Investigating the effect of radiotherapy to the
SubVentricular Zone (SVZ) in high-grade glioma
patients

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Abstract

Glioblastoma multiforme (GBM) is the most aggressive type of malignant brain tumour. Despite treatments including surgery, chemotherapy and radiotherapy, GBM recurrence rates are high and patient prognosis remains poor. The SubVentricular Zone (SVZ) may have a role in tumour recurrence as it is theorised to provide a sanctuary or 'niche' for migrating glioma stem cells, whose escape and survival from therapeutic intervention are thought to drive future disease relapse. On this basis, inclusion of the SVZ within the radiotherapy target volume has been proposed by several authors, however there is conflicting current evidence on the benefits of SVZ irradiation and the subject remains controversial. Amidst calls for randomised prospective clinical trials on SVZ radiotherapy, the potential role of the SVZ in GBM still requires some clarification.

This thesis describes the investigation into the effect of radiotherapy to the SVZ for patients in the author's centre. A retrospective study of 57 patients examined the prognostic impact on patient Overall Survival of the mean incidental dose delivered to the SVZ during radiotherapy, analysed alongside other prognostic covariables such as age and tumour genetic factors. SVZ dose appeared to have no significant impact on survival as only increasing patient age, unmethylated O^6-methylguanine-DNA-methyltransferase (MGMT) gene status and absence of chemotherapy proved to be detrimental to Overall Survival in this cohort. A subsequent radiological study further categorised patients using a novel classification methodology based on tumour proximity to and invasion of the SVZ, finding also that this had no significant impact on Overall Survival. This study also investigated inter-observer variability when contouring the SVZ and the considerable discordance seen in the results highlights the importance of clear delineation protocols, though reported dosimetric findings were not affected.

Though the results of this investigation are currently inconclusive on the potential role of SVZ radiotherapy in improving survival in GBM, a potential area of renewed research focus has been identified through the novel classification methodology introduced in this work. Future studies should perhaps aim to investigate the potential role of radiotherapy in improving survival for a subset of patients with SVZ-invasive tumours, where complete surgical resection is often not possible.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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1) MSci Mathematical Physics, University of Nottingham.
   Dissertation Title: 'Spin Diffusion during hyperpolarisation'
2) MSc Medical Physics, University of Surrey
   Dissertation Title: 'A comparison of linear accelerator-based treatment and planning systems for the radiosurgery of metastatic brain lesions'

All the work performed in this investigation was performed by the author, with three exceptions that are further described in the text but also listed here:

(i)   The obtaining of information related to MGMT-methylation status, surgical extent, details of chemotherapy treatment and patient Performance Status was performed by Oncology Speciality Registrar Dr Najah Nizam.

(ii) The radiological classification of tumours based on their anatomical location within the brain, was performed by an experienced Consultant Radiologist Dr Amish Lakhani.

(iii) The contouring of the SubVentricular Zone that are termed contours by 'Observer B' throughout the text, was performed by an experienced Consultant Clinical Oncologist Dr Anup Vinayan.

The author has written all text in this thesis.

Foreword

Twenty-five years ago, my grandfather was diagnosed with advanced bowel cancer and my mother's explanation to me at the time was simply "it's cancer, so we've been told he may live for a year but don't be too hopeful". Since being told of my grandfather's prognosis as a child, I've always been intrigued to find out more about this disease: to understand what it is, why people with a cancer diagnosis can have such a poor prognosis and what can be done to improve this. This interest in oncology is one of the fundamental reasons for my chosen career as a clinical scientist in radiotherapy and though oncology in general remains a broad area of interest for me, brain tumours in particular have always held a certain curiosity. I again recall as a child watching a distressing episode of one of my favourite shows in the 1990s (Byker Grove) where a major teenage character collapsed in her kitchen and eventually died of a brain tumour. Once again, when I asked my mother what a brain tumour was, her answer that it was a terminal condition that unfortunately couldn't really be cured sparked an interest in me that lasts to this day. Brain tumours involve arguably the most complex organ of the human body and to the average lay person, the term 'brain tumour' represents a condition with a terminal outcome. Given the opportunity to perform research as part of the HSST programme, a project involving brain tumours was my favoured option and I hope that this project undertaken on a consultant clinical scientist training scheme is able to provide a small contribution to this area of research.

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1. Introduction

1.1. Prologue

"I got into a taxi, but I couldn't speak. I had two powerful seizures. I was taken to hospital. Two days later I was told I had a brain tumour, a glioblastoma multiforme"

[Dame Tessa Jowell, House of Lords, 2018. Quoted in *The Guardian* by Sparrow (2018)]

These compelling, poignant words were spoken by a frail-looking Dame Tessa Jowell to a packed House of Lords on 25th January 2018 as she described the dramatic events that led to her brain tumour diagnosis. There followed a four-minute speech to a captivated audience in which she described her diagnosis and explained how her disease was unfortunately very difficult to treat. With her terminal prognosis and very little that could be done for her, she set out her vision for greater research collaboration to improve our understanding of this disease and to seek improved treatments to reduce the number of patients dying from glioblastoma multiforme. When she had finished, such was the impact and power of her words, that the Chamber rose to give her an unprecedented standing ovation. Five months later Dame Tessa Jowell sadly passed away, almost precisely a year after her diagnosis (Elgot, 2018).

1.2. Glioblastoma Multiforme

Glioblastoma Multiforme (GBM) is a type of glioma, a form of brain cancer that accounts for 80% of malignant primary brain tumours (Aparicio-Blanco and Torres-Suarez, 2017, p.66). Gliomas originate from glial cells in the brain and are classified by the World Health Organisation (WHO) in to four grades of ascending malignancy (Hamerlik, 2014, p.3). A grade IV glioma is termed a GBM and their categorisation as the highest grade indicates that GBMs are the most aggressive form. This aggressive nature is characterised by widespread invasion of surrounding tissue, a great potential for rapid proliferation and a high chance of recurrence following treatment. Given these features and as sadly reflected in Dame Tessa Jowell's case, an ominous prognosis often accompanies a GBM diagnosis for a patient. Such a diagnosis can be emotionally shattering to hear as a patient and many newly confirmed GBM patients experience psychological distress as a result (Boele *et al.*, 2015). As one senior neurosurgeon remarked to a junior doctor after diagnosing a patient with the disease in clinic:

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"It's a dreadful disease, it doesn't respond to any chemotherapy, resection never cures it and I'm not sure radiotherapy makes all that much difference, basically it's a death sentence"

(Jackson, 2011).

This quote is so powerful as it reflects not only the poor prognosis of patients with GBM but also the relative absence of truly effective treatment options. Despite available combinations of the mainstays of cancer treatment (surgery, chemotherapy and radiotherapy), outcomes remain poor and there is clearly much work to be done improve this current state of affairs.

1.3. Research Motivation

One in two people in the UK born after 1960 are now expected to be diagnosed with some form of cancer in their lifetime (Cancer Research UK, 2021a). Whilst prostate, breast, lung and bowel are the most common forms of cancer diagnosed in the UK, brain and other intracranial tumours remain relatively uncommon by comparison and account for approximately 3% of all new cancer cases as illustrated in figure 1.1. Despite their rare occurrence, there were 12,100 new cases of brain tumours in the UK each year between 2015 and 2017 and incidence rates are projected to rise by 6% by 2035 (Cancer Research UK, 2021b). Though relatively rare in incidence compared to other cancers, the significantly poorer mortality rates of brain tumours are reflected in a five-year survival rate of only 12%, a figure that has seen little improvement over the course of a decade (Cancer Research UK, 2021b).
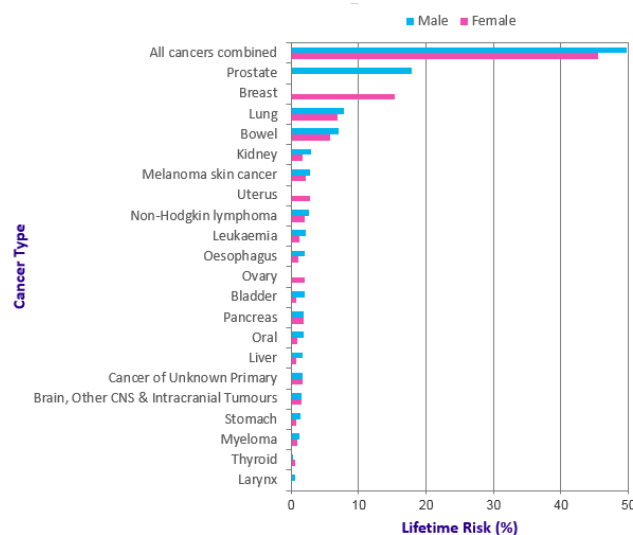
Figure 1.1. Lifetime risk of cancer categorised by cancer type, showing relatively low percentage risk of brain tumours (fifth row from the bottom) versus other types (Source: Cancer Research UK, 2021a, with copyright permission).

There are many underlying and complex reasons for the discrepancy in mortality rates between different tumour types. Whilst brain tumour mortality continues to be high, by contrast testicular cancer mortality rates have fallen by 84% since the 1970s (Cancer Research UK, 2021c) with the improved survival data for these tumours being attributed to the development of improved and standardised chemotherapy regimens through dedicated research (Shanmugalingam *et al.*, 2013). The case study of testicular cancer is not unique and it is evident that improvements in mortality rates can only be achieved by committing resources to dedicated research programmes.

Investment in cancer research varies across the different tumour types and despite the variations in incidence and mortality, a recent systematic analysis revealed that the amount of investment made into cancer research for each site is not proportional to the relative burden of each disease (Marupthappu *et al.*, 2017). Examining this analysis reveals that brain tumours rank amongst the lowest in terms of funding per disease burden. It was this apparent shortfall in brain tumour research investment that was so strongly emphasised by Dame Tessa Jowell in her landmark House of Lords speech, where she outlined how brain tumour research was lagging behind the advances made in other more common forms of cancer and identified that mortality rates remained high. This claim is further supported by a report from the Brain Tumour Research charity which identified that only 1% of the national spending on cancer research was allocated to brain tumour research (Brain Tumour Research, 2016).

In light of Dame Jowell's impassioned speech and the apparent shortfall in funding for brain tumour research, in the months that followed her death the UK Government pledged to double its original funding on brain tumour research to £40m over the following five years (Department of Health, 2018) with a further £25m coming from Cancer Research UK. This figure still compares unfavourably with the average annual spend of £33m on breast cancer research over the past two decades and it is estimated and that at the current rate of spending it would take 100 years for brain cancer to catch up with developments in other cancer sites (Brain Tumour Research, 2016). Two years later and the total investment into brain tumour research since the Government's pledge stood only at just over £4m to September 2020 (Argar, 2020), leaving significant room for catching up. However, national research budgets across Europe are now threatened by the economic impact of the COVID-19 pandemic (Tsagakis and Papatriantafyllou, 2020) and Cancer Research UK experienced a £45m funding cut alone in the first year of the pandemic (Cancer Research UK, 2020). The economic

impact of COVID-19 only adds to the continued challenges for brain tumour research as it competes for funding with other sites.

This research project funded through the Higher Specialist Scientific Training (HSST) scheme, presented an opportunity to make a contribution to an area of brain tumour research within the author's own area of scientific expertise, working as a clinical scientist in radiotherapy physics. As the following paragraphs will introduce, this research will investigate radiotherapy treatment for GBMs and specifically focus on an area of the brain where there is much current interest in its potential role in patient outcomes – the SubVentricular Zone (SVZ).

1.4. Introducing the Research Project: Treating GBM using radiotherapy and introducing the Sub-Ventricular Zone

Radiotherapy is a form of cancer treatment which aims to destroy malignant tumour cells using ionising radiation delivered most commonly as high-energy megavoltage photons, electrons or protons. Surgery remains the first line of treatment for GBM patients who are suitable, however complete resection of the tumour is not always possible and surgery is often limited to debulking of the tumour or in some cases only a biopsy. Radiotherapy delivered to GBM patients in a post-operative setting seeks to eradicate those remaining tumour cells not removed by surgery. The benefits of post-operative radiotherapy for GBM have been identified by many studies and the literature review in Chapter 2 of this work will provide an overview of this evidence base. Guidelines from Laperriere *et al.* (2002) recommended a total dose of 50-60Gy to a target volume comprising the enhancing tumour plus a margin (as opposed to whole brain radiotherapy), delivered as 1.8Gy-2Gy fractions and this prescribed dose range is still in use to this day. After the results of the landmark EORTC-NCIC trial (Stupp *et al.,* 2009), post-operative radiotherapy is now delivered with chemotherapy in the form of concomitant and adjuvant temozolomide for eligible patients and the standard of care for GBM treatment is now widely accepted to be the multidisciplinary triumvirate of surgery, radiotherapy and chemotherapy (Mann, 2018). Chapter 3 of this thesis will provide a detailed technical introduction to the radiotherapy treatment modality for GBM.

Technical advances in radiotherapy in the past decade have included the widespread use of Intensity-Modulated Radiotherapy (IMRT) often involving the delivery of the rotational form of IMRT - Volumetric Modulated Arc Therapy (VMAT). Greater confidence in treatment accuracy is provided by the adoption of Image-Guided Radiotherapy (IGRT) with most

modern radiotherapy treatment machines being capable of imaging patients to verify target position before delivering a radiotherapy fraction. These advances in technology permit the increasingly precise delivery of highly conformal radiation doses and improved sparing of Organs At Risk (OAR) across a range of clinical sites whilst treatment planning systems are capable of producing increasingly complex treatment plans that can be tailored to a patient's clinical situation. Furthermore, these technological innovations open the possibility for accurate targeting and dose-escalation of sub-volumes of radiotherapy targets that are identified based on their underlying biological characteristics (Corwin *et al.,* 2013). Indeed, this technique has been used to increase the dose to dominant intraprostatic lesions in prostate cancer with promising results (Feutren and Herrera, 2018). The ability to identify biological features within a tumour that may warrant variation of the prescribed radiation dose is in itself only possible through technological advances in diagnostic imaging, particularly in Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) where functional imaging can also be utilised to highlight areas of active disease pathophysiology. It seems logical therefore to consider extending the application of such techniques to the radiotherapy treatment of GBM patients to target active cellular proliferations that could be driving the disease.

One area of current interest in GBM radiotherapy is the irradiation of the SVZ, a part of the brain whose concentration of Neural Stem Cells (NSCs) is theorised to be the origin of gliomas (Sanai, Alvarez-Buylla and Berger, 2005). By targeting the SVZ as the origin of the tumour and eliminating cancer stem cells as part of the radiotherapy treatment plan, it is theorised that the chances of tumour recurrence can be reduced. This research project aims to investigate the potential notion of targeting the SVZ within a patient's radiotherapy treatment and seeks to answer the primary research question that is:

"Is there evidence that the inclusion of the ipsilateral SVZ as a target in a radiotherapy treatment plan could lead to improved survival for patients with GBM?"

In order to address this question, this work is sub-divided into several areas of research, each of which forms a separate chapter within this thesis. These topics of investigation are as follows:

1. A formal retrospective study of patients treated in the author's centre to investigate whether Overall Survival correlates with dose delivered to the ipsilateral SVZ.

2. An examination of how tumour location with respect to the SVZ impacts on Overall Survival and whether a subset of tumour types can be identified that may benefit from SVZ irradiation.

3. A study which examines the contouring accuracy when defining the ipsilateral SVZ and how this can affect reported survival outcomes.

The main body of this work describing the research methodology and results will be divided to focus on each of these areas as separate chapters. A discussion chapter will then provide unification of the key themes identified and provide a reflection on the research project with consideration given to further work to be carried out. Ahead of these sections, the two chapters that now begin the main body of this thesis will first provide a review of the literature related to this research topic. A technical background chapter then follows to provide the scientific theoretical basis to the concepts being investigated in this work.

2. Literature Review

In Chapter 1 of this thesis, the motivation and rationale for this research project was established. Shortfalls in brain tumour research were identified when compared to other cancer sites and the funding challenge as a result of the COVID-19 pandemic was also highlighted. The slim proportion of cancer research dedicated to brain tumours comes despite continued poor patient outcomes, however an area of current interest is the potential significance of the SVZ and the role of glioma stem cells. Many authors have suggested that targeting the SVZ as part of radiotherapy treatment could improve patient outcomes. In this literature review chapter, a critical examination of the scientific literature covering this subject area is provided, describing the work done to date, summarising the findings and highlighting the key conclusions and outstanding controversies.

Much of this chapter incorporates sections from the standalone literature review that was submitted for assessment as part of the C1 component of HSST in February 2019. This chapter builds on the previous review by giving further consideration and analysis to works within the scientific literature on the topic of GBMs, the SVZ and the effect of radiotherapy and will include some additional studies that have been published since the submission of the original literature review.

This review chapter starts by first describing the search strategy employed to research the existing scientific literature on the subject. The main review then begins with a context-setting examination of the clinical studies that first made steps towards improving patient outcomes for GBM: identifying that prolonged Overall Survival (OS) and Progression-Free Survival (PFS) could be achieved when GBM patients are treated with post-operative radiotherapy. The role of chemotherapy agents is introduced including temozolomide, whose role in improving outcomes was identified through a series of landmark clinical trials that identified the benefits of this chemotherapy agent and cemented the current multi-disciplinary treatment approach of surgery, radiotherapy and chemotherapy for GBM patients. As the poor prognoses of GBM patients are universally recognised across the GBM literature, attention is turned to further attempts at improving outcomes through modified radiotherapy fractionation and the use of novel radiosensitising agents. Focus then shifts to the specific area of interest for this research project as an introduction is provided to the NSC theory of glioma origins. Studies are critiqued that have investigated the significance of NSC regions in tumour recurrence patterns and survival analysis following treatment with radiotherapy, investigating

both the significance of proximity of the tumour to the SVZ and the dose received during treatment. Through a critical examination of the methodology used in such studies and by considering the controversies that remain unresolved, the aims, methodology and areas of focus for this research project are identified and explored.

2.1. Search Strategy

This chapter aims to construct a coherent narrative that supports and justifies the chosen area of research through a review of the existing scientific literature on the subject. The literature review described in this chapter does not claim to include all possible published work on the subjects in question, as a full systematic literature review on the subject of radiotherapy for GBM was beyond both the achievable timescales and requirements of this HSST-based research project. Notwithstanding the absence of a formal systematic review approach, a documentation of the search strategy employed for this literature review chapter is provided in this section for clarity. The approach suggested by Kable, Pich, and Maslin-Prothero (2012) has been used as a guide for documenting the search details which are provided in table 2.1.

Purpose Statements	*Overall: Radiotherapy dose delivered to the SVZ may have a significant effect on patient survival in GBM* *Section 2.2:* Identify literature supporting the current multi-disciplinary treatment approach for GBM. *Section 2.3:* Identify literature on the subject of Glioma origin theories, Glioma stem cells and the possible role of the SVZ in tumour recurrence. *Section 2.4:* Identify literature that correlates survival with tumour proximity to the SVZ or dose delivered to the SVZ in patients treated with radiotherapy for GBM.
Databases Searched and Dates	Google Scholar, PubMed®, Science Direct. Main literature review performed October 2018-February 2019 ahead of HSST C1 Literature Review Submission in February 2019. Follow-up literature search performed May 2020-June 2020. Final literature search performed August-September 2021.
Limits Applied	Section 2.2: English language articles published 1970-2021 (for historical review of the establishment of the multidisciplinary approach over the past 50 years).

	Other Sections: English language articles published 2001-2021 (for identifying more contemporary research reflecting modern treatment techniques).
Inclusion Criteria	Original research studies: prospective and retrospective. Published Systematic reviews of research studies on the subject.
Exclusion Criteria	Studies reporting on paediatrics or low-grade gliomas.
Search Terms Used	In all sections, both the full names 'Glioblastoma multiforme' and 'Subventricular Zone' were searched together with their respective acronyms 'GBM' and 'SVZ'. In the lists that follow, only the acronyms are included for succinctness. *Section 2.2:* 'GBM radiotherapy', 'GBM resection', 'GBM surgical resection', 'GBM chemotherapy', 'GBM temozolomide', 'GBM nitrosoureas', 'Stupp trial GBM', 'GBM adjuvant radiotherapy'. *Section 2.3:* 'SVZ stem cells', 'SVZ glioma stem cells', 'SVZ cancer stem cells', 'SVZ niche GBM', 'SVZ tumour recurrence', 'SVZ neural stem cells' *Section 2.4:* 'SVZ dose GBM survival', 'SVZ proximity GBM survival', 'SVZ contact GBM survival', 'SVZ radiotherapy GBM'.
Search Strategies Employed	Sequential searching of databases with search terms above, followed by manual searching of reference lists within individual manuscripts.

Table 2.1. Literature search details and strategy.

2.2. Establishing the Multidisciplinary Treatment Approach

As stated in the introduction to this thesis, radiotherapy has now been firmly established within a multidisciplinary approach to GBM treatment. In order to understand this and before consideration is given to the notion of potentially targeting the SVZ as a sub-volume within the radiotherapy treatment, it is logical that this literature review should begin with a historical examination of those studies that first recognised the survival benefits of post-operative radiotherapy for GBM. Studies are also reviewed which sought to optimise radiotherapy treatments by adjusting fractionation schedules and using radio-sensitisers, before consensus guidelines are established that reflect the current radiotherapy treatment protocol for GBM. Consideration is also given to those papers that recognised the additional benefits gained by supplementing post-operative radiotherapy with chemotherapy with particular attention paid to those that recognised the role of temozolomide - an alkylating chemotherapy agent that can cross the blood-brain barrier and is toxic to tumour cells due to

inhibition of DNA replication (National Institute for Health and Care Excellence, 2001, p.8.).

2.2.1. Post-Operative Radiotherapy Improves Survival

Historically, GBM treatments generally involved maximal safe surgical resection followed by administration of a nitrosourea, a form of alkylating anti-cancer drug that can cross the blood-brain barrier (Mann *et al.,* 2018). The multidisciplinary treatment approach involving radiotherapy that is now employed for patients was promoted as early as the 1970s by Bloom (1975) who hypothesised that the future of cerebral glioma treatment lay in a combined strategy of radiotherapy in conjunction with surgery and chemotherapy. Bloom's comments conclude a review of previous clinical studies that found higher recurrence rates in glioblastoma patients who had not received radiotherapy following surgical resection (Bloom, 1975). This study was amongst several others that decade including Onoyama *et al*. (1976) and Sheline (1977) who similarly identified survival benefits in patients treated post-operatively with radiotherapy.

Given the technological limitations of radiotherapy delivery at the time, many of these authors advise caution as the required radiation dose was close to the observed limits of normal brain tissue tolerance of 50-60Gy (Bloom, 1975). Compared to the Multileaf Collimators (MLCs) that are standard on modern linear accelerators (linacs) and their ability to deliver VMAT, accelerators of the 1970s lacked both the capability to deliver highly conformal precise radiation fields and the image guidance to verify them. Though delivering such high doses to a precise target area was beyond technological capabilities at the time, modern radiotherapy technology now permits such therapeutic strategies, as Chapter 3 of this work will explain.

The success of radiotherapy treatment relies on the optimisation of the therapeutic index, that is selecting a target dose based on the respective dose-response curves for the tumour (the tumour control probability, TCP) and the surrounding normal tissue (the Normal Tissue Complication Probability, NTCP). As radiation dose increases, the probability of achieving tumour control is improved, though the risk of side effects from normal tissue damage also arises as illustrated in figure 2.1. Optimising the therapeutic index – destroying tumour cells whilst minimising toxicity from damage to surrounding healthy tissue cells, can be achieved through dose fractionation, that is delivering the radiotherapy in small doses (fractions) which

allows normal tissue to repair in between treatments. A more thorough introduction to the radiobiology of radiotherapy is covered in Chapter 3 of this thesis.
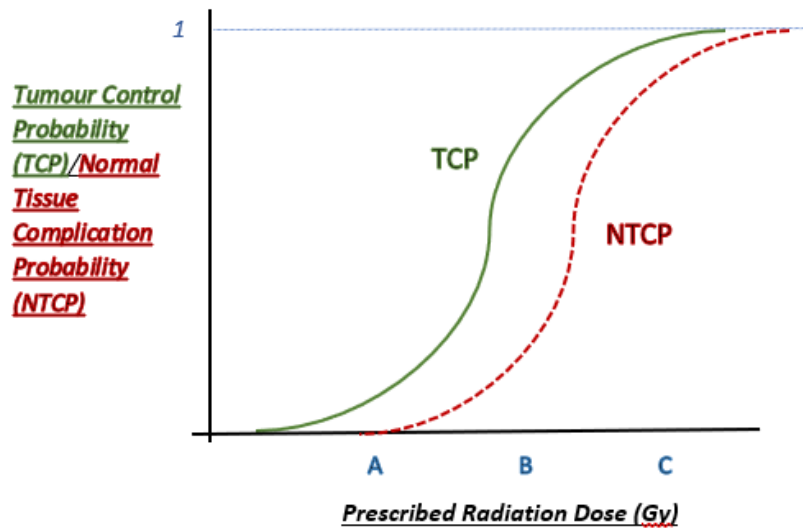
Figure 2.1. Theoretical illustration of the therapeutic index- increasing tumour control probability (TCP) and normal tissue complication probability (NTCP) from increasing radiation dose. Prescription doses at A and C provide insufficient TCP or unacceptable NTCP respectively. Prescription dose B may offer a suitable compromise.

The search for the ideal dose prescription for GBM radiotherapy was assisted by the discovery of the radiobiological dose-effect relationship for glioblastoma by Walker, Strike and Sheline (1979) who noted significantly increased survival for patients receiving higher radiation doses (median survival of 42 weeks for 60Gy versus a median survival of only 28 weeks for 50Gy and only 18 weeks for no radiotherapy) implying that the slope of the glioblastoma TCP curve perhaps lay in this dose range. The shoulder of the TCP curve appeared to be discovered later as Salazar *et al.* (1979) found that further increases in radiation dose up to 75Gy did not improve patient survival and theorised that such increased doses would lead to greater risk of brain necrosis. The importance of post-operative radiotherapy for GBM was conclusively seen at the turn of the millennium when the systematic review performed by Laperriere *et al.* (2002) detected a significant survival benefit from post-operative radiotherapy (risk ratio for death of 0.81 versus no radiotherapy) and established radiotherapy guidelines for adults with malignant glioma. These guidelines stipulate a total dose of 50-60Gy to a target volume comprising the enhancing tumour plus a margin (as opposed to whole brain radiotherapy), delivered as 1.8-2.0Gy daily fractions. Linac technology now permitted the safe delivery of such a targeted approach and this strategy continues to underpin the current radiotherapy guidelines used to this day (Royal College of Radiologists, 2019).

