Aus der Neurochirurgischen Klinik und Poliklinik

Klinikum der Ludwig-Maximilians-Universität München

Stellenwert und Komplikationsraten stereotaktischer Probenentnahmen in der Gliomdiagnostik und -therapie

Dissertation

zum Erwerb des Doktorgrades der Medizin

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität München

vorgelegt von

Sophie Jutta Theresia Katzendobler

aus

München

Jahr

2023

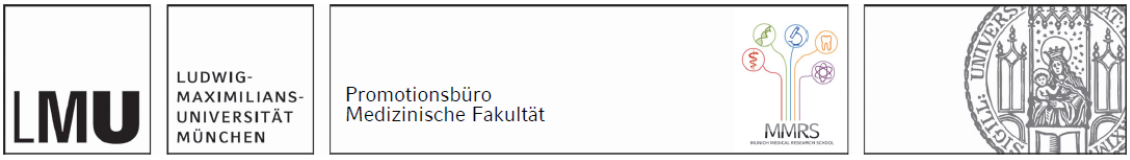
Mit Genehmigung der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

Erster Gutachter:	Prof. Dr. Jörg-Christian Tonn
Zweiter Gutachter:	Prof. Dr. Maximilian Niyazi
Dritter Gutachter:	Prof. Dr. Nathalie Albert
ggf. weitere Gutachter:	_____
	_____
Mitbetreuung durch den promovierten Mitarbeiter:	_____
Dekan:	Prof. Dr. med. Thomas Gudermann

Tag der mündlichen Prüfung: 25.04.2023

Affidavit



Eidesstattliche Versicherung

Katzendobler, Sophie Jutta Theresia

Name, Vorname

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Titel:

Stellenwert und Komplikationsraten stereotaktischer Probenentnahmen in der Gliomdiagnostik und -therapie

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 25.04.2023
Ort, Datum

Sophie Katzendobler
Unterschrift Doktorandin bzw. Doktorand

Inhaltsverzeichnis

Abkürzungsverzeichnis

CTCAE	-	Common Terminology Criteria for Adverse Events
IDH	-	Isocitrat-Dehydrogenase
MGMT	-	O6-Methylguanin-DNA-Methyltransferase
PCV	-	Procarbazine, CCNU/Lomustin und Vincristin
TERT	-	Telomerase Reverse Transcriptase
Wt	-	Wildtyp
1p/19q-codel	-	Kombinierter Verlust der Chromosomen 1p und 19q

Publikationsliste

Publikation I

Diagnostic Yield and Complication Rate of Stereotactic Biopsies in Precision Medicine of Gliomas.

Katzendobler S, Do A, Weller J, Dorostkar MM, Albert NL, Forbrig R, Niyazi M, Egensperger R, Thon N, Tonn JC, Quach S.

Front Neurol. 2022 Mar 30;13:822362.

doi: 10.3389/fneur.2022.822362.

PMID: 35432168

Publikation II

Limited efficacy of temozolomide alone for astrocytoma, IDH-mutant, CNS WHO grades 2 or 3.

Weller J, Katzendobler S, Blobner J, Thiele F, Becker H, Quach S, Egensperger R, Niyazi M, Suchorska B, Thon N, Weller M, Tonn JC.

J Neurooncol. 2022 Sep 16.

doi: 10.1007/s11060-022-04128-y.

PMID: 36112301

1. Beitrag zu den Veröffentlichungen

1.1 Beitrag zu Publikation I

In der Publikation mit dem Titel „Diagnostic Yield and Complication Rate of Stereotactic Biopsies in Precision Medicine of Gliomas" wird der Stellenwert der stereotaktischen Biopsie in der Diagnostik und Therapie hirneigener Tumore in einem Kollektiv von 1‘214 Patienten über einen Zeitraum von 5 Jahren untersucht.

Mein Beitrag zu dieser Publikation beinhaltete in einem ersten Schritt die Erstellung der Datenbank. Hierfür führte ich nach positivem Ethikvotum zunächst eine interne Suchabfrage basierend auf ICD-10- (International Statistical Classification of Diseases and Related Health Problems) sowie OPS-Codes (Operationen- und Prozedurenschlüssel) durch. Eingeschlossen wurden Patienten mit der Verdachtsdiagnose eines Glioms, die zu einem beliebigen Zeitpunkt im Behandlungsverlauf mindestens eine stereotaktische Probenentnahme erhalten hatten. Im Anschluss an die Durchsicht der elektronischen Patientenakten aller Patienten, die eine stereotaktische Probenentnahme erhalten hatten, identifizierte und inkludierte ich 1‘214 Patienten zur weiteren Datenanalyse. In meinem Aufgabenbereich lag im Anschluss die Erstellung einer Excel-basierten Datentabelle, in der patientenbezogene, histologische, molekulargenetische und klinische Informationen der Patienten erfasst wurden. Berücksichtigt wurden von mir beispielsweise folgende Parameter: Alter, Geschlecht, Datum der Intervention, klinischer Zustand (Karnofsky Index), Menge des gewonnenen Probematerials, histopathologische Diagnose sowie molekulargenetische Charakteristika, Lokalisation der Gliome, Sequenzierungsdaten, periprozedurale Komplikationen sowie Dauer und Kosten der Prozedur und des Klinikaufenthalts. Im Anschluss analysierte ich die Daten, indem ich die diagnostische Genauigkeit anhand der Fehlbiopsierate betrachtete. Die Komplikationsraten wurden von mir anhand der Common Terminology Criteria for Adverse Events (CTCAE Version 5) untersucht, indem der postoperative, klinische Zustand erfasst und die postoperative CT-Bildgebung analysiert wurden. Die Erstellung der Grafiken und Tabellen des Manuskripts erfolgte durch mich (PMCID: PMC9005817; Figure 1 und 2; Tabelle 1-5).

In Rahmen der Manuskriptverfassung habe ich die Abschnitte „Introduction", „Materials and Methods" und „Results" erstformuliert und gemeinsam mit den Co-Autoren überarbeitet. Die im Rahmen des Review-Verfahrens gestellten Nachfragen wurden durch mich beantwortet und Anpassungsvorschläge eingearbeitet.

1.2 Beitrag zu Publikation II

In der Publikation mit dem Titel „Limited efficacy of temozolomide alone for astrocytoma, IDH-mutant, CNS WHO grades 2 or 3" wird die Wirksamkeit der Temozolomidtherapie in Astrozytomen mit Mutationen des Isozitrat-Dehydrogenase-Gens (IDH) im Vergleich zu anderen, postoperativen Strategien untersucht. Nach positivem Ethikvotum wurde eine interne Suchanfrage an unser Klinikinformationssystem sowie die Datenbanken der Klinik für Neuropathologie gestellt. Nachdem alle Patienten mit IDH-mutierten Astrozytomen der WHO-Grade 2 oder 3 identifiziert wurden, stellte ich eine Datentabelle zusammen und erfasste folgende Parameter für die gesamte Patientenkohorte: Alter, Geschlecht, Geburtsdatum, Datum der Erstdiagnose, klinischer Zustand (Karnofsky Index), Art der histologischen Sicherung, histologische Diagnose, molekulargenetische Charakteristika, Ausmaß der Tumorresektion, postoperative Therapiestrategie, Anzahl absolvierter Chemotherapiezyklen und/oder Dosis der applizierten Strahlentherapie, Komplikationsraten, Progressdaten, Progresstherapie, Sterbedatum und Follow-Up-Daten. Um die initiale Tumorgröße als prognostischen oder prädiktiven Faktor zu analysieren, segmentierte ich sämtliche verfügbaren, initialen MRT-Datensätze (T1-Sequenzen mit Kontrastmittel sowie T2-Sequenz) mithilfe der Smartbrush Software (Elements Smartbrush, Brainlab Gruppe, München, Deutschland). Im Rahmen des Review-Verfahrens wurden weitere, molekulargenetische Eigenschaften, nämlich der Methylierungsstatus des MGMT-Gens der Tumore, sowie Details zur Therapie bei Progression nachgefordert. Diese wurden von mir ausgewertet und in das Manuskript eingearbeitet.

2. Einleitung

2.1 Hintergrund

Gliome sind hirneigene Tumore, die nach der aktuellen Klassifikation der Weltgesundheitsorganisation (WHO, World Health Organization) aus dem Jahr 2021 eingeteilt werden. Der Begriff „Gliom“ umfasst eine heterogene Gruppe von Neoplasien. Manche Gliome, beispielsweise pilozytische Astrozytome WHO Grad 1, werden häufig im jungen Erwachsenen- oder Kindesalter erstdiagnostiziert und sind je nach Lokalisation operativ potenziell heilbar (1). Glioblastome hingegen, deren altersadjustierte Inzidenzrate bei 0.59 bis 3.69 Personen pro 100‘000 liegt, verlaufen meist letal (2, 3). Die 5-Jahres-Überlebensrate liegt bei unter 5% (4). Oligodendrogliome und Astrozytome mit einer Mutation des Isozitrat-Dehydrogenase Gens 1 oder 2 (IDH) können unterschiedliche, histologische Tumorgrade aufweisen. Der klinische Verlauf ist variabel. Diese Tumore sprechen initial meist auf Chemotherapien und Strahlentherapie an, rezidivieren jedoch häufig und sind trotz multimodaler, wiederholter Therapien meist lebenszeitlimitierend (5). Bildmorphologisch sind Gliome in der kontrastmittelgestützten MRT-Untersuchung (Magnetresonanztomographie) auch durch erfahrene Neuroradiologen oder -chirurgen und künstliche Intelligenz bislang nicht eindeutig klassifizierbar (6, 7). Die Standardtherapie in der Behandlung hirneigener Tumore der CNS WHO Grade 2, 3 und 4 beinhaltet in einem ersten Schritt die Tumorresektion, falls diese unter Berücksichtigung des Patientenwunschs, des klinischen Zustands, der Tumorausdehnung und -lokalisation mit einem niedrigen Risiko vertretbar ist (8). Falls die Tumorresektion kontraindiziert ist, sollte eine Biopsie zur Diagnosefindung durchgeführt werden. Eine histologische Sicherung wird im Falle einer neu diagnostizierten, tumorverdächtigen Signalalteration in der MRT-Untersuchung dringlich empfohlen, da insbesondere die Molekulargenetik, aber auch die Histologie, die weiterführende Therapie bestimmen. Ein entscheidender, molekulargenetischer Parameter ist der Mutationsstatus des Isozitrat-Dehydrogenase Gens 1 und 2. Liegt eine Punktmutation in einem dieser beiden Gene vor, handelt es sich entweder um ein Oligodendrogliom oder ein Astrozytom. Die Differenzierung dieser beiden Entitäten erfolgt über die Bestimmung des 1p/19q-Status. Liegt eine Co-Deletion der beiden Chromosomenabschnitte vor, handelt es sich um ein Oligodendrogliom (1). Die Histologie entscheidet über die Einteilung in den jeweiligen WHO-Grad. Wenn keine IDH-Mutation vorliegt, handelt es sich möglicherweise um ein Glioblastom. Bildmorphologisch unscheinbare Läsionen ohne pathologische Kontrastmittelaufnahme können frühzeitig diagnostizierte Glioblastome sein (9-11). Eine frühe Behandlung mittels Resektion und Radiochemotherapie mit konkomitanter und adjuvanter Temozolomidtherapie ist für die lokale Kontrolle und Prognose der PatientInnen notwendig (1, 8, 12). Molekulargenetisch wird die Diagnose eines Glioblastoms neben einem IDH-wildtyp-Status im Falle einer Mutation im Promoter des Telomerase-Reverse-Transkriptase-Gens (TERT), einer Amplifikation des „Epidermal Growth Factor Recptor"

(EGFR) oder eines Gewinns des Chromosoms 7 kombiniert mit einem Verlust des Chromosoms 10 gestellt (8).

2.2 Biopsien in der Hirntumordiagnostik

Bei Kontraindikationen für eine Tumorresektion sollte die histologische Sicherung einer verdächtigen, intrazerebralen Läsion durch eine Biopsie erfolgen (8, 13-15). Es existieren unterschiedliche Biopsie-Techniken, die sich im Wesentlichen in rahmen-basierte und nicht-rahmen-basierte Verfahren unterteilen lassen. Rahmen-basierte, stereotaktische Probenentnahmen beruhen auf der Durchführung einer intraoperativen Bildgebung, meist mittels kontrastmittelgestützter Computertomographie (CT) des Schädels, das mit der präoperativ angefertigten MRT-Untersuchung des Patienten fusioniert wird (16). Der Rahmen fixiert hierbei den Kopf mit Dornen, von denen 2 im Bereich des Hinterkopfes und 2 frontal angebracht werden, sodass eine Achsenrotation innerhalb des Rahmens nicht möglich ist. Der Vorteil dieses Verfahrens ist die intraoperative, bildmorphologische Darstellung des Gehirnparenchyms inklusive dessen Blutversorgung und die Berechnung einer Trajektorie im Submillimeterbereich, die weder mit Gefäßen noch eloquenten Arealen interferiert. Rahmenlose Biopsietechniken beruhen auf Neuronavigationstechniken oder visueller, makroskopischer Identifikation pathologischen Gewebes, von dem freihändig oder auch mit geschienten Systemen Proben entnommen werden. Als Vorteile dieser Techniken werden insbesondere die schnelle Durchführung unter Verzicht einer intraoperativen Bildgebung und ebenfalls niedrige Komplikationsraten genannt (17, 18). Biopsien sind im Vergleich zur operativen Tumorresektion mit einem niedrigeren periprozeduralen Risiko assoziiert (13-15).

Biopsien kommen im klinischen Alltag nicht nur in der initialen Diagnostik zur Anwendung, sondern spielen auch in der Rezidivdiagnostik eine wichtige Rolle. Eine mögliche Folge der Bestrahlung zentralnervöser Tumore ist die Strahlennekrose, auch Pseudoprogress genannt, die MR-morphologisch oft nicht sicher von einem Tumorprogress unterschieden werden kann (19, 20). Nuklearmedizinische Verfahren mittels aminosäure-basierter Positronen-Emissions-Tomographie (PET) (O-(2-18Ffluorethyl)-L-tyrosine (^{18}F-FET PET)) kommen in der Differenzierung strahlentherapeutisch induzierter Veränderungen von Tumorrezidiven zur Anwendung (21, 22). Diese Untersuchungen helfen in der differenzialdiagnostischen Einordnung unklarer, MR-morphologischer Befunde und in der Planung der Probenentnahmen. Sie können zudem intraoperativ mit der MRT- und CT-Bildgebung fusioniert werden (16).

Patienten mit WHO Grad 2 bis 4 Gliomen, ob IDH-wildtyp oder IDH-mutiert, erleiden typischerweise wiederholte, zunehmend aggressivere und therapierefraktäre Rezidive. Zielgerichtete Therapien gewinnen in der Neuroonkologie zunehmend an Bedeutung. Um molekulare Ziele mittels Sequenzierung und Methylomanalysen zu identifizieren, ist die Probegewinnung unerlässlich (23, 24). Eine sichere, wiederholbare, minimalinvasive Methode zur Gewebesicherung ist für dieses Patientenkollektiv potenziell wertvoll. In dieser Promotionsarbeit wird der diagnostische Mehrwert stereotaktischer Biopsien

sowohl im Rahmen der Erstmanifestation als auch im weiteren, klinischen Verlauf mit einem standardisierten Verfahren an einem neuroonkologisch spezialisierten Zentrum untersucht. Neben der diagnostischen Wertigkeit wird ein besonderes Augenmerk auf die Komplikationsraten des Verfahrens gelegt.

