Aus der Klinik für Neurochirurgie

der Ludwig-Maximilians-Universität München

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Stellenwert der Verlaufsbeobachtung, operativen Tumorresektion und Monochemotherapie bei Oligodendrogliomen WHO Grad II

Dissertation

zum Erwerb des Doktorgrades der Medizin

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität München

vorgelegt von

Jonathan Sebastian Weller

aus

Männedorf in der Schweiz

Jahr

2022

Mit Genehmigung der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

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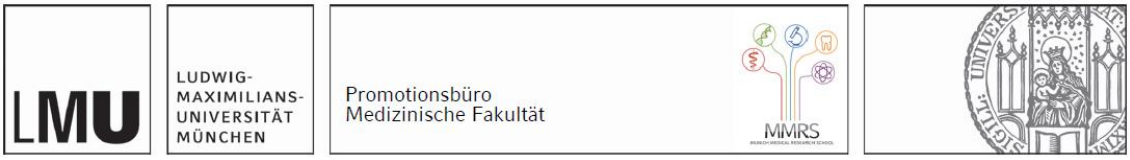

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Inhaltsverzeichnis

Abkürzungsverzeichnis

Gy	-	Gray
MGMT	-	O6-Methylguanin-DNA-Methyltransferase
mut	-	mutiert
n.r.	-	not reached, nicht erreicht
OS	-	overall survival, Gesamtüberleben
PCR	-	polymerase chain reaction, Polymerare-Kettenreaktion
PCV	-	Procarbazine, CCNU/Lomustin und Vincristin
PFS	-	progression-free survival, progressionsfreies Überleben
RES	-	Resektion
TERT	-	Telomerase Reverse Transcriptase
TMZ	-	Temozolomid
TTM	-	time-to-malignization, Zeit bis zur histologischen Malignisierung
Vs	-	versus
W&S	-	wait-and-scan, Abwarten und bilmorphologische Kontrollen
Wt	-	Wildtyp
1p/19q-codel	-	Kombinierter Verlust der Chromosomen 1p und 19q

Publikationsliste

Publikation I

PCV chemotherapy alone for WHO grade 2 oligodendroglioma: prolonged disease control with low risk of malignant progression.

Weller J, Katzendobler S, Karschnia P, Lietke S, Egensperger R, Thon N, Weller M, Suchorska B, Tonn JC.

J Neurooncol. 2021 Jun;153(2):283-291. doi: 10.1007/s11060-021-03765-z. Epub 2021 May 1.

PMID: 33932195

Publikation II

Extent, pattern, and prognostic value of MGMT promotor methylation: does it differ between glioblastoma and IDH-wildtype/TERT-mutated astrocytoma?

Teske N, Karschnia P, Weller J, Siller S, Dorostkar MM, Herms J, von Baumgarten L, Tonn JC, Thon N.

J Neurooncol. 2021 Dec 13. doi: 10.1007/s11060-021-03912-6. Online ahead of print.

PMID: 34902093

1. Beitrag zu den Veröffentlichungen

1.1 Beitrag zu Publikation I

In der Publikation mit dem Titel „PCV chemotherapy alone for WHO grade 2 oligodendroglioma: prolonged disease control with low risk of malignant progression" werden unterschiedliche Therapiestrategien und deren Auswirkung auf die Prognose molekularer Oligodendrogliome untersucht. Mein Beitrag zu dieser Publikation umfasste folgende Aufgaben:

- Verfassen des Ethikantrags
- Identifizierung aller Patienten, die zwischen den Jahren 2003 und 2019 die Erstdiagnose eines WHO Grad 2 Oligodendroglioms in der Klinik und Poliklinik für Neurochirurgie des Klinikums der Universität München erhalten haben
- Erstellung einer Excel-basierten Datentabelle mit klinischen, histologischen, molekulargenetischen und bildmorphologischen Daten der betroffenen Patienten
- Ausmessen des prä- und posttherapeutischen MRT-Tumorvolumens mittels manueller Segmentierung
- Statistische Analysen (deskriptive Statistik, Matched-Pair-Analysen, Kaplan-Meier-Kurven, univariate Analysen)
- Erstellung der Diagramme und Tabellen
- Verfassen des Manuskripts
- Korrektur des Manuskripts im Anschluss an das Peer-Review-Verfahren

1.2 Beitrag zu Publikation II

In der zweiten Publikation, an der ich als Co-Autor mitgearbeitet habe, wurden Gliome untersucht, die zwar histologisch einem Astrozytom WHO Grad 2 oder 3 entsprechen, molekulargenetisch jedoch Eigenschaften eines Glioblastoms WHO Grad 4 aufweisen. Mein Beitrag zu dieser Publikation umfasste folgende Aufgaben:

- Identifizierung aller Patienten, die zwischen den Jahren 2004 und 2014 die Diagnose eines IDH-Wildtyp-Glioms WHO Grad 2 oder 3 mit nachgewiesener Mutation des TERT-Promoters erhielten
- Erstellung einer Excel-basierten Datentabelle mit klinischen, histologischen, molekulargenetischen und bildmorphologischen Daten der betroffenen Patienten
- Korrekturlesen des finalen Manuskripts

2. Einleitung

2.1 Hintergrund

Das zentrale Nervensystem, das im Menschen Gehirn und Rückenmark umfasst, enthält Neurone und Stützzellen. Neurone sind Nervenzellen und für die Erregungsweiterleitung und -verarbeitung verantwortlich. Die Stützzellen des zentralen Nervensystems werden Gliazellen genannt und erfüllen Supportivfunktionen. Hirntumore, die mutmaßlich den Gliazellen entstammen, werden Gliome genannt (1, 2). Die jährliche Inzidenz pro 100'000 Einwohner in den USA beträgt ca. 6-7 (3). Die Ursache für die Entstehung von Gliomen ist nicht abschließend geklärt, das Erkrankungsrisiko steigt jedoch mit dem Alter. Strahlenexposition, beispielsweise durch eine vorhergehende Bestrahlungstherapie oder ein Atomunglück, ist ein Risikofaktor. Familiäre Häufungen sind selten, können aber insbesondere im Rahmen von Tumor-Syndromen auftreten, beispielsweise bei Patienten mit Neurofibromatose, Li-Fraumeni-, Lynch- oder Turcot-Syndrom (4). Häufig erstmanifestieren sich Gliome mit epileptischen Anfällen oder neurologischen Defiziten, beispielsweise Aphasie, Paresen, Gesichtsfelddefekte oder neurokognitiven Einschränkungen. Weitere klinische Zeichen können Kopfschmerzen, Übelkeit und Erbrechen sein (5).

Die Klassifikation der Gliome basiert auf Kriterien der Weltgesundheitsorganisation (World Health Organization; WHO), die im Jahr 2021 aktualisiert wurden (2). Die integrierte Diagnose eines Glioms basiert auf der histologischen Tumorart (I), dem histologischen Tumorgrad zwischen 1 und 4 (II) und dem molekulargenetischen Profil (III) (6). Gliome des WHO Grades 1, beispielsweise pilozytische Astrozytome, entstehen häufig im Kindesalter und sind je nach Lokalisation und Ausdehnung potenziell kurativ behandelbar. Astrozytome und Oligodendrogliome der WHO Grade 2 und 3 manifestieren sich häufig im jungen und mittleren Erwachsenenalter (1, 5, 7). Die Prognose dieser Tumoren ist heterogen. Trotz effektiver Therapien rezidivieren diese Gliome häufig und histologische Malignisierungen sind nicht selten (5, 8). Glioblastome entsprechen dem WHO Grad 4 und sind die häufigsten und prognostisch ungünstigsten malignen, primären Neoplasien des zentralen Nervensystems. Das Gesamtüberleben betroffener Patienten nach Diagnosestellung umfasst in klinischen Studien ca. 14-17 Monate trotz multimodaler Therapie (9, 10).

2.2 WHO Klassifikation und Molekulargenetik

In der WHO Klassifikation aus dem Jahr 2016 wurden molekulargenetische Eigenschaften erstmalig als maßgebliches Definitionskriterium diffuser und anaplastischer Gliome eingeführt (1). Entscheidend für die Namensgebung, Prognose und Therapie ist insbesondere der Mutationsstatus des Isocitrat-Dehydrogenase-Gens 1 und 2 (*IDH*). Die Isocitrat-Dehydrogenase ist ein Enzym des Citratzyklus, das die Metabolisierung von Isocitrat zu alpha-Ketoglutarat katalysiert. In Gliomzellen führt eine

IDH1- oder *IDH2*-Mutation (*IDHmut*) zu einer Metabolisierung des alpha-Ketoglutarats zu D-2-Hydroxyglutarat. Die Akkumulation des D-2-Hydroxyglutarats bedingt intrazelluläre, metabolische Veränderungen sowie und epigenetische Veränderungen durch globale DNA- und Histon-Hypermethylierung (11-14). Innerhalb der *IDHmut* Gliome erfolgt die weitere Stratifizierung anhand des 1p/19q-Status. Eine unbalancierte Translokation zwischen den Chromosomen 1 und 19 führt zu einem Verlust des kurzen Arms des Chromosoms 1 und des langen Arms des Chromosoms 19, der als 1p/19q-Co-Deletion bezeichnet wird (1). *IDHmut* Gliome ohne 1p/19q-Co-Deletion werden gemäß der WHO Klassifikation aus dem Jahr 2021 Astrozytome genannt und histologisch in Grad 2, 3 oder 4 eingeteilt. *IDHmut* Gliome mit einer 1p/19q-Co-Deletion entsprechen den Oligodendrogliomen, die entweder dem histologischen Grad 2 oder 3 zugewiesen werden (Fig. 1).

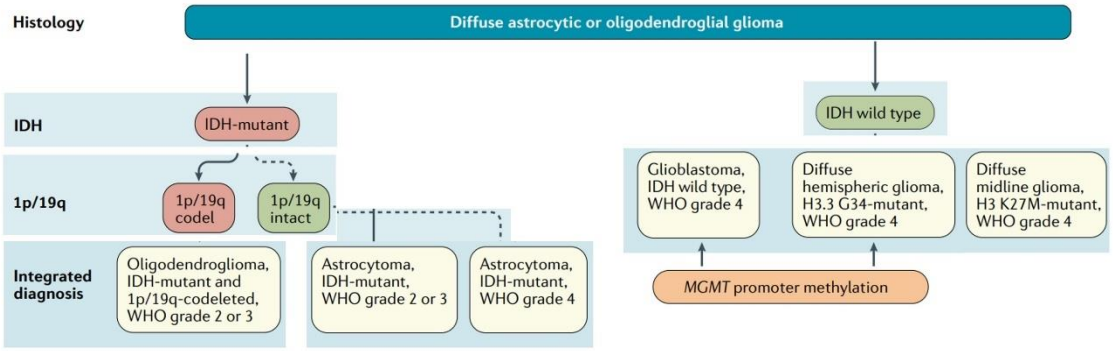

Fig. 1. Vereinfachter, diagnostischer Algorithmus zur Klassifizierung der diffusen Gliome anhand des IDH- und 1p/19q-Co-Deletions-Status. Angepasst von *Weller M et al., 2021, Nature Reviews*, Referenz 27. *IDH*, isocitrate dehydrogenase gene; *codel*, Co-Deletion; *WHO*, World Health Organization.

In der Behandlung des Glioblastoms ist der Promoter-Methylierungsstatus des O^6-Methylguanin-DNA-Methyltransferase-Gens (MGMT) prädiktiv für ein Ansprechen des Tumors auf eine Temozolomid-Therapie (15, 16). MGMT ist ein DNA-Reparaturprotein, das Alkylgruppen an der O^6-Position des Guanins entfernt. Eine Promoter-Methylierung des MGMT-Gens führt zu einer verminderten Genexpression und konsekutiv zu einer erhöhten Anfälligkeit gegenüber alkylierenden Chemotherapeutika wie Temozolomid (16).

In den vergangenen Jahren wurden zunehmend eine Gruppe von Gliomen ohne IDH-Mutation abgegrenzt, die histologisch zwar niedriggradigen, hirneigenen Tumoren entsprechen, molekulargenetisch jedoch Marker eines Glioblastoms WHO Grad 4 aufweisen, beispielsweise eine Mutation im Telomerase-Reverse-Transkriptase

Promoter (*pTERTmut*) bei *IDH*-Wildtyp-Status (*IDHwt*) (17-20). Telomere sind DNA-Abschnitte an den Enden der Chromosomen, die eine wichtige Rolle in der Zellstabilisierung und -alterung spielen (21). Bei der Zellteilung verkürzen sich die Telomere, wodurch die Lebensdauer der meisten, normalen Körperzellen im Menschen limitiert ist. In gewissen Zellen, beispielsweise Keim- oder Stammzellen, kann dieser Verlust durch das Enzym Telomerase revertiert werden. In Tumoren führt eine vermehrte Telomerase-Aktivität, beispielsweise durch *pTERTmut*, zu einer Immortalisierung der Zellen. In ca. 60-70% der Glioblastome wird eine vermehrte Telomerase-Aktivität beobachtet (22-24).

