.001
Macrovascular invasion = 1 (%)	6 (1.7)	21 (6.4)	160 (45.7)	29 (49.2)	<0.001
Metastases = 1 (%)	1 (0.3)	7 (2.1)	140 (40.1)	10 (17.2)	<0.001
Lymph Node involvement = 1 (%)	12 (3.5)	33 (11.6)	79 (38.3)	4 (23.5)	<0.001
Gruppe (%)					<0.001
Spleen-Diameter cranio-caudal (mean (SD))	9.96 (3.75)	10.42 (3.71)	10.80 (3.66)	13.45 (3.68)	<0.001
AFP (ng/mL) (median [IQR])	7.45 [3.80, 72.35]	19.95 [5.20, 189.00]	168.10 [9.55, 4038.50]	108.15 [5.05, 5070.25]	<0.001
Bilirubin (mg/dl) (median [IQR])	0.90 [0.60, 1.30]	1.00 [0.70, 1.50]	1.00 [0.60, 1.51]	3.36 [2.59, 4.23]	<0.001
Alkaline Phosphatase (U/L) (median [IQR])	112.00 [83.00, 151.50]	144.00 [102.00, 202.00]	159.50 [116.00, 243.75]	208.50 [160.00, 295.75]	<0.001

(*Continued*)

Table 1 (Continued).

Variable	BCLC A	BCLC B	BCLC C	BCLC D	p-value
INR (median [IQR])	1.10 [1.00, 1.14]	1.17 [1.08, 1.26]	1.10 [1.02, 1.30]	1.40 [1.30, 1.45]	<0.001
Albumin in g/dl (median [IQR])	4.10 [3.50, 4.50]	3.80 [3.40, 4.30]	3.84 [3.34, 4.20]	2.76 [2.59, 3.08]	<0.001
Platelet count (median [IQR])	157.50 [103.25, 220.75]	142.00 [97.00, 206.50]	180.00 [117.00, 260.25]	94.50 [71.25, 159.50]	<0.001

Notes: Values are shown as mean (standard deviation, SD) if normally distributed or median [interquartile range, IQR] if non-normally distributed; categorical data are presented as percentages. Statistical comparisons between BCLC stages used ANOVA for normally distributed variables, Kruskal–Wallis test for non-normally distributed variables, and Chi-square (χ^2) test for categorical variables.
Abbreviations: BMI, Body Mass Index; ECOG, Eastern Cooperative Oncology Group Performance Status; BCLC, Barcelona Clinic Liver Cancer Staging System; Childscore, Child-Pugh Score; NASH, Non-Alcoholic Steatohepatitis; AFP, Alpha-Fetoprotein; INR, International Normalized Ratio.

the previous model. Upon inclusion of varice status the overall model the performance does not improve (Concordance with varices 0.72 versus 0.72 without varices) and the multivariate Cox-Regression analysis results remain nearly unchanged spp Table 2. We also assessed the effect of antiplatelet medication (data available for 593 patients, with 112 receiving treatment); the hazard ratio was 1.14 (p = 0.391), and the concordance index remained 0.72 (spp Table 3). Bootstrapping, performed with 1000 resamples, validated the robustness of the model's coefficient estimates and the predictive accuracy of platelet counts as a prognostic marker (spp Table 4).

Discussion

Low platelets count in HCC has been extensively researched as surrogate of portal hypertension, particularly in patients with early-stage disease (BCLC A and B). Thrombocytopenia is considered in this setting a negative predictor of the outcome of surgical or interventional procedures. In contrast, recent research highlighted an association between lower platelet count and favorable outcome in patients with advanced-stage disease (BCLC C and D) (Scheiner et al, 2018). Chen et al recently underscored the association with poor prognosis of elevated platelet counts for BCLC B patients as well.[16] However, only few studies have linked elevated platelet counts to shorter survival.[7,8,16] The findings of Scheiner et al and Chen et al prompted us to examine the precise role of platelet counts in HCC across different BCLC stages, adjusting for the effects of portal hypertension, a factor not considered in previous studies to validate these observations and to find clinically relevant cutoff values.

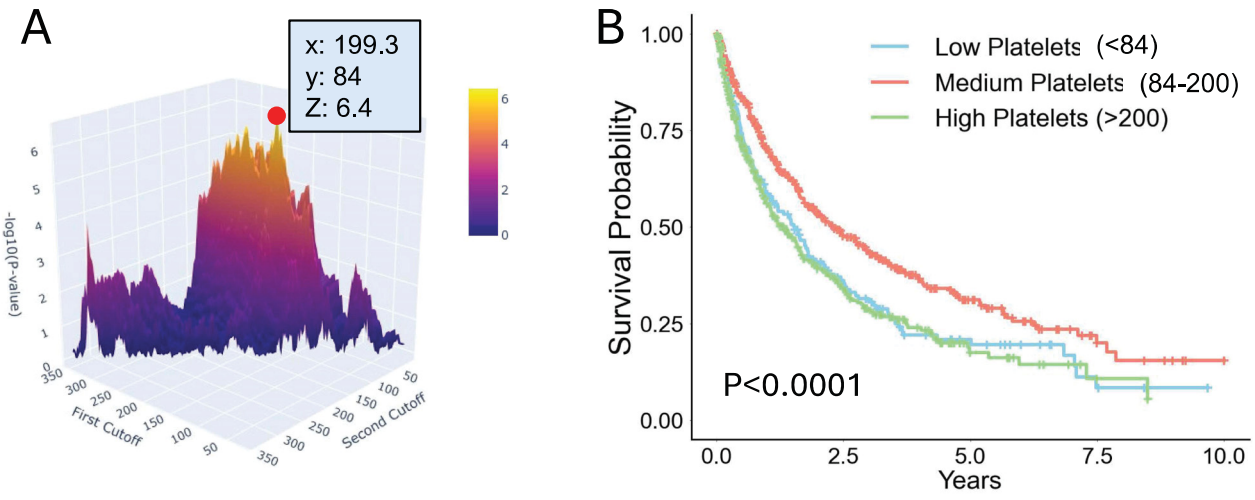

Figure 1 (**A**) Heatmap depicting the statistical significance (z-axis) of various cutoff pairs tested with the Logrank survival test (z-axis), hereby the best survival discrimination was found for an upper cutoff of 200000 platelets/µL and lower cutoff at 84000 platelets/µL (**B**) Kaplan-Meier survival analysis with newly defined platelet count cutoffs, comparing survival probabilities of patients within the new platelet range (84–200 × 10^9/L) vs outside ranges (Logrank, p<0.0001).
Abbreviation: BCLC, Barcelona Clinic Liver Cancer Staging system.

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Table 2 Baseline Characteristics of Patients According to the Newly Defined Platelet Ranges

Variable	Low Platelets (<84 x10^9L) (n=182)	Normal Range (84–200 x10^9L) (n=545)	High Platelets (>200 x10^9L) (n=364)	p-value
ECOG (%)				0.311
0	120 (58.0)	314 (60.4)	194 (53.4)	
1	74 (35.7)	171 (32.9)	140 (38.6)	
2	13 (6.3)	31 (6.0)	28 (7.7)	
3	0 (0.0)	4 (0.8)	1 (0.3)	
Height (mean (SD))	1.73 (0.09)	1.74 (0.08)	1.73 (0.08)	0.585
Weight (mean (SD))	81.64 (17.98)	81.83 (15.61)	78.61 (14.20)	0.012
BMI (mean (SD))	27.10 (4.88)	27.05 (4.62)	26.06 (3.68)	0.004
Age (mean (SD))	62.15 (9.60)	66.80 (9.50)	67.70 (11.60)	<0.001
Sex = Female (%)	45 (21.7)	81 (15.6)	73 (20.1)	0.081
Cirrhosis = Y (%)	199 (96.1)	441 (84.8)	205 (56.3)	<0.001
Child-Score (%)				<0.001
1 – Child-Pugh A	80 (38.6)	372 (71.5)	291 (79.9)	
2 – Child-Pugh B	85 (41.1)	112 (21.5)	63 (17.3)	
3 – Child-Pugh C	42 (20.3)	36 (6.9)	10 (2.7)	
Etiology (%)				<0.001
Alcoholic	76 (36.7)	203 (39.0)	83 (22.8)	
Viral	71 (34.3)	142 (27.3)	85 (23.4)	
Other	35 (16.9)	71 (13.7)	41 (11.3)	
Unknown	25 (12.1)	104 (20.0)	155 (42.6)	
Ascites = Y (%)	104 (50.2)	162 (31.2)	91 (25.0)	<0.001
Portal vein thrombosis = Y (%)	35 (22.4)	66 (16.0)	58 (20.4)	0.142
Portal hypertension = Y (%)	187 (90.3)	350 (67.4)	135 (37.2)	<0.001
BCLC Stage (%)				<0.001
A	61 (29.5)	180 (34.6)	117 (32.1)	
B	70 (33.8)	172 (33.1)	85 (23.4)	
C	49 (23.7)	147 (28.3)	152 (41.8)	
D	27 (13.0)	21 (4.0)	10 (2.7)	
Macrovascular invasion = Y (%)	41 (20.0)	94 (18.2)	76 (21.1)	0.549
Metastases = Y (%)	17 (8.3)	63 (12.2)	76 (21.4)	<0.001
Lymph node involvement = Y (%)	16 (10.3)	51 (12.5)	60 (21.4)	0.001
Spleen diameter cranio-caudal (mean (SD))	13.97 (3.52)	10.53 (3.31)	8.49 (3.08)	<0.001

(*Continued*)

Table 2 (Continued).

