A REVIEW ON DIFFUSE GLIOMA AND GENE TRANSFER TECHNOLOGIES AND THE IMPACT OF COVID-19 ON NEUROSURGICAL ONCOLOGY SERVICE PROVISION

BY

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Abstracts

1) Diffuse glioma is one of the most devastating cancers know in terms of morbidity and mortality. During the last decade, the mortality rate has seen a very modest change. Due to the rapid improvement in understanding the glioma-genesis, evolution, and genetic landscape, a new classification has

emerged, improving the diagnostic accuracy. However, the new knowledge acquired has not been translated into novel therapeutic therapies. The treatment regime has mostly relied on maximal surgical resection and chemo/radiotherapy. Nevertheless, gross macroscopic surgical resection is allowing for a significant number of tumour cells to be left behind. Glioma Stem Cells (GSCs) left behind can acquire chemo-resistant properties and exhibit a plethora of characteristics that enable cell survival and growth, leading to an aggressive tumour recurrence. It is now increasingly recognised that IDH mutations are linked to the development of glioma and tumour reoccurrence. IDH mutations can lead to genetic and epigenetic changes promoting cell proliferation, tumour invasion and immune evasion as well as preventing differentiation. Understanding these pathways will allow for new rational therapeutic interventions in an attempt to improve patient outcomes.

2) Gene delivery is the process whereby foreign genomic material is inserted into a host cell or organism. A number of viral and non-viral gene transfer system have been developed in the last few decades. However, no system to date has been used without certain limitations. Here we critically appraise and outline, the advantages, disadvantages, and common uses of viral and non-viral methods.

3) The severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) pandemic brings new challenges to the management of neuro-oncology patients. In the West Midlands (WM) a population of 5.7 million is served by three neuro-oncology centres. NHS service delivery was reconfigured to cope with SARS-CoV-2 infections. Here we report the impact at three centres with

low (C_{LOW}), medium (C_{MED}) and high (C_{HIGH}) levels of SARS-COV-2 mortality on referrals and diagnosis, surgical safety and quality and clinical management during the pandemic.

Data were collected either prospectively or retrospectively using electronic clinical records from each centre during period 1 (before lockdown) and period 2 of SARS-CoV-2 (complete lockdown).

Referral into specialist care pathways fell by 40% leading to reductions in diagnostic surgery of over 30% as a result of SARS-COV-2 related changes in healthcare provision and help-seeking behaviour. For SARS-COV-2 negative patients, surgical morbidity, 30-day readmission and 30-day mortality were unaffected by changes in management, but operations took longer, and there was an increase in patients with post-operative residual disease. There were no readmissions with SARS-COV-2 infection within 30 days of surgery. Access to radiotherapy and chemotherapy was reduced with patients suffering from the most aggressive cancers being more severely impacted.

Future planning should consider accelerating access to advanced molecular diagnostic technologies to refine clinical decision making and the use of chemotherapy. Regional networking solutions could optimise the use of resources and maintain a higher standard of care, allowing patients to continue to receive the best possible care.

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1. **Royal College of Surgeons of England Intercalated Degree Award**

   A highly competitive award, open to all medical students in the UK and Ireland offered by the college only to a handful of candidates, designed to support medical student who want to pursue the career of academic surgery.

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Abbreviations

α-KG	Alpha-Ketoglutarate
5-ALA	5-Aminolevulinic Acid
5-caC	5-carcboxylcytosine
5-hmC	5-hydroxymethylcytocine
A	Diffuse Astrocytoma (Grade: II)
AA	Anaplastic Astrocytoma (Grade: III)

ABC	ATP Binding Cassette
AO	Anaplastic Oligodendroglioma (Grade: III)
APE1	Apurinic/apyrimidinic Endonuclease One
BBB	Blood-Brain Barrier
BER	Base Excision Repair
BNOS	British Neuroncology Society
CET	Contrast Enhanced Tumour
CIC	Capicua Transcriptional Regressor gene
CNS	Central Nervous System
CRUK	Cancer Research UK
CSF	Cerebrospinal Fluid
CTCF	CCCTC-Binding Factor
D-2HG	D-2-Hydroxyglutarate
DT	Dendritic Cells
DTI	Diffusion Tensor Imaging
EGFR	Epidermal Growth Factor Receptor
EORTC	European Organisation for Research and Treatment of Cancer
F.D.A.	Food and Drug Administration
G-CIMP	Glioma CpG Island Methylator Phenotype
GB	Glioblastoma
GSC	Glioma Stem Cells
GTR	Gross Total Resection
HGG	High Grade Glioma
HIF	Hypoxia Inducible Factor
IDH	Isocitrate Dehydrogenase Enzyme
IL-10	Interleukin Ten
iMRI	Intraoperative MRI
iUS	Intraoperative Ultrasound
KDM	Lysine-specific Demethylase
LDH	Lactate Dehydrogenase
LGG	Low Grade Glioma
MGMT	O^6-methylguanine-DNA Methyltransferase

MHC	Major Histocompatibility Complex
MMR	Mismatch Repair
MRI	Magnetic Resonance Imaging
NADP+	Nicotinamide Adenine Dinucleotide Phosphate
NAMPT	Nicotine Phosphoribosyl Transferase
NAPRT1	Nicotinamide Phosphoribosyl Transferase One
NF1	Neurofibromin One
NICE	National Institute of Clinical Excellence
NOS	Not Otherwise Specified
O	Oligodendroglioma (Grade: II)
OS	Overall Survival
PARP	Poly-ADP Ribose Polymerase
PCNSL	Primary Central Nervous System Lymphoma
PDGFRA	Platelet Derived Growth Factor Receptor Alpha
PEG	Polyethene Glycol
PFS	Progression Free Survival
PGE_2	Prostaglandin E two
PHD	Prolyl Hydroxylase
PHE	Public Health England
PpIX	Protoporphyrin Nine
PTEN	Phosphatase and Tensin Homolog
RCT	Randomised Control Trial
ROS	Reactive Oxygen Species
RTOG	Radiation Therapy Oncology Group
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBNS	Society of British Neurological Surgeons
STAT3	Signal Transducer and Activator of Transcription three
STR	Subtotal Resection
TERT	Telomerase Reverse Transcriptase gene
TET	Ten-eleven Translocation Methyl Cytosine Dioxygenase
TGF-ß	Tissue Growth Factor Beta
TSG	Tumour Suppressor Genes

VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation

Chapter 1

DIFFUSE GLIOMA: A LITERATURE REVIEW ON CHEMORESISTANCE TUMOUR RECURRENCE IDH MUTATION RELATED MECHANISM AND THERAPEUTIC TARGETING

1.1 Introduction

1.1.1 Brain Tumours and Glioma

A brain tumour is developed when there are aberrant proliferation and growth of cells, leading to a space-occupying lesion, confined within and/or surrounding the brain. The brain tumours can be broadly classified into the cancerous (malignant), which are associated with poor prognosis and the non-cancerous (benign), which are most of the time curable. Cancerous tumours can be further classified into primary and secondary. Secondary malignant tumours are lesions arising from cancerous cells that have haematologically or otherwise spread to the brain, also commonly referred to as metastatic brain tumours. On the other hand, primary malignant tumours originate from cells within the central nervous system (CNS). Composing the CNS, neurones and glia (also referred to as glial or neuroglia), are the two main cell types. Neurones act as communicators, conveying signals as electrical impulses, whereas glia forms the myelin sheath surrounding neurones providing support and protection as well as maintaining homeostasis [1]. In the CNS, astrocytes, oligodendrocytes, microglia and ependymal cells, are all types of glial cells. Tumours arising from glial cells are termed as gliomas.

According to the World Health Organisation (WHO), gliomas can fall under four broad categories: i) diffuse astrocytic and oligodendroglioma tumour, ii) other astrocytic tumours iii) ependymal tumours and iv) other gliomas [2] (**Table 1.1**). The WHO 2007 classification, has grouped tumours of the CNS based on histopathological criteria, cell type and grading (Grades: I-IV), referring to similarities on known cells of origin and stages of differentiation [3]. Diffuse astrocytic and oligodendroglioma tumours are

otherwise known as “diffuse gliomas”, were classified into: i) grade II oligodendroglioma (O) and diffuse astrocytoma (A), with low proliferative and metastatic potential, commonly seen to grow 2-4 mm per year [4], with 50-70% transforming into a higher grade tumour within 7-8 years [5], ii) grade III anaplastic oligodendroglioma (AO) and astrocytoma (AO), which are aggressive, spreading and invading nearby tissues, iii) grade IV glioblastoma (GB), which is the most aggressive and incurable. Further, gliomas can be commonly classified into low-grade glioma (LGG), grades I-II, and high-grade glioma (HGG), grades III-IV.

1.1.2 Epidemiology

Out of all primary malignant brain tumours in adults, gliomas are the most common (75%) [6]. Regards to primary malignant tumours of the CNS, between 2012-2016, GB, accounted for 48.3% of the cases in the U.S.A, while astrocytoma and oligodendroglioma, 16.7% and 4.5% respectively [6]. It is estimated that 15,832 people are diagnosed with a “diffuse glioma” in the U.S.A every year. 11,833 patients are diagnosed with the most aggressive and currently incurable brain tumour, the GB [6], while 2,903 with an astrocytoma and 1,096 with oligodendroglioma. Recently, a study found that there was a significant increase in the incidence rate of GB from 1995 to 2015, more than doubling from 2.4 to 5.0 per 100,000 people in the UK, while the rest of diffuse gliomas remained stable [7]. In addition, the future in the U.S.A is also disheartening, with an estimated rise in GB diagnosis for the next 30 years [8].

The devastating prognosis of “diffuse gliomas” is well known. Between 2000 to 2015, the 5-year survival rate of GB patients was 6.8%. On the contrary, patients with

histological diagnosis of O or AO, have the best prognosis with 82.7% and 60.2% 5-year survival rate, respectively. Astrocytomas have a worse prognosis than oligodendrogliomas. The 5-year survival rate of A was 51.6% whereas 30.2% of AA. Despite advances in management, the prognosis has not been drastically altered [9] (**Table 1.2**). To date, clinical trials, testing new therapeutic options, fail to show a survival benefit. In an attempt to develop rational therapeutic strategies, the scientific community has made significant progress in understanding the function of the associated genetic mutations and the role of the immune environment in the complex interplay of tumour formation, progression and evolution.

Diffuse Astrocytic and Oligodendroglia Tumours		Other Astrocytic Tumours		Ependymal Tumours			Other Gliomas
Diffuse Astrocytoma (IDH mutant or IDH wildtype or IDH NOS)	9400/3	Pilocytic Astrocytoma	9421/1	Sub ependymoma	9383/1	Choroid Glioma of the Third Ventricle	9444/1
Anaplastic Astrocytoma (IDH wildtype or IDH mutant or IDH NOS)	9401/3	Subependymal Giant Cell Astrocytoma	9384/1	Myxopapillary ependymoma	9394/1	Angiocentric Glioma	9431/1
Glioblastoma (IDH wildtype or IDH mutant or IDH NOS)	9440/3 9445/3 9440/3	Pleomorphic xanthoastrocytoma	9424/3	Ependymoma (papillary or clear cell or tanycytic)	9391/3 9393/3 9391/3 9391/3	Astroblastoma	9430/3
Diffuse Midline Glioma (H3 K27M-mutant)	9385/3	Anaplastic Pleomorphic Xanthoastrocytoma	9424/3	Ependymoma (RELA fusion-positive)	9396/3		
Oligodendroglioma (IDH mutant and 1p19q co-deleted or NOS)	9450/3			Anaplastic Ependymoma	9392/3		
Anaplastic Oligodendroglioma (IDH mutant and 1p19q co-deleted or NOS)	9451/3						
Oligoastrocytoma (NOS)	9382/3						
Anaplastic Oligoastrocytoma (NOS)	9382/3						

Table 1.1: This table shows the four groups of glia tumour according to the World Health Organisation 2016 Classification of the Tumours of the Central Nervous System and the codes assigned for each tumour type [2].

		Diffuse Astrocytoma	Anaplastic Astrocytoma	Oligodendroglioma	Anaplastic Oligodendroglioma	Glioblastoma
2000-2015	5-year Survival	51.6%	30.2%	82.7%	60.2%	6.8%
	2-year Survival	64.1%	46.0%	90.6%	74.3%	18.5%
1995-2010	5-year Survival	47.3%	26.5%	79.1%	50.7%	4.7%
	2-year Survival	60.6%	47.1%	89.5%	67.7%	13.7%

Table 1.2: This table shows the 5-year and 2-year mortality rates for two periods, 1995-2010 Versus 2000-2015, of "diffuse gliomas" based on histological classification, according to the Central Brain Tumour Registry of the United States of America [6,9].

1.2 New Genetic Classification of Adult Diffuse Gliomas

Since the rapid emergence of a plethora of genomic and molecular pathology advancements, a new era of CNS tumour neurobiology has been demarcated. In response to the gain of knowledge and understanding of the disease, the WHO has allowed for major reconstruction of categorisation of CNS tumours, leading to the WHO updated 2016 classification. While in the past, the classification has grouped together tumours, based on the histological diagnosis only [3], since 2016 all diffusely infiltrated gliomas have been grouped primary based on their shared genetic driver mutations in the Isocitrate Dehydrogenase genes one and two (*IDH1,IDH2*) (**Table 1.3**). The new classification now combines genetic and histopathological nomenclature, where the phenotypic name precedes the genetic classification (i.e. glioblastoma, *IDH* wildtype) [10]. The importance of the new genetic classification can be appreciated further since the WHO 2016 recommendation clarifies that in the case of discordance between histological and genetic description, the latter overrides. The concept that genomic classification trumps histopathological observation allows us to move towards a new diagnostic and therapeutic approach based on objective genotypic identification rather than a subjective observational description.

Based on a hallmark alteration, “diffuse gliomas” are now classified into two groups, *IDH* wildtype (non-mutant) and mutants. *IDH* mutations are somatic changes that are found to be mostly acquired during the primitive stages of glioma-genesis [11] with the most common being a heterozygous mutation on the Arg 132 (R132H) of the *IDH* one gene. Normally, *IDH* converts isocitrate in α-Ketoglutarate (α-KG) but when mutated this enzyme acquires neomorphic activity by reducing α-KG to D-2-Hydroxyglutarate

(D-2HG) [12]. D-2HG is primarily competitively inhibiting α-KG-dependent dioxygenases and acting as an oncometabolite via histone and DNA hypermethylation, inhibiting differentiation, aiding the production of reactive oxygen species (ROS), contributing to tumour invasion and micro-vessel formation [13-15].

90% of GBs, which are formed *de novo*, 30% of DAs and AAs are falling under the *IDH* wildtype "diffuse glioma" arm, [16,17]. Further, it has been established that IDH wildtype GB is usually accompanied by other mutations. 72% have a mutation within the core promoter of the *telomerase reverse transcriptase* (*TERT*) gene [18,19], which leads to aberrant telomerase activity, an important step towards cell immortalisation [20]. It has been shown that *IDH* wildtype GB has 27% mutation rate in the tumour suppressor *TP53* gene, 24% *Phosphatase and Tensin homolog* (*PTEN*) deletion, and 35% mutation and thus aberrant activation in the absence of signalling of *epidermal growth factor receptor* (*EGFR*) [21,22]. In addition, *IDH* wildtype GB has alterations in the pro-oncogenic pathway such as dysregulation of the receptor tyrosine kinase (RTK), Ras and Phosphatidyl Inositol 3-kinase (PI3K) pathways have been reported as a result of *PTEN*, *EGFR*, as well as mutations in the Neurofibromin 1 (*NF1*) gene and the platelet-derived growth factor receptor-A (*PDGFRA*) [21]. *IDH* wildtype "diffuse gliomas" are correlated with worse prognosis compared to *IDH* mutants [2-3,23].

All Os and AOs are found to fall under the *IDH* mutant arm, in addition to 70% of DAs and AAs as well as 10% of GBs. *IDH* mutants can be further subclassified into two prognostic and diagnostic groups. The first subgroup corresponds to oligodendrogliomas both the diffuse and anaplastic (grade II-III), of which 100% show

a co-deletion of chromosome 1p and 19q, leading to a transformation of a non-balanced centromeric translocation *t(1:19)(q10;p10).* The codeletion has been correlated to better response to temozolomide (TMZ) chemotherapy and better overall prognosis [2,17,23-25]. Despite the fact that 1p/19q co-deletion was originally mentioned about two decades ago, little progress has been made to understand its functional impact on tumour development [26]. Further, the literature suggests that *TERT* promoter mutation is also prevalent within 1p/19q co-deleted *IDH* mutant oligodendrogliomas [27]. The mutation leads to an increase in the TERT promoter transcriptional activity, leading to an increase in telomerase activity and thus ultimately a decisive progression to immortalisation [20,28]. Whole exosome studies have further shown that the *Capicua transcriptional repressor gene (CIC),* is mutated the majority of times along with *1p/19q* co-deleted oligodendrogliomas [29,30]. *1p/19q* co deleted *CIC* mutant oligodendrogliomas are found to proliferate and grow faster than the *non-CIC* mutated tumours [31,32], thus the *CIC* mutation is speculated to be of prognostic significance. However, to date, the WHO 2016 did not incorporate this mutation into the new classification. The second subgroup lacks *1p/19q* codeletion. However, *IDH* mutant DAs, AAs and GBs, which belong to this subgroup carry a transcriptional regulator *ATRX* mutations and a *TP53* mutations [33]. The role of *ATRX* mutations regards to tumour formation, propagation and invasion have not been deciphered to date. There is some evidence to suggest that its role might be related to cell cycle regulation, histone regulation and chromatin remodelling [34].