2.2.2. Nitrosoureas – breaking the blood-brain barrier

With post-operative radiotherapy recognised as being significant to prolonging patient survival, further research studies examined the effects of combining the post-operative radiotherapy with chemotherapy agents. Though frequently employed for other tumour sites, the presence of the blood brain barrier has long been a hindrance to the effective delivery of chemotherapy drugs for glioblastoma (Bhowmik, Khan and Ghosh, 2015). Nitrosoureas are DNA alkylating agents that can penetrate the blood brain barrier and prior to 1999 were commonly used as the first-line treatment of glioblastoma (Weller *et al.,* 2013). Walker *et al.* (1980) investigated the effect of post-operative radiotherapy with or without nitrosoureas, randomising 358 patients into one of four groups. Two groups received radiotherapy in combination with a nitrosoursea (semustine or carmustine), with a further two groups receiving either radiotherapy or semustine alone respectively. The three groups receiving radiotherapy showed a significant survival improvement with no significant differences in survival between the two nitrosourea drugs. A meta-analysis of 16 randomised clinical trials involving over 3000 patients by Fine *et al.* (1993) concluded that there was a 10% absolute increase in one-year survival for patients treated with combination radiation and chemotherapy following surgery. A more recent meta-analysis and systematic review of 12 clinical trials involving similar patient numbers by Stewart (2002) also reported an absolute survival increase at 1 year for these patients (6%). Nitrosourea agents have since become less desirable owing to their significant haematologic toxicity and the availability of more effective agents such as temozolomide (Weller *et al.,* 2013). The success of temozolomide in replacing nitrosourea agents will be described later in this chapter.

2.2.3. Refining the Approach

With strong evidence established for the importance of the multi-disciplinary approach to GBM treatment involving post-operative radiotherapy in conjunction with chemotherapy, proposed refinements of radiotherapy regimes were explored in some studies but to limited success. Combinations of nitrosoureas with both conventionally fractionated and hyperfractionated radiotherapy were the subject of a randomised trial involving 603 patients reported by Deutsch *et al.* (1989). Four treatment groups received radiotherapy with a chemotherapy agent, three using conventional fractionation and one using hyperfractionation (smaller dose fractions of <2Gy). No significant differences in Overall Survival were noted between the groups with the authors concluding that hyperfractionated radiotherapy regimes

showed no advantage over conventional fractionation. These findings were reinforced by the follow-up Phase III RTOG9006 trial which randomised 712 patients between 60Gy in 30 daily fractions or 72Gy in 60 fractions twice daily between 1990 and 1994 and no significant differences in OS, PFS or toxicity were observed (Ali *et al.,* 2018).

Improved treatment responses have also been sought through external radio-sensitising agents administered during radiotherapy, including the use of anti-hypoxia agents. A small pilot study by Van der Maazen *et al.* (1995) administered carbogen and nicotinamide during radiotherapy for 16 patients but revealed significant toxicity effects including deterioration of liver enzymes and psychiatric disorders including hallucinations and paranoia, whilst showing no benefit in survival. Pickles *et al.* (1996) and a Phase I/II trial (EORTC 22933) reported by Miralbell *et al.* (1999) found equally concerning toxicity amongst patients with reported hepatic toxicity, persistent nausea and vomiting together with an intolerance of the breathing apparatus but crucially no benefits in Overall Survival. Though Fatigante *et al.* (1997) noted a trend towards improved survival despite these toxicities that they deemed to be acceptable, the use of such radiosensitisers to treat GBMs has not seen much recent interest and studies have largely concentrated on identifying the most effective concurrent and adjuvant chemotherapy agent to be used with conventionally fractionated radiotherapy.

2.2.4. Role of Temozolomide

The Phase III EORTC 26981 clinical trial reported by Stupp *et al.* (2009) established concomitant and adjuvant temozolomide with radiotherapy as the standard of care for GBM following surgical resection. This trial followed a series of promising results from Phase I trials including Newlands *et al.* (1992) who found good toleration and ease of use amongst patients and recommended a dose of 150mgm^{-2} administered orally for five days. Further encouraging results from Phase II trials such as Bower *et al.* (1997), Yung *et al.* (2000) and Stupp *et al.* (2002) paved the way for larger, multi-centre Phase III trials to test radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone. EORTC 26981/22981 recruited 573 patients across 85 centres which were randomised between two groups: 60Gy in daily fractions of 2Gy treated over 6 weeks (standard fractionation) of radiotherapy alone, versus radiotherapy with daily concomitant 75mgm^{-2} temozolomide taken 7 days a week, followed by six cycles of adjuvant temozolomide (150-200mgm^{-2}). The primary endpoint was Overall Survival and the initial results reported by Stupp *et al.* (2005) after a median follow-up of 28 months showed a statistically significant survival benefit with

minimal additional toxicity in the temozolomide group: median survival being 14.6 months versus 12.1 months with an unadjusted hazard ratio for death in the temozolomide group being 0.63. Consistent findings were also found in the 5-year follow up data (Stupp *et al.*, 2009). The relative benefits of adjuvant and concurrent temozolomide are dependent on tumour mutations (Van Den Bent *et al.,* 2019) and the methylation status of the methyl-guanine-methyltransferase gene proved to the strongest predictor of outcome and benefit for temozolomide in the Stupp trial (Stupp *et al.*, 2009). Nonetheless, the delivery of standard fractionation radiotherapy with eligible patients receiving concomitant and adjuvant temozolomide has now become established as the standard of care for eligible GBM patients.

Whilst the EORTC 26981 trial provided substantive evidence of the survival benefits of temozolomide, the absence of patients aged over 70 years from the trial was a notable limitation, especially as a significant increase in the number of cases of GBM has been predicted due to an increasingly aging population (Perez-Larraya and Delattre, 2014). The 'Nordic' Phase III trial (Malmstrom *et al.*, 2012) however, has since identified the benefits of hypofractionated radiotherapy (40Gy in 15 fractions) and temozolomide in these patients, finding improved outcomes versus standard radiotherapy.

2.3. GBM Origins: Glioma Stem Cells, Neural Stem Cells and The SVZ Niche

Despite the establishment of multi-disciplinary treatment regimens for GBM and the relative success of trials involving temozolomide, it is still clear that patient prognosis remains poor with tumour recurrence a common problem. Several studies have sought to examine the reasons for tumour recurrence and this section of the literature review now introduces the concepts of Cancer Stem Cells (CSCs) and explores the principal focus of this research project – the perceived importance of the subventricular zone in glioma recurrence and radiotherapy treatment failure.

Retrospective patterns of failure analysis performed by several authors including Gebhardt *et al.* (2014), Minniti *et al.* (2010) and Sheriff *et al.* (2013) found that close to 80% of their approximately 100 patient cohorts recurred close to the original tumour site, despite surgery and chemoradiation. Petrecca *et al.* (2013) even noted that patients who have undergone complete surgical resection suffer from tumour recurrence. These recurrence observations suggest a survivorship of tumour cells, possibly resistant to the effects of radiotherapy and chemotherapy, that drive tumour growth and recurrence and are contributing to the poor prognosis of GBM. As the evidence reviewed in the previous section showed, there are poor

outcomes or insignificant survival benefits from the use of radio-sensitising agents and different fractionation regimes when seeking improved tumour response to radiotherapy. Attention should therefore perhaps turn towards cell survivorship observations and glioma origin theories that may prove a more successful focus for seeking improved radiotherapy outcomes in the near future. It is possible that targeting these glioma precursors and surviving cells during the radiotherapy treatment could prevent tumour recurrence and improve patient prognosis.

2.3.1. Glioma Origin Theories

Stem cells are cells that can generate mature cells of a particular tissue through differentiation (Reya *et al.,* 2001). A glioma origin theory proposed by Friedmann-Morvinski *et al.* (2012) was based on observed cellular behaviour in mice, supposing that oncogene-induced de-differentiation of mature neurons and astrocytes to NSCs or progenitor states eventually led to the formation of gliomas. However, the work of Sanai, Alverez-Buylla and Berger (2005) casts doubt on this theory given its inadequate explanation of the origin of mixed histology high grade gliomas such as oligoastrocytomas. Rather than mature cells de-differentiating, these authors acknowledge the recent recognition of NSCs and glial progenitor cells as actively proliferating cell populations in the brain and propose that this may lead to a more accurate picture of GBM origins, citing evidence that chemical-induced oncogenesis in animal studies was greatest in NSC regions where there are high levels of cellular proliferation. With findings in the literature such as Marsh *et al.* (2012) reporting that 99% of 104 retrospectively-analysed patients had tumours involving a NSC region, NSCs are therefore thought to be highly susceptible to neoplastic transformation and are a likely source of GBMs. Following the recognition by Doetsch *et al.* (1999) and Quinones-Hinojas *et al.* (2006) that the SVZ contained such niches of NSCs that were capable of self-proliferation, NSCs residing in the SVZ have been implicated as possible precursors of GBMs (Lee J-H *et al.,* 2018) and their ability to repopulate proliferating tumour cells may explain patterns of treatment failure. NSCs and malignant glioma cells share a capacity to migrate through mature parenchyma and a preference for white matter tracts, as well as sharing several gene-expression profiles including the tumour suppressor gene PTEN that is commonly mutated in GBM (Smith, Mehta and Wernicke, 2016). The proximity of the original tumour to these neurogenic regions of the brain, including the SVZ, may therefore have a prognostic significance.

2.3.2. NSCs and Cancer Stem Cells in Recurrence

In the same way that stem cells renew healthy tissue, the growth and recurrence of tumours is proposed to be fuelled by dedicated cancer stem cells (Batlle and Clevers, 2017) whose capability for self-renewal and tumorigenesis gives them a critical role in cancer relapse (Yu *et al.,* 2012). Lombard *et al.* (2021) attribute the first proposal of CSCs to Virchow 150 years ago, who suggested that a quiescent sub-population of embryonic stem cells was able to generate tumours. Since then, the existence of CSCs for glioma has been demonstrated by several authors including Ignatova *et al.* (2002), Singh *et al.* (2004), and Galli *et al.* (2004) and it is proposed that it is these cells that contribute to tumour recurrence and treatment failure.

The process by which NSCs become malignant is thought to involve an intermediate step where NSCs transform to CSCs and the role of CSCs in glioblastoma has been extensively reviewed by Sundar *et al.* (2014) with the authors concluding that CSCs not only contribute to tumour propagation but possess the intrinsic properties that promote treatment resistance and encourage tumour recurrence. Lathia *et al.* (2015) explain this concept further, reasoning that in the same way that normal stem and progenitor cells promote tissue development and repair, CSCs support the development and growth of tumours, though in doing so also require the supportive microenvironment (the 'niche') of normal stem cells. Glioma stem cells (GSCs) have been identified in the SVZ (Goffart *et al.* (2017) and Hira *et al.* (2021)) and it is therefore postulated that the SVZ is a niche for GSCs, the survival of which is a driver for tumour recurrence and relative failure of GBM treatments.

2.3.3. Glioma CSC Niches – The SVZ

Doetsch *et al.* (1999), Doetsch (2003) and Lombard *et al.* (2021) describe the features of the SVZ GSC niche, defining it anatomically as a layer of dividing cells extending along the lateral wall of the lateral ventricle and identifying features including vasculature, the specialised basal lamina and extracellular matrix that form an integral part of the stem cell niche. The cellular composition and detailed cytoarchitecture of the SVZ has been further described by Quinones-Hinojosa *et al.* (2006) and a schematic diagram of the human SVZ is shown in figure 2.2.
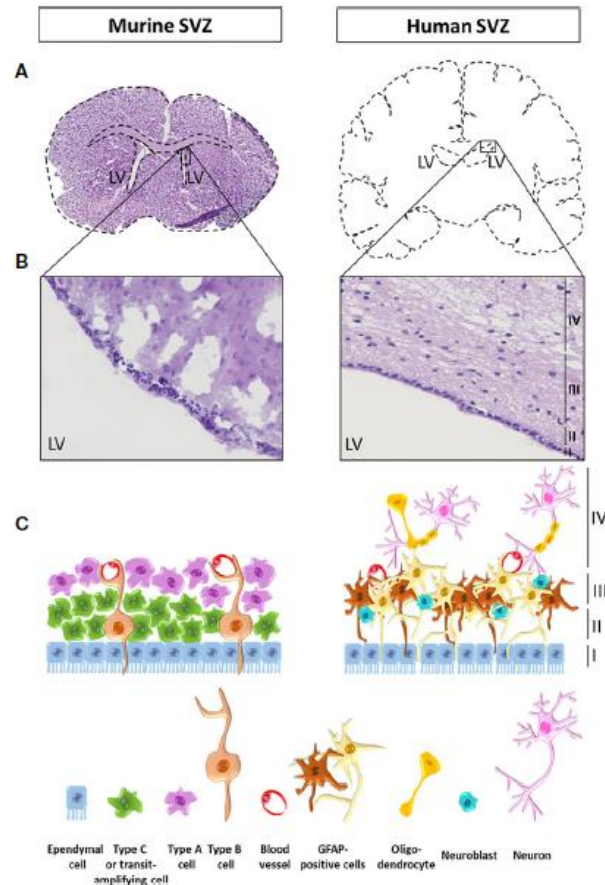
Figure 2.2. Comparison of rodent (left) and human (right) subventricular zone anatomy, showing cellular composition amongst four distinct layers I-IV (Source: Lombard et al., 2021, with copyright permission).

Lombard *et al.* (2021) summarise the evidence that shows GSCs migrating via white matter tracts to reach the sanctuary of the SVZ. Facilitating this migration is the expression of the chemokine CXCL12 by the SVZ, which binds to CXCL4 proteins expressed by GSCs. Heddleston *et al.* (2009) argue that therapeutic strategies should target such microenvironmental GSC niches as well as the tumour in order to prevent tumour recurrences caused by GSC-supported tumour regrowth. Furthermore, both Steinbicher *et al.* (2018) and Zhao *et al.* (2018) have proposed such GSC targeting strategies as the future direction of cancer therapies. However, specifically targeting the SVZ as a GSC niche during radiotherapy would be a novel and controversial approach, especially as normal NSCs in the SVZ have been implicated in repairing damage in the brain (Ming and Song, 2005) and directly contrasting studies have examined SVZ-sparing in radiotherapy to reduce neurotoxicity (Barani *et al.,* 2007). This controversy is not lost on Elicin *et al.* (2014) who warn of the dangers of changing clinical practice on the basis of retrospective studies reporting contradictory results. Targeting with radiotherapy would be further hindered by

the presence of CXCL12 in the SVZ which is known to facilitate the repair of DNA double-strand breaks (DSBs) and potentially cause GSC radio-resistance (Lombard *et al.,* 2021). The following section now explores the existing literature on this subject and examines the contradictory results and unresolved controversies within this subject.

2.4. Recurrence and Survival Analysis – Significance of the SVZ

The SVZ has been identified as a potential niche for GSCs and there is a great number of scientific studies that have sought to clarify the potential role played by the SVZ in driving tumour recurrence. The following sections review literature on the subject in two key areas. Firstly, studies are examined that propositioned the idea that tumour proximity to the SVZ impacts on survival before studies are examined that have sought to determine the prognostic significance of the dose received by the SVZ during radiotherapy. Through a summary of the key findings and the contradictions and controversies that remain, the key areas of research focus for this project will be established.

2.4.1. Correlating Survival: Proximity to the SVZ

Lombard *et al.* (2021) describe how GSCs migrate from the tumour mass to reach the niches of the SVZ where they escape surgical and radiotherapy treatment before triggering tumour recurrence. Given this supposed migration, many authors have theorised that the physical distance from tumour to SVZ may be of significance with SVZ-contact thought to be detrimental to survival. Many of these retrospective studies employ a methodology established by Lim *et al.* (2007) where patients are grouped according to the spatial localisation of the tumour with respect to the SVZ and cortex as visualised on pre-operative MRI. Patients are assigned according to one of four categories defined in table 2.2 and illustrated in figure 2.3.

	Group 1	Group 2	Group 3	Group 4
Contacting SVZ	✓	✓	✗	✗
Infiltrating Cortex (Ctx)	✓	✗	✓	✗

Table 2.2. Tumour classification methodology established by Lim et al. (2007).
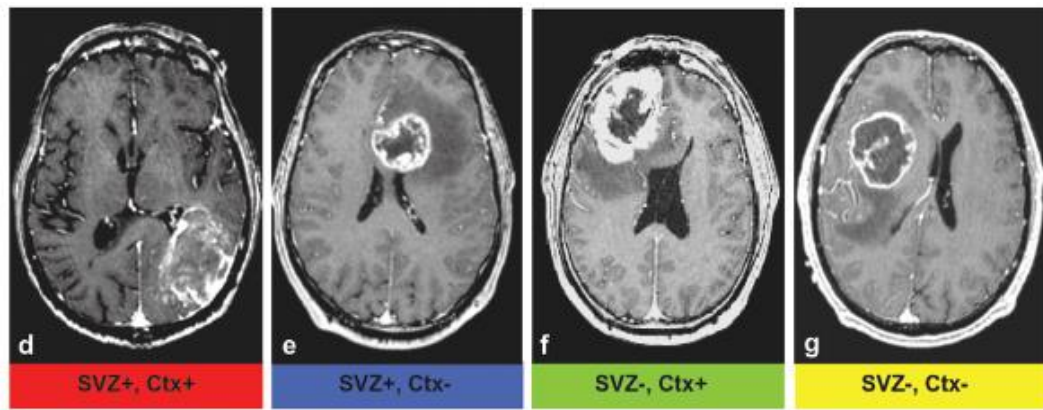
Figure 2.3. Four categories of tumour according to the Lim et al. (2007) classification in table 2.2 (Sub-figures d-g = Group 1-4). (Source: Lim et al., 2007, with copyright permission).

In the original Lim *et al.* (2007) study, the main focus was on recurrence patterns and incidence of multifocal disease. Patient treatment details were excluded and there was no analysis of PFS and OS between the groups. All Group 1 patients suffered tumour recurrence and showed the highest incidence of multifocal tumours at presentation. Moreover, Group 1 patients were more likely to recur at greater distances from the initial site. All Group 4 patients by contrast recurred within the resection margin. This finding supports the NSC theory of gliomagenesis by suggesting an increased migratory ability of tumour cells contacting NSC regions compared to their Group 4 counterparts.

A cohort study reported by Chaichana *et al.* (2008) applied the same classification as the Lim study but in contrast focussed only on reporting survival data rather than analysing recurrence patterns. Treatment details are this time provided and analysis reveals a survival detriment for Group 1 tumours contacting the SVZ at presentation. A curious observation of this study is the deliberate exclusion of patients with multifocal tumours from the analysis, which by definition are more likely to have migratory cell types. With the Lim study omitting survival data and Chaichana *et al.* omitting recurrence analysis, Jafri *et al.* (2013) included both in their study though once again details of the radiotherapy dose are omitted. Once more it was found that Group 1 patients had a significantly reduced PFS (only 39% of Group 1 patients had PFS at 6 months versus 67% for groups 2-4, p=0.01) whilst the OS at 2 years was only 23% in patients with tumours involving the SVZ versus 48% in those without SVZ involvement (p=0.002). The main limitations of these three studies were the fewer than 100 patients included in each and a much larger study of 607 patients was reported by Adeberg *et al.* in 2014. Equally significant reductions in PFS and OS for SVZ-contacting tumours were found (PFS: 4.8 months versus 6.9 months; OS: 12.3 months versus 16.3 months, each p<0.001),

though there were inconsistencies in chemotherapy and radiotherapy treatments between patients and a large range of SVZ doses of 10 to 68Gy was reported. This study agreed with the Lim *et al.* findings that tumours contacting the SVZ were more likely to recur at distant locations and be multifocal at progression. Table 2.3. describes the respective methodologies and findings from these four studies.

Other studies of note on this subject include Chen *et al.* (2015) who examined recurrence patterns with respect to neurogenic regions (NRs) in the brain but included the sub-granular zone (SGZ) as well as the SVZ in the analysis. More detailed information on patient treatments is provided in this paper, with patients undergoing surgical resection and receiving concomitant temozolomide with 60Gy in 30 fractions IMRT. It was found that 86/102 patients recurred in contact with a NR including all those who contacted a NR at presentation (49/102). Sonoda *et al.* (2014) retrospectively examined 61 patients to establish if SVZ involvement at recurrence led to reduced survival following repeat surgery, finding significantly reduced survival in patients whose recurrent tumours involved the SVZ (median survival 10 months versus 14 months, p=0.022). Patients in this study were further categorised by extent of surgical resection and post-surgical therapy which included chemotherapy and stereotactic radiotherapy, though once again details of dose and target volumes in the latter are omitted.

Despite the consistent survival findings amongst these studies, by contrast Kappadakurnel *et al.* (2010) actually found that Group 3 tumours had the highest rates of multifocal disease, though the authors readily acknowledge that their small sample size (n=47) may account for these discordant findings and reassuringly their survival analysis followed the same trend as the others. A meta-analysis of 15 such studies conducted by Mistry *et al.* (2017a) confirmed that patients with GBM contacting SVZ had lower survival. In the past two years (and since the submission of the initial literature review for this research project) there have been further studies published on this subject (table 2.4), all of which show the same detrimental survival with SVZ-contacting lesions, though as in the case of Kappadakurnel *et al.* there are conflicting findings on recurrence patterns.

Study	Patients Included	Treatment + RT Dose	Imaging Details & Methodology	Follow-Up Data Availability + Range	Recurrence Analysis	Survival Analysis	Multifocal Incidence
Lim *et al.* (2007)	53 G1: 16 G2: 9 G3: 14 G4: 14	Surgery. No mention of chemo or RT.	Classified on Pre-Op MRI [Axial FLAIR, SE T2W, DWI, SPGR] by neuroradiologist + 1 other.	G1: 11/16 patients (5-23 months) G2: Not reported G3: Not reported G4: 13/14 patients (3-38 months)	G1: 11/11 with 9/11 in distant locations. G2: Not reported G3: Not reported G4: 10/13 all within resection cavity	Not reported	Highest in G1 (9/16). 0/14 in G4.
Chaichana *et al.* (2008)	69 patients reviewed 2 matched cohorts of 26 (Bordering SVZ or not)	Surgery + RT: 60Gy/ 30#. No mention of chemo.	As for Lim *et al.* (2007)	Not reported. Patients treated 1999-2004	Not reported.	**Lower median survival in SVZ-contacting lesions (8 months vs. 11 months, p=0.02)**	**Multifocal tumours excluded from study**
Jafri *et al.* (2013)	91 G1: 31 G2: 18 G3: 28 G4: 14	Surgery, chemo (Temodar) and RT (dose not reported)	As for Lim *et al.* (2007)	July 2012 (Patients treated 2000-2008)	59/75 progressions had SVZ involvement.	<u>PFS at 6 months:</u> **39% for G1 vs. 67% G2-G4 (p=0.01)** <u>OS at 2 years:</u> **23% for G1/G2 vs. 48% G3/G4 (p=0.002)**	Group 1 had higher % of multifocality at presentation.
Adeberg *et al.* (2014)	607	Range of biopsy/partial resection/GT R/Chemo/RT	As for Lim *et al.* (2007)	6 weeks post treatment, 3-month intervals until death	Tumours contacting SVZ had higher risk of distant progression	<u>PFS</u> **G1/G2 4.8 months vs 6.9**	Higher incidence of multifocal recurrence in

| | | : 10-68Gy (median 51.7Gy). | | | | **months (p<0.001).**

OS
G1/G2 12.3 months vs 16.3 months (p<0.001). | SVZ-contacting lesions |

Table 2.3. Summary of studies published prior to February 2019 that have retrospectively analysed the spatial distribution of GBMs and recurrences with respect to the SVZ, most using the Lim et al. (2007) classification. Abbreviations: G=group, RT=Radiotherapy, FLAIR= Fluid Attenuated Inversion Recovery, T2W=T2 Weighted, T1W= T1 weighted. Notable findings highlighted in bold.

Study	Patients Included	Treatment + RT Dose	Imaging Details & Methodology	Follow-Up Data Availability + Range	Recurrence Analysis	Survival Analysis	Multifocal Incidence
Yamaki *et al.* (2020)	167	Surgery + chemorad. RT dose not reported	MRI T1W and T2 FLAIR and SPGR, categorised by Lim criteria.	Median of 24.3 months	No sig. diff. in recurrence patterns between 4 groups. Distant recurrence most frequent in G3.	Contacting SVZ **Lower median OS (7 months v 12 months, p=0.048)**	Included in reported distant recurrences. **59/105 local recurrences for SVZ-contact versus 12/35 distant recurrences, p=0.03)**
Mistry *et al.* (2020)	502	Surgery + TMZ-based chemorad. RT dose not reported.	Measured distance to SVZ on Pre-Op MRIs.	Not reported	Not reported	Contacting SVZ **Decreased survival on SVZ contact**, actual distance not significant.	Not reported
Hallaert *et al.* (2020)	214	Surgery + TMZ-based chemorad 60Gy in 30#	Evaluated SVZ contact on Pre-OP MRI.	Treated 2003-2014	Not reported	Contacting SVZ **Decreased PFS 5.9 months v 10.3 months (p=0.007).** **Decreased OS 12.2 months v 16.9 months (p=0.016)**	Not reported

Comas *et al.* (2021)	133	Surgery+ TMZ chemorad 60Gy/30# or 40Gy/15# for low performance status or elderly patients	Examined pre-op MRI. Follow-up MRIs every 3 months T1WI and FLAIR/T2 registered to planning CT and recurrence related to isodose line to classify relapse. Categorised by Lim criteria.	Median of 18.6 months	97.7% had disease progression. Anatomical relation to SVZ had no impact on relapse pattern. Higher rates of CL relapses in SVZ contacting lesions	Contacting SVZ **Lower median PFS (6.1 months vs. 8.7 months, p=0.006)** **Lower median OS (10.6 months vs. 17.9 months, p=0.037)**	G2 lesions had highest rate of multifocality at diagnosis. No association with SVZ contact for multifocality at recurrence.