Variable	Low Platelets (<84 x10^9L) (n=182)	Normal Range (84–200 x10^9L) (n=545)	High Platelets (>200 x10^9L) (n=364)	p-value
AFP (ng/mL) (median [IQR])	20.60 [5.20, 210.95]	14.90 [5.10, 310.50]	77.20 [4.70, 2693.00]	0.002
Bilirubin (mg/dl) (median [IQR])	1.70 [1.15, 2.76]	1.00 [0.70, 1.50]	0.70 [0.50, 1.10]	<0.001
Alkaline phosphatase (U/L) (median [IQR])	135.50 [104.00, 186.00]	136.00 [95.00, 189.00]	150.00 [106.75, 257.25]	<0.001
INR (median [IQR])	1.29 [1.18, 1.40]	1.13 [1.08, 1.30]	1.10 [1.00, 1.20]	<0.001
Albumin in g/dl (median [IQR])	3.40 [2.90, 3.90]	3.90 [3.40, 4.40]	4.00 [3.50, 4.33]	<0.001
Platelet count (median [IQR])	66.00 [52.00, 79.00]	139.00 [112.00, 168.00]	261.00 [226.00, 325.00]	<0.001

Notes: Baseline characteristics of patients according to the newly defined platelet ranges: low (<84,000/μL), normal (84,000–200,000/μL), and high (>200,000/μL). Values are shown as mean (standard deviation, SD) if normally distributed or median [interquartile range, IQR] if non-normally distributed; categorical data are presented as percentages. Statistical comparisons between platelet count groups used ANOVA for normally distributed variables, Kruskal–Wallis test for non-normally distributed variables, and Chi-square (χ^2) test for categorical variables.

Abbreviations: ECOG, Eastern Cooperative Oncology Group Performance Status; BMI, Body Mass Index; BCLC, Barcelona Clinic Liver Cancer Staging System; AFP, Alpha-Fetoprotein; INR, International Normalized Ratio.

Table 3 Multivariate Cox Regression Analysis by BCLC Stages

Variable	All BCLC (n=1112)	BCLC A	BCLC B	BCLC C	BCLC D
ECOG	1.38 (1.21–158), p < 0.0001	1.46 (1.10–1.94), p = 0.010	1.18 (0.92–1.52), p = 0.202	1.49 (1.20–1.86), p < 0.001	1.02 (0.63–1.66), p = 0.920
Age (increase in 5 years)	1.09 (1.05–1.15), p < 0.0001	1.24 (1.11–1.38), p < 0.001	1.04 (0.96–1.12), p = 0.333	1.05 (0.98–1.14), p = 0.165	1.23 (0.99–1.54), p = 0.066
Child-Score	1.20 (1.01–1.40), p=0.0236	1.46 (0.94–2.26), p = 0.093	1.83 (1.34–2.51), p < 0.001	1.27 (0.99–1.63), p = 0.062	1.74 (0.65–4.65), p = 0.272
Ascites	1.68 (1.37–2.05), p < 0.0001	1.13 (0.64–2.00), p = 0.673	1.36 (0.92–2.01), p = 0.125	1.68 (1.22–2.32), p = 0.002	4.06 (1.57–10.47), p = 0.004
BCLC Stage	1.60 (1.42–1.80) p < 0.0001	n.a.	n.a.	n.a.	n.a.
Macrovascular invasion	1.16 (0.94–1.44) p = 0.1766	0.64 (0.15–2.74), p = 0.549	0.86 (0.49–1.52), p = 0.609	1.32 (0.99–1.75), p = 0.055	0.82 (0.40–1.69), p = 0.586
Metastases	1.27 (1.00–1.62), p = 0.0509	3.24 (0.44–23.91), p = 0.249	1.75 (0.70–4.39), p = 0.236	1.29 (0.97–1.72), p = 0.081	2.75 (1.12–6.77), p = 0.028
Spleen Diameter (increase in 5 cm steps)	1.01 (0.99–1.04), p = 0.2690	1.12 (0.84–1.48), p = 0.434	1.06 (0.86–1.30), p = 0.588	1.26 (1.00–1.58), p = 0.046	1.02 (0.69–1.52), p = 0.921
Platelets (increase in 50/nl steps)	1.10 (1.05–1.15), p < 0.0001	1.08 (0.99–1.18), p = 0.070	1.24 (1.12–1.36), p < 0.001	1.07 (1.01–1.14), p = 0.029	1.43 (1.14–1.79), p = 0.002
Group: Low Platelets (<84/nl)	1.18 (0.94–1.49), p = 0.1563	2.17 (1.25–3.76), p = 0.006	1.16 (0.77–1.74), p = 0.471	1.02 (0.66–1.58), p = 0.922	0.93 (0.47–1.84), p = 0.830
Group: High Platelets (>200/nl)	1.65 (1.35–2.02), p < 0.0001	1.57 (0.99–2.48), p = 0.054	1.93 (1.35–2.77), p < 0.001	1.66 (1.20–2.29), p = 0.002	2.55 (0.84–7.69), p = 0.097

Notes: Multivariate Cox regression analysis by BCLC stages. Model variables from the previous model across all stages were included. Hazard ratios (HR) with 95% confidence intervals (CI) and p-values are presented, showing the stage-dependent prognostic significance of platelet counts, specifically the detrimental impact of elevated platelet counts (>200 000/μL) in BCLC B and C stages.

Abbreviations: ECOG, Eastern Cooperative Oncology Group Performance Status; BCLC, Barcelona Clinic Liver Cancer Staging System; HR, Hazard Ratio; CI, Confidence Interval.

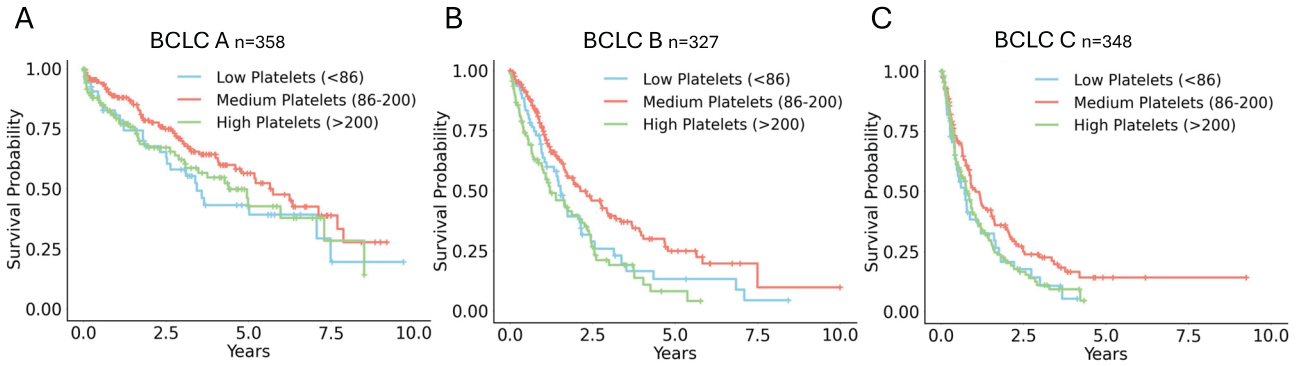

Figure 2 (**A**) Kaplan-Meier survival analysis with the newly defined platelet count cutoffs for BCLC stage A patients (**B**) Kaplan-Meier survival analysis with the newly defined platelet count cutoffs for BCLC stage B patients (**C**) Kaplan-Meier survival analysis with the newly defined platelet count cutoffs for BCLC stage C patients.
Abbreviation: BCLC, Barcelona Clinic Liver Cancer Staging system.

To further investigate this apparently conflicting information on the prognostic role of platelet counts and given that conventional platelet cutoffs did not adequately discriminate the different populations, we tested various cutoff values for discriminatory potential and found better discriminatory potential upon using two cutoff values (spp. Table 1). We identified that platelet counts above a cutoff of 200/nl indicated poorer survival in the patient population. The multivariate analyses confirmed that elevated platelet counts, according to the new cutoff of 200/µL, were negatively associated with survival in HCC patients. This finding aligns with the results of Pinter et al and Chen et al,[9,16] showing that elevated platelet counts are associated with poorer survival in BCLC B and C stages (Table 3). The new lower cutoff (below 84/µL) closely approximates the commonly used 100/µL cutoff for assessing surgical risk in BCLC A patients hereby validating the study cohort and methodology (Table 3).[5,17,18] A recent meta-analysis further supports our data, demonstrating that while low platelets (<100/nl) predict poorer prognosis in curative-intent HCC patients, elevated platelet counts are associated with worse outcomes in palliative treatment settings (BCLC B and C).[19]

Other prognostic markers, in addition to or in combination with platelet counts, can further support prognostic estimation in HCC. Inflammatory markers like the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are associated with poorer outcomes, especially in HCC with BCLC B stage.[20–24] The aspartate aminotransferase to platelet ratio index (APRI) helps assess liver fibrosis, while C-reactive protein (CRP) reflects systemic inflammation and correlates with advanced disease.[25–27] Lymphocyte-to-monocyte ratio (LMR) and red cell distribution width (RDW) have additionally shown a linkage between hematologic changes to prognosis.[20,28] Additionally, Shi et al demonstrated that integrating platelet counts with CD8+ T cell levels improves prognostic accuracy in HCC patients with portal vein tumor thrombosis (PVTT).[29] Combining these markers with platelet counts could create more comprehensive models for risk assessment and treatment planning in HCC.