Klinische Studien haben gezeigt, dass sich das Gesamtüberleben von Patienten mit *IDHwt pTERTmut* Astrozytomen WHO Grad 2 und 3 vermutlich nicht wesentlich von Patienten mit Glioblastomen unterscheidet (20, 25, 26). Dies wirft die Frage auf, ob histologische Astrozytome mit molekularen Markern eines Glioblastoms möglicherweise über die genannten Mutationen hinaus auch ähnliche, epigenetische Eigenschaften wie Glioblastome aufweisen. Diese Fragestellung wird in der vorliegenden Promotionsarbeit aufgegriffen (27). In dieser Arbeit wird der MGMT Promoter von *IDHwt pTERTmut* Astrozytomen und histopathologischen Glioblastomen mittels PCR und Sanger-Sequenzierung auf ihre MGMT-Promoter-Methylierungsmuster analysiert, um eine mögliche Verwandtschaft der genannten Subgruppen zu untersuchen. Darüber hinaus soll untersucht werden, ob das Ausmaß der Methylierung mit der Prognose korreliert.

2.3 Therapie

Nachdem die Verdachtsdiagnose eines Glioms anhand einer MRT-Untersuchung mit und ohne Kontrastmittelgabe gestellt wurde, sollte möglichst zeitnah die Gewebsuntersuchung erfolgen. Sofern eine operative Tumorresektion mit einem geringen Risiko für den Patienten möglich ist, sollte diese als diagnostische und therapeutische Maßnahme am Anfang der Tumortherapie stehen (28). Falls eine Tumorresektion nicht zielführend erscheint, beispielsweise aufgrund eines bereits präoperativ deutlich eingeschränkten, klinischen Zustands des Patienten, einer eloquenten Lage des Tumors oder aufgrund der Größe des Befunds, sollte eine stereotaktische Probenentnahme erfolgen, um die Verdachtsdiagnose zu sichern.

Abhängig vom Resektionsausmaß, den neurologischen Symptomen und dem Patientenalter erfolgt die Indikationsstellung zur weiterführenden Therapie im Anschluss an die Klassifizierung des Glioms (7, 28-31). In der klinischen Praxis existieren unterschiedliche Chemotherapien, die in der Behandlung molekularer Astrozytome und Oligodendrogliome Anwendung finden. Temozolomid ist ein alkylierendes Zytostatikum, das insbesondere in Kombination mit einer Strahlentherapie in der Behandlung höhergradiger Gliome etabliert und standardmäßig angewandt wird (16, 32). Die Kombinationsbehandlung mittels Procarbazin, CCNU/Lomustin und Vincristin (PCV) ist besonders in der Behandlung der Oligodendrogliome etabliert und kann sequenziell mit

einer Strahlentherapie kombiniert werden. Die Strahlentherapie, ob als Monotherapie oder in Kombination mit einer Chemotherapie, ist ein entscheidender Bestandteil der Gliombehandlung. Die Standardtherapie umfasst in der Regel die Applikation von 50-60 Gy in 1.8-2 Gy-Fraktionen über 6 Wochen (31-33).

Bei Patienten mit Oligodendrogliomen der WHO Grade 2 und 3, die bei Diagnosestellung jünger als 40 Jahre alt sind und eine Totalresektion des Tumors erhalten haben, kann ein abwartendes Verhalten mit regelmäßigen, klinischen und bildmorphologischen Verlaufskontrollen vertreten werden. Anderenfalls wird eine Radiotherapie mit sequenzieller PCV-Polychemotherapie empfohlen (28, 31). Spätfolgen der Strahlentherapie beinhalten neurokognitive Defizite und potenziell sekundäre Neoplasien. Eine bekannte Nebenwirkung der Vincristin-Therapie ist die periphere Neuropathie (7, 34-36). Der Vorteil einer frühen, aggressiven Tumortherapie muss insbesondere bei Patienten mit Oligodendrogliomen, die in der Regel lange Überlebenszeiten aufweisen, vorsichtig gegen die potenziellen Nebenwirkungen der frühen, tumorspezifischen Therapie abgewogen werden.

In dieser Promotionsarbeit wird untersucht, ob weniger aggressive, initiale Tumortherapien ebenfalls mit einem Tumoransprechen und gutem, klinischen Verlauf assoziiert sind. Verglichen werden das progressionsfreie Überleben und Malignisierungsraten unterschiedlicher Therapie-Kohorten: reine Beobachtungsstrategie nach histologischer Sicherung durch minimalinvasive Biopsie (I) versus alleinige Tumorresektion (II) versus PCV-Polychemotherapie nach Biopsie (III) versus TMZ nach Biopsie (IV).

3. Zusammenfassung

Im Rahmen des Promotionsvorhabens erfolgte eine retrospektive Datenanalyse von Patienten mit WHO Grad 2 und 3 Gliomen, die im Klinikum der Universität München erstdiagnostiziert und behandelt wurden. Es wurde der Einfluss unterschiedlicher Einzeltherapien auf die Prognose von Patienten mit molekularen Oligodendrogliomen untersucht. Des Weiteren wurden epigenetische Eigenschaften des MGMT-Promoters in *IDHwt pTERTmut* Astrozytomen untersucht und mit dem Gesamtüberleben korreliert.

Zusammenfassend suggerieren die erhobenen und publizierten Daten einen Vorteil der PCV-Therapie gegenüber einer alleinigen Tumorresektion oder einer alleinigen Temozolomid-Therapie in der Behandlung molekularer Oligodendrogliome des WHO Grads 2. Patienten mit einer PCV-Therapie im Anschluss an eine Biopsie zeigten ein progressionsfreies Überleben von 9.1 Jahren im Vergleich zur 4.4 Jahren nach einer Tumorresektion ohne weiterführende Therapie und 3.6 Jahren im Falle einer Temozolomid-Monotherapie (p=0.05). In der PCV-Patientenkohorte traten signifikant seltener histologische Tumormalignisierungen auf als bei Patienten, die im Anschluss an die Probesicherung durch Biopsie oder Tumorresektion keine weitere Therapie bis zum Zeitpunkt des ersten Progresses erhielten (p=0.01). Der beobachtete Vorteil der PCV-Therapie gegenüber einer Temozolomid-Therapie wurde durch Matched-Pair-Analysen und Vergleiche in Tumorrezidiven gestützt. In der Analyse der Tumorvolumina zeigte sich eine Korrelation zwischen kleinem, initialen Tumorvolumen und verlängertem, progressionsfreien Überleben. Durch PCV-Behandlung ließ sich das Tumorvolumen im Median um 49% im Vergleich zum prätherapeutischen Tumorvolumen verkleinern.

Die Ergebnisse sprechen für eine PCV-Therapie bei Patienten, bei denen eine Tumorresektion oder Strahlentherapie ungünstig ist oder abgelehnt wird. Dies könnte beispielsweise junge Patienten mit großen Tumorvolumina in eloquenten Arealen betreffen. Prospektive Studien hierzu stehen aus. Eine neoadjuvante PCV-Therapie könnte evaluiert werden, um das Tumorvolumen vor einer Resektion oder Strahlentherapie zu verringern.

Die epigenetischen Analysen des MGMT-Promoters bei histologischen Astrozytomen des WHO Grads 2 und 3 mit molekularen Markern eines Glioblastoms zeigte ähnliche Ausmaße und Muster der Promoter-Methylierung des MGMT-Gens wie histopathologische Glioblastome und ein verbessertes Gesamtüberleben in Patienten mit ausgeprägter Methylierung im Vergleich zu Patienten mit fehlender oder geringer Methylierung.

4. Abstract

INTRODUCTION

Gliomas of histological WHO grades 2 and 3 show a heterogenous prognosis that largely depends on molecular tumor characteristics. Presence of 1p/19q codeletions distinguishes oligodendroglioma from astrocytoma among *IDHmut* gliomas. Both entities are associated with favourable outcome when compared to *IDHwt* WHO grade 2 and 3 astrocytomas with molecular features of glioblastomas, e.g., *pTERTmut* (which are now referred as glioblastoma). We here investigated different therapies in WHO grade 2 oligodendrogliomas (Publication I) and assessed clinical and epigenetic similarities between *IDHwt pTERTmut* WHO grade 2 and 3 astrocytomas and glioblastoma (Publication II).

METHODS

In this single-centre, retrospective study, 420 patients were included, including 142 patients with oligodendroglioma, 54 patients with *IDHwt* astrocytoma WHO grade 2 or 3 with *pTERTmut* and 224 patients with histopathological glioblastoma. In Publication I, different therapeutic approaches in the treatment of oligodendroglioma WHO grade 2 were investigated, including surveillance strategies after biopsy (wait-and-scan; n=59) or tumor resection (n=27), temozolomide chemotherapy after biopsy (n=26) or the combination therapy of procarbazine, CCNU/lomustine and vincristine (PCV; n=30) after biopsy. In Publication II, extent and pattern of MGMT promoter methylation in *IDHwt pTERTmut* astrocytomas WHO grade 2 and 3 and in histological glioblastoma were investigated through PCR and Sanger sequencing.

RESULTS

The retrospective outcome analysis of WHO grade 2 oligodendrogliomas showed superior progression-free survival in patients receiving PCV therapy after biopsy when compared to wait-and-scan or resection only or temozolomide. (in years, 9.1 (PCV) vs 5.1 (wait-and-scan) vs 4.4 (resection) vs 3.6 (temozolomide); p=0.05). Longer progression-free survival in patients treated with PCV when compared to temozolomide was also seen in a matched-pair analysis (p=0.03) and in patients that were treated with PCV or temozolomide at first progression (p=0.04). Histological progression occurred at significantly lower rate in the PCV cohort when compared to all other cohorts (p=0.01). Epigenetic analyses of the MGMT promoter showed similar pattern and extent of methylation in *IDHwt pTERTmut* astrocytomas and glioblastomas. Overall survival and progression-free survival were similar in both groups irrespective of lower initial histological grade.

CONCLUSION

PCV chemotherapy prolongs progression-free survival and potentially delays malignant transformation in oligodendroglioma WHO grade 2. Extent and pattern of MGMT promoter methylation in *IDHwt pTERTmut* astrocytomas and glioblastomas are similar.

5. Publikation I

Journal of Neuro-Oncology
https://doi.org/10.1007/s11060-021-03765-z

CLINICAL STUDY

PCV chemotherapy alone for WHO grade 2 oligodendroglioma: prolonged disease control with low risk of malignant progression

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Received: 27 February 2021 / Accepted: 22 April 2021

Abstract

Introduction The role of chemotherapy alone in newly diagnosed WHO grade 2 oligodendroglioma after biopsy, incomplete or gross total resection remains controversial. We here analyze the clinical outcome of four patient cohorts being treated with either procarbazine, CCNU and vincristine (PCV) or temozolomide (TMZ) after biopsy, resection only, or wait-and-scan after biopsy.

Methods Patients (n = 142) with molecularly defined oligodendroglioma (WHO 2016) were assigned to four cohorts: W&S, wait-and-scan after stereotactic biopsy (n = 59); RES, surgical resection only (n = 27); TMZ, temozolomide after biopsy (n = 26) or PCV (n = 30) after biopsy. Presurgical MRI T2 tumor volumes were obtained by manual segmentation. Progression-free survival (PFS), post-recurrence PFS (PR-PFS) and rate of histological progression to grade 3 were analyzed.

Results PFS was longest after PCV (9.1 years), compared to 5.1 years after W&S, 4.4 years after RES and 3.6 years after TMZ. The rate of histological progression from grade 2 to 3 within 10 years was 9% for the PCV, 29% for the W&S, 67% for the RES and 75% for the TMZ group ($p = 0.01$). In the W&S group, patients treated with PCV at first relapse had a longer PFS from intervention than those treated with TMZ (7.2 vs 4.0 years, $p = 0.04$). Multivariate analysis identified smaller tumor volume prior to any intervention ($p = 0.02$) to be prognostic for PFS.

Conclusions PCV chemotherapy alone is an effective treatment for WHO grade 2 oligodendroglioma, with long PFS and low rate of histological progression.

Keywords Resection · Chemotherapy · Imaging · Low-grade glioma

Introduction

Oligodendrogliomas occur predominantly in young to middle adulthood and often show a prolonged, indolent clinical course. The World Health Organization (WHO) histologically distinguishes diffuse, WHO grade 2 from anaplastic, WHO grade 3 oligodendroglioma, but grading criteria to distinguish grades 2 and 3 remain controversial [1–4]. Mutations of the isocitrate dehydrogenase (*IDH*) genes 1 or 2 and codeletion of chromosomes 1p and 19p have become molecular prerequisites for a diagnosis of oligodendroglioma in 2016 [5].