Table 2.4. Summary of studies published since February 2019 that have retrospectively analysed the spatial distribution of GBMs and recurrences with respect to the SVZ, most using the Lim et al. (2007) classification. Abbreviations: G=group, RT=Radiotherapy, FLAIR= Fluid Attenuated Inversion Recovery, T2W=T2 Weighted, T1W= T1 weighted, CL=Contralateral. Notable findings highlighted in bold.

Many of these studies either lack sufficient clarity or consistency on radiotherapy doses received by patients. Furthermore, a significant drawback of many reported studies is the lack of consistency in SVZ delineation, with many lacking robust SVZ delineation protocols either through inconsistent definition or through the use of human observers as detailed in table 2.5. A more quantitative method for identifying SVZ involvement using diffusion weighted imaging has been outlined by Van Dijken *et al.* (2017) which appears to be more robust to inter- and intra-observer variability. Notwithstanding the limitations identified, there is seemingly clear evidence that SVZ involvement of the initial tumour or tumour recurrence is associated with poorer outcomes and Young *et al.* (2011) calculate a significant four-fold increase in mortality risk with initial SVZ involvement.

Study	Imaging Used	Observers	Definition of SVZ Involvement
Lim *et al.* (2007)	Pre-Op MRI: 3 plane localiser Axial FLAIR Axial T2W Axial DWE Post Contrast SPGR T1W Post-Op MRI: As above, plus additional post-contrast axial/coronal.	1 x neuroradiologist 1 x MRI research assistant	Measured distance of tumour to nearest ventricle
Chaichana *et al.* (2008)	As for Lim *et al.* (2007)	Neurosurgeon blinded to clinical outcome	Involved if contacting lining of ventricle
Jafri *et al.* (2013)	MRI sequences not reported. ADC measured if DWI available.	Not reported	Involved if contacting lining of ventricle
Adeberg *et al.* (2014)	Contrast-enhanced T1W axial/coronal	2 x experienced radiology specialists	Involved if contacting lateral wall of lateral ventricle
Chen *et al.* (2015)	Pre-op MRI, post-op planning MRI and suspected recurrence diagnostic MRIs.	Not reported	Ipsi, contra and bilateral SVZ contoured as 5mm margin along lateral wall of lateral ventricles. Measured distance from contrast-enhancing tumour on post-contrast T1W.
Yamaki *et al.* (2020)	Pre-Op MRI: T1WI + T2 FLAIR + SPGR	Not reported	Not reported
Mistry *et al.* (2020)	Pre-Op MRI: T1WI	1 Neuroradiologist + 1 other	Measured distance of tumour to nearest ventricle, involved if

			contacting lateral ventricular ependyma.
Hallaert *et al.* (2020)	Pre-Op MRI: T1 MPRAGE	Not reported	Not reported
Comas *et al.* (2021)	Pre-Op MRI: T1WI + FLAIR/T2	Not reported	Defined on CT as 4mm lateral margin along LVs including both temporal horns.

Table 2.5. Variations in imaging sequences, definitions of the SVZ and observers in studies assessing SVZ involvement in GBM. T1W= T1 weighted, T2W = T2 weighted, TSE= Turbo Spin Echo, FLAIR=Fluid Attenuated Inversion Recovery.

Despite the overwhelming evidence that SVZ-contacting lesions have reduced survival outcomes, there is some inconsistency in observed recurrence patterns with respect to lesions contacting the SVZ. The Yamaki study for example finding that SVZ-contacting lesions were actually more likely to recur locally rather than distant as found by several other studies.

As the literature demonstrates that SVZ-contact is of prognostic significance, the next section reviews the concept of actively targeting the SVZ with radiotherapy treatment by correlating patient outcomes with the radiotherapy dose received by the SVZ.

2.4.2. Correlating Survival: Dose delivered to the SVZ during Radiotherapy

Given the apparent significance of tumour proximity to the SVZ, several retrospective analyses have further sought to correlate PFS and OS with the dose delivered to the SVZ during radiotherapy and summaries of such studies are provided by Smith, Mehta and Wernicke (2016), Nourallah *et al.* (2017) and Lombard *et al.* (2021). Despite the evidence that proximity to the SVZ appears to be a detrimental prognostic factor in GBM, studies investigating dosimetric effects have varying methodologies and generate conflicting evidence on the effect of dose to the SVZ. If the SVZ is to be considered as a target for radiotherapy in a prospective randomised controlled trial, this conflicting evidence presents problems for the design and patient inclusion criteria. Table 2.6 contains a summary of the retrospective studies summarised in the previously submitted literature review together with data presented in an alternative format to the previous tables 2.3 and 2.4 in order to provide clarity on PFS and OS findings. Within this table, findings that support or oppose the active targeting of the SVZ in radiotherapy are coloured in green and red respectively. Table 2.7 shares the same formatting properties as its predecessor, but contains a review of studies that have been published since the initial literature review.

Study	Type	Patients	Histology	Patient Treatment Details	SVZ Contouring Method	Survival Findings
Evers *et al.* (2010)	Retrospective	55	WHO III or IV	Radiotherapy – no details of surgery or chemo. Median prescribed RT dose 59.4Gy. No further details.	CT only. SVZ = 3-5mm lateral margin to lateral ventricles. Note: Hippocampal formation (HF) regions also contoured.	PFS BL SVZ> 43Gy (median): Median PFS 15 months vs 7.2 months (p=0.03) OS not reported (heterogeneous patient population)
Slotman *et al.* (2011)*	Retrospective	40	GBM	Surgery, chemotherapy, radiotherapy prescription 60Gy/30# but no further details on technique.	Bilateral SVZ contoured on MRI, method not reported	PFS: No correlation of ipsi, contra or BL SVZ dose. OS: No correlation of ipsi, contra or BL SVZ dose. Instances of distant recurrences correlated with contralateral SVZ dose <43Gy (p=0.016)
Gupta *et al.* (2012)	Retrospective	40	GBM	Surgery, chemotherapy, 60Gy/30# single phase 3DCRT	CT-only. SVZ defined as 5mm margin along lateral ventricles.	PFS 10 months if BL SVZ>57.9Gy v 14 months (p=0.06) Worse if CL SVZ dose > 53.6Gy OS Higher mean dose to ipsi SVZ improved OS (HR 0.87, p=0.025) Worse if CL SVZ dose > 53.6Gy

Chen et al. (2013)	Retrospective	116	GBM	Surgery, radiotherapy, chemotherapy IMRT 60Gy/30#	Co-registered MRI and CT. 5mm margin along lateral ventricles.	PFS Mean Ipsi SVZ>40Gy and GTR: 15.1 months vs 10.3 months (p=0.028) OS: Mean Ipsi SVZ>40Gy and GTR: 17.5 months vs 15.6 months (p=0.027) No association amongst patients with STR or biopsy.
Lee et al. (2013)	Retrospective	173	GBM	Surgery and radiotherapy, some received chemotherapy. Centre A: 45-46Gy to resection cavity +2cm; 59-60Gy to resection cavity+1.5-2cm. Centre B: 60Gy/30# to CTV=resection cavity+2cm. PTV margin edited.	CT only. Ipsi and CL SVZ defined as 3-5mm lateral margin to the lateral ventricles.	PFS >59.4Gy to ipsi SVZ: median 12.6 months vs 9.9 months, p=0.042. OS >59.4Gy to ipsi SVZ: median 25.8 months vs 19.2 months, p=0.173
Elicin et al. (2014)†	Retrospective	60	GBM	Surgery, chemotherapy and 60Gy/30# 3DCRT.	CT only. 3-5mm lateral to lateral ventricles.	PFS CL SVZ < 59.2Gy 10.4 months vs. 7.1 months (p=0.009) OS CL SVZ>59.2Gy HR:4.83 p=0.004
Sakuramachi et al. (2015)*	Retrospective	74	WHO Grade III/IV	Radiotherapy, some with surgery	CT, definition of SVZ not reported	PFS and OS: No significant correlation with SVZ dose. High Ipsi SVZ and subtotal resection gave shorter PFS.

Ravind, Prameela and Dinesh (2015)*	Retrospective	50	GBM	Maximum safe resection, concurrent chemoradiation and adjuvant chemotherapy. Radiotherapy dose not reported	CT only 3mm and 5mm auto lateral margin from lateral ventricles.	PFS: Not reported OS: D>50Gy to Ipsi SVZ: 19.83 months vs 6.07 months (p=0.031) D>37Gy to CL SVZ (3mm) and CL SVZ (5mm): 16.73 months vs 8.73 months (p=0.305) 19.83 months vs 8.73 months (p=0.118)
Adeberg et al. (2016)	Retrospective	65	GBM	Surgery, TMZ-chemotherapy, 3DCRT median dose 60Gy (Range 40Gy-68Gy).	5mm lateral to lateral ventricle contoured on CT and MRI	PFS Mean IL SVZ dose >40Gy 8.5 months v 5.2 months, p=0.013. Mean CL SVZ dose > 30Gy 10.1 months v 6.9 months, p=0.025. OS No significance found
Comas et al. (2016)*	Retrospective	106	GBM	n=84: 60Gy/30# with temozolomide. n=22: 40Gy/15# with temozolomide	Not reported	SVZ dose > 43Gy did not influence PFS or OS.
Kusumawidjaja et al. (2016)	Retrospective	49 DE 23 Std	GBM	Radiotherapy, concurrent and adjuvant temozolomide DE: 70Gy/30# to GTV+5mm 60Gy/30# to GTV+2cm. Std: 60Gy/30# to GTV+2cm.	Co-registered MRI-CT SVZ defined as 5mm margin along lateral ventricles.	PFS High dose to Ipsi SVZ led to superior PFS: HR=0.95, p=0.052 V50Gy=100% for ipsi SVZ: Superior PFS: HR=0.52, p=0.055 OS: High dose to Ipsi SVZ: HR=1.03, p=0.352

						No relation for OS: HR=0.76, p=0.446.
Arnalot *et al.* (2017)	Retrospective	65	GBM	Surgery, TMZ-chemo, 3DCRT 60Gy/30# in 2 phases	As per Evers *et al.* (2010)	PFS: Greater in patients receiving >48.8Gy to CL SVZ (HR=0.46, p=0.028) OS: No significance
Blumenfeld *et al.* (2017)*	Retrospective	109	GBM	Surgery, TMZ-chemo, 60Gy/30# IMRT	Co-registered MRI-CT SVZ defined as 5mm margin along lateral ventricles.	PFS Not reported OS Worse for mean ipsi SVZ dose > 57.8Gy: 14.5 months vs 19.4 months, p=0.06
Khalifa *et al.* (2017)	Retrospective	43	GBM	Surgery, TMZ-chemotherapy + 60Gy/30# 3DCRT	iSVZ, cSVZ and bSVZ contoured as 5mm lateral margin to lateral ventricles on planning CT. SVZ contoured with and without temporal horn.	PFS SVZ without temporal horn: BL SVZ >40Gy 9.4 months v 4.6 months p=0.023 OS No significance SVZ contact poor prognostic factor for TTP (HR=3.07, p=0.017)
Chaudry and Goenka (2018)*	Retrospective	45	GBM	Surgery, TMZ-chemo and 60Gy/30# radiotherapy	Bilateral SVZ contoured as 3mm margin around ventricles and subdivided to 4 zones: frontal,	PFS No difference in local control at 6 months.

					insular, parietal occipital and anterior temporal.	Dose >60Gy to the anterior-temporal SVZ: LC at 6 months: 38% vs 56% p=0.07 OS Dose >60Gy to the anterior-temporal SVZ: OS at 6 months: 37% vs 73% p=0.01
Mathew *et al.* (2018)	Retrospective	47	GBM	Surgery, TMZ-chemo and 3DCRT/IMRT with median prescribed dose 59.4Gy at 1.8Gy/#.	Co-registered MRI-CT SVZ defined as 5mm margin along lateral ventricles.	PFS Ipsi SVZ D>56Gy: No significance CL SVZ D>50Gy: Better PFS (HR=0.64, p=0.14) OS Ipsi SVZ D>56Gy: HR=0.61 p=0.116 CL SVZ D>50Gy: HR=0.65, p=0.16
Murchison *et al.* (2018)	Retrospective	370	GBM	Surgery, TMZ-chemo + 60Gy/30# 3DCRT/IMRT	Co-registered MRI-CT SVZ defined as 5mm margin along lateral ventricles.	No association of dose to SVZ with PFS or OS.
Weinberg *et al.* (2018)	Retrospective	50	GBM	Surgery, TMZ-chemo, 60Gy/30# radiotherapy.	CT with pre/post op MRI. Criteria of Lim *et al.* (2007) used.	No significant difference in survival with SVZ dose threshold 60Gy.

Table 2.6. Summary of studies investigating dose to the SVZ in GBM. * indicates abstract form only. Abbreviations: LC=local control, Ipsi=ipsilateral, CL=contralateral, DE=dose escalated, Std=standard fractionation 60Gy/30#, 3DCRT=3D Conformal Radiotherapy, GTR=Gross Total Resection, HR= Hazard Ratio, TTP= Time to Progression. Notable findings that support SVZ irradiation highlighted in green, those that oppose it highlighted in red.

†Erratum in Elicin et al. (2014): acknowledgement given to multiple signage errors on figure captions in the original paper. Inequality signs reported here are the corrected versions.

Study	Type	Patients	Histology	Patient Treatment Details	SVZ Contouring Method	Survival Findings
Susman *et al.* (2019)	Meta-analysis	328	GBM	Range of patient treatments across 12 studies	Range of methods used	PFS: High dose to ipsi SVZ HR=0.58 p=0.002 High dose to CL SVZ HR not significant OS: High dose to ipsi SVZ not significant
Valiyaveetil *et al.* (2020)	Prospective	74	GBM	Trial – include SVZ in PTV. SVZ included as target with mean dose >50Gy.	5mm zone surrounding lateral wall of lateral ventricle	No difference in PFS or OS between low and high dose groups
Bender *et al.* (2021)	Retrospective	200	GBM	Surgery, chemotherapy and radiotherapy (60Gy/30#, some patients had SIB 66Gy/30#, some treated with accelerated hyperfractionated 2x1.6Gy per day to 59.2Gy)	5mm zone surrounding lateral wall of lateral ventricle	No correlation SVZ dose and OS.
Hallaert *et al.* (2021)	Retrospective	139	GBM	Surgery, chemotherapy, 3DCRT/IMRT median dose 60Gy/30#	5mm zone surrounding lateral wall of lateral ventricle, including temporal horn	No correlation SVZ dose and OS. Higher mean cSVZ dose led to worse OS (HR=1.029, p=0.032) but relationship lost on multivariate analysis.

*Table 2.7. Summary of studies investigating dose to the SVZ in GBM published since 2019. * indicates abstract form only. Abbreviations: LC=local control, Ipsi=ipsilateral, CL=contralateral, DE=dose escalated, Std=standard fractionation 60Gy/30#, 3DCRT=3D Conformal Radiotherapy, GTR=Gross Total Resection, HR= Hazard Ratio, TTP= Time to Progression. Notable findings that support SVZ irradiation highlighted in green, those that oppose it highlighted in red.*

As evident from tables 2.6-2.7, there is conflicting evidence over the survival impacts of radiotherapy dose to the SVZ. Indeed, some papers present conflicting results within their own study as to the significance of the SVZ dose on patient survival with Gupta *et al.* (2012) reporting that a higher mean dose to the ipsilateral SVZ led to improved OS, yet similarly high doses to the contralateral SVZ made survival worse. As described earlier, the definition of the SVZ remains inconsistent between studies, a point which Lee *et al.* (2013) readily acknowledge (figure 2.4). Furthermore, SVZ dose is likely highly dependent on tumour location with those residing close to the SVZ likely to receive higher doses.

(a)

(b)
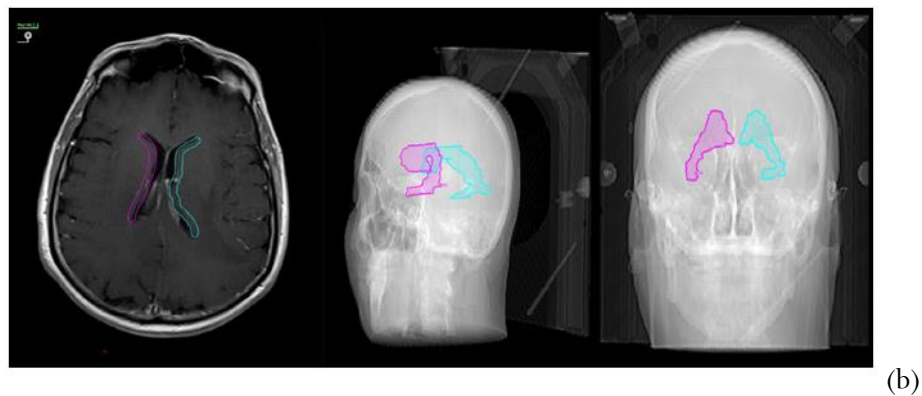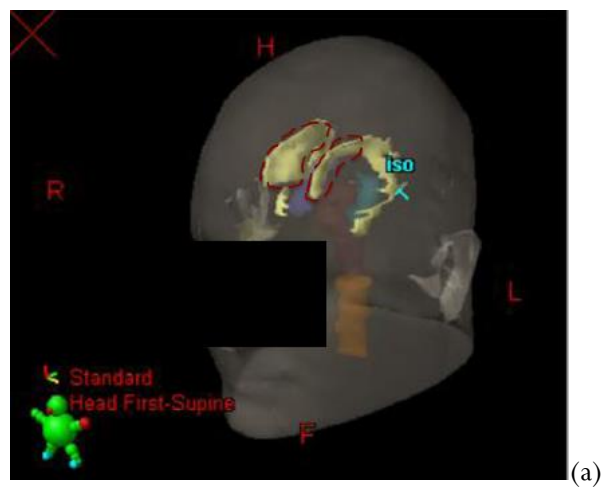
Figure 2.4. (a) Comparison of delineated SVZ by Lee et al. (2013) [yellow structure] and that employed by Gupta et al. (2012) [dark red outline]. From Lee et al. 2013, with copyright permission (b) 4mm lateral margin from lateral ventricle as SVZ definition from Comas et al. (2021), with copyright permission.

Many of the studies may be affected by confounding factors for which there are limited details, for example, extent of surgical resection, chemotherapy dose and regimen and genetic status. These covariates have prognostic significance in GBM, though they are not always included in multivariate analyses in the literature which the following section now explores.

2.4.3. Inconsistencies in Survival Analysis Methodology

The contrasting findings and contradictions found in the studies listed in tables 2.6 and 2.7 are exacerbated by the often-inconsistent approaches taken to survival analysis. As an example, the dose to the SVZ is a continuous variable and yet is often treated as categorical with dosimetric thresholds being employed to dichotomise the data followed by survival comparisons using the Kaplan-Meier method and log-rank tests. In order to illustrate the variation in survival analyses performed, the papers summarised in tables 2.6 and 2.7 were further investigated to examine the respective methodologies employed for assessing the effect on OS of the mean dose to the ipsilateral SVZ. Those studies that were in abstract-form only lacked the methodological detail for performing this assessment, hence were excluded from this part of the review. Table 2.8 summarises this review, showing that the majority of studies used dosimetric thresholds for OS analysis. Only the two studies published most recently (Bender *et al.*, 2021 and Hallaert *et al.*, 2021) incorporated mean dose as a continuous variable in Cox Regression analysis. Two older studies (Murchison *et al.,* 2018 and Gupta *et al.,* 2012) included dose as a continuous variable only in the Cox multivariate analysis. In those studies that used dosimetric thresholds, there was little consistency seen as a range of dosimetric thresholds are reported.

Most studies incorporated a consideration of the effect of other prognostic variables that could influence survival through multivariate analysis. However, there were again methodological inconsistencies noted in the selection of variables for inclusion in these analyses, as summarised in table 2.9 and displayed in figure 2.5. The use of thresholds was also noted as being applied to another continuous variable in patient age, however some more recent studies did include age as a continuous variable. Whilst surgical extent was included by all studies examined, chemotherapy treatment was considered by only 3 studies; a surprising omission given the significance of temozolomide chemotherapy that has been outlined in section 2.2.4. of this review. Comments on the selection of other prognostic covariables are provided in the following paragraphs.

Surgical Resection
Survival outcomes are greatly improved in patients with Gross Total Resection (GTR) as Brown *et al.* (2016) found decreased mortality in GTR versus Sub-Total Resection (STR) up to 2 years post-surgery, plus a decreased likelihood of disease progression at 6 months and 1 year. However, attempting to completely resect a tumour involving the SVZ would require entry in the ventricles, a procedure that risks hydrocephalus and further tumour dissemination via the cerebrospinal fluid (Elliott *et al.,* 1994). Behling *et al.* (2017) demonstrated that ventricular

opening during resection was not a negative predictor of outcome (risk ratio 1.09, p=0.77) and argue that as resection extent is such a strong predictor of Overall Survival, neurosurgeons should consider ventricular opening, when necessary, in order to achieve maximal tumour resection when in contact with the subventricular zone. Saito *et al.* (2020) further support this by finding that a small lateral ventricle opening compared to a wide (>23.2mm) opening was a significant poor prognostic factor (HR=3.674, p<0.0001), concluding that this was due to the removal of a greater proportion of tumour stem cells from the SVZ. Such surgical procedures are challenging for neurosurgeons and remain a controversial subject.

Methylation Status

The O^6-Methylguanine-DNA-methyltransferase (MGMT) gene has an important and well-investigated role in predicting patient prognosis in GBM which the EORT-NCIC trial by Stupp *et al.* (2009) recognised. The MGMT gene encodes a DNA-repair protein that counteracts the effect of chemotherapy agents such as temozolomide, hence silencing of the gene by methylation of its promoter may disable this repair mechanism (Olson, Brastianos and Palma, 2011). Epigenetic silencing of this gene via promoter methylation has been associated with longer OS in patients receiving concomitant chemo-radiotherapy (Hegi *et al.,* 2005) and patients with methylated MGMT genes have been found to have longer OS in prospective clinical trials (Hegi *et al.,*2004). Given the prognostic significance of the methylation status of this gene, it is surprising to note that many studies in the literature do not include this variable in their analyses (figure 2.5). Note that for succinctness, future references to the promoter methylation and silencing of the MGMT gene in this thesis will be referred to as 'MGMT-methylation status'.

Performance Status

The extent to which disease affects everyday activities for a patient is measured by a Performance Status (PS) scale such as the Eastern Cooperative Oncology Group (ECOG) scales (Oken *et al.,* 1982) or Karnofsky Performance Status (KPS, Karnofsky *et al.,* 1948). PS has been seen to be of prognostic significance in many cancers including GBM (Lee J-H *et al.,* 2013) yet is not included in many of multivariate analyses of OS with respect to SVZ irradiation (figure 2.5).

The choice of many studies to omit key prognostic variables in their survival analyses does however present a potential opportunity for this research project.