The WHO 2016 classification has majorly refined the definition of the type of tumour that has always been controversial, the oligoastrocytoma. Previously,

histopathologists have heterogeneously reported the existence of a tumour that shares common histological characteristics of oligodendroglioma and astrocytoma, and thus not been able to comment for sure as to whether it is one nor the other [3]. However, based on the new classification, the oligoastrocytomas and anaplastic oligoastrocytomas fall under an IDH not otherwise specified (NOS) category. The importance of the new genotypic and phenotypic description has been demonstrated since now only rare reports exist able to classify a tumour as a “true” oligoastrocytoma, which consists of astrocytic (*ATRX-mutated*) and oligodendroglia (*1p/19q* co-deleted) mixed population of cells that are genetically and histologically distinct [35,36]. Previously classified astrocytomas can now be reported as pure oligodendrogliomas/astrocytomas with the aid of genetic testing [37,38]. Therefore the genetic advancements led to oligoastrocytomas only to be identified if the diagnostic molecular testing is absent or inconclusive or when tumour sample shares histological and genetic features of both oligodendrogliomas and astrocytomas - the genetic and molecular understanding of the disease allowed for tumour diagnosis objectively. However, despite the progress made, the knowledge acquired has not been translated into effective therapies, which can ultimately have a strong impact on the prognosis of these tumours.

Histological Classification	Histological Grading	Molecular Classification
Diffuse Astrocytoma (A)	II	IDH wildtype (30%) IDH mutant (70%)
Anaplastic Astrocytoma (AA)	III	
Oligodendroglioma (O)	II	IDH mutant (100%) 1p19q codeleted (100%)
Anaplastic Oligodendroglioma (AO)	III	
Oligoastrocytoma (OA)	II	IDH NOS
Anaplastic Oligoastrocytoma (AOA)	III	IDH NOS
Glioblastoma (GB)	IV	IDH wildtype (90%), TERT mutation (72%), TP53 mutation (27%), EGFR mutation (35%), PTEN mutation (24%) IDH mutant (10%)

Table 1.3: In the 2016 WHO classification of "diffuse gliomas" (grades II-IV), are classified in two groups based on the Isocitrate Dehydrogenase genes (IDH1 and 2), wildtype versus mutant status. This table summarises the percentage of mutations for every tumour with distinct histological classification as reported in the literature to date [16-38].

1.3 Current Treatments for Diffuse Glioma

1.3.1 Surgery for Diffuse Glioma

The current management of GB is based on three pillars: surgery, radiotherapy and chemotherapy. “Diffuse glioma” surgery has been drastically improved and to an extent, transformed over the last ten years. This is due to a combination of major improvement in brain tumour visualisation and imaging, aiding preoperative surgically planning as well as intraoperative surgical resection. Intraoperative technologies and neurosurgical techniques have also been improved. The philosophy of treatment is maximal safe resection, aiding to improve symptoms but also prolong progression-free survival (PFS) and overall survival (OS). Currently, the relationship of the extent of surgical resection (EOR) for LGG and clinical outcomes is incompletely understood through a number of articles emerged to show that EOR prolongs survival [39,40]. However, the consensus regards to HGG resection is that resection is correlated with increased survival [41,20]. With as little at 78% of resection of contrast-enhancing tumour (CET) on T1-weighted Magnetic Resonance Imaging (MRI), an exponential increase in survival has been shown, from 12 months median survival to 16 months [41,42]. Nevertheless, the way the EOR is assessed in randomised control trials (RCT) has been questioned, hindering achievement of level-one evidence linking EOR and survival. For HGG, although near-complete resection of CET is correlated to better outcomes, the operating neurosurgeon is challenged to preserve non-cancerous neurological tissue, minimising the risk of impact on morbidity, postoperatively. The fine balance between the gain of a few extra months of survival should be balanced against a functional outcome. Failure to preserve eloquent regions of the brain can compromise patients’ quality of life and possibly preventing them from being eligible

for chemo and/or radiotherapy with consequent prognostic implications [43,44]. The maximal resection for both LGG and HGG has been encouraged and incorporated into European and UK guidelines [45,46]. Numerous intraoperative technologies have now been developed and are still under evaluation, primarily aiming to equip better surgeons' ability to identify and resect tumour boundaries, while preserving "normal" eloquent brain tissue.

Fluorescence guided surgery for HGG allows for real-intraoperative identification of tumour. Currently, the gold-standard and recommended intraoperative fluorescence imaging technology is 5-aminolevulinic acid (5-ALA) [47]. 5-ALA is orally administered 4 hours prior to the operation resulting in accumulation of protoporphyrin IX (PpIX) in proliferating HGG cells, which under the violet-blue excitation light is seen to fluoresce in a bright pink colour. The colour is used to guide the resection of cancerous tissue and leaving behind "normal" tissue. The first RCT, conducted in 2006, showed a 29% reduction in the proportion of HGG patients who had residual enhancing disease on postoperative T1-weighted MRI imaging with contrast [48]. This led to an increase in PFS at six months and OS.

Functional organisation of the cortex varies considerably amongst individuals. In addition, a growing space-occupying lesion like a glioma is likely to distort normal and thus the expected neuroanatomy, and/or lead to reorganisation of neural networks as such surgical tools have been developed to help identify eloquent brain regions. Intraoperative neurophysiological monitoring and mapping, as well as direct electrical stimulation of brain regions in question either during an awake or under anaesthesia

patients, aim to identify reliable cortical and subcortical pathways involved in higher cognitive functions locations such as motor, sensory and language areas [49-51]. Subsequently, these areas can be avoided by preserving the normal physiological function of the brain. A systematic review and meta-analysis have evaluated the benefit of intraoperative stimulation, leading to a conclusion that upon usage, severe neurological deficits are reduced without compromising EOR [52]. Furthermore, 5-ALA combined with neurophysiological monitoring resulted in an increase in the EOR versus 5ALA alone, reducing mortality [53]. Combining subcortical mapping of motor areas and 5-ALA has also been shown to enable an increase in the EOR [54].

Recent developments in surgical intraoperative tumour visualisation technologies, such as intraoperative MRI (iMRI), intraoperative ultrasound (iUS) and neuronavigation have offered an additional and important group of tools in the surgeon's quiver in an attempt to achieve safe resection of CET [55-59]. A novel integrated imaging modality is the multimodal neuronavigation whereby a single software is able to combine functional and structural imaging data with the end goal to allow a surgeon to know the precise location of the tumour in real-time. This technology is ultimately assisting safe, maximal resection of a "diffuse glioma" adjacent to eloquent brain regions. Furthermore, functional MRI and diffusion tensor imaging (DTI) MRI enable the neurosurgeon to incorporate functional data into the software aiding pre-operative surgical planning as well as intraoperative navigation [60]. The choice of the aforementioned intraoperative adjuncts lies within the discretion of the operative surgeon. Interestingly, a recent Cochrane review by Jenkinson et al., recently concluded that while iMRI and 5-ALA evidently aid maximal safe resection.

However, the quality of evidence with regards to the impact of OS and PFS is low [61]. Technologies continue to improve and are refined, minimising possible disadvantages and maximising the surgical benefit.

1.3.2 Chemotherapy and Radiotherapy for Diffuse Glioma

Following resection, “diffuse glioma” surgery patients may undergo through cycles of chemotherapy and/or radiotherapy. DA and O patients are usually stratified into low-risk and high-risk patients. Though the stratification of those patients is highly variable and still lacks concrete evidence [62], a number of criteria exist to help with this process. Patients who are under 40 years of age and underwent complete resection with subsequent good, postoperative neurological function, which are found to have a mutation in the IDH gene are deemed as low-risk [63,64]. For this group of patients, the European Organisation for Research and Treatment of Cancer (EORTC) 22845 analysis, suggests watch-and-wait with regular MRI scans policy [63]. The current evidence relies on a retrospective comparison, which showed similar overall survival for patients who had immediate radiotherapy versus differed radiotherapy [46]. No prospective evidence exists to solidify these data. More, RCTs are needed to provide level-one evidence for this group of patients. According to the EORTC 22033-26033 [65] and the Radiation Therapy Oncology Group (RTOG) 9802 [66,67], patients who are older than 40 years of age, had a large tumour (>5cm) and reduced post-operative function or did not have complete resection are deemed as high-risk. The genetic addition to the WHO 2016 has a pivotal impact on the LGG patients’ treatment regime. LGG tumours, which are found to be IDH wildtype, are expected to behave like an HGG tumour, in terms of metastatic potential and reoccurrence [10]. A such, patients

with an LGG IDH wildtype tumour are deemed as high-risk. The high-risk patients who had surgery are advised to undergo radiotherapy, with does between 50 to 54 Gray, followed by adjuvant procarbazine, lomustine and vincristine (PCV), therapy of 6 cycles [68,69].

HGG patients are deemed as high-risk. The standard post-operative treatment regime for these tumours is predominantly based on the results from a phase 3 RCT and the CATNON trial interim analysis data [17,71]. This included 60 Gray radiotherapy with adjuvant six cycle PCV or concurrent and adjuvant TMZ. The CATNON interim analysis has demonstrated that *1p19q* co-deleted AAs can potentially benefit from 12 cycles of TMZ instead of 6 as well. Currently, the RCT called CODEL is investigating whether PCV is to be replaced with TMZ in the treatment of *1p19q* co-deleted oligodendrogliomas without sacrificing survival [72]. Furthermore, the POLCA trial, which is in progress, is currently evaluating whether PCV can replace radiotherapy for *1p19q* co-deleted oligodendrogliomas [73]. For GB patients, a treatment combination of 60 Gray radiotherapy for a cycle of 6 weeks with concurrent daily TMZ followed by another six cycles TMZ therapy has been the gold-standard regime, also known as the “Stupp” protocol [74]. In addition, recently, it has been reported that adding electrical field therapy to adjuvant TMZ may lead to an excess of 5-month additional survival benefit [75,76]. The survival benefit shown was independent of subgroup prognostic co-factors, i.e. complete resection of enhancing disease. The Nordic and NOA-08 trials have presented important information regarding patients who are older than 70 years of age who have been histologically diagnosed with GB [77,78]. For this group of patients, TMZ therapy can only show a survival benefit for those who harbour

the methylated promoter region of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), an important prognostic marker.

Currently, studies have shown that the concentration of TMZ within the tumour might not be high enough to successfully eliminate glioma stem cells (GSC) left behind after surgical resection [79]. As such novel drug delivery systems are under development aiming to improve spatial and temporal delivery of TMZ within the CNS. These include implantable controlled-release polymer systems, injectable nanoparticles and convention-enhanced delivery using a catheter-based approach to an agent placed directly to the tumour cavity [80]. Furthermore, a Food and Drug Administration (F.D.A.) – approved delivery system, called the Carmustine (BCNU) loaded wafer has been developed and used. Wafers are placed along the surface of the resected cavity delivering chemotherapy locally for a period of days to weeks [81]. This technology was primarily based on the assumption that a local delivery chemotherapeutic agent is most likely to achieve higher concentration levels and thus eliminate the remaining of the tumours cells left behind. A John Hopkins based group led by Henry Brem has shown that BCNU usage can show significant improvement in median survival (31 weeks) compared to placebo (23 weeks) [82]. The usage of the BCNU wafers has been controversial due to concerns regarding infection rates and the need for complete resection for glioma patients [83,84]. However, more studies have emerged to show survival benefit upon usage [85]. Despite the number of studies conducted and workforce/resources invested towards identifying the best treatment regime for “diffuse glioma” patients, the prognosis remains devastating and largely unchanged. Systematic non-targeted therapy seems to have reached its limits. The new molecular

paradigm has allowed for a new classification of tumours. However, no targeted translational therapies, such as direct drug inhibitors have been licensed to date.

1.3.3 Immunotherapy and Targeted Therapy

During the past decade, the brain has been considered an immune-privileged organ. This leads to the conclusion that the full effects of the immune system are not expected to be seen for any lesion within the blood-brain barrier (BBB) [86]. This has been derived from the notion than no lymphatics exist in the brain and that the BBB efficiently and selectively prevent an influx of immune-related chemokines and cells. However, it is now appreciated that the brain has an interaction with the immune system and *vice-versa* [87]. Importantly, it has been increasingly recognised that the brain tumours, including gliomas, are actively evading and suppressing the immune system, by minimising the expression of the major histocompatibility complex (MHC) receptors, reducing cell activation and expressing pro-apoptotic factors [88]. Numerous studies have shown that gene expression, evolution and aggressiveness of gliomas are regulated by a complex interplay between cancer cells and dynamic tumour microenvironment [89-91]. Consequently, delineating and overcoming the immune evading mechanisms is an emerging field of exciting new research.

Active immunotherapy, cytokine therapy and passive immunotherapy are the three main methods currently studied for overcoming the tumour-induced immunosuppression. Active immunotherapy has primarily been aiming to prime the immune response against know tumour targets by vaccination and adoptive T-cells therapeutic regimes [92-94]. On the other hand, cytokine therapy research has mainly

concentrated on activating and enhancing the immune system, which will concomitantly fight tumour cells [95]. Currently, passive immunotherapy research is focusing on utilising conjugated antibodies targeting tumour expressing antigens, with the second antibody inhibiting downstream effectors or indirectly killing the cells [96].

To date, direct pathway inhibition either by extracellular receptor binding or downstream effector inhibition such as tyrosine kinase molecules has been an emerging field of study. However, oncogenic pathway inhibition involving PDGFR and EGFR and intracellular downstream pathways have been trialled without meeting primary outcomes [97]. A number of ongoing trials exist; however, to date, no RCT has reported positive results.

Several clinical trials have been conducted to date, testing the efficacy of new drugs. The vast majority were unable to progress from phase II to phase III, and/or meeting their primary endpoints, failing to show superior outcomes versus conventional therapy in terms of OS and/or progression-free survival PFS [98-102]. Despite substantial progress in revealing the underpinnings of GB [103], few effective therapies remain, and thus new therapeutic strategies are needed.

1.4 Glioma Stem Cell Resistance to Chemotherapy and Glioblastoma Recurrence

1.4.1 Glioma Stem Cell

Despite the current evidence-based optimal therapy, the prognosis for “diffuse glioma”, especially for the HGG, is devastating. GB is the most common “diffuse

glioma” and has the worst prognosis. In the light of the aggressiveness of this tumour, GB patients undergo surgery and chemo/radiotherapy. However, malignant recurrence is seen and identified as the hallmark of poor prognosis [104]. For a new tumour to be formed, there has to be a group of cells that are able to resist therapy and survive. Thereafter they should be able to proliferate, multiply, invade nearby tissue leading to tumour re-genesis. GB formation is a result of the complex interplay between a dynamic microenvironment and new epi/genetic mutations leading to a heterogeneous group of cell types across space and time, with various proliferating and differentiating potentials, within the same bulk of tumour [105-110]. GB formation occurs through evolution across time, whereby a phenotypically and genetically heterogeneous cluster of cells, the clones, are generated, with a subsequent rise to daughter cells from each clone and/or combination of clones, within the same tumour [105,111,112]. For a tumour to form, there has to be a subpopulation of cells, which preserve higher stem-like properties compared to the rest.

The last two decades, a number of manuscripts have emerged proposing the notion of functional intra-tumour heterogeneity, implying the existence of a hierarchal model of tumorigenesis, whereby a cell type, the GSC, can demonstrate multi-potency and aberrant self-renewal capacity [106,113-115]. Thus it was proposed the GSC is the most tumorigenic and hence the founder clone [116-119]. GSC is able to rapidly proliferate, and self-renew, leading the conclusion that is the perfect candidate to be responsible for therapeutic resistance and malignant reoccurrence [120,121].

1.4.2 Glioma Stem Cell in Relation to Surgical Resection and Temozolomide Administration

The current surgical paradigm includes using intraoperative technologies for the resection of CET. It is widely agreed that one of the main reasons explaining poor prognosis despite optimal surgical resection, is the existence of GSC, leading to progression and malignant recurrence [117,122]. After complete resection of CET and adjuvant therapy, recurrence is commonly seen at the peripheries of the tumour cavity [122,123], at which GSC are commonly found as distinct “niches” [124,125]. Recently, a large comparative study from Mayo Clinic, University Californian San Francisco and Cleveland Clinic highlighted the need to resect tumour beyond the contrasted enhanced margin, as seen on T1-weighted MRI with contrast, since it can prolong survival regardless of molecular subgroup [126]. Taking into consideration that GB recurrence requires a cell type capable of tumorigenesis, there is speculation that GSCs may sit and extent beyond the CET tumour. Therefore a population of GSCs may remain unresected, allowing for tumour re-genesis following therapy. Further evidence exists, suggesting that infiltrating GSCs are found beyond the CET, supporting the notion that resection up to the CET margin may hinder survival outcomes [127-130]. Thus the philosophy of surgical resection for this tumour is currently under reconceptualisation.

TMZ is the main chemotherapeutic agent used for the GB management, which is systemically administered; however, not exhibit the first-pass metabolism. BBB is very highly selective, preventing a significant amount of the agent from reaching the tumour cavity [131]. Studies have shown that the concentration of TMZ found within the cerebrospinal fluid (CSF) surrounding the CNS ranges from 17.8% to 20% compared

to plasma [132-134]. In addition, other studies have found that 5uM of TMZ found in CSF compared to 50uM found in plasma is not able to have a drastic impact on cell death [135,136]. This is due to the fact the 5uM was shown to deplete on half of GB cell *in vitro* [135,136]. The BBB is disrupted at the tumour cavity. However, GSCs residing adjacent to the tumour may be surrounded by an intact BBB, and thus subjected to 5uM of TZM only [137]. Therefore understanding whether the concentration of TMZ within the tumour is reaching the expected and proposed levels are important. Nevertheless, quantifying the actual concentration and evaluate its efficacy in eradicating the remaining tumour should be prioritised.

1.4.3 Glioma Stem Cell Biology Favouring Survival Tumour Recurrence

For any cell type supposed to be responsible to tumour reoccurrence, it is paramount to retain properties enabling resistance to therapy and ultimately survival. Pertinent to their therapeutic role, surgical resection and chemo/radiotherapy can create conditions within the tumour cavity that are unfavourable to cell growth and survival. Disruption of blood supply can create low-tissue oxygen tension. At the extremes, oxygen tension can be significantly impaired and thus being low. This leads to inadequate ATP production and thus, lack of energy for the cells. It has been shown that GSCs are found within regions of mild to moderate hypoxia, consequently impaired oxygen tension [138]. Within these regions, these cell types were found to express increased hypoxia-inducible factors (HIF1/2), both at their very baseline and in recognition of decreased oxygen availability. Hypoxia has led to promote GSC expansion through activation of HIF-1a [139] as well as regulating tumorigenic potential [118]. Therefore GSCs are able to utilise the HIF signalling pathway for

survival. Furthermore, low oxygen tension increased the expression of stem-cell markers and induced clonogenicity [140]. Through upregulation of downstream effectors and induction of GSC-associated genes, HIFs are able to enhance the GSC potential. Thus, resistance to hypoxia is pivotal to promote GSC survival to therapy.