2.3 Therapie

Die Behandlungsempfehlung für Patienten mit Gliomen erfolgt unter Berücksichtigung des klinischen Zustands des Patienten, der Lokalisation und Größe des Tumors, des histologischen Tumorgrads und der Molekulargenetik. In der Behandlung IDH-mutierter Gliome wird zwischen Patienten mit niedrigem oder hohem Risiko für einen ungünstigen, klinischen Verlauf unterschieden (8, 25, 26). Ein niedriges Risiko weisen junge Patienten ohne neurologische Defizite, deren Gliom operativ vollständig entfernt werden konnte, auf. In diesen Fällen kann auf eine weiterführende Therapie verzichtet werden und es erfolgt eine Verlaufsbeobachtung mit regelmäßigen MRT-Untersuchungen. Eine Hochrisikokonstellation liegt im Umkehrschluss vor, wenn der Patient bei Diagnosestellung älter als 40-45 Jahre ist, postoperativ residuales Tumorvolumen verbleibt oder neurologische Defizite über eine symptomatische Epilepsie hinaus vorhanden sind. Sind eine oder mehrere dieser Kriterien erfüllt, wird eine weiterführende Therapie über die initiale Tumorresektion hinaus empfohlen, die sich nach Tumorentität und histologischem Grad richtet (8). In der Behandlung IDH-mutierter Astrozytome sollte in einer Hochrisikokonstellation eine Radiotherapie, gefolgt von einer Temozolomid-Therapie oder einer sequenziellen Kombinationstherapie der Chemotherapeutika Procarbazin, CCNU und Vincristin (PCV) durchgeführt werden. Die Radiotherapie ist der Radiochemotherapie unterlegen (8, 25, 26). Da eine alleinige Chemotherapie mit Temozolomid oder PCV in anaplastischen Gliomen keine signifikanten Unterschiede im Vergleich zu einer alleinigen Strahlentherapie zeigte und Temozolomid möglicherweise in der Subgruppe der IDH-mutierten Astrozytome WHO Grad 3 sogar mit einem kürzeren progressionsfreien Überleben assoziiert ist, geht man in logischer Konsequenz auch von einer Unterlegenheit der Chemotherapie gegenüber einer Radiochemotherapie aus (27-29). Junge Patienten mit ausgedehnten, inoperablen Tumoren können dennoch initial leitliniengerecht mittels alleiniger Chemotherapie behandelt werden, um Nebenwirkungen einer großflächigen Strahlentherapie zu vermeiden (8). In der Literatur finden sich wenige klinische Daten zur Langzeitprognose dieser Patientengruppe.

In den letzten Jahren haben mehrere Studien potenzielle, schädliche Nebenwirkungen durch das alkylierende Chemotherapeutikum Temozolomid beschrieben, die in Rezidivtumoren mittels Sequenzierung festgestellt wurden (30-32). Mechanistisch kann durch die Temozolomid-Therapie eine Hypermutation induziert werden. Diese führt möglicherweise in manchen Patienten zu einer Dysfunktion der DNA-Fehlpaarungsreparatur, die aggressivere Rezidiven zur Folge haben kann (31). Temozolomid wird, zusammen mit der Radiotherapie, in der Glioblastom-Therapie angewendet. Da Glioblastome trotz multimodaler Therapie bei einem medianen Überleben von etwa 15 Monaten nach Diagnosestellung mit einer sehr schlechten

Prognose vergesellschaftet sind, fällt hier ein schädlicher Langzeiteffekt möglicherweise nicht gleichermaßen ins Gewicht (12, 33, 34). IDH-mutierte Astrozytome und Oligodendrogliome zeichnen sich jedoch typischerweise durch einen langen, klinischen Verlauf aus. In diesem Kontext wird in dieser Promotionsarbeit in der Subgruppe der WHO Grad 2 und 3 Astrozytome, die in unserem Zentrum diagnostiziert und behandelt wurden, die Langzeitprognose nach Temozolomidtherapie analysiert und mit anderen Therapiestrategien verglichen.

3. Zusammenfassung

In dieser Promotionsarbeit wurde die Sicherheit und diagnostische Wertigkeit einer standardisierten Biopsietechnik anhand einer retrospektiven, konsekutiven Kohorte von 1‘214 Patienten in einem Zeitraum von 5 Jahren mit der Verdachtsdiagnose eines Glioms untersucht. Die Biopsietechnik beruht auf einem Rahmensystem mit einer intraoperativ angefertigten CT-Bildgebung, die mit der präoperativ durchgeführten MRT-Untersuchung und gegebenenfalls ^{18}FET-PET-Untersuchung fusioniert werden kann. Komplikationsraten der Prozedur wurden retrospektiv anhand der „Common Terminology Criteria for Adverse Events“ Version 5 (CTCAE) erfasst. In einem zweiten Projekt wurde die Wirksamkeit und die Langzeitprognose unterschiedlicher Therapiestrategien in der Behandlung von Patienten mit IDH-mutierten Astrozytomen der WHO-Grade 2 oder 3 untersucht. Hier wurden Patienten eingeschlossen, deren Erstdiagnose im Rahmen einer stereotaktischen Probenentnahme oder Tumorresektion gestellt wurde. Der Fokus der Überlebenszeitanalysen wurde auf die alleinige Temozolomid-Therapie gelegt, da mehrere Publikationen eine mögliche, schädliche Wirkung im Sinne aggressiverer Rezidive und eines verkürzten Überlebens durch Temozolomid in IDH-mutierten Gliomen beschrieben haben.

Bei 617 Patienten (51%) wurde die Biopsie im Rahmen einer Erstdiagnose durchgeführt. In 597 Patienten (49%) handelte es sich um Biopsien, die im Rahmen bereits bekannter Gliomerkrankungen durchgeführt wurden und die der differenzialdiagnostischen Einordnung unklarer, bildmorphologischer Befunde oder der Gewinnung Tumormaterials zur molekulargenetischen Analyse dienten. In 99.3% der durchgeführten Biopsien wurde eine MRT-Untersuchung mit der intraoperativ angefertigten, kontrastmittelgestützten CT-Untersuchung fusioniert. In 34.3% der Fälle wurde zusätzlich eine ^{18}FET-PET-Untersuchung zur Biopsieplanung genutzt. In 96.3% der Fälle konnte durch die Biopsie eine integrierte Diagnose inklusive Molekulargenetik, immunhistochemischer Untersuchung und Tumorgradierung gestellt werden. Bei 13 Patienten (1.1%) wurden Gliome diagnostiziert, die sich auch nach der gültigen WHO-Einteilung aus dem Jahr 2021 nicht weiter klassifizieren ließen. Diagnosen, die nicht zu der Familie der Gliome gehörten, wurden in 73 Patienten (6%) gestellt. Schwere Nebenwirkungen („severe adverse events“), beispielsweise revisionsbedürftige Einblutungen oder bleibende, neurologische Defizite, traten in 1.2 % der biopsierten Patienten auf.

In unserer Klinik wurden zwischen den Jahren 2001 und 2019 insgesamt 183 Patienten mit IDH-mutierten Astrozytomen WHO Grad 2 oder 3 behandelt, die in folgende, postoperative Therapiegruppen unterteilt werden konnten: Verlaufsbeobachtung nach Tumorresektion oder stereotaktischer Biopsie („Wait-and-scan-Strategie“) (n=98), Temozolomid-Monotherapie (n=48) oder alleinige Strahlentherapie (n=37). Es zeigte sich ein signifikant längeres progressionsfreies (6.2 versus 3.4 Jahre, p=0.02) und Gesamtüberleben (14.4 versus 10.7 Jahren, p=0.02) bei Patienten, die mittels alleiniger Strahlentherapie behandelt wurden im Vergleich zur alleinigen Temozolomid-Therapie. Patienten, die initial keine Therapie erhalten hatten und lediglich mittels regelmäßigen Ambulanzterminen und MRT-Verlaufskontrollen beobachtet wurden, zeigten ebenfalls

ein signifikant längeres Überleben (nicht erreicht versus 10.7 Jahre, $p<0.01$) bei ähnlichem progressionsfreiem Überleben (4 versus 3.4 Jahre, p=0.65). Ähnliche Ergebnisse zeigten sich in einer Subgruppenanalyse der WHO Grad 2 Astrozytome. Patienten aus der Wait-and-scan-Kohorte, die nach dem ersten Progress Temozolomid erhielten, zeigten ebenfalls ein signifikant kürzeres Überleben als Patienten, die bei Progress mit alternativen Strategien behandelt wurden.

Zusammengefasst konnte in der Promotionsarbeit und den damit einhergehenden Publikationen gezeigt werden, dass stereotaktische Biopsien risikoarm sind und eine hohe diagnostische Wertigkeit aufweisen. Darüber hinaus stützen die Daten eine kritische Beurteilung einer alleinigen Temozolomid-Therapie in der Behandlung IDH-mutierter Astrozytome.

4. Abstract

BACKGROUND: Histopathological and molecular characterization of gliomas is necessary to determine subtype and therapy of the tumor. Tissue sampling through open resection or biopsy is possible. Different biopsy techniques are used when resection is deemed not safely feasible. Postoperative strategies for treatment of isocitrate dehydrogenase (IDH)-mutant astrocytomas are controversial. We first aimed at determining the safety and efficacy of stereotactic biopsies in patients with suspected gliomas overall and secondly investigated outcome of patients with IDH-mutant astrocytomas according to different treatment strategies after diagnosis through stereotactic biopsy or tumor resection: wait-and-scan, radiotherapy or temozolomide. Temozolomide has been reported to be associated with poor outcome in some patients with IDH-mutant astrocytomas.

METHODS: We retrospectively screened our databases for patients that had undergone stereotactic biopsies for suspected glioma between January 2016 and March 2021. Patient characteristics and procedural data were assessed. Complication rates were determined according to the Common Terminology Criteria for Adverse Events Version 5 (CTCAE). Additionally, patients diagnosed with IDH-mutant astrocytomas WHO grade 2 or 3 (n=183), either diagnosed by biopsy or resection, were further investigated for progression-free and overall survival. The cohort was stratified according to the postoperative strategy: wait-and-scan (n=98), radiotherapy (n=37) or temozolomide alone (n=48). Subgroup and matched-pair analyses were conducted.

RESULTS: Within the biopsy cohort of 1’214 individual patients, 617 patients (50.8%) received a biopsy for a newly diagnosed lesion and 597 patients (49.2%) for a suspected recurrence. An integrated diagnosis was possible in 96.3% of all patients. IDH wildtype glioblastoma was the most frequent diagnosis with 596 patients (49.2%) affected, followed by oligodendroglioma WHO grade 2 (n = 109; 9%), astrocytoma WHO grade 3 (n = 108; 8.9%), oligodendroglioma WHO grade 3 (n = 76; 6.3%), and astrocytoma WHO grade 2 (n = 66; 5.4%). Peri-procedural complications CTCAE grade 3 or higher occurred in 1.2% of patients overall with no fatal outcome. In the treatment of IDH-mutant astrocytomas, radiotherapy alone was associated with better overall survival than temozolomide alone (14.4 vs 10.7 years, p=0.02). Patients from the wait-and-scan cohort also lived significantly longer than patients treated with temozolomide (not reached vs 10.7 years, p<0.01). This association was confirmed in subgroup and matched-pair analyses.

CONCLUSION: Image-guided, stereotactic biopsies yield diagnoses with high accuracy and low peri-procedural complication rates initially and during clinical course. The implication of temozolomide chemotherapy alone in IDH-mutant astrocytomas remains uncertain and potential detrimental effects on outcome need to be further evaluated.

5. Publikation I

frontiers in Neurology

ORIGINAL RESEARCH
published: 30 March 2022
doi: 10.3389/fneur.2022.822362

Diagnostic Yield and Complication Rate of Stereotactic Biopsies in Precision Medicine of Gliomas

*Sophie Katzendobler[1], Anna Do[1], Jonathan Weller[1], Mario M. Dorostkar[2], Nathalie L. Albert[3,4], Robert Forbrig[5], Maximilian Niyazi[4,6], Rupert Egensperger[2], Niklas Thon[1], Joerg Christian Tonn[1,4] and Stefanie Quach[1]**

[1] Department of Neurosurgery, University Hospital, LMU Munich, Munich, Germany, [2] Center for Neuropathology and Prion Research, LMU Munich, Munich, Germany, [3] Department of Nuclear Medicine, University Hospital, LMU Munich, Munich, Germany, [4] German Cancer Consortium (DKTK), Partner Site Munich, German Cancer Research Center (DKFZ), Heidelberg, Germany, [5] Institute of Neuroradiology, University Hospital, LMU Munich, Munich, Germany, [6] Department of Radiation Oncology, University Hospital, LMU Munich, Munich, Germany

OPEN ACCESS

Edited by:
Philip De Witt Hamer,
Amsterdam University Medical Center, Netherlands

Reviewed by:
Anna Luisa Di Stefano,
Hôpitaux Universitaires Pitié Salpêtrière, France
Mark Ter Laan,
Radboud University Nijmegen Medical Centre, Netherlands

***Correspondence:**
Stefanie Quach
stefanie.quach@med.uni-muenchen.de

Specialty section:
This article was submitted to Neuro-Oncology and Neurosurgical Oncology, a section of the journal Frontiers in Neurology

Received: 25 November 2021
Accepted: 23 February 2022
Published: 30 March 2022

Citation:
Katzendobler S, Do A, Weller J, Dorostkar MM, Albert NL, Forbrig R, Niyazi M, Egensperger R, Thon N, Tonn JC and Quach S (2022) Diagnostic Yield and Complication Rate of Stereotactic Biopsies in Precision Medicine of Gliomas. Front. Neurol. 13:822362. doi: 10.3389/fneur.2022.822362

Background: An integrated diagnosis consisting of histology and molecular markers is the basis of the current WHO classification system of gliomas. In patients with suspected newly diagnosed or recurrent glioma, stereotactic biopsy is an alternative in cases in which microsurgical resection is deemed to not be safely feasible or indicated. In this retrospective study, we aimed to analyze both the diagnostic yield and the safety of a standardized biopsy technique.

Material and Methods: The institutional database was screened for frame-based biopsy procedures (January 2016 until March 2021). Only patients with a suspected diagnosis of glioma based on imaging were included. All tumors were classified according to the current WHO grading system. The clinical parameters, procedural complications, histology, and molecular signature of the tissues obtained were assessed.

Results: Between January 2016 and March 2021, 1,214 patients underwent a stereotactic biopsy: 617 (50.8%) for a newly diagnosed lesion and 597 (49.2%) for a suspected recurrence. The median age was 56.9 years (range 5 months–94.4 years). Magnetic resonance imaging (MRI)-guidance was used in 99.3% of cases and additional positron emission tomography (PET)-guidance in 34.3% of cases. In total, stereotactic serial biopsy provided an integrated diagnosis in 96.3% of all procedures. The most frequent diagnoses were isocitrate dehydrogenase (IDH) wildtype glioblastoma (n = 596; 49.2%), oligodendroglioma grade 2 (n = 109; 9%), astrocytoma grade 3 (n = 108; 8.9%), oligodendroglioma grade 3 (n = 76; 6.3%), and astrocytoma grade 2 (n = 66; 5.4%). A detailed determination was successful for IDH 1/2 mutation in 99.4% of cases, for 1p/19q codeletion in 97.4% of cases, for TERT mutation in 98.9% of cases, and for *MGMT* promoter methylation in 99.1% of cases. Next-generation sequencing was evaluable in 64/67 (95.5%) of cases and DNA methylome analysis in 41/44 (93.2%) of cases. Thirteen (1.1%) cases showed glial tumors that could not be further specified. Seventy-three tumors were different non-glioma entities, e.g., of infectious or inflammatory nature. Seventy-five out of 597 suspected recurrences turned out to be post-therapeutic changes only. The rate of

post-procedural complications with clinical symptoms of the Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher was 1.2% in overall patients and 2.6% in the subgroup of brainstem biopsies. There was no fatal outcome in the entire series.

Conclusion: Image-guided stereotactic serial biopsy enables obtaining reliable histopathological and molecular diagnoses with a very low complication rate even in tumors with critical localization. Thus, in patients not undergoing microsurgical resection, this is a valuable tool for precision medicine of patients with glioma.

Keywords: stereotactic biopsy, glioma, recurrent glioma, pseudoprogression, precision medicine, molecular diagnostics, image-guided procedures

INTRODUCTION

Gliomas represent a heterogeneous group of neoplasms of the central nervous system. Classification and subsequent management decisions depend on histological and molecular features. The WHO provides the framework for classification which leads to the guidelines for clinical management (1–5).

Hence, both histology and molecular diagnosis are mandatory in newly diagnosed intracerebral lesions suspicious for glioma. This can be obtained either by tumor resection or stereotactic biopsy. Whether the patient should undergo an open, microsurgical tumor resection or just a biopsy depends mainly on the clinical status of the patient, location and extent of the lesion, and the patients' preference. Gross total resection is associated with better long-term outcome but also inherits a risk of perioperative and postoperative complications despite modern neurosurgical techniques (6–8). Conversely, biopsies are not used for the reduction of tumor volume and but are administered for tissue-based diagnosis only (9). They can be minimally invasive, provide both histological and molecular diagnosis, and may be more suitable for multimorbid or frail patients with very high surgical risk factors for midline tumors or patients with gliomas in highly eloquent areas of the brain bearing a high functional risk in case of extensive tumor reduction.

Especially in *MGMT* methylated glioblastomas, and also in IDH mutated gliomas, treatment-induced changes on conventional magnetic resonance imaging (MRI) are not always easily distinguishable from true tumor progression, a phenomenon termed pseudoprogression (10, 11). Despite the added value of advanced MRI including MR perfusion and MR spectroscopy and positron emission tomography (PET) using radiolabeled amino acids (e.g., O-(2-^{18}Ffluorethyl)-L-tyrosine ([^{18}F]FET PET)) to assess the real tumor burden (12–14), tissue sampling provides the gold standard of information for further management of these uncertain cases.