	Kaplan–Meier Survival Analysis for OS	Cox Regression OS: IL SVZ Dose			
Study	Dose Thresholds Used and Findings	UVA/MVA Dose as Continuous	UVA/MVA Dose as Thresholds	UVA OS Results	MVA OS Results
Bender *et al.* (2021)	X	✓/✓	✓/✓ Range of thresholds used	Cont: NS ≥43Gy v <43Gy 0.021	Cont: NS ≥43Gy v <43Gy: NS
Hallaert *et al.* (2021)	X	✓ / X	X/ X	NS	X
Murchison *et al.* (2018)	Multiple. NS	X/ ✓	✓ / ✓ (60Gy)	NS	NS
Arnalot *et. al.* (2017)	X	X/ X	75th percentile (52.7Gy)	NS	X
Khalifa *et al.* (2017)	Multiple.NS	X/ X	✓multiple (no Šidák correction)/ X	NS	X
Adeberg *et.al.* (2016)	≥40Gy v <40Gy. NS	X / X	≥40Gy v <40Gy	NS	NS
Elicin *et al.* (2014)	✓ 25th, 50th, 75th percentiles NS	X / X	✓ 25th, 50th, 75th percentiles	NS	X
Chen *et al.* (2013)	≥40Gy v <40Gy * In GTR patients: 17.5 v 15.6 p=0.027	X / X	>=40Gy v <40Gy	UVA: NS	MVA: 0.385 p=0.027 in GTR patients
Lee *et al.* (2013)	X	X / X	>59.4Gy	NS	HR 0.45 (0.25-0.82), p=0.009

Gupta *et al.* (2012)	≤59.9Gy v >59.9Gy. NS	X/ ✔	X/ X	X	HR 0.87 (0.77-0.98), p=0.025

*Table 2.8. Methodological variations in survival analysis performed to assess effect on OS of mean dose to ipsilateral SVZ. Abstract-only studies omitted due to absence of methodological details, as were any papers with unclear methodology. Grey cells indicate not included in analysis. Abbreviations: OS- Overall Survival, UVA – Univariate Analysis, MVA- Multivariate Analysis, HR – Hazard Ratio, NS- Not Significant, Cont.- Continuous. Key: ✔- performed, X – not performed. *: multiple dichotomisations by surgical type.*

	Log-Rank or Cox Regression Analysis Performed for Prognostic Variables - OS								
Study	MVA Inclusion	Sex	Age Thresholds	Age Continuous	MGMT	Chemo	Surgery	PS	SVZ Contact
Bender *et al.* (2021)	All UVA variables	✔ UVA: NS ✔ MVA NS	Median 61 ✔UVA: NS ✔MVA: significant	X	✔UVA: p<0.001 ✔MVA: p<0.001	X	✔ UVA: p=0.009 ✔ MVA: p<0.05	✔ UVA: p=0.001 ✔ MVA: p=<0.001 (KPS 70% threshold)	✔ UVA: p=0.009 ✔ MVA: p<0.05
Hallaert *et al.* (2021)	Not specified	X	X	✔UVA: NS ✔MVA:1.034 (1.014-1.054), p=0.001	✔UVA: p<0.001 ✔MVA: p<0.001	X	✔ UVA: GTR v biopsy 0.417 (0.261-0.668), p<0.001 ✔ MVA: GTR+STR v biopsy 0.432 (0.278-0.669) p<0.001	✔ UVA: 0.979 (0.964-0.994), p=0.007 ✔ MVA: NS [KPS continuous]	✔ UVA: NS X MVA

Murchison *et al.* (2018)	All UVA variables	X	✓ 50 years UVA: NS MVA: NS	X	X	✓ UVA:adjuvant >26 weeks p<0.001 X MVA	✓ UVA p<0.001 ✓ MVA: biopsy v GTR 1.62 (1.15-2.28), p<0.01	✓ UVA: <70 11.3m v 17.1m p=0.001 ✓ MVA: <70 1.51 (1.16-1.06) p<0.01 [KPS 70 threshold]	X
Arnalot *et. al.* (2017)	If significant on UVA	✓ UVA: NS	X	✓ UVA: NS X MVA	X	UVA: adjuvant 0.09 p=0.000 MVA: adjuvant 0.11 p=0.000	✓ UVA: NS X MVA	X	X
Khalifa *et al.* (2017)	MVA not performed for OS	✓ UVA: NS X MVA for OS	✓ 60 years UVA: <60 NS X MVA for OS	X	✓ UVA: NS X MVA for OS	X	✓ UVA: NS X MVA for OS	✓ UVA: NS X MVA for OS [ECOG used]	✓ UVA:18.7 m v 41.7m p=0.014 X MVA for OS
Adeberg *et.al.* (2016)	All UVA variables	X	X	X	✓ UVA: NS ✓ MVA: NS	✓ UVA: 0.49 (0.27-0.90) p =0.02 ✓ MVA: NS	✓ UVA: NS ✓ MVA: NS	✓ UVA: NS (KPS>80) ✓ MVA: NS (KPS>80)	X

Elicin *et al.* (2014)	If significant on UVA	✓ UVA: NS X MVA	✓ 54 years UVA: NS X MVA	X	X	X	✓ UVA: NS X MVA	✓ UVA: NS X MVA (KPS>90)	✓ UVA:NS X MVA
Chen *et al.* (2013)	If known prognostic indicator	X	✓ 70 years UVA: <70, HR 0.409 in STR&GTR p=0.006 MVA: HR 0.400 in STR&GTR p=0.007	X	X	X	✓ UVA: NS ✓ MVA: NS	✓ UVA: NS (KPS>90) ✓ MVA: NS (KPS>90)	✓ UVA: NS X MVA
Lee *et al.* (2013)	Not specified	✓ UVA: NS X MVA	✓ 50 years UVA: >50 HR 1.61 (1.05-2.48) p=0.03 MVA: NS	X	X	X	✓ UVA:STR v GTR 1.81 (1.22-2.70), p=0.003 Bx v GTR 2.57 (1.31-5.02), p=0.006 ✓ MVA: STR v GTR 1.90 (1.28-2.84), p=0.002 Bx v GTR 2.97 (1.49-5.91), p=0.002	X	X

Gupta et al. (2012)	If known prognostic indicator	✓ UVA: NS X MVA	✓ 50 years UVA: >50 p=0.003 X MVA	X UVA ✓ MVA: NS	✓ UVA: NS X MVA	X	✓ UVA: NS ✓ MVA: NS	✓ UVA: NS ✓ MVA: NS (KPS high v low)	✓ UVA: NS X MVA

Table 2.9. Variations in inclusion of prognostic variables for Cox Regression Multivariate Analysis to assess effect on OS across a selection of the literature. Grey cells indicate variable not included. Abstract-only studies omitted due to absence of methodological details, as were any papers with unclear methodology. Abbreviations: UVA – Univariate Analysis, MVA- Multivariate Analysis, HR – Hazard Ratio, NS- Not Significant, Cont- Continuous, KPS- Karnofsky Performance Status. Key: ✓- performed, X – not performed. *: multiple dichotomisation by surgical type.
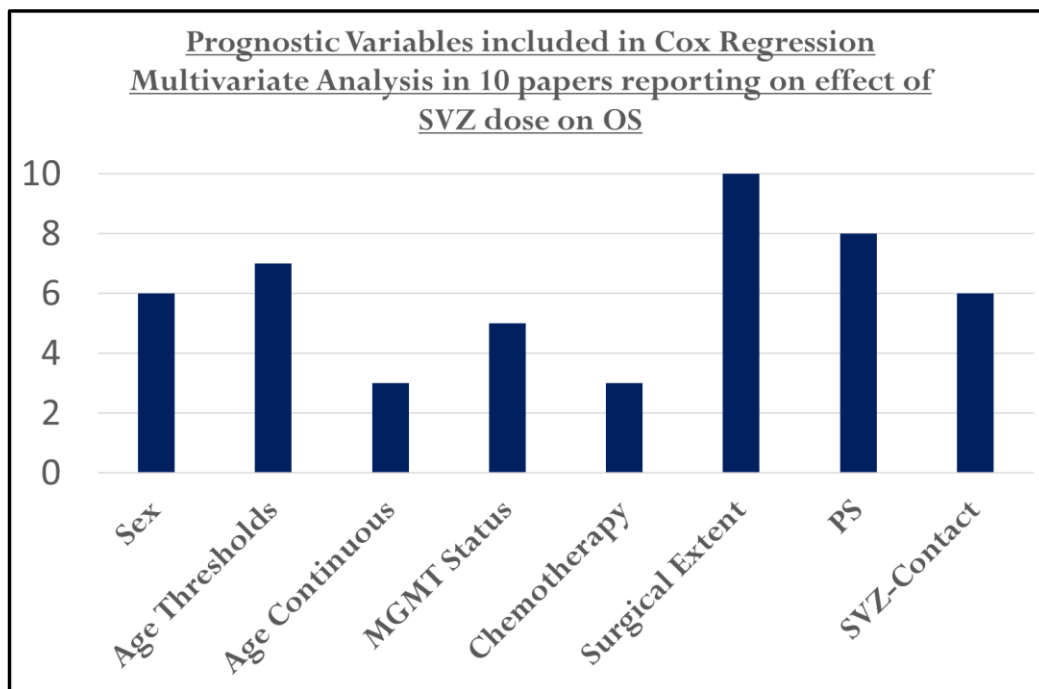
Figure 2.5. Variation in inclusion of key prognostic variables in Cox Regression Multivariate Analysis.

2.5. SVZ: Target or Avoidance? Conflicting Clinical Trials in SVZ Irradiation

It is clear from the literature summarised above that the SVZ has a prominent, if poorly understood, role in patient prognosis. Both proximity of tumour to the SVZ at presentation and the dose received in radiotherapy treatment may have a potential impact on patient PFS and OS. Many of the studies are retrospective which have the usual limitations of bias, confounding factors and uncontrolled patient conditions in the analysis of their data. The conflicting evidence reviewed so far raises the question of the potential benefit to targeting the SVZ during treatment, but moreover there is also evidence in the literature that the SVZ should in fact be actively spared.

Given these conflicting results, the case for a prospective controlled clinical trial was outlined by Nourallah *et al.* (2017) who found only a single, uncontrolled prospective study (Malik *et al.,* 2015) in their review, which reported a dose of >58Gy to the ipsilateral SVZ correlated positively with survival (median OS 16 months versus 14 months, p=0.03). Despite the growing calls for clinical trials, Mann *et al.* (2018) cast doubt on the benefits of SVZ-targeted radiotherapy in such a trial, stating that it is "unlikely to be of significant benefit given the biology of GBM CSCs" though they express confidence that a current randomised Phase II trial (NCT02177578) should provide important information.

Building on the comments in Mann *et al.* (2018), this section describes a further search that was performed to seek details on any current or past clinical trials where SVZ radiotherapy was the key subject of the research question.

2.5.1. List of Clinical Trials and Search Strategy

With increased interest in the potential for clinical trials to clarify the subject in recent years, this section of the chapter describes a review of current and past clinical trials on the subject of SVZ radiotherapy. The details of the search performed on the 'clinicaltrials.gov' database are first provided in table 2.10 with the results of that search summarised in table 2.11, showing the conflicting trial aims and findings of these studies.

Database Search Details	
Database Searched	https://clinicaltrials.gov
Status/Country	All Studies/All countries
Condition or Disease	Sequential Searches for: GBM, Glioblastoma, Glioblastoma Multiforme. Additional search with none specified.
Other terms	Sequential Searches for: SVZ, Radiotherapy Subventricular zone, Radiotherapy, Neural stem cell, Radiotherapy
Country	[None specified]
Results Filtering	
Inclusion Criteria	External beam photon radiotherapy involving inclusion or sparing of the SVZ.
Results	NCT02177578 (Recruiting) NCT01478854 (Closed 2018) NCT02039778 (Terminated)
Excluded Results	NCT01111396 (No radiotherapy) NCT0395670 (Stereotactic radiosurgery)

Table 2.10. Details of search strategy employed for clinicaltrials.gov database.

2.5.2. Clinical Trials Targeting the SVZ

The NCT02177578 trial is recruiting 60 patients randomised to receive a two-phase IMRT treatment of 46Gy in 23 fractions to the bilateral SVZ and enhancing tumour or tumour alone, plus a 14Gy in 7 fraction boost to the enhancing tumour and ipsilateral SVZ or tumour alone (to give 60Gy in 30 fractions to the tumour as per standard treatment), both arms in conjunction with concomitant and adjuvant temozolomide. The primary endpoint is PFS with secondary endpoints including an assessment of neurocognitive function changes. Unfortunately, a further prospective clinical study (NCT02039778, STRONG trial) which aimed to recruit 83 patients to receive the same dose regimen as NCT02177578 with 1-year OS the primary end point, was terminated due to poor recruitment in December 2015. No other trials could be found that met the inclusion criteria.

Trial ID	Study Title/SVZ target or avoidance	Phase	Sponsor	Enrolment	Design	Start	Estimated Completion	Design	End Point
NCT02177578 (Recruiting)	*A Randomised Phase II Study of Subventricular Zone (SVZ) Irradiation Plus Temozolomide in Newly Diagnosed Glioblastoma Multiforme.* **SVZ Target.**	2	Sidney Kimmel Comprehensive Cancer Centre, John Hopkins, Baltimore MD, USA	60	Randomised Parallel Assignment Open Label	8th July 2014	December 2022	Control: Concomitant + adjuvant TMZ and 60Gy/30# RT. Test Arm: Tumour+BL SVZ 46Gy/23# then tumour + IL SVZ 14Gy/7#.	PFS
NCT02039778	*STRONG Trial – Stem Cell Radiotherapy (ScRT) and Temozolomide for Newly Diagnosed High-grade Glioma (HGG): A Prospective, Phase I/II Trial.* **SVZ Target.**	N/A	Roosevelt Hospital, NY, USA	Actual n=4	Single group assignment	Dec'13-Dec-'15	[Terminated]	60Gy/30# IMRT + TMZ concomitant only. Tumour + IL SVZ 46Gy/23# + 14Gy/7# tumour only.	OS
NCT01478854	*A Prospective Trial of Neural Progenitor Cell Sparing Radiation Therapy plus Temozolomide for Newly Diagnosed Glioblastoma Multiforme.* **SVZ Avoidance.**	N/A	Sidney Kimmel Comprehensive Cancer Centre, John Hopkins, Baltimore MD, USA	30	Single group assignment	2011-2018	Completed	SVZ-sparing radiotherapy: 60Gy/30#	Local recurrence in spared niches. 0/30 reported.

Table 2.11. List of clinical trials results obtained from search of clinicaltrials.gov database.

2.5.3. Should the SVZ be spared?

Kut and Redmond (2014) outlined the evidence in the literature that sparing NSC regions may preserve neurocognitive function in patients. The Clinical Trial NCT01478854 sought to investigate such a theory and deduce if neurocognitive outcomes were improved by sparing the niches of neural progenitor cells, aims that were in complete contrast to those of NCT02177578. Out of 30 patients recruited to the neural stem cell sparing trial NCT01478854, none had reported recurrences within the spared- SVZ and it was found (Gui *et al.*, 2020) that higher doses to the SVZ led to a greater decline in verbal memory ($p<0.01$). The results from this trial support the findings from earlier animal studies, including Achanta *et al.* (2012) who examined the effect of radiation on the function of healthy NSCs within mice, noting that radiation may compromise the ability of neuroblasts to migrate to the sites of damage and participate in repair. Furthermore, the study reported by Valiyaveetil *et al.* (2020) where 89 patients were recruited to a prospective study which included the ipsilateral SVZ within the radiotherapy target found no significant correlations between dose and survival amongst the 74 patients that were available for analysis. The authors concluding that future studies should instead focus on sparing these areas to preserve neurocognitive function.

Notwithstanding the potential benefits to patient PFS and OS from irradiating the SVZ, the conclusion of the Valiyaveetil group is not unique and their concerns are shared by many others authors given the potential toxicity that can arise from such treatment strategies, particularly when it comes to normal tissue repair that is so crucial to maintaining the therapeutic index of radiotherapy outlined in the introduction to this chapter.

2.6. Areas of Potential Research

The retrospective studies detailed in section 2.3 were limited by inconsistent methodologies and often contradictory results and there remains scope to explore these gaps in this and potential future research projects.

2.6.1. Resolving Inconsistencies in Methodologies

As section 2.4.3. reported, there is a high degree of variation in the applied methodologies in the published works examined, especially when it comes to survival analysis. Older studies often choose to dichotomise patients according to traditional dosimetric thresholds, rather than perform survival analysis with dose as a continuous variable. This methodology is frequently also applied to another continuous variable in patient age at diagnosis.

Furthermore, whilst a range of prognostic variables are included in reported multivariate analyses – many studies omit patient Performance Status and MGMT-methylation status which are both widely reported to be of prognostic significance. The work carried out in this project will aim to address these methodological issues.

2.6.2. Objectives and Hypotheses for this project

Many of the studies choose to focus only on either dose received or tumour proximity to SVZ rather than considering both. This project will investigate the importance of both elements individually but also seek to establish if the two are linked. Hypotheses for the project are set out as follows:

Hypothesis 1: A higher mean incidental dose delivered to the SVZ during radiotherapy leads to longer OS in GBM patients.

Hypothesis 2: Tumour location with respect to the SVZ has a significant impact on OS in GBM patients.

One further area of uncertainty is the delineation of the SVZ which remains somewhat ambiguous, being defined using generic 3-5mm margins using either CT or MRI with inherent differences in soft-tissue contrast between the two. Given the steep dose gradients associated with IMRT and intrinsic co-registration inaccuracies, the delineation of the SVZ must be robustly and unambiguously defined in any future research with the quantitative method proposed by Van Dijken *et al.* (2017) offering some promise. The impact of delineation precision on reported dosimetric statistics to the SVZ will be investigated in this project which gives the final hypothesis:

Hypothesis 3: Delineation precision has a significant effect on reported dosimetric metrics for the SVZ.

2.6.3. Future Research Opportunities

Whether the SVZ should be targeted or avoided, there is potential radiotherapy technology now available via the use of IMRT and IGRT where selective 'dose painting' and accurate on-set matching of the SVZ sub-volumes can potentially be achieved. Hippocampal-sparing whole brain radiotherapy techniques have been established at Mount Vernon Cancer Centre (MVCC) which aims to preserve neurocognitive function in patients undergoing whole brain radiotherapy with palliative intent. Further techniques to achieve such highly complex IMRT

plans can be investigated and could include non-coplanar RapidArc techniques or variations of dynamic couch rotation treatment techniques (Smyth *et al.,* 2013; Lyu *et al.,* 2018).

One further area of current interest is adaptive radiotherapy for GBM patients with Vegvary *et al.* (2020) demonstrating an increased survival in patients whose volumes were adapted and reduced during treatment, albeit with a small cohort of 43 patients. Adaptive radiotherapy is long established as a technique to modify the initial treatment plan in response to changes in the patient during treatment and is in routine use at MVCC. It could well be the case that the main limitations of the studies reviewed in section 2.3. are a failure to adapt the radiotherapy treatment plan to changes in treatment – therefore compromising the correlations between dose received by the SVZ and the patient outcome. The 'actual' dose received by the SVZ compared to the planned dose received could be quite different, a point emphasised by Darazs *et al.* (2019).

2.7. Conclusion

This work has set the context for a research project investigating the effects of radiotherapy to the SVZ in patients with GBM. The background to the use of radiotherapy in GBM has been provided through a review of the evidence which highlights the benefits of using radiotherapy as part of a multi-disciplinary treatment approach. Despite many attempts at improving outcomes from radiotherapy treatment, including changes in fractionation and the use of radiosensitisers, the prognosis remains poor and research into improved survival for these patients has turned to theories on cellular origins of the disease which highlight the SVZ as being of particular importance.

Studies that have examined the origins of GBMs have described the potential importance of NSCs in the SVZ in driving tumour recurrence which present a possibility for future targeted therapies. Several retrospective reports have either sought to correlate tumour recurrence and patient survival with spatial location with respect to the SVZ or compared the dose received by the SVZ in radiotherapy with OS and PFS. Methodologies and findings in these studies are inconsistent and limitations have been identified. Amidst the controversy, there are also ongoing prospective clinical trials with conflicting aims, either actively sparing or boosting these NSC regions to avoid neurotoxicity and improve survival. Given the evidence reviewed in this report, there remains much scope and potential for research into this area and three hypotheses have been generated to guide the aims and methodology of this research project.

3. Technical Background

Before proceeding to the main body of this research, the thesis now provides a brief technical background to the concepts introduced in the work so far and that will be frequently referred to in the remaining chapters of this thesis. A short introduction to cancer and GBM pathophysiology is first provided before the technical concepts and terminology that are used in radiotherapy are described.

3.1. Cancer and Glioblastoma Multiforme

The term 'cancer' refers to a group of diseases characterised by uncontrolled cellular proliferation. Mutations in a patient's DNA can disrupt the normal cellular mitotic processes and lead to uninhibited cell division, creating often rapidly growing masses of cells called tumours. Malignant tumours can invade surrounding tissues and eventually spread to other parts of the body via the lymphatic and circulatory systems where they can seed metastatic deposits that go on to create further secondary tumours away from the primary site. Once established, secondary tumours also compete for space and nutrients at the new site and tumours eventually form their own vasculature: developing their own network of blood vessels to provide further nutrients for growth (Tortora and Anagnostakos, 1987, p.70). The development of metastases account for a vast majority of morbidity and mortality in cancer patients (Zubair and Ahmed, 2017, p.3) and patients with metastatic disease have significantly poorer prognoses, with treatment options often focussed on symptom palliation rather than being of curative intent.

Tumours are named based on the cellular origin and location of the disease. Carcinomas for example arise from epithelial cells which line organs in the body such as the intestinal lumina (Lever, 1985, p.3.) and account for 85% of cancers diagnosed in the UK (Cancer Research UK, 2021d). Gliomas, the subject of this research project, originate from glial cells in the brain. The WHO categorises gliomas into four categories of increasing malignancy (I-IV) with low grade gliomas (I-II) being relatively slow-growing and composed of well-differentiated tumour cells whilst high grade gliomas (Grade IV, GBM) are the most aggressive. GBMs are infamous for a marked tumour histologic heterogeneity (hence the 'multiforme' in the name) and are characterised by diffusively infiltrative growth within the brain parenchyma (Perry and Wesseling, 2016, p.72). Metastases outside the brain for GBM are very rare, though have been reported (Seo *et al.,* 2012).

3.2. Radiotherapy for GBM

As outlined in the literature review in Chapter 2, post-operative radiotherapy forms part of the treatment pathway for patients with GBM with patients prescribed a dose of 60Gy in 30 daily fractions (30#) as per the current RCR guidelines (Royal College of Radiologists, 2019). As this thesis will frequently discuss technical concepts in the radiotherapy process, this section is intended to provide the necessary background information to underpin and support the remainder of this work.

3.2.1. Fractionation

Radiotherapy aims to destroy malignant tumour cells by using ionising radiation in the form of protons, electrons or most commonly high-energy x-ray photons. Ionising radiation can damage the DNA through direct ionisation and through indirect action via the production of free-radicals. Radiation-induced damage occurs due to DNA-strand breaks which if not repaired lead to the death of the cell. The unit of absorbed dose of ionising radiation is the gray (Gy) and patients are prescribed a total dose in Gy to be delivered over a set number of treatments (fractions, often denoted by a hash #). A prescription of 60Gy in 30 fractions is often written as 60Gy/30# for short.

Treatments are fractionated in order to optimise the therapeutic index introduced in section 2.2. of this work, maximising the probability of tumour cell death whilst minimising the damage to surrounding healthy tissue. The magnitude of the prescribed dose per fraction depends on the relative radiosensitivities of the tumour and adjacent normal tissues. Fractionated radiation doses allow normal cells to repair in between fractions (delivered once per day, five days per week) at a presumed faster rate than the tumour cells can repair whilst also allowing time for tumour cells to redistribute to more radiosensitive parts of the cell cycle (such as the mitotic phase) for targeting in subsequent fractions. Fractionation also enables the potential reoxygenation of tumour cells making them more susceptible to radiation damage, although GBMs have been found to be chronically hypoxic and indeed these hypoxic states have been seen to support the survival of GSCs (Yang *et al.*, 2012).

3.2.2. The Basics of Radiotherapy Treatment Planning

Linear Accelerators and Output Calibration

GBM patients undergoing radiotherapy are treated with high-energy megavoltage photons delivered using a linear accelerator (linac). Linac radiation outputs are calibrated in terms of

the delivery of monitor units (MUs) where 1MU equals 1cGy of dose in specific reference conditions. For the 6MV photon beams used for VMAT treatments, these reference conditions are a depth of 1.5cm (the depth of maximum dose, d_{max}) in water with a 10cm square field and 100cm source to phantom surface distance. In those specific reference conditions, 200MUs from the linac would equal 2Gy at the reference point - the prescribed dose per fraction for a 60Gy/30# GBM. However, the required MUs to deliver 2Gy per fraction in a GBM patient requires a treatment plan calculation that accounts for the geometry and densities associated with an individual patient, as these will clearly be different compared to the water phantom geometry of machine calibration described above. The plan will also account for the specific clinical circumstances such as target size and location, the requirement to minimise the dose to adjacent critical structures and the mechanisms of treatment delivery (for example varying gantry angles) as the next section will describe.

Treatment Planning Overview

An individualised treatment plan is produced for each patient in order to precisely deliver the prescribed radiation dose to the desired target whilst minimising the dose to nearby critical structures. In order to minimise patient motion and therefore ensure the accurate delivery of each daily fraction, GBM patients are immobilised on a flat treatment couch with their head firmly secured in a thermoplastic shell. Patients undergo a planning CT scan in this treatment position which is imported into the Treatment Planning System (TPS). The CT scan dataset is a representation of the relative electron densities of different tissues in the patient and therefore supplies the TPS with the density information required for the calculation of dose via a calibration curve that converts CT Hounsfield Units (HUs) to relative electron density. CT has relatively poor soft-tissue contrast for imaging the brain so the planning CT dataset is co-registered to a planning MRI which has much improved soft-tissue contrast and facilitates more accurate target volume definition.

Target Volumes and Margins

The recipe for the final target volume begins with the delineation of the Gross Tumour Volume (GTV), that is the visible enhancing tumour seen on the MRI. In order to ensure that sub-clinical microscopic disease is included in the radiotherapy treatment, the GTV is expanded to form the Clinical Target Volume (CTV) as per the definitions set out in ICRU Report 50 (International Commission on Radiation Units and Measurements, 1993) and can be tailored according to the routes of presumed tumour spread and edited to anatomical boundaries such as the skull. A further margin is added to the CTV to account for the

geometrical uncertainties in the radiotherapy process to form the final Planning Target Volume, the PTV. The addition of the PTV margin ensures that the dose delivered to the CTV is not compromised by the day-to-day setup variations or from the technical limitations and tolerances of the radiotherapy equipment. The PTV margin for GBM patients is relatively small at 5mm, due to the relative absence of inter and intra-fractional changes in the target position in large part due to the rigid immobilisation of the patient in a thermoplastic shell. By contrast, some targets in the pelvis have a PTV margin of up to 10mm owing to the contrasting absence of a rigid immobilisation device such as a thermoplastic shell and the potential for significant inter and intra-fractional changes from physiological processes such as variable bladder and bowel contents.

Critical Structures and Planning Aims

Organs At Risk (OAR) are also contoured for planning which are structures where the dose should be minimised to avoid causing side-effects. Each OAR has a dose constraint that is based on the magnitude of the dose delivered that would cause a particular side-effect. A further margin is applied to any OARs where the exceeding of a maximum dose constraint would risk terminating the organ function (termed 'serial-like' organs) to form Planning organ at Risk Volumes (PRVs). The brain has several critical 'serial-like' structures which require the addition of a PRV for planning including the optic nerves, spinal cord and brainstem. Once the required structures have been delineated, a treatment plan is created comprising combinations of linac gantry angles and beam apertures shaped by the Multileaf Collimators (MLCs) of the linac. On a treatment plan, isodose lines show lines of equal dose with percentages expressed relative to the prescribed dose. The PTV should be encompassed by the 95% isodose with a homogenous dose distribution across the target varying to only 95%-107% of the prescribed dose. The plan must also avoid exceeding OAR tolerances and maximum dose constraints for PRVs.

3.2.3. Volumetric Modulated Arc Therapy for GBM

At Mount Vernon Cancer Centre, GBM patients are treated using VMAT which enables highly conformal treatment plans to be created and delivered using intensity modulated photon fluences. VMAT treatments involve the linac gantry continuously rotating around the patient whilst delivering a photon beam whose collimation and intensity is modulated by dynamically varying the MLC positions and dose rate. The modulated intensity of the photon

beam facilitates the conforming of the 95% isodose to even highly complex PTV shapes and potentially improved sparing of adjacent OARs.

Due to the complexity of the delivery (with continuous gantry rotation and dynamically varying MLC apertures), VMAT treatments are 'inversely planned' using an optimiser within the TPS which creates a plan based on the objectives specified by the treatment planner. Each contoured structure is assigned at least one objective, for example for the PTV: 100% of the volume to receive 99% of the dose. Each objective is also assigned a relative priority – a measure of how important the objective is relative to the objectives for the other structures in the plan. A mathematical combination of all objectives and their priorities forms the 'objective function' of the plan.