Resistance to hypoxia is not the only attribute needed for survival and ultimately tumour recurrence. An element of immune evasion and suppression has to be in place, to prevent tumour cells from being recognised by the immune system whilst the patient is under treatment as well as after the duration of the therapy. GSCs are known to be able to manipulate the tumour microenvironment [141]. A plethora of literature exists describing how GSCs are able to evade the cancer-immunity cycle at various stages. GSCs are able to utilise the signal transducer and activator of transcription 3 (STAT3) pathway and via the release of Interleukin 10 (IL10) to shape gene expression, activation and proliferation of resting primary macrophages (IL-10) [142,143]. Further, IL10 release is able to regulate the cytotoxicity potential of natural killer cells (NK) and neutrophils, which are the first few cells to arrive at the site of tumour cavity following therapy responsible for recognising engulfing tumour cells [143]. The STAT3 pathway activation is held responsible for the release of a number of other anti-inflammatory mediators such as prostaglandin (PG) E_2, tissue growth factor-beta (TGF-ß), which have a unique role in i) downregulating the Th1-mediated cytotoxicity and ii) inducing Th17 cells activation which is immunosuppressive and iii) inducing T-reg and inhibit T-cell proliferation, respectively [144-147]. Further, they can manipulate the expression of the cell-surface receptors of dendritic cells (DT) such as MHC II, CD80 and IL-12, preventing the DT-T-cell interaction, which can generate antitumor

immunity [148]. GSCs are able to evade the immune response increases the probability of survival.

For a new tumour to be formed, there has to be adequate blood supply. GSCs are found in perivascular niches, having a close interaction with blood vessels [149]. It is established that vascular endothelial cells are supervising survival of GSCs [150-152]. However, GSCs might also interact with adjacent vascular endothelial cells embracing vascular bed formation within the growing tumour [153,154]. Skog and colleagues have shown that tumour cells are able to secrete extracellular vesicles containing angiogenic factors [155]. A recent paper found that vascular endothelial growth factor A (VEGF-A) is also released in extracellular vesicles by the perivascular GSCs [156]. Indeed, a scoping review on the "crosstalk between glioma stem cells and their microenvironment", concluded that GSCs play a dominant role in tumour angiogenesis via i) releasing extracellular pro-angiogenic vesicles ii) recruitment of endothelial cells and iii) direct differentiation into endothelial cells [157]. Therefore, GSCs are aiding tumour reoccurrence via angiogenesis.

A key feature enabling tumour recurrence is the GSC plasticity and intra-tumour genetic, phenotypic and functional heterogeneity. Within an HGG, three main phenotypes of cells exist: proneural, mesenchymal and classical [110]. The proneural, common in young adults, corresponds to a secondary GB subtype, associated with *IDH1* mutation or *PDGFR* an amplification and a *p53* mutations, which are linked to favourable outcomes [110]. On the contrary, mesenchymal and classical phenotypes are associated with the worst outcomes [158]. The classical phenotype was found to

be associated with amplified EGFR signalling pathway, while the mesenchymal that is associated with older adults harbour no mutations on *IDH1* but rather an *NF1* deregulated gene expression. It has been shown that a phenotypic shift within the same tumour is possible. Tumour recurrence and thus, worst outcomes were associated with a shift from proneural to mesenchymal [159]. In addition, it is possible that more than two different phenotypes exist within the same tumour [159]. Concomitantly, phenotypic heterogeneity within the same tumour has been linked to worse survival [107]. Moreover, the same study showed that it is possible for cells can simultaneously "score" highly for two phenotypes. This means that a cell can be a product of two different phenotypic cells. Therefore this leads to the conclusion that the existence of "hybrid" states is possible, reflecting aberrant developmental programmes. Thus, within the same tumour, there might be a number of different GSC subpopulations. The deletion of one could mean the enrichment of another.

During optimal, surgical and adjuvant therapy, a certain subpopulation of cells will show greater vulnerability than others. This applies a selective pressure to the rest of the subpopulations, which may remain. Meaning a new set of subpopulations will arise and proliferate. These new population could have survived due to drug induce mutations, making them resistant to treatment or existence of resistant clones due to 'natural' tumour progression even before therapeutic intervention. In fact, GB resistant subclones were found to acquire the cellular information pertinent to tumour reoccurrence at the early stages of their development, rather than the accumulation of mutations over time [160]. The subclones will persist after treatment and repopulate over time, leading to tumour reoccurrence.

Interestingly, recent discoveries denote that non-GSCs can acquire proliferative potential [155,161]. It has also been demonstrated in animal models that glioma cells, which did not express stem cells markers were able to de-differentiate and gain stem cell properties [162]. Dahan et al., have discussed the importance of cellular plasticity in chemoresistance [163]. They showed that cells that had no GSC-like properties were able to dedifferentiate into GSCs following radiation. Similarly, other studies proposed that GSCs were able to interconvert to non-GSCs induced by TMZ therapy also [164]. In fact, TMZ may create cellular stressors such as hypoxia and acidity, which may be responsible for the GSC conversion. Furthermore, the loss of *von Hippel-Lindau* and/or *PTEN,* have been proposed to induce GSC conversion [165,166]. It can be concluded that even if all GSCs subpopulations are eliminated, the surrounding cells can be induced to acquire oncogenic potentials, which may lead to tumour recurrence (**Figure 1.1**). Therefore understanding why systemic therapies have failed and developing inhibitors based on the new molecular classification, which reflects the biology of the disease, will allow us to move towards an era of precision medicine with fewer side effects and hopefully improved outcomes.

Figure 1.1: Illustration showing four unique abilities of Glioma Stem Cells (GSCs) suitable for survival, allowing for tumour recurrence.

1.4.4 Glioma Stem Cells and TMZ Chemoresistance

GSCs remain at the centre of attention, held responsible for current treatment failures and therapeutic resistance. GSCs were shown to circumvent chemotherapy, the hallmark of HGG treatment, in a plethora of ways. Preventing the high accumulation of TMZ within the cell, increasing expression of specific enzymes counteracting cytotoxic effects of TMZ as well as altering expression of DNA repair mechanisms are but a few examples (**Figure 1.2**). It has been questionable whether TMZ's concentration reaching the remnants of the tumour is adequate for the eradication of remaining tumour cells. Cellular bioavailability is further hindered by a unique cell surface transported, the ATP binding cassette (ABC) transporter channels. The ABC transporter was found to be increasingly expressed in GSCs [167,168]. Its main function is to actively carry molecules such as TMZ, using ATP, out of the cell as well as the BBB [169]. ABC-G2 is the main transporter of reference, which has been associated with small stem-like subpopulations showing multidrug resistance and ultimately, survival [170]. Denoting to its functions in cancer progression, ABC transported has also been described as a cancer driver [171].

GB cells exhibit TMZ cytotoxicity via methylation of the O6-position of guanine base in DNA. This creates a change in DNA structure, which can be detected by other cellular mechanisms and if the damage cannot be repaired, inducing apoptotic pathways and thus cell death. However, the methyl residue added by TMZ on the DNA molecule can be removed by an MGMT repair enzyme that is variably expressed. MGMT's promoter region can be arbitrarily methylated leading to inactivation of the MGMT enzyme and thus inability to repair the methylation gain in DNA molecule. Cell

cycle checkpoint inhibitors can detect the DNA change, and if not repaired, cell cycle arrest can be induced [172-174]. Whereas if methylation of the MGMT promoter is low, an increased MGMT ability to remove methyl residue from DNA will reduce cytotoxicity, leading to poor prognosis. MGMT repair enzyme is found to be disproportionally increased in GSCs, leading to TMZ resistance [175]. Notably, TMZ was shown unable to block the renewal properties of GSCs, expressing MGMT [176]. On the contrary, TMZ was found to be effective in eradicating MGMT negative GSCs. [177].

DNA repair aberrations have been previously described and accounted for treatment resistance and recurrence. TMZ leads to methylation of a guanine base. This base pair change is a common target for intercellular DNA repair mechanisms [178]. The mismatch repair (MMR) mechanism, is responsible for recognising the base-pair mismatches and deletions, including the O6-methylguanine produced by TMZ [179]. Thereafter if a base change is irreparable, the DNA breaks, followed by the initiation of apoptotic pathways for self-destruction [178]. In GSCs, MMR is found to be inactivated or at least dysfunctional [180]. TMZ main mechanism of action is thus bypassed. This allows for cell cycle and replication to continue without correction, leading to an accumulation of mutations.

Via the poly ADP-ribose polymerase (PARP-1) pathway, another way of recognising DNA damage, cells can activate the DNA repair systems [180]. PARP-1 was also found to be upregulated in glioma [181]. A base excision repair (BER) mechanism is aiding the correction of the base pairs detected [182]. BER is able to excise the

common chemo/radiotherapeutic-induced DNA changes detected [182]. Subsequently, damaged DNA base pairs can be removed via a DNA glycosylate and/or apurinic/apyrimidinic endonuclease (APE1) enzyme. Following this, a DNA polymerase will add the correct bases and a ligase with enable collect closure of the DNA molecule [183]. PARP-1 is affecting both the MMR and BER systems. These systems are hyperactive in malignancy, herein GB cells, leading to a cumulative effect of a drastic increase in repair DNA damage induced by therapy.

The role of checkpoint inhibitors in cancer biology has been well-known [184]. DNA checkpoints, specifically ChK1 and Chk2 kinases, are known to detect possible DNA alterations, prevent cycle progression and activate genes aiding correction of DNA changes [185]. Chk1 is active, in the S and G2 cycle phase, without the detection of a DNA change. On the contrary, Chk2 protein is activated upon the detection of DNA damage at any point in the cell cycle. Chk1 has found to be overexpressed in a number of tumours including breast and colon as well as being correlated with tumour grade and recurrence [186-190]. Enhanced Chk1 has led to chemo/radiotherapeutic resistance of cancer cells, not only in GB but also in prostate and lung [191-194]. Increased expression of Chk1 has also been specifically linked to resistance to chemotherapy [194]. Chk1 is known to be essential for cell survival and viability. The increased expression may lead to increased survival because the higher the Chk1 protein concentration is, the higher the ability to detect and handle DNA damage induced by therapies [195]. Cell cycle checkpoint proteins have an overwhelming role in therapeutic resistance, and thus are extensively studied, hoping to provide future targets for therapy.

Combining a variety of intracellular counteracting chemotherapeutic mechanisms, specifically to TMZ, the GSC antitherapeutic abilities, are an area of research and discovery. Despite the finding of myriad chemo-resistant mechanisms, no clinical translation improving overall prognosis has been made. New drug targets, combined with precision medicine protocols, may offer hope to one of the most feared malignancies known to humankind.

GSCs exist in quiescence and active states, able to switch from between functional states influenced by the micro-environment. They play a key role in tumour reoccurrence. They are able to release key pro-angiogenic mediators and induce blood vessel formation. Further, they can downregulate the expression of pro-inflammatory mediators and suppress cell induce cytotoxicity and antibody production. Their properties allow for new tumour to be formed. TMZ is the gold-standard chemotherapeutic agent for GB. However, adequacy of its bioavailability within a tumour is debatable. GSCs are not only able to survive but can resist the action of TMZ. Firstly, they can increase the expression of ABC transporters, effectively pumping out TMZ of the cells. Secondly, they show increase expression of MGMT, an enzyme which can efficiently correct TMZ-methylation induced changes to the DNA. Thirdly, they demonstrated an alteration of MMR mechanism allowing for the accumulation of TMZ-induced DNA changes without arresting the cell cycle. However, for the DNA changes that are detrimental to the cell, they exhibit an increase in ChK1 and Chk2 kinase expression, and thus an enhanced ability to handle pertinent DNA chemotherapeutic-induced damage.

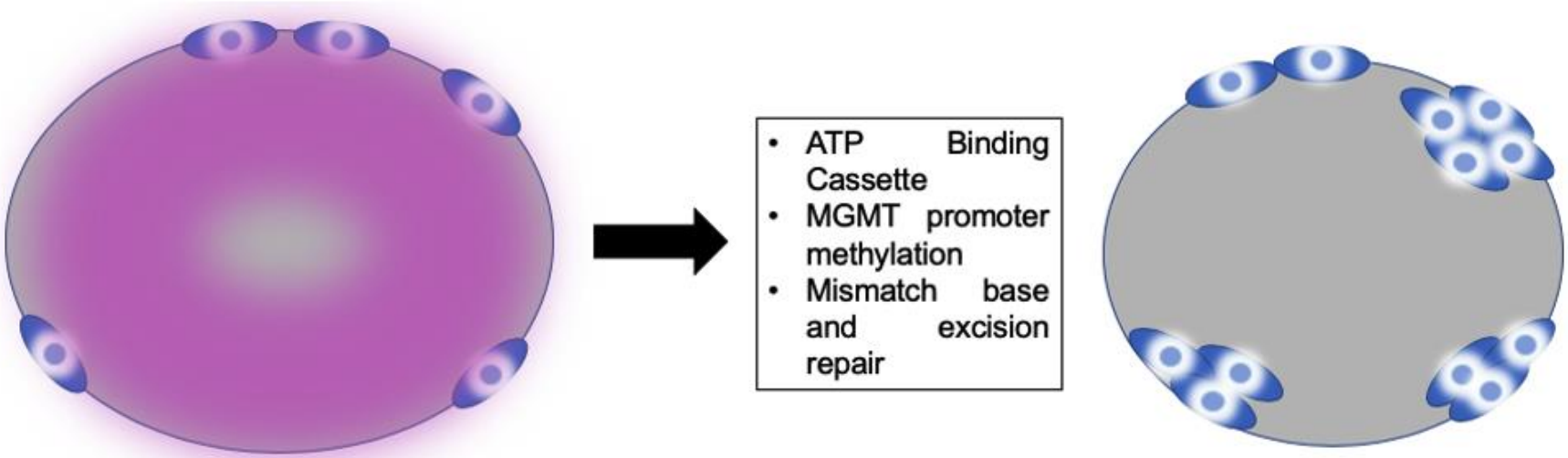

Figure 1.2: Illustration showing the unique molecular pathways leading to temozolomide resistance and thus allowing for cell survival and proliferation.

1.5 IDH Mutation

1.5.1 Introduction

The prevalence of *IDH* mutations in “diffuse gliomas” has been previously mentioned. It is now increasingly recognised that *IDH* mutations are linked to the development of glioma and tumour reoccurrence. Taking into consideration that current therapies fail to demonstrate improvement in outcomes, *IDH* induced biochemical alterations should be adequately understood and assessed as potential targets.

IDH enzymes play a role in the Krebs cycle, lipogenesis, glutamine metabolism and redox regulation [196-198]. There are three isoforms: i) *IDH1* that is located in the cytoplasm and peroxisomes; ii) *IDH2* and *IDH3*, which are found in the mitochondrial matrix [199]. All *IDH* enzymes catalyse the same chemical reaction, the oxidative decarboxylation of isocitrate to α-KG. However, while the chemical reaction catalysed by IDH1/2 id reversible and require binding of the nicotinamide adenine dinucleotide phosphate (NADP+) as a co-factor, which is converted to NADPH [200]. On the contrary, the chemical reaction of IDH3 is irreversible, and requires the binding of NAD+, which is reduced to NADH. IDH1/2 work as homodimer, while IDH3 is heterotetrameric enzyme, composed by two IDH3α catalytic subunits, one IDHβ and one IDH3γ, which are involved in the allosteric regulation of the enzyme function [201]. The active site is frequently mutated in many cancers, including gliomas. In addition to diffuse LGG and HGG, *IDH* mutations have been described in chondrosarcomas (56%), intrahepatic cholangiocarcinomas (23%), acute myeloid leukaemia (16% prevalence), as well as to a lower extend, myelodysplastic syndrome and

angioimmunoblastic T-cell lymphoma, as detected by RNA, DNA and antibody patient-sample analyses [202-207].

IDH cancer-related mutations affect predominantly *IDH1/2*, most of the times tending to localise to the arginine residues of *IDH1* (R132) and *IDH2* (R172, R140) [208]. The arginine residue is crucial for the recognition of isocitrate. A highly positively charged arginine residue at position 132, is replaced by lower polarity amino acids such as histidine, cysteine or lysine. This missense mutation intervenes with the alpha and beta carboxyl sites of isocitrate [209, 210]. This results in a weak affinity for isocitrate in addition to raised NADPH levels, since less is going to be used as a co-factor for a forward reaction. In gliomas, the *IDH* mutations are heterozygous, most commonly affecting the amino acid residue R132H. *IDH* mutations produce a dimeric enzyme composed of a wildtype and mutant monomer. Consequently, in *IDH* mutant cells, the wildtype part of the dimer leads to the conversion of isocitrate to α-KG producing NADPH, whereas the mutant monomer by using the NADPH as a co-factor, converts α-KG to D-2-hydroxyglutarate (D-2-HG) exhibiting neomorphic activity [211]. *IDH* mutations have a biological impact both intracellularly as well as part of the tumour microenvironment favouring tumour formation and recurrence.

1.5.2 Metabolic Alterations

The accumulation of D-2HG as a result of *IDH* enzyme mutation leads to significant reduction and drainage of Krebs Cycle substrates and thus carbohydrate sources [211,212]. Consequently, the Krebs cycle is forced to adjust and drain other sources to yield ATP [213]. The loss of α-KG and depletion of cellular metabolism, results in

the recruitment of other carbohydrate sources [214,215]. A study found, that glutamate dehydrogenase 2 catalysing the conversion of glutamate to α-KG, is highly expressed in *IDH* mutant brain cells [216]. In addition, *IDH*-mutated glioma cells were found to be highly sensitive to the inhibition of glutaminase, an enzyme that contributes to the lysis of glutamate, further adding to the notion that *IDH* mutated cells are glutamate-dependent [217]. Furthermore, the depletion of NADPH for the formation of D-2HG, leads to the reduction of intracellular lipogenesis, resulting in a great dependence in exogenous lipid sources [197] and as such, partly explaining the slow but steady growth of *IDH* mutant gliomas.