Preclinical evidence suggests a complex, potentially detrimental role of platelets in hepatocarcinogenesis. For example, platelet-derived GPIbα, which plays a crucial role in platelet adhesion to blood vessels, has been shown to be critical for HCC development in a model of non-alcoholic steatohepatitis.[30] Additionally, platelet releasates have been found to enhance the proliferative response of HCC cells.[31] Other in vitro findings reinforce the importance of platelets in HCC pathogenesis,[32] indicating that platelets themselves may play a harmful role. Padickakudy et al reported that platelet-derived serotonin promotes tumor angiogenesis with elevated serotonin levels predisposing patients to early HCC recurrence, collectively linking platelet-driven hypercoagulability to venous thromboembolic events and poorer prognosis.[33] Further studies are needed to delineate the impact of platelets on HCC progression to advance biomarker discovery and therapeutic targeting.

Despite its multicentric nature, our study has several limitations. Platelet counts were not adjusted for inflammatory markers such as C-reactive protein (CRP) or leukocyte numbers, which could influence the results. Additionally, the absence of a validation cohort means that these findings require further confirmation through subsequent studies. Therapy-related variables (eg, sorafenib, SIRT) could further refine prognosis and survival outcomes. Data collection protocols across centers

may introduce variability and could impact reliability and standardization. Further the study design is retrospective and missing information (ie variceal status) or inconsistencies could affect the reliability of the conclusions.

The impact of platelet count was adjusted for surrogate markers of portal hypertension and comprehensively analyzed across different BCLC stages. This study revealed a stage-dependent association of elevated platelet counts with poor HCC prognosis and suggests a new platelet cutoff (200/μL) for assessing prognosis in BCLC B and C patients, which could enhance risk stratification and guide therapeutic decision-making.

Disclosure

Alexander B Philipp and Enrico N De Toni are co-senior authors for this study. Najib Ben Khaled reports meeting attendance fees and travel reimbursements from Eisai; lecture honorarium from Falk Foundation, AstraZeneca; consultant for Ipsen, Roche; research support from Genentech, outside the submitted work. Florian P Reiter has received honoraria for lectures, consulting activities and travel support from the Falk Foundation, AbbVie, Gilead, Ipsen, AstraZeneca, Roche, and Novartis. Daniel Roessler reports personal fees from Ipsen, Bayer, and Falk, outside the submitted work. Bernhard Scheiner reports grants, personal fees for speaker honoraria from Eisai, travel supports from Ipsen, Gilead, AbbVie, Roche; speaker honoraria, consulting fee and travel supports from AstraZeneca, outside the submitted work. Matthias Pinter reports grants, personal fees from AstraZeneca, Bayer, BMS, Eisai, Roche, Ipsen, Eli Lilly, MSD, outside the submitted work. Max Seidensticker reports grants from Astra Zeneca, Boston Scientific, SIRTEX Medical; personal fees for lectures from Cook Medical, Siemens Healthineers, Balt, outside the submitted work. Alexander Philipp reports grants and/or personal fees from AstraZeneca, Roche, MSD, Falk Foundation, Ipsen, Pfizer, outside the submitted work. Enrico De Toni is an employee of Boehringer-Ingelheim; he reports personal fees from AstraZeneca, Bayer, BMS, EISAI, Lilly, MSD, Pfizer, IPSEN, Terumo, Roche; travel grants from Arqule, AstraZeneca, BMS, Bayer, Eli Lilly, IPSEN and Roche. The authors report no other conflicts of interest in this work.

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Use of checkpoint inhibitors in liver transplant recipients

Stefan Munker and Enrico N De Toni

Abstract

In spite of their major impact in cancer therapy, immune checkpoint inhibitors are considered to be contraindicated in liver transplant recipients due to fear of rejection and fatal liver failure. Nevertheless, an increasing number of instances of liver transplant recipients treated with checkpoint inhibitors is being published. We reviewed the reports on 14 known cases of liver transplant recipients who underwent treatment with checkpoint inhibitors and discuss factors likely to determine susceptibility to organ rejection including the choice of the agent and the immunosuppression employed, the assessment of Programmed cell death 1 ligand 1 (PD-L1) status in liver graft biopsies, and the time of treatment initiation.

Keywords

Checkpoint inhibition, hepatocellular carcinoma, liver transplantation, pembrolizumab, nivolumab, liver transplant recipients, rejection

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The approach to systemic treatment of many malignancies has been changed drastically by the recent advent of immune checkpoint inhibitors (CPIs).[1] These agents are designed to enhance the tumour-specific activity of immune cells and do not possess intrinsic cytotoxicity; their mechanism of action explains the lack of adverse events typically associated with the use of conventional chemotherapy, however it also accounts for the frequent occurrence of autoimmune-related unwanted effects associated with their use as well as the reluctance to use them in transplant recipients.[2]

Owing to long-term immunosuppression, organ transplant recipients are an at-risk population for the development of a wide spectrum of malignancies comprising melanoma and haematological cancers.[3] This risk is further increased in patients who underwent transplantation due to hepatocellular carcinoma (HCC) due to a post-transplant recurrence risk of approximately 10% within five years.[4,5] Despite this increased need for effective cancer treatment options, CPIs are considered as contraindicated in organ transplant recipients for fear of organ rejection.

Nevertheless, due to their potential clinical benefit CPIs have, since their approval, been used as a salvage option in a number of transplanted patients. Accounts of these cases, which at present amount to less than 40 published reports, confirm that organ rejection is a frequent occurrence in this setting.[6–8] However, good tolerability and signs of significant anticancer efficacy were observed in some cases.[6]

As of today, to our knowledge 14 cases of liver transplant recipients who have been treated with immune CPIs have been published (Table 1). Altogether, liver graft rejection was reported in four of 14 reported cases; in three cases with lethal outcome, rejection occurred within three weeks since the initiation of therapy.[9] Overall survival was available in 12 cases and amounted to a median value of 1.2 months. However, in four patients showing a response to treatment, survival ranged between at least four[10] and 18[11] months.

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Table 1. Patients' characteristics and reported outcome of published case reports on treatment with checkpoint inhibitors in liver transplant recipients, listed according to publication date.

Patient	Reference	Malignancy	Compound	Transplant to immunotherapy in years	Response	OS (months)	Graft rejection	PD-L1 status	status	Immunosuppression
1	[17]	Melanoma	Ipilimumab	8	No	>5[a]	No	N/A	N/A	Low dose tacrolimus
2	[10]	Melanoma	Ipilimumab	8	Yes	>4[a]	No	N/A	N/A	Low dose sirolimus
3	[7]	HCC	Nivolumab	1	Yes	10	No	0%	N/A	Low dose tacrolimus
4	[14]	Fibrolamellar HCC	Nivolumab	4	N/A	1	Yes, lethal	Positive	Positive	Sirolimus
5	[14]	Fibrolamellar HCC	Nivolumab	3	N/A	1	Yes, lethal	Positive	Positive	Tacrolimus
6	[9]	Melanoma	Pembrolizumab	N/A	N/A	N/A	Yes, lethal	N/A	N/A	Ciclosporine
7	[19]	HCC	Pembrolizumab	8	No	3	No	N/A	N/A	Low dose tacrolimus
8	[15]	HCC	Nivolumab	2.7	No	1.2	No	N/A	10%	Tacrolimus
9	[15]	Melanoma	Pembrolizumab	5.5	Yes	9.5	No	0%	5%	Everolimus, MMF
10	[15]	HCC	Nivolumab	7.8	No	1.1	No	0%	N/A	Sirolimus, MMF
11	[15]	HCC	Nivolumab	3.7	No	1.3	No	0%	0%	Tacrolimus
12	[15]	HCC	Nivolumab	1.2	N/A	0.3	No	N/A	0%	Tacrolimus
13	[15]	HCC	Nivolumab	1.1	N/A	0.9	Yes	30%	0%	N/A
14	[11]	Melanoma	Ipilimumab/ pembrolizumab	6	Yes/yes	18[a]	No	N/A	N/A	Sirolimus

HCC: hepatocellular carcinoma; OS: overall survival; N/A: not available.
PD-L1 or PD1 - status of the transplant liver; MMF: Mycophenolate Mofetil; PD1: Programmed cell death 1; PD-L1: Programmed cell death 1 ligand 1.
[a]Based on report on response duration.

Data from these reports are scanty and too heterogeneous to draw definitive conclusions on the factors determining graft rejection; they tell us, however, that while some patients experience graft loss upon treatment with CPIs, others do not and that, besides individual host- and donor-related genetic factors, other modifiable factors might determine the susceptibility to organ rejection. We discuss below a possible role of such factors which include the choice of the agent and the immunosuppression employed, the availability of biopsies, and the time of treatment initiation.