The optimiser creates a treatment plan (comprising MLC positions, dose rates and gantry positions) through an iterative process. At each iteration, the objective function is computed by calculating the difference between the structure dose in the current plan iteration and that required from its objective. The larger the difference between the structure dose in the current plan state and the dose required by the objective, the larger the contribution to the objective function. The optimisation continues to iterate as it seeks to minimise the value of the composite objective function for all structures i.e., minimising the difference between the current treatment plan state and the desired goals. The treatment planner must carefully specify the priorities and objectives for each structure in order to manipulate the optimiser to produce a suitable treatment plan that meets the clinical goals.

3.2.4. GBM Radiotherapy at Mount Vernon Cancer Centre

In order to provide context for the results and analysis to come in later chapters, table 3.1 provides an overview of the GBM radiotherapy procedure at MVCC with figures 3.1-3.4 providing illustrations of the concepts introduced in the previous paragraphs.

Parameter	Description
Prescription	60Gy in 30# for radical patients. 40Gy in 15# or 30Gy/6# for palliative patients.
Treatment Technique	VMAT (Varian RapidArc) delivered using Varian Truebeam or Clinac linear accelerators.
TPS	Varian Eclipse v15.6.
Patient Database	Varian Aria Record & Verify System v15.1.
Treatment Fields	6MV photons. Two ipsilateral arcs used.
GTV	Gross Tumour Volume delineated using co-registered MRI.

CTV	GTV+1.5-2.0cm margin for subclinical spread, edited to anatomical boundaries.
PTV	5mm isotropic expansion of CTV.
OARs	Brainstem, optic chiasm, optic nerves, globes, pituitary gland, cochleas, lens, spinal cord.
PRVs	Brainstem, spinal cord, optic nerves, globes, pituitary gland. All 3mm isotropic expansions.

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Table 3.1. Overview of GBM radiotherapy treatment and planning protocol at Mount Vernon Cancer Centre.
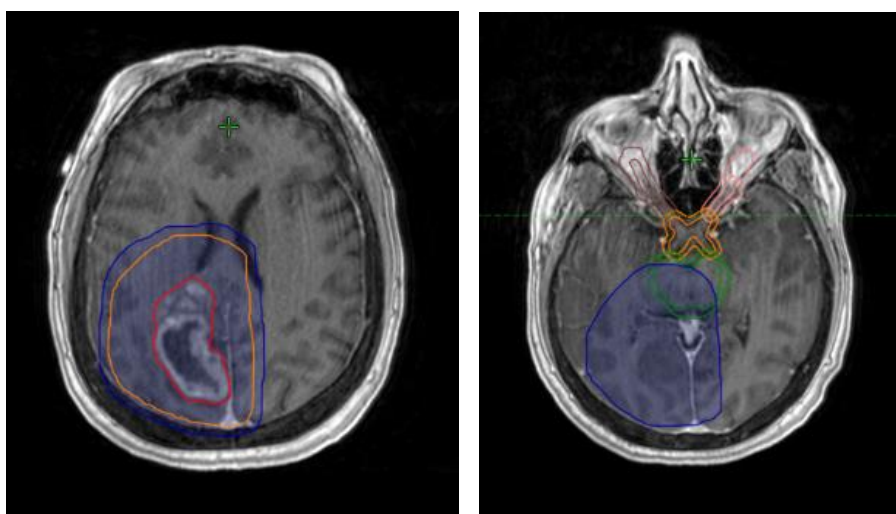
Figure 3.1. Left: Axial co-registered planning MRI showing delineated GTV (red), CTV (orange) and 5mm isotropic margin PTV (blue) for a GBM patient. Right: PTV (blue) shown together with contoured OARs including the optic chiasm and its PRV (orange), bilateral optic nerves and their PRVS (brown and pink) and the brainstem and its PRV (dark green).
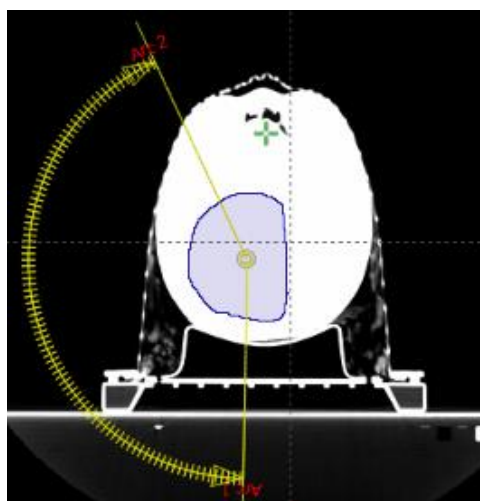
Figure 3.2. Axial planning CT slice with window/level chosen to show thermoplastic shell immobilisation. Also shown are the PTV (blue) and planned treatment arcs: Arc 1 starting at 180 degrees (patient's posterior) and ending at 335 degrees with Arc 2 covering the same angles but in reverse.
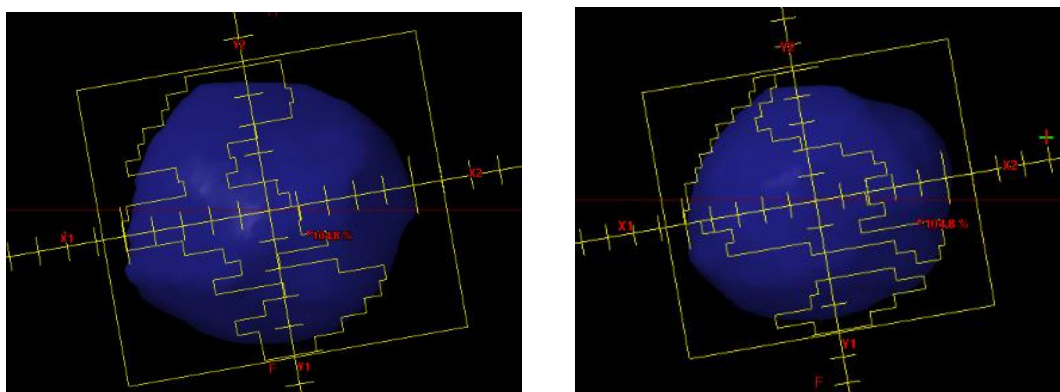
Figure 3.3. Beams-eye-view of VMAT treatment field at two arc positions highlighting the movement of the MLCs (indicated by yellow outline) to modulate the dose to the PTV (3D blue structure).
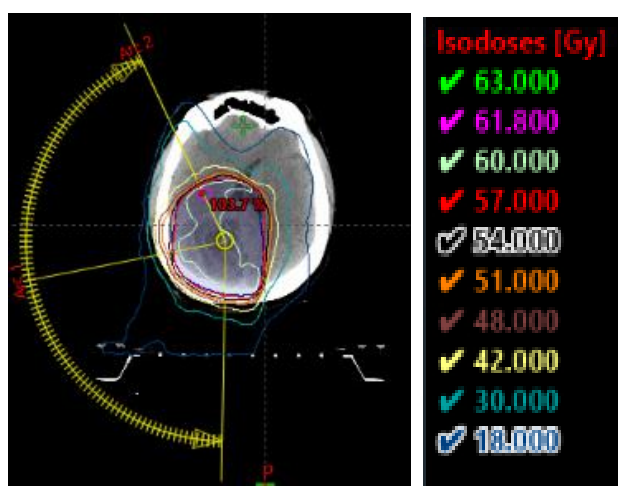
Figure 3.4. Dose distribution obtained from the TPS. Note high conformality of 95% (57Gy) isodose to PTV (blue) and steep dose gradient (close proximity of isodose lines).

3.3. Summary

This chapter has provided a brief clinical background to cancer and GBM to go with the extensive discussions in the literature review of Chapter 2. A technical introduction to radiotherapy has been provided with details described of the radiotherapy treatment planning procedure for GBM with particular reference to the process at MVCC. Chapter 4 now goes on to introduce the formalities of the research project itself.

4. Research Project Overview

The initial three chapters of this thesis have set the foundations for this research project by providing a clinical context and justification for the work, critically examining the literature on the subject area and providing a technical background to the scientific concepts referred to throughout the investigation. The main body of this thesis now begins with this brief introductory chapter which confirms the principal areas of research focus for this work and provides the formal research governance details for this project.

4.1. Principal Areas of Research Project

The literature review of Chapter 2 identified the conflicting conclusions and unresolved controversies with respect to the effect of radiotherapy to the SVZ. Given these findings, the three main areas of focus for this project are confirmed as follows:

1) Does the incidental dose that was delivered to the ipsilateral SVZ during radiotherapy correlate with patient Overall Survival?
2) How does tumour location with respect to the SVZ correlate with patient Overall Survival? Are there are a subset of tumours where SVZ irradiation may be of benefit?
3) How critical is the precision of SVZ delineation in reported dosimetric metrics?

Each of these three areas will have a separate chapter in this work. Each chapter will describe the methodology and results for the area of investigation and provide an interim discussion on the key findings identified. A final overview discussion and reflection chapter will bring together all the key findings and reflect on the outcomes of the project.

4.2. Formal Registration of Research Project

This research project underwent formal approval procedures through the Research and Development (R&D) department at Mount Vernon Cancer Centre (part of East and North Hertfordshire NHS Trust) and through the Health Research Authority. A structured study protocol was devised and approved by the Trust's R&D Steering Group. The project was submitted through the Integrated Research Application System (IRAS) and received confirmation of approval from the Health Research Authority (HRA) on 9th October 2020. The project was deemed exempt from review by a Research Ethics Committee (REC) due to its retrospective nature and absence of patient interventions. Formal acknowledgement of capacity and capability for the project was received from East and North Hertfordshire NHS

Trust on 29th October 2020. Table 4.1 provides a summary of the governance elements of this research work.

Long Title	Investigating the effect of radiotherapy to the subventricular zone (SVZ) in high-grade glioma patients – a retrospective analysis
Short Title	IER-SVZ Study
Study Chief Investigator	Dr Anup Vinayan
Study Principal Investigator	Thomas Hague [Thesis author]
Study Sponsor	East and North Hertfordshire NHS Trust (incorporating the Mount Vernon Cancer Centre) of Lister Hospital, Coreys Mill Lane, Stevenage, SG1 4AB
IRAS ID	279555
Protocol RD Number	RD2020-08

Table 4.1. Overview of formal registration of the research project with the Research and Development department at Mount Vernon Cancer Centre, East and North Hertfordshire NHS Trust.

5. Investigating the effect of Ipsilateral SVZ Dose on Overall Survival in GBM.

The review of the scientific literature presented in Chapter 2 found particularly conflicting evidence when it came to the potential for targeting the SVZ as part of the radiotherapy treatment plan, as many authors found contradictory results in their studies compared to the existing data. This chapter aims to address the first hypothesis in this thesis which is:

Hypothesis 1: A higher mean incidental dose delivered to the SVZ during radiotherapy leads to longer OS in GBM patients.

With many studies lacking detail on radiotherapy treatments and methodology for contouring the SVZ, one initial aim of this arm of the project is to provide a much more robust delineation protocol for the SVZ. The results and analyses presented in this chapter also aim to provide greater clarity on the details of patient's radiotherapy treatments in contrast to the omissions often noted in the literature. Furthermore, as GBM patients undergo treatment following a multi-disciplinary approach, details of patient chemotherapy treatments and extent of surgical resection are included in the survival analyses that follow. The survival analyses presented are further strengthened by the inclusion of other key prognostic variables such as age, performance status and MGMT-methylation status. Note that in the analysis that follows in this chapter, only the ipsilateral SVZ is considered and therefore references to the 'SVZ' should be taken to meaning the ipsilateral SVZ only.

The chapter begins with a general methodology section which describes the creation of the project database and provides an overview of the materials and methods used throughout this retrospective study to obtain data from patients within the treatment planning system. Following this introduction, a more specific methodology for this arm of the project is provided which includes details of the statistical analyses performed. Results are then described before an interim discussion is held to outline the initial findings suggested by this part of the study.

5.1. General Methodology – Materials and Methods

The general method for all arms of the study was to create additional SVZ contours within each patient's database record in the TPS and recalculate their treatment plan to provide dosimetric and volumetric information for these new additional structures. The subsections

that follow now provide details of the creation of this project database, the methods employed for the plan recalculations and a description of the contouring protocol and dataset co-registrations employed for the delineation of the ipsilateral SVZ on each patient.

5.1.1. Creation of Project Database

A search of the Aria database was performed in order to identify all GBM patients treated in the author's centre in the three years between September 2016 and September 2019. The database was searched for patients coded as C71 – malignant neoplasm of brain, according to the ICD-10-CM coding system (World Health Organisation, 2021). This first search yielded a total of 159 records which were then checked for eligibility based on the criteria in the IER-SVZ study protocol. Further exclusion criteria were applied in order to keep the patient population as homogeneous as possible to avoid confounding factors influencing the analysis. Patients were excluded if they were replanned during their radiotherapy (to avoid uncertainties arising from the plan summation process and contouring on multiple datasets) or if they went on to have further radiotherapy delivered using simple, palliative treatment techniques whose data would not be available in the TPS. The inclusion and exclusion criteria applied to this initial database are listed in table 5.1.

Inclusion Criteria	- Adults aged ≥18 years. - Diagnosis of WHO Grade IV Glioma (GBM). - Treated with External Beam Radiotherapy (EBRT) using fixed-field IMRT or RapidArc between September 2016 and September 2019. - Patient data available and accessible within Eclipse TPS.
Exclusion Criteria	- Planned with fewer than 30 fractions (in order to exclude patients treated with palliative intent whose treatment plans would vary significantly and whose dosimetry would not be accurately calculated). - Patients with multiple treatment courses (in order to avoid uncertainties arising from summation of treatment plans).

Table 5.1. Inclusion and exclusion criteria applied to the results of the initial search of the Aria clinical database according to WHO coding criteria.

Applying these criteria yielded a final database of 57 patients. A master database was created to store all the identifiable patient information and assign a unique study number to each patient. This database was stored securely on the Mount Vernon Cancer Centre Radiotherapy Physics Local Area Network (LAN) as per the terms of the IER-SVZ protocol, was password-

protected and not transferred to any other device. A further master results database was created comprising pseudo-anonymised results data with only the study numbers used to identify each patient. The pseudo-anonymised data could be accessed and stored on multiple devices for data analysis under the agreed terms of the study.

5.1.2. Radiotherapy Treatment Planning and Recalculation of Treatment Plans

All patients in the study had been treated with external beam photon radiotherapy at Mount Vernon Cancer Centre in the period specified in the eligibility criteria. An upgrade to the TPS occurred between the end of the study inclusion period and the time of the project commencing. As a result, a newer version of TPS software was used for the study recalculations compared to that used for the original treatment planning, which therefore required additional considerations (see section 5.1.4). Table 5.2 provides technical details of the treatment planning system software and linear accelerator hardware used for the production and delivery of the radiotherapy treatments for the patients in the study, including details of the software versions prior to and after the TPS upgrade. Also included in table 5.2 are the treatment planning protocol details for GBM patients, including descriptions and margin formalism for GTV, CTV and PTV and a brief description of the treatment planning technique that was more thoroughly described in Chapter 3.

Technology		Details
Oncology Management System	*Patient Treatment*	Varian Aria v11 [September 2016-October 2019]
	Recalculations	Varian Aria v15.1 [October 2019 until present]
Linear Accelerators		Varian Clinac + Varian Truebeam
Treatment Planning System	*Patient Treatment*	Varian Eclipse v11 [September 2016-October 2019]
	Recalculations	Varian Eclipse v15.6 [October 2019 until present]
GTV, CTV, PTV Details		**GTV** = enhancing tumour. **CTV** = GTV +1.5-2cm isotropic margin, edited to anatomical boundaries. **PTV_Orig** = CTV + 0.5cm isotropic expansion. **PTV** = PTV_Orig cropped by 5mm from skin to avoid fluence boosting in buildup region.
Treatment Planning Technique		Fixed Field sliding window IMRT with 5,6 or 7 fixed gantry beams. RapidArc with 1-2 partial arcs. Each planned using inverse-planning optimisation module within Eclipse TPS.

Table 5.2. Details of hardware and software employed for the planning and delivery of patient radiotherapy treatments at Mount Vernon Cancer Centre during the study period.

5.1.3. Organisation of Patient Data

The Varian Eclipse TPS organises patient data in a tree structure, beginning with treatment course and cascading through treatment plan, structure set and CT dataset. In order to isolate the research study from the clinical data, a separate treatment course was created within each patient's database and named 'NFT_IER-SVZ' where 'NFT' stands for 'Not for Treatment' and is the locally agreed acronym used for the labelling of non-clinical data. The patient's radiotherapy treatment plan was copied into the new research course and labelled as 'CPlan' (short for 'Clinical Plan'). A duplicate of the clinical structure set was created in order to enable the contouring of the additional SVZ structures that would be needed to extract the relevant dosimetric information required of the study. The duplication of the structure set also created a linked duplication of the CT dataset and both were named 'NFT_IER-SVZ'. Finally, a copy of the clinical plan was pasted onto the new structure set to create the final plan, once again labelled 'NFT_IER-SVZ'. In figure 5.1 the tree structure organisation of the Eclipse TPS is demonstrated and shows the creation of the course, plans, structure set and CT datasets for each patient in the study.
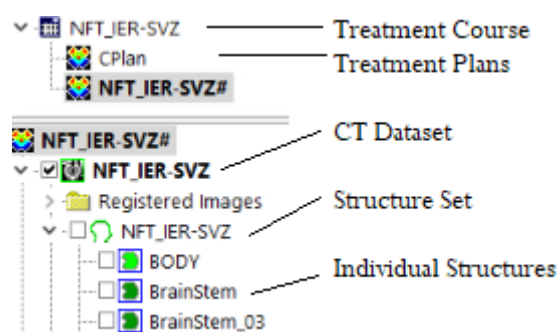
Figure 5.1. Structure of an individual patient's database within the Eclipse TPS. The IER-SVZ project treatment course, plan copies, structures and CT datasets are labelled. Note the tree structure of data organisation. Only a few individual structures are shown to aid clarity, the actual structure sets contain 20-25 structures.

5.1.4. Considerations following TPS Upgrade

Due to the upgrade of the Eclipse TPS that took place at the end of the study selection period, the plan recalculations would be performed on a different version of Eclipse from that used for the calculation of the patient's original clinical plans. Each patient therefore required an updated CT-density calibration table to be linked to the duplicated CT dataset. Furthermore, due to differences in the Eclipse calculation engine between the two versions, there were small differences observed in the calculated monitor units (MUs) on some plans. It was not possible to manually edit calculated MUs so in order to minimise these MU differences, plan

normalisations were changed to identically match the clinical plan that was originally calculated and delivered. A consideration of the effect of the MU differences on calculated plans is included in section 5.3.1.

5.1.5. Dataset Details, Co-Registration and Associated Uncertainties

All patient primary CT datasets had been acquired on one of the two pre-treatment CT scanners at MVCC: a Toshiba Aquilion large-bore scanner and a Siemens Somatom Definition AS scanner. Conversion of HUs to electron densities for dose calculation is achieved via a scanner-specific calibration table. All patients were scanned using the standard 'CT Head' treatment planning scan protocol on each scanner, which comprise 3mm slice spacing. All patients were immobilised on a flat-top couch using a three-point fixated thermoplastic shell.

SVZ contours would be added to the project 'NFT_IER-SVZ' CT dataset within each patient's database (figure 5.1) which would serve as the primary dataset for dose recalculation. Though the required additional SVZ structures would be added to this CT dataset to enable dose reporting following recalculation, the CT dataset itself offers poor soft-tissue contrast for contouring within the brain. Image registration with higher contrast MRI sequences would therefore be required to ensure accurate delineation of the SVZ using the MRI as a secondary dataset.

The secondary MRI sequences required for SVZ delineation were already available in each patient's database in Eclipse, having been imported and subsequently co-registered to the primary CT dataset as part of the clinical radiotherapy planning pathway. The MRI datasets comprised multiple T1 and T2-weighted sequences with slice thicknesses ranging from 0.5mm to 5mm. Duplication of the clinical CT dataset (section 5.1.3.) did not preserve these registrations and image co-registrations therefore needed to be repeated. Registrations were performed using the automatic Varian Rigid Registration tool which employs a Downhill Simplex optimisation and Mutual Information Matching algorithm to co-register the datasets. The algorithm applies both translational and rotational transformations of the secondary dataset (the MRI sequences) to anatomically fuse to the primary CT dataset which remains in a fixed coordinate system. Separate MRI sequences acquired within the same imaging series are grouped together automatically such that a single co-registration applies to all sequences in the same series. Figure 5.2 shows the dataset structures in Eclipse, with connecting lines indicating the dataset co-registrations and an arrow denoting the fixed primary dataset.

Figure 5.2. Dataset co-registration arrangement in Eclipse. Primary CT datasets highlighted with green borders. Secondary MRI sequences from the same imaging series enclosed by dashed orange border. Lines and arrows indicate dataset co-registration with arrow pointing to the fixed primary dataset in the registration. The thicker white arrow shows dataset registration performed for this project for each patient: fusing the secondary MRI sequences to the duplicated primary CT dataset.

The application of an image registration algorithm can introduce geometric uncertainties to the planning process as described in detail by Brock *et al.* (2017). Quality control checks are therefore required to ensure accuracy is optimised. The Varian Rigid Registration algorithm has been in clinical use for brain radiotherapy planning at MVCC for several years and has been shown to be accurate to within 1mm (Kang *et al.,* 2017). As a qualitative quality control check, each clinical dataset fusion is manually inspected to ensure accurate co-registration between the secondary MRI and primary CT datasets by using image overlay and split-screen displays (figure 5.3) to verify the validity of the anatomical match. This procedure was replicated in this study to verify the registrations on each patient, assessing the accuracy around the ventricles in particular to check for anatomical changes between CT and MRI that could affect SVZ delineation and using the skull boundaries to assess geometrical displacement. Accurate reporting of the SVZ dosimetric statistics in this study is therefore reassured, as these checks are in line with routine clinical practice.
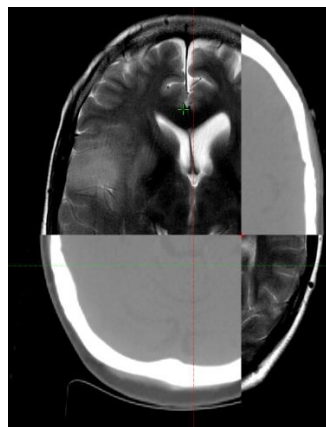
Figure 5.3. Qualitative checks performed to verify accuracy of rigid image registration between MRI sequences and primary CT dataset using the split-screen tool. Note appearance of bone as black on T2 MRI and white on CT.

5.1.6. Practical Delineation Details

The required SVZ contours were contoured using an image overlay feature in Eclipse (the 'blend' function) whereby the secondary dataset is superimposed over the primary dataset to a degree determined by the user's adjustment of a sliding bar (figure 5.4). This function therefore allows the use of the co-registered MRI sequences for higher accuracy delineation whilst retaining the contoured structure on the primary CT dataset for dose reporting. T2-weighted turbo-spin echo sequences were preferred for contouring but if T2 images were not available, contouring was performed using the best available T1 weighted imaging.
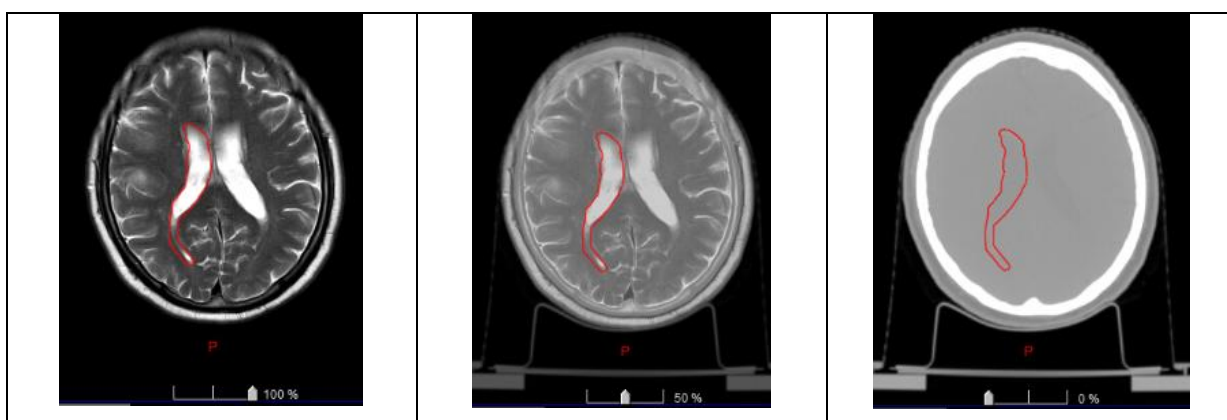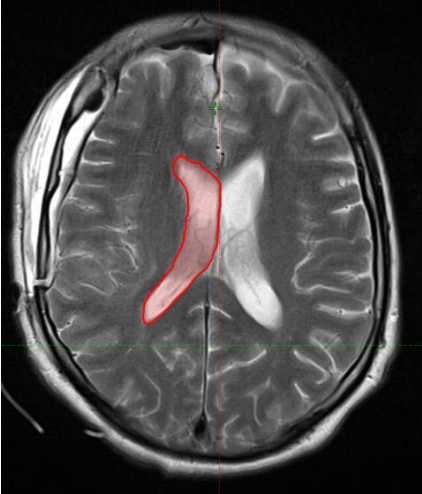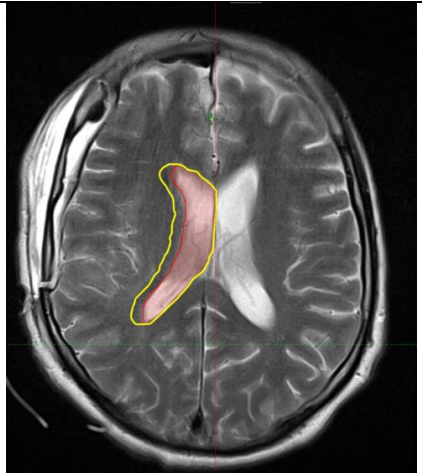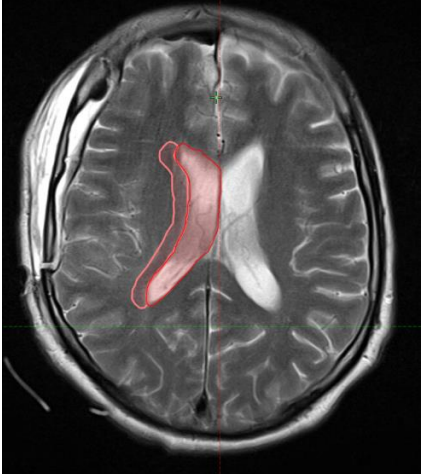
Figure 5.4. Illustration of the 'blend' contouring function in Eclipse. The three sub-figures show the same CT dataset slice but with varying degrees of secondary data superimposition from 100% (left, showing only the superimposed MRI dataset on which the ventricle can be easily visualised) through to 0% (right, showing only the primary CT dataset and the contoured structure).