In the majority of cancers, the need for an adequate supply of energy is mediated by the increased expression of lactate dehydrogenase (LDH) [218]. LDH catalyses the transformation of pyruvate, the end product of glycolysis, to L-lactate [219]. L-lactate can serve as immediate fuel for the increased demands for energy, to match the rapid proliferative potential of the cancer cells. On the contrary, in *IDH* mutated glioma patient-derived samples, the LDH is silenced [220, 221]. Hypermethylation of the promoter region of the LDH gene was found to be the main reason for the lack of LDH expression [220, 221]. This epigenetic silencing might explain the slow-growing nature of *IDH* mutants compared to *IDH* wild-type gliomas [221, 222].

IDH mutations result in significant alterations and reprogramming of the cellular metabolic pathways. Glutamine derivatives can serve as key substrates to the Krebs cycle compensating for the depletion of isocitrate. *IDH* mutate gliomas show a distinctive metabolic behaviour compared to other tumours, most notably reduced

glycolysis, which not only partially explains the slow-growing nature but provides useful metabolic targets for future glioma therapies.

1.5.3 Epigenetic Modifications

Several studies have reported that *IDH* mutant glioma is associated with CpG island hypermethylation [223,224]. Neomorphic *IDH1* mutant activity leads to histone as well as DNA hypermethylation. However, the extend of hypermethylation is highly variable [225]. Understanding the pathogenesis of *IDH* mutant gliomas in relation to the hypermethylation patterns might lead to rational therapeutic targets identified. Methyltransferases and demethylases are able to control the DNA methylation pattern. Within the demethylation process, 5-methylcytosine is converted to 5-hydroxymethylcytocine (5-hmC) catalysed by the ten-eleven translocation methyl cytosine dioxygenase (TET), in an α-KG and iron-dependent manner. In addition, TET concomitantly catalyses cytosine demethylation steps by converting 5hmC to 5-carcboxylcytosine (5-caC) and -formyl cytosine. Thymine DNA glycosylase BER enzymes will eventually convert 5ac-C to cytosine [226]. However, in *IDH* mutant gliomas, the TET activity is stopped due to D-2HG structural similarity to α-KG [227,228]. Therefore the demethylation process cannot take place. *IDH* mutations are adequate in inducing a hypermethylated phenotype [229,230]. Follow up studies have shown that once the hypermethylation happens, it is irreversible and thus playing a pivotal role in malignant transformation and recurrence [231].

Furthermore, D-2-HG is aiding histone methylation by inhibiting histone demethylases. A notable example will be lysine-specific demethylase (KDM) [227,232]. Histone

methylation is predominantly regulated via histone methyltransferases, which add the methyl group as well as demethylases (such as KDM), which remove the methyl group. Due to the fact that D-2HG is playing a catalytic role in these reactions, in a similar way as with the aforementioned TET, the increased levels of α-KG competitively block the reactions [232]. It is now recognised that these histone and CpG island hypermethylation patterns are predominantly found in *IDH* mutant GSCs [233]. Studies have shown that CpG hypermethylation leads to inactivation of tumour suppressor genes (TSG) as well as altered gene expression related to cell differentiation [234]. Thus the *IDH* mutations ultimately block cell differentiation and cell cycle regulation, leading to uncontrolled proliferation, with concomitant accumulation of new somatic mutations, which are acquired across time and remain undetected. However, the development of glioma not only requires seeds (GSCs) with uncontrolled proliferation but also a fertile soil (tumour micro-environment).

1.5.4 Redox Imbalance

IDH mutations lead to the increased affinity for NADPH and α-KG, leading to the conclusion that the mutant glioma cells will prefer using NADPH and not NADP+ [211,235]. The high consumption of NADPH disrupts the reducing equivalents of biochemical reactions that are needed for important ROS processes, leading to the accumulation of ROS [236,237]. ROS are involved in genomic instability, cellular motility and acquisition of invasive characteristics [238,239]. Excessive ROS leads to DNA and protein damage, disrupting enzymatic reactions and gene expression. The alteration of gene expression might lead to new mutations, which are oncogenic. Consequently, the ROS accumulation is fundamental and a hallmark to cancer

biology, especially for the *IDH* mutated gliomas. A number of studies have shown that cells derived from *IDH1* mutated gliomas exhibit strong oxidative stress, evident by the increased expression of ROS such as manganese superoxide dismutase [240]. The elevated stress was confirmed with further evaluation shown that *IDH* mutated cells are prone to oxidative damage [241,242]. In the face of the raised oxidative profile enhancing antioxidant pathways, such as synthesis of glutathione, maybe be a valuable strategy to downplay the oncogenic effects of ROS [243].

1.5.5 Tumour Microenvironment

IDH mutant glioma cells alter not only an intercellular biochemical cascade of events but also their surrounding environment. It has been documented that *IDH1/2* mutant cells are able to promote the genesis of new micro-vessel bed formation via the increase of VEGF [244]. Aberrant proliferation requires constant energy supply, which in turn will be supplied by nutrients and oxygen delivered by the bloodstream. Studies found that the expression of VEGF was significantly higher in *IDH* mutated gliomas cells versus *IDH* wildtype [245,246]. The HIF-1α released by GSCs in hypoxic conditions has been correlated to increased transcription of the *VEGF* gene [246]. As previously mentioned, GSCs are able to release pro-angiogenic factors. IDH mutated cells can upregulate *VEGF* to promote angiogenesis as well as inhibiting the breakdown of HIF-1α, leading to more VEGF production [247]. *IDH1/2* mutations can positively affect their surroundings to create favourable conditions for aberrant cell proliferation and propagation.

1.5.6 Propagation and Invasion

Glioma invasiveness is one of the main reasons for tumour recurrence and thus poor prognosis. As previously discussed, GSCs resist therapy, and some are left behind even after surgical resection. *IDH1/2* mutations play a pivotal role in this process. The rapid growth of cells and the uncontrolled proliferation leads to pressure for nutrients. Therefore the invasive process of GSCs and their daughter cells allow them to escape the adversity of the surrounding environment. *IDH2* mutations may lead to HIF-1a and beta-catenin accumulation, which have been correlated to tumour invasion [248]. Furthermore, *IDH* mutant cells lead to an increased expression of *PDGF*. *PDGF*-induced glioma models exhibit invasive properties [249,250]. Further, the invasion of nearby tissue leads to rapid accumulation of microglia, a factor that has also been correlated to the invasive potential of glioma cells [249,250]. It is speculated that both PDGR and HIF-1α are released in response to tumour hypoxia. These factors contribute towards resistance to lack and acquisition of more oxygen. It is worth highlighting that HIF-1a is partially regulated via the prolyl hydroxylase PhD (PHD), which is an α-KG dependent dioxygenase [247]. *IDH* mutations cause α-KG-dependent dioxygenase to be inhibited. [211]. As such, the HIF-1α regulation is lost due to *IDH* mutations. In addition, the aberrant expression of *HIF-1*α is found in cells at the peripheries of the necrotic tumour centre areas, which are commonly described to exhibit migration patterns [251]. One can hypothesise that glioma tumour cells are acquiring *HIF-1*α and *PDGF* expression to escape the necrotic core and invade nearby tissues. The exact mechanisms, which link *HIF-1*α and *PDGF*, are yet to be delineated.

1.6 Novel Therapeutic Options for IDH Mutant Glioma in the Molecular Era

1.6.1 Direct Inhibition of IDH

The mutant *IDH* gene and subsequently the enzyme have been correlated as explained above, with neomorphic activity and tumour recurrence. As such, in an attempt to identify novel but rational drug targets, direct inhibition of the mutant *IDH* has been pursued. AGI-5198 has been reported as the first novel, synthetic, direct enzyme inhibitor of the *IDH* mutant enzyme [252]. This drug was able to block the generation of D-2-HG, which is aberrantly produced in *IDH* mutant gliomas, impairing xenograft progression *in vivo* [252]. As reported, the drug was able to induce the expression of a gene that is related to differentiation, leading to reduced proliferation. AG-120 (ivosidenib) and AG-881 (vorasidenib) and AG-221 (enasidenib) are the second generations selective, reversible drug inhibitors produced, which are approved by F.D.A. for the treatment of acute myeloid leukaemia [253,254]. AG-221 was the first drug to be approved by F.D.A in 2017. Interestingly, the drug was tested in a clinical trial for gliomas and other *IDH* mutant tumour in 2014. The drug showed inhibitory effects for these tumours. However, appropriate dosing was an issue. A number of clinical trials are underway, currently evaluating the efficacy and safety profile of AG-120 and AG-881 [255]. Since the discovery that *IDH1* R132H is the most common mutation in “diffuse” gliomas, more clinical trials have emerged [256-261]. Recently, Agios Pharmaceuticals has conducted a multicentre clinical study on recurrent LGG with *IDH* mutation using an AG-120 and AG-881 [256]. The primary outcome was to compare the D-2-HG concentrations in surgically removed tumours, which were treated versus not treated with the drug inhibitors. Clinical safety, dosage, tolerance

and pharmacokinetics will also be studied. This safety, feasibility trial will provide appropriate dosing for future studies. It is worth noting that the new inhibitors exhibit a good CSF-plasma ratio [262]. On the contrary, the main issue with the most common chemotherapeutic agent for HGG, the TMZ, was whether the amount of the drug reaching the tumour might not be enough to eliminate the remaining of the cells. BAY1436032 is also a new inhibitor, predominantly tested in myeloid leukaemia, showing tumour-suppressing potential in experimental trials [263, 264].

Despite the positive results, the success of *IDH*-mutant inhibitors is found to have a plethora of limitations. A study showed that despite the fact that AGI-5198 reduces neomorphic activity, it also does not alleviate the DNA and histone hypermethylation phenotype since histone methylation was found to be high [265]. Further, Sulkowski and colleagues demonstrated that AGI-5198 is preventing DNA damage in cancer cells, leading to the conclusion that this might allow for resistance to DNA damage agents like current chemo and radiotherapeutic options [266]. This has also been confirmed by another study, suspected that *IDH1* mutated cells under the action of AGI-5198 gain radioprotective abilities [267]. Currently, a number of other novel molecular inhibitors are tested. Those can be combined with *IDH* inhibitors to overcome possible drawbacks of each.

1.6.2 IDH Vaccine

Currently, there are three trials under investigation for and *IDH1* peptide vaccine [259-261]. Initially, a spontaneous immune response to the *IDH* mutation has been documented [268]. The researchers used a 15 amino acid-base construct to generate

an *IDH1* peptide, with the R132H mutation and injected to mice. In animal models, it was reported that *IDH1* mutated cells could be prevented from growing in the CNS and the vaccine preserved the normal physiological function of IDH1 wildtype gene [268]. To date, the German National Cancer Centre, Duke University and the Tiantan Hospital in China initiated 3 RCTs for *IDH1* vaccines.

1.6.3 Modulating Epigenetic Alterations

As previously mentioned, the *IDH* mutation leads to histone and DNA hypermethylation patterns [223-234]. The hypermethylation phenotype might lead to neomorphic and oncogenic activities. Therefore intervening with the epigenetic changes has been postulated as a potential therapeutic option for *IDH* mutant gliomas. Flavahan and colleagues have demonstrated that glioma CpG island methylator phenotype (G-CIMP) is linked to hypermethylation at sites for cohesion and CCCTC-binding factor (CTCF), leading to the reduced affinity of this protein [249]. The CTFC reduced binding affinity allows for enhancer mediated expression of PDGFR-A. PDGFR-A is a known mitogen that has been linked to glioma-genesis [269]. By administrating a demethylating agent, the showed that the CTCF binding is partially restored and the PDGFR-A expression is reduced. The notion that by inhibiting hypermethylation, might be beneficial has also been documented by another study. Decitabine, a DNA methyltransferase inhibitor was able to suppress the proliferation both *in vitro* and *in vivo,* of *IDH* mutant glioma cells [270]. Concomitantly, with the usage of 5-azacytidine, an analogue that controls the DNA methyltransferase activity, has led to reduced proliferation of *IDH*-mutated xenograft glioma model [271].

However, epigenetic changes are only a piece of the puzzle. Combinatorial therapies might be needed to tackle the oncogenic potential induced by *IDH* mutations.

1.6.4 Inhibiting Metabolic Pathways

IDH mutated glioma cells develop new metabolic changes in response to depletion of carbohydrates from the Krebs cycle and the decrease in α-KG. Therefore understanding and delineating the new metabolic alterations, will allow us to evaluate potential druggable target as novel therapeutic options. In *IDH* mutated glioma, the *de novo* production of NAD is reduced due to epigenetic silencing of nicotinamide phosphoribosyltransferase (NAPRT1). NAD is an important co-factor, pertinent to electron transport and metabolism of redox reactions, as it is able to carry H+ ions and is derived from *de novo* and salvage pathways [272]. As a result, the only option for *IDH* mutant cells is to rely on salvage pathways to generate NAD [272,273]. It can be concluded that mutated cell can be potentially be influenced by blockage of the pathway that is the only option for NAD production. A small molecule that potentially inhibits the production of NAD is nicotine phosphoribosyltransferase (NAMPT) [274].

Moreover, glutaminolysis has been shown to be the major pathway of metabolic compensation [216] due to lack of isocitrate and thus targeting glutamine/ate metabolism might deplete the energy sources and thus inhibit major anabolic functions of the cell. For instance, bis-2-[5-9phenylacetamide)-1,3,4-thiadiazol-2y]ethyl sulfide has been shown to block glutaminase and thus blocking the glutamate metabolism and reducing proliferation and growth in *IDH* mutant AML cell [217,275]. Further, another drug called Zaprinast was able to block glutaminase and reduce the

proliferation of *IDH*-mutated cells [276]. Further, a drug called telaglenastat (CB-839) is a glutamine inhibitor that was shown to cause reduced D-2-HG production inducing glioma differentiation [277]. A phase 1 RCT is about to start recruiting, combining TMZ and radiotherapy and CB-839, a glutaminase blocker, in *IDH* mutated DAs and AAs [278]. By suppressing the glutaminolysis, tumour growth, proliferation might cease, and differentiation might take place.

1.6.5 Modulating Redox Homeostasis

ROS are predominantly elevated in *IDH* mutated tumours [235-243]. It was found that glutamine/ate and glutathione are reduced in *IDH*-mutated glioma cell compared to adjacent areas of normal tissues. D-2-HG is negatively correlated with the levels of glutathione, implying that glutathione is essential for the maintenance of redox homeostasis [279]. Increased consumption and thus, reduction of glutathione, suggests the increased burden of ROS scavenging. Therefore understanding these relationships will allow for therapies able to intervene with redox homeostasis. Limiting the ROS scavenging, which is driven by glutathione, could be an add-on therapy to the existing or under trial therapies. As previously mentioned, CB-839 can lead to blockage of glutamine metabolism and thus impaired redox homeostasis as well as sensitisation to radiotherapy [280].

1.6.6 Inhibiting DNA Repair

D-2-HG is able to compromise DNA repair mechanisms, such as inhibiting AlkB homologue 2/3 in addition to the homologous recombination DNA repair process [266,281,282]. D-2-HG is not able to compromise all repair mechanism, is thus

inhibiting some of the remaining will be detrimental. This might be a good alternative therapeutic option in addition to others as a combinatorial therapy. Several groups have shown that combining the effect of small drug inhibitors of the poly-ADP ribose polymerase (PARP) could be an important and effective strategy [283,284]. Serious DNA repair defects could be seen due to suppression of the homologous recombination pathways. Genomic integrity under stress is highly dependent on the PARP mediated BER [285,286]. Enhanced apoptotic changes can be with PARP inhibitor usage on *IDH*-mutant gliomas due to homologous recombination DNA repair [287]. In another study, it was demonstrated that by depleting NAD+, which is needed for PARP during TMZ induced BER, using GMX1778 as well as inhibiting NAMPT using FK866, eliminates the PARP remaining of repair activity [273,288]. This induces a specific metabolic stress response to TMZ-induced DNA damage and improves the duration of therapy response. *IDH*-mutated gliomas develop unique DNA repair mechanism compared to *IDH* wild-type cells. For instance, RAD51 recombinase is involved in the homologous recombination and protects TMZ induced DNA alterations [289]. Nunez and colleague, have shown that by depleting the TP53 and/or ATRX in *IDH* mutated cells, the DNA undergoes damage, highlighted by upregulating ATM signalling and resistance to radiotherapy [290]. It can be concluded that genomic instability and glioma metabolism is interrelated and thus offer a unique area to explore therapeutic strategies.

1.7 Conclusion

The substantial progress made in delineating the neurobiology of glioma formation has allowed for the new genetic and histopathological glioma classification, which

profoundly revolutionised the diagnostic accuracy of the disease. Despite the significant translation of the scientific knowledge to clinical care, the gliomas continue to be mostly incurable. GSCs play a crucial role in tumour recurrence. They are able to release key pro-angiogenic mediators and induce blood vessel formation. Further, they can downregulate the expression of pro-inflammatory mediators and suppress cell induce cytotoxicity and antibody production. Their properties allow for new tumour to be formed. TMZ is the gold-standard chemotherapeutic agent for GB. However, adequacy of its bioavailability within a tumour is debatable. GSCs are not only able to survive but can resist the action of TMZ. Firstly, they can increase the expression of ABC transporters, effectively pumping out TMZ of the cells. Secondly, they show increase expression of MGMT, an enzyme which can efficiently correct TMZ-methylation induced changes to the DNA. Thirdly, they demonstrated an alteration of MMR mechanism allowing for the accumulation of TMZ-induced DNA changes without arresting the cell cycle. However, for the DNA changes that are detrimental to the cell, they exhibit an increase in ChK1 and Chk2 kinase expression, and thus an enhanced ability to handle pertinent DNA chemotherapeutic-induced damage.