Tumor relapse is not only a hallmark of IDH wild type glioblastoma but also occurs frequently in lower grade, IDH mutant gliomas (15–17). Patients, thus, are often subjected to a multitude of therapies over time given the fact that, so far, no standard treatment for recurrent gliomas exists. Individualized, targeted therapy is an emerging field in the treatment of gliomas and tissue sampling is necessary to identify the druggable targets using next-generation sequencing. Drugs directed against receptor tyrosine kinases (RTK) and downstream molecules like PI3K/AKT/mTOR as well as drugs targeting the mitogen-activated protein kinase (MAPK) signaling pathway are currently under investigation (2, 18, 19). Small-molecule inhibitors targeting IDH mutations are being tested in clinical trials (NCT02073994, NCT02481154). As mutational landscapes of gliomas may change during therapy and disease course, a safe and efficient way to obtain glioma tissue for identification of targetable molecular alterations would be of great benefit (20).

Thus, there is a growing need to obtain a tissue-based diagnosis even at multiple points in time during the clinical course of glioma. A minimally invasive approach would be desirable to accomplish the goal of having maximally informative specimens with minimal risk and burden for the patient. Whether risks and gains of stereotactic biopsies are well-balanced has been a matter of debate for a long time (21). However, the diagnostic yield in the framework of a molecular-driven brain tumor diagnosis and the associated complication rates of biopsies initially and during clinical course have not yet been investigated comprehensively. In this retrospective study, we aimed at analyzing both the diagnostic yield and the safety of a standardized biopsy technique between 2016 and 2021 in a single high-volume center with a high number of tertiary referrals.

MATERIALS AND METHODS

Patient Evaluation

The local database of the Department of Neurosurgery of the University Hospital Munich (Ludwig-Maximilians University) was screened for all biopsy procedures in a 5-year period between January 2016 and March 2021. Only patients with a suspected diagnosis of glioma were included. After histological confirmation of a glioma through biopsy, molecular analyses were performed. Clinical parameters such as age at diagnosis, Karnofsky Performance Status (KPS), initial symptoms, date of stereotactic biopsy, postoperative clinical course, and last follow-up were assessed retrospectively. All patients or caregivers gave written informed consent. The local ethics committee of the University Hospital Munich approved the study (project number 325-2011).

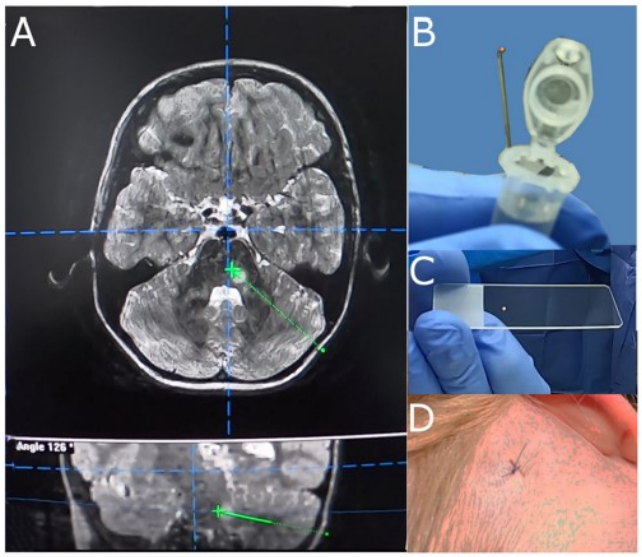


FIGURE 1 | Biopsy trajectory planning **(A)**, sample size of acquired specimen [arrows, **(B,C)**], and skin incision **(D)**.

Biopsy Technique

A standardized frame-based imaging-guided stereotactic biopsy technique was used in all patients. The preoperative workup comprised a 1.5 or 3T MRI scan (with T2 and T1 sequences before and after application of a Gadolinium-based contrast agent and MR-angiography sequences) that was acquired 1 day prior to surgery and fused with an intraoperative, contrast-enhanced CT angiography scan (**Figure 1**). If available, the PET imaging data based on [^{18}F]FET PET was included in the triplanar trajectory planning (**Figure 2**). Each trajectory was meticulously planned to avoid any risk of vascular damage, contact to sulci, or drainage of the cerebrospinal fluid (CSF), which may lead to an intraoperative brain shift with a subsequent mismatch between planning MRI and real anatomy. A phantom frame was used to confirm the correct 3-dimensional angulation prior to the surgery in all patients. If present, the T1 contrast-enhancing lesions and/or suspicious [^{18}F]FET PET foci were targeted. After attaching the frame under sterile conditions, a skin incision of 4–6 mm is made and followed by a frame-guided burr hole trepanation with a diameter of 3 mm. After perforation of the dura through advancing a sharp trocar, a blunt trocar inside a guiding tube (1.4 mm guide tube and trocar, Medical High Tech GmbH, Bad-Krozingen-Biengen, Germany) is used to reach the lesion. Subsequently, with the guide tube in place, multiple small tissue samples of 1 mm^3 each are taken by utilizing the designated biopsy forceps (Medical High Tech GmbH, Bad-Krozingen-Biengen, Germany) inserted into the guide tube. Usually, 5–30 individual specimens per trajectory were taken depending on tumor size and the relation between solid tumor and necrosis. Thereafter, the skin is closed with a single stitch. The average length of the procedure, including the intraoperative CT scan, is 50.4 min.

An experienced neuropathologist is on site in the OR during the procedure to check *via* smear preparation whether the material obtained is sufficient in terms of quantity and quality for diagnosis.

Complications and Follow-Up

Complications were classified according to the Common Terminology Criteria for Adverse Events (CTCAE 5.0; **Supplementary Table 1**) (22). Complications receding within 3 months were classified as transient, else they were classified as permanent. The routine follow-up after biopsy consisted of a postoperative CT scan on the first day after the procedure and an MRI follow-up in 3–6 months intervals for high-grade gliomas and low-grade gliomas, respectively.

Histology and Molecular Markers

All glioma specimens were classified according to the WHO 2016 at the Center for Neuropathology and Prion Research of the University Hospital Munich and retrospectively re-classified according to the WHO 2021 (3). Routine molecular analysis at

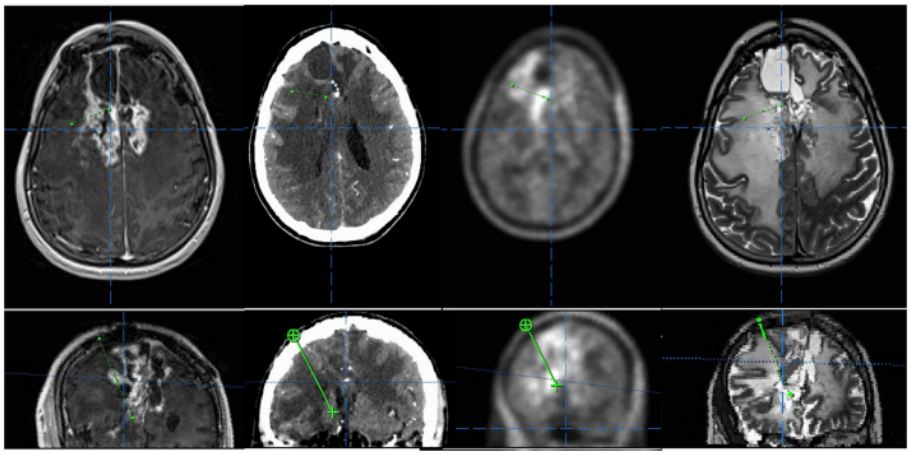


FIGURE 2 | Example of a multimodal trajectory planning targeting both contrast- and (fluorethyl)-L-tyrosine (FET)-enhancing areas in a case of a suspected recurrence of a multimodally treated oligodendroglioma, IDH mutated and 1p/19q co-deleted, the central nervous system (CNS) WHO grade 3. **(Upper panel)** Axial view of contrast enhanced T1, CT, FET positron emission tomography (PET), and T2. **(Lower panel)** Inline view depicting the trajectory plane.

first diagnosis comprised immunohistochemical staining against R132H-mutated IDH1 and ATRX and PCR-based analysis of the IDH1 and 2 mutational hotspots, R312 and R172, respectively (PyroMark Q24 System, Pyro Gold reagents kit, Qiagen, Hilden, Germany); a microsatellite marker analysis was used for the detection of 1p and 19q deletions (23, 24). The mutations within the TERT promoter sequence were detected by the Sanger sequencing utilizing the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), the BigDye Terminator V3.1 Cycle Sequencing kit (Life Technologies, Carlsbad, USA), the DyeEX 2.0 Spin Kit (Qiagen, Hilden, Germany), and 3130 Genetic Analyzer (Life Technologies, Carlsbad, USA) (25). The DNA methylation status of the *MGMT* promoter was determined by bisulfite modification and subsequent nested methylation-specific PCR and sequencing analysis. Tumors were classified binarily as methylated or unmethylated (26). Further molecular analyses were initiated when the results were inconclusive or when aiming at identifying targetable mutations in patients with conventional treatment failure. In these cases, next-generation sequencing was performed using a combined DNA and RNA panel (Trusight Oncology 500, Illumina, San Diego, CA, USA). The DNA methylation profiling was performed for tumor not classifiable by other means or to detect clinically or diagnostically relevant copy number alterations such as homozygous CDNK2A/B deletions. The methylation profiling was done using an Illumina Infinium MethylationEPIC BeadChip array (Illumina, San Diego, CA, USA) with subsequent data analysis using the DNA methylation-based brain tumor classifier provided by the Deutsche Krebsforschungszentrum (v11b4) (27).

Statistics

The final database contained patient-related, clinical, and tumor-specific information such as patient age at diagnosis, gender, clinical status utilizing the KPS, localization of the tumor, histological and molecular glioma features, and postinterventional complication rates. Based on this data, descriptive statistical analyses were performed utilizing the SPSS Statistics 25 software (IBM, Armonk, New York, USA).

RESULTS

Patients and Procedural and Tumor Characteristics

In total, 1,214 consecutive biopsy procedures were analyzed. The median age of patients was 56.9 years (range 5 months−94.4 years). Of the total patients, 58.6% were men and 41.4% were women. A KPS of 80 or higher was reported in 82.1% of all patients. In 50.8% of cases, a biopsy was performed to obtain tissue in a newly diagnosed tumor and in 49.2% of cases for suspected recurrence. Image guidance was based on MRI in 99.3% cases and on CT in 0.7% cases due to contraindications for MRI imaging. Additionally, [^{18}F]FET PET was used in 34.3% cases.

A total of 596 tumors (49.1%) were located on the left and 535 (44.1%) on the right side, and 83 patients (6.8%) had a bilateral midline tumor. The tumor site was lobar in 1,011 (83.3%), deep seated (insula, thalamus, corpus callosum, pineal region) in 123 (10.1%), cerebellar in 40 (3.3%), and

TABLE 1 | Biopsy location in primary and recurrent diseases.

Location		First diagnosis *n* (%)	Recurrence *n* (%)	Total *n* (%)
Lobar	Frontal	155 (12.8)	232 (18.4)	378 (31.1)
	Temporal	158 (13.0)	161 (13.3)	319 (26.3)
	Parietal	79 (6.5)	78 (6.4)	157 (12.9)
	Occipital	15 (1.2)	12 (1.0)	27 (2.2)
	Pre-/postcentral gyrus	67 (5.5)	63 (5.2)	130 (10.7)
Deep-seated	Callosal	12 (1.0)	3 (0.2)	15 (1.2)
	Insular	27 (2.2)	26 (2.1)	53 (4.4)
	Thalamic	31 (2.6)	6 (0.5)	37 (3.0)
	Pineal	15 (1.2)	3 (0.2)	18 (1.5)
	Cerebellar	25 (2.1)	15 (1.2)	40 (3.3)
Brainstem	Mesencephalon	8 (0.7)	3 (0.2)	11 (0.9)
	Pons	14 (1.2)	4 (0.3)	18 (1.5)
	Medulla oblongata	11 (0.9)	0 (0.0)	11 (0.9)
Total		617 (50.8)	597 (49.2)	1,214 (100.0)

brainstem in 40 (3.3%) patients (for detailed location see **Table 1**).

The most common diagnosis was glioblastoma IDH wild type with 596 cases (49.2%), followed by oligodendroglioma grade 2 ($n = 109$; 9.2%), astrocytoma grade 3 ($n = 108$; 8.9%), oligodendroglioma grade 3 ($n = 76$; 6.4%), astrocytoma grade 2 ($n = 66$; 5.4%), IDH 1/2 mutated astrocytoma WHO grade 4 ($n = 45$; 3.7%), and diffuse midline glioma, H3K27M- or FGFR1-mutated ($n = 15+1$; 1.3%) (**Table 2**).

Diagnostic Yield and Molecular Analyses

Among all newly diagnosed lesions, histopathology and molecular analyses provided a definite diagnosis in 595/617 cases (96.4%). Among the 22 unclear results, 14 patients were followed up by MRI imaging, as a low-grade tumor in an eloquent location was histologically and clinically the most likely diagnosis. None of these patients experienced tumor progression during a mean follow-up of 21 months. In six cases, the treatment was initiated based on recommendations by our interdisciplinary tumor board according to the most likely diagnosis (3 glial tumors without further subclassification; 3 diagnoses other than glioma). In only two cases, a second invasive procedure was required for obtaining the diagnosis: one patient underwent re-biopsy after 2 weeks, confirming IDH wild type glioblastoma, and another patient underwent open tumor resection revealing ganglioglioma.

Among all suspected recurrences, vital tumor was detected in 522 out of 597 cases (87.1%), while predominantly post-therapeutic changes were found in 75 cases (12.6%). In 3 cases (4% of all tissues showing post-therapeutic changes), recurrence within 3 months suggested a false negative sampling. In three cases with histologically diagnosed tumor recurrence (0.6%), further clinical course suggested mainly post-therapeutic changes, i.e., false-positive sampling. This amounts to a positive predictive value of 99.4% and a negative predictive value of 96%.

The standard molecular analyses, required by the WHO 2021 grading system, were successfully obtained in the vast majority of tumors being identified as gliomas by histology. The molecular status was informative for IDH 1/2 mutation in 99.4%, for 1p/19q codeletion in 97.4%, for TERT mutation in 98.9%, and for *MGMT* promoter methylation in 99.1%. Next-generation sequencing was attempted in 67 cases and evaluable in 64. The DNA methylation analysis was attempted in 44 cases and evaluable in 42. Twelve, thereof, showed no match with known methylation classes. Altogether, a successful molecular characterization for integrated diagnosis was obtained in 93% of all newly diagnosed and in 88.3% of all recurrent lesions.

Complications

The routine postoperative CT showed no visible conspicuity in 816 (67.2%) cases, a minimal (<5 mm) hemorrhage in 305 (25.1%) cases, a local (>5 mm) hemorrhage in 51 (4.2%) cases, and a space-occupying hemorrhage in 10 (0.8%) cases. In 30 cases, no postoperative CT scan was performed in young patients without relevant deficit. **Table 3** lists clinical complications in relation to imaging features. No clinical sequelae of the stereotactic biopsy were observed in 1,164 (95.9%) of procedures. Mild complications (CTCAE grade 1) were documented in 14 (1.2%) and moderate (CTCAE° 2) in 21 (1.7%) cases. Complications of CTCAE grade 3 occurred in 11 procedures (5 hemiparesis, 4 seizure series, 3 cases of delirium, 1 reduced level of consciousness, total 0.9%). Four patients (0.3%) required urgent intervention (CTCAE grade 4): three patients with postoperative bleeding required craniotomy and hematoma evacuation. One of these patients re-bled a second time after an initially successful hematoma evacuation and needed a second revision craniotomy, possibly due to a decreased level of fibrin stabilizing factor (factor XIII) diagnosed after the second revision surgery. All three patients with hematoma evacuation improved to CTCAE grade 1 or 0 within 3 months. One superficial wound infection required local debridement. Regarding the subgroup of brainstem lesions, two patients (5.3%) experienced mild complications and one (2.6%) a moderate complication (local hemorrhage with transient aggravation of a preexisting

TABLE 2 | Histological diagnoses.