Treatment options for WHO grade 2 oligodendroglioma comprise neurosurgical resection if safely feasible, radiotherapy (RT), chemotherapy, surveillance strategies, and combinations thereof. If gross total resection is achieved, a wait-and-scan strategy might be pursued. In patients with incomplete resection or considered at high risk for tumor progression, e.g., because of higher age, radiotherapy (RT) followed by chemotherapy consisting of procarbazine, lomustine and vincristine (PCV) is recommended [6]. On a cautionary note, benefits in terms of overall survival need to be weighed carefully against long-term adverse effects including neurocognitive decline or secondary hematological neoplasms [7–11]. In patients with incompletely resected or progressive oligodendrogliomas after resection, TMZ

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Published online: 01 May 2021

alone showed promising results in deferring RT [12, 13]. In anaplastic oligodendrogliomas, TMZ did not achieve the same outcome as RT alone or RT plus TMZ [14, 15]. Studies investigating long-term outcome of patients suffering from WHO grade 2 oligodendroglioma treated with TMZ or PCV alone are lacking.

Deferring multimodal and aggressive therapies in young patients with intention to delay treatment-induced toxicity without jeopardizing long-term outcome would be of great benefit. Due to institutional multidisciplinary tumor board decisions adhering to such treatment concepts, we could investigate long-term outcome of four different treatment strategies omitting initial RT in WHO grade 2 oligodendroglioma in this study: resection only or, after stereotactic biopsy, either a wait-and-scan strategy or chemotherapy using TMZ or PCV only.

Patients and methods

Patient evaluation and treatment

The database of the Department of Neurosurgery at the University Hospital Munich was screened for patients with newly diagnosed 1p/19q-codeleted WHO grade 2 gliomas between 2003 and 2019 (Fig. 1). Ethics approval was obtained by the Ethics Committee of the Ludwig Maximilian University of Munich (project number 20-513). Diagnosis was defined as first histological confirmation of oligodendroglioma through frame-based stereotactic biopsy or tumor resection. Initial symptoms leading to the diagnosis were assessed. In patients diagnosed due to reasons other than neurological symptoms consistent with site and volume of the oligodendroglioma, the diagnosis was termed "incidental". Treatment decisions were based on interdisciplinary brain tumor board recommendations and patient's preference.

After exclusion of small treatment cohorts, four patient cohorts could be defined (Table 1; Fig. 1):

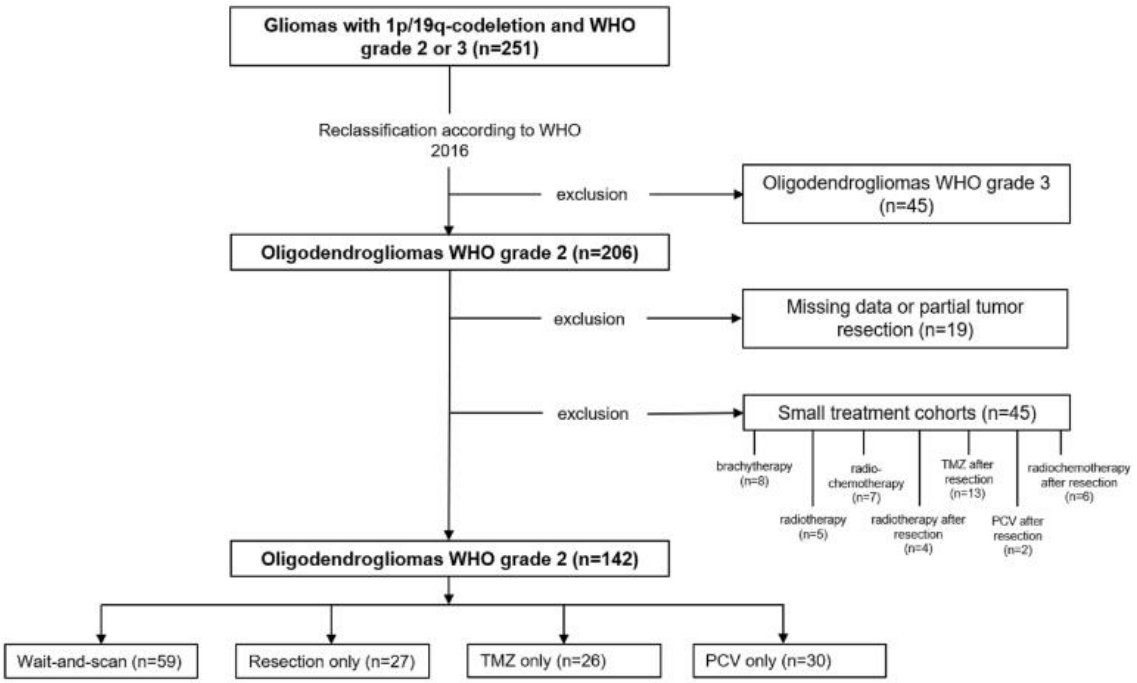

Fig. 1 Histological oligodendrogliomas, oligoastrocytomas and astrocytomas with 1p/19q codeletion between 2003 and 2019 were reclassified. WHO grade 3 oligodendrogliomas, patients with partial tumor resection, patients with missing data and patients from small treatment cohorts were excluded. Partial resection was defined as > 50% residual, postoperative T2 tumor volume. *WHO* World Health Organization, *PC(V)* procarbazine, CCNU and vincristine, *TMZ* temozolomide

Table 1 Clinical and patient characteristics

Parameter	All (n = 142)	Wait-and-scan (n = 59)	Resection only (n = 27)	TMZ only (n = 26)	PC(V) only (n = 30)	*p*-value
Age (years)						
Median	42	40	39	42	46	0.37
Range	20–80	20–80	20–64	26–70	27–64	
Sex, n (%)						
Female	74 (52%)	34 (58%)	7 (26%)	14 (54%)	13 (43%)	0.04*
Male	68 (48%)	25 (42%)	20 (74%)	12 (46%)	17 (57%)	
KPS						
≥ 80	138 (97%)	57 (97%)	27 (100%)	24 (92%)	30 (100%)	0.26
< 80	4 (3%)	2 (3%)	0 (0%)	2 (8%)	0 (0%)	
Trigger for diagnostic work-up						
Incidental finding	29 (20%)	16 (27%)	4 (15%)	3 (12%)	6 (20%)	
Seizure	98 (69%)	40 (68%)	20 (74%)	18 (69%)	20 (67%)	0.36
Neurological deficit	15 (11%)	3 (5%)	3 (11%)	5 (19%)	4 (13%)	
Localization, n (%)						
Frontal	74 (52%)	24 (41%)	21 (78%)	17 (65%)	12 (40%)	
Temporal	33 (23%)	19 (32%)	2 (7%)	4 (15%)	8 (27%)	
Insular	16 (11%)	7 (12%)	1 (4%)	3 (12%)	5 (17%)	
Parietal	15 (11%)	7 (12%)	3 (11%)	0 (0%)	5 (17%)	0.09
Occipital	1 (1%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	
Cingulate	1 (1%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	
Midline	2 (1%)	1 (2%)	0 (0%)	1 (4%)	0 (0%)	
Laterality, n (%)						
Left	70 (49%)	34 (58%)	12 (44%)	10 (39%)	14 (47%)	
Right	67 (47%)	25 (42%)	15 (56%)	13 (50%)	14 (47%)	0.09
Bilateral	5 (4%)	0 (0%)	0 (0%)	3 (12%)	2 (7%)	
Initial T2 volume (cm^3)						
Median	42	24	47	76	52	< 0.01*
Range	3–374	3–247	10–171	13–374	6–311	

- patients with biopsy and wait-and-scan strategy (W&S),
- patients with tumor resection (RES) and no further therapy,
- patients with biopsy and PCV chemotherapy (PCV) and
- patients with biopsy and TMZ chemotherapy (TMZ).

Patients who received maximal safe resection within 3 months after histological diagnosis through stereotactic biopsy were allocated to the resection only group. Extent of resection (EOR) was defined as absence of residual tumor volume on postoperative MRI scans that had to be obtained within 72 h after surgery. If residual tumor volume was less than 50% of the initial T2 tumor volume, EOR was termed "subtotal". If no residual tumor volume was observed, EOR was termed "gross total resection" (GTR). "Partial" resection referred to residual tumor volumes of more than 50%. All patients from the WS, TMZ and PCV cohorts fall into the high-risk low grade glioma group, because all were diagnosed through biopsy and thus had high postoperative tumor burden [7]. In the RES group, 10 patients had undergone GTR and 5 were younger than 40 years of age (range 31–39 years). Because of the risk of polyneuropathy and perceived lack of efficacy of tumors protected by the blood brain barrier, vincristine was not routinely added to procarbazine and CCNU. Patients receiving PC or PCV were pooled (PC(V)). PC(V) was given for 6 cycles if tolerated [16, 17]. TMZ was given for 6 cycles according to standard protocols if tolerated [18]. In the TMZ cohort, a median of 6 and a mean of 8 cycles was completed (range 3–20 cycles). In the PCV cohort, a median and mean of 6 cycles was administered (range 1–8 cycles). Clinical and imaging routine follow-up intervals were 3 to 6 months. Progression was defined retrospectively in accordance with RANO guidelines for low-grade gliomas as either clinical deterioration not attributable to other causes apart from the tumor (I) or tumor growth (increase in perpendicular diameters of 25% or more; increase in 25% tumor volume or more)

on T2 weighted MRI (II) [19]. In patients with suspicious, new MRI foci, e.g. new contrast enhancement, progression was assumed if a stereotactic biopsy of the focus confirmed glioma tissue (III). Adverse events were classified retrospectively according to the Common Terminology Criteria for Adverse Events 5.0 (CTCAE 5.0).

Histology and molecular status

All oligodendrogliomas in this study were re-evaluated by an experienced neuropathologist (R.E.) according to the WHO classification 2016 and only patients with tumors classified histologically and molecularly as oligodendroglioma WHO grade 2 were included (Fig. 1) [5]. Microsatellite markers were utilized for confirmation of 1p/19q codeletion (chromosome 1p: D1S1608, D1S1592, D1S548, D1S1161, D1S1184; chromosome 19q: D19S718, D19S433, D19S601, D19S559, D19S431). For the *IDH1* gene, an 88 base-pair long fragment and for *IDH2* gene, an 83 base-pair long fragment were subjected to pyrosequencing to detect hotspot mutations at codon 132 for *IDH1* or codon 172 for *IDH2* [20]. MGMT promoter methylation has been investigated in 120 of 144 patients from our cohort through methylation specific PCR analysis [21]. Malignant progression was defined as histologically confirmed progression from WHO grade 2 to WHO grade 3.

Volumetric assessment, matching and statistics

Pre-therapeutic tumor volumes were obtained through manual segmentation of T2-weighted sequences utilizing BrainLab Elements Smartbrush Software. In patients treated with chemotherapy, posttherapeutic MRI scans obtained within 1 month after completion of the last cycle were investigated. In our center, post-therapeutic imaging is not routinely transferred to the databases, hence T2 imaging for comparative pre- and post-therapeutic manual volume segmentation were only obtained in 27 patients (48%) treated with chemotherapy (Supplementary Fig. 2). Radiological reports of volume status were available in 53 out of 56 patients receiving chemotherapy (95%).

For a matched-pair analysis of the TMZ and PC(V) groups, oligodendrogliomas were matched according to initial T2 tumor volume. Patients were paired only if the initial tumor volumes did not differ by more than 10% of the larger volume of the pair (Supplementary Table 1). Malignant progression rates and progression-free, post-recurrence and overall survival were evaluated through Kaplan–Meier estimator method. T-tests and ANOVA were used for parametric comparative testing of continuous variables in two or more groups. For categorical variables, chi-square test was used. Results were termed significant if p-values were lower than 0.05. For comparison of interactions, log-rank tests and Cox regression hazards models for multivariate analyses were used. Tests were performed using IBM SPSS 25.0 and GraphPad Prism 8.4.2 software.

Results

Study population and baseline characteristics

We identified 142 patients. Median age at diagnosis was 42 years (range 20–80 years). 137 Patients (97%) had an initial Karnofsky performance status (KPS) of 80 or higher (Table 1). All patients were retrospectively assigned to the following groups: wait-and-scan surveillance after biopsy (n = 59); surgical tumor resection (n = 27, including 10 patients with complete resection and 17 patients with subtotal resection); TMZ chemotherapy after biopsy (n = 26); and PCV chemotherapy after biopsy (n = 30; including 20 patients who received PC only). Methylated MGMT promotor was investigated in 120 patients and detected in 117 patients.

Preoperative MRI scans were available in 113 patients (80%). Initial tumor volumes were largest in the TMZ and smallest in W&S group ($p < 0.001$). In W&S, the proportion of patients with incidental diagnoses was higher than in the other groups. Seizures were the most prevalent trigger for diagnostic work-up. Male patients were more often managed by resection alone than female patients (Table 1).

Outcome

Progression was documented in 88 patients (62%) and determined through tumor growth on conventional MRI in 64 patients. In 6 patients, progression was determined through neurological decline and in 18 patients through stereotactic biopsies of new T2 hyperintense or T1 contrast-enhancing foci confirming recurrence or progression. 54 Patients did not progress after a median follow-up of 67 months. During the overall clinical course, repeated histological sampling through biopsy or resection was performed in 64 patients. Histological progression from WHO grade 2 to 3 was observed in 20 of these patients.