Agent used

Out of 14 cases of liver graft recipients treated with CPIs, all but two patients, who received the cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4) inhibitor ipilimumab, underwent treatment with Programmed cell death 1(PD1)/Programmed cell death 1 ligand 1(PD-L1) blocking agents. Organ rejection was reported in four out of 10 patients treated with nivolumab or pembrolizumab but in neither of the two patients treated with ipilimumab, although in these latter cases immunosuppression was tapered at subtherapeutic levels. Data from these reports are insufficient to draw the conclusion that CTLA4 inhibitors are safer than PD-1/PDL-1 inhibitors in liver transplant recipients. However, some authors suggest that the lack of organ rejection during ipilimumab treatment reflects a predominant role of PD-1 in determining graft tolerance.[8,12] Nevertheless, a recent survey reported kidney graft rejection occurring under treatment with either CTLA-4 or PD-1 inhibitors.[8] There is therefore, at this time, no evidence supporting the use of a particular CPI in liver allograft recipients and, until more data become available, it seems reasonable that the choice of the agent should be primarily guided by the data available on effectiveness in the respective tumour entities.

Liver biopsies

Another relevant point regards the issue of whether assessment of immune checkpoint regulators in liver biopsies might serve to predict rejection in the same way their assessment predict response in some tumour entities.[13] Each of the three available biopsies from patients with acute graft rejection showed elevated PD-L1 expression,[9,14,15] whereas none of the four biopsies available from patients without rejection (including

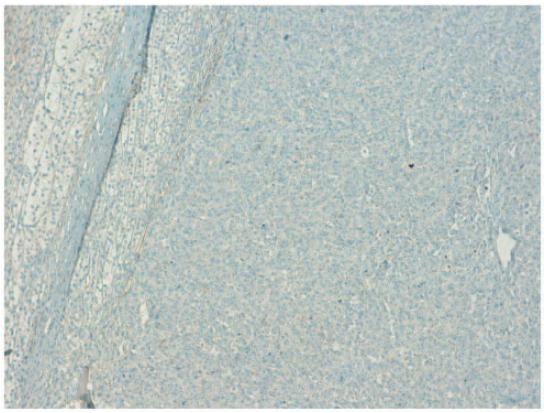

Figure 1. Negative anti-programmed cell death 1 ligand 1 (PD-L1) staining (100x) of a pretherapeutic liver biopsy from a liver graft recipient treated with Nivolumab without signs of organ rejection[7] (Courtesy Prof. Jens Neumann).

a pre-treatment biopsy performed in one of our patients – Figure 1) showed positive PD-L1 staining. It is therefore highly suggestive that PD-L1 expression might predict graft rejection. However, biopsies from patients experiencing rejection were reported to be obtained after initiation of therapy and it cannot be ruled out that positive PD-L1 staining reflects a consequence of organ rejection. It seems therefore reasonable to suggest that graft liver biopsies are preformed routinely prior to therapy with CPI in liver transplant recipients and that staining for PDL-1 is taken into consideration for the choice of whether a PD1/PDL-1- or a CTLA4-blocking agent should be employed.

Immunosuppression

Immunosuppression plays a potentially detrimental role in determining the efficacy of CPIs, since their effect requires an intact T-cell response. Whether concurrent steroid treatment counteracts the efficacy of CPIs in non-transplanted patients is still an issue of debate, a recent survey suggests that concomitant use of steroids does not necessarily influence the efficacy of CPI treatment.[16] In addition, immunosuppression did not prevent response in four patients (cases 1, 3, 8, and 13, Table 1) and a report has recently shown that preventive treatment with high-dose steroids, intended to avoid CPI-mediated rejection, did not prevent a remarkable response in a kidney transplant recipient.[6] Therefore, although reduction of immunosuppression to sub-therapeutic doses was not associated with organ rejection,[7,10,17] pre-treatment with steroids could be attempted in the absence of contraindications and immunosuppression tapered during the course of treatment whenever possible.

Initiation of treatment

Due to the potentially lethal consequences of organ rejection, the decision to employ CPIs is usually delayed until unequivocal signs of therapy failure are observed during treatment with conventional agents. Although response to CPIs are expected to occur soon after treatment initiation (most occurring within three months of initiation of therapy),[18] a delayed onset of treatment might prevent the efficacy of CPIs to become evident before untreatable disease progression or organ dysfunction occur; this might at least in part account for the dismal survival so far reported for liver transplant patients undergoing treatment with CPIs (Table 1). It is therefore advisable that, if CPI treatment is considered as an option, a close follow-up is performed during first-line conventional treatment to recognise signs of disease progression early, and that the decision to initiate treatment with a CPI is taken in a timely manner.

In summary, the decision to initiate CPI treatment in liver transplant patients should be made on an individual basis based on consideration of the patient's performance status, the potential oncological benefits and the tentative nature of this possibility. We suggest that liver biopsies of liver allografts are taken routinely before treatment initiation and that, if staining for PD-L1 is detected, initiation of treatment with a CTL4-blocking agent is considered. Pre-treatment with steroids can be attempted in the absence of obvious contraindications, as recently suggested,[6] and immunosuppression progressively tapered under close surveillance.[7] Close follow-up to detect signs of progression under conventional therapy should trigger a timely choice to begin CPI treatment.

Finally, it cannot be emphasised enough that at the present state of knowledge, the decision to treat transplant recipients with immune CPI is to be considered as *ultima ratio* to be weighed against the possibility of graft loss and fatal organ failure. However, an accurate account of the above factors in future reports will contribute to understanding of the factors determining rejection upon treatment with CPI and possibly guide the design of appropriate prospective studies.

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Declaration of conflicting interests

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EpiCO (Epirubicin, Cyclophosphamide and Vincristine) as treatment for extrapulmonary high-grade neuroendocrine neoplasms

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Key Words:

Neuroendocrine neoplasms, high grade neuroendocrine carcinoma, mixed-neuroendocrine-non-neuroendocrine neoplasm, mixed adeno-neuroendocrine carcinoma, epirubicin, cyclophosphamide, vincristine

Einleitung:

High-grade neuroendokrine Neoplasien (NEN) sind eine seltene Entität. Bisher sind keine randomisiert kontrollierten Studien durchgeführt worden. Therapieempfehlungen sind hauptsächlich von dem pulmonalen Analog, dem kleinzelligen Bronchialkarzinom (SCLC), extrapoliert und in retrospektiven Fallserien validiert. Die multizentrische Nordic NEC Studie von gastroenteropathischen (GEP) und "cancer of unknown primary" (CUP) high-grade neuroendokrinen Neoplasien zeigte ein signifikantes Therapieansprechen auf Etoposide und Platinhaltige Kombinationen [1]. Diese Kombination ist Standard Erstlinientherapie für high-grade GEP/CUP NEN. High-grade gemischtzellige-neuroendokrine-nicht-neuroendocrine Neoplasien (MiNEN), früher auch als gemischtzellige adenoneuroendocrine Karzinome (MANEC) bezeichnet, zeigen ebenfalls eine schlechte Prognose und werden wie andere high-grade NEN behandelt. Das Carboplatin/Etoposide (CE) Protokoll zeigt ein Ansprechen von von 50-60% in high-grade NEN und MiNEN, aber Remissionen sind häufig von kurzer Dauer [1]. Zweitlinien Therapien sind nicht allgemein etabliert.

Eine Therapie mit Doxorubicin, Cyclophosphamid und Vincristine (CAV) zeigte Effektivität beim SCLC und galt als Standardchemotherapie vor der Ära von Etoposid und platinhaltigen Zytostatika Kombinationen. Aufgrund eines besseren Toxizitätsprofils wurde Doxorubicin durch Epirubicin ersetzt, das hieraus resultierende Regime mit Epirubicin, Cyclophosphamid und Vincristin wurde EpiCO oder CEV abgekürzt. [4,5,6]

In Analogie zum SCLC wurden ausgewählte Patienten mit high-grade NEN mit EpiCO in einem Zweitlinien Setting am Universitätsklinikum Regensburg behandelt. In dieser retrospektiven Arbeit berichten wir von 5 Patienten mit metastasiertem high-grade GEP/CUP NEN/MiNEN, die eine Chemotherapie nach diesem Schema erhielten.

Introduction:

High-grade neuroendocrine neoplasms (NEN) comprise a rare entity. Due to the lack of randomized controlled trials, therapy recommendations were mainly extrapolated from its pulmonary analog, small cell lung cancer and mostly validated in small retrospective case series. The multicentric Nordic NEC Study of gastro-entero-pancreatic (GEP) and cancer of unknown primary (CUP) high-grade neuroendocrine neoplasms showed a significant disease control upon treatment with etoposide and platinum-based chemotherapies [1]. Such a combination with etoposide and a platinum (CE) compound is currently considered standard first-line treatment for high-grade GEP/CUP NEN. High-grade mixed-neuroendocrine-

non-neuroendocrine neoplasms (MiNEN) formerly termed mixed adreno-neuroendocrine carcinomas (MANEC) also have a poor prognosis and are generally treated like other high-grade NEN. The CE protocol has significant activity in high-grade NEN and MiNEN, but the response is short-lived in most cases with response rates around 50-60% [1]. Second-line treatment alternatives are not established so far. The need for additional treatment options is evident.