The discovery of the *IDH* mutation not only adds to the landscape of glioma genetics but also allows us to understand its role in oncogenesis and tumour recurrence deeply as well as to develop rational therapeutic approaches to intervene with the intracellular genetic, epigenetic and metabolic changes. *IDH* mutations are able to inhibit the differentiation of GSC, upregulated the tumour micro-environment and enable tumour invasion and propagation. Further, the *IDH*-related biochemical changes allow for compensatory metabolic and redox alterations. These changes potentially contribute

to tumour formation. However, more research is needed to establish the precise pathways in which these changes lead to tumour genesis. Nevertheless, *IDH* is an important target for the treatment of gliomas. The upcoming years, clinical trials conducted evaluating new therapies are likely to be increased. *IDH* vaccine trials are now underway, and it remains to be established whether these attempts are going to yield desirable outcomes. Furthermore, exploiting the metabolic and redox imbalance as a result of the *IDH* mutation is another therapeutic option. Without further randomised studies, based on current knowledge, we will never be able to provide hope to glioma patients. The scientific community will continue to explore new avenues in pursuit of improving patient outcomes.

Chapter 2

CRITICAL APPRAISAL OF GENE TRANSFER TECHNOLOGIES

2.1 Introduction

Since 2004 when the human genome was sequenced [291], a number of new genes have been identified, playing a role in disease progression and maintenance. In 2013, the somatic genomic landscape of gliomas had been defined [110]. From this point onwards, the study of genes identified became a global effort. Techniques are allowing the study novel genes were instrumental in delineating the functional role in tumour progression, invasion, evolution and reoccurrence. To do so, manipulating genes and by either deleting some or inserting others was a common practice. However, the means to transfer genes became available, only a couple of decades ago [292]. Herpes and adenoviral gene transfer techniques were proven effective in transducing neurons, which were subsequently used to study the function of genes in animal systems [292]. Thereafter, scientists have begun to develop a number of ways optimising the technologies and thus the vectors for gene transfer. The gold standard gene transfer vector should have specific characteristics such as i) being able to transfer genes in infant and adult animal, ii) having high efficiency in transducing, iii) enabling high expression but also a long-term feeling of genes, iv) causing minimal or no toxicity, v) not generating an elevated immune response, vi) allowing for long DNA to be inserted and thus incorporated to the host genome so that the transgene of interest to be accommodated and vii) mediating regulated expression. Nevertheless, these characteristics are optimal and thus not common to find in single gene transfer vector system. Therefore a plethora of delivery systems have been developed with their own advantages and disadvantages. These can be broadly classified into viral and non-viral methods.

2.2 Non-Viral Methods for Gene Transfer

Non-viral technologies consist of physical and chemical means to transfer genes, such as liposomes and polymers, encapsulate the genetic information, thereafter incorporating it to the host cell. The efficiency of this vector is much less than viral methods. However, they are much more cost-effective, readily available, and their crucial characteristic is that they do not induce an immune response. Moreover, they usually allow for a large DNA to be incorporated and delivered [293,294]. Physical methods can be applied both *in vivo* and *in vitro*. The permit for cell membrane contact and immediately penetrating through, via mechanical, electrical, hydrodynamic, ultrasonic or laser-based energy providing a force for the DNA to enter the cell.

2.2.1 Gene Gun

Naked DNA particle can be bombarded by a gene gun whereby is pushed through and injected into the cell. In order for the DNA to be heavy and hard enough, gold or tungsten particles (1-3μm) are coating the DNA particle, and then with the aid of a pressurised gas allowing for acceleration in a very high speed, the particle can penetrate through the target tissue [295]. This technique is a similar concept with the “biolistic” technique, which was initially developed for plant transgenesis [296].

2.2.2 Electroporation

Electroporation is a method whereby two pair of electrodes are inserted onto the cell membrane, passing on electricity and as such destabilising the lipid membrane. DNA is found in particles in a media surrounding the tissue. Once the membrane is destabilised, DNA is able to penetrate into the cytoplasm [297,298]. However, this

method is highly inefficient, with only 0.01% of the DNA able to be integrated into the host genome [299]. This method has been predominantly used *in vivo,* for muscle, lung and skin tissue as well as tumour treatment [300-303]. Significant drawbacks of this method also include controlling the voltage not to damage the tissue as well as the genomic DNA stability [304].

2.2.3 Hydrodynamic

A highly efficient and straightforward method is hydrodynamic delivery. This method allows for water-soluble molecules to be directly delivered to the cell and particles to organs [305]. It is predominantly used *in vivo,* with higher efficiency than alternative non-viral techniques. This gene transfer system has demonstrated high efficiency for rodent liver gene transfer allowing the expression of inflammatory factors [306], erythropoietin [307] and growth factors in mice [308] but it has never been used in humans yet.

2.2.4 Ultrasound

Ultrasound energy is utilised to make nonameric pores through the cell membrane allowing for and facilitating intercellular gene delivery in cells. The size of the pores formed is usually limited; thus, the size of the plasmid used to deliver the DNA should also be limited [309,310]. A significant drawback includes low in vivo efficiency.

2.2.5 Magnetofection

Magnetofection is a relatively new method that combines the advantages of the biochemical non-viral techniques (cationic lipids) and physical transfection methods

(electroporation, gene gun) with high efficiency, in a single system. In addition, the drawbacks of low efficiency and toxicity are very much minimised. In this technique, there is the usage of magnetic fields allow of a concentrate bulk of magnetic particles encapsulating or attached to the nucleic acid to be landed on cell membranes of target cells [311,312]. The magnetic field allows for a force to be generated, which is exerted on the membrane so that 100% of the cells can attach on the membrane of the cells. Magnetofection can be used for all types of nucleic acids such as DNA, RNA and mRNA. It has been used for a range of cell lines and primary cells [313,314].

2.2.6 Cationic Liposomes

Cationic liposomes are a prevalent and commercially available non-viral gene transfer method whereby negatively charged nucleic acids form nanomeric complexes. The formation of cationic liposomes allows for the incorporation of both hydrophilic and hydrophobic molecules, minimal activation of the immune system and negligible toxicity as well as targeted delivery [315-317]. However, there are two significant disadvantages that need to be considered when using such a system. The liposomes can be easily degraded via the reticuloendothelial system, thus preventing the prolonged sustained delivery. Nevertheless, the modification of the liposomes using polyethene glycol (PEG) on the surface and the integration of pre-encapsulated liposomes withing polymer-based systems have entertained the major drawbacks [318]. The liposomes consist of fatty acids and alkyl moieties, which can be 12-18 carbons long, with a positively charged polar head group. Since 1987 [319], when the first cationic lipid was synthesised, a plethora of new micelle systems have emerged to be used for gene transfer. To date, the efficiency of the system depends on the

specific structure, the size and the charge ratio between the DNA and liposome. Cationic systems allow for membrane fusion between lipoplexes and endosomal membrane, allowing for high gene expression [320,321]. This technique is ideal for *in vivo* when the cationic liposome/nucleic acid ratio is >1 [322-323] and in vitro, when it is closer to 1[324-327]. Liposomes have been used for gene transfer in a variety of cells (lung, skeletal, spleen, kidney) [328-335]. The liposome-based technologies continue to improve from the first generation vesicles to stealth and targeted liposomes [336,337].

2.3 Viral Methods

Viruses have been evolving since their existence. Independent of their family or order, their strategies to evade cells have been fine-tuned over the millennia. Their innate ability to efficiently do so and express genetic information into the host cell has been exploited by scientists over the decades. The viruses used for gene deliver are replication-defective vectors [338] most likely derived from wildtype viruses, in which the genes of interest are inserted replacing the genes needed for the replication. This prevents the cytotoxic effects of aberrant exploitation of the host genome. The lytic cycle genes can be replaced by trans-acting factors through specific cell line production or via the aid of helped virus in the manufacturing process [339-341]. The majority of viral vectors now used for targeting cells of the CNS have been readily derived from retroviruses, adeno and adeno-associated viruses and rarely now herpes simplex viruses [342-344]. These vectors have many differences with regards to their cell tropism, payload capacity and efficiency that can inevitably affect the duration of

the transgene expression. In addition, each virus has advantages and disadvantages if to be used for gene therapy and CNS applications.

2.3.1 Retroviral/Lentiviral Vectors

The *retroviridae* family consists of small RNA viruses that commonly replicate through a DNA intermediate. Gamma-Retrovirus and lentivirus belong to the aforementioned family [345]. Retroviral vectors are one of the most common viral method utilised for gene delivery in somatic and germline therapy. Due to the fact that retroviruses are able to linearly integrate into the host genome as well as not being very efficient in infecting non-dividing cells and thus difficult to reach high viral titers, are readily used for ex vivo delivery of somatic cells [346]. An example can be their usage in human gene therapy for the x-linked recessive single-gender disorder [347-352]. Retrovirus is able to transfect dividing cells because they are able to enter the cells by passing through the nuclear pores, making them good candidates for in situ treatment [353,354]. In addition, it is worth mentioning that if all viral genes are removed, there is 8kb space for transgenic incorporation. Notable limitations of retroviruses included low *in vivo* efficiency, immunogenic stimulation and inability to transduce non-dividing cells and risk of insertion, which might lead to oncogene activation or tumour suppressor inactivation [347-352].

Lentiviruses, however, are very good at infecting both proliferating and quiescence cells ensuing long-term stable expression of the transgene without immune reaction [355-358]. Lentiviral vectors can also deliver 8kb of sequence. Due to the fact that lentivirus has a strong tropism for neural stem cells, they have been commonly used

for the ex vivo gene delivery in the CNS with minimal side effects. Examples include animal models of a neurological disorder such as Parkinson's, Alzheimer's and Huntington's disease as well as spinal injury models [359-364]. High infection efficiency though comes with the drawback of insertional mutagenesis. Nevertheless, gene editing has allowed developing safe lentiviruses [365]. To prevent the potential of recombinant virus fenestration and thus increase the safety, the viral genome can be split in multiple plasmids [366]. Additionally, the envelope glycoproteins can be pseudo-typed to direct the viral load to other targets [367,368].

2.3.2 Adenoviral Vectors

Adenoviruses are DNA viruses that are double-stranded encoding for 30-40 genes, and their size varies between 35-40kb. Around 57 adenoviruses are able to infect humans. They are classified into seven groups from A to G based on their differences in cellular tropism [369,370]. Group C virus [371] and types 2 and 5 are the ones, which are commonly used for gene delivery and therapy [372]. Adenoviruses are used as vectors, able to deliver large DNA particles, up to 38kb, transducing efficiently non-dividing and dividing cells with minimal risk of integration in the host cell genome [370]. Adenoviral vectors have been readily used for the study of tumours [373,374] as well as developing preclinical rodent models of neurodegenerative disorders [375,376]. Their main disadvantages are the short-term, transient gene expression [377], high immunogenicity and significant cytotoxicity [378]. However, the new generation of viruses is able to overcome some of these limitations [379].

2.3.3 Adeno-Associated Viral Vectors

Adeno-associated viruses belong to the Parvoviridae family number. They are relatively small single-stranded DNA viruses that are non-enveloped. Despite their small size, they are very promising vectors for gene transfer therapies for the CNS. Their main advantages include being safe and highly efficient in transducing both non-dividing and dividing cells while maintaining long-term gene expression [380]. There are more than 150 clinical trials showing that the adeno-associated viruses have a good safety profile and clinical benefit for gene therapies [381]. Their genome has only three genes: one for replication, assembly and capsid, needed for viral replication, integration and packaging [382,383]. To date, nearly 12 different viruses have been isolated [384], and each is having unique features. These include differences in cellular tropism based on their unique surface proteins [385-387], differences in transduction efficiency and differences in the immunogenicity [388,389]. A number of serotypes are able to transduce neurones glial cells with high efficiency [390,391]. The viruses have been significantly refined, enabling increased uptake via mixing genome and serial genotypes, insertion of capsid proteins and peptide motifs from phage libraries and enrichment of capsids through the incorporation of peptide motifs [392,393]. A major limitation is their small sizes genome not allowing to accommodate more than 5kb of DNA [394,395]. However, a plethora of strategies are under development to enable incorporation of larger genes such as using truncated genes and promoters that retain the necessary protein expression of the full-length counterpart [396,397]. Another strategy is to take advantage of the ability of the virus to undergo intermolecular recombination that can lead to head-to-tail DNA concatamerisation [398]. In this way, the DNA sequence needed can be split and

package in two or three vectors [399-401]. Thereafter, upon transduction, multiple vectors can give rise to DNA concatemers, that are able to form the whole cassette [401]. As expected, the efficiency of this strategy is compromised [402]. The therapeutic potential of adeno-associated viral gene therapy has been tested in a variety of neurological disease [403-408].

2.3.4 Herpes Simplex Viral Vectors

Herpes viral vectors are enveloped double-stranded DNA viruses. They are characterised by the short replication cycle and their innate ability to infect the CNS and via retrograde axonal transport to attach the sensory neurones, establishing life-long latency [409]. To date, there are three main forms of herpes viral vectors developed: i) replication-competent, ii) replication-defective and iii) amplicon vectors [410]. The replication-competent viral vectors are predominantly used in oncology since they are able to complete lytic cycles in the presence of permissive environments [411]. On the other hand, replication-defective viruses are used for gene delivery in the CNS [412]. Herpes virus encodes for approximately 80 genes (152kb). Regards to replication-defective viruses, half are usually removed and thus accommodate around 50kb of foreign DNA, whereas amplicon vectors almost all genes (150kb) can be removed [413]. Herpes viruses are very infective [414]. However, major limitations include toxicity [415] and short term expression of transgene due to silencing mechanisms [416].

2.5 Conclusion

The last three decades a number of non-viral and viral gene transfer systems have been developed, all of them having their unique advantages and disadvantages. To date, no perfect delivery system is yet to be designed for gene therapy and/or gene delivery for in vivo and in vitro applications. The process of developing optimal non-viral vectors is still at its infancy and will most likely continue to evolve rapidly. In general, the key steps in developing effective gene transfer methods include the following: i) improving extracellular targeting, ii) improving intracellular delivery, iii) enable long-term gene expression, iv) reduce cytotoxicity and v) minimise immunogenicity. However, a key lesson learned throughout the last decade is that not a single delivery tool is able to adapt to all applications, but rather each should be chosen based on the specific situation's needs and requirements.

Chapter 3

THE IMPACT OF COVID-19 ON NEUROSURGICAL ONCOLOGY PATHWAY IN THE WEST MIDLANDS UK: A COMPARATIVE STUDY OF NEUROSURGICAL OUTCOMES AND CLINICAL DECISION

3.1 Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) pandemic brings new challenges to the management of neuro-oncology patients. The rapid increase of confirmed SARS-COV-2 test positive cases in the UK [417,418], resulted in lockdown measures being introduced on 23/03/2020. In response to the anticipated surge of SARS-COV-2 positive patients [419] requiring early intubation and respiratory support, hospitals augmented their critical care capacity, including converting operating theatres/recovery areas into overflow critical care units. To support the high volume of intubated patients, specialist health care professionals, including neurosurgeons, were redeployed to support critical care units. As per Public Health England (PHE) advice, healthcare workers who were vulnerable were required to shield at home, and those with suspected SARS-COV-2 symptoms required self-isolating for 14 days [420]. These changes led to a profound decrease in the availability of specialist services and healthcare professionals. Consequently, cancer care was limited to clinical emergencies only, throughout the whole clinical pathway. In the face of the anticipated decrease in availability of neurosurgical specialty services, we hypothesised that the number of patients referred for a specialist review to the neuro-oncology multidisciplinary meeting (MDT or tumour board) would change. Accordingly evaluating the impact of SARS-COV-2 measures on neuro-oncology referral patterns and clinical management would provide more robust evidence describing management changes and clinical complications. These data will allow clinicians, hospital managers and policy makers to objectively anticipate the clinical demand during phase 2 recovery from SARS-COV-2 and facilitate future planning.

In order to aid MDT decision making and stratify patients with brain tumours requiring surgery during the SARS-COV-2 pandemic the Society of British Neurological Surgeons (SBNS), British Neuro-oncology Society (BNOS), the National Institute of Health and Care Excellence (NICE) and NHS England (NHS) released new guidance [421-424]. The objective was to identify high-priority patients and facilitate optimal, timely active treatment including prioritization of patients undergoing systemic cancer therapy and radiotherapy [421]. As per the NHS coronavirus action plan all cancer patients should expect to receive the best possible clinical care [425]. For brain cancer patients this includes maximal surgical resection followed by radiotherapy and chemotherapy. However, a number of patients may not have been offered the standard of care previously provided. Clinical care could be compromised by pressures to delay or reduce the duration of operations, lack of support from allied health professionals such as speech therapists or neurophysiologists, restricted access to surgical adjuncts and reduced radiotherapy and chemotherapy resources.

The West Midlands region of the UK has a population of 5.7 million [426] and experienced one of the highest mortalities from SARS-COV-2 in the UK with age standardised mortality of 92.6 per 100,000 population compared to 81.9 per 100,000 for England as a whole [427]. Brain tumour patients within this region are served by three neurosurgical oncology centres that were differently affected by SARS-COV-2. These three centres experienced an estimated mortality of 102 (C_{HIGH}), 54 (C_{MED}) and 27 (C_{LOW}) deaths per 100,000 population respectively[1]. From 23/03/20 onwards

[1] *Estimated unadjusted morbidity for each centre was calculated up to 6/6/2020 using published NHS trust specific COVID-19 death rates (https://www.england.nhs.uk/statistics/statistical-work-areas/covid-19-daily-deaths/) normalised by the corresponding trust population footprint (https://www.england.nhs.uk/digitaltechnology/connecteddigitalsystems/digital-roadmaps/footprints/) accessed 24/6/20*

(complete lockdown), all three centres adopted the SARS-COV-2 pandemic neuro-oncology NICE and BNOS guidelines [421-424].