Entity		Newly diagnosed lesion n (%)	Recurrence *n* (%)	Total *n* (%)
Glioma	Glioblastoma, IDH wild type	354 (29.2)	243 (20.1)	596 (49.2)
	Midline glioma, H3K27M-mutated	12 (1.0)	3 (0.2)	15 (1.2)
	Astrocytoma WHO grade 4, IDH-mutant	4 (0.3)	41 (3.4)	45 (3.7)
	Astrocytoma WHO grade 3, IDH-mutant	19 (1.6)	89 (7.3)	108 (8.9)
	Astrocytoma WHO grade 2, IDH-mutant	34 (2.8)	32 (2.6)	66 (5.4)
	High-grade astrocytoma with piloid features	3 (0.2)	1 (0.1)	4 (0.3)
	Oligodendroglioma WHO grade 3, IDH-mutant and 1p/19q-codeleted	8 (0.7)	68 (5.6)	76 (6.3)
	Oligodendroglioma WHO grade 2, IDH-mutant and 1p/19q-codeleted	37 (3.0)	72 (5.9)	109 (9.0)
	Ganglioglioma	7 (0.6)	4 (0.3)	11 (0.9)
	Pilocytic astrocytoma	11 (0.9)	13 (1.1)	24 (2.0)
	Pleiomorphic xanthoastrocytoma	0 (0.0)	1 (0.1)	1 (0.1)
	Pleiomorphic astroglial tumor	2 (0.2)	0 (0.0)	2 (0.2)
	Ependymoma	1 (0.1)	1 (0.1)	2 (0.2)
	Anaplastic ependymoma	2 (0.2)	3 (0.2)	5 (0.4)
Other gliomas, not elsewhere classified (NEC)	*Glioma (NEC)*	1 (0.1)	0 (0.0)	1 (0.1)
	Glial tumor	11 (0.9)	1 (0.1)	12 (1.0)
	Glioneural tumor	7 (0.6)	0 (0.0)	7 (0.6)
	Neuroepithelial tumor	7 (0.6)	1 (0.1)	8 (0.7)
Other	Initially suspected glioma, diagnosis other than glioma	41 (3.4)	4 (0.3)	45 (3.7)
	Metastasis	31 (2.6)	2 (0.2)	33 (2.7)
	Medulloblastoma	3 (0.2)	4 (0.3)	7 (0.6)
	Meningioma	6 (0.5)	4 (0.3)	10 (0.8)
	Neurocytoma	3 (0.2)	2 (0.2)	5 (0.4)
	Germinoma	6 (0.5)	1 (0.1)	7 (0.6)
	Other entities (pineocytoma, neurinoma, diffuse leptomeningeal glioneuronal tumor, papillary tumor of the pineal region, pineoblastoma, solitary fibrous tumor, craniopharyngioma, yolk sac tumor)	7 (0.7)	7 (0.7)	14 (1.3)
Total		617 (50.8)	597 (49.2)	1,214 (100.0)

hemiparesis). In total, 74% of all clinical complications were resolved within 3 months (**Table 4**). There were no procedure-related deaths in the overall cohort.

Brainstem Biopsies

A subgroup of 40 patients underwent a stereotactic biopsy of a brainstem lesion, whereof 13 were pediatric patients. The most frequent diagnosis was diffuse midline glioma, H3K27M mutated ($n = 8$), glioblastoma IDH wild type ($n = 5$), IDH 1/2 mutated astrocytoma ($n = 7$), and pilocytic astrocytoma ($n = 5$). All diagnoses of brainstem tumors are detailed in **Table 5**. In six cases, another diagnosis other than tumor was made, which was confirmed also by a further clinical course. NGS and DNA methylation analysis was attempted and successfully performed in three cases each. Two patients (5.3%) experienced mild complication and one (2.6%) patient had a moderate complication (local hemorrhage which transient aggravation of a preexisting hemiparesis).

DISCUSSION

With the help of image-guided stereotactic biopsy, we could establish a histopathological and molecular diagnosis and distinguish true progression from pseudoprogression in a consecutive series of 1,214 patients with suspected glioma with a very high diagnostic accuracy of 96.4% in terms of histology, over 97% for molecular markers, and over 95% in 850 k/NGS arrays. The rate of non-gliomas among all suspected gliomas was low, possibly reflecting that an interdisciplinary tumor board with dedicated experienced neuroradiologists and nuclear medicine physicians had put forward the biopsy indications. Most previously published studies comprised sample sizes of a few dozen to a couple hundred patients (28–36). The largest retrospective monocentric study comprised 622 patients biopsied over the course of 20 years as compared to a sample size of 1,214 patients over 5 years reported in our study (28, 30). The rate of biopsies investigating

TABLE 3 | Complications according to postoperative imaging and severity.

Blood on postoperative CT scan (*n*, % of total)	Clinical complications (CTCAE grade)	Newly diagnosed lesions; *n* (%)	Recurrent lesions; *n* (%)	Total; *n* (%)
No visible blood (*n* = 816; 67.2%)	0 (none)	395 (98.0)	406 (98.3)	801 (98.2)
	1 (mild)	2 (0.5)	3 (0.7)	5 (0.6)
	2 (moderate)	6 (1.5)	2 (0.5)	8 (1.0)
	3 (severe)	0	2 (0.5)	2 (0.2)
Minimal (<5 mm) hemorrhage (*n* = 305; 25.1%)	0 (none)	149 (96.1)	142 (94.7)	291 (95.4)
	1 (mild)	0	4 (2.7)	4 (1.3)
	2 (moderate)	5 (3.2)	4 (2.7)	9 (3.0)
	3 (severe)	1 (0.6)	0	1 (0.3)
Local (>5 mm) Hemorrhage (*n* = 51; 4.2%)	0 (none)	26 (81.3)	16 (84.2)	42 (82.4)
	1 (mild)	2 (6.3)	1	3 (5.9)
	2 (moderate)	1 (3.1)	1 (5.3)	2 (3.9)
	3 (severe)	2 (6.3)	1 (5.3)	3 (5.9)
	4 (life-threatening)	1 (3.1)	0	1 (2.0)
Space occupying hemorrhage (*n* =10;0.8%)	2 (moderate)	1 (16.7)	1 (25.0)	2 (20.0)
	3 (severe)	2 (33.3)	3 (75.0)	5 (50.0)
	4 (life-threatening)	3 (50.0)	0	3 (30.0)
Ischemia (*n* = 2;0.8%)	0 (none)	0	1 (50.0)	1 (50.0)
	1 (mild)	0	1 (50.0)	1 (50.0)
No imaging available (*n* = 30; 2.5%)	0 (none)	20 (95.2)	9 (100)	29 (96.7)
	1 (mild)	1 (4.8)	0	1 (3.3)
Total (*n* = 1,214; 100%)	0 (none)	590 (95.6)	574 (96.1)	1,164 (95.9)
	1 (mild)	5 (0.8)	9 (1.5)	14 (1.2)
	2 (moderate)	13 (2.1)	8 (1.3)	21 (1.7)
	3 (severe)	5 (0.8)	6 (1.0)	11 (0.9)
	4 (life-threatening)	4 (0.6)	0	4 (0.3)

TABLE 4 | Fraction of transient or permanent complications among all complications.

Clinical complications (CTCAE grade)	Transient *n* (% of total)	Permanent *n* (% of total)	Total *n* (% total)
1	12 (0.9)	2 (0.2)	14 (1.2)[a]
2	17 (1.4)	4 (0.3)	21 (1.7)
3	4 (0.3)	7 (0.6)	11 (0.9)
4	4 (0.3)	0 (0.0)	4 (0.3)
Total	37 (3.0)	13 (1.1)	50 (4.1)

[a]*Percentages do not add up due to rounding.*

TABLE 5 | Diagnoses of brainstem biopsies in adult and pediatric patients.

	Adult *n* (%)	Pediatric *n* (%)	Total *n* (%)
Midline glioma	3 (11.1)	5 (38.5)	8 (20.0)
Glioblastoma, IDH wildtype	3 (11.1)	2 (15.4)	5 (12.5)
Astrocytoma, IDH mutated	6 (22.2)	1 (7.7)	7 (17.5)
Astrocytoma with piloid features	1 (3.7)	0 (0.0)	1 (2.5)
Oligodendroglioma, IDH mutated, 1p/19q codeleted	1 (3.7)	0 (0.0)	1 (2.5)
Pilocytic astrocytoma	3 (11.1)	2 (15.4)	5 (12.5)
Glial tumor, NEC	3 (11.1)	0 (0.0)	3 (7.5)
Glioneuronal tumor	1 (3.7)	0 (0.0)	1 (2.5)
Papillary tumor of the pineal region	0 (0.0)	1 (7.7)	1 (2.5)
Metastasis	2 (7.4)	0 (0.0)	2 (5.0)
Other diagnoses than tumor	4 (14.8)	2 (15.4)	6 (15.0)
Total	27 (100)	13 (100)	40 (100)

suspected tumor recurrence is relatively high, as we provide an effective, low-risk stereotactic biopsy technique and have many patients with suspected recurrences coming to our tertiary referral center for second opinions and to get a tissue-based diagnosis, which is decisive to maintain a successful therapy or enable an informed change of therapy. Unspecific therapy-related changes and pseudoprogression phenomena mimicking tumor relapse gain more importance in light of emerging immunotherapies (37). In our series, more than one in ten (12.5%) of suspected tumor recurrences showed only therapy-induced changes histologically, obviating the need for more invasive procedures in this patient collective. In addition, in analogy to solid cancers and brain metastases, the search for druggable targets in newly diagnosed and recurrent gliomas just embarks and will increase in the future. As new therapies being recommended by a molecular tumor board become available, tissue diagnosis of possible druggable targets should not be withheld from "biopsy-only" patients. Consequently, in all cases where open microsurgical resection is not deemed feasible or medically justified and in all "diagnostic-only" situations, the need for a minimal invasive and maximal effective technique to obtain an informative diagnostic material is beyond doubt. This has also been adopted now for diffuse brainstem gliomas (38, 39).

Earlier, small biopsies did not yield enough viable tissue for obtaining a valid and, presently, mandatory molecular diagnosis; however, the contemporary refined technologies of molecular biology enable the analysis of a panel of different molecular markers even from very small specimens (40, 41). Only with access to elaborate the neuropathological technique and expertise, stereotactic biopsies are adequate to gain all diagnostic information in case open resection is not deemed feasible or justified. In our series, over 96% of biopsies were informative concerning histology and the molecular signature of the tumor. Prerequisite for a proper molecular diagnosis is to obtain the material out of the solid parts of the tumor since any "contamination" of the specimen with either normal adjacent brain or else tumor necrosis might hamper diagnostic yield and accuracy. Moreover, the neuropathologist has to be experienced in working up these small samples. In our practice, the pathologist is on site in the OR during the procedure to check *via* smear preparation whether the material obtained is sufficient in terms of quantity and quality for diagnosis.

Serial sampling with multiple specimens along the trajectory allows to "map" the tumor, including its infiltration zone. This is extremely useful in heterogeneously composed tumors where one single biopsy might lead to a sampling error like misdiagnosing or undiagnosed. MR features such as contrast enhancement on T1-weighted imaging or cell density on T2-weighted sequences can highlight the suspicious areas that should be targeted preferentially. PET with amino acid tracers such as [^{18}F]FET, [^{11}C]Methionine, or [^{18}F]FDOPA are particularly useful to detect the relevant areas for diagnostic biopsies in either diffuse, non-contrast enhancing gliomas or in multimodally pretreated lesions with differential diagnosis of recurrent tumor vs. treatment-related phenomena (12, 13, 42, 43). While [^{18}F]FET PET and perfusion MRI can give important hints about the likelihood of true progression vs. pseudoprogression (12), our data support the continued use of histology as the gold standard for identifying both with high reliability and low risk. Furthermore, image-guided biopsies allow to precisely target and sample different areas within heterogeneously composed tumors to address the mutational and clonal analyses with a high spatial resolution.

As long as molecular alterations within the tumor are homogeneously distributed, sampling errors are not an issue. Referring to this, the homogeneous distribution of the alteration has to be shown in a systemical order to elucidate whether a risk of sampling error might be relevant for a given particular marker. This has been demonstrated for most of the relevant basic molecular signatures in gliomas (26, 43, 44). The earlier a molecular alteration appears in the timeline of tumor evolution, the more likely it can appear homogeneously within the tissue (45). Conversely, especially for late events, more heterogeneous patterns evolve, which have to be taken into account for biopsy (46).

The patterns of either diagnostic or therapeutic targets may change during the course of disease, so recurrent tumors may have a completely different pattern compared to the original newly diagnosed tumor. Again, early events in the tumorigenesis may not change, whereas new subclones during tumor progression may carry new mutations (45). Especially, therapy-driven alterations and an increase in mutational burden may necessitate re-biopsy (47–50). Whereas, *MGMT* promoter methylation does not change over time (51), other therapy relevant markers do (52, 53). Hence, it may not justified to include patients with recurrent tumors into clinical trials for targeted therapy just on the basis of the initial specimen. Instead, dependent on the target, the molecular status has to be newly defined by either resection or biopsy (54, 55).

The complication rate was low with only 0.6% permanent and 0.6% transient severe complications overall. In the subgroup of brainstem lesions, moderate or severe complications occurred at a slightly higher rate of 2.6%. Thus, even in patients with gliomas located in delicate areas such as the brainstem or the midbrain, tissue can be acquired with a low risk of permanent deficit and a high diagnostic yield. The low complication rate reported in this study justifies the application of stereotactic biopsies less reluctantly whenever diagnostic uncertainties occur during the course of disease and treatment. The low number of symptomatic hemorrhages suggests waiving the routine CT scan. Previous series of frame-based biopsies report mortality rates of 0.7–4% (28, 30–36). Post-procedural morbidity (i.e., transient or permanent neurological deficits, epileptic seizures, coma) ranged from 3 to 13%. Asymptomatic bleedings on postoperative CT scans have been reported in up to 60% of patients and symptomatic bleedings occurred in up to 8.6% of cases. In our series with no mortality, the rate of severe transient and permanent complications was much lower. In previous studies, brain biopsies typically yielded diagnoses at rates of 89–92% and even higher when intraoperative histological smears were carried out (21, 28, 31, 56–59). By comparing frame-based with frameless biopsies, no clear advantage of either technique regarding complication rates or diagnostic yield could be shown so far (29, 32, 57–60). In our experience, a high personal and interdisciplinary expertise is required to obtain

constant procedural safety and efficiency. A high caseload being taken care of by a group of few dedicated neurosurgeons is, in our opinion, important. In addition, high-resolution vascular imaging, including MR and CT angiography, meticulous planning of the trajectories by avoiding vessels, ventricular puncture, and arachnoidal contact, as the subarachnoid space is especially prone to hemorrhage, is required. Furthermore, the presence of a dedicated neuropathologist on site not only ensures specimen quality but also prevents an unnecessary high number of specimens, which is especially important in delicate locations. Also, as always in neurosurgery, proper selection of indications and patients is key. Despite low complication rates, the indication for brain biopsy must be strict as it still is an invasive procedure.

In the future, determination of changes in the molecular signature of gliomas and very early detection of therapy response or failure will gain further importance. Whether several techniques and concepts of "liquid biopsy" using CSF, plasma, or even urine may complement or even replace stereotactic biopsies for at least some indications remains yet uncertain (61–66). Also, molecular imaging using novel specific tracers might help to non-invasively better characterize gliomas in the future (67, 68).

With a mean duration of 50 min, frame-based biopsy in a streamlined setting is a time- and cost-efficient procedure. At our institution, we can perform up to five biopsies in the same OR within the regular working hours. We could obtain a high diagnostic yield with a very low rate of either inconclusive biopsies or complications. This leads to a low rate of re-biopsies, which is an important factor for both the safety and the effectiveness in the process of decision making and patient management. Hence, we consider the balance between the complexity and the costs on one side and the benefit for the patient/patient management on the other side to be in due proportion.

CONCLUSION

In conclusion, a streamlined stereotactic biopsy procedure proved to be time-effective and low-risk in primary and recurrent glioma. A high diagnostic yield enables the diagnostics of molecular markers, as required by the current WHO classification, as well as in the increasingly important context of molecular tumor boards. A postoperative CT scan should only be performed when clinically indicated. A good technical setup with easily accessible CT and a specialized team for trajectory planning and neuropathological analysis are recommended.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of national and institutional laws to protect patient confidentiality. Requests to access the datasets should be directed to the Center for Neuropathology and Prion Research of the University Hospital of Munich.