Follow-up time ranged from 0.2 to 16 years with a mean FU of 6.5 years and a median FU of 5.9 years. 34 patients had a FU of 10 years or longer. Median overall survival was not reached. 5 Patients have died overall [PC(V), n = 0; W&S, n = 1; RES, n = 3; TMZ, n = 1]. Causes of death were tumor-related in all patients. Progression-free survival (PFS) and malignization rates (MR) at different time points per group are summarized in Table 2.

Patients treated with PC(V) showed the best outcome with a median PFS of 9.1 years. Only 1 out of 30 patients underwent histological progression from WHO grade 2 to 3.

Table 2 Progression-free survival and time-to-malignization—outcome by initial strategy

Outcome parameter	All (n = 142)			Strategy											
				Wait-and-scan (n = 59)			Resection only (n = 27)			TMZ only (n = 26)			PCV only (n = 30)		
	Median			Median			Median			Median			Median		
Progression-free survival (years)	5			5.1			4.4			3.6			9.1		
	n	Events	Rate	n	Events	Rate	n	Events	Rate	n	Events	Rate	n	Events	Rate
1 year	122	17	14	49	7	14	24	4	17	24	2	8	25	4	16
2 years	116	34	29	48	13	27	23	7	30	23	8	35	22	6	27
5 years	98	63	64	42	28	67	21	14	67	17	14	70	18	7	39
10 years	93	83	89	40	37	93	19	18	95	17	17	100	17	11	65
15 years	90	88	98	40	38	95	18	18	100	17	17	100	15	15	100
Outcome parameter	All (n = 142)			Strategy											
				Wait-and-scan (n = 59)			Resection only (n = 27)			TMZ only (n = 26)			PCV only (n = 30)		
	Median			Median			Median			Median			Median		
Time-to-malignization (years)	n.r			n.r			8.7			n.r			n.r		
Malignization rate (%)	n	Events	Rate	n	Events	Rate	n	Events	Rate	n	Events	Rate	n	Events	Rate
1 year	122	1	1	49	0	0	24	1	4	25	0	0	24	0	0
2 years	112	4	4	47	1	2	21	1	5	23	1	4	21	1	5
5 years	77	10	13	36	3	8	15	3	20	14	3	21	12	1	8
10 years	45	16	36	21	6	29	9	6	67	4	3	75	11	1	9
15 years	27	20	74	15	9	60	7	7	100	3	3	100	2	1	50

TMZ temozolomide, *PC(V)* procarbazine, CCNU and vincristine, *n.r.* not reached

The shortest PFS was seen in TMZ (median 3.6 years). PFS of W&S and RES was 5.1 years and 4.4 years (Fig. 2). Patients with gross total resection (n = 10) had a PFS of 6.1 years versus 2.5 years in patients with subtotal resection (n = 17) ($p = 0.27$) and residual tumor volumes ranged from 0.31 to 53.00 cm^3 (median 6.28 cm^3; mean 12.54 cm^3). In the PC(V) group, malignant transformation from WHO 2 to 3 occurred at a significantly lower rate than in the W&S and RES groups. In RES, malignant transformation occurred more often than in W&S ($p = 0.04$).

Small initial T2 volumes were associated with an overall favorable outcome. A matched-pair analysis of tumor volumes in patients treated with TMZ or PC(V) was performed (Fig. 2c; Supplementary Table 1). The results suggested superiority of PC(V) over TMZ with a median PFS of 9.1 vs 4.7 years ($p = 0.03$, HR = 3.0, 95% CI for HR 1.2–8.1). An overall analysis of oligodendrogliomas smaller than 80 cm^3 showed longest PFS in PC(V) (in years, 5.1 in W&S versus 3.2 in RES versus 6.8 in TMZ versus 10.9 in PC(V)), however, this analysis did not reach statistical significance potentially due to small sample size (p = 0.27).

An analysis of salvage therapy for progressive gliomas of the W&S group showed superiority of PC(V) over TMZ (Fig. 2d) at first recurrence. In W&S, treatment choice in case of recurrence was mostly chemotherapy with 17 patients (44% of progressive oligodendrogliomas in W&S) receiving TMZ and 10 patients (26%) receiving PC(V) at first progression (Supplementary Fig. 1). Post-recurrence PFS (PR-PFS) was 4.0 years for TMZ and 7.2 years for PC(V) ($p = 0.04$) (Fig. 2d).

Univariate analyses were performed for PFS and time-to-malignization including the factors age, KPS, initial therapy and initial T2 tumor volume. Smaller initial T2 volumes correlated with longer PFS. PC(V) therapy was associated with longer PFS as compared to resection only or TMZ only and showed significantly longer time-to-malignization than resection only (Table 3). Subsequent multivariate analyses confirmed initial tumor volume to be prognostic for PFS ($p = 0.02$, HR = 1.01, 95% CI 1.01–1.02). Multivariate analyses for TTM were not performed due to the low number of events.

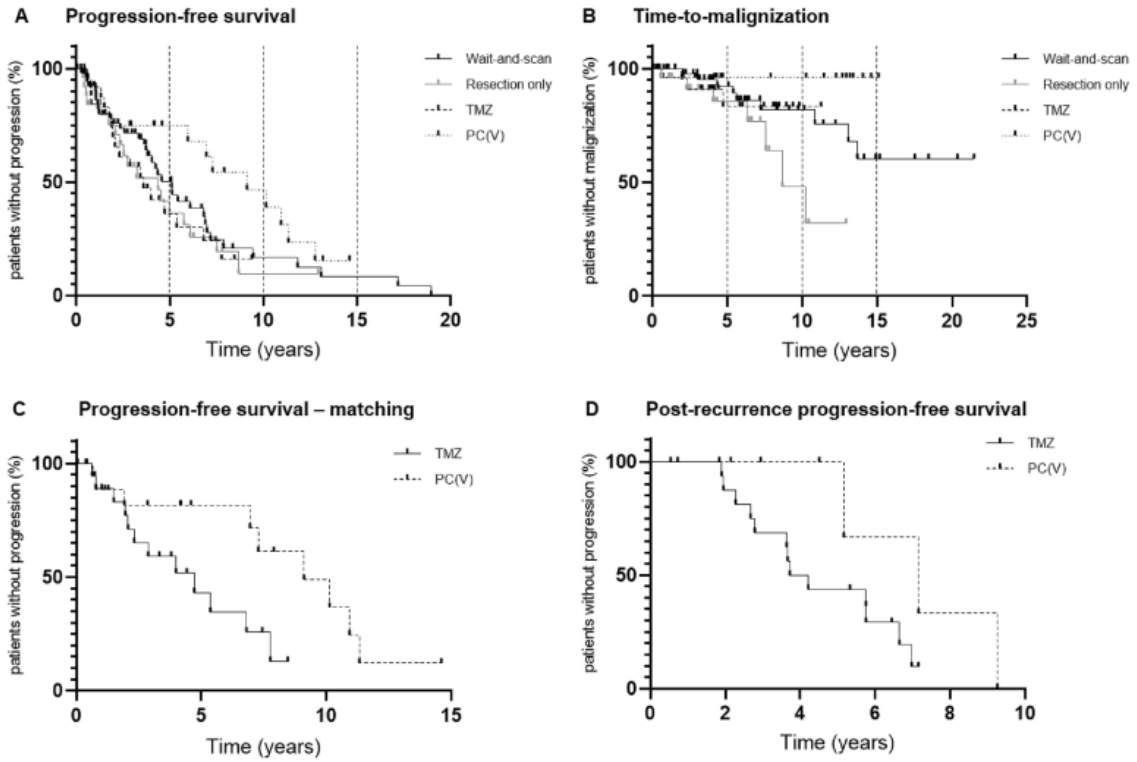

Fig. 2 Overall progression-free survival (PFS, $p = 0.05$) (**a**), time-to-malignization (TTM, $p = 0.04$*) (**b**), PFS of T2-volume matched patients treated with temozolomide (TMZ) or procarbazine + CCNU (+/− vincristine) (PC(V)) ($p = 0.03$*) (**c**) and post-recurrence PFS ($p = 0.04$*) (**d**) of patients from the wait-and-scan cohort treated with TMZ or PC(V) at first progression. For **a** and **b**, four different groups were compared: wait-and-scan versus resection only versus temozolomide only versus procarbazine + CCNU (+/− vincristine) (PC(V)) only. For **c** and **d** patients treated with TMZ or PC(V) were compared

Volume change during chemotherapy

There was no reported tumor growth during chemotherapy. In patients treated with PC(V), a stable tumor volume after therapy completion was reported in 5 patients (17%). Stable was defined as no apparent volume change on MRI. Volume reduction, defined as median proportional decrease of T2 tumor volume after therapy when compared to pre-therapeutic imaging in percentages, was 49% (range 12–71%). In the TMZ cohort, a stable tumor volume was reported in 4 patients (15%) and median volume reduction of those with available post-therapeutic MRI was 39% (range 9–62%) (Supplementary Fig. 2).

Adverse events

Adverse events were documented in 23 of 56 patients (41%). Severe adverse events (SAE), i.e. CTCAE grade 3–5 AE, that led to transient or permanent discontinuation of therapy, were seen in 11 patients (20%). Out of 10 patients treated with PCV, 4 (40%) developed SAEs and out of 20 patients treated with PC, 3 (15%) developed SAEs. SAEs were seen in 4 patients (15%) out of 26 receiving TMZ (Supplementary Fig. 3).

In patients receiving PCV, SAEs were reported in 40%, whereas only 15% of patients treated with PC or TMZ developed SAEs (Supplementary Fig. 3). This higher percentage could be attributed to peripheral sensory neuropathy, a well-known potential side effect from vincristine.

Discussion

Patients suffering from oligodendroglioma can survive for decades [22]. Histological and molecular diagnostic criteria have changed over the last years. Several treatment options including different surgical strategies, radiotherapy techniques and alkylating agent chemotherapy protocols

Table 3 Uni- and multivariate analyses

Univariate analysis of PFS and TTM						
Parameter	PFS			TTM		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	0.99	0.97–1.01	0.44	1.00	0.97–1.04	0.84
KPS	0.99	0.96–1.03	0.77	1.03	0.94–1.12	0.58
Wait-and-scan vs resection	0.78	0.43–1.42	0.42	0.29	0.09–0.96	0.04*
Resection vs chemotherapy	1.47	0.79–2.74	0.22	4.99	1.37–18.22	0.02*
Resection vs TMZ	1.03	0.53–2.00	0.94	2.10	0.60–7.39	0.25
Resection vs PC(V)	2.12	1.02–4.40	0.04*	7.38	1.75–31.05	0.01*
TMZ vs PC(V)	2.29	1.05–4.98	0.04*	2.66	0.37–18.85	0.33
Other strategy vs PC(V)	1.67	1.01–2.75	0.05*	2.84	1.01–8.11	0.05*
T2 volume	1.01	1.01–1.02	0.05*	1.00	0.99–1.01	0.94

Multivariate analysis of PFS: backwards (Wald)			
Parameter	PFS		
	HR	95% CI	*p*-value
T2 volume	1.01	1.01–1.02	0.02*

Other strategy = wait-and-scan, resection only and TMZ. Statistical significance ($p < 0.05$) is depicted by asterisks (*)

KPS Karnofsky performance score, *TMZ* temozolomide, *PC(V)* procarbazine, CCNU and vincristine

are available and increasingly used in combination [6]. Treatment-induced toxicity must be weighed carefully when selecting type and timing of tumor-specific treatment. Here, we investigated long-term clinical course with different therapeutic approaches.

Our data strongly suggest that among the preferred treatments for WHO grade oligodendroglioma at our site, PC(V) is the best initial monotherapy. We find that PC(V) after biopsy leads to better PFS than resection only or than TMZ after biopsy. PC(V) was associated with significantly lower malignization rates than wait-and-scan strategies or tumor resection with only one affected patient. Superiority of PC(V) over TMZ was further supported by a matched-pair analysis of patients treated with PC(V) versus TMZ. Additionally, patients from the W&S cohort treated with PC(V) at first progression showed a longer PFS than those treated with TMZ at first progression.

Smaller initial tumor volumes were associated with longer PFS, matching results from a prior publication of oligodendroglioma volumes [23]. This may in part explain the poor overall outcome in the TMZ group that had the largest median initial tumor volume. Worse outcome in patients with larger initial tumor volumes may also explain why, counterintuitively, PFS was shorter in RES than in W&S with a median initial tumor volume twice as large. The criterion that any new lesion is considered as progressive disease might lead to a quicker call of progression in patients receiving tumor resections, e.g. when observing new lesions bordering the resection cavity. Conversely, marginal, 3-dimensional tumor growth in patients with large tumors is not accounted for if not exceeding 25% or more of largest perpendicular diameters.