Here we report the impact of the SARS-COV-2 pandemic on: i) Changes in referral patterns and clinical workload in specialist care pathways for brain cancer patients, ii) Surgical safety, management and quality of surgical outcomes, iii) Changes in clinical oncology management. We report the initial impact of reconfiguration of health care delivery pathways in response to the SARS-COV-2 pandemic. We reveal how the health care restrictions due to the pandemic have impacted on clinical management of brain cancer patients and pave the way for future long term outcome studies. We hope that our data will inform clinical policy making and guideline formation for future SARS-COV-2 surges or new pandemics.

3.2 Methods

3.2.1 Study Design

A West Midlands UK ambi-spective study was conducted in three parts. All datasets were collected from the three neurosurgical centres (C_{HIGH}, C_{MED}, C_{LOW}) across the West Midlands region. In Part 1, we collected data reporting the number of neuro-oncology MDT referrals and surgical operations. In Part 2, we collected data reporting surgical outcomes, morbidity and 30-day mortality. In the datasets from both Part 1 and Part 2, comparison was made between a one-month baseline period before the pandemic lockdown (Period 1, retrospective data collection 03/02 – 28/02/2020) and one-month period during the complete pandemic lockdown (Period 2, prospective data collection 01/04 – 28/04/2020). In Part 3, we prospectively recorded changes in MDT decision making and oncology management over a 7-week period (23/03 - 08/05/20) during period 2, complete lockdown, at the height of the UK pandemic.

3.2.2 Patient Identification

For Part 1 of the study, we included any patient referred to and discussed at the neuro-oncology MDT and any adult patient who underwent surgical resection with subsequent diagnosis of a tumour of the CNS. For Part 2 of the study, we included adult patients who underwent surgical resection, with a histological diagnosis of a malignant or benign tumour of primary neuroepithelial tissue, or the meninges. In addition primary CNS lymphoma and metastatic brain tumour were included. Tumours of the sellar region were excluded. For Part 3 of the study, we included all newly diagnosed brain tumour patients or new recurrence/progression from previously

diagnosed brain tumours that were discussed in the MDT together with all patients seen in the neuro-oncology clinic with a histological diagnosis of a brain tumour.

3.2.3 Data Collection

Data were collected from hospital electronic MDT records, clinic letters, operative and imaging notes. A trainee was responsible for the initial data collection at each centre. Prospective MDT data required members of the authorship team to be present at every MDT in each centre to capture data. Consultants were able to verify information case-by-case to ascertain accuracy of data collection. For Part 2 of the study, pre-operative, operative and post-operative/follow-up data were collected for both time periods. Pre-operative data included, gender and age as well as functional status (WHO 0-4). Operative data included duration of the operation. Postoperative data and follow up data collected included: i) post-operative deficit and complications, ii) resection rate, gross total resection (GTR) or subtotal resection (STR), iii) length of stay in hospital, iv) 30-day readmission, v) complications, vi) histological diagnosis and vii) 30-day mortality. GTR was defined as greater than 90% resection of contrast enhancing disease seen on the post-operative MRI scan obtained within 72 hours of operation as per NICE guidelines [428]. For Part 3 of the study, baseline demographics, radiological, histopathological and molecular diagnosis were documented.

3.2.4 Statistical Analysis

Continuous variables were reported using medians and interquartile ranges due to the non-normality of the data and categorical variables were reported as numbers and percentages. Tables and bar charts were generated to display relevant data.

Analysis and plots were generated using Excel sheets and SPSS (IBM) version 26. Data distributions were assessed visually using a histogram. The Sankey plot was created in R studio (Version 1.3.959) using the *riverplot* and *Rcolorbrewer* packages.

3.2.5 Ethical Approval and Consent

This study has been registered as a qualitative improvement study/audit under the local Research and Audit department and thus no ethical approval was needed. No patient identifiable factors were shared amongst centres. No consent was required for this manuscript.

3.3 Results

3.3.1 SARS-COV-2 in the West Midlands

A comparison between the 3 neurosurgical oncology centres serving the West Midlands is summarised in **Table 3.1**. Briefly, the centre with the highest number of SARS-CoV-2 patients admitted to hospital (C_{HIGH}) reported 3251 cases with a mortality of 102/100,000 compared to 694 cases and a mortality of 57/100,000 in C_{MED} and 964 cases and a mortality of 27/100,000 for the centre with the lowest number of SARS-CoV-2 patient deaths (C_{LOW}). Referral patterns into specialist care pathways at the height of the pandemic in April 2020 were reduced in each centre with the greatest impact in the centre with the highest impact from SARS-CoV-2 infections (**Table 3.1**).

3.3.2 Neurosurgical Workload during Phase 1 of COVID

Across all three centres, 183 patients were referred to the neuro-oncology MDT in period 1 (before lock down; February), compared to 111 in period 2 (complete lockdown April), with a 39.3% overall decrease, (**Table 3.1**). Comparison between period 1 and 2 showed that $C_{HIGH,}$ C_{MED} and C_{LOW} had a 48.3%, 23.1% and 40.5% decrease in the number of referrals respectively, with C_{HIGH} facing the largest reduction (**Figure 3.1**). Regarding the neurosurgical oncology procedures: 56 were conducted in period 1 (February) and 36 in period 2 (April) across all three centres, representing a 35.7% reduction overall, (**Table 3.2a, 3.2b**). C_{HIGH} and C_{LOW} showed large reductions in the number of neurosurgical operations between February and April, 37.0% and 52.9% respectively, whereas for C_{MED} only a small reduction was recorded, 8.3% (**Figure 3.2**).

		Neurosurgical Centre			All centres
		C_{HIGH}	C_{MED}	C_{LOW}	
Cumulative SARS-CoV-2 hospital inpatients during complete lockdown (23/03/2020-06/06/2020)		3251	678	964	4909
SARS-CoV-2 related NHS Trust inpatient mortality per 100 000 population		102	54	27	
Period 1: Before Lockdown (February 2020)	**MDT referrals (Average per week)**	89 (22.3)	52 (13)	42 (10.5)	183 (45.8)
Period 2: Complete Lockdown (April 2020)	**MDT referrals (Average per week)**	46 (11.5)	40 (10)	25 (6.3)	111 (27.8)
Percentage change in MDT referrals (%)		↓48.3	↓23.1	↓40.5	↓39.3

Table 3.1: Demographic data for three West Midland centres with SARS-CoV-2 related high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of mortality and impact on neuro-oncology MDT referrals.

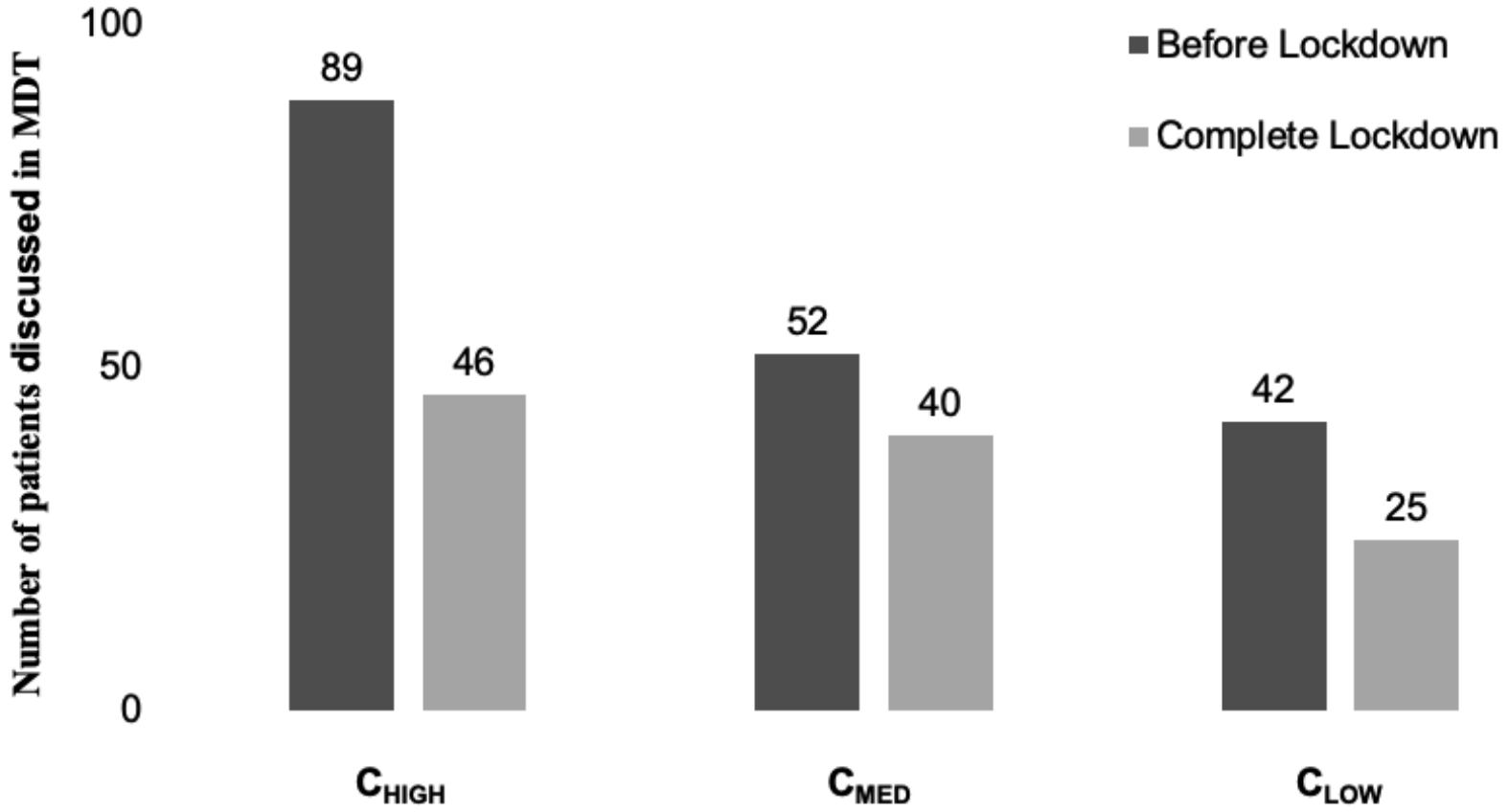

Figure 3.1: Number of new patients discussed in neuro-oncology MDT Before Lockdown (February 2020; dark grey) versus Complete Lockdown (April 2020; light grey), in three West Midland centres with SARS-CoV-2 related high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of mortality.

			Neurosurgical Centre			All centres
			C_{HIGH}	C_{MED}	C_{LOW}	
Period 1: Before Lockdown (February 2020)	Neurosurgical procedures		27	12	17	56
	Median age in years (IQR)		54.0 (9.0)	51.5 (13.8)	60.0 (10.0)	55 (12.0)
	Male : Female percentage ratio		63:37	50:50	65:35	63:37
	Operative time (%)	1-2 hours	7.4	33.3	5.9	12.5
		2-3 hours	63	66.7	82.4	69.6
		3-4 hours	29.6	0	12.8	17.9
		4-5 hours	0	0	0	0
		>5 hours	0	0	0	0
	Gross total resection achieved/eligible for (%)		14/17 (82.3%)	6/6 (100%)	8/13 (61.5%)	28/33 (77.8%)
	Median length of stay in days(IQR)		2 (1-14)	7.5 (2-14)	3 (1-14)	2.5 (1-14)
	Complications		Number and Type		Management/Outcome	
			1 wound dehiscence (Readmitted overnight)		Re-sutured on ward, oral antibiotics,	
			1 pneumonia in patient with COPD and Non-Small Cell lung Cancer		Died within 30 days despite active management	

Table 3.2a: Demographics of patients undergoing neurosurgical oncology procedures at three West Midland centres with high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of SARS-CoV-2 related mortality

Period 2: Complete Lockdown (April 2020)	**Neurosurgical procedures**		**17**	**11**	**8**	**36**
	Median age in years (IQR)		59.0 (5.0)	60.5 (1.9)	53.5 (9.8)	59 (4.5)
	Male: Female percentage ratio		59:41	60:40	62:38	60:40
	Operative time (%)	1-2 hours	23.5	0	0	11.1
		2-3 hours	23.5	0	0	11.1
		3-4 hours	35.3	81.8	87.5	61.1
		4-5 hours	11.8	18.2	12.5	13.9
		>5 hours	5.9	0	0	2.8
	Gross total resection achieved/eligible for (%)		6/15 (40.0%)	5/8 (62.5%)	2/4 (50.0%)	13/27 (48.1%)
	Median length of stay in days (IQR)		3 (2-9)	4.5 (2-14)	2.5 (1-6)	4 (1-14)
	Complications		**Number and Type**		**Management/Outcome**	
			1 wound dehiscence (Readmitted overnight)		Resutured on ward, oral antibiotics	
			1 post-operative seizure		Anti-epileptics	
			1post-operative oedema with reduced conscious level		Decompressive craniectomy Delayed discharge but complete recovery	
Percentage change in neurosurgical procedures (%)			↓37.0%	↓8.3	↓52.9	↓35.7

Table 3.2b: Demographics of patients undergoing neurosurgical oncology procedures at three West Midland centres with high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of SARS-CoV-2 related mortality

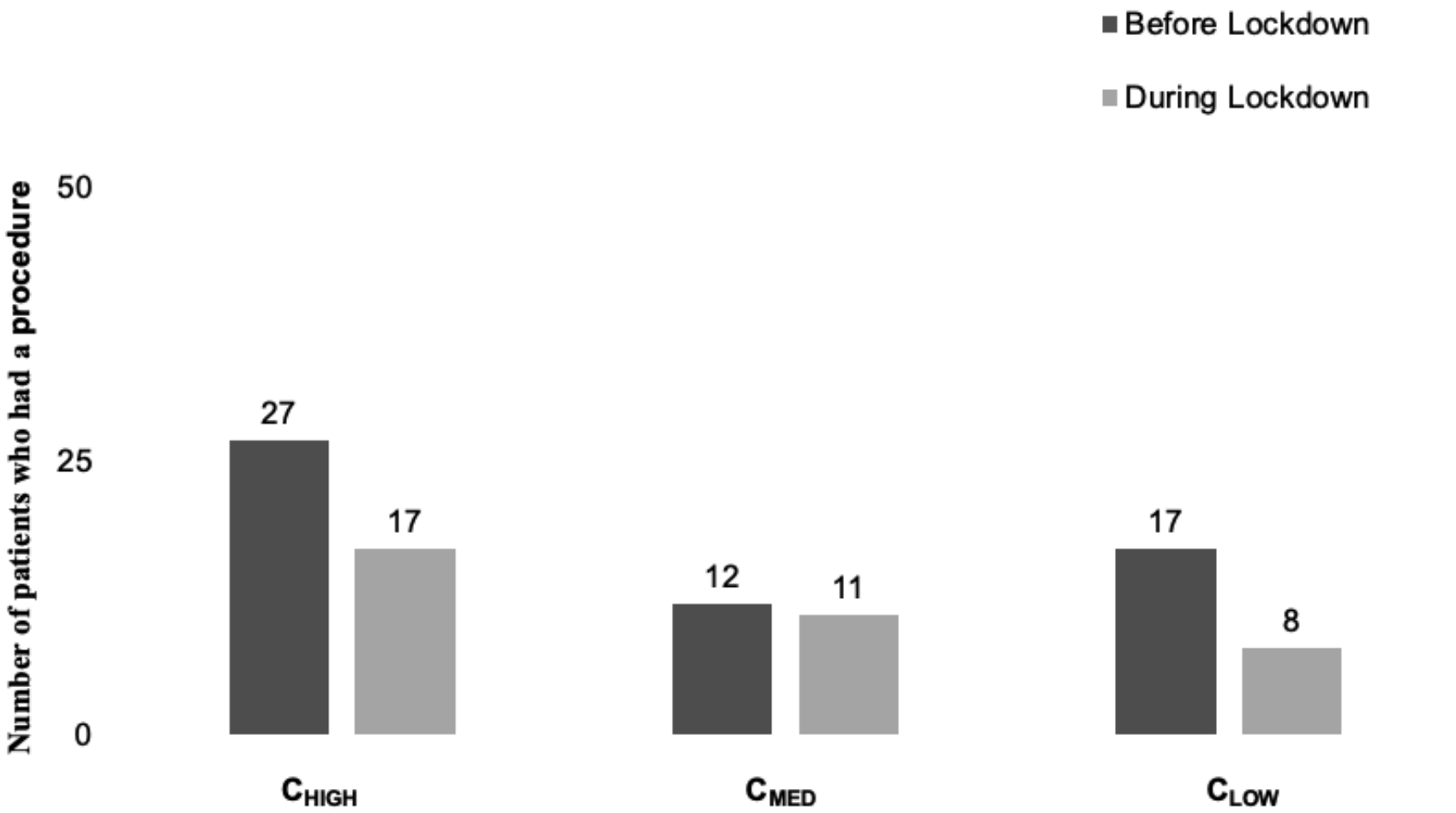

Figure 3.2: Number of patients who had a neurosurgical oncology procedure Before Lockdown (February 2020; dark grey) versus during complete lockdown (April 2020; light grey), in three West Midland centres with SARS-CoV-2 related high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of mortality.

3.3.3 Surgical Management during Phase 1 of COVID

Across centres, demographics of patients who had surgery in period 1 (February) versus period 2 (April), were fairly similar in terms of age and gender (**Table 3.2a, 3.2b**). Operations during the complete lockdown (April) lasted longer than in February. (**Table 3.2a, 3.2b**, **Figure 3.3**). 77.8% of operations lasted longer than 3 hours in April, versus 17.9% in February. The number of patients with GTR in February was higher (77.8%) compared to April (48.1%), (**Table 3.2a, 3.2b**). We found limited differences with regards to length of stay in hospital (**Figure 3.4**) or histological diagnosis. Across all centres during both period 1 and period 2, high-grade glioma was the most common histological diagnosis, with metastatic tumour being second most common. Across centres, two recognized post-operative complications occurred in February (2.9%): one patient with non-COVID pneumonia who died within 30 days and one patient with wound dehiscence. Three recognized post-operative complications occurred in April (8.3%): one wound dehiscence, one post-operative seizure and one with post-operative oedema with reduced consciousness that required a decompressive craniectomy. Regarding 30-day readmissions: one patient was readmitted in February and one in April (with a wound dehiscence). No patients died within 30 days of surgery that took place in Period 2 (April). No patients in these cohorts were SARS-COV-2 positive at the time of operation and no patients were readmitted within thirty days of surgery with SARS-COV-2 infections.