AUTHOR CONTRIBUTIONS

JT and SQ contributed to the conception and design of the study. AD, SK, and SQ organized the database, evaluated the clinical courses, and performed the image analyses. SQ carried out the statistical analysis. SK, SQ, JW, and JT wrote the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

FUNDING

This project was partly funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) (FOR 2858 Project Number 421887978).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.822362/full#supplementary-material

REFERENCES

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 world health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.* (2016) 131:803–20. doi: 10.1007/s00401-016-1545-1
2. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol.* (2020). doi: 10.1038/s41571-020-00447-z
3. Louis D, Wiestler OD, Cavenee *WK. World Health Organization Classification of Tumours of the Central Nervous System*, 5th ed. Lyon: International Agency for Research on Cancer (2021).
4. Wen PY, Weller M, Lee EQ, Alexander BM, Barnholtz-Sloan JS, Barthel FP, et al. Glioblastoma in adults: a society for neuro-oncology (SNO) and european society of neuro-oncology (EANO) consensus review on current management and future directions. *Neuro Oncol.* (2020) 22:1073–113. doi: 10.1093/neuonc/noaa106
5. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol.* (2021) 23:1231–51. doi: 10.1093/neuonc/noab106
6. Jakola AS, Myrmel KS, Kloster R, Torp SH, Lindal S, Unsgard G, et al. Comparison of a strategy favoring early surgical resection vs a strategy favoring watchful waiting in low-grade gliomas. *JAMA.* (2012) 308:1881–8. doi: 10.1001/jama.2012.12807
7. Southwell DG, Birk HS, Han SJ, Li J, Sall JW, Berger MS. Resection of gliomas deemed inoperable by neurosurgeons based on preoperative imaging studies. *J Neurosurg.* (2018) 129:567–75. doi: 10.3171/2017.5.JNS17166
8. Molinaro AM, Hervey-Jumper S, Morshed RA, Young J, Han SJ, Chunduru P, et al. Association of maximal extent of resection of contrast-enhanced and non-contrast-enhanced tumor with survival within molecular subgroups of patients with newly diagnosed glioblastoma. *JAMA Oncol.* (2020) 6:495–503. doi: 10.1001/jamaoncol.2019.6143
9. Karschnia P, Vogelbaum MA, van den Bent M, Cahill DP, Bello L, Narita Y, et al. Evidence-based recommendations on categories for extent of resection in diffuse glioma. *Eur J Cancer.* (2021) 149:23–33. doi: 10.1016/j.ejca.2021.03.002
10. Wen PY, Chang SM, Van den Bent MJ, Vogelbaum MA, Macdonald DR, Lee EQ. Response assessment in neuro-oncology clinical trials. *J Clin Oncol.* (2017) 35:2439–49. doi: 10.1200/JCO.2017.72.7511
11. Okada H, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, et al. Immunotherapy response assessment in neuro-oncology: a

report of the RANO working group. *Lancet Oncol.* (2015) 16:e534–42. doi: 10.1016/S1470-2045(15)00088-1
12. Albert NL, Weller M, Suchorska B, Galldiks N, Soffietti R, Kim MM, et al. Response assessment in neuro-oncology working group and european association for neuro-oncology recommendations for the clinical use of PET imaging in gliomas. *Neuro Oncol.* (2016) 18:1199–208. doi: 10.1093/neuonc/now058
13. Law I, Albert NL, Arbizu J, Boellaard R, Drzezga A, Galldiks N, et al. Joint EANM/EANO/RANO practice guidelines/SNMMI procedure standards for imaging of gliomas using PET with radiolabelled amino acids and [F]FDG: version 1. 0. *Eur J Nucl Med Mol Imaging.* (2019) 46:540–57. doi: 10.1007/s00259-018-4207-9
14. Chuang MT, Liu YS, Tsai YS, Chen YC, Wang CK. Differentiating radiation-induced necrosis from recurrent brain tumor using mr perfusion and spectroscopy: a meta-analysis. *PLoS ONE.* (2016) 11:e0141438. doi: 10.1371/journal.pone.0141438
15. Lassman AB, Iwamoto FM, Cloughesy TF, Aldape KD, Rivera AL, Eichler AF, et al. International retrospective study of over 1000 adults with anaplastic oligodendroglial tumors. *Neuro Oncol.* (2011) 13:649–59. doi: 10.1093/neuonc/nor040
16. Lietke S, Schmutzer M, Schwartz C, Weller J, Siller S, Aumiller M, et al. Interstitial photodynamic therapy using 5-ALA for malignant glioma recurrences. *Cancers.* (2021) 13:1767. doi: 10.3390/cancers13081767
17. Weller J, Katzendobler S, Karschnia P, Lietke S, Egensperger R, Thon N, et al. PCV chemotherapy alone for WHO grade 2 oligodendroglioma: prolonged disease control with low risk of malignant progression. *J Neurooncol.* (2021) 153:283–91. doi: 10.1007/s11060-021-03765-z
18. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. *Cell.* (2013) 155:462–77. doi: 10.1016/j.cell.2013.09.034
19. Touat M, Idbaih A, Sanson M, Ligon KL. Glioblastoma targeted therapy: updated approaches from recent biological insights. *Ann Oncol.* (2017) 28:1457–72. doi: 10.1093/annonc/mdx106
20. Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrson CL, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature.* (2020) 580:517–23. doi: 10.1038/s41586-020-2209-9
21. Dhawan S, Venteicher AS, Butler WE, Carter BS, Chen CC. Clinical outcomes as a function of the number of samples taken during stereotactic needle biopsies: a meta-analysis. *J Neurooncol.* (2021) 154:1–11. doi: 10.1007/s11060-021-03785-9
22. US Department of Health and Human Services; National Institutes of Health NCI. *Common Terminology Criteria for Adverse Events (CTCAE) Version 5.* Washington, DC: U.S. Department of Health and Human Services (2017).
23. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. *Int J Cancer.* (2005) 113:379–85. doi: 10.1002/ijc.20575
24. Suchorska B, Schuller U, Biczok A, Lenski M, Albert NL, Giese A, et al. Contrast enhancement is a prognostic factor in IDH1/2 mutant, but not in wild-type WHO grade II/III glioma as confirmed by machine learning. *Eur J Cancer.* (2019) 107:15–27. doi: 10.1016/j.ejca.2018.10.019
25. Remke M, Ramaswamy V, Peacock J, Shih DJ, Koelsche C, Northcott PA, et al. TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma. *Acta Neuropathol.* (2013) 126:917–29. doi: 10.1007/s00401-013-1198-2
26. Grasbon-Frodl EM, Kreth FW, Ruiter M, Schnell O, Bise K, Felsberg J, et al. Intratumoral homogeneity of MGMT promoter hypermethylation as demonstrated in serial stereotactic specimens from anaplastic astrocytomas and glioblastomas. *Int J Cancer.* (2007) 121:2458–64. doi: 10.1002/ijc.23020
27. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. *Nature.* (2018) 555:469–74. doi: 10.1038/nature26000
28. Riche M, Amelot A, Peyre M, Capelle L, Carpentier A, Mathon B. Complications after frame-based stereotactic brain biopsy: a systematic review. *Neurosurg Rev.* (2021) 44:301–7. doi: 10.1007/s10143-019-01234-w
29. Hall WA. The safety and efficacy of stereotactic biopsy for intracranial lesions. *Cancer.* (1998) 82:1749–55. doi: 10.1002/(sici)1097-0142(19980501)82:9<1756::aid-cncr23>3.0.co;2-2
30. Kongkham PN, Knifed E, Tamber MS, Bernstein M. Complications in 622 cases of frame-based stereotactic biopsy, a decreasing procedure. *Can J Neurol Sci.* (2008) 35:79–84. doi: 10.1017/S0317167100007605
31. Ferreira MP, Ferreira NP, Pereira Filho Ade A, Pereira Filho Gde A, Franciscatto AC. Stereotactic computed tomography-guided brain biopsy: diagnostic yield based on a series of 170 patients. *Surg Neurol.* (2006) 65(Suppl. 1):S1:27–1:32. doi: 10.1016/j.surneu.2005.11.036
32. Dammers R, Haitsma IK, Schouten JW, Kros JM, Avezaat CJ, Vincent AJ. Safety and efficacy of frameless and frame-based intracranial biopsy techniques. *Acta Neurochir.* (2008) 150:23–9. doi: 10.1007/s00701-007-1473-x
33. Chen CC, Hsu PW, Erich Wu TW, Lee ST, Chang CN, Wei KC, et al. Stereotactic brain biopsy: single center retrospective analysis of complications. *Clin Neurol Neurosurg.* (2009) 111:835–9. doi: 10.1016/j.clineuro.2009.08.013
34. Burns JD, Cadigan RO, Russell JA. Evaluation of brain biopsy in the diagnosis of severe neurologic disease of unknown etiology. *Clin Neurol Neurosurg.* (2009) 111:235–9. doi: 10.1016/j.clineuro.2008.10.003
35. Bernstein M, Parrent AG. Complications of CT-guided stereotactic biopsy of intra-axial brain lesions. *J Neurosurg.* (1994) 81:165–8. doi: 10.3171/jns.1994.81.2.0165
36. Bai HX, Zou Y, Lee AM, Lancaster E, Yang L. Diagnostic value and safety of brain biopsy in patients with cryptogenic neurological disease: a systematic review and meta-analysis of 831 cases. *Neurosurgery.* (2015) 77:283–95; discussion 95. doi: 10.1227/NEU.0000000000000756
37. Galldiks N, Lohmann P, Werner JM, Ceccon G, Fink GR, Langen KJ. Molecular imaging and advanced MRI findings following immunotherapy in patients with brain tumors. *Expert Rev Anticancer Ther.* (2020) 20:9–15. doi: 10.1080/14737140.2020.1705788
38. Chen LH, Pan C, Diplas BH, Xu C, Hansen LJ, Wu Y, et al. The integrated genomic and epigenomic landscape of brainstem glioma. *Nat Commun.* (2020) 11:3077. doi: 10.1038/s41467-020-16682-y
39. Hoffman LM, DeWire M, Ryall S, Buczkowicz P, Leach J, Miles L, et al. Spatial genomic heterogeneity in diffuse intrinsic pontine and midline high-grade glioma: implications for diagnostic biopsy and targeted therapeutics. *Acta Neuropathol Commun.* (2016) 4:1. doi: 10.1186/s40478-015-0269-0
40. Eigenbrod S, Trabold R, Brucker D, Eros C, Egensperger R, La Fougere C, et al. Molecular stereotactic biopsy technique improves diagnostic accuracy and enables personalized treatment strategies in glioma patients. *Acta Neurochir.* (2014) 156:1427–40. doi: 10.1007/s00701-014-2073-1
41. Thon N, Tonn JC, Kreth FW. The surgical perspective in precision treatment of diffuse gliomas. *Onco Targets Ther.* (2019) 12:1497–508. doi: 10.2147/OTT.S174316
42. Kunz M, Albert NL, Unterrainer M, la Fougere C, Egensperger R, Schuller U, et al. Dynamic 18F-FET PET is a powerful imaging biomarker in gadolinium-negative gliomas. *Neuro Oncol.* (2019) 21:274–84. doi: 10.1093/neuonc/noy098
43. Kunz M, Thon N, Eigenbrod S, Hartmann C, Egensperger R, Herms J, et al. Hot spots in dynamic FET-PET delineate malignant tumor parts within suspected WHO grade II gliomas. *Neuro Oncol.* (2011) 13:307–16. doi: 10.1093/neuonc/noq196
44. Thon N, Eigenbrod S, Grasbon-Frodl EM, Ruiter M, Mehrkens JH, Kreth S, et al. Novel molecular stereotactic biopsy procedures reveal intratumoral homogeneity of loss of heterozygosity of 1p/19q and TP53 mutations in World Health Organization grade II gliomas. *J Neuropathol Exp Neurol.* (2009) 68:1219–28. doi: 10.1097/NEN.0b013e3181bee1f1
45. Korber V, Yang J, Barah P, Wu Y, Stichel D, Gu Z, et al. Evolutionary trajectories of IDH(WT) glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. *Cancer Cell.* (2019) 35:692–704 e12. doi: 10.1016/j.ccell.2019.02.007
46. Diplas BH, He X, Brosnan-Cashman JA, Liu H, Chen LH, Wang Z, et al. The genomic landscape of TERT promoter wildtype-IDH wildtype glioblastoma. *Nat Commun.* (2018) 9:2087. doi: 10.1038/s41467-018-04448-6
47. van Thuijl HF, Mazor T, Johnson BE, Fouse SD, Aihara K, Hong C, et al. Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol.* (2015) 129:597–607. doi: 10.1007/s00401-015-1403-6
48. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science.* (2014) 343:189–93. doi: 10.1126/science.1239947

49. Yu Y, Villanueva-Meyer J, Grimmer MR, Hilz S, Solomon DA, Choi S, et al. Temozolomide-induced hypermutation is associated with distant recurrence and reduced survival after high-grade transformation of low-grade IDH-mutant gliomas. *Neuro Oncol.* (2021) 23:1872–84. doi: 10.1093/neuonc/noab081
50. Pham TV, Goodman AM, Sivakumar S, Frampton G, Kurzrock R. Intra-patient stability of tumor mutational burden from tissue biopsies at different time points in advanced cancers. *Genome Med.* (2021) 13:159. doi: 10.1186/s13073-021-00979-8
51. Felsberg J, Thon N, Eigenbrod S, Hentschel B, Sabel MC, Westphal M, et al. Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas. *Int J Cancer.* (2011) 129:659–70. doi: 10.1002/ijc.26083
52. van den Bent MJ, Gao Y, Kerkhof M, Kros JM, Gorlia T, van Zwieten K, et al. Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas. *Neuro Oncol.* (2015) 17:935–41. doi: 10.1093/neuonc/nov013
53. Felsberg J, Hentschel B, Kaulich K, Gramatzki D, Zacher A, Malzkorn B, et al. Epidermal growth factor receptor variant III (EGFRvIII) positivity in EGFR-amplified glioblastomas: prognostic role and comparison between primary and recurrent tumors. *Clin Cancer Res.* (2017) 23:6846–55. doi: 10.1158/1078-0432.CCR-17-0890
54. French PJ, Eoli M, Sepulveda JM, de Heer I, Kros JM, Walenkamp A, et al. Defining EGFR amplification status for clinical trial inclusion. *Neuro Oncol.* (2019) 21:1263–72. doi: 10.1093/neuonc/noz096
55. Draaisma K, Chatzipli A, Taphoorn M, Kerkhof M, Weyerbrock A, Sanson M, et al. Molecular evolution of IDH wild-type glioblastomas treated with standard of care affects survival and design of precision medicine trials: a report from the EORTC 1542 study. *J Clin Oncol.* (2020) 38:81–99. doi: 10.1200/JCO.19.00367
56. Mathon B, Amelot A, Mokhtari K, Bielle F. Increasing the diagnostic yield of stereotactic brain biopsy using intraoperative histological smear. *Clin Neurol Neurosurg.* (2019) 186:105544. doi: 10.1016/j.clineuro.2019.105544
57. Woodworth GF, McGirt MJ, Samdani A, Garonzik I, Olivi A, Weingart JD. Frameless image-guided stereotactic brain biopsy procedure: diagnostic yield, surgical morbidity, and comparison with the frame-based technique. *J Neurosurg.* (2006) 104:233–7. doi: 10.3171/jns.2006.104.2.233
58. Bekelis K, Radwan TA, Desai A, Roberts DW. Frameless robotically targeted stereotactic brain biopsy: feasibility, diagnostic yield, and safety. *J Neurosurg.* (2012) 116:1002–6. doi: 10.3171/2012.1.JNS111746
59. Barnett GH, Miller DW, Weisenberger J. Frameless stereotaxy with scalp-applied fiducial markers for brain biopsy procedures: experience in 218 cases. *J Neurosurg.* (1999) 91:569–76. doi: 10.3171/jns.1999.91.4.0569
60. Lu Y, Yeung C, Radmanesh A, Wiemann R, Black PM, Golby AJ. Comparative effectiveness of frame-based, frameless, and intraoperative magnetic resonance imaging-guided brain biopsy techniques. *World Neurosurg.* (2015) 83:261–8. doi: 10.1016/j.wneu.2014.07.043
61. Gatto L, Franceschi E, Di Nunno V, Tosoni A, Lodi R, Brandes AA. Liquid biopsy in glioblastoma management: from current research to future perspectives. *Oncologist.* (2021) 26:865–78. doi: 10.1002/onco.13858
62. Mouliere F, Smith CG, Heider K, Su J, van der Pol Y, Thompson M, et al. Fragmentation patterns and personalized sequencing of cell-free DNA in urine and plasma of glioma patients. *EMBO Mol Med.* (2021) 13:e12881. doi: 10.15252/emmm.202012881
63. Billard P, Guerriau C, Carpentier C, Juillard F, Grandin N, Lomonte P, et al. The TeloDIAG: how telomeric parameters can help in glioma rapid diagnosis and liquid biopsy approaches. *Ann Oncol.* (2021). doi: 10.1016/j.annonc.2021.09.004
64. Mathios D, Phallen J. Circulating biomarkers in glioblastoma: ready for prime time? *Cancer J.* (2021) 27:404–9. doi: 10.1097/PPO.0000000000000541
65. On J, Natsumeda M, Watanabe J, Saito S, Kanemaru Y, Abe H, et al. Low detection rate of h3k27m mutations in cerebrospinal fluid obtained from lumbar puncture in newly diagnosed diffuse midline gliomas. *Diagnostics.* (2021) 11:681. doi: 10.3390/diagnostics11040681
66. Kang KM, Muralidharan K, Yekula A, Small JL, Rosh ZS, Jones PS, et al. Blood-based detection of BRAF V600E in gliomas and brain tumor metastasis. *Cancers.* (2021) 13:1227. doi: 10.3390/cancers13061227
67. Schnell O, Krebs B, Carlsen J, Miederer I, Goetz C, Goldbrunner RH, et al. Imaging of integrin alpha(v)beta expression in patients with malignant glioma by [18F] Galacto-RGD positron emission tomography. *Neuro Oncol.* (2009) 11:861–70. doi: 10.1215/15228517-2009-024
68. Unterrainer M, Fleischmann DF, Vettermann F, Ruf V, Kaiser L, Nelwan D, et al. TSPO PET, tumour grading and molecular genetics in histologically verified glioma: a correlative F-GE-180 PET study. *Eur J Nucl Med Mol Imaging.* (2020) 47:1368–80. doi: 10.1007/s00259-019-04491-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6. Publikation II

Journal of Neuro-Oncology
https://doi.org/10.1007/s11060-022-04128-y

RESEARCH

Limited efficacy of temozolomide alone for astrocytoma, IDH-mutant, CNS WHO grades 2 or 3

Jonathan Weller[1] · Sophie Katzendobler[1] · Jens Blobner[1] · Frederic Thiele[1] · Hannes Becker[2] · Stefanie Quach[1] · Rupert Egensperger[3] · Maximilian Niyazi[4] · Bogdana Suchorska[5] · Niklas Thon[1] · Michael Weller[6] · Joerg-Christian Tonn[1,7]

Received: 1 August 2022 / Accepted: 30 August 2022

Abstract

Purpose The role of temozolomide chemotherapy alone in isocitrate dehydrogenase (IDH)-mutant astrocytomas has not been conclusively determined. Radiotherapy might be superior to temozolomide. Recent studies have linked temozolomide with induction of hypermutation and poor clinical course in some IDH-mutant gliomas.