Combination chemotherapy with doxorubicin, cyclophosphamide and vincristine (CAV) showed efficacy in small cell lung carcinoma (SCLC) and was considered standard first-line therapy before the era of etoposide and platinum combinations[2, 3]. Due to a better toxicity profile, doxorubicin was replaced by epirubicin, resulting in the combination of epirubicin, cyclophosphamide and vincristine (abbreviated as EpiCO[4] or CEV [5, 6]).

In analogy to SCLC, selected patients with high-grade NEN were treated with the EpiCO regimen in second-line (or in one patient first line) at our center. In this report, we present the retrospective series of 5 cases with metastatic high-grade GEP/CUP NEN/MiNEN who received chemotherapy according to this protocol.

Methods

Study Design

Epidemiological data and survival were obtained of patients diagnosed with NEN and MINEN between 2004 and 2017 who received chemotherapy at University Medical Center Regensburg. Additional clinical and survival data were kindly provided by Dr. Gerken from the population-based clinical cancer registry at the Regensburg Tumor Center in Eastern Bavaria, Germany.
Data were anonymized in accordance with the Declaration of Helsinki and the Bavarian Law of Cancer Registration. Due to the retrospective nature of this archival study, the local ethics committee deemed individual consent as not necessary.

Treatment

Patients received Vincristine 2mg as a bolus, epirubicin 70mg/m² as a 15min infusion followed by cyclophosphamide 1000mg/m² as a 1h infusion. The drugs were given (including antiemetic prophylaxis according to local guidelines) on day one of a three-week cycle. According to local standards, restaging was performed after 2 or 3 cycles of EpiCo.

Results

In order to identify patients who received EpiCO chemotherapy as second-line for neuroendocrine neoplasms, we performed a hospital-wide search of our patient database. For the keyword “NET”, we received 334 results. We screened for high-grade NEN and found 31 patients fitting these criteria. Of these, 20 patients received CE and were followed in our clinic.

In this group of 20 patients receiving CE, disease control rate was 50% (10/20; 7 patients with partial responses and 3 patients with stable disease). The median time to progression was 3.5 months. Upon progression, the patients received various second-line chemotherapy regimens. Five patients received topotecan single-agent therapy, four patients received pioglitazone, rofecoxib and capecitabine as part of a study, two patients received combination therapy with folinic acid, fluorouracil and irinotecan (FOLFIRI), five patients received EpiCO, and five patients received best supportive care. Patient characteristics and outcomes of the patients receiving EpiCO are shown in **Table 1**.

In summary, EpiCO treatment was given to three patients with metastatic CUP/GEP high-grade NEN and two patients with high-grade MiNEN. One patient (no. 4) received upfront EpiCO without prior CE.

Patient 5 was not eligible for analysis due to an early death. Out of the four patients eligible for analysis, one patient showed partial remission, two patients had stable disease, and one patient had progressive disease.

Discussion

Extrapulmonary high-grade neuroendocrine neoplasm or NEC is rare; therefore, the development of new treatments has not been prioritized, and clinical studies for such a low incidence disease are challenging to establish. It is a highly malignant cancer entity manifesting in all ages with a rapid growth pattern leading to a poor prognosis. When encountering a patient with high-grade NEN, clinicians often face the difficulty of being forced to choose from several marginally studied treatment alternatives. In the past, treatment recommendations were often extrapolated from SCLC [7].

In 2013 Sorbye *et al.* published a large multicenter retrospective analysis, de facto defining CE as standard chemotherapy regimen in the first-line setting of high-grade NEN [1]. In their subgroup analysis, it was evident that NEN with a Ki-67 index <55% had a better prognosis but simultaneously a worse response to CE. In the WHO classification of 2010, no substratification of G3 NEN is included. But recently, the biological and prognostic differences of the NEN G3 group were more clearly defined [7]. This was in concordance with the current ENETS guidelines [8]. As evident from Sorbye *et al.*, high-grade NEN should be further sub-stratified into either morphologically well-differentiated neuroendocrine tumors with a Ki-67 rate usually below 55% (G3-NET) and neuroendocrine carcinoma (NEC) with poor differentiation (small or large cellular) and a Ki-67 index above 55% [8, 9]. Due to biological and prognostic similarities of G3 NET with G2 NET responsiveness to chemotherapy of both entities is assumed to be comparable.

In a case series of 25 patients with high-grade NEN, Welin et al. showed the effectiveness of temozolomide with or without capecitabine or bevacizumab in a second-line setting. They achieved a disease control rate of roughly 71% (33% responsive disease, 38% stable disease). Of their 25 patients, 16 (64%) had a Ki-67 index lower than 60%. It is likely that a majority of those patients were among the G3 NET group [10]. Temozolomide single-agent therapy showed limited efficacy with a disease control rate of 36% with disease control in 6 out of 16 evaluable patients of 28 treated patients with G3 NEN [11]. In this study, however, the proportion of NEC was higher (Ki-67 > 50% in 17 of 28 cases), 9 patients received temozolomide as third-line and in contrast to the aforementioned study capecitabine was not used.

In a single-center retrospective study, Hentic *et al.* described the outcome of the FOLFIRI regimen in 19 gastrointestinal NEC patients that progressed upon treatment with a platinum-etoposide combination. An objective response rate of 31% and stable disease in 31% could be observed [12]. Another study published in 2015 showed comparable response rates for FOLFOX in the second-line setting [13].

In their multicenter study, Sorby *et al.* presented data of a small subgroup (n=28) with vincristine in combination with CE the response rate 68% (with 44% PR and 24% SD) appeared promising; however, this subgroup lacked statistical significance as for survival [1].

Taken together, platinum-based therapies are considered the standard first-line choice for high-grade extrapulmonary NEC, whereas no standard exists in second- or third-line. The EpiCO regimen consists of epirubicin, cyclophosphamide, and vincristine. To our knowledge, only one other publication describes the use of an anthracycline containing regimen for high-grade NEN in the second-line setting.

In this study from Japan, 3/16 patients (18.8%) responded to single-agent amrubicine [13]. In our case series, we describe a heterogeneous group of three patients with metastasized extrapulmonary NEN (two high-grade NET and one NEC) and two patients with metastasized high-grade MiNEN. In three out of 5 patients, disease control was achieved (2 SD, 1PR). As shown in **Table 1**, the response (time to progression) compares favorably to the first line CE treatment in 2 of the 3 evaluable patients. However, the limitation of this study is the small number of cases treated with EpiCO.

With the advent of immunotherapy and whole-genome sequencing [14], further new treatment options will become available. Klempner *et al.* showed in their case series the feasibility of BRAF-MEK inhibition in BRAF mutated NEC [16]. Two patients with colorectal NEC harboring BRAF V600E mutation were treated with MEK inhibitors dabrafenib or vemurafenib and trametinib. Both patients experienced a profound and lasting response. In an analysis of 109 colorectal NETs, they found 10 cases (9%) with BRAF mutations (8/10 with BRAF V600E). More recently, Girardi *et al.* reviewed the molecular pathways of poorly differentiated GEP- NECs. They reported commonly TP53 alterations, microsatellite instability in about 10% of patients as well as alterations of p16/RB1/cyclin D1, Hedgehog, and Notch pathways [17].

In conclusion, an anthracycline-containing regimen (EpiCO) may be effective in some patients with GEP/CUP high-grade NEN and MiNEN (especially if no targetable mutations are found). Therefore, EpiCO could be an option in the second or third-line setting, especially if contraindications for FOLFIRI or FOLFOX are present. Evaluating EpiCO in a larger series is indicated.

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Disclosure Statement

The authors declare no conflict of interest

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Table 1 Clinical characteristics and outcome of patients treated with EpiCO

#	Age	Gender	PS at Diag-nosis	Primary site	Metastatic sites	Histology	Ki67 Index	Time to Progression upon CE in months	Best Response to EpiCo	Time to Progression upon EpiCo in months	Overall Survival in months
1	45	F	0	Unknown	Lymph nodes	NEC	90%	3	PD	3	6
2	51	M	0	Stomach	Liver, lymph nodes	NET G3	45%	4	SD	9	22
3	46	M	0	Unknown (pancreas)	Lymph nodes, bone	NET G3	25-30%-> 60% (Upgrading) *	2	SD	6	24+ (since diagnosis and 9+ since “upgrading”)
4	72	F	0-1	Rectum	Liver	High-grade MiNEN	70%	N/A	PR	6	8
5	48	M	0	Rectum	Liver	High-grade MiNEN	40%	6	N/A	N/A	7

Abbreviations: Not available: (N/A); progressive disease: (PD); partial remission: (PR); stable disease: (SD) *lung resection was performed due to progressive pulmonary infiltrate, pathology report confirmed pulmonary metastasis with a Ki-67 of 60%

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Chemotherapy for metastatic colon cancer: No effect on survival when the dose is reduced due to side effects

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Abstract

Background: 5-Fluorouracil (5FU), Folinic acid (FA), and Oxaliplatin (FOLFOX) or 5FU, FA, and Irinotecan (FOLFIRI) are standard regimens for palliative chemotherapy of metastatic colon cancer. Since data showing the influence of dose reduction in palliative treatment are rare, the objective of this single center, retrospective study was to further characterize the influence of dose reduction on efficacy of these therapeutic regimens.