Regarding differences between centres: C_{HIGH} recorded the largest reduction in the number of patients with a GTR (42.3%) in April compared to February, whereas C_{MED} and C_{LOW} recorded 37.5% and 11.5%, respectively, (**Table 3.2a, 3.2b**). C_{HIGH} was the

only centre that demonstrated an increase in median length of post-operative stay in hospital, 2 days in February versus 3 days in April (**Table 3.2**). A low number of post-operative complications, 30-day readmissions and 30-day morality rates was seen across centres.

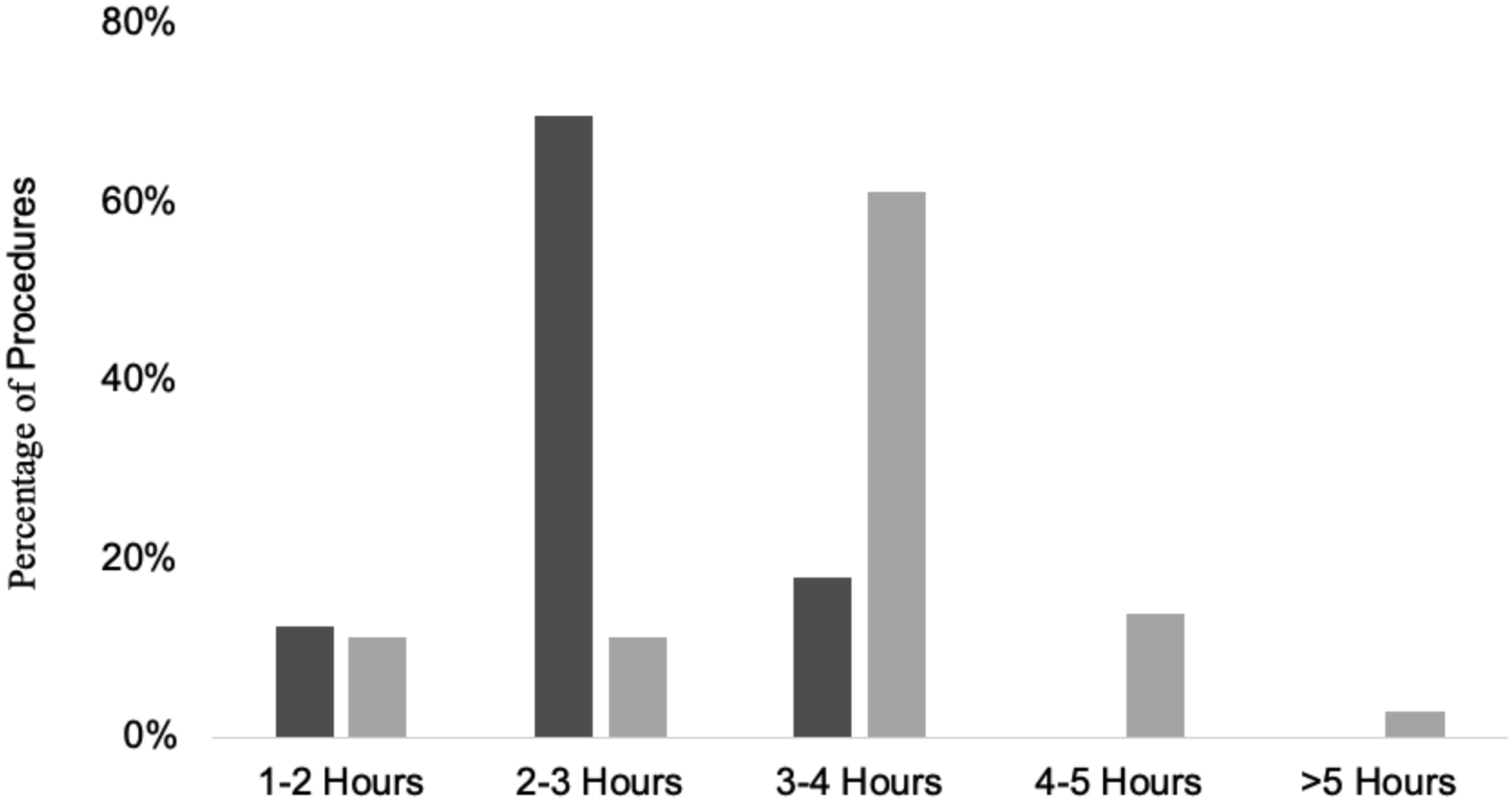

Figure 3.3: Duration of neurosurgical oncology operations in hours Before SARS-CoV-2 Lockdown (dark grey; February) versus Complete Lockdown (light grey; April).

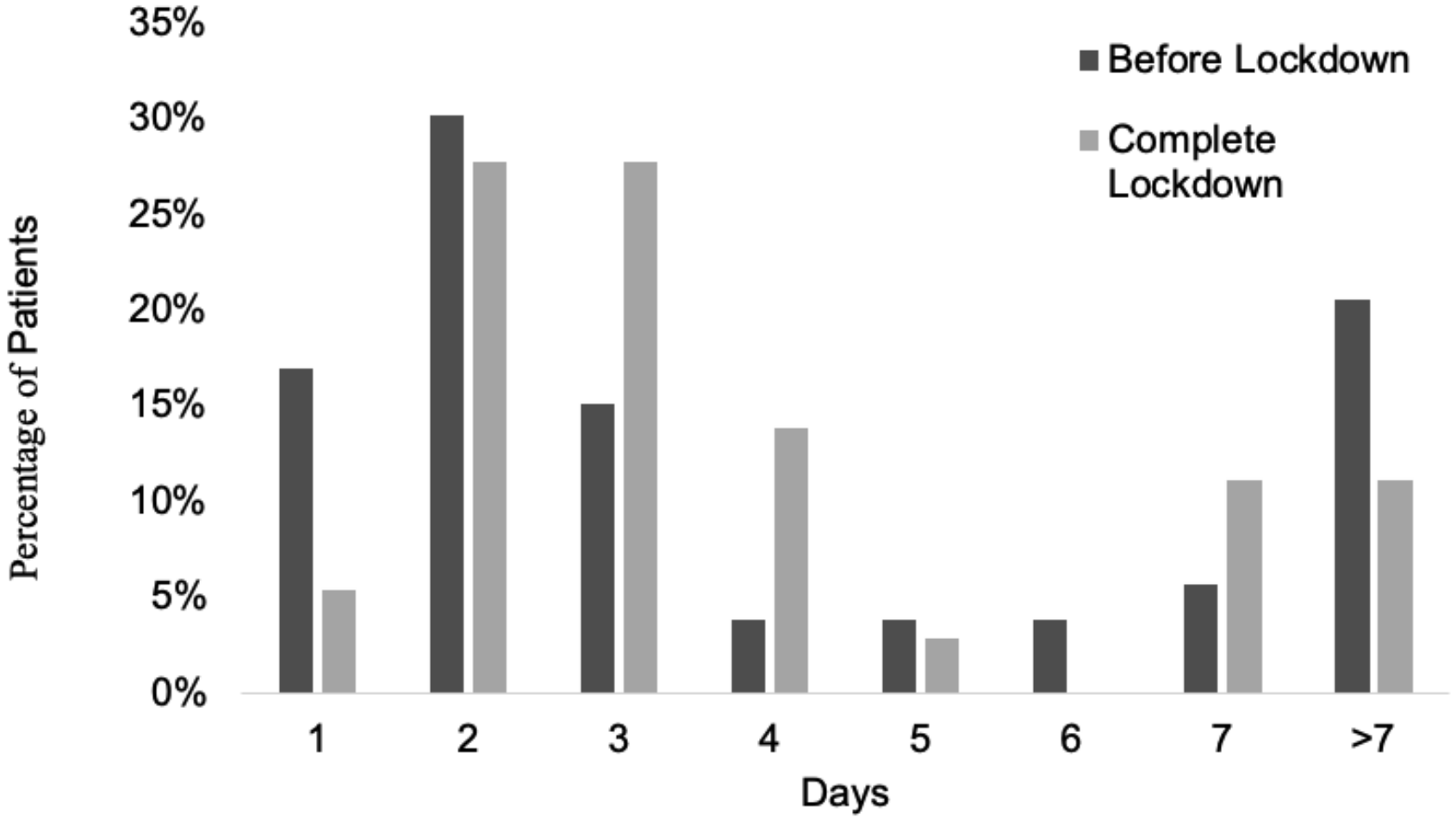

Figure 3.4: Length of stay (days) of patients undergoing operation for brain tumour Before SARS-CoV-2 Lockdown (February, dark grey) and during Complete Lockdown (April, light grey).

3.3.4 MDT Decisions and Management during Phase 1 of COVID

Across the centres over the seven weeks from 23/03/2020 (complete lockdown) 152 new patients were discussed at the neurosurgical oncology MDT, of which 23 (15.1%) had a change in MDT management, (**Table 3.3**). The MDT decision before and during complete lockdown due to the SARS-CoV-2 pandemic are depicted in the Sankey diagram in **Figure 3.5**. Surgery was the most common management plan before lockdown (n=56, 36.8%), and represented the largest proportion of patients who experienced change in clinical management due to SARS-CoV-2. Out of 56 patients considered for surgery (biopsy n=13, resection n=43), 19 (33.9%) were offered an alternative including radiotherapy or stereotactic radiosurgery (SRS; n=12, 21.4%), chemotherapy (n=1, 1.8%) or interval monitoring (n=1). Out of 6 patients considered for chemotherapy before SARS-CoV-2, 50% were offered an alternative including best supportive care (BSC, n=2, 33.3%) or radiotherapy (n=1, 16.6%), (**Table 3.4)**. Age and gender appeared similar for those who had a change in MDT management, compared to those who didn't. Of the patients impacted in this way, 19 (83%) had cerebral metastatic disease or suspected HGG. The commonest change in management 52.2% (n=12) was to offer radiotherapy (RT) or stereotactic radiosurgery (SRS) without a histological diagnosis (**Table 3.4**). A further 21.7% (n=5), were offered best supportive case (BSC) instead of any other 'active' treatment, (**Table 3.4**). These changes in management, away from standard of care, were predominantly in C_{HIGH} where 18.6% patients in C_{HIGH} (n=18) were affected compared to 8.3% in C_{MED} (n = 2) and 9.7% in C_{LOW} (n=3) (**Table 3.3**).

		Neurosurgical Centre			All centres
		C_{HIGH}	C_{MED}	C_{LOW}	
MDT outcomes during Complete Lockdown (23/03-08/05/2020)	MDT referrals total (Average per week)	97 (13.9)	24 (3.4)	31 (4.4)	152 (21.7)
	Median age (Years ±IQR)	65 (±9.5)	67.5 (±7.4)	64 (±10.5)	65.5 (±10.0)
	Male : Female percentage ratio	54:46	54:46	52:48	53:47
	Change in MDT decision N (%)	18 (18.6%)	2 (8.3%)	3 (9.7%)	23 (15.1%)
Oncology clinic outcomes during Period 2 Complete Lockdown (23/03-08/05/20)	Number of patients (Average per week)	104 (14.9)	47 (6.7)	36 (5.1)	187 (26.7)
	Median age (Years ±IQR)	58 (±10.5)	46 (±11.5)	56 (±8.5)	56 (±11)
	Male : Female percentage ratio	43:57	45:55	55:45	46:54
	Change in oncology decision N (%)	27 (26.0%)	3 (6.4%)	26 (72.2%)	56 (30.0%)

Table 3.3: Change in MDT and oncology management decisions during complete lockdown of SARS-CoV-2 pandemic procedures at three West Midland centres with high (C_{HIGH}), medium (C_{MED}) and low (C_{LOW}) magnitude of SARS-CoV-2 related mortality.

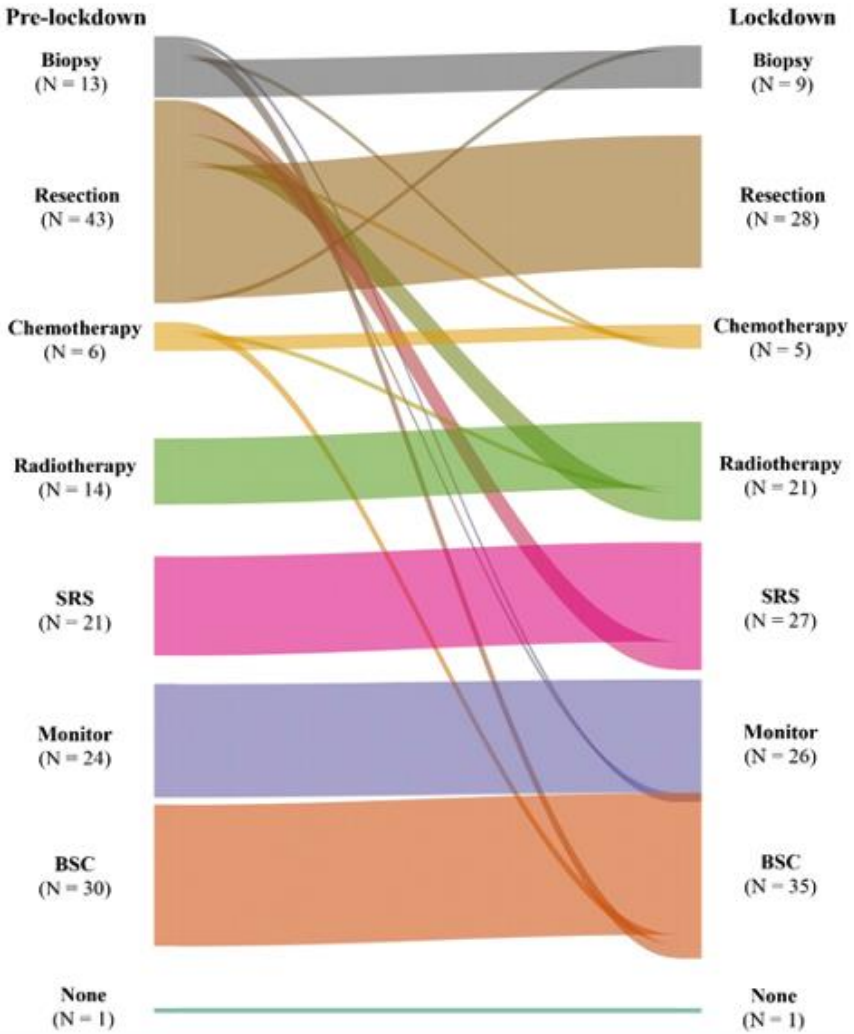

Figure 3.5: Sankey diagram of change in management at neuro-oncology MDT during lockdown due to SARS-CoV-2 pandemic. SRS = Stereotactic radiosurgery, BSC = Best Supportive Care, None = No intervention required

MDT Decision Before SARS-CoV-2 lockdown	MDT Decision During SARS-CoV-2 complete lockdown	Number of Patients	Presumed Radiological Diagnosis of Tumour			
			HGG (%)	LGG (%)	Metastasis (%)	PCNL (%)
Resection	SRS/RT	12	5		7	
	Biopsy	1	1			
	Chemotherapy	1			1	
	Interval Monitoring	1		1		
Biopsy	Chemotherapy	1				1
	BSC	3	2			1
	Interval Monitoring	1		1		
Chemotherapy	RT	1	1			
	BSC	2	1		1	
Total number patients (%)		23 (100%)	10 (43.5)	2 (8.7)	9 (39.1)	2 (8.7)

Table 3.4: Change in neuro-oncology MDT clinical management correlated with presumed radiological diagnosis before and during complete lockdown of SARS-CoV-2 pandemic, out of a total of 152 patients discussed at MDT.

3.3.5 Oncology Management during Phase 1 of COVID

187 patients were seen by a clinical oncologist over a 7-week period during complete lockdown (23/03 - 08/05/20), with 56/137 (30%) having a change of management following consideration of UK SARS-COV-2 guidelines (**Table 3.5**). C_{LOW} (72.2%) had the largest change in oncology management followed by C_{HIGH} (26.0%) and C_{MED} (6.4%). There was no difference in the baseline demographics of age, gender and histological diagnosis between patients who did and did not have a change in clinical management between centres. Patients with a diagnosis of GB were more likely to have treatment changed (**Figure 3.5**), which usually involved postponing alkylating chemotherapy, 36/56 patients (63.2%; **Table 3.5**). MGMT promotor methylation status was not available to inform clinicians when these decisions were made at the post-op MDT meetings or in clinic. Interestingly, clinical decision making was impacted by the IDH status. Patients with less aggressive isocitrate dehydrogenase mutant (IDH mutant) tumours were more likely to have chemotherapy postponed or stopped or radiation dose reduced compared to IDH wild type tumours (**Figure 3.7**). This was particularly noticeable for oligodendroglia tumours. One patient became infected with SARS-CoV-2 during chemotherapy for a primary CNS lymphoma (PCNSL). No other infections during chemotherapy were reported

Change in oncology management during SARS-CoV-2	Patient number	Histological Tumour diagnosis		
		HGG	LGG	Other
Temozolomide Postponed	36	33	1	2
PCV Stopped/Postponed	10	1	8	1
Radiotherapy Dose or Duration Reduced	8	8		
Lomustine/Carboplatin Stopped	2	1	1	
Total patient number	56	43	10	3

Table 3.5: Tumour diagnosis of patients who had a change in oncology treatment during Complete Lockdown of SARS-CoV-2 pandemic out of a total of 187 patients reviewed in neuro-oncology clinics. HGG= High grade glioma, LGG=low grade glioma.