Methods In this retrospective study, 183 patients with astrocytoma, IDH-mutant, CNS WHO grade 2 or 3 and diagnosed between 2001 and 2019 were included. Patients initially monitored by wait-and-scan strategies or treated with radiotherapy or temozolomide alone were studied. Patient data were correlated with outcome. Matched pair and subgroup analyses were conducted.

Results Radiotherapy was associated with longer progression-free survival than temozolomide (6.2 vs 3.4 years, $p=0.02$) and wait-and-scan strategies (6.2 vs 4 years, $p=0.03$). Patients treated with radiotherapy lived longer than patients treated with temozolomide (14.4 vs 10.7 years, $p=0.02$). Survival was longer in the wait-and-scan cohort than in the temozolomide cohort (not reached vs 10.7 years, $p<0.01$). Patients from the wait-and-scan cohort receiving temozolomide at first progression had significantly shorter survival times than patients treated with any other therapy at first progression ($p<0.01$). Post-surgical T2 tumor volume, contrast enhancement on MRI and WHO grade were associated with overall survival in univariate analyses ($p<0.01$).

Conclusion The results suggest superiority of radiotherapy over temozolomide and wait-and-scan strategies regarding progression-free survival and superiority of radiotherapy over temozolomide regarding overall survival. Our results are consistent with the notion that early temozolomide might compromise outcome in some patients.

Keywords Astrocytoma · Temozolomide · Radiotherapy · Hypermutation

Introduction

Patients with IDH-mutant (IDHmut) WHO grade 2 or 3 astrocytomas often show a long clinical course and are subjected to a multitude of therapeutic interventions over time. This poses a challenge to health care professionals, patients and caregivers as to determine time point and modality of tumor specific treatment. Adverse effects from therapy need to be weighed against tumor control. Gold standard for IDHmut astrocytomas is initial maximum safe

✉ Jonathan Weller
jonathan.weller@med.uni-muenchen.de

[1] Department of Neurosurgery, Medical Center of the University of Munich, Marchioninistrasse 15, 81377 Munich, Germany

[2] Department of Neurosurgery, University Hospital of Tubingen, Tübingen, Germany

[3] Center for Neuropathology and Prion Research, Medical Center of the University of Munich, Munich, Germany

[4] Department of Radiotherapy and Radiation Oncology, Medical Center of the University of Munich, Munich, Germany

[5] Department of Neurosurgery, Sana Hospital Duisburg, Duisburg, Germany

[6] Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland

[7] German Cancer Consortium (DKTK), Partner Site Munich, Munich, Germany

Published online: 16 September 2022

resection [1]. Postoperative radiotherapy (RT) followed by procarbazine, CCNU and vincristine (PCV) in WHO grade 2 astrocytomas or maintenance temozolomide (TMZ) in WHO grade 3 astrocytomas should be initiated in high-risk patients defined as older than 40–45 years of age, after incomplete resection or with neurological deficits beyond seizures [1–3]. Of note, predictive and prognostic implication of grading in IDHmut gliomas remains controversial since data from large cohorts showed only marginal differences in overall survival between WHO grade 2 and 3 [4].

The role of surveillance strategies versus RT alone, PCV alone, TMZ alone or combined modality treatment in the treatment of IDHmut gliomas has not been conclusively determined. Surveillance strategies can be pursued in younger patients and after gross total resection [5]. Early RT as opposed to RT at first progression was shown to be associated with prolonged progression-free survival, but not overall survival in WHO grade 2 gliomas [6]. A major concern of early RT in young patients is the potential long-term neurocognitive decline as a complication of treatment [7, 8]. The role of chemotherapy alone remains investigational, and data are scarce. Guidelines give leeway for chemotherapy regimens, omitting RT, with either PCV or TMZ if the tumor volume is large, the patient is young, or RT is not indicated for other reasons [1]. In high-risk WHO grade 2 IDHmut astrocytomas, TMZ monotherapy was associated with shorter progression-free survival than RT alone [9]. Similar outcome of chemotherapy alone versus RT has been reported in WHO grade 3 gliomas with a possible superiority of PCV over TMZ [10, 11]. The CODEL study (NCT00887146) was closed and redesigned prematurely due to inferior outcome of patients with WHO grade 3 oligodendroglioma treated with TMZ, as compared with the other study groups of RT alone or RT with concomitant and maintenance TMZ [12]. A retrospective analysis failed to demonstrate benefit for progression-free survival of TMZ alone over resection only or active surveillance after biopsy in WHO grade 2 oligodendrogliomas [13]. In addition to the rather discouraging clinical data on TMZ alone for lower grade gliomas, molecular analyses have reported induction of hypermutation by TMZ therapy in subgroups of IDHmut gliomas [14, 15]. TMZ-driven hypermutational genotypes have been associated with shorter survival times and distant recurrence in initially low grade, IDHmut gliomas [16, 17]. In this context, it is unclear whether TMZ alone is beneficial or even potentially detrimental in the treatment of IDHmut gliomas in the long-term.

Here we set out to investigate the long-term outcome of a consecutive single-center series of 183 patients diagnosed with CNS WHO grade 2 or 3 IDHmut astrocytomas either monitored postoperatively by means of active surveillance or treated with TMZ alone or treated with RT alone.

Patients and methods

Patient evaluation

Patients with newly diagnosed *IDH1*- or *IDH2*-mutant astrocytomas without 1p/19q-codeletion diagnosed at the Department of Neurosurgery of the University Hospital Munich between 2001 and 2019 were included in this study. The study was waived by the institutional Ethics committee (project number 21-0612). Patients with histological CNS WHO grade 4 astrocytomas according to WHO 2021 were excluded [18]. Patient-related and clinical parameters as well as complications from therapy, classified according to CTCAE version 5.0 (Common Terminology Criteria for Adverse Events), were assessed (Table 1) [19]. If initial imaging was performed due to symptoms not explicable by the lesion, the diagnosis was termed "incidental". In patients with multiple infiltrated lobes, the location in Table 1 referred to the most infiltrated lobe. Therapy recommendations were given based on multidisciplinary tumor board decisions and options were discussed with the patients. Besides wait-and-scan (W&S) strategies that had been pursued in many patients with histological WHO grade 2 gliomas at our department, tumor board recommendations often left the option for post-surgical monotherapy strategies.

The date of histological sampling through stereotactic biopsy or tumor resection was set as date of diagnosis. Progression-free survival was defined as the time interval from diagnosis to first progression according to RANO (Response Assessment in Neuro-Oncology) criteria [20]. TMZ was given in a 5/28-schedule and a 150–200 mg/m^2 dose [1, 9]. In the RT cohort, only patients who had received involved-field RT with 50.4–60 Gy in 1.8–2.0 fractions were included. Overall survival was defined as the time interval between the date of diagnostic surgery and date of death. Patients were monitored through regular visits with up-to-date MRI (magnetic resonance imaging) in the outpatient clinic of the Department of Neurosurgery. Outpatient visits were scheduled every 6 months in case of stable disease and after completion of therapy. When progression was suspected, but RANO criteria had not been met, intervals were 2–3 months. During TMZ, patients without compromising side effects were seen every 2–3 months. Patients treated with RT were first seen 6 weeks after completion of the treatment.

Histopathology and molecular analyses

Histological samples were classified according to the WHO 2021 classification [18]. Sequencing of sodium

Table 1 Patient-related and clinical characteristics overall and in different cohorts

Parameter	All patients (n = 183)	Wait-and-scan (n = 98)	TMZ (n = 48)	RT (n = 37)	*p* value
Age (years)					
Median	37	35	37	38	0.02*
Range	16–76	16–69	21–76	25–56	
Sex, n (%)					
Female	84 (46)	48 (49)	19 (40)	17 (46)	0.57
Male	99 (54)	50 (51)	29 (60)	20 (54)	
KPS at first admission, n (%)					
100	7 (4)	6 (6)	1 (2)	0 (0)	0.04*
90	149 (81)	82 (84)	34 (71)	33 (89)	
80	27 (15)	10 (10)	13 (27)	4 (11)	
< 80	0 (0)	0 (0)	0 (0)	0 (0)	
Trigger for diagnostic workup, n (%)					
Incidental finding	46 (25)	32 (33)	10 (21)	6 (16)	0.1
Epileptic seizure	103 (56)	51 (52)	24 (50)	27 (73)	
Neurological deficit	35 (19)	15 (15)	14 (29)	4 (11)	
CNS WHO grade, n (%)					
Grade 2	116 (63)	86 (88)	17 (35)	13 (35)	< 0.01*
Grade 3	67 (37)	12 (12)	31 (65)	24 (65)	
Localization, n (%)					
Frontal	92 (50)	46 (47)	24 (50)	22 (60)	0.87
Temporal	55 (30)	34 (35)	16 (33)	5 (14)	
Insular	13 (7)	7 (7)	2 (4)	4 (11)	
Parietal	16 (9)	9 (9)	4 (8)	3 (8)	
Occipital	1 (1)	1 (1)	0 (0)	0 (0)	
Midline	6 (3)	1 (1)	2 (4)	3 (8)	
Laterality, n (%)					
Left	97 (53)	54 (55)	24 (50)	19 (51)	0.67
Right	78 (43)	43 (44)	20 (42)	15 (41)	
Bilateral	8 (4)	1 (1)	4 (8)	3 (8)	
Post-surgical T2 tumor volume (cm^3)					
Median	22	11	58	16	< 0.01*
Range	0–319	0–200	0–319	0–64	
CE on initial MRI, n (%)	55 (30)	14 (14)	27 (56)	14 (38)	< 0.01*

The right column shows *p*-values when comparing numbers and values of the different cohorts. Statistically significant values, i.e., *p*-values of 0.05 or below (ANOVA and Kruskal–Wallis tests), are depicted with asterisks

KPS Karnofsky Performance Status; *CNS* central nervous system; *WHO* World Health Organization; *CE* contrast enhancement; *MRI* magnetic resonance imaging

bisulfite-modified DNA was performed to determine MGMT promoter (O6-methylguanine-DNA methyltransferase) methylation status. Microsatellite markers were utilized to assess allelic loss on chromosomes 1p and 19q. Assessment of codon 132 and codon 172 for IDH genes 1 and 2 was performed in all patients through pyrosequencing [21, 22].

Tumor volumes

Initial and postoperative tumor volumes on T2 weighted MRI were manually segmented utilizing Brainlab Elements Smartbrush Software (Brainlab Elements Smartbrush, Munich, GER). In case of tumor resection, patients are subjected to postoperative MRI during inpatient treatment and

within 48–72 h after histological sampling at our institution. Matched-pair analyses were conducted to eliminate residual, postoperative tumor volume as a potential confounder. Matching was done manually according to the segmented, postoperative T2 volume and contrast enhancement on MRI, before initiation of chemotherapy or RT. Pairing was accepted if the absolute difference between the volumes did not exceed 10% of the larger volume of the match [13].

Statistical analysis

Statistical tests were performed and figures designed utilizing GraphPad PRISM software version 9.3.1. Kaplan–Meier estimator method, Mantel–Cox (Log-rank) and Gehan–Breslow–Wilcoxon tests were used for comparison of progression-free and overall survival. Continuous and categorial variables were compared by conventional t-tests, ANOVA and χ^2 tests respectively. Kruskal–Wallis test was used for comparison of non-parametric, ordinal variables. For univariate analyses, log-rank tests and Cox proportional hazards regression models were used. Associations were considered statistically significant when p-values were 0.05 or below.

Results

Study population and clinical parameters

Of 213 patients with IDHmut WHO grade 2 or 3 astrocytomas treated at our center between 2001 and 2019, 183 patients could be allocated to three different cohorts according to the post-surgical management strategy: W&S, TMZ alone or RT alone (Fig. 1, Table 1). 30 patients were treated otherwise, e.g., with radiochemotherapy, brachytherapy, photodynamic therapy, or combinations thereof. Initial tumor resection was performed in 100 patients (55%), 83 patients (45%) received a stereotactic biopsy. The median age at diagnosis was 37 years (range 16–76). MGMT promoter methylation was present in 139 patients (76%). Partially methylated promoters were seen in 27 patients (15%). In 17 patients (9%), MGMT promoter methylation was not detected. After biopsy or tumor resection, 98 patients (54%) were not further treated but monitored through regular outpatient visits until progression, 48 patients (26%) received TMZ alone and 37 patients (20%) were treated with RT alone. At the time of database closure, 135 patients (74%) had experienced tumor progression and 33 patients (18%) had died from tumor-related causes. There was no significant difference in sex distribution, trigger for diagnostic workup, tumor location or affected brain hemisphere between the cohorts. Patients were eldest in the RT cohort ($p = 0.02$). The proportion of patients with KPS of 80 was highest in the TMZ cohort ($p = 0.04$). The median postoperative T2 tumor volume was highest in the TMZ cohort ($p < 0.01$). The relative distribution of WHO grade 2 and WHO grade 3 astrocytomas was identical in the RT and TMZ cohort, but the proportion of WHO grade 2 astrocytomas was higher in the W&S cohort ($p < 0.01$) (Table 1). In the TMZ cohort, a median of 6 cycles of chemotherapy was completed (mean and range of cycles completed: 8, 2–15). The mean dose

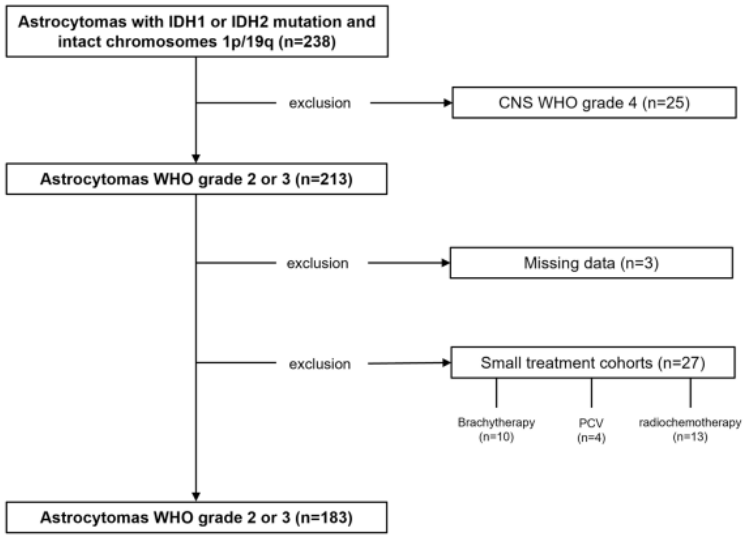


Fig. 1 CONSORT figure. Patients having received the diagnosis of an IDH-mutant glioma between the years 2001 and 2019 were stratified according to initial histology. Exclusion criteria were a histological WHO grade 4 at initial diagnosis (n = 25), missing data (n = 3) or small treatment groups (n = 27). *CNS* central nervous system; *WHO* World Health Organisation; *PCV* procarbazine + CCNU + vincristine

applied to the patients who were initially treated with RT was 60 Gy (range 50.4–60 in fractions of 1.8–2.0). In 2 patients (4%) treated with TMZ, CTCAE grade 3 thrombocytopenia demanded hospitalization. No grade 3 or higher toxicity was reported in the RT cohort.