Standard therapy for oligodendroglioma grade 2 by consensus is tumor resection if safely feasible [18]. Whether or not treatment beyond surgery is initiated depends on various factors, including the extent of residual tumor volume. Many patients included in this study would be treated differently nowadays, e.g. patients with frontal oligodendrogliomas and biopsy only. As many patients included were diagnosed before the era of molecular classification and KPS was generally high, interdisciplinary tumor boards back then oftentimes left scope for mono-chemotherapies which was openly discussed with the patients. Cultural differences might have played a role in deferring surgery or radiotherapy in many patients. A retrospective study found that greater extent of resection (EOR) correlated with favorable outcome in oligodendroglioma grade 2 but did not delay time to malignant transformation [24]. This is in line with our findings. The fact that malignization rates were significantly higher in RES than in W&S might be explained by the fact that patients in the W&S group were treated far more often with PCV at first progression than patients in RES (Supplementary Fig. 1) and that RES patients had larger volumes initially.

PCV alone or following radiotherapy is a well-established therapy in the treatment of oligodendroglioma. In this study, superiority of PC(V) over TMZ and RES in terms of PFS was observed in the long-term. There were multiple crossovers in Kaplan–Meier curves of PFS within the first 2 years

of diagnosis, e.g. between W&S and PC(V). This might hint at a subgroup of WHO grade 2 oligodendrogliomas that are refractory to PC(V) chemotherapy or to challenges in determining progression in patients exhibiting minor changes on MRI.

As PC(V) and TMZ both reduced initial T2 tumor volume, their potential as "neoadjuvant" presurgical therapy and the question if volume decrease after chemotherapy might be a marker for outcome require further evaluation. Radiotherapy with sequential PCV chemotherapy is an exceptionally effective therapy for WHO grade 2 and 3 oligodendroglioma as shown by prospective, clinical trials [16, 25–27]. The question whether early radiotherapy in initially smaller tumors is superior as compared to delayed radiotherapy in larger tumors remains unanswered. In our data set of oligodendroglioma grade 2 diagnosed between 2003 and 2019, only 5 patients (4%) died from tumor-related death and the question remains if invasive treatment regimens comprising resection and radiotherapy with sequential chemotherapy are warranted in these tumors.

Important limitations of the present study are its retrospective nature and the small sample size in some subgroups. Although overall survival was not reached in this study, prospective clinical trials of anaplastic oligodendroglioma have shown that in the long-term, prolonged PFS translates into superior OS [16, 25]. Strengths of this study comprise the standardized histological and molecular classification of all oligodendrogliomas according to WHO 2016, investigation of initial tumor volumes, long follow-up intervals and high rate of repeated histological sampling, providing information on malignant transformation.

In summary, our data strongly suggest that PCV chemotherapy is an important compound in the treatment of WHO grade 2 oligodendroglioma after biopsy or resection due to its superiority in prolonging PFS and potentially delaying malignant progression.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11060-021-03765-z.

Author contributions JW: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Validation; Visualization; Roles/Writing—original draft. SK: Data curation; Formal analysis; Resources; Software. PK: Formal analysis; Writing—review and editing. SL: Data curation. RE: Data curation; Investigation; Methodology, Resources. NT: Writing—review and editing. MW: Conceptualization; Formal analysis; Investigation; Supervision; Writing—review and editing. BS: Investigation; Methodology. J-CT: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Roles/Writing—original draft; Writing—review and 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.

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

Declarations

Conflict of interest Jonathan Weller, Sophie Katzendobler, Philipp Karschnia, Stefanie Lietke, Rupert Egensperger, Niklas Thon and Bogdana Suchorska have no disclosures. Michael Weller 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.

Ethical approval Ethics approval was obtained by the Ethics Committee of the Ludwig Maximilian University of Munich (Project Number 20-513).

Human and Animal Rights The present study was conducted retrospectively.

Informed consent 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, institutional prospective tumor registry.

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6. Publikation II

Journal of Neuro-Oncology
https://doi.org/10.1007/s11060-021-03912-6

CLINICAL STUDY

Extent, pattern, and prognostic value of MGMT promotor methylation: does it differ between glioblastoma and IDH-wildtype/TERT-mutated astrocytoma?

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Received: 25 September 2021 / Accepted: 23 November 2021

Abstract

Introduction The cIMPACT-NOW update 6 first introduced glioblastoma diagnosis based on the combination of IDH-wildtype (*IDHwt*) status and TERT promotor mutation (*pTERTmut*). In glioblastoma as defined by histopathology according to the WHO 2016 classification, MGMT promotor status is associated with outcome. Whether this is also true in glioblastoma defined by molecular markers is yet unclear.

Methods We searched the institutional database for patients with: (1) glioblastoma defined by histopathology; and (2) *IDHwt* astrocytoma with *pTERTmut*. MGMT promotor methylation was analysed using methylation-specific PCR and Sanger sequencing of CpG sites within the MGMT promotor region.

Results We identified 224 patients with glioblastoma diagnosed based on histopathology, and 54 patients with *IDHwt* astrocytoma with *pTERTmut* (19 astrocytomas WHO grade II and 38 astrocytomas WHO grade III). There was no difference in the number of MGMT methylated tumors between the two cohorts as determined per PCR, and also neither the number nor the pattern of methylated CpG sites differed as determined per Sanger sequencing. Progression-free (PFS) and overall survival (OS) was similar between the two cohorts when treated with radio- or chemotherapy. In both cohorts, higher numbers of methylated CpG sites were associated with favourable outcome.

Conclusions Extent and pattern of methylated CpG sites are similar in glioblastoma and *IDHwt* astrocytoma with *pTERTmut*. In both tumor entities, higher numbers of methylated CpG sites appear associated with more favourable outcome. Evaluation in larger prospective cohorts is warranted.

Keywords WHO CNS 2021 · cIMPACT · TERT · IDH wildtype · Glioma

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Introduction

In 2016, the World Health Organization (WHO) revised the classification of central nervous system (CNS) tumors which, for the first time, incorporated both histological characteristics as well as molecular features [1]. Since the introduction of the WHO 2016 classification, ongoing advances have led to an increasing understanding of brain tumor molecular pathogenesis and its clinical impact on patients' outcome. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy—(cIMPACT-NOW) was founded to review and integrate those advances into clinical practice between WHO updates. The cIMPACT-NOW update 6 has proposed to reclassify isocitrate dehydrogenase 1/2 wildtype (*IDHwt*) diffuse astrocytomas as glioblastoma if they present with either (1) telomerase reverse

Published online: 13 December 2021

transcriptase (TERT) promotor mutation (*pTERTmut*); (2) epidermal growth factor receptor (EGFR) gene amplification; or (3) whole chromosome 7 gain and whole chromosome 10 loss (+7/− 10) [2]. Such tumors were found to have clinical outcomes similar to those of glioblastoma as defined per histopathology, and as expected, the recently published WHO 2021 classification has incorporated the diagnosis of glioblastoma based on molecular markers [3, 4].

Methylation of the promotor region of the O6-methylguanine-DNA-methlytransferase (MGMT) gene is another molecular marker associated with favourable prognosis and response to alkylating chemotherapy in glioblastoma [5, 6]. In the prospective CATNON trial (which compares radiotherapy with or without chemotherapy), a subgroup analysis of *IDHwt* astrocytoma with molecular features of glioblastoma demonstrated improved survival for tumors with MGMT promotor methylation (but surprisingly did not find evidence for beneficial effects of alkylating chemotherapy among methylated tumors) [7]. We recently reported on a large cohort of gliomas WHO grade II, and found that a higher number of methylated CpG sites within the MGMT promotor region also represents a positive prognostic factor for outcome in gliomas of lower grades [8]. Of note, a subgroup analysis of our cohort showed that the prognostic value of MGMT promotor methylation was only retained in *IDHwt* astrocytomas, but not in IDH-mutant glioma with or without 1p19q co-deletion. However, the subgroup of *IDHwt* astrocytomas included in our previous study was limited given its small sample size of only 20 patients and missing TERT status.

In the present study, we describe a molecularly well-defined cohort of 57 *IDHwt* astrocytoma with *pTERTmut* who were consecutively treated at a single academic neuro-oncology centre. Based upon the comparison with a group of 224 glioblastoma patients defined per histopathology according to the WHO 2016 classification, we aim to describe pattern and extend of MGMT promotor methylation in *IDHwt* astrocytoma with *pTERTmut* and its association with survival in the presence of chemo- and radiotherapy.

Materials and methods

Study population

Study design and methods were approved by the Institutional Review Board of the Ludwig Maximilians University in Munich, Germany, and patient consent was waived (AZ 20-650). We retrospectively searched the institutional database of the Center for Neuro-Oncology at the Ludwig Maximilians University School of Medicine for adult patients seen between 2004 and 2014 with: (1) *IDHwt* glioblastoma WHO grade IV as defined by histopathology according to the WHO 2016 classification [1]; and (2) *IDHwt* astrocytoma with *pTERTmut* in the absence of classical histological hallmarks (corresponding to WHO grade II and III *IDHwt* according to the WHO 2016 classification, but to WHO grade 4 according to the cIMPACT-NOW update 6 and WHO 2021 classification) [2, 4]. Histopathologic diagnosis was based upon tissue sampled during microsurgical tumor removal, or stereotactic biopsy in lesions where safe resection appeared not feasible. Patients with IDH1/2 mutations or in which IDH status was unavailable for review were excluded from the study. Diagnostic and treatment decisions were based upon interdisciplinary brain tumor board recommendations and patient preference. Follow-up imaging and surveillance scans were obtained per institutional guidelines with follow-up imaging every three to six months or in case of any clinical detoriation [9]. We collected demographic and clinical information, histopathology, molecular markers and other diagnostic findings, treatment specifics and clinical outcome. Complete resection of contrast-enhancing tumor was defined as previously proposed in the classification by Karschnia et al. [10] Database closure in this study was December 1, 2020.

MGMT promotor methylation and molecular markers

MGMT promotor status was analysed using the following two methods: (1) methylation-specific polymerase chain reaction (MSP) and (2) Sanger sequencing of the Cytosine-Guanine dinucleotide (CpG) sites 74–98 within the MGMT promotor region as previously described [11, 12]. CpG site methylation was defined as ratio of cytosine/thymine peak > 50%. The total number of methylated CpG sites was calculated for each patient.

IDH 1/2 mutation status was assessed per pyrosequencing, and TERT promotor mutation status was retrospectively analysed using Sanger sequencing as previously described for the purpose of the present study [13, 14]. TERT promotor mutation status was not routinely tested in histopathological GBMs.

Statistical analysis

Categorical variables are described in absolute numbers and percent points. Relationships between two or more categorical variables were assessed using the chi-square test. For numerical data, the D' Agostino-Pearson omnibus normality test was used to test for normal distribution. In case of parametric data, differences between two groups were analysed by the unpaired Student's t test. Mann–Whitney U-test was used to assess differences between two groups in case of non-parametric data. Differences among more than two groups were analysed by ANOVA. If not indicated otherwise, all

values are expressed as mean ± standard error of the mean and range is given. For survival analyses, patients were followed until day of database closure (December 1, 2020) or death. Patients lost to follow-up were censored at day of last follow-up. Date of diagnosis was set as date of pathological diagnosis. Date of radiographic progression was defined as date when diagnosis of radiographic progression according to RANO criteria was made, or tumor-related death. Overall survival was defined as interval from diagnosis to tumor-related death. Kaplan–Meier survival analysis and log-rank test were used to calculate follow-up, survival, and predictors of outcome. Statistical analyses were performed using Prism statistical software (Prism 9.0; GraphPad Software Inc., San Diego, CA, USA). The significance level was set at $p \leq 0.05$.

Results

Study population

A total of 281 patients were identified and included in the present study. We encountered 224 glioblastomas WHO 2016 grade IV (80%, all *IDHwt*; hereafter referred to as ‚histopathological GBM′) and 57 *IDHwt* astrocytomas with *pTERTmut* (20%; hereafter referred to as ‚molecular GBM′) (Table 1). The latter cohort consisted of 18 diffuse astrocytomas *IDHwt* WHO 2016 grade II (18/57 patients, 32%), 38 anaplastic astrocytomas *IDHwt* WHO 2016 grade III (38/57 patients; 67%), and 1 gemistocytic astrocytoma *IDHwt* WHO 2016 grade II (1/57 patients; 2%).

Demographic and clinical findings

Among the two cohorts, median patient age at diagnosis was similar (histopathological GBM: 59 ± 0.8 years, range 13–86 years and molecular GBM: 59 ± 1.4 years, range 39–81; $p = 0.695$). Male-to-female ratio was 1:0.6 in histopathological GBM and 1:0.7 in molecular GBM ($p = 0.518$). Karnofsky performance score was significantly higher in patients with molecular GBM (90%; range 60–90% vs. 80%; 40–100 in histopathological GBM) (*$p = 0.027$).