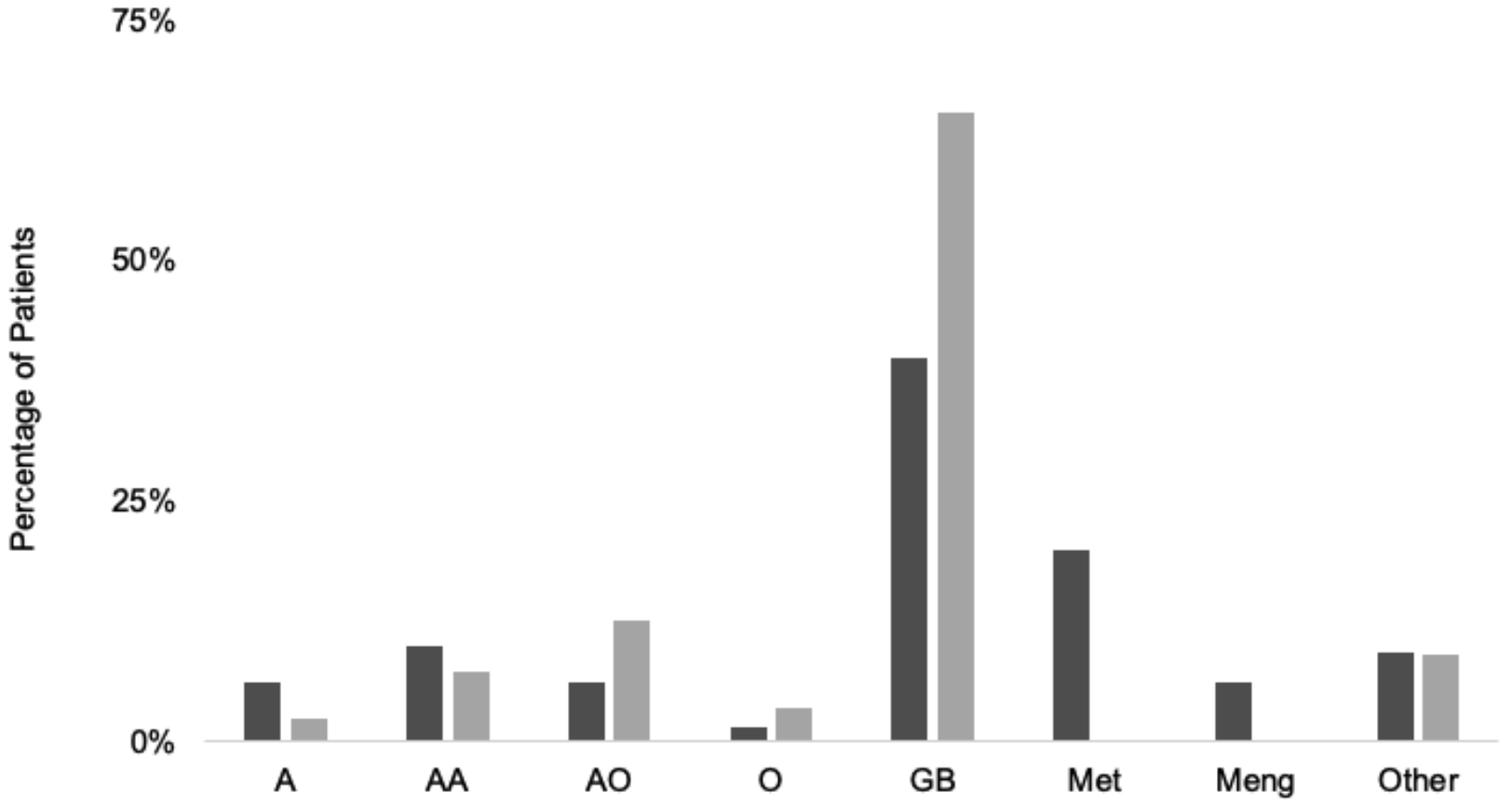

Figure 3.6: Brain tumour tissue diagnosis of patients who had no change (dark grey) versus change (light grey) of oncology treatment plan at oncology clinic during the seven weeks of Complete Lockdown for the SARS-CoV-2 pandemic. (A: Astrocytoma WHO II; AA: Anaplastic Astrocytoma WHO III; O: Oligodendroglioma; AO: Anaplastic Oligodendroglioma WHO III; GB: Glioblastoma WHO IV; Meng: Meningioma WHO I, Met: Metastasis; Other: other brain tumour).

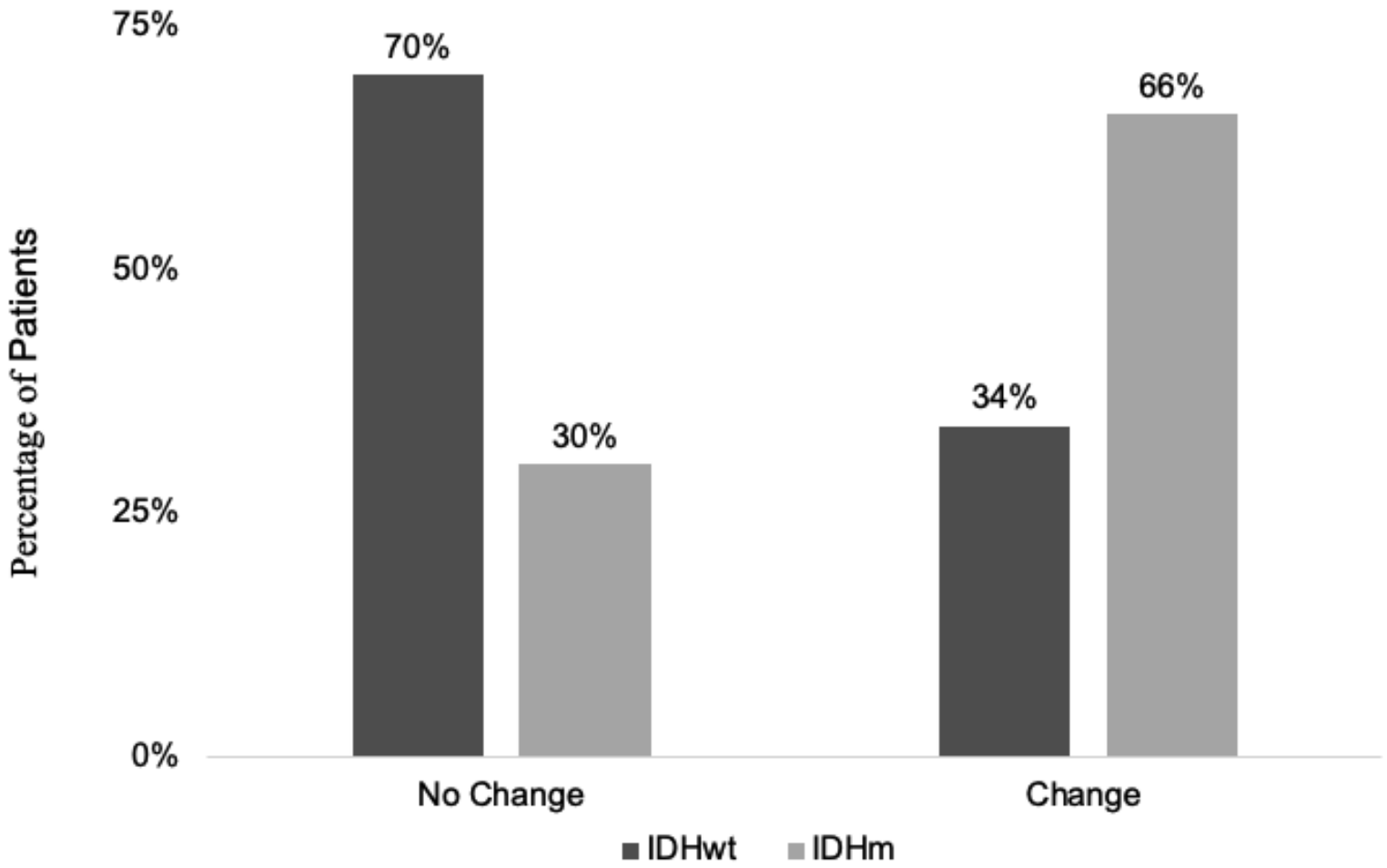

Figure 3.7: Comparing neuro-oncology patients who had no change versus change in their oncology management when seen in clinic during SARS-CoV-2 pandemic (complete Lockdown) and were molecularly diagnosed with Isocitrate Dehydrogenase wild type (IDHwt) or mutation (IDHm).

3.3.6 Illustrative Case-Study of Patient Impacted by SARS-COV-2

A 57 year old patient was referred to the specialist neuro-oncology MDT, just prior to the implementation of period 2 SARS-COV-2 (complete lockdown). MRI demonstrated a right periventricular contrast enhancing lesion and following clinical review the patient was recommended for a burr-hole biopsy for tissue diagnosis. However, due to SARS-COV-2 guidelines the management plan was altered. The patient was presumptively treated with one cycle of chemotherapy for suspected PCNSL based on radiological evaluation and discussion at the neuro-oncology MDT. The patient failed to respond to chemotherapy and deteriorated with neutropenic sepsis and a seizure. Repeat imaging revealed disease progression in keeping with a glioblastoma. Following an image guided stereotactic biopsy the patient was formally diagnosed with a glioblastoma (WHO IV astrocytoma, IDH wild type, MGMT promotor unmethylated). The patient had a fifty-day delay between initial presentation and formal histopathological diagnosis. This was compounded by inappropriate chemotherapy and treatment-related neutropenia. Only after formal tissue diagnosis was the patient able to receive appropriate chemoradiotherapy.

3.4 Discussion

Globally, the UK is one of the most heavily affected by the SARS-COV-2 pandemic [429]. Reports are emerging on how routine clinical care has been affected in different disciplines [430-432]. However, a comparison of the management of brain cancer patients before and during the pandemic has yet to be described. Here we report the impact on patients with brain cancer of NHS service delivery reconfiguration to manage SARS-CoV-2 infections for an ethnically diverse population of 5.7 million people. We compare three cancer centres managing brain tumour patients that report significantly different SARS-COV-2 mortality including one centre managing one of the highest mortality rates in Western Europe.

3.4.1 Specialist referral and diagnosis

Our study confirms that the SARS-COV-2 related changes in healthcare provision such as the decrease in available manpower, the redeployment of staff and the conversion of operating theatres to intensive care units led to a significant decline of nearly 40% in referrals into the specialist care pathways and a reduction of surgical workload by over 30%. Our findings are consistent with Cancer Research UK (CRUK) estimates of 75% reduction of urgent cancer referrals was seen the first 4-weeks post-lockdown with a subsequent 50% reduction in weeks 4 to 10 of the lockdown and an estimated 60% reduction in cancer surgery [433]. A national report from the Netherlands reported up to 26% reduction in cancer diagnosis [434] and a global, large-scale study estimated that elective cancer surgery cancellation rates were between 35.3% to 39.6%, with 2.3 million elective cancer cases cancelled worldwide [435]. Two international surveys compiling data from 96 and 60 countries respectively,

reported an up to 57.5% and 52.5% cancellation of neurosurgical operation and clinics across the globe [436,437]. The significant delays of cancer surgery are likely to impact on survival. A 3-month delay in surgery across all Stage 1-3 cancers is predicted to result in over 4,700 attributable deaths per year in England [438].

We postulate that in response to the "*Stay at home, Stay safe, Protect the NHS*" [439] strategy patients did not want go to hospital due to the perceived risk of developing corona virus infections. Our data suggests that this is unlikely even in centres managing very high SARS-CoV-2 caseloads.

The reduction of referrals and operative cases has the additional potential to impact on neurosurgical training [440-443] as well as having major financial burden due to the surgical backlog in the upcoming months [444]. Our study is the first to document the decrease in diagnostic workload for neuro-oncology services using real-time evidence. Regional variation in the impact of SARS-CoV-2 on diagnostic surgery for brain cancer patients has implications for future network-based strategies to mitigate the reduction in access to resources in future SARS-CoV-2 surges. There is growing concern amongst clinicians that in the coming weeks and months there will be an increase in late presentation of brain cancer patients who may no longer be eligible for treatment. A flexible strategy of referring patients from regions of high impact to regions of lower impact will improve opportunities for all cancer patients to receive the best possible clinical care [425].

3.4.2 Delivering standard of care for patients

During period 2, SARS-CoV-2 complete lockdown, regional brain cancer care shifted to an emergency-based pathway in each centre in accordance with national guidelines [421-424]. By comparing the clinical management of patients during period 1, before lockdown with period 2, complete lockdown we were able to examine the effect these changes had on clinical management. These data may inform future planning and update clinical guidelines.

Despite loss of specialist clinical care pathways surgical complications, 30-day readmission rates and 30-day mortality rates were not impacted confirming that the safety of surgery was maintained. The perceived increased risk of SARS-COV-2 infection among patients going to hospital for diagnostic surgery was not substantiated. All patients were SARS-COV-2 negative at the time of surgery and no patient returned with a SARS-COV-2 infection within 30 days of surgery. Diagnostic surgery for brain cancer patients is safe and fear of SARS-CoV-2 infection should not deter patients from going to hospital for help.

Our data confirmed that operations lasted longer and the number of GTR achieved was reduced in the emergency-based system compared to a normal urgent elective standard of care pathway. The benefits of elective care pathways for brain cancer patients are well established [445] and the unit that had the largest SARS-COV-2 caseload, C_{HIGH}, suffered the greatest impact on surgical services. The changes were not due to variation in age, gender or radiological diagnosis. Possible contributing factors to longer operating times may include the impact on the theatre team of

wearing full PPE and increased complexity of some of the urgent cases that were prioritised.

Complete surgical resection of brain cancers including HGG and cerebral metastasis is associated with improved overall and progression-free survival, [446-448] and SARS-COV-2 related consensus management guidance, suggested that patients should still be offered GTR when deemed feasible [449]. The reduction in GTR rates seen during the pandemic is likely to be multifactorial including: 1) cases being managed on an emergency list with inherent difficulty utilizing surgical adjuncts, 2) difficulty providing 5-ALA 2-4 hours pre-operatively, 3) limited access to neurophysiological monitoring and awake craniotomy with speech mapping and 4) some operations being performed by consultant surgeons not in the oncology core team. It has been suggested that awake surgery and extended operation times may lead to more complications and extend the post-operative hospital stay in SARS-CoV-2 hot environments [450]. However, our comparative data before & during SARS-CoV-2 does not show any differences in complication rates or 30-day mortality.

Our data reveal changes to standard of surgical care in approximately 15% of patients, with those with high grade gliomas or metastasis being more adversely affected. Whilst age was a well-recognised risk for fatal SARS-COV-2 infection [451,452], we observed no age related differences in referral patterns or MDT planning, suggesting this was not a relevant factor in neuro-oncology SARS-COV-2 decision making.
The centre with highest SARS-COV-2 caseload and morbidity reported the greatest number of changes from normal standard of care. The most important prognostic

factor, especially for HGG is surgical resection [446] and surgery plus RT has superior prognostic outcomes than RT alone for brain metastasis [447]. Despite this, approximately three quarters of patients who had a change in management were not offered surgery but rather another form of active treatment typically RT/SRS without a tissue diagnosis. We report a clinical case-study illustrating the profound dangers of this approach.

Similarly, there was a change in oncology standard of care management in 30% of patients, with HGG patients again the most adversely affected. Typical changes involved postponement in their chemotherapy due to concerns over pancytopenia and increased susceptibility to corona virus infection. These data are consistent with a study of 800 cancer patients from 55 cancer centres in the UK showing that 22% of patients had a change of oncology management [453]. In our study, a high proportion of patients with IDH-1 mutations received radiotherapy alone with any chemotherapy being deferred. The rationale was based on the better prognosis of IDH mutant tumours and the possible increased risk of SARS-CoV-2 infection if there was myelosuppression. A shift toward surveillance may be acceptable for low-grade brain tumours in the short term. However, moves away from standard of care for aggressive brain tumours (defined by NICE guidance NG99 [428]) should be avoided whenever possible [425]. Importantly, faster access to molecular diagnostic stratification tools for brain tumour patients will aid critical clinical decision making in a timely manner at the MDT meetings in future surges.

Overall these data suggest that SARS-COV-2 related changes in resources and care pathways have impacted the quality but not the safety of clinical management of brain cancer patients. Importantly, the correct usage of PPE and early adoption of hospital protocols provided high levels of protection for both patients and healthcare professionals. Follow-up studies will be needed to evaluate the long term impact of these changes in clinical management. Despite the ethical challenges of clinical decision making during the pandemic, our results demonstrate that oncologists effectively used the existing evidence and guidelines to manage changes in oncology management

3.4.3 Limitations of this study

Our scientific approach had to be developed rapidly in the face of the evolving SARS-COV-2 pandemic. We recognize the inherent limitations of retrospective data and a small sample size. Nevertheless, these data provide an initial “snapshot” of the impact of SARS-COV-2 on the management of brain cancer patients in the UK that has the potential to inform future guidelines and strategies to mitigate the impact on brain cancer patients of future surges and new pandemics. For patients undergoing a change in management further follow-up studies are required to understand if their treatment during SARS-COV-2 will impact on their overall survival.

3.5 Conclusion

Here we report the impact on patients with brain cancer of NHS service delivery reconfiguration to manage the SARS-CoV-2 pandemic. We compared three cancer centres managing brain tumour patients for a population of 5.7M that reported

significantly different SARS-COV-2-related mortality. We compared period 1 before the pandemic lockdown (February 2020) with a period at the height of the SARS-CoV-2 complete lockdown in the UK (April 2020). Our data show that referral of brain cancer patients into specialist care pathways fell by 40% leading to reductions in diagnostic surgery of over 30% as a result of SARS-COV-2 related changes in healthcare provision and help-seeking behaviour. Surgical safety was not impacted by high levels of SARS-CoV-2 infections, but the quality of surgical results was reduced by the shift to emergency-based care leading to increased incidence of residual disease post-operatively and longer operation times. Clinical decision making and management after diagnosis was compromised with patients suffering from the most aggressive cancers being most severely affected by reduced access to chemotherapy. In the future we recommend that accelerated access to molecular diagnostics will refine clinical decision making and help balance risk/benefit evaluations for individual patients. Regional networking could provide a flexible strategy of referring patients from regions of high SARS-CoV-2 to regions of lower SARS-CoV-2 incidence and improve opportunities for all cancer patients to receive the best possible clinical care.

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