5.1.7. SVZ Contouring Protocol

As table 2.5 highlighted, there is much variation in the definition of the SVZ in the current literature and contouring methodologies often appear to lack reproducibility. The contouring protocol outlined below in figure 5.5 was therefore devised in order to facilitate the consistent delineation of the SVZ between each patient. SVZ delineation follows the recommendations of the majority of the literature, suggesting a 5mm lateral expansion of the lateral ventricle. However, rather than manually contouring the SVZ itself, the higher contrast lateral ventricle structure was contoured with margin-growing and Boolean operations subsequently employed to create the SVZ structure. Though some manual editing was still required at the end, it was thought that contouring the higher contrast structure would minimise contouring error and be more reproducible between patients. The ipsilateral SVZ was defined using this protocol for all 57 patients in the study.

1. Create new structure named 'Ventricles_MR'. Select post-operative T2-weighted turbo-spin echo axial imaging sequence and the smallest brush size (0.4cm) for optimum precision. 2. Contour the entire ipsilateral lateral ventricle bounded medially by the brain midline and extending inferiorly into the temporal horn. Contouring guided by axial MR anatomical maps from Moeller and Reif (2007).	
3. Expand the 'Ventricles_MR' structure by 5mm laterally on the ipsilateral side, forming the structure indicated by the yellow contour in the figure on the right:	
4. Crop the 5mm expansion volume away from 'Ventricles_MR' to form new structure 'SVZ_Ipsi' shown in salmon pink on the left of the red lateral ventricle: Examine the new contour and manually edit with a 5mm brush to join any areas of non-contiguous contour or other areas of cropping-induced artefact to ensure a continuous SVZ contour. Try to minimise the amount of manual editing.	
5. Using Boolean operations, create the required sub-structures for data-analysis including 'IpsiSVZinGTV' and 'IpsiSVZinPTV':	

| GTV (red), Ipsilateral SVZ (salmon pink) and Boolean Union − Ipsilateral SVZ in GTV (navy blue). | PTV (navy blue), Ipsilateral SVZ (salmon pink) and Boolean Union − Ipsilateral SVZ in PTV (green). |
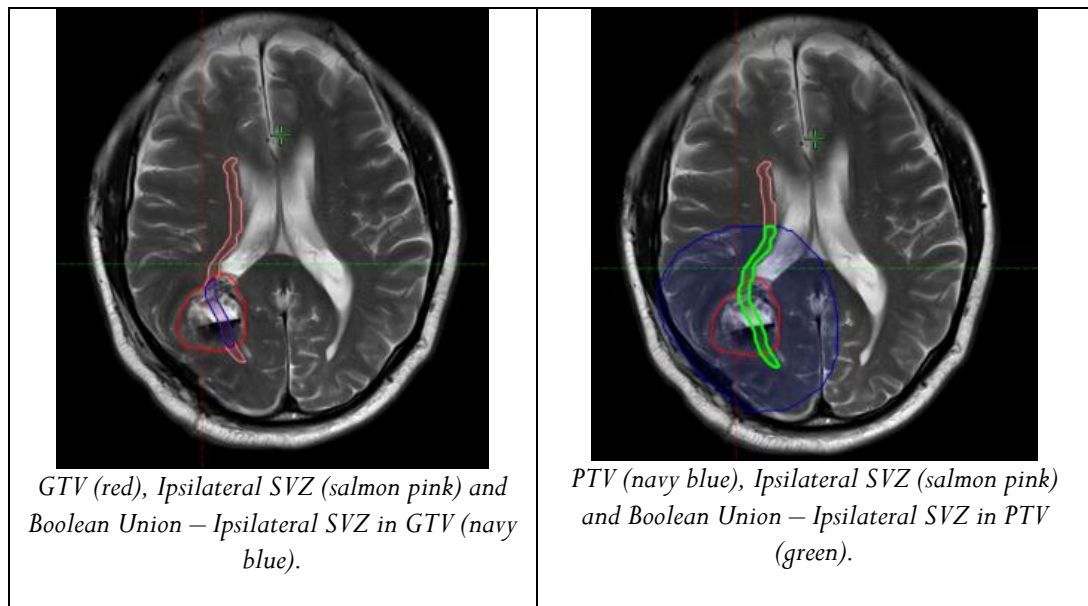
Figure 5.5. Contouring protocol for delineation of SVZ and creation of volumes required for this study.

5.2. Methodology – Effect of SVZ Dose on Overall Survival

The following subsections now provide a detailed methodology related to the specific arm of the project that is the subject of this Chapter. As outlined in the Chapter's introduction, the aim of this part of the project was to perform survival analyses to investigate the effect of SVZ dose on survival but that also accounted for patient demographics, the effect of other treatments received by the patient and other key prognostic variables.

Initial data recording and processing were performed in Microsoft Excel (Microsoft Office 2016) before subsequent data analysis, graph plotting and statistical analysis were carried out using SPSS (v28.0.0.0).

5.2.1. Patient Demographics, Recorded Deaths and Data Censoring

Patient sex and date of birth were taken as those recorded in the Aria system databases. For each patient, the date of decision to treat (DDT) on the radiotherapy referral form was taken as the 'date of diagnosis' and used to calculate patient age at the time of diagnosis. Due to potential time lags in recording patient dates of death in the Aria system, the NHS Spine Portal (NHS Digital, 2021) was used to obtain the patient's date of death and hence calculate the age at death. Overall Survival for deceased patients was calculated as the difference in months between the DDT and the date of death. The time of data analysis was taken as the 1st June 2021. Those patients still alive at the time of analysis were censored from the survival analysis.

5.2.2. Patient Radiotherapy Treatment Details and Prognostic Variables

The Aria database was inspected for each patient to confirm the total number of delivered radiotherapy treatment fractions. Information on patient chemotherapy and surgical treatments together with patient Performance Status and MGMT-methylation status were obtained from the patient medical records for inclusion in the survival analysis. Due to the need to interpret clinical information such as surgical and histopathology reports which are not routinely part of the author's work as a Clinical Scientist in Radiotherapy Physics, this information was obtained and recorded by an Oncology Speciality Registrar in order to ensure that data collection was accurate and clinically consistent. The data was then passed on to the author of this thesis for analysis. The specific methodology for obtaining the information on each of these prognostic variables is stated below:

Patient Performance Status: Patient PS was taken as that recorded on the typed clinic letter from the patient's first consultation appointment and was classified using the ECOG Performance Status scale (ECOG-ACRIN, 2022) as defined in table 5.3. Note that the KPS reported by many of the studies in Chapter 2 is not used to classify patients at MVCC, hence was not obtainable or recorded.

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities, up and about more than 50% of waking hours.
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair.
5	Dead.

Table 5.3. Definitions of ECOG Performance Status used at MVCC and referred to in this study (ECOG-ACRIN, 2022).

Surgical Extent: The extent of surgery was determined by reviewing the post-operative surgical notes, the summary from the patient's multidisciplinary team (MDT) treatment management proforma and histopathology results. Patients were categorised into one of three categories: Biopsy-only (Bx), SubTotal Resection (STR) and Gross Total Resection (GTR).

Chemotherapy Treatment: All GBM patients at MVCC are considered for first-line temozolomide chemotherapy if eligible. Details of chemotherapy treatments were taken from the Trust's 'ChemoCare' prescribing system and categorised according to receipt of concomitant and/or adjuvant temozolomide.

MGMT-methylation status: This was taken from the patient's histopathology reports. Where this was not recorded on the results report, contact was made directly with the Pathology department to obtain the information where possible.

5.2.3. Volumetric and Dosimetric Data

Volumetric data was obtained from the TPS using the Eclipse 'measure volume' tool which recorded the structure volumes in cubic centimetres (cc) to two decimal places. The volume of the ipsilateral SVZ and the Boolean generated structures 'Ipsilateral SVZ in GTV' and 'Ipsilateral SVZ in PTV' were recorded and used to calculate the percentage overlap using equation 5.1 and 5.2.

$$\% \ Overlap \ SVZ \ in \ GTV = 100\% \times \frac{Volume \ of \ Ipsilateral \ SVZ \ in \ GTV \ (cc)}{Volume \ of \ Ipsilateral \ SVZ \ (cc)} \quad [5.1]$$

$$\% \ Overlap \ SVZ \ in \ PTV = 100\% \times \frac{Volume \ of \ Ipsilateral \ SVZ \ in \ PTV \ (cc)}{Volume \ of \ Ipsilateral \ SVZ \ (cc)} \quad [5.2]$$

Dosimetric data for the mean dose to the ipsilateral SVZ was obtained using the dose-volume histogram (DVH) functionality in Eclipse. For the study cohort, the mean ipsilateral SVZ dose and standard deviation were computed together with the median and range. Mean SVZ dose was chosen as the measure in this study in order to permit comparisons with existing studies where it is the chosen measure amongst multiple authors in the literature including Evers *et al.* (2010), Gupta *et al.* (2012), Lee *et al.* (2013), Chen *et al.* (2013) and Adeberg *et al.* (2016). Using minimum SVZ dose as an alternative measure would be subject to uncertainties arising from reported low doses in the Eclipse TPS that are derived from largely 'out-of-field' parameters, where modelling of the MLC 'tongue and groove' effect and rounded MLC ends presents a significant challenge to dose calculation accuracy (Kielar *et al.,* 2012). With a significant proportion of patients likely to have SVZ overlapping with PTV, the maximum SVZ dose would likely be very similar for all patients with a narrow range centred around 60Gy, hence was also disregarded as a measure.

5.2.4. Survival Analysis I: Kaplan-Meier & Cox Regression Analysis

Overall Survival was chosen as the endpoint for this study due to ease of data collection. Estimates of Overall Survival for cohorts of patients within the study were provided using Kaplan-Meier survival analysis. The effect on Overall Survival of SVZ dose and the other covariates was deduced via Cox Regression univariate analysis. Patient age and mean dose to the SVZ were considered as continuous variables in the analysis.

A maximum of 5 covariates were included in the Cox multivariate analysis on the advice of a statistician and following the 'rule of thumb' for multivariate analysis of 10 events per variable (Peduzzi *et al.,* 1995). Together with the inclusion of mean dose to the SVZ as the main subject of this investigation, the four other chosen covariates were surgical extent and chemotherapy treatment (such that all three treatment modalities were included) together with age and MGMT-methylation status which were chosen based on significance seen on univariate analysis. Hazard ratios were reported with 95% confidence intervals and p-values below 0.05 were considered as significant.

5.2.5. Survival Analysis II: Literature Comparison and Šidák Correction

In order to facilitate comparisons with results from the literature where patients are often dichotomised by thresholds into two groups (see section 2.4.3.), additional survival analysis was also performed according to this technique. Kaplan-Meier survival curves were constructed for patients dichotomised into comparison groups based on a range of age and dosimetric thresholds. The Kaplan-Meier curves were used to compute the estimated median Overall Survival for each group and the significance of the difference in median Overall Survival were assessed using log-rank tests and the Chi-squared distribution.

As multiple tests were performed on the same data for the different thresholds of age and dose, a Šidák correction was required to reduce the chances of obtaining a Type I error. Statistical significance is usually considered at p values below 0.05, however for a number of tests C on the data, this value is corrected using the Šidák equation [5.3] where α is the new value for statistical significance and α_0 is the original p value (0.05). For small test numbers C, the Šidák equation can be approximated by the Bonferroni correction in equation [5.4] (Abdi, 2007).

$$\alpha = 1 - (1 - \alpha_0)^{\frac{1}{C}} \qquad [5.3]$$

$$\alpha = \frac{\alpha_0}{C} \qquad\qquad [5.4]$$

As six threshold values for age and SVZ mean dose were tested ($C=6$), statistical significance in this part of the analysis was considered only for p values below 0.008 [equation 5.3].

5.3. Results – Effect of SVZ Dose and Prognostic Variables on Overall Survival

The following results section is divided into sub-sections in line with the methodology described in the previous subsection.

5.3.1. Radiotherapy Plan Recalculations and Treatment Details

All plans were recalculated on Eclipse v15.6 following the TPS upgrade with plan normalisations set to be identical to that in the original treatment plan. Identical MUs between the recalculation and the original plan were found in 37 patient plans. Of the 20 plans with MU differences, the median difference was 2MU (range $0.1MU - 3.6MU$). For the plan with the maximum difference in MUs observed (3.6MUs), the difference accounted for 0.5% of the total MUs in the plan. Differences in MUs between plan recalculations and the original treatment plans were therefore assumed negligible for the study cohort and are disregarded during any further analysis.

All patients were planned using either fixed field IMRT or RapidArc. Out of 57 patients in the study, 3 did not complete their planned 30 fractions due to progressing symptoms making them unable to attend for radiotherapy. These 3 patients were excluded from all subsequent analysis to avoid the confounding factor of partial treatment influencing survival results. Table 5.4 summarises the plan and treatment details.

Description	Value
Completed 30#s of Radiotherapy: Final Patient Cohort	54 (95%)
Median Overall Treatment Time and Range (days) for completed treatments	41 (39-44)
Number Treated with Fixed Field IMRT	39 (68%)
Modal Number of Fixed Fields (range)	5 (4-9)
Number Treated with RapidArc	15
Mean Total Arc Length (± 1 standard deviation) (degrees)	361 ± 65.7

Table 5.4. Summary of radiotherapy treatment parameters for the 57 patients in study.

5.3.2. Patient Demographics

Details of patient demographics, treatments received (including surgery, chemotherapy and radiotherapy) and prognostic variables were recorded in the project database. Table 5.5 lists

the age and sex characteristics of the study population. The majority (63%) of patients in the study were aged over 60 at the time of diagnosis with a median population age of 63 (figure 5.6). Almost two-thirds of the patients in the study population were male.

Characteristic		Value
Age at diagnosis	*Median Age*	63 (range 26-73)
	Number Age < 60	20
	Number Aged >=60	35
Sex	*Male*	35
	Female	19

Table 5.5. Demographics of the study population.
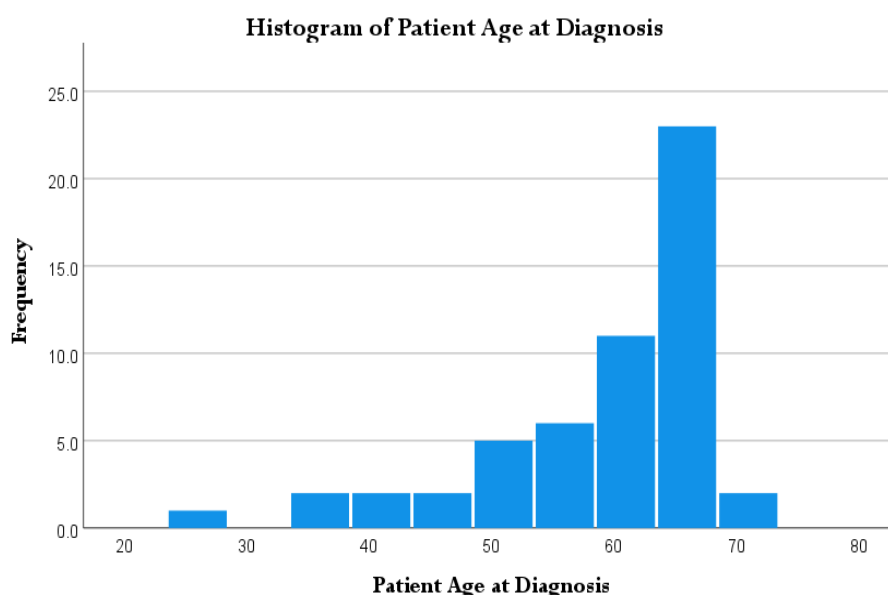
Figure 5.6. Population age histogram for the IER-SVZ study.

5.3.3. Patient Treatment Details and Prognostic Variables

Data was obtained on prognostic variables including surgical extent, chemotherapy regime, Performance Status and MGMT-methylation status. Aside from data on MGMT-methylation status being unavailable for 4 patients, all patients had information recorded for these four variables. Figure 5.7 shows these results for the study cohort.

The vast majority of patients (50/54) received concomitant and adjuvant temozolomide chemotherapy following the standard clinical protocol at MVCC derived from the EORTC 26981 trial (Stupp *et al.,* 2009). Within the 50 patients receiving both concomitant and adjuvant temozolomide (from now on referred to as the 'Stupp' regime), one patient unfortunately died whilst receiving their adjuvant chemotherapy and another had their

adjuvant chemotherapy terminated due to a pneumothorax. Of the 4 patients that did not receive the combined 'Stupp' chemotherapy regime: 1 patient was unsuitable for chemotherapy completely due to thrombocytopenia, 1 patient refused adjuvant chemotherapy having completed concomitant chemotherapy and 2 patients received adjuvant-only chemotherapy.

The majority of patients were Performance Status 0 or 1 with only 3 patients recorded as being of worse PS. In regard to surgery, only 9 patients were recorded as undergoing a GTR with the majority recorded as STR (26 out of 54). 19 patients had a biopsy-only surgical procedure. Patient methylation was a roughly even split with a slim majority (52%) of the patients having unmethylated MGMT status.
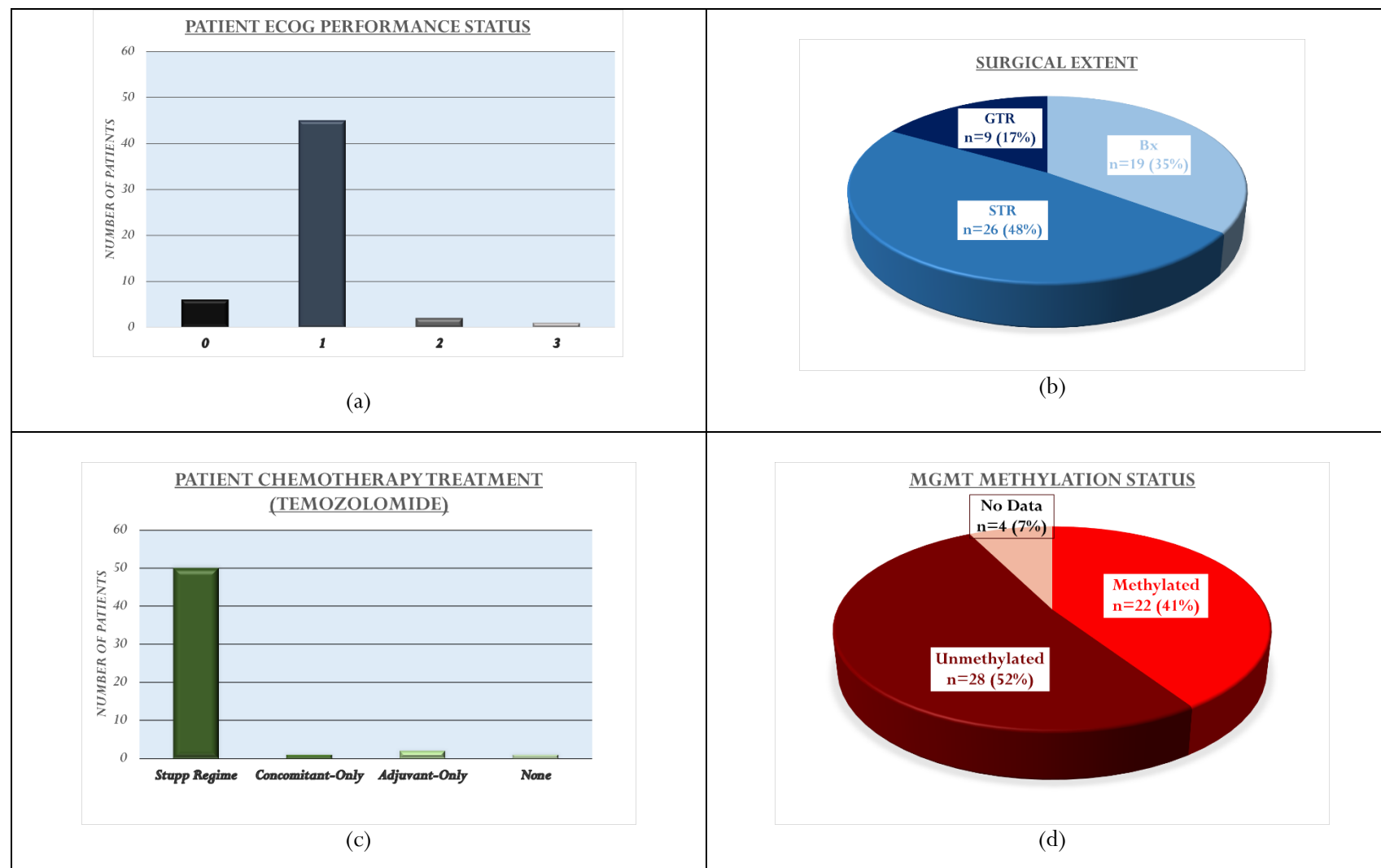
Figure 5.7. Summary of prognostic variables for study cohort including (a) Performance Status, (b) Surgical Extent, (c) Chemotherapy Regime and (d) MGMT-methylation status.

5.3.4. Degree of SVZ Overlap

The ipsilateral SVZ was delineated following the protocol outlined in section 5.1.7. with Boolean operations used to compute volume statistics for the degree of overlap with the Gross Tumour Volume (GTV) and Planning Target Volume (PTV). The error on the reported volumes in Eclipse for a 3mm CT slice thickness is estimated to be 20% for volumes <10cc and 1% for those ≥10cc (Srivastava, Cheng and Das, 2016). Table 5.6 summarises target volume and SVZ volume statistics and the degree of overlap. The range of volumes is across the study cohort. Error analysis is excluded from the table for clarity.

	Median Volume (cc)	Volume Range (cc)
GTV	29.8	3.8 -165.5
PTV	351.8	114.2 – 725.5
Ipsilateral SVZ	8.0	4.3-18.4
	Median Overlap (%)	Range (%)
Ipsilateral SVZ in GTV	0.8	0-37.7
Ipsilateral SVZ in PTV	70.0	1.6-99.6

Table 5.6. Volumetric information for radiotherapy target volumes, SVZ and percentage of SVZ overlap.

5.3.5. Correlating Overlap with Dose

The mean ipsilateral SVZ dose across the cohort was 50.1 ± 11.0Gy whilst the median dose was 54.6Gy (range 9.8Gy-60.6Gy). The mean dose to the ipsilateral SVZ correlated reasonably strongly with the degree of SVZ overlap with PTV (figure 5.8) using linear regression (R=0.886, p<0.001). No such correlation was found for degree of overlap with GTV (R=0.118, p=0.009).
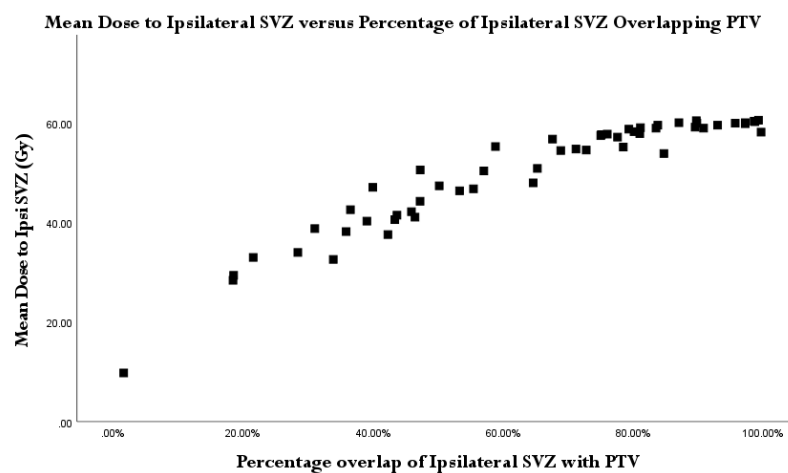
Figure 5.8. Correlation of mean dose to ipsilateral SVZ with percentage of ipsilateral SVZ overlapping PTV.

The plot in figure 5.8 resembles a growing saturation curve rather than a linear correlation. Using non-linear regression and curve-fitting tools in Excel, an estimated equation of best fit for this relationship is given in equation [5.5] to relate the percentage of SVZ overlap with PTV (x) with the mean dose (D). Good agreement between this model and the observed data was seen when plotted (figure 5.9) and tested using Chi-squared statistics (p=0.986).

$$D(Gy) = 60 + \frac{0.4}{x} + 20 \ln x \qquad [5.5]$$
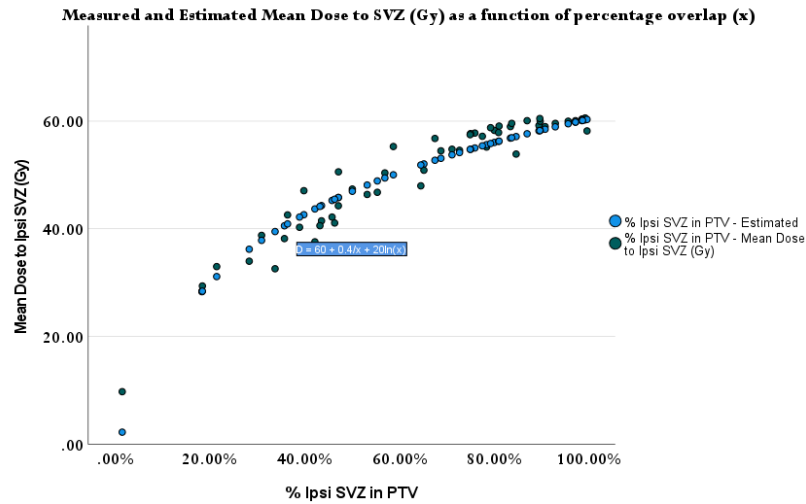
Figure 5.9. Estimation of mean dose to ipsilateral SVZ using curve-fitted equation [5.3], comparison to measured data.