Progression-free and overall survival

PFS was longer in patients treated post-surgically with RT alone than in patients treated with TMZ alone (6.2 vs 3.4 years, $p=0.02$) and patients monitored by W&S strategies (6.2 vs 4 years, $p=0.03$) (Fig. 2A). Superiority of RT over TMZ in terms of PFS was confirmed in a matched-pair analysis that comprised 16 pairs (7.8 vs 2.8 years, $p=0.03$) (Fig. 3A). OS was significantly longer in the RT cohort than in the TMZ cohort (14.4 vs 10.7 years, $p=0.02$), but not in the matched-pair analysis ($p=0.27$; number of pairs/events: 8/16) (Figs. 2C, 3B). Comparing W&S with TMZ, patients treated with TMZ alone showed similar PFS, but significantly shorter survival times overall (not reached vs 10.7 years, $p<0.01$) and in a subgroup analysis of WHO grade 2 astrocytomas only ($p=0.02$) (Fig. 4D, E). Patients with WHO grade 3 astrocytomas receiving radiotherapy lived significantly longer than patients treated with temozolomide (14.3 vs 10.7 years, p = 0.01). For WHO grade 3 gliomas, there was an association by trend between longer overall survival and wait-and-scan strategies as opposed to temozolomide (18.3 vs 10.7, p = 0.08).

In a matched pair analysis, in which 28 pairs were identified, TMZ was associated with poorer survival by trend ($p=0.09$) when compared to W&S strategies (Fig. 4F). In a subgroup analysis according to the mode of initial histological sampling, there was an association between longer overall survival and W&S strategies after biopsy as opposed

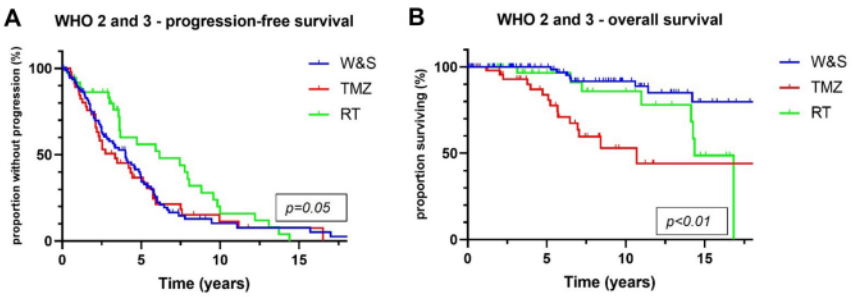


Fig. 2 Overall, radiotherapy was associated with longer progression-free survival than wait-and-scan strategies or temozolomide alone (p = 0.05) (**A**). Patients treated with temozolomide monotherapy showed significantly shorter overall survival than patients with other postoperative strategies (p < 0.01) (**B**). WHO World Health Organization; W&S wait-and-scan strategy; TMZ temozolomide; RT radiotherapy

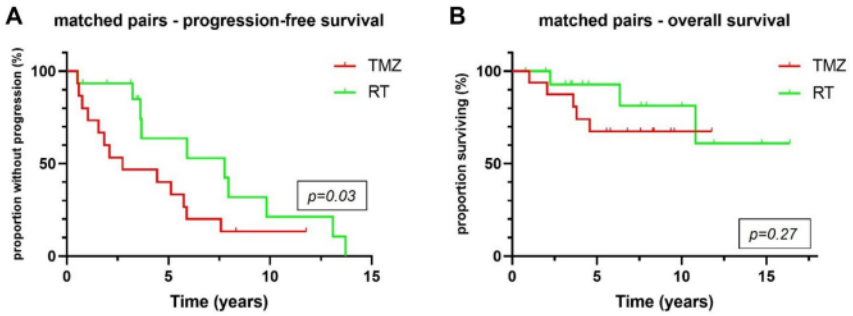


Fig. 3 In a matched-pair analysis accounting for post-surgical tumor volume and WHO grade, radiotherapy was superior in terms of progression-free survival (p = 0.03) (**A**), but not overall survival (p = 0.27) (**B**). *TMZ* temozolomide; *RT* radiotherapy

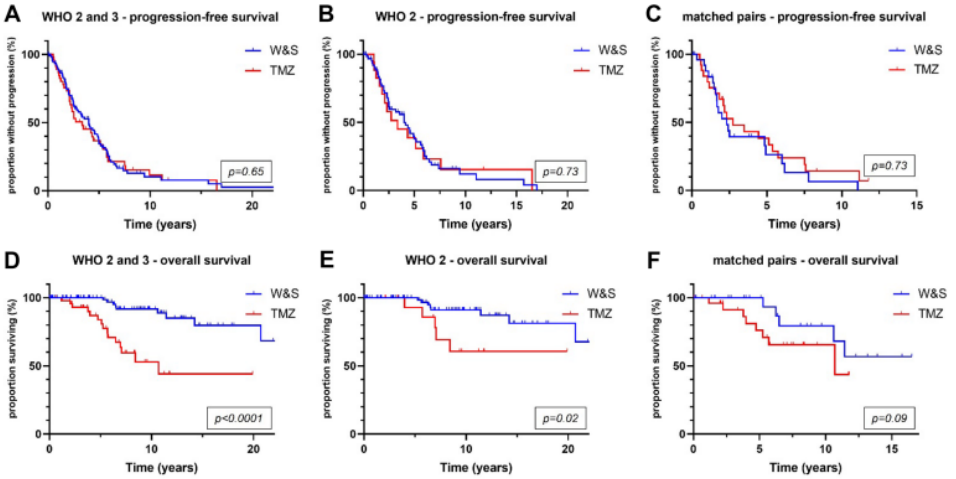


Fig. 4 Wait-and-scan strategies and temozolomide chemotherapy showed similar progression-free survival (**A**–**C**). Waitand-scan strategies were associated with longer overall survival ($p < 0.01$) and in WHO grade 2 gliomas ($p = 0.02$) (**D**, **E**). In a matched-pair analysis accounting for post-surgical T2 tumor volume and WHO grade, there was an association with prolonged overall survival by trend ($p = 0.09$) (**F**). WHO World Health Organization; W&S wait-andscan; TMZ temozolomide

to TMZ alone after biopsy by trend ($p = 0.07$) (Fig. 5A). This difference was significant in patients initially receiving tumor resection ($p < 0.01$) (Fig. 5B). Patients with tumor progression from the W&S cohort were stratified according to the first treatment after progression. Here, TMZ alone was associated with shorter OS than other treatments ($p < 0.01$), although mean tumor volumes were smaller in the TMZ subgroup with 46 cm^3 versus 54 cm^3 (Fig. 5D).

In addition to the Kaplan–Meier estimates determined for the different treatment cohorts, several variables commonly associated with PFS and OS were tested in univariate analyses. None of the tested variables (patient age at diagnosis, sex, CNS WHO grade at diagnosis, KPS, post-surgical T2 volume, contrast enhancement on initial MRI, MGMT promoter methylation) were associated with PFS (Table 2). CNS WHO grade, post-surgical T2 tumor volume and contrast enhancement on initial MRI, however, were associated with OS (Table 2). Multivariate analysis for OS did not show significant associations, but the number of events was low with 33 tumor-related deaths, limiting the interpretation (Table 3).

Discussion

Patients with lower grade IDHmut gliomas are often subjected to repetitive treatment over time due to frequent recurrences at a relatively young age [1, 23]. The roles of W&S strategies and chemotherapy regimens alone, e.g., to defer RT, have not been determined conclusively. Especially studies addressing TMZ chemotherapy alone have yielded controversial results, some of which imply potential negative effects on IDHmut tumors due to induction of hypermutation [14–17].

Table 3 Multivariate analysis of factors showing significant associations with overall survival in univariate analyses

Factor	OS		
	HR	95% CI	*p* value
Post-surgical T2 tumor volume[x]	1.01	1.00–1.02	0.07
CE on MRI yes vs no	1.71	0.65–4.62	0.28
Postsurgical strategy (TMZ vs W&S vs RT)	2.02	0.46–8.76	0.36
CNS WHO grade 3 vs 2	0.38	0.10–1.26	0.14

Vs versus; *CE* contrast enhancement; *MRI* magnetic resonance imaging; *OS* overall survival; *TMZ* temozolomide; *W&S* wait-and-scan; *RT* radiotherapy; *CNS* central nervous system; *WHO* World Health Organization

[x]Continuously scaled

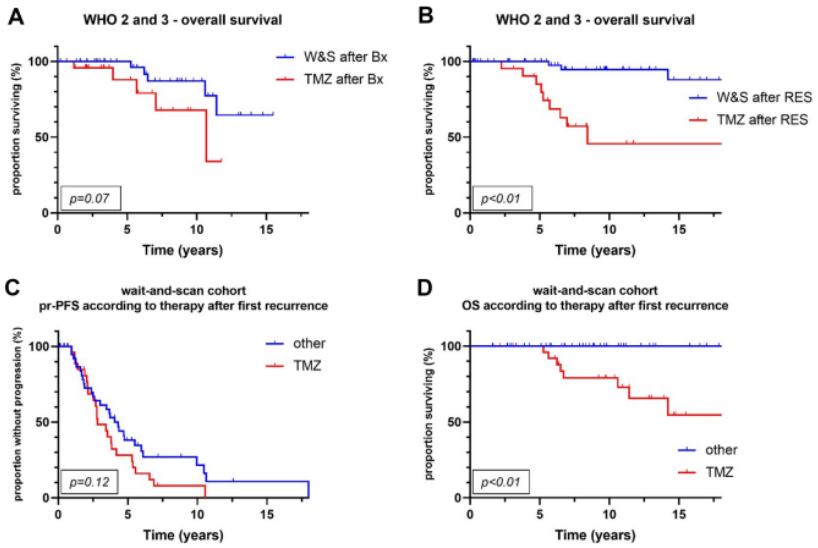


Fig. 5 Wait-and-scan strategies after stereotactic biopsy were associated with longer overall survival than temozolomide alone after stereotactic biopsy by trend (p = 0.07) (**A**). Patients monitored by means of wait-and-scan strategies after tumor resection showed significantly longer overall survival times than patients treated with temozolomide alone after tumor resection (p < 0.01) (**B**). Patients initially monitored by active surveillance (wait-and-scan cohort) and experiencing tumor progression were further stratified according to the initiated therapy at first recurrence. There was no significant difference in progression-free survival between patients treated with temozolomide alone and any other treatment strategy at first progression (p = 0.12) (**C**). Temozolomide alone at first recurrence was associated with shorter OS (p < 0.01) (**D**). *WHO* World Health Organization; *W&S* wait-and-scan; *Bx* stereotactic biopsy; *TMZ* temozolomide; *RES* tumor resection; *pr-PFS* post-recurrence progression-free survival

Table 2 Univariate analysis of patient-related factors potentially associated with progression-free survival or overall survival

Factor	PFS			OS		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age[x]	0.99	0.97–1.02	0.64	1.0	0.97–1.03	0.94
Sex, female vs male	1.35	0.75–2.47	0.33	1.14	0.56–2.33	0.72
KPS[x]	1.01	0.94–1.09	0.9	0.98	0.89–1.08	0.63
Post-surgical T2 tumor volume[x]	1.00	0.99–1.01	0.86	1.01	1.00–1.02	< 0.01
CE on MRI yes vs no	1.38	0.93–2.03	0.11	2.81	1.30–6.29	< 0.01
Postsurgical strategy (TMZ vs W&S vs RT)	0.83	0.38–1.68	0.62	6.78	2.87–17.25	< 0.01
CNS WHO grade 3 vs 2	1.16	0.63–2.21	0.65	3.23	1.59–6.63	< 0.01
MGMT promoter status	0.45	0.19–1.33	0.10	0.86	0.25–5.93	0.84

Vs versus; *KPS* Karnofsky Performance Status; *CNS* central nervous system; *WHO* World Health Organization; *MGMT O6*-methylguanine-DNA methyltransferase; *CE* contrast enhancement; *MRI*, magnetic resonance imaging, *PFS* progression-free survival; *OS* overall survival; *W&S* wait-and-scan; *TMZ* temozolomide; *RT* radiotherapy

[x]Continuously scaled

We here present a retrospective outcome analysis of 183 patients with WHO grade 2 or 3 IDHmut astrocytomas initially monitored through a W&S strategy or treated with TMZ alone or RT alone. We accounted for WHO tumor grade, postoperative T2 tumor volumes and contrast enhancement and report superiority of RT over TMZ in

terms of PFS and OS. Furthermore, patients initially treated with TMZ showed significantly shorter survival times than patients initially monitored through W&S alone despite identical PFS. This association was observed in multiple subgroup analyses (Figs. 3, 4, 5). Of note, the latter analysis was subjected to substantial selection bias because of significant differences in tumor volume, contrast enhancement and WHO grades between groups that could only be in part accounted for in our analyses (Fig. 3).

Prospective trials respecting the contemporary, molecular classification of adult gliomas and investigating surveillance or monotherapy strategies are scarce. The NOA-04 randomized phase 3 trial investigated RT alone versus chemotherapy alone with either PCV or TMZ in anaplastic gliomas [10, 11]. Here, no differential benefit of RT alone versus chemotherapy alone was reported. In the subgroup of gliomas with 1p/19q-codeletion, i.e., oligodendrogliomas, and a CpG island methylator phenotype, TMZ was inferior to RT and PCV in terms of PFS and there was a trend towards shorter time-to-treatment failure and overall survival [11]. Superiority of RT over TMZ alone regarding PFS in the treatment of IDHmut low-grade astrocytomas was reported in the prospective, phase 3 EORTC 22033-26033 study [9]. The prospective EORTC 22845 randomized trial compared early RT versus RT that was delayed to the timepoint of first progression in lower grade gliomas. The study demonstrated prolonged PFS in the RT cohort, but no significant improvement in OS [6]. These results were mirrored by our data. Prospective trials comparing W&S strategies with chemotherapy alone within the framework of molecularly defined gliomas have yet to be conducted. Whereas the question of whether any of these strategies is superior to the others remains unanswered, clinical trials have demonstrated benefit of combination therapy of RT and alkylating chemotherapy over monomodality treatment in the treatment of IDHmut gliomas. For IDHmut astrocytomas WHO grade 2, radiochemotherapy with sequential PCV after radiation therapy is recommended in older patients or those with residual, posteroperative tumor. This recommendation is based on the RTOG 9802 study that demonstrated prolongated PFS and OS in the radiochemotherapy cohort when compared to RT alone in a large, prospective cohort of WHO grade 2 gliomas [2]. Therapy for WHO grade 3 astrocytomas is mainly determined by the CATNON trial (EORTC study 26053-22054) and consists of RT followed by 12 cycles of maintenance TMZ [24]. Clinical trials investigating RT followed by TMZ versus RT followed by PCV in IDHmut astrocytomas have not been conducted so far.

Despite the positive results from the CATNON study, recently published studies have slightly tempered expectations from TMZ therapy in IDHmut gliomas. Barthel et al. described hypermutator phenotypes after treatment with alkylating agents in diffuse gliomas [25]. In 2020, Touat et al. published sequencing data, analyzing mutational burden in 10′249 gliomas. Here, a therapy-driven induction of hypermutation through acquisition of a mismatch repair deficiency after TMZ therapy was described [15]. Yu et al. sequenced recurrent IDHmut gliomas and confirmed hypermutation in some patients treated with TMZ. They also reported an association with shorter survival and discontiguous, recurrent disease in patients previously treated with TMZ [16]. Still, these data do not warrant a change of current guidelines. The data provided here and within the framework of recent studies investigating TMZ-induced hypermutation, does, however, raise the question whether TMZ is the right choice in patients deemed not eligible for RT and believed to show a long, relatively good clinical course. PCV chemotherapy, or even PC regimens omitting vincristine to limit side effects, might be appropriate alternatives if W&S strategies are not justifiable. Multiple studies have shown efficacy of PCV chemotherapy after RT [2, 3, 26]. Data on surveillance strategies cannot be expected soon. The wait-or-treat study (IWOT; NCT03763422) sought to investigate W&S strategies versus radiochemotherapy after gross total resection, but was closed prematurely because of poor accrual. Chemotherapy alone is not the gold standard in the treatment of IDHmut gliomas, but there will be patient subgroups not eligible for standard therapy with RT, e.g., due to extensive tumor volumes. These patients are subjected to a selection bias and will have to be treated based on recommendations supported by comparably low levels of evidence. Prospective studies on monochemotherapy are not likely to be initiated in the future.