Methods: One hundred nine patients, diagnosed with stage IV colon cancer between 2004 and 2012 and receiving palliative first-line chemotherapy with either FOLFOX or FOLFIRI regimens in our outpatient clinic were analyzed for treatment efficacy. Patients who received dose reductions due to side effects usually received doses of 80% or lower of per protocol dose. Survival data were obtained from the Regensburg Tumor Registry. Survival analysis was performed using Kaplan-Meier statistical analysis and multivariable analysis.

Results: A dose reduction due to side effects was necessary in 46 (42%) patients. Dose reduction was independent of age. Major reasons for dose reduction were neutropenia (30%) followed by polyneuropathy (16%) and diarrhea (14%). Dosage was more often reduced in patients receiving FOLFOX based therapy. Comparison of patients with dose reduction versus patients with full dosage showed no significant difference on overall survival ($p = 0.430$). Subgroup analysis revealed dose reduction in patients with N2 stage disease was associated with improved survival. Patients who underwent dose reduction received more cycles of chemotherapy (13.7 vs. 10.8 cycles) and cumulative dosage was similar in both groups.

Conclusion: Contrary to our expectations, the need to reduce chemotherapy dosage due to side effects does not indicate a worse prognosis in our retrospective analysis. We believe this can in part be explained by better adaption to interindividual pharmacokinetics and longer time of treatment.

Keywords: Dose reduction, Cancer, Colorectal cancer, Chemotherapy

Background

Colon cancer is the third most common cancer and a major cause of morbidity and mortality worldwide [1]. During the last few decades, improvement in therapeutic regimens for advanced colorectal cancer led to a dramatic increase in efficacy, reduction of mortality rates, and improved survival. Upon diagnosis, 20% of newly diagnosed colorectal cancer patients present with metastatic disease with no curative treatment options currently available. Among the chemotherapy regimens considered effective in palliative treatment, Irinotecan or Oxaliplatin in combination with 5-Fluorouracil regimens are standard back bones of current systemic treatment [2, 3].

These chemotherapy regimens were initially tested for efficacy in well-defined study populations not necessarily reflecting average (multimorbid older) patients in real

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life settings [4, 5]. Clinicians increasingly realize the shortcoming of the initial studies [6] since these mostly included younger patients better able to cope with adverse side effects or toxicities [7]. But especially in elderly and comorbid patients, side effects, organ toxicities and therefore potentially limited survival need to be considered [8]. In clinical practice, these effects are prevented or mitigated by a dose reduction of chemotherapy with the suspected consequence of worse tumor related survival [9]. We therefore believe that there is a need to further investigate the impact of dose reduction of the currently used therapeutic "standard" regimens on survival and side effects in different subgroups. Hence, we performed a retrospective analysis of our patient cohort with advanced stage colorectal cancer patients to assess outcome of reduced chemotherapy dosage.

Methods

Study design

A retrospective analysis was performed based on clinical data obtained from the population-based clinical cancer registry at the Regensburg Tumor Center in Eastern Bavaria, Germany. Epidemiological data and survival were investigated in a cohort of colon cancer diagnosed between 2004 and 2012 receiving chemotherapy in the outpatient clinic of the University Medical Center Regensburg. Patients with stage IV colorectal cancer undergoing palliative combination chemotherapy were divided into dose reduction (≤ 80%) and full dosage (100%) groups. We intend to analyze survival time by Kaplan Meier method and to estimate 2- and 3-year survival rates and mean and median survival time. Data collection and retrospective analysis of patient information were anonymized in accordance with the Declaration of Helsinki, and in line with the Bavarian Law of Cancer Registration.

Background and data collection

The baseline cohort of the present study consisted of patients with the ICD-10-GM (http://www.dimdi.de/static/de/klassi/icd-10-gm/index.htm) diagnosis C18, i.e. a malignant neoplasm of colon. 109 patients with advanced stage colon carcinoma (UICC Stage IV) with histologically confirmed adenocarcinoma of the colon between 2002 and 2012 receiving palliative chemotherapy in our University Medical Center were included in the study. Neuroendocrine tumors were excluded. 3 Patients receiving only 5FU based chemotherapy regimens without Irinotecan or Oxaliplatin were excluded. Prior to palliative chemotherapy, 72 patients received palliative or oncological tumor resection. In this subgroup, the diagnosis of metastasized colon cancer was made during surgery or in follow up examinations. Patients were routinely assessed prior to chemotherapy by a physician trained in oncology. Efficacy evaluation was performed by CT scans in 3-month intervals. Patients who died of cancer unrelated causes and patients with death within one month after start of palliative chemotherapy were censored.

Baseline characteristics are shown in Table 1. For assessing comorbidities a scoring according to the Charlson Score comorbidities index [10] was performed Additional file 1: Table S1. Patients receiving either FOLFOX, FOLFIRI or sequentially both chemotherapy regimens were included in this study. Mode of administration was exclusively i.v.. Chemotherapy dosing values were calculated and registered with a chemotherapy planning software (OnkoDAT®) [11]. A patient was considered receiving reduced dosage of chemotherapy if he received ≥3 cycles of less than 80% of 5FU, Oxaliplatin, or Irinotecan. Survival information were obtained from the Regensburg Tumor Center founded in 1991. The Cancer registry includes epidemiological and clinical data from all consenting patients with malignancies diagnosed and treated in Eastern Bavaria (2.1 million population). All data were extracted, recorded, and fed into a central database by trained personnel. The patients' life status and disease recurrence were ascertained from clinical reports, death certificates issued by the local public health departments, and the registration offices of the respective residential districts. Data were processed and secured according to the Bavarian Law of Cancer Registration.

Statistical analysis

Continuous data were described as means, median, minimum, maximum values and standard deviation, and categorical data were expressed as absolute frequencies and relative percentages. Patient characteristics were compared with t-tests for normally distributed continuous data, otherwise by means of non-parametric Mann-Whitney-U- and Chi-square tests for categorical variables. Tumor-specific survival rates (OS) were analyzed from the start of palliative chemotherapy until the event of death or last patient contact. Survival rates of patients with and without dose reduction were described by Kaplan-Meier analysis. Survival differences were tested for statistical significance by the two-sided Log Rank in Kaplan-Meier analyses; the level of significance was set to 0.05. The follow-up period and survival times were right censored using December 31, 2012 as cut-off date. To determine the influence of further co-variables on overall survival, we performed univariable and multivariable regression analysis using Cox proportional hazard models. In multivariable analysis, the hazard ratio (HR) was adjusted for the co-variables age, sex, TNM status, grading, lymph vessel invasion, vein invasion, Charlson score for comorbidities, surgery and

Table 1 Patient characteristics according to dosage reduction and in total

		Dosage reduction						
		Yes (<=80% dosage)		No (=100% dosage)		Total		T-test Chi^2
		N	(%)	*N*	(%)	*N*	(%)	*p* value
Age	Mean/Median	56.5	59.0	58.4	58.0	57.6	59.0	.440
	< 65	32	69.6%	42	66.7%	74	67.9%	.749
	> = 65	14	30.4%	21	33.3%	35	32.1%	
Sex	Male	29	63.0%	46	73.0%	75	68.8%	.267
	Female	17	37.0%	17	27.0%	34	31.2%	
T stage	T3	17	37.0%	23	36.5%	40	36.7%	
	T4	22	47.8%	22	34.9%	44	40.4%	.205
	Tx	7	15.2%	18	28.6%	25	22.9%	
N stage	N0	4	8.7%	5	7.9%	9	8.3%	
	N1	14	30.4%	16	25.4%	30	27.5%	.796
	N2	18	39.1%	23	36.5%	41	37.6%	
	Nx	10	21.7%	19	30.2%	29	26.6%	
Grading	G2	27	58.7%	40	63.5%	67	61.5%	
	G3	18	39.1%	19	30.2%	37	33.9%	.419
	Gx	1	2.2%	4	6.3%	5	4.6%	
L stage	L0	6	13.0%	7	11.1%	13	11.9%	
	L1	23	50.0%	15	23.8%	38	34.9%	.010
	Lx	17	37.0%	41	65.1%	58	53.2%	
V stage	V0	13	28.3%	11	17.5%	24	22.0%	
	V1	14	30.4%	10	15.9%	24	22.0%	.030
	Vx	19	41.3%	42	66.7%	61	56.0%	
Charlson Comorbidity Score	6	32	69.6%	44	69.8%	76	69.7%	
	7	7	15.2%	13	20.6%	20	18.3%	
	8	6	13.0%	4	6.3%	10	9.2%	.336
	9	0	0.0%	2	3.2%	2	1.8%	
	10	1	2.2%	0	0.0%	1	0.9%	
Oncological resection/Surgery	Yes	35	76.1%	37	58.7%	72	66.1%	.059
	No	11	23.9%	26	41.3%	37	33.9%	
No. of CTX lines	1	11	23.9%	17	27.0%	28	25.7%	
	2	23	50.0%	34	54.0%	57	52.3%	
	3	11	23.9%	11	17.5%	22	20.2%	.587
	4	0	0.0%	1	1.6%	1	0.9%	
	5	1	2.2%	0	0.0%	1	0.9%	
Biological	Yes	21	45.7%	26	41.3%	47	43.1%	.648
	No	25	54.3%	37	58.7%	62	56.9%	
CTX regimen sequence	FOLFOX- > FOLFIRI	30	65.2%	27	42.9%	57	52.3%	
	FOLFIRI- > FOLFOX	3	6.5%	13	20.6%	16	14.7%	.044
	FOLFOX	10	21.7%	13	20.6%	23	21.1%	
	FOLFIRI	3	6.5%	10	15.9%	13	11.9%	
	Total	46	100.0%	63	100.0%	109	100.0%	

further chemotherapy, i.e. number of lines and regimens. Again, a two-sided *p*-value of 0.05 was considered to indicate statistical significance. Hazard ratios and corresponding 95% confidence intervals (CI) were calculated and considered statistically significant when the CI excluded 1. 0. All analyses were performed using IBM SPSS Statistics, version 23.0.