The correlation seen between degree of SVZ-PTV overlap and mean SVZ dose in figures 5.8 and 5.9 is supported conceptually, as patients with a high degree of SVZ overlap with the PTV will likely have a higher mean SVZ dose. As this relationship has been derived and conceptually verified, the survival analyses that follow are limited only to consideration of the SVZ dose and not the degree of overlap.

5.3.6. Survival Analysis I(a): Univariate Analysis

The median follow-up time for the study population was 15.5 months. At the time of analysis taken to be 1st June 2021, 45 patients were deceased. A Kaplan-Meier survival curve showed an estimated median OS of the study cohort to be 15 months. Of those patients who had died, most (69%) were aged over 60 with the median age at death being 65 (range 41-75).

Further Kaplan-Meier survival analysis was performed to estimate the median OS for the patients categorised by prognostic indicators that could be classed as categorial variables (table 5.7). A significant improvement in OS was seen for patients with methylated MGMT

promoter status (21 months versus 13 months, p=0.028) and a significant difference was also seen between survival curves categorised by chemotherapy regime, though likely heavily influenced by the short 5-month OS of the patient who received no chemotherapy (p=0.017). Categorisation by patient sex, Performance Status and surgical extent did not show a significant difference between survival curves.

Covariate	Number of Patients	Median OS (months)	p-value
Sex			0.741
Female	19	19	
Male	35	14	
Performance Status			0.960
0	6	14	
1	45	15	
2	2	13	
3	1	17	
Surgical Extent			0.219
Bx-only	19	17	
STR	26	14	
GTR	9	21	
Chemotherapy Regime			_0.017_
'Stupp' Regime	50	15	
Adjuvant-Only	2	12	
Concomitant-Only	1	16	
No Chemotherapy	1	5	
MGMT Methylation*			_0.028_
No	28	13	
Yes	22	21	

*Table 5.7. Kaplan-Meier estimations of median OS for patients categorised according to prognostic variables. *indicates missing data for 4 patients in cohort. Significant results italicised and underlined.*

Further univariate analysis was performed using Cox Regression to calculate Hazard Ratios for each prognostic covariable (table 5.8). Increasing patient age at diagnosis proved to be significant (HR 1.055 (1.018-1.092), p=0.003). Patient sex and Performance Status were both confirmed to not be significant indicators of poor Overall Survival in this cohort. In terms of patient treatments, surgical extent also did not prove to be significant and in respect of chemotherapy treatment it was only the absence of chemotherapy completely that proved to be a significant survival detriment. A significant survival benefit was found with MGMT-methylation (HR 0.510 (0.271-0.959), p=0.037). Most crucially for this part of the study, no significant survival impact was found for mean dose to SVZ (p=0.482).

Covariate	HR (95% Confidence)	p-value
Sex (male versus female)	1.104 (0.603-2.023)	0.748
Age	_1.055 (1.018-1.092)_	_0.003_
Mean SVZ Dose (Continuous)	1.010 (0.982-1.038)	0.482
Performance Status		0.963
0	Reference Category	
1	0.858 (0.334-2.203)	0.751
2	1.154 (0.223-5.968)	0.865
3	1.092 (0.127-9.406)	0.936
Surgical Extent		0.248
Bx-only	Reference Category	
STR	1.445 (0.749-2.788)	0.272
GTR	0.727 (0.278-1.896)	0.514
Chemotherapy Regime		0.130
'Stupp' Regime	Reference Category	
Adjuvant-Only	1.411 (0.338-5.895)	0.637
Concomitant-Only	1.425 (0.193-10.520)	0.729
**No Chemotherapy**	_13.562 (1.513-121.574)_	_0.020_
MGMT Methylation*		
No	Reference Category	
**Yes**	_0.510 (0.271-0.959)_	_0.037_

Table 5.8. Results from Cox Regression univariate analysis. Only MGMT-methylation status, absence of chemotherapy and age proved to be significant (italicised and underlined). Mean SVZ dose was not found to be significant. *4 patients had unrecorded methylation status and were excluded from this analysis.

5.3.7. Survival Analysis I(b): Multivariate Analysis

The combined effect of multiple covariates was assessed in a multivariate analysis performed using Cox Regression. A total of 45 deaths (events) were recorded for the study cohort which permitted the inclusion of up to five covariates in the multivariate analysis on advice from a statistician. With mean dose to the SVZ being an important variable for the multivariate analysis as the subject of this investigation, the remaining four variables had to be selected. Age, MGMT-methylation and chemotherapy treatment had been identified as having significant prognostic indication on univariate analysis, so were also included in the multivariate analysis. Whilst neither Performance Status nor surgery showed significant impacts on univariate analysis, surgical extent was chosen as the final variable for two reasons. Firstly, together with radiotherapy dose and chemotherapy, surgical extent inclusion would ensure the full multidisciplinary treatment triumvirate is represented in the multivariate analysis. Secondly, surgical extent was the most commonly included covariant in the multivariate analyses seen in the literature, hence its inclusion would permit comparisons with existing studies. The 4 patients for which MGMT-methylation status was unavailable were also excluded from the model, giving a total of 50 patients to be included. The

conservative approach of excluding the patients with missing data from the multivariate analysis was verified by a statistician. Table 5.9. lists the results from this multivariate analysis.

Covariate	HR (95% Confidence)	p-value
Age	*1.070 (1.024-1.117)*	*0.002*
Mean SVZ Dose (Continuous)	0.982 (0.948-1.017)	0.304
Surgical Extent		0.360
Bx-only	Reference Category	
STR	1.075 (0.495-2.334)	0.855
GTR	0.480 (0.154-1.493)	0.205
Chemotherapy Regime		0.138
'Stupp' Regime	Reference Category	
Adjuvant-Only	1.879 (0.401-8.809)	0.423
Concomitant-Only	4.065 (0.384-43.083)	0.244
No Chemotherapy	10.464 (1.051-104.149)	*0.045*
MGMT Methylation		*0.009*
No	Reference Category	
Yes	*0.405 (0.206-0.799)*	*0.009*

Table 5.9. Results from a Cox Regression multivariate analysis. Only MGMT-methylation status and age proved to be significant (italicised and underlined). Mean SVZ dose was found to be not significant.

Increasing age and complete absence of chemotherapy treatment retained their significance as prognostic indicators of poor Overall Survival. MGMT-methylation status also retained significance as an indicator of improved OS. Surgical extent remained non-significant in the analysis, as did the mean dose to the SVZ (HR 0.982 (0.948-1.017), p=0.304).

5.3.8. Survival Analysis II: Specific Literature Comparisons and Gaps

In this section, it should be emphasised that the author recognises that mean SVZ dose and age are continuous variables and that stratifying patients by thresholds within these variables is not statistically rigorous. Dichotimisation of the data is performed purely to enable comparison with existing studies in the literature.

Increasing patient age has been identified on both univariate and multivariate Cox Regression analysis as being of significant survival detriment. In order to complete a specific gap identified in the literature where age thresholds are often employed for survival analysis rather than age being treated as a continuous variable, the patient cohort was divided into two groups either side of a threshold of 55 years of age, a cut-off not seen previously in the literature. Kaplan-Meier survival curves were constructed (figure 5.10) for patients either side of this threshold with a log-rank test showing a lower median OS for patients aged 55 and

above which was found to be statistically significant (14 months versus 38 months, p=0.006). Further age thresholds were investigated in order to make comparisons to other thresholds seen in the literature (table 5.10) with a Šidák correction applied to account for multiple comparisons within the same data (section 5.2.5). In the other thresholds, more than half of the 9-patient cohort aged under 50 were still alive at the time of analysis which prevented an estimate of median OS, though the difference in the curves was reported as statistically significant. The thresholds of 54 and 61 years of age also proved to be statistically significant.
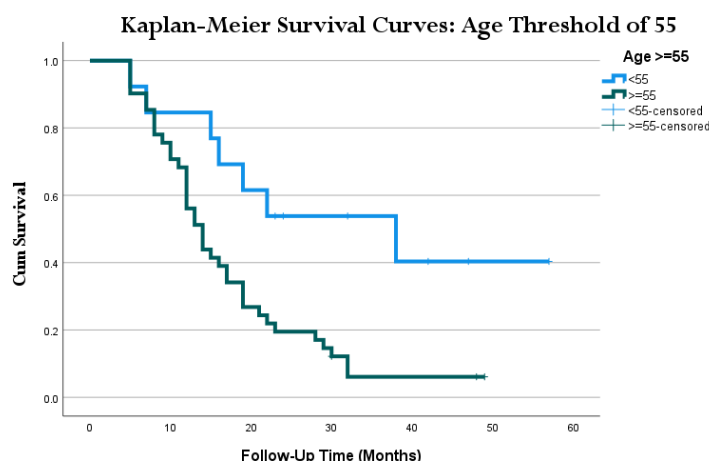
Figure 5.10. Kaplan-Meier survival curves based on patient age at diagnosis with a threshold of 55. Note that Kaplan-Meier analysis not suited to age as a continuous variable, analysis performed for literature comparisons only.

Age	Literature References	Results in this study				p value (<0.008 significance [Šidák])
		< Threshold		≥Threshold		
		Number of Patients (n)	*Median OS (months)*	*Number of Patients (n)*	*Median OS (months)*	
50	Murchison *et al.* (2018) Lee *et al.* (2013) Gupta *et al.* (2012)	9	Not reached	45	14	<0.001
54	Elicin *et al.* (2014)	12	38	42	14	*0.005*
55	[None]	13	38	41	14	*0.006*
60	Khalifa *et al.* (2017)	20	19	34	13	0.058
61	Bender *et al.* (2021)	24	19	30	12	*0.007*
70	Chen *et al.* (2013)	53	15	1	23	0.835

Table 5.10. Kaplan-Meier survival estimates and log-rank comparison tests for a range of age thresholds taken from the literature, and a threshold of 55 not found in previous studies. Šidák correction applied to significance values.

Further literature comparisons were made with patients divided into groups either side of a dosimetric threshold. Kaplan-Meier estimates of OS were calculated for each group and compared using log-rank tests (table 5.11). A Šidák correction was once again applied to adjust the p-value for which statistical significance was recognised.

No statistically significant differences in median OS were observed between any of the groups with p-values < 0.008 being considered significant following the application of the Šidák correction. It should be noted that unlike the earlier Cox Regression Analysis, the effect of prognostic variables such as surgery, age, PS and MGMT-methylation status is not included in this analysis.

Threshold: Mean Dose to Ipsilateral SVZ (Gy)	< Threshold		≥Threshold		p value (<0.008 significance [Šidák])
	Number of Patients (n)	Median OS (months)	Number of Patients (n)	Median OS (months)	
40	9	15	45	16	0.422
50	21	14	33	17	0.873
Median (54.6)	26	15	28	16	0.651
55	28	15	26	13	0.659
57	31	15	23	16	0.618
60	46	15	8	16	0.768

Table 5.11. Comparison of median OS in patients stratified according to the mean dose to the ipsilateral SVZ. Survival curves statistically compared using log-rank Chi square statistics (1 degree of freedom).

5.4. Commentary on Results

Hypothesis 1: A higher mean incidental dose delivered to the SVZ during radiotherapy leads to longer OS in GBM patients.

5.4.1. Completion of Radiotherapy

Unlike many of the studies examined in the literature review in Chapter 2, full details of patient radiotherapy treatments have been provided in this work. In particular, the first part of the study examined radiotherapy treatment records to check that all patients completed their radiotherapy treatment. With 3 out of the 57 patients in the cohort failing to complete their fractionation as planned, it was important that these patients were removed from the subsequent data analysis, as a significant confounding factor on the extent of survival would likely have been the failure to complete the full radiotherapy treatment leading to reduced dose to the CTV. Subsequent survival analysis was limited to the 54 patients who completed

their entire fractionation. Having confirmed that patients completed all 30 planned treatment fractions, planned dose data from the TPS could be used with the confidence that it would closely match the delivered dose.

5.4.2. Patient Demographics

Having performed survival analysis on the final cohort of 54 patients, a number of initial conclusions can be drawn for this part of the study. In regard to patient age, Stupp *et al.* (2005) have previously shown that increasing age is detrimental to survival in GBM. In this study, the majority of patients were aged over 60 (median age of 63) and of those patients that had died the majority were in this age range. On statistical analysis, increasing age was confirmed to be prognostically significant for worse Overall Survival for the patients in this cohort, therefore showing consistency with a well-established prognostic factor for GBM survival. In the survival analyses in this study, age was treated as a continuous variable, an approach taken by only three other studies in the literature in their methodologies (Hallaert *et al.* (2021), Arnalot *et al.* (2017) and Gupta *et al.* (2012)). These three papers actually found that increasing age was not a significant prognostic indicator on univariate analysis, in contrast to this present study which showed increasing age to be detrimental to Overall Survival on univariate Cox Regression with a HR of 1.055 (1.018-1.092), p=0.003. This present study therefore appears to be reasonably unique amongst SVZ studies in finding that increasing age is a significant prognostic indicator for OS when treating as a continuous variable.

Patient sex did not prove to be of prognostic significance for Overall Survival (male versus female HR=1.104 (0.603-2.023), p=0.748) with no significant difference seen between the median OS (14 months versus 19 months, p=0.741). These findings echo those of previous studies including Arnalot *et al.* (2017), Khalifa *et al.* (2017), Elicin *et al.* (2014), Lee *et al.* (2013), Gupta *et al.* (2012) and a recent study from Bender *et al.* (2021) who all found sex to be a non-significant prognostic indicator on Cox Regression univariate analysis. This present study therefore appears consistent with published works in this respect.

5.4.3. Correlation of Dose with Overlap

Mean dose to the ipsilateral SVZ strongly correlated with the degree of overlap of the SVZ with the PTV. Close agreement between these two variables is expected, as a higher dose to the SVZ should be expected if overlapping with the planned radiotherapy target volume. However, as can be seen in the figures, the trend is not linear and reaches a saturation level at the median prescribed dose to the PTV. Again, this saturation behaviour is expected

conceptually, as the mean dose approaches the radiotherapy prescription but would not exceed this in a normal radiotherapy treatment plan where the PTV dose is homogeneously distributed. Using a non-linear regression and curve-fitting techniques, a model for this relationship was estimated and provides a means to estimate the mean dose to the SVZ given the degree of overlap with the PTV. This is significant for potential therapeutic targeting of the SVZ as the required prescription dose for the remaining SVZ can be calculated.

5.4.4. Effect of Surgery: Univariate Analysis

Amongst the wide and inconsistent choices of covariates included in the studies reported in Chapter 2, the extent of patient surgery was common to all papers as a covariate on survival analysis. The widespread inclusion of surgery as a covariate reflects the acknowledged importance that surgical extent has on patient outcomes as verified in the meta-analysis from Brown *et al.* (2016) who found decreased mortality in GTR versus STR up to 2 years post-surgery, plus a decreased likelihood of disease progression at 6 months and 1 year. Despite the reported significance of GTR on patient prognosis, surgical extent was not found to be significant in this present study. There was a trend towards improved OS for patients in this study who had GTR versus biopsy or STR with a median OS of 21 months versus 17 months versus 14 months respectively, however this was not significant ($p=0.219$) and neither STR nor GTR showed as being significant prognostic indicators versus biopsy-alone on Cox univariate analysis. The non-significance of the results in this study could be due to low patient numbers as only 9 patients were recorded as having a GTR. The failure to detect a significant difference on univariate analysis is not unique to this study and previous works including Adeberg *et al.* (2016), Arnalot *et al.* (2017) and Khalifa *et al.* (2017) found similar results with their similar-sized patient cohorts (n=65, 65 and 43 respectively). Interestingly a larger study from Chen *et al.* (2013) of 116 patients also found no significant difference between surgical types. Those studies that did find a significant difference in OS between surgical extents include Hallaert *et al.* (2021), Bender *et al.* (2021) and Murchison *et al.* (2018) which had larger patient cohorts of 137, 200 and 360 respectively. Even with the smallest of these three cohorts (Hallaert *et al.,* 2021) a significant HR for GTR versus biopsy of 0.417 (0.261-0.668) was detected ($p<0.001$, n=137). This present study was perhaps therefore hindered by low patient numbers in failing to detect an effect from patient surgical extent.

5.4.5. Effect of Chemotherapy: Univariate Analysis

Chemotherapy treatment results showed that the majority of patients were recorded as having the full 'Stupp' regime of concomitant and adjuvant temozolomide. A significant difference in median OS was seen between the survival curves for the different regimes and absence of chemotherapy treatment altogether was shown to be significant for poor prognosis, albeit with only 1 patient in this category. Turning to the literature for comparison, chemotherapy was excluded from the survival analysis in all but three studies and comparison is hindered further by inconsistent categorisation. Adeberg *et al.* (2016) used only temozolomide therapy as a category (omitting sub-categorisation by concomitant, adjuvant or a combination) but finding a significant HR of 0.49 (0.27-0.90), p=0.02. In contrast, Arnalot *et al. (*2017) and Murchison et al. (2018) both considered adjuvant and concomitant separately but did not include a category for patients who had both, as in this study. Both of these papers report favourable prognosis with adjuvant chemotherapy. Notwithstanding the differences in categorisation, chemotherapy as a treatment was generally found to be a significant prognostic indicator in all three, in line with the results in this present study, though the categorisation methodology in this present work appears to be fairly unique.

5.4.6. Effect of Performance Status: Univariate Analysis

The majority of patients in the study were PS1 with low numbers of PS0, PS2 and PS3. There was no significant difference in median OS between these patient categories (p=0.96) and Performance Status was not a significant prognostic indicator on Cox univariate analysis. Direct comparison to the literature is made difficult by the almost exclusive use of the KPS for patient Performance Status evaluations in existing studies, a metric which is not utilised in the author's centre. Though the two metrics can be compared to each other, Buccheri, Ferrigno and Tamburini (1996) outline that such a process is not easy and can be prone to error. With this in mind, comparisons of the results in the present study to those in the literature should be treated with caution.

Performance Status was considered in all but two of the studies examined in Chapter 2. Most studies used a KPS of 70 as a threshold to dichotomise their patients where a KPS of >70 approximately equates to an ECOG PS of 0 or 1 (ECOG-ACRIN, 2022). A higher KPS is reported to be a significant indicator of improved prognosis in the larger studies, such as Bender *et al.* (2021) who report significance (p<0.001) without quoting a HR and Murchison *et al.* (2018) who report a difference in median OS of 17.1 months versus 11.3 months

(p=0.001) for KPS>70 versus ≤70. The use of the ECOG scale for categorising PS was found in one paper (Khalifa *et al.*, 2017) who also reported no significant effect on Overall Survival (PS0-1 v PS2-4: 24.7m v 14.6m, p=0.465). Whilst this present study may appear to be limited by patient numbers, as the non-significant findings echo those of the studies with smaller patient numbers such as Elicin *et al.* (2014) and Adeberg *et al.* (2016) with n=60 and 65 respectively, the non-significant findings in the Khalifa *et al.* (2017) study (ECOG scale) was for a much larger cohort of 360 patients. In respect of the effect of PS, findings therefore remain inconclusive.

5.4.7. Effect of Methylation Status: Univariate Analysis

Presence of MGMT-methylation was found to be a significant prognostic indicator in this study (HR 0.510 (0.271-0.959), p=0.037) with a significant improvement in median OS seen in these patients (21 months versus 13 months, p=0.028). These findings are consistent with the landmark findings reported by Stupp *et al.* (2009) who found that patients with methylated MGMT-promoter status had the most benefit from post-operative chemotherapy and radiotherapy. Curiously, despite its widely reported importance in determining GBM OS, univariate analysis of MGMT-methylation status was not performed in all comparable studies to this investigation, with only four such papers found. This is a surprising oversight in the literature given the prognostic significance of MGMT promoter methylation that has also been reported in multiple meta-analyses including Binabaj *et al.* (2017). In detecting a significant prognostic effect of MGMT-methylation, this present work with only 50 patients (54 patient cohort minus the 4 patients with no data on MGMT-methylation) actually reflects the findings of the two larger studies who included it too (Bender *et al.*(2021) and Hallaert *et al.* (2021)). In contrast, the comparably sized cohorts to this investigation of Khalifa *et al.* (2017) and Adeberg *et al.* (2016) both reported non-significant findings.

5.4.8. Effect of SVZ Dose on Overall Survival, Univariate Analysis

Crucially, in Cox Regression univariate analysis, mean SVZ dose was not found to be a significant prognostic indicator, with a non-significant Hazard Ratio of 1.010 (0.982-1.038), p=0.482. Hypothesis 1 (restated below) appears to not hold true for the patients in this study.

Hypothesis 1: A higher mean incidental dose delivered to the SVZ during radiotherapy leads to longer OS in GBM patients.

The consideration of mean SVZ dose as a continuous variable in Cox Regression univariate analysis was found in only two other papers in the literature review which interestingly were two of the more recent published works on the subject (Bender *et al.,* 2021 and Hallaert *et al.,* 2021). Reassuringly, the non-significance of mean SVZ dose as a prognostic indicator found in this investigation when using this methodology is consistent with the results reported in these two papers. Though Bender *et al.* (2021) do not report their Hazard Ratio (only the non-significance of the result, p=0.138), the HR reported above for this investigation is very similar to that from the Hallaert *et al.* (2021) study (1.014 (0.987-1.043), p=0.313). Both the Bender *et al.* (2021) and Hallaert *et al.* (2021) cohorts were much larger than the current investigation (200 and 137 patients respectively), hence the explanation of low patient numbers does not necessarily hold true in this case. More detailed investigations are clearly required and the uncertainties on the effect of radiotherapy to the SVZ remain unresolved for now.

5.4.9. Multivariate Analysis

The multivariate analysis considers how multiple factors influence survival, rather than each individually. The Cox Regression multivariate analysis performed in this study incorporated factors from the other GBM treatment techniques (chemotherapy regime received and extent of surgical resection) alongside the mean SVZ dose. Age and MGMT-methylation status were also included given their significance seen on univariate analysis. In the multivariate model, both age and MGMT-methylation status retained their significance with increasing age giving a HR of 1.070 (1.024-1.117, p=0.002) and methylated MGMT status HR of 0.405 (0.206-0.799, p=0.009). Surgical extent and mean SVZ dose continued to be non-significant prognostic indicators whilst chemotherapy lost significance in this multivariate analysis. The following subparagraphs provide comment on the relative significance of each covariate.

Overall Findings

The overall findings in this study are similar to that of Bender *et al.* (2021) which investigated a much larger patient cohort (n=200) and hence included several other covariates in the model. Their analysis revealed, like this study, that whilst mean SVZ dose (when analysed as a continuous variable) was not a significant prognostic indicator, age and MGMT status were found to be significant, albeit with age incorporated in terms of stratification by age thresholds. Disappointingly the Hazard Ratios are not reported in this paper which prevents direct results comparison, nonetheless the findings in the present study appear to be

reassuringly consistent with this much larger patient cohort in terms of the relative significance of the covariates.

Age

The absence of age as a continuous variable is not unique to the Bender *et al.* (2021) study and most other studies used age stratification thresholds for their data. Nonetheless, there were three other papers found (Hallaert *et al.* (2021), Arnalot *et al.* (2017) and Gupta *et al.* (2012)) who like this investigation incorporated continuous age into their multivariate analyses. The latter two both found age to be non-significant for cohort sizes of 65 and 40 respectively with only Hallaert *et al.* (2021) reporting age as being significant for their 137-patient cohort (HR of 1.034 (1.014-1.054, p=0.001)). This present investigation therefore contributes a rare statistically significant result for the use of age as a continuous variable in an SVZ-related multivariate analysis for Overall Survival.

Dose as a Continuous Variable

In the literature, the Bender *et al.* study is one of only two that could be found that like this present investigation performed a multivariate analysis with mean SVZ dose as a continuous variable. Whilst the more recent paper from Bender *et al.* (2021) report similar findings to this study, with mean SVZ dose being a non-significant prognostic indicator (p=0.512), Gupta *et al.* (2012) actually report a HR of 0.87 (0.77-0.98) with p=0.025 for a smaller cohort of 40 patients. The reasons for this finding are not clear, although interestingly the patients in the Gupta study all had conformal radiotherapy rather than IMRT.

Use of Dosimetric Thresholds

The majority of studies who performed multivariate analysis for Overall Survival did so using dosimetric thresholds to dichotomise the SVZ data, making it difficult to make direct comparisons to the reported results. Moreover, the Bender *et al.* (2021) paper employed multiple dosimetric thresholds in the multivariate analysis to go with their use of dose as a continuous variable, though all thresholds reported non-significant findings. Non-significance is also seen in the Adeberg *et al.* (2016) paper using a 40Gy threshold and in Murchison *et al.* (2018) who used a 60Gy threshold. Significance is seen by Lee *et al.* (2013) who use a 59.4Gy threshold and report a HR of 0.45(0.25-0.82), p=0.009 in the high dose group, but included only sex, age (50 years threshold) and surgery as their other covariates.

MGMT-Methylation Status

Despite its well-renowned prognostic significance, MGMT-methylation status was not included in the multivariate analyses of many studies in the literature. Those that did include this variable once again includes the 2021 studies from Bender *et al.* and Hallaert *et al.* who both reported it as significant as in the case of this present study. The three other papers that incorporated MGMT-methylation status all found it to be non-significant in multivariate analysis (Khalifa *et al.* (2017), Adeberg *et al.* (2016) and Gupta *et al.* (2012)).

Chemotherapy Treatment

Chemotherapy treatment regime as a category lost its significance in the multivariate analysis performed in this investigation, though complete absence of chemotherapy was still seen as being significantly detrimental to prognosis. Only one other paper included chemotherapy in their multivariate analysis for OS: Arnalot *et al.* (2017) reporting a significant HR of 0.11 (0.05-0.24, p=0.000) for patients who received adjuvant chemotherapy. Comparison with the Arnalot *et al.* (2017) study is difficult due to the difference in classification methodology. Whilst Adeberg *et al.* (2016) performed a multivariate analysis that included chemotherapy, this was for PFS only and was not performed for OS. This present study appears fairly unique amongst SVZ studies in its classification methodology for chemotherapy treatments, categorisation patients according to those who received partial, complete or none of the 'Stupp' chemotherapy regime.