MGMT promotor methylation

MSP and Sanger sequencing data was available for review for all patients. Binary analysis of MGMT promotor methylation with MSP showed comparable methylation rates with 48.4% methylation in the entire cohort, 47.8% in histopathological GBM, and 50.9% in molecular GBM ($p = 0.675$) (Fig. 1A). In the entire cohort, the mean number of methylated CpG sites was 11.3 ± 0.5 (45 ± 2.1% of 25 CpG sites) and did strongly vary between individual patients (range 0–25). The mean number of methylated CpG sites in histopathological GBM was 11.9 ± 1.2 (47.6 ± 4.7%; range 0–25) and did not significantly differ when compared to 11.1 ± 0.6 (44.3 ± 2.7%; range 0–25) in molecular GBM ($p = 0.545$) (Fig. 1B). Also, the range in the individual number of methylated CpG sites was identical in both groups (0–25 CpG sites). Moreover, mean number of methylated CpG sites was similar when allocating gliomas according to WHO 2016 classification with 11.4 ± 2.1 (45.7 ± 8.5% of 25 CpG sites) in WHO grade II, 12.2 ± 1.4 (48.6 ± 5.7% of 25 CpG sites) in WHO grade III, and 11.1 ± 0.6 (44.3 ± 2.7%; range 0–25) in WHO grade IV ($p = 0.784$).

Of note, histopathological and molecular GBM showed a similar methylation pattern with some CpG sites such as number 87 and 91 being more frequently found to be methylated than others (Fig. 1C).

Treatment and outcome

Diagnosis was made by microsurgical tumor resection or stereotactic biopsy. Following tissue-based diagnosis, first-line therapeutic management of all gliomas included chemotherapy (temozolomide or procarbazine/lomustine), involved-field radiotherapy, radiochemotherapy, interstitial brachytherapy, and wait-and-scan approaches (Table 1). Radiochemotherapy with temozolomide was most often provided in histopathological GBM (216/224 patients, 96%), followed by alkylating chemotherapy with temozolomide in the absence of radiotherapy (7/224 patients, 3%). Interstitial brachytherapy was provided in 1 patient (< 1%). Molecular GBM received a more diverse first-line therapy, most often consisting of alkylating chemotherapy (overall: 18/57 patients, 32%; temozolomide: 16/57 patients, 28%; procarbazine/lomustine: 2/57 patients, 4%) or radiochemotherapy (15/57 patients, 26%). Of note, 10/57 (18%) patients with a molecular GBM received wait-and-scan approaches after initial biopsy, the majority being assigned as WHO grade II tumors (8/57 patients, 14%). Microsurgical tumor resection was more frequently provided in histopathological GBM (94/224 patients, 42%) than in molecular GBM (3/57 patients, 5%). In resected tumors, gross total tumor resection was most often achieved (histopathological GBM: 53/94 patients, 56%; molecular GBM: 2/3 patients, 66%) All other patients received stereotactic biopsy for diagnostic purposes. There was no clear difference in regard of therapy provided after tumor progression between patients in both cohorts.

Median follow-up was 24 months (range 0–142 months). In the entire group, median time to radiographic progression was 9 months (range 0–71 months) and median overall survival was 19 months (range 1–142 months). Next, we aimed to compare overall survival and radiographic progression free survival in patients with first-line medical therapy including radio- or chemotherapy of any kind

Table 1 Patient characteristics for glioblastoma and *IDHwt* astrocytoma with *pTERTmut*

	Glioblastoma WHO°IV	*IDHwt* astrocytomas with *pTERTmut*	Total	*p*-value
Overall, n (%)	224 (80%)	57 (20%)	281	
Age, years				
< 18	1 (0%)	0	1 (0%)	0.695
18–35	11 (5%)	0	11 (4%)	
36–50	34 (15%)	16 (28%)	50 (18%)	
51–65	101 (45%)	22 (39%)	123 (44%)	
> 65	77 (34%)	19 (33%)	96 (34%)	
Gender				
Female	80 (36%)	23 (40%)	103 (37%)	0.518
Male	144 (64%)	34 (60%)	178 (63%)	
KPS, %				
< 90	127 (57%)	23 (40%)	150 (53%)	***0.027**
90–100	97 (43%)	34 (60%)	131 (47%)	
Histopathology				
Diffuse AST WHO°II	0	18 (32%)	18 (6%)	
Anaplastic AST WHO°III	0	38 (67%)	38 (14%)	
Gemistocytic AST WHO°II	0	1 (2%)	1 (0%)	
GBM WHO°IV	224 (100%)	0	224 (80%)	
Methylated CpG sites				
0–8	99 (44%)	21 (37%)	120 (43%)	0.527
9–16	40 (18%)	13 (23%)	53 (19%)	
17–25	85 (38%)	23 (40%)	108 (38%)	
First-line therapy				
Chemotherapy				***0.001**
TMZ	7 (3%)	16 (28%)	23 (8%)	
PC	0	2 (4%)	2 (1%)	
Radiotherapy	0	10 (18%)	10 (4%)	***0.001**
Radiochemotherapy	216 (96%)	15 (26%)	231 (82%)	***0.001**
Brachytherapy	1 (0%)	4 (7%)	6 (2%)	***0.001**
Wait-and-scan	0	10 (18%)	10 (4%)	***0.001**

Characteristics are given for patients with glioblastoma, IDH-wildtype, WHO grade IV (n = 224) and *IDHwt* astrocytoma with *pTERTmut,* WHO grade II and III (n = 57); and are summarized for all patients (total; n = 281)

CpG: cytosine-guanine dinucleotide, *IDHwt*: isocitrate dehydrogenase 1/2 wildtype, *KPS*: Karnofsky performance score, *MGMT*: O6-methylguanine-DNA methyltransferase promotor, *PC*: procarbazine, lomustine, *TMZ:* temozolomide, *TERT*: telomerase reverse transcriptase promotor, *pTERTmut:* TERT promotor mutation, *TMZ*: temozolomide

Asterisks indicate $*p \leq 0.05$

(radiochemotherapy, alkylating chemotherapy, radiotherapy, interstitial brachytherapy). Median overall survival was identical in both cohorts with 19 months ($p = 0.356$); median time to radiographic progression was similar in both cohorts with 9 months in histopathological GBM vs. 8 months in molecular GBM (n = 224 vs. 47; $p = 0.327$) (Fig. 1D, E).

In patients treated with any chemotherapy (including radiochemotherapy) at first line, molecular GBM showed a similar median overall survival and progression free survival in comparison to histopathological GBM (molecular GBM n = 33, histopathological GBM n = 223; OS 22 vs. 19 months, $p = 0.625$; PFS 8 vs. 9 months, $p = 0.179$).

Association of MGMT promotor methylation with outcome

To analyse whether MGMT promotor methylation was associated with outcome in the presence of radio- or chemotherapy, patients who received medical treatment including radiochemotherapy, radiotherapy, chemotherapy and brachytherapy were stratified according to number of methylated

CpG sites. A larger number of methylated CpG sites was associated with favourable outcome in glioblastoma patients (Table 2): a total of > 18 methylated CpG sites was a significant cutoff for improved overall survival (15 vs. 30 months, hazard ratio 0.49, $p = *0.001$) and the most significant cutoff for longer time to radiographic progression (8 vs. 20 months, hazard ratio 0.48, $p = *0.001$) (Fig. 2A, B).

In patients with molecular GBM treated with radio-/chemotherapy, no significant correlation between number of methylated CpG sites and overall survival or time to radiographic progression was seen, respectively (Table 3); but a relevant trend towards better outcome was seen in tumors with a total of > 18 methylated CpG sites (HR 0.65 for overall survival, $p = 0.171$; Fig. 2C, D). To minimize bias from extent of resection we also compared patients with molecular GBM treated with radio-/chemotherapy who only received stereotactic biopsy (and no surgical tumor resection). Here, no significant difference in overall survival (22 vs. 16 months, HR 0.67, $p = 0.216$) nor time to radiographic progression (11 vs. 6 months, HR 0.67, $p = 0.238$) was seen for patients with more or less than 18 methylated CpG sites.

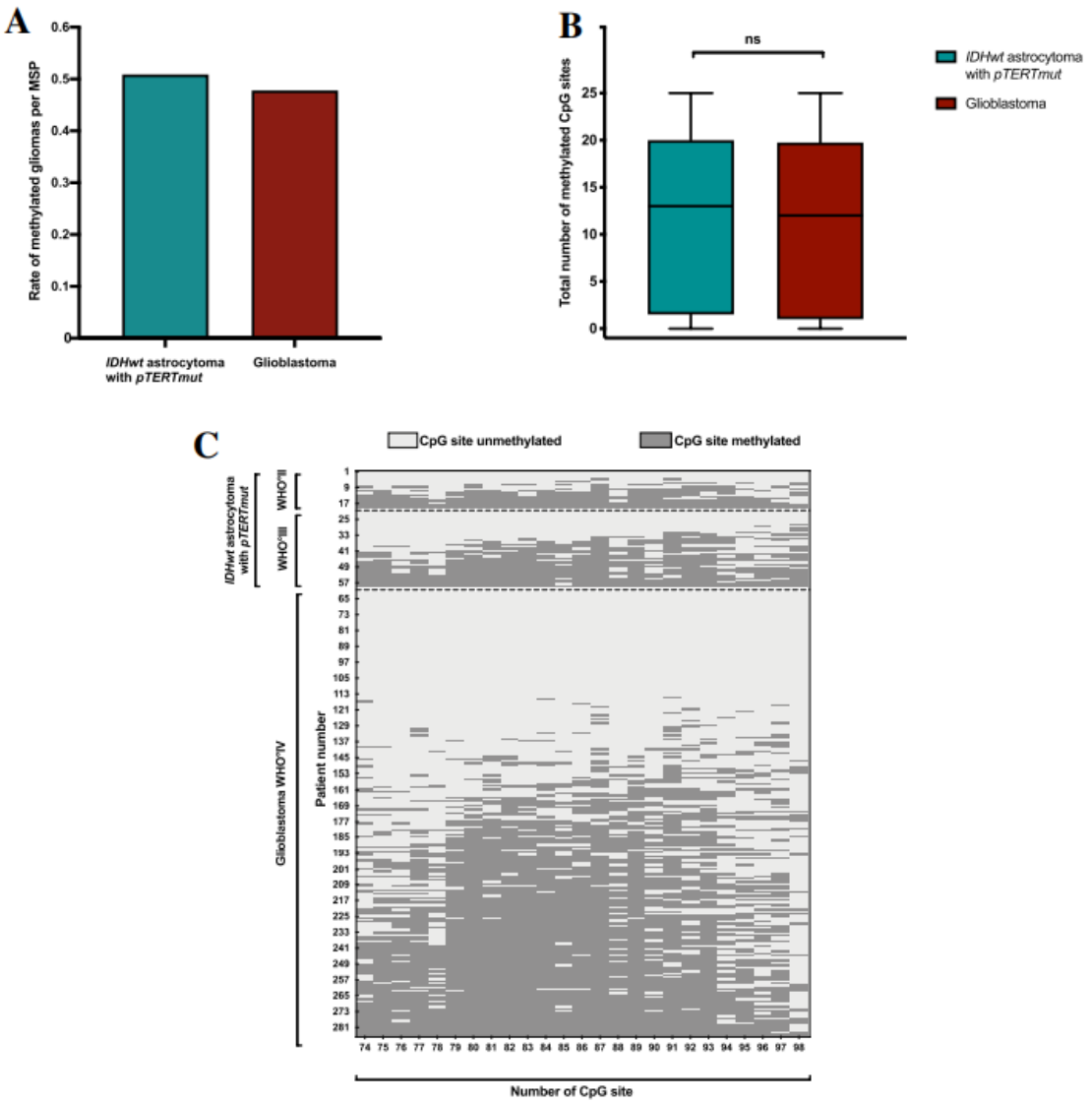
Next, we aimed to analyse whether MGMT promotor methylation was associated with outcome in patients receiving alkylating chemotherapy (including radiochemotherapy) in molecular GBM. We divided the cohort into individuals with msore (n = 13) or less (n = 20) than 18 methylated CpG sites (in analogy to the optimal cut-off, see above; Fig. 2E, F). Of interest, higher number of methylated CpG sites were associated with longer time to radiographic progression (12 vs. 6 months, $*p = 0.007$). Overall survival was longer in patients with > 18 methylated CpG sites compared with ≤ 18 methylated CpG sites, but without statistical significance (22 vs. 17 months, $p = 0.319$). Similar findings were made when only comparing patients who received stereotactic biopsy without microsurgical tumor resection (OS 22 vs. 17 months, $p = 0.366$; PFS 12 vs. 6 months, $p = *0.034$).

In addition, all patients with molecular GBM with re-exposure to alkylating chemotherapy (including radiochemotherapy) for post-progression therapy (n = 28) were stratified according to number of methylated CpG sites as above. Here, no significant difference in outcome between patients with > 18 methylated CpG sites (n = 9) and patients with ≤ 18 methylated CpG sites (n = 19) was seen (15 vs. 13 months, $p = 0.917$).