Results

Reduction of chemotherapy dosage

Among 109 patients 67% (73/109) of the patients received both (FOLFOX and FOLFIRI) regimens sequentially. The majority 78% (57/73) began with FOLFOX and changed during the course of disease to FOLFIRI, 21% (23/109) of the patients received exclusively FOLFOX, whereas FOLFIRI chemotherapy regimens were used exclusively in 12% (13/109) of patients. 43% (47/109) received additional treatment with a biological agent.

Upon discretion of the treating physician, in most cases dosage was reduced to 80% of the protocol dosage. We therefore used reduction to 80% of protocol dosage as a cut off to investigate the clinical effectiveness of standard chemotherapy in patients receiving reduced dosage.

In 42% (46/110) of our patients a dose reduction to 80% or less was performed during the course of chemotherapy. Those 46 patients who received a dose reduction, received in average 14 chemotherapy cycles in our clinic (Mean: 13.7 cycles; + − 12.3 cycles). Patients continuing on protocol chemotherapy doses, received 10.5 cycles (stdv 10.8, $p = 0.149$). In more detail, the majority of chemo cycles (72%, median: 81%; stdv: 27%) in these patients were applied with reduced dosage. Average cumulative dosage and dose intensities for 5-Flourouracil, Irinotecan and Oxaliplatin were calculated for full dosage and dose reduction subgroups (Table 2). As expected, relative dose intensities were significantly different. Even though a trend to a higher cumulative dosage can be observed, t-test revealed no significant differences for cumulative dosages.

Dose reduction in subgroups – analysis of distribution

In preparation to compare survival rates, we compared the distribution of the co-variables age, sex, TNM status, grading, lymph vessel invasion, vein invasion, Charlson score for comorbidities, surgery and further chemotherapy, i.e. number of lines and regimens in the dose reduction and full dosage group.

No significant difference was seen except for lymph vessel and vein invasion, CTX regimen and adverse side effects, when Chi-square test for independence was performed. As expected, subgroups of patients suffering from severe symptoms/side effects related to chemotherapy such as diarrhea ($p < 0.001$), PNP ($p < 0.001$), or neutropenia ($p = 0.02$) received dose reduction almost exclusively. Detailed summary of the distribution of dose reductions in different subgroups is given in Table 1.

Reasons for dose reduction

The most common reason for dose reduction of chemotherapy was neutropenia (30%). Other common side effects leading to dose reduction were polyneuropathy (16%) and diarrhea (14%). Polyneuropathy was generally due to treatment with Oxaliplatin (10 patients, 14%). Diarrhea was observed at similar rates in both Oxaliplatin (6/48 patients, 13%) and Irinotecan treated patients (4/30 patients, 13%). Less common reasons for dose reduction included mostly symptom-related causes, hyperemesis, worsening of general condition, hand foot syndrome and mucositis. A complete overview of the reasons for dose reduction of chemotherapy is shown in Table 3.

Table 2 Dose intensity and cumulative dosage for the dose-reduction and full dosage subgroups

	Substance	Dose group	Mean in mg	standard deviation	*p*-value
Cumulative Dosage	5-Fluorouracil	100%	54,934	55,617	0.42
		< 80%	64,321	61,824	
	Oxaliplatin	100%	1046	852	0.34
		< 80%	1245	976	
	Irinotecan	100%	2861	3295	0.77
		< 80%	3099	3296	
Dose intensity	5-Fluorouracil	100%	5383	1450	0.002
		< 80%	4547	1178	
	Oxaliplatin	100%	173	31	0.03
		< 80%	156	35	
	Irinotecan	100%	311	74	0.04
		< 80%	274	65	

P-values were calculated with t-test

Table 3 Reasons for dose reduction

Distribution of adverse effects	N	%
Leukopenia	20	30%
Polyneuropathy	11	16%
Diarrhea	10	14%
Worsening of general condition	6	9%
Hand-foot-syndrome	5	7%
Thrombocytopenia	5	7%
Hyperemesis	3	4%
Elevated bilirubin	2	3%
Deterioration of kidney function	1	1%
Mucositis	1	1%
Not specified	5	7%

Survival in dose reduced versus full dose 5-FU based regimens

In terms of tumor-specific survival, we did not observe any differences between patients receiving full dose and reduced dose chemotherapy (Log Rank, $p = 0.430$) (Fig. 1). Median survival for patients receiving full dosage was 13.0 months (Mean 19.1), for patients with dosage reduction 14.9 months (Mean 21.2). Two-year survival was 19.5% (full dosage) vs 35.8% (reduced dosage). Three-year survival rate of patients with full dose and reduced dose chemotherapeutic treatment was 19.5% and 9.1%. A univariable Cox regression rendered a hazard ratio of 0.841 (95% CI 0.547–1.294; $p = 0.431$) for the dose reduction group versus full reduction. After adjustment for age, sex, TNM status, grading, lymph vessel invasion, vein invasion, Charlson score for comorbidities, surgery and further chemotherapy, i.e. number of lines and regimens in a multivariable Cox regression analysis the hazard ratio for patients with dose reduction was 0.861 (95% CI 0.492–1.506; $p = 0.600$) Table 4.

Efficacy – subgroup analysis

We performed a subgroup analysis to identify potential subgroups in which reduction of chemotherapy might be beneficial or harmful. Table 5 summarizes survival in several subgroups with respect to dose reduction. In most subgroups, no significant differences in survival were observed. Unexpectedly, in the stage N2 lymph node subgroup with 41 patients dose reduction was associated with improved survival (Log Rank $p = 0.024$).

Discussion

Over the past decades a considerable progress has been made in the management of colorectal cancer by medical oncologists. The studies leading to these advances were predominantly performed on young and healthy populations; hence the common practice of dose reduction in elderly or frail patients was not a primary issue. Thus, these studies were mainly performed on a subgroup not suffering from relevant co-morbidities and being in good performance status.

In recent years, the need to investigate real life populations and the common practice of dose reduction has been recognized by the scientific community. However, (retrospective) studies investigating the effects of dose reduction in a palliative setting have been published but are still sparse. In 2001, a retrospective analysis of

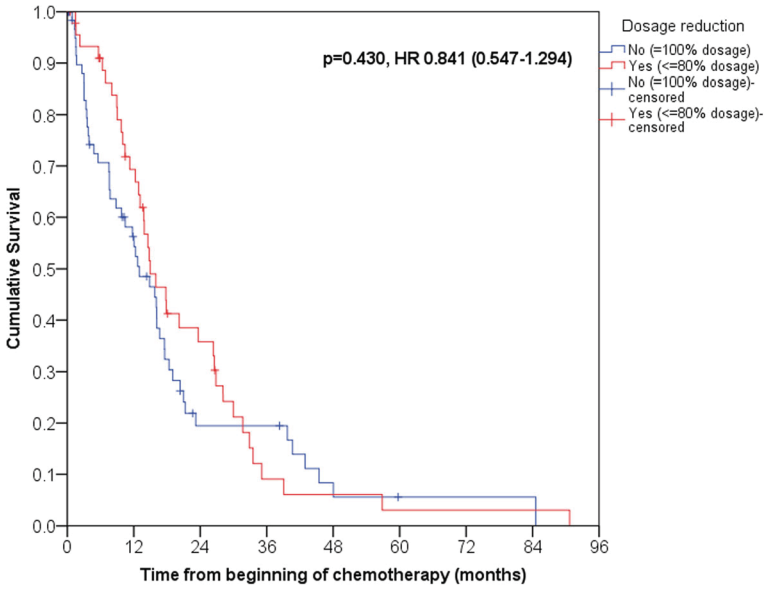

Fig. 1 Kaplan Meier analysis of survival in patients with reduced and full dosage of standard chemotherapy back bone (FOLFIRI, FOLFOX). Survival analysis showed no difference in survival ($p = 0.430$, Log Rank)

Table 4 Results of univariable and multivariable Cox-regression for survival according to dosage reduction