The major shortcoming of this study is its retrospective nature and thus selection bias. Some patient characteristics differed significantly between the cohorts. In the RT cohort, patients were older at diagnosis. In the TMZ cohort, clinical status was worse and more importantly, post-surgical T2 tumor volumes were larger. The proportion of WHO grade 2 as opposed to grade 3 astrocytomas was higher in the W&S cohort, favouring this cohort. These differences were in part corrected for through matched-pair and subgroup analyses (Figs. 3, 4).

Conclusions from retrospective data must be drawn with caution, but our data within the framework of recent studies favor RT over TMZ alone and justify a critical view on initial TMZ monotherapy for IDHmut astrocytomas [15, 16, 25]. The results on RT match prospective data. Even though the TMZ cohort is at a disadvantage, especially because of the comparably large tumor volumes, the inferiority as compared to the W&S cohort in terms of survival despite identical initial PFS was clear and highly significant. The fact that there was no difference in PFS might point towards a long-term effect of potential genetic alteration through TMZ therapy. This might not become clinically relevant in more aggressive, high-risk gliomas or glioblastomas. If

IDHmut astrocytomas previously treated with TMZ recur, caretakers might consider conducting a biopsy if the patient is not eligible for tumor resection to investigate mutational burden and potentially find molecular alterations that allow for targeted therapy [27]. So far, there is only indirect data on whether there is any tumor specific benefit from TMZ monotherapy when compared to no further therapy beyond surgery. Taking the retrospective nature of this study into account, one might at least hope for similar, if not better, clinical outcome in a cohort of patients that had received a median of 6 months of TMZ chemotherapy as compared to no therapy at all after histological sampling.

Author contributions JW: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Validation; Visualization; Roles/Writing—original draft. SK: Data curation; Methodology; Resources; Software; Validation; Formal analysis; Resources; Software. JB: Data curation; Formal analysis. FT: Data Curation. HB: Data curation; Investigation. SQ, BS: Investigation; Methodology. RE: Data curation; Investigation; Methodology, Resources. MN: Investigation; Resources. NT: Writing—review & editing. MW: Conceptualization; Formal analysis; Investigation; Supervision; Writing—review & editing. J-CT: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Roles/Writing—original draft; Writing—review & editing.

Funding Open Access funding enabled and organized by Projekt DEAL. No funding to report.

Data availability Clinical and molecular data on all patients are anonymized and stored in local data bases secured by passwords.

Code availability Not applicable.

Declarations

Conflict of interest Maximilian Niyazi—MN has received honoraria for lectures or advisory board participation or consulting from Brainlab and Novocure. Michael Weller—MW has received research grants from Abbvie, Adastra, Apogenix, Merck, Sharp & Dohme (MSD), Merck (EMD), Novocure and Quercis, and honoraria for lectures or advisory board participation or consulting from Abbvie, Adastra, Basilea, Bristol Meyer Squibb (BMS), Celgene, Medac, Merck, Sharp & Dohme (MSD), Merck (EMD), Nerviano Medical Sciences, Novartis, Orbus, Philogen, Roche, Tocagen and yMabs. Joerg-Christian Tonn—Consultant/speaker honoraria from BrainLab and Carthera, and royalties from Springer Publisher Intl. Jonathan Weller, Sophie Katzendobler, Jens Blobner, Frederic Thiele, Hannes Becker, Stefanie Quach, Rubert Egensperger, Bogdana Suchorska, Niklas Thon—No disclosures.

Ethical approval Ethics approval was obtained by the ethics committee of the Ludwig Maximilian University of Munich (Project Number 21-0612).

Consent to participate Consent to participate in retrospective studies is given prospectively by all patients treated at the Department of Neurosurgery of the Ludwig Maximilian University of Munich through a local prospective tumor registry.

Consent for publication All authors have consented in submitting this manuscript for publication in the Journal of Neuro-Oncology.

Research involving humans and/or animals participants The present study was conducted retrospectively.

References

1. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G et al (2021) EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol 18(3):170–186
2. Bell EH, Zhang P, Shaw EG, Buckner JC, Barger GR, Bullard DE et al (2020) Comprehensive genomic analysis in NRG Oncology/RTOG 9802: a phase III trial of radiation versus radiation plus procarbazine, lomustine (CCNU), and vincristine in high-risk low-grade glioma. J Clin Oncol 38(29):3407–3417
3. Buckner JC, Chakravarti A, Curran WJ Jr (2016) Radiation plus chemotherapy in low-grade glioma. N Engl J Med 375(5):490–491
4. Reuss DE, Mamatjan Y, Schrimpf D, Capper D, Hovestadt V, Kratz A et al (2015) IDH mutant diffuse and anaplastic astrocytomas have similar age at presentation and little difference in survival: a grading problem for WHO. Acta Neuropathol 129(6):867–873
5. Pignatti F, van den Bent M, Curran D, Debruyne C, Sylvester R, Therasse P et al (2002) Prognostic factors for survival in adult patients with cerebral low-grade glioma. J Clin Oncol 20(8):2076–2084
6. van den Bent MJ, Afra D, de Witte O, Ben Hassel M, Schraub S, Hoang-Xuan K et al (2005) Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults: the EORTC 22845 randomised trial. Lancet 366(9490):985–990
7. Douw L, Klein M, Fagel SS, van den Heuvel J, Taphoorn MJ, Aaronson NK et al (2009) Cognitive and radiological effects of radiotherapy in patients with low-grade glioma: long-term follow-up. Lancet Neurol 8(9):810–818
8. Klein M, Heimans JJ, Aaronson NK, van der Ploeg HM, Grit J, Muller M et al (2002) Effect of radiotherapy and other treatment-related factors on mid-term to long-term cognitive sequelae in low-grade gliomas: a comparative study. Lancet 360(9343):1361–1368
9. Baumert BG, Hegi ME, van den Bent MJ, von Deimling A, Gorlia T, Hoang-Xuan K et al (2016) Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033–26033): a randomised, open-label, phase 3 intergroup study. Lancet Oncol 17(11):1521–1532
10. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F et al (2009) NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine,

lomustine, and vincristine or temozolomide. J Clin Oncol 27(35):5874–5880
11. Wick W, Roth P, Hartmann C, Hau P, Nakamura M, Stockhammer F et al (2016) Long-term analysis of the NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. Neuro Oncol 18(11):1529–1537
12. Jaeckle KA, Ballman KV, van den Bent M, Giannini C, Galanis E, Brown PD et al (2021) CODEL: phase III study of RT, RT + TMZ, or TMZ for newly diagnosed 1p/19q codeleted oligodendroglioma. Analysis from the initial study design. Neuro Oncol 23(3):457–467
13. Weller J, Katzendobler S, Karschnia P, Lietke S, Egensperger R, Thon N et al (2021) PCV chemotherapy alone for WHO grade 2 oligodendroglioma: prolonged disease control with low risk of malignant progression. J Neuro-Oncol 153(2):283–291
14. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY et al (2014) Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 343(6167):189–193
15. Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrson CL et al (2020) Mechanisms and therapeutic implications of hypermutation in gliomas. Nature 580(7804):517–523
16. Yu Y, Villanueva-Meyer J, Grimmer MR, Hilz S, Solomon DA, Choi S et al (2021) Temozolomide-induced hypermutation is associated with distant recurrence and reduced survival after high-grade transformation of low-grade IDH-mutant gliomas. Neuro Oncol 23(11):1872–1884
17. Varn FS, Johnson KC, Martinek J, Huse JT, Nasrallah MP, Wesseling P et al (2022) Glioma progression is shaped by genetic evolution and microenvironment interactions. Cell 185(12):2184-2199 e16
18. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2021) World Health Organization classification of tumours of the central nervous system, 5th edn. International Agency for Research on Cancer, Lyon
19. US Department of Health and Human Services; National Institutes of Health NCI (2017) Common Terminology Criteria for Adverse Events (CTCAE) version 5
20. Wen PY, Chang SM, Van den Bent MJ, Vogelbaum MA, Macdonald DR, Lee EQ (2017) Response assessment in neuro-oncology clinical trials. J Clin Oncol 35(21):2439–2449
21. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G (2005) Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. Int J Cancer 113(3):379–385
22. Suchorska B, Schuller U, Biczok A, Lenski M, Albert NL, Giese A et al (2019) Contrast enhancement is a prognostic factor in IDH1/2 mutant, but not in wild-type WHO grade II/III glioma as confirmed by machine learning. Eur J Cancer 107:15–27
23. Lassman AB, Iwamoto FM, Cloughesy TF, Aldape KD, Rivera AL, Eichler AF et al (2011) International retrospective study of over 1000 adults with anaplastic oligodendroglial tumors. Neuro Oncol 13(6):649–659
24. van den Bent MJ, Baumert B, Erridge SC, Vogelbaum MA, Nowak AK, Sanson M et al (2017) Interim results from the CATNON trial (EORTC study 26053-22054) of treatment with concurrent and adjuvant temozolomide for 1p/19q non-co-deleted anaplastic glioma: a phase 3, randomised, open-label intergroup study. Lancet 390(10103):1645–1653
25. Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E et al (2019) Longitudinal molecular trajectories of diffuse glioma in adults. Nature 576(7785):112–120
26. Shaw EG, Wang M, Coons SW, Brachman DG, Buckner JC, Stelzer KJ et al (2012) Randomized trial of radiation therapy plus procarbazine, lomustine, and vincristine chemotherapy for supratentorial adult low-grade glioma: initial results of RTOG 9802. J Clin Oncol 30(25):3065–3070
27. Katzendobler S, Do A, Weller J, Dorostkar MM, Albert NL, Forbrig R et al (2022) Diagnostic yield and complication rate of stereotactic biopsies in precision medicine of gliomas. Front Neurol 13:822362

7. Literaturverzeichnis

1. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021;23(8):1231-51.

2. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014;16(7):896-913.

3. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. Neuro Oncol. 2015;17 Suppl 4:iv1-iv62.

4. Burgenske DM, Yang J, Decker PA, Kollmeyer TM, Kosel ML, Mladek AC, et al. Molecular profiling of long-term IDH-wildtype glioblastoma survivors. Neuro Oncol. 2019;21(11):1458-69.

5. Lassman AB, Iwamoto FM, Cloughesy TF, Aldape KD, Rivera AL, Eichler AF, et al. International retrospective study of over 1000 adults with anaplastic oligodendroglial tumors. Neuro Oncol. 2011;13(6):649-59.

6. Tatekawa H, Hagiwara A, Uetani H, Bahri S, Raymond C, Lai A, et al. Differentiating IDH status in human gliomas using machine learning and multiparametric MR/PET. Cancer Imaging. 2021;21(1):27.

7. Lee MH, Kim J, Kim ST, Shin HM, You HJ, Choi JW, et al. Prediction of IDH1 Mutation Status in Glioblastoma Using Machine Learning Technique Based on Quantitative Radiomic Data. World Neurosurg. 2019;125:e688-e96.

8. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol. 2021;18(3):170-86.

9. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. Cell. 2016;164(3):550-63.

10. Brat DJ, Aldape K, Colman H, Holland EC, Louis DN, Jenkins RB, et al. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". Acta Neuropathol. 2018;136(5):805-10.

11. Tesileanu CMS, Dirven L, Wijnenga MMJ, Koekkoek JAF, Vincent A, Dubbink HJ, et al. Survival of diffuse astrocytic glioma, IDH1/2 wildtype, with molecular features of glioblastoma, WHO grade IV: a confirmation of the cIMPACT-NOW criteria. Neuro Oncol. 2020;22(4):515-23.

12. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96.

13. Jakola AS, Myrmel KS, Kloster R, Torp SH, Lindal S, Unsgard G, et al. Comparison of a strategy favoring early surgical resection vs a strategy favoring watchful waiting in low-grade gliomas. JAMA. 2012;308(18):1881-8.

14. Southwell DG, Birk HS, Han SJ, Li J, Sall JW, Berger MS. Resection of gliomas deemed inoperable by neurosurgeons based on preoperative imaging studies. J Neurosurg. 2018;129(3):567-75.

15. Molinaro AM, Hervey-Jumper S, Morshed RA, Young J, Han SJ, Chunduru P, et al. Association of Maximal Extent of Resection of Contrast-Enhanced and Non-Contrast-Enhanced Tumor With Survival Within Molecular Subgroups of Patients With Newly Diagnosed Glioblastoma. JAMA Oncol. 2020;6(4):495-503.

16. Katzendobler S, Do A, Weller J, Dorostkar MM, Albert NL, Forbrig R, et al. Diagnostic Yield and Complication Rate of Stereotactic Biopsies in Precision Medicine of Gliomas. Front Neurol. 2022;13:822362.

17. Hall WA. The safety and efficacy of stereotactic biopsy for intracranial lesions. Cancer. 1998;82(9):1749-55.

18. Dammers R, Haitsma IK, Schouten JW, Kros JM, Avezaat CJ, Vincent AJ. Safety and efficacy of frameless and frame-based intracranial biopsy techniques. Acta Neurochir (Wien). 2008;150(1):23-9.

19. Bodensohn R, Forbrig R, Quach S, Reis J, Boulesteix AL, Mansmann U, et al. MRI-based contrast clearance analysis shows high differentiation accuracy between radiation-induced reactions and progressive disease after cranial radiotherapy. ESMO Open. 2022;7(2):100424.

20. Gutin PH, Iwamoto FM, Beal K, Mohile NA, Karimi S, Hou BL, et al. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. Int J Radiat Oncol Biol Phys. 2009;75(1):156-63.

21. Suchorska B, Jansen NL, Linn J, Kretzschmar H, Janssen H, Eigenbrod S, et al. Biological tumor volume in 18FET-PET before radiochemotherapy correlates with survival in GBM. Neurology. 2015;84(7):710-9.

22. Jansen NL, Suchorska B, Wenter V, Schmid-Tannwald C, Todica A, Eigenbrod S, et al. Prognostic significance of dynamic 18F-FET PET in newly diagnosed astrocytic high-grade glioma. J Nucl Med. 2015;56(1):9-15.

23. Tan MSY, Sandanaraj E, Chong YK, Lim SW, Koh LWH, Ng WH, et al. A STAT3-based gene signature stratifies glioma patients for targeted therapy. Nat Commun. 2019;10(1):3601.

24. Sathornsumetee S, Reardon DA, Desjardins A, Quinn JA, Vredenburgh JJ, Rich JN. Molecularly targeted therapy for malignant glioma. Cancer. 2007;110(1):13-24.

25. Buckner JC, Chakravarti A, Curran WJ, Jr. Radiation plus Chemotherapy in Low-Grade Glioma. N Engl J Med. 2016;375(5):490-1.

26. Bell EH, Zhang P, Shaw EG, Buckner JC, Barger GR, Bullard DE, et al. Comprehensive Genomic Analysis in NRG Oncology/RTOG 9802: A Phase III Trial of Radiation Versus Radiation Plus Procarbazine, Lomustine (CCNU), and Vincristine in High-Risk Low-Grade Glioma. J Clin Oncol. 2020;38(29):3407-17.

27. Baumert BG, Hegi ME, van den Bent MJ, von Deimling A, Gorlia T, Hoang-Xuan K, et al. Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033-26033): a randomised, open-label, phase 3 intergroup study. Lancet Oncol. 2016;17(11):1521-32.

28. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. J Clin Oncol. 2009;27(35):5874-80.

29. Wick W, Roth P, Hartmann C, Hau P, Nakamura M, Stockhammer F, et al. Long-term analysis of the NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. Neuro Oncol. 2016;18(11):1529-37.

30. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science. 2014;343(6167):189-93.

31. Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrson CL, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. Nature. 2020;580(7804):517-23.

32. Yu Y, Villanueva-Meyer J, Grimmer MR, Hilz S, Solomon DA, Choi S, et al. Temozolomide-induced hypermutation is associated with distant recurrence and reduced survival after high-grade transformation of low-grade IDH-mutant gliomas. Neuro Oncol. 2021;23(11):1872-84.

33. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014;370(8):709-22.

34. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014;370(8):699-708.

Danksagung

An dieser Stelle möchte ich meinen Dank aussprechen an alle Menschen, die zur Anfertigung dieser Dissertation beigetragen haben.

In erster Linie gilt mein Dank meinem Doktorvater Herrn Prof. Jörg-Christian Tonn für die Möglichkeit, meine Promotion unter seiner Leitung durchzuführen und die stets hervorragende Betreuung.

Herrn Prof. Maximilian Niyazi und Herrn Prof. Niklas Thon danke ich für die Unterstützung und Hilfsbereitschaft während des gesamten Promotionsprozesses.

Besonders bedanken möchte ich mich bei Herrn Jonathan Weller für die maßgebliche Beteiligung an der Manuskripterstellung und das tiefgreifende Verständnis in jeder Hinsicht.

Zuletzt danke ich meinen Eltern, die mir meine Ausbildung ermöglicht haben und mich stets in jeder Entscheidung bedingungslos unterstützt haben.