Discussion

cIMPACT-NOW first introduced diagnosis of glioblastoma, WHO grade 4 based upon the presence of molecular features in *IDHwt* astrocytoma WHO grade II and III (according to WHO 2016) even in the absence of classical histological hallmarks. Also, the recently published WHO 2021 classification now incorporates grading of gliomas based on such molecular markers [4]. Methylation of the MGMT promotor region is an essential molecular marker for outcome and response to alkylating chemotherapy in histologically defined glioblastoma [5, 15]. However, its role in *IDHwt* astrocytoma with *pTERTmut* is less well established. A subgroup analysis of the CATNON trial with 154 *IDHwt* astrocytoma with molecular features of glioblastoma showed that MGMT promotor methylation was prognostic for overall survival but not predictive for temozolomide chemotherapy in this cohort [7]. Data from the NOA-04 trial demonstrated the positive predictive value of MGMT promotor methylation in *IDHwt* astrocytomas WHO grade III but did not test for molecular features of glioblastoma like TERT promotor mutation [6]. Of interest, several studies found a prognostic interaction of TERT promoter mutation with MGMT promoter methylation in patients with *IDHwt* glioblastoma treated with radiochemotherapy [16, 17]. Furthermore, a recent meta-analysis with low-grade glioma as well as glioblastoma patients including the aforementioned studies showed that among TERT promotor mutated low-grade gliomas, MGMT promotor methylation was associated with improved overall survival [18]. However, no IDH-status was evaluated in this retrospective subgroup analysis. Here, we focused on detailing our institutional experience on the role of MGMT promotor methylation in *IDHwt* astrocytomas with *pTERTmut*.

We found that extent of MGMT promotor methylation was similar in both histologically defined glioblastoma as well as *IDHwt* astrocytomas with *pTERTmut*. Methylation rates were comparable to those for glioblastoma reported in the literature [19]. A larger number of methylated CpG sites was prognostic for improved overall survival as well as longer time to radiographic progression in glioblastoma. In molecular GBM treated with alkylating chemotherapy including radiochemotherapy, MGMT promotor methylation was associated with improved progression free survival (statistical significance for overall survival was not reached in patients with molecular GBM, potentially due to the relatively small sample size). Interestingly, MGMT methylation of CpG sites 74–98 using Sanger sequencing showed a similar pattern across histopathological and molecular GBM patients with certain CpG sites such as 87 being more frequently found to be methylated, thus underlining their molecular similarities. In a retrospective study of histopathological GBM patients treated with radiochemotherapy, Sanger sequencing analysis showed a potential linear correlation of methylated CpG sites with outcome highlighting its additional value in contrast to conventional methods [20]. Of note, Sanger sequencing and other methods assessing MGMT promotor methylation status correlate with low MGMT mRNA expression levels but not necessarily MGMT protein levels, indicating


A
Rate of methylated gliomas per MSP
0.6
0.5
0.4
0.3
0.2
0.1
0
IDHwt astrocytoma with pTERTmut
Glioblastoma
B
ns
Total number of methylated CpG sites
25
20
15
10
5
0
IDHwt astrocytoma with pTERTmut
Glioblastoma
C
CpG site unmethylated
CpG site methylated
IDHwt astrocytoma with pTERTmut
WHO°II
WHO°III
Glioblastoma WHO°IV
Patient number
Number of CpG site
D
First-line RC/R/C/B therapy
p = 0.351
Glioblastoma
IDHwt astrocytoma with pTERTmut
Overall survival
Survival probability (%)
Months
E
First-line RC/R/C/B therapy
p = 0.327
Glioblastoma
IDHwt astrocytoma with pTERTmut
Progression free survival
Probability of progression-free survival (%)
Months

◂**Fig. 1** Survival and extent of MGMT promotor methylation in glioblastoma and *IDHwt* astrocytoma with *pTERTmut*. **A** Rate of methylated tumors per MSP in patients with *IDHwt* astrocytoma with *pTERTmut*, WHO grade II and III (cyan) and glioblastoma (red). **B** Number of methylated CpG sites in patients with *IDHwt* astrocytoma with *pTERTmut*, WHO grade II and III (cyan) and glioblastoma (red). Median, interquartile range, and total range are given. **C** Methylation pattern of CpG sites 74–98 within the MGMT promotor region in patients with *IDHwt* astrocytoma with *pTERTmut*, WHO grade II (n = 19) and WHO grade III (n = 38), and glioblastoma WHO grade IV (n = 224). Each row corresponds to an individual patient, and each column to a different CpG site. Dark grey rectangles represent methylated sites and light grey rectangles represent unmethylated sites. **D/E** Kaplan–Meier estimates of overall survival (**D**) and radiographic progression-free survival (**E**) in the entire cohort treated with any medical therapy. Patients were stratified into *IDHwt* astrocytoma with *pTERTmut*, WHO grade II and III (cyan) and glioblastoma, WHO grade IV (red). *B therapy:* brachytherapy; *C therapy:* chemotherapy; *CpG:* Cytosine-Guanine dinucleotide; *IDHwt:* isocitrate dehydrogenase 1/2 wildtype; *pTERTmut*: TERT promotor mutation; *R therapy:* radiotherapy; *RC therapy:* radiochemotherapy

post-transcriptional regulation of MGMT that can affect predictive and prognostic value of MGMT promotor methylation status [21]. Overall, extent of MGMT promotor methylation was comparable between histopathological and molecular GBM treated with radiotherapy or radiochemotherapy. However, particularly its predictive role for response to alkylating chemotherapy seems less clear and warrants evaluation in large prospective trials.

Furthermore, we aimed to define a MGMT promotor methylation cut-off point for strongest prognostic value in molecular GBM as well as histopathological GBM patients using Sanger sequencing of 25 CpG sites. Interestingly, the calculated MGMT promotor methylation cut-off point predicting longer progression-free survival was higher (≥ 18/25 CpG sites, ≥ 72%) in comparison to the cut-off point predicting improved overall survival (≥ 11/25 CpG sites, ≥ 44%) in histopathological GBM patients. A prognostic cut-off point in molecular GBM patients could not be calculated but a as a trend, a non-significant better outcome was also seen in tumors with a total of > 18 methylated CpG sites (≥ 76%), regardless of extent of resection. Other cut-off points which have been validated in studies by others range from a mean MGMT promotor methylation of 7% to 30% [22, 23] which is lower than our calculated cut-off points. Of interest, mean number of methylated CpG sites in glioblastoma as well as *IDHwt* astrocytoma with *pTERTmut* were comparable to mean number of methylated CpG sites in a cohort of glioma WHO grade II recently described by our group [8], suggesting extent of MGMT promotor methylation to be independent of histopathological WHO grade and may rather depend on molecular markers. Technical cut-off values for the distinction of methylated versus unmethylated cases usually would be set at the nadir of the distribution. However, given the heterogenous methylation patterns in gliomas, there appears to be prognostic and predictive uncertainty for patients with an intermediate number of methylated CpG sites. Finding consensus on reliable cut-off values remains to be found and will need prospective validation in the future. [24].

As it is the case in our neuro-oncology center for all glioma patients, Wick et al. proposed to test for MGMT promotor methylation using two distinct methods for a better discrimination in patients with a "grey zone" methylation status [19]. Consequently, results from both methods can then be added to guide therapeutic management. In our cohort, MGMT promotor methylation status was analysed by two commonly used methods comprising of 1) MSP and 2) Sanger sequencing. Both showed comparable methylation rates in our entire cohort, as well as subgroup analysis. Of note, both methods described in this study are semi-quantitative analysis methods to assess MGMT promotor methylation and thus underlie a more subjective interpretation rather than fully quantitative methods such as pyrosequencing. A recent comprehensive meta-analysis examining studies using different methods for MGMT promotor methylation testing in glioblastoma patients treated with temozolomide showed MSP and pyrosequencing to be superior to immunohistochemistry for MGMT protein but did not provide evidence for best CpG site threshold [25]. A gold standard to distinguish between patients with and without MGMT promotor methylation remains to be defined in *IDHwt* astrocytomas with *pTERTmut*, but a combination of different methods seems to be a feasible approach.

Next, we reviewed treatment-algorithms in our cohort. Management was distinctly different in both subgroups. Whereas histopathological GBM patients most commonly received microsurgical tumor resection followed by radiochemotherapy with temozolomide, treatment of molecular GBM patients was more diverse. An unusually high amount of 18% received a wait-and-scan approach until first recurrence. In part, patient's preference and thus shared decision making in treatment management has to be accounted for. More importantly, however, most patients with a wait-and-scan approach in our cohort were diagnosed during a time when significance of molecular markers like IDH mutation and TERT promotor mutation were less well established and treatment strategies in low grade gliomas varied from wait-and-scan to complete resection of all visible tumor on MRI. Consequently, tumor tissue from initial biopsy or surgery was most often tested for TERT promotor mutation at time of recurrence or even retrospectively for the purpose of the present study (9/10 patients with wait-and-scan approach, 90%). In addition, most gliomas in this subgroup were classified as WHO grade II thus explaining treatment decisions in these patients. IDH 1/2 mutation status was assessed per pyrosequencing, and TERT promotor mutation status was retrospectively analysed using Sanger sequencing as

Table 2 Number of methylated CpG sites within the MGMT promotor region as a prognostic factor in glioblastoma

Number of methylated CpG sites (*patients at risk*)	Radiographic progression-free survival			Overall survival		
n = 224	Hazard ratio	95% confidence interval of HR	*p*-value	Hazard ratio	95% confidence interval of HR	*p*-value
0 (44) vs. ≥ 1 (180)	0.60	0.4–0.9	***0.001**	0.55	0.4–0.8	***0.001**
≤ 1 (62) vs. ≥ 2 (162)	0.59	0.4–0.8	***0.001**	0.52	0.4–0.7	***0.001**
≤ 2 (71) vs. ≥ 3 (153)	0.53	0.4–0.7	***0.001**	0.45	0.3–0.6	***0.001**
≤ 3 (75) vs. ≥ 4 (149)	0.56	0.4–0.8	***0.001**	0.47	0.3–0.7	***0.001**
≤ 4 (80) vs. ≥ 5 (144)	0.53	0.4–0.7	***0.001**	0.44	0.3–0.6	***0.001**
≤ 5 (86) vs. ≥ 6 (138)	0.53	0.4–0.7	***0.001**	0.45	0.3–0.6	***0.001**
≤ 6 (91) vs. ≥ 7 (133)	0.53	0.4–0.7	***0.001**	0.45	0.3–0.6	***0.001**
≤ 7 (92) vs. ≥ 8 (132)	0.53	0.4–0.7	***0.001**	0.45	0.3–0.6	***0.001**
≤ 8 (99) vs. ≥ 9 (125)	0.51	0.4–0.7	***0.001**	0.44	0.3–0.6	***0.001**
≤ 10 (103) vs. ≥ 11 (121)	0.49	0.4–0.6	***0.001**	0.43	0.3–0.6	***0.001**
≤ 11 (107) vs. ≥ 12 (117)	0.48	0.4–0.6	***0.001**	0.43	0.3–0.6	***0.001**
≤ 12 (116) vs. ≥ 13 (108)	0.47	0.4–0.6	***0.001**	0.45	0.3–0.6	***0.001**
≤ 13 (118) vs. ≥ 14 (106)	0.47	0.4–0.6	***0.001**	0.46	0.3–0.6	***0.001**
≤ 14 (123) vs. ≥ 15 (101)	0.46	0.4–0.6	***0.001**	0.49	0.4–0.6	***0.001**
≤ 15 (129) vs. ≥ 16 (95)	0.46	0.4–0.6	***0.001**	0.48	0.4–0.6	***0.001**
≤ 16 (139) vs. ≥ 17 (85)	0.45	0.3–0.6	***0.001**	0.50	0.4–0.7	***0.001**
≤ 17 (147) vs. ≥ 18 (77)	0.45	0.3–0.6	***0.001**	0.47	0.4–0.6	***0.001**
≤ 18 (157) vs. ≥ 19 (67)	0.48	0.4–0.6	***0.001**	0.49	0.4–0.7	***0.001**
≤ 19 (168) vs. ≥ 20 (56)	0.53	0.4–0.7	***0.001**	0.52	0.4–0.7	***0.001**
≤ 20 (180) vs. ≥ 21 (44)	0.50	0.3–0.7	***0.001**	0.48	0.4–0.7	***0.001**
≤ 21 (193) vs. ≥ 22 (31)	0.57	0.4–0.8	***0.002**	0.60	0.4–0.9	***0.013**
≤ 22 (204) vs. ≥ 23 (20)	0.50	0.3–0.7	***0.001**	0.53	0.3–0.8	***0.012**
≤ 23 (215) vs. ≥ 24 (9)	0.55	0.3–0.9	0.057	0.57	0.3–1.0	0.123
≤ 24 (220) vs. ≥ 25 (4)	0.46	0.2–0.9	0.092	0.69	0.3–1.6	0.447

Univariate analysis for radiographic progression-free and overall survival was performed among patients with glioblastoma, IHD-wildtype, WHO grade IV (n = 224). Number of methylated CpG sites was tested as dichotomous variable. Number of patients at risk is indicated. Hazard ratio, 95% confidence interval of hazard ratio, and *p*-value are given

CpG: cytosine-guanine dinucleotide, *HR*: hazard ratio

Asterisks indicate $*p \leq 0.05$

previously described for the purpose of the present study. As significance of molecular markers in our daily treatment decisions increase and suitable diagnostics are more frequently incorporated in the routine pathological tumor workup, it will become increasingly important to address treatment differences and compare similar molecular tumor signatures in future studies to minimize bias.