				95% CI for HR	
		p value	Hazard ratio	Lower	Upper
Univariable Cox-regression					
Dosage reduction	No		1.000		
	Yes	.431	.841	.547	1.294
Multivariable Cox-regression					
Dosage reduction	No		1.000		
	Yes	.600	.861	.492	1.506
Age	continuous	.118	.981	.958	1.005
Sex	Male		1.000		
	Female	.446	1.264	.692	2.310
T stage	T3		1.000		
	T4	.229	1.541	.761	3.117
	TX	.890	.916	.265	3.170
N stage	N0		1.000		
	N1	.981	.988	.345	2.824
	N2	.713	1.225	.415	3.615
	NX	.927	.922	.161	5.266
Grading	G2		1.000		
	G3	.302	1.354	.761	2.407
	GX	.458	.576	.134	2.474
L stage	L0		1.000		
	L1	.726	1.215	.408	3.620
	LX	.289	.469	.116	1.901
V stage	V0		1.000		
	V1	.872	.920	.334	2.531
	VX	.164	2.620	.675	10.165
Charlson Score	6		1.000		
	7	.750	.892	.443	1.797
	8	.909	.954	.426	2.138
	9	.609	1.605	.262	9.822
Oncological resection/Surgery	No		1.000		
	Yes	.227	.483	.149	1.570
No of CTX lines	continuous	.797	1.053	.712	1.556
Biological CTX	No		1.000		
	Yes	.178	.663	.364	1.207
CTX regimen	FOLFOX- > FOLFIRI		1.000		
	FOLFIRI- > FOLFOX	.691	1.177	.527	2.627
	FOLFOX	.001	3.601	1.656	7.827
	FOLFIRI	.921	.945	.309	2.892

patients with Stage II-III colon cancer demonstrated that in an adjuvant setting 5-FU based chemotherapy may be safely administered in the elderly, but this study did not elaborate the dose reduction needed [12]. In 2011 Langley et al. published the Focus II study, a first randomized controlled trial including only the frail and old patients with colorectal cancer. This study incorporated primary dose reduction as the standard treatment for all treatment arms. However, it was not designed to investigate whether dose

Table 5 Subgroup analysis. Comparison of the survival after dose reduction versus full dosage in different subgroups analyzed by Kaplan-Meier procedure and Log-Rank test (Chemotherapy (CTX))

		Dosage reduction		Log-rank
		Yes (≤ 80%)	No (100%)	
		Mean (Median) survival in months		*p*-value
Age	< 65	22.0 (14.6)	17.1 (12.0)	.170
	≥65	18.8 (14.9)	21.2 (15.8)	.764
Sex	Male	23.5 (16.0)	18.6 (14.9)	.339
	Female	17.2 (13.1)	17.4 (12.7)	.170
T stage	T3	27.8 (26.4)	25.8 (14.9)	.525
	T4	15.6 (13.8)	14.1 (12.7)	.960
	Tx	24.3 (10.4)	14.1 (7.7)	.273
N stage	N0	25.9 (23.6)	30.5 (14.9)	.851
	N1	20.7 (13.9)	24.1 (12.3)	.669
	N2	21.3 (26.4)	13.3 (16.7)	.024
	Nx	20.4 (16.0)	13.2 (7.7)	.294
Grading	G2	24.1 (17.8)	22.0 (16.1)	.570
	G3	17.5 (14.5)	10.6 (10.4)	.062
	Gx	26.8 (26.8)	25.5 (3.0)	.808
L stage	L0	19.7 (17.8)	30.7 (21.0)	.541
	L1	23.3 (13.9)	16.9 (17.5)	.485
	Lx	18.1 (14.8)	16.3 (12.0)	.561
V stage	V0	28.8 (23.6)	26.9 (21.0)	.828
	V1	20.3 (13.9)	15.7 (16.7)	.311
	Vx	16.9 (14.5)	15.9 (11.8)	.671
Charlson Score for Comorbidities	6	20.6 (14.8)	19.6 (12.0)	.424
	7–10	23.3 (15.0)	19.6 (16.1)	.632
Oncological resection/Surgery	Yes	22.1 (14.9)	23.8 (17.5)	.997
	No	19.6 (12.9)	12.9 (7.6)	.301
No. of CTX lines	1	10.1 (10.4)	8.4 (3.0)	.565
	2	23.1 (17.9)	23.5 (15.8)	.896
	3	24.7 (23.6)	16.6 (12.3)	.238
Biological CTX	Yes	29.4 (26.8)	23.5 (11.8)	.371
	No	13.2 (13.1)	14.6 (14.9)	.823
CTX regimen sequence	FOLFOX- > FOLFIRI	22.0 (20.2)	26.5 (17.5)	.425
	FOLFIRI- > FOLFOX	12.3 (12.3)	16.4 (14.9)	.631
	FOLFOX	9.7 (12.9)	7.7 (3.6)	.829
	FOLFIRI	62.3 (90.7)	13.0 (3.9)	.300

reduction itself could be performed if required without affecting PFS or OS [13].

In order to evaluate whether dose reduction has an effect on survival in patients with advanced colorectal cancer and suffering from side effects under standard treatment dose, we performed this retrospective analysis of such patients in our outpatient clinic. In our current study, we chose a cutoff for dose reduction of 80% since in our experience a dose reduction to 80% is a commonly performed reduction in case of adverse reactions. Since only very few patients received further dose reduction ($n = 4$), we chose not to include these patients as a separate group with dose reduction of ≤50% but rather included these patients in the group of patients with reduction of chemotherapy dosage. Thus, the overall average dose reduction in this patient group was even more than 20%. In these patients, a dose reduction was applied to the

majority of cycles (72%), emphasizing that this group predominantly received a reduced chemotherapy dosage throughout the course of therapy.

In daily clinical routine, clinicians are more likely to reduce the dose of elderly patients. Therefore, one might assume that in the elderly (> 65 years) in our collective dose reduction would be more common. However, dose reduction was evenly distributed among younger and elderly patients and thus independent of age. Also, additional subgroup analysis (T-stage, N-stage, M-stage, gender, and chemotherapy regimen) showed an even distribution of dose reduction.

Since clinical trials showed the effectiveness of per protocol chemotherapy, in theory a reduced dosage of chemotherapy would be expected to affect survival, which has also been confirmed by several publications for other entities [9]. It is therefore common belief, that dose reduction should be avoided. Additionally, clinicians choose a dose reduction often due to symptom-related causes or deterioration of laboratory findings. Many of these reasons (recurring neutropenia, deterioration of general condition) are associated with a poor clinical outcome. Therefore, one might assume, that dose reduction is more common in patients with clinical features suggesting a poor prognosis. Interestingly, statistical analysis of our data showed that a moderate dose reduction does not affect survival significantly.

In a recently published manuscript, the influence of the relative dose intensity (RDI, of adjuvant 5FU and Oxaliplatin combination treatment in veterans with Stage III colon cancer had been further investigated. Aspinalli et al. showed that a major reduction of RDI (< 70%) to be associated with worse overall survival in this patient group [14]. In addition to the bias of the patient group which consisted mainly of male veterans, there was a bias towards dose reduction in the frail and elderly. In comparison with our study also the magnitude of dose reduction was more pronounced.

When Oxaliplatin was introduced to the treatment of CRC, a retrospective analysis of three phase II studies of pretreated colorectal cancer showed that higher dose intensity leads to improved survival [15]. In contrast to our study, these study populations had been pretreated and in two out of the three studies patients' inclusion age was limited to younger patients. In another more recent study, Nakayama et al. showed that a dose reduction in metastasized CRC led to poorer survival of the respective (Irinotecan) patient group. A comparison with our study population is difficult, since per protocol Irinotecan dosage in Japan is already 12% lower than in the western countries and further dose reduction adds up to even more pronounced dose reductions. For the patient group receiving Oxaliplatin only PFS was significantly associated to RDI [16].

Nevertheless, in clinical practice, patients often experience side effects and need dose reduction. For these patients, our data in treatment-naive patients suffering from stage IV colorectal cancer, suggest that a moderate dose reduction does not necessarily result in less efficacy. Until now, only limited data were reported on this issue, which we believe to be of high clinical relevance. Thus, we suggest further randomized studies potentially leading to more personalized treatment strategies depending on tolerance of treatment and co-morbidities and a more side effect oriented approach on chemotherapy dosing.

Conclusion

In our group of patients with colorectal cancer treated in a palliative setting, the need for a moderate reduction of chemotherapy due to side effects has no measurable effect on survival. This may be in part due to better adaption to interindividual pharmacokinetics and to a longer treatment of patients with reduced chemotherapy dosage if side effects cause dose reduction.

Additional file

Additional file 1: Table S1. Summary of comorbidities. (DOCX 13 kb)

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Comment

Part of this study was presented at the ASCO GI Meeting 2017 San Francisco. Permission was obtained for final publication [17].

Authors' contributions

SM, MG, AT carried out the primary data analysis. SM, AT, SFF, CS, HJS, CO, ES, PW, MV, WH, ME, MR, PF, MKS were responsible for the treatment of patients with CRC at the Regensburg University Medical Center. SM, AT, MG, PW and HJS helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Due to the analysis of data from a clinical cancer registry, no ethics approval was necessary. This was confirmed by the Ethics Committee at the Regensburg University, Regensburg, Germany.

Competing interests

The authors declare that they have no competing interest.

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