Furthermore, stereotactic biopsy was the preferred choice for tumor diagnosis in molecular GBMs whereas histopathological GBM patients often received microsurgical tumor resection. To what extent differences in extent of resection (biopsy vs. gross total resection vs. subtotal resection) may play a role in patients' outcome as well as its association with MGMT promotor methylation remains uncertain. Also, different terminology to describe extent of resection across clinical trials have made it difficult to perform comparative analysis between study centers. [10].

Overall survival as well as radiographic progression-free survival was similar in both cohorts and consistent with previously reported data [26, 27]. On a cautionary note, progression-free survival was similar in both subgroups although glioblastoma patients more often received aggressive therapy with tumor resection and radiochemotherapy. Patients with *IDHwt* astrocytoma with *pTERTmut* WHO grade II and III, in turn, received a diverse treatment regimen spanning wait-and-scan approaches to tumor resection with following radiochemotherapy. None of the different treatment approaches demonstrated a benefit in patient's outcome when compared to other treatments. It remains to be noted that our sample size was limited. Prospective studies will need to address treatment approaches in such patients in the future.

In conclusion, our data show a similar extent of MGMT promotor methylation in patients with molecular GBM and

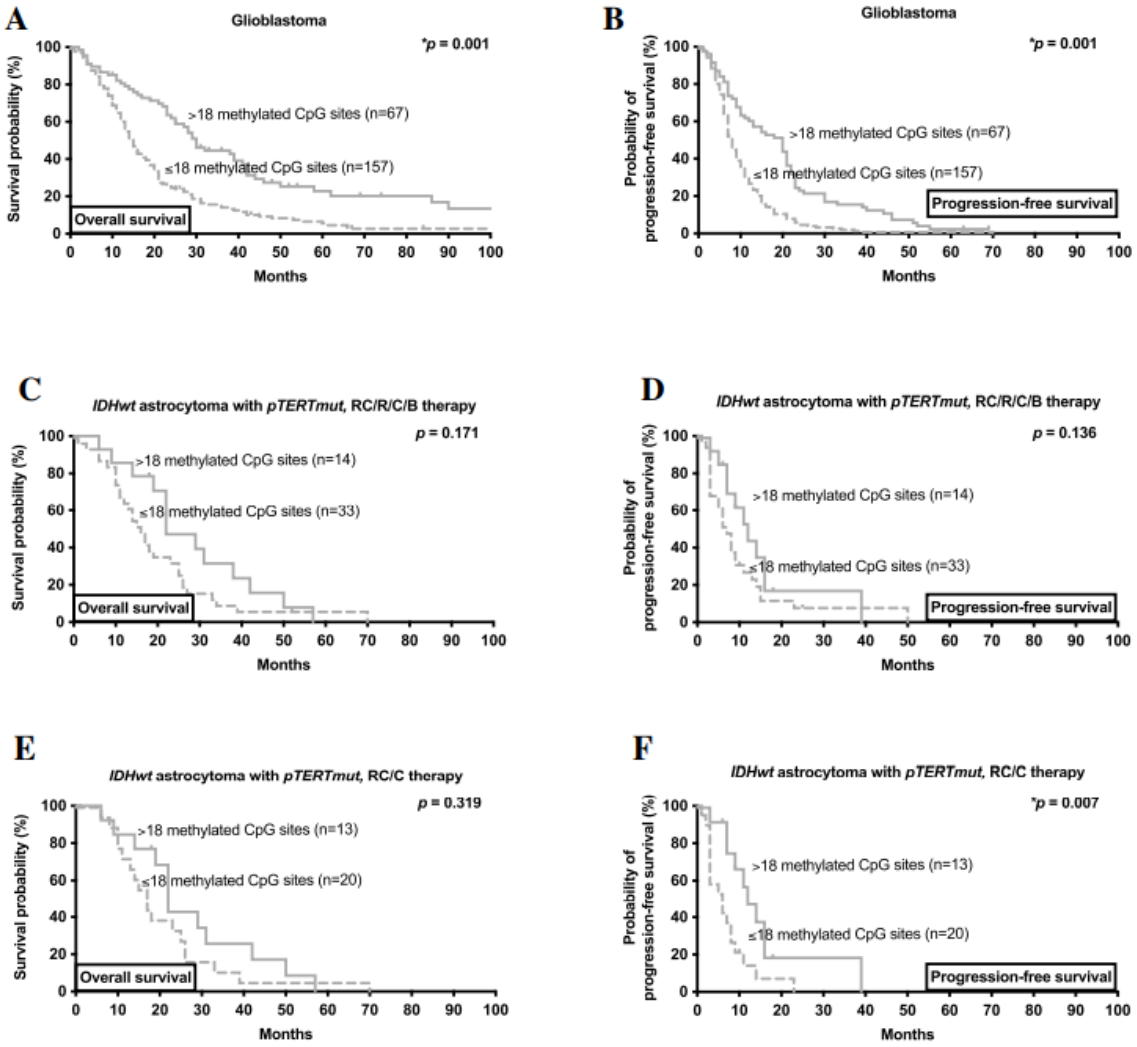


Fig. 2 MGMT as a marker for survival and disease progression in glioblastoma and *IDHwt* astrocytoma with *pTERTmut*. **A/C** Kaplan–Meier estimates of overall survival in glioblastoma and *IDHwt* astrocytoma with *pTERTmut* treated with any form of radio-/chemotherapy. Curves are displayed for patients with > 18 methylated CpG sites (straight lines) and ≤ 18 methylated CpG sites (dottes lines) **B/D** Kaplan–Meier estimates of radiographic progression-free survival in glioblastoma and *IDHwt* astrocytoma with *pTERTmut* treated with any form of radio-/chemotherapy. Curves are displayed for patients with > 18 methylated CpG sites (straight lines) and ≤ 18 methylated CpG sites (dottes lines). **E/F** Kaplan–Meier estimates of overall survival (**E**) and radiographic progression-free survival (**F**) in *IDHwt* astrocytoma with *pTERTmut* treated with first-line radiochemotherapy or chemotherapy. Curves are displayed for patients with > 18 methylated CpG sites (straight lines) and ≤ 18 methylated CpG sites (dottes lines). Tick marks indicate censored patients. *B therapy:* brachytherapy; *C therapy:* chemotherapy; *CpG:* Cytosine-Guanine dinucleotide; *IDHwt:* isocitrate dehydrogenase 1/2 wildtype; *pTERTmut*: TERT promotor mutation; *R therapy:* radiotherapy; *RC therapy:* radiochemotherapy

patients with histopathological GBM. Methylation rates were similar in both cohorts defined by Sanger sequencing as well as MSP. MGMT methylation was associated with improved outcome in patients with histopathological GBM and showed a non-significant trend for improved outcome in molecular GBM patients treated with radio- or chemotherapy. However, in both cohorts higher numbers of methylated CpG sites were associated with a significant longer time to radiographic progression in case of first line treatment with alkylating chemotherapy. Randomized prospective studies of treatment algorithms accounting for MGMT promotor methylation status are urgently needed in

Table 3 Number of methylated CpG sites within the MGMT promotor region in *IDHwt* astrocytoma with *pTERTmut*

Number of methylated CpG sites (*patients at risk*) n = 47	Radiographic progression-free survival			Overall survival		
	Hazard ratio	95% confidence interval of HR	*p*-value	Hazard ratio	95% confidence interval of HR	*p*-value
0 (8) vs. ≥ 1 (39)	1.46	0.7–3.0	0.311	0.94	0.4–2.1	0.871
≤ 1 (12) vs. ≥ 2 (35)	1.38	0.7–2.7	0.334	0.88	0.4–1.8	0.699
≤ 2 (14) vs. ≥ 3 (33)	1.17	0.6–2.3	0.615	0.82	0.4–1.7	0.557
≤ 4 (15) vs. ≥ 5 (32)	1.12	0.6–2.3	0.610	0.74	0.4–1.5	0.344
≤ 5 (17) vs. ≥ 6 (30)	1.08	0.6–2.1	0.808	0.73	0.4–1.4	0.304
≤ 7 (18) vs. ≥ 8 (29)	1.06	0.6–2.0	0.856	0.72	0.4–1.4	0.271
≤ 9 (19) vs. ≥ 10 (28)	0.97	0.5–1.9	0.931	0.66	0.3–1.3	0.163
≤ 10 (21) vs. ≥ 11 (26)	0.83	0.4–1.6	0.537	0.92	0.5–1.7	0.783
≤ 11 (23) vs. ≥ 12 (24)	0.89	0.5–1.7	0.681	0.95	0.5–1.7	0.864
≤ 13 (24) vs. ≥ 14 (23)	0.86	0.5–1.6	0.606	0.90	0.5–1.6	0.732
≤ 14 (25) vs. ≥ 15 (22)	0.83	0.4–1.6	0.535	0.91	0.5–1.7	0.751
≤ 15 (27) vs. ≥ 16 (20)	0.76	0.4–1.4	0.351	0.80	0.4–1.5	0.448
≤ 17 (30) vs. ≥ 18 (17)	0.62	0.3–1.2	0.125	0.69	0.4–1.3	0.218
≤ 18 (33) vs. ≥ 19 (14)	0.61	0.3–1.2	0.136	0.65	0.4–1.2	0.171
≤ 19 (34) vs. ≥ 20 (13)	0.72	0.4–1.4	0.326	0.78	0.4–1.5	0.450
≤ 20 (37) vs. ≥ 21 (10)	0.75	0.4–1.6	0.459	1.05	0.5–2.2	0.888
≤ 21 (39) vs. ≥ 22 (9)	0.58	0.3–1.3	0.216	1.13	0.5–2.6	0.759
≤ 22 (42) vs. ≥ 23 (5)	0.31	0.1–0.8	0.068	1.34	0.4–4.3	0.561
≤ 23 (43) vs. ≥ 24 (4)	0.34	0.1–0.9	0.095	1.02	0.3–3.3	0.970
≤ 24 (45) vs. ≥ 25 (2)	n.a	n.a	0.071	1.24	0.1–11.2	0.828

Univariate analysis for radiographic progression-free and overall survival was performed among patients with *IDHwt* astrocytoma with *pTERTmut* treated with radio- or chemotherapy of any kind (n = 47). Number of methylated CpG sites was tested as dichotomous variable. Number of patients at risk is indicated. Hazard ratio, 95% confidence interval of hazard ratio, and *p*-value are given

CpG: cytosine-guanine dinucleotide, *HR*: hazard ratio, *n.a.:* not applicable, *pTERTmut*: TERT promotor mutation

patients with molecular GBM. Understanding the biological role of MGMT promotor status and its clinical impact in the presence of TERT promotor mutations and absence of IDH mutations in gliomas formerly assigned to WHO grade II and III may be of great importance for future therapeutic management of such patients.

Acknowledgements The authors thank the patients and their families. The generous financial support of Dr. Dirk Ippen and Mrs. Marlene Ippen for bearing the publication charges is thankfully acknowledged.

Author contributions Study concept and design: NT, PK, JCT, NT. Data collection: NT, PK, JW, SS, MMD. Analysis and interpretation of data: NT, PK, LVB, JCT, NT. Drafting of the manuscript: NT, PK, JCT, NT. Revising of the manuscript: NT, PK, JW, SS, MMD, JH, LVB, JCT, NT.

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

Data availability Data and material can be made available by request to the corresponding author.

Declarations

Conflict of interest Joerg-Christian Tonn: research grants from Novocure and Munich Surgical Imaging, honoraria for lectures from BrainLab and CarThera, and royalties from Springer Publisher Intl. The authors Nico Teske, Philipp Karschnia, Jonathan Weller, Sebastian Siller, Mario M. Dorostkar, Jochen Herms, Louisa von Baumgarten, and Niklas Thon declare that they have no conflict of interest.

Ethical approval Study design and methods were approved by the Institutional Review Board of the Ludwig Maximilians University in Munich, Germany, and patient consent was waived (AZ 20-650).

Consent for publication We have full access to all of the reported data, including the right to publish any and all reported data.

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Danksagung

Mein Dank gilt in erster Linie Herrn Prof. Dr. Jörg-Christian Tonn für die kontinuierliche und wegweisende Betreuung des Promotionsvorhabens sowie der damit einhergehenden Publikationen. Herrn Prof. Dr. Peter Bartenstein und Herrn Prof. Dr. Niklas Thon danke ich für die Hilfsbereitschaft und zielorientierte Betreuung dieser Promotion.

Darüber hinaus danke ich Frau Sophie Katzendobler, die maßgeblich an der Datenerhebung, den Bildanalysen und der Manuskripterstellung beteiligt war.

Ich bedanke mich bei Frau PD Dr. Bogdana Suchorska, die insbesondere mit ihrem Wissen zu statistischen Analysen einen großen Beitrag geleistet hat.

Ganz besonders bedanken möchte ich mich bei meiner Familie für die durchgehende Unterstützung, nicht nur im Rahmen dieses Promotionsvorhabens, sondern auch darüber hinaus in allen privaten und beruflichen