Surgical Extent

Surgery was surprisingly not found to have prognostic significance in the multivariate analysis in this investigation, despite the widespread acknowledgement that patients with increasing resection extent have favourable outcomes (Brown *et al.*, 2016). Surgery was included in the multivariate analysis for OS in seven similar studies seen in the literature with divided results. Three papers (Gupta *et al.* (2012), Chen *et al.* (2013) and Adeberg *et al.* (2016)) also all report non-significance of surgery in line with the results of this investigation (p=0.360). There is consistency amongst the remaining four papers as findings universally show that increasing extent of surgical resection is of favourable prognostic indication, though once again these are in much larger patient cohorts than in this study.

Another factor that could affect the interpretation of results is the relative subjectivity of the reported categories, as the definitions of 'Gross' and 'Sub-total' could be interpreted differently. This is a point identified by Karschnia *et al.* (2021) who recognise that inconsistent nomenclature of surgical extent often hinders comparisons between studies and

argue for more objective measures based on percentage of absolute residual tumour volume and relative reduction in tumour volume. Such objective measures were unavailable for the data collection in this study, and patients were categorised according to written information in the surgical notes (see section 5.2.2).

MVA Summary

The inconsistencies in reported findings, inclusion of covariates and methodologies for multivariate analyses on OS were outlined in section 2.4.3 of Chapter 2. Those papers that report significance of mean SVZ dose do so for a relatively limited number of covariates and notably both excluding MGMT-methylation status. This present study is placed into context with the existing literature in table 5.12, entered as the final row of the table for comparison and listing those studies that, like this one, performed a multivariate analysis to assess the effect on OS of ipsilateral SVZ mean dose.

IL SVZ Dose Significant	Dose Continuous or Threshold	Patients	Covariates Included
Gupta *et al.* (2012) HR: 0.87 (0.77-0.98) p=0.025	Continuous	40	Age (continuous), KPS, Surgery
Lee *et al.* (2013) HR: 0.45 (0.25-0.82) p=0.009	>59.4Gy	173	Age (thresholds), Surgery
IL SVZ Dose Non-Significant	Continuous or Threshold	Patients	Covariates Included
Bender *et al.* (2021)	Continuous & Range of Thresholds	200	Age (thresholds), Sex, MGMT, PS, Surgery, SVZ-Contact.
Murchison *et al.* (2018)	60Gy	360	Age (thresholds), Chemo., PS, Surgery.
Adeberg *et al.* (2016)	40Gy	65	MGMT, Chemo., Surgery, PS
[Present Study]	Continuous	50	Age (continuous), MGMT, Chemo, Surgery

Table 5.12. Comparison of included covariates amongst literature reporting significant or non-significance of mean SVZ dose on OS. Abbreviations: Chemo. – Chemotherapy, PS- Performance Status, HR- Hazard Ratio.

The current controversies, inconsistencies and contradictions when it comes to the potential role of SVZ radiotherapy therefore continue to remain unresolved, at least at this point.

5.4.10. Survival Analysis II: Stratification of Patients by Age and Dose Thresholds

The final part of this chapter looked at stratifying patients by age and dose thresholds in line with many of the studies in literature. This analysis was univariate only, did not include the covariates listed above and was performed purely to make comparisons with the same methodologies applied in many papers.

Age Thresholds

Increasing age was already shown to be of prognostic significance for the patients in this study in the Cox Regression analysis performed previously. The threshold-based analysis performed and discussed here was to facilitate comparisons to the literature where age dichotomisation was a common methodology for survival analysis.

The dataset in this study was stratified by a variety of age thresholds ranging from 50 to 70 and estimates of median OS were compared between two groups. Owing to the number of comparisons being made on the same dataset, the p-value for significance was reduced to 0.008 according to the Šidák equation. Significant differences in median OS were observed for thresholds of 50 (as used by several authors), 54 years (as used by Elicin *et al.,* 2014), 55 years (a chosen threshold unique to this current study) and 61 years (as used by Bender *et al.,* 2021). The choice of 55 years of age as a threshold adds to the list of thresholds used in the literature (table 5.13) and indeed shows a marked difference in Kaplan-Meier survival curves as evident in figure 5.10.

Age Threshold	Literature References	Results (below versus above threshold)	
		Literature	This Study
50	Murchison *et al.* (2018)	Not significant	*p<0.001 [<50 median OS not reached]*
	Lee *et al.* (2013)	*>50: HR 1.61 p=0.03*	
	Gupta *et al.* (2012)	*p=0.003 [<50 median OS not reached]*	
54	Elicin *et al.* (2014)	Not significant	*38m v 14m, p=0.005*
55	[None]	-	*38m v 14m p=0.006*
60	Khalifa *et al.* (2017)	Not significant	Not significant
61	Bender *et al.* (2021)	Not significant	*19m v 12m p=0.007*
70	Chen *et al.* (2013)	Not significant	Not significant

Table 5.13. Comparison of results from current study versus literature for 6 different age thresholds used for data dichotomisation. Abbreviation: m-months. Non statistically significant results not reported. 95% confidence interval on HR omitted for clarity. Significant results in bold and italics.

Dose Thresholds

Finally, stratification was performed on patients according to the mean dose to the ipsilateral SVZ either side of a threshold in order to facilitate comparisons with existing studies in the literature that were reported on in Chapter 2. No significant difference was observed between Overall Survival in either group for a variety of thresholds, echoing the findings of the Cox Regression analysis that SVZ mean dose does not appear have a significant impact on Overall Survival.

The absence of a correlation between SVZ dose and Overall Survival echoes the findings of a large number of other retrospective studies including Slotman *et al.* (2011), Sakuramachi *et al.* (2015), Comas *et al.* (2016), Khalifa *et al.* (2017), Weinberg *et al.* (2018), Murchison *et al.* (2018), Bender *et al.* (2021) and Hallaert *et al.* (2021). Most of these studies had similar patient numbers to the present study, ranging from 40 (Slotman study) to 74 (Sakuramachi study) but even the much larger cohorts of the Hallaert (139), Bender (200) and Murchison (370) studies showed no significant differences between their groups.

As seen in the literature review in Chapter 2, the effect of radiotherapy dose to the SVZ is a contentious topic and in contrast to the studies listed above which found, like this one, that

there was no significant difference in Overall Survival for groups stratified by dose, there are some that champion the effect on survival of a higher ipsilateral SVZ dose. Of some interest is a similar sized study from Ravind, Prameela and Dinesh (2015) who did report that Overall Survival in a cohort of 50 patients was markedly improved for doses higher than 50Gy to the ipsilateral SVZ. The survival differences are quite stark between groups, being 19.8 months versus 6.0 months with p=0.031, though it is not clear whether the 50Gy dichotomising threshold is the maximum, mean or median dose to the ipsilateral SVZ. Lee *et al.* (2013) and Mathew *et al.* (2018) report similar improvements for almost identical thresholds of 59.4Gy and 56Gy respectively, though in neither case are the findings significant (p=0.173 and p=0.116 respectively).

Though disappointing, it should be emphasised that these results show only non-significance of SVZ dose with respect to Overall Survival, rather than showing a significant detriment. This is in contrast to the Chaudry and Goenka study (2018) who found that a dose > 60Gy to the anterior temporal SVZ led to a significantly worse Overall Survival at 6 months (37% versus 73%, p=0.01) and Blumenfeld *et al.* (2017) who reported worse Overall Survival for mean ipsilateral SVZ dose >57.8Gy albeit not significant (14.5 months versus 19.4 months, p=0.06).

The findings of this study would be further strengthened with the inclusion of toxicity data to aid the comparisons. It is possible that though no difference in survival was seen, there could be a difference in neuro-cognitive side-effects experienced by patients, something that studies including Gui *et al.* (2020) and Valiyaveetil *et al.* (2020) advise as a significant caveat to potential SVZ radiotherapy.

5.5. Chapter Summary

This chapter has sought to address the first hypothesis of this project by investigating if the mean dose to the ipsilateral SVZ has a significant effect on patient Overall Survival. Dosimetric data for the SVZ has been acquired and analysed for the study cohort and a relationship has been derived between the degree of SVZ overlap with the PTV and the mean SVZ dose. Information on key prognostic variables such as age, Performance Status, surgery, chemotherapy and MGMT-methylation status has also been collected. On univariate analysis, it was found that increasing patient age, absence of chemotherapy treatment and absence of MGMT-promoter methylation were detrimental prognostic indicators for Overall Survival, however mean SVZ dose showed no significant impact. Incorporation of multiple covariates

in a Cox multivariate analysis showed similar findings as age, MGMT-methylation status and absence of chemotherapy treatment retained their prognostic significance whilst mean SVZ dose remained non-significant. Both age and mean SVZ dose were entered as continuous variables in the analysis, a method that has been seen very rarely in the reported literature to date. Finally, dataset stratification by age and dosimetric thresholds was also performed to make comparisons with the more commonly used methodologies in the existing literature. Increasing age continued to be shown as detrimental to Overall Survival using the threshold method, whilst mean SVZ dose continued to show no significant impact.

In terms of potential for supporting the active inclusion of the SVZ in radiotherapy treatment, there is very little evidence at least at this stage, for irradiating the SVZ to improve patient Overall Survival. The next phase of the project will aim to investigate if there is a potential survival benefit depending on the specific characteristics of the tumour and in particular, the proximity and invasive properties of the tumour with respect to the SVZ.

6. Investigating the Effect of Tumour Location and Invasive Properties on Overall Survival in GBM

In Chapter 5, it was found that the incidental mean dose delivered to the ipsilateral SVZ during radiotherapy for GBM seemingly had no significant impact on patient Overall Survival, as multivariate analysis revealed this variable to be a non-significant prognostic indicator. The survival analysis performed in the chapter used only the dosimetric data obtained from the TPS DVH together with information on the prognostic covariables of age, MGMT-methylation status, Performance Status, chemotherapy treatment and surgical extent. The anatomical location and invasive properties of the tumour were not specifically included in the analysis.

Several authors including Chaichana *et al.* (2008) and Adeberg *et al.* (2014) have identified a prognostic significance of tumour location with respect to the SVZ, suggesting that those patients whose tumours contacted the SVZ at presentation had poorer survival outcomes. However, the anatomical location of the tumour with respect to the SVZ cannot be derived from the analysis performed so far in Chapter 5. In radiotherapy planning, the relationship between GTV (the enhancing tumour seen on imaging) and CTV (the final Clinical Target Volume used to expand to the PTV) is not consistent for all patients, as the CTV can be individually tailored by the oncologist based on the anatomical boundaries and routes of presumed tumour spread. As a result of inconsistent CTV definition between patients, the degree of PTV overlap with the SVZ does not necessarily correlate with the proximity of the tumour (GTV) with respect to the SVZ and should not be used as a surrogate for tumour anatomical location. Classifying patients by their individual disease anatomy therefore requires further work involving radiological interpretation of tumour location. This chapter describes the work performed to investigate the significance of tumour location with respect to the SVZ on Overall Survival for the patients in this study cohort and addresses the question posed by hypothesis 2:

Hypothesis 2: Tumour location with respect to the SVZ has a significant impact on OS in GBM patients.

Furthermore, rather than just tumour proximity, this work will also specifically examine the invasive properties of the tumour with respect to the SVZ by classifying patients in the study according to these radiological features.

As outlined in the literature review of Chapter 2, the classification of tumour location with respect to the SVZ has been performed by several other studies. The majority of these studies employed a classification methodology first proposed by Lim *et al.* (2007), in which patients are assigned one of four groups according to the radiological presentation with respect to the SVZ and cerebral cortex (figure 2.3).

This study goes one step further in its patient classification, defining a 'Modified-Lim' criteria as a novel classification methodology by introducing two additional categories according to the invasive property of the tumour with respect to the SVZ. Under this novel classification system, the survival analysis performed in Chapter 5 is revisited to include tumour location as classified by this methodology whilst also incorporating the dosimetric information already obtained. The aim of this chapter is therefore to provide a more complete picture of the effect of both incidental SVZ dose and tumour proximity to SVZ on survival outcomes in GBM, whilst also accounting for the other known prognostic variables.

Inclusion of SVZ invasion as a further criterion for classification is based on a hypothesised theory from Lombard *et al.* (2021) that glioma stem cells seek refuge from radiotherapy treatment by migrating to the SVZ-niche. A reduced physical distance from the SVZ would clearly benefit GSCs seeking sanctuary and may explain the reduced survival seen in those patients contacting SVZ at presentation. In this study, the author hypothesises that tumour invasion of the SVZ rather than merely contacting, further aides this process and may be of prognostic significance by the same theory.

In order to perform this study, as the author of this thesis has no radiological qualifications, an experienced consultant radiologist was asked to perform the classification. This chapter follows the format of its predecessor with a methodology section preceding the results and an interim commentary on the findings.

6.1. Methodology

This section of the study took place in parallel to the work performed in Chapter 5. The original four Lim groups were used as a basis for the new categories. For tumours contacting the SVZ (Groups 1 and 2), two further groups were proposed to sub-categorise these patients based on whether the contact included macroscopic invasion of the SVZ as well (based on imaging characteristics) to form groups 1A and 2A, with those that only contacted but did not invade the SVZ being categorised in groups 1B and 2B. The additional groups to include SVZ invasion status were added to those proposed by the Lim study to form the 6 group categories

used for the classification in this work. Table 6.1 defines the six categories with illustrative examples of each provided in figure 6.1.

Modified Lim Criteria	Group 1A	Group 1B	Group 2A	Group 2B	Group 3	Group 4
GTV Contacting SVZ	Yes	Yes	Yes	Yes	No	No
If Contacting SVZ − Invading SVZ also?	Yes	No	Yes	No		
GTV Contacting cerebral Cortex	Yes	Yes	No	No	Yes	No

Table 6.1. Definitions of the novel 'Modified-Lim' classification criteria for the patients in this study, modified from the original criteria proposed by Lim et al. (2007).

An experienced consultant radiologist was asked to examine each patient's pre-operative MRI sequences and assign the patient to one of the six groups based on the location of the enhancing tumour with respect to the SVZ and the cerebral cortex. The radiologist was asked to select the most appropriate imaging sequence to make their decision rather than stipulating a required sequence. The radiologist was blinded to the outcomes of the patients and to the results from the previous work analysed in Chapter 5. Invasion of the SVZ was decided based only on the macroscopic imaging features and defined as macroscopic involvement of the ventricular ependymal surface. Microscopic tumour physiology was not available for this part of the study. Data was recorded to a database within Microsoft Excel (Microsoft Office, 2016).

Group 1A Tumour
[SVZ Contact + Invasion, Cortex Contact]

Group 2A Tumour
[SVZ Contact + Invasion, No Cortex Contact]

Group 3 Tumour
[Cortex Contact Only]

Group 1B Tumour
[SVZ Contact, no invasion, Cortex Contact]

Group 2B Tumour
[SVZ Contact, No SVZ Invasion, No Cortex Contact]

Group 4 Tumour
[Contact with neither SVZ or Cortex]
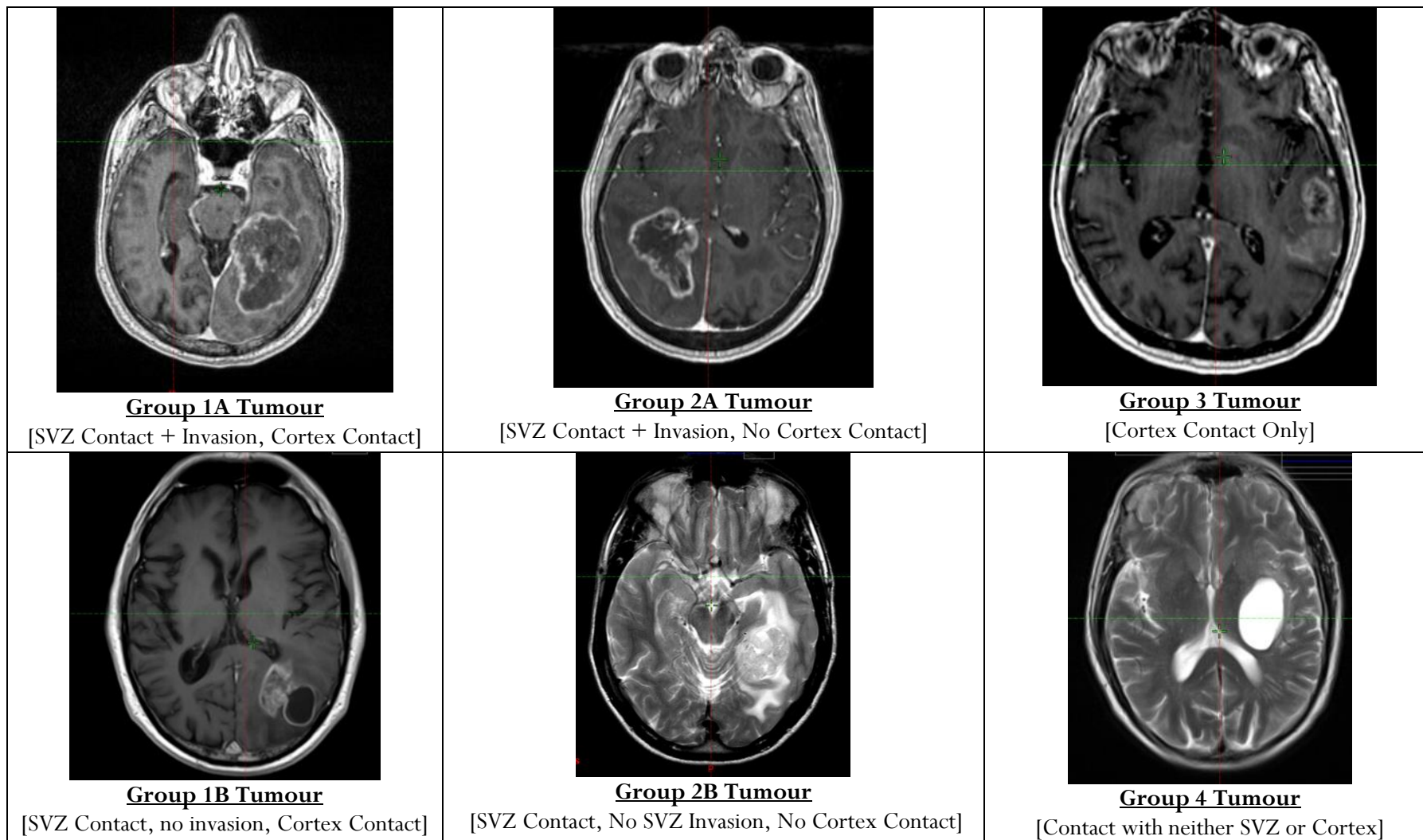
Figure 6.1. Examples of tumours categorised according to the novel 'Modified-Lim' criteria.

Once classification was completed, the database was returned to the author of this thesis to perform the analysis which involved merging the group classification database with patient follow-up data gathered during the work of Chapter 5. Sequential comparisons were made between patients categorised according to four classifications:

1) The original 'Lim' criteria.
2) The novel 'Modified Lim' criteria.
3) Tumour contact with the SVZ
4) Tumour invasion of the SVZ

Categorisation according to criteria (1), (3) and (4) was performed by combining patients categorised by the radiologist according to the Modified Lim classification into new categories. The radiological classification from the Radiologist was only performed once.

Statistical analysis of Overall Survival was again performed using SPSS (v28.0.0.0) with the same methodology employed for calculating follow-up time as used in Chapter 5 (date of analysis being 1st June 2021). Those still alive at the time of analysis were censored from the Overall Survival calculations. Kaplan-Meier survival analysis was performed to compare the median OS between the different groups with log-rank tests used to test for statistical significance between the survival curves of each group. Cox Regression univariate analysis was performed to assess the relative prognostic significance of each group, with Hazard Ratios reported together with 95% confidence intervals. Significance was considered for p-values <0.05. Cox Regression multivariate analysis was performed to include the dosimetric data for mean dose to the ipsilateral SVZ from Chapter 5 together with the other prognostic covariables.

6.2. Results

6.2.1. Patient Database and Exclusions

The entire original 57-patient cohort was analysed by the radiologist. The three patients excluded from the analysis in Chapter 5 were also excluded from this data analysis due to failure to complete their radiotherapy fractionation. Of the remaining 54 patients (those analysed in Chapter 5), 13 more were excluded due to a lack of available imaging, whilst a further 2 were excluded as their tumour was non-enhancing and thus difficult to accurately categorise. The final cohort for this part of the study was 39 patients.

6.2.2. Original Lim Criteria Classification

Kaplan-Meier survival analysis estimated the median OS for the entire cohort to be 15 months. In order to make comparisons to other studies, analysis was first performed by recombining groups 1A and 1B and 2A and 2B in to groups 1 and 2 respectively according to the original Lim criteria. Categorising patients this way yielded similar median OS calculations for all groups (table 6.2) with no statistically significant differences between the groups (figure 6.2, p=0.906).

Group Number	Tumour Contact Points (SVZ/Cerebral Cortex)	Number of Patients	Median OS (Months)
Original Lim Groups p=0.906			
1	SVZ + Cerebral Cortex	15	16
2	SVZ Only	7	17
3	Cerebral Cortex Only	12	14
4	None	5	13

Table 6.2. Kaplan-Meier estimates of median OS between patients categorised by Lim criteria into one of four groups.
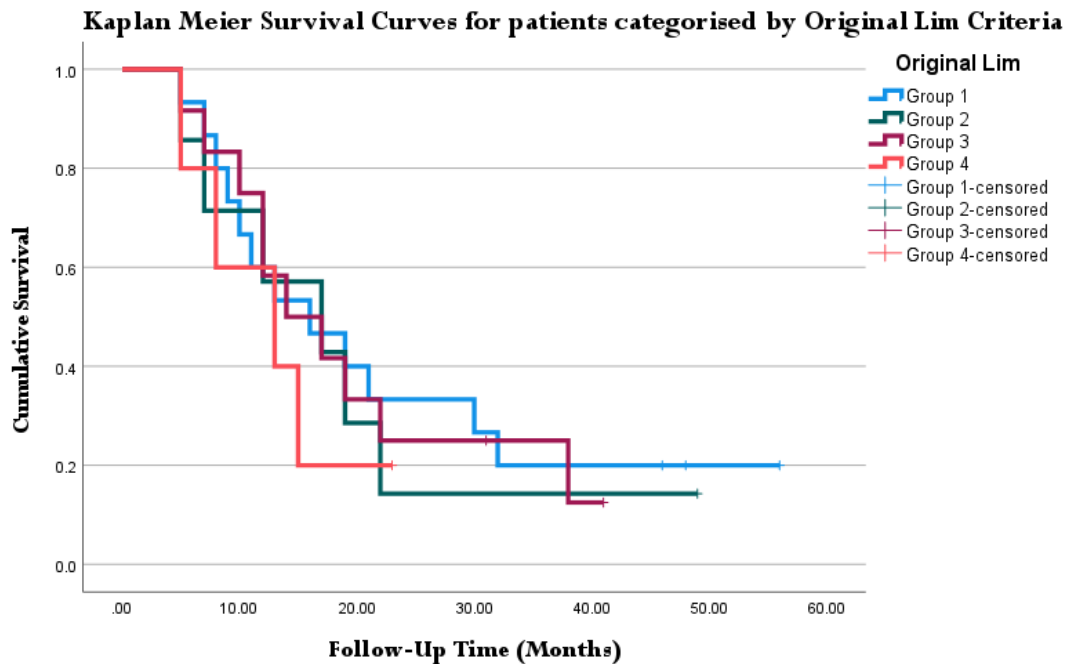
Figure 6.2. Comparison of median OS between patients categorised according to original four-group Lim criteria. No significant difference seen in median OS between the four groups.

Cox Regression univariate analysis was performed to generate Hazard Ratios for each group with group 1 being the reference group. None of the groups were seen to be of prognostic significance for OS (table 6.3).

Covariate	HR (95% Confidence)	p-value
Original Lim Group		0.914
1	Reference Category	
2	1.155 (0.432-3.086)	0.774
3	1.050 (0.453-2.432)	0.910
4	1.503 (0.477-4.731)	0.486

Table 6.3. Cox Regression univariate analysis for original Lim criteria group classification.

Cox Regression multivariate analysis was performed to incorporate the impact of dose and the prognostic variable data obtained from Chapter 5. Of the 4 patients with absent MGMT-methylation data, 1 had already been excluded with the earlier exclusion criteria. The remaining 3 were also excluded from this Cox model to give a further reduced cohort of 36 patients. With this reduced cohort, only a maximum of 4 covariates could be included in the analysis. Together with group classification and dose (as the key variables in this investigation), age and MGMT-methylation were selected as the other covariates due to their consistent significance seen in the analysis in Chapter 5. Likewise, surgical extent and Performance Status were omitted due to their non-significance on survival seen in Chapter 5. Unfortunately to achieve the maximum of 4 covariates, chemotherapy treatment also had to be omitted as a variable in this analysis. Age and MGMT-methylation continued to show prognostic significance for OS whilst mean SVZ dose continues to be non-significant (table 6.4) as found in Chapter 5. Classification by the original Lim groups did therefore not prove to be a significant prognostic indicator.

Covariate	HR (95% Confidence)	p-value
Original Lim Group		0.702
1	Reference Category	
2	0.809 (0.292-2.242)	0.683
3	1.049 (0.341-3.224)	0.934
4	1.799 (0.531 – 6.098)	0.346
Mean SVZ Dose (Continuous)	0.980 (0.934-1.029)	0.423
Age	*1.070 (1.024-1.117)*	*0.002*
MGMT Methylation		0.014
No	Reference Category	
Yes	*0.365(0.163-0.816)*	*0.014*

Table 6.4. Cox Regression multivariate analysis for original Lim criteria group classification.

6.2.3. Modified Lim Criteria Classification

Categorising patients by the 'Modified-Lim' criteria introduced by this study did show some more marked, though not statistically significant differences in OS (figure 6.3), therefore providing some justification for the usage of this novel methodology. Though there was no

significant difference in OS between the groups when analysed as a whole cohort (p=0.424, table 6.5), there was a noticeably reduced median OS in patients from group 1A, being only 9 months. Patients contacting but not invading the SVZ (groups 1B and 2B) had noticeably higher overall median OS (21 and 22 months respectively) though there was only 1 patient in group 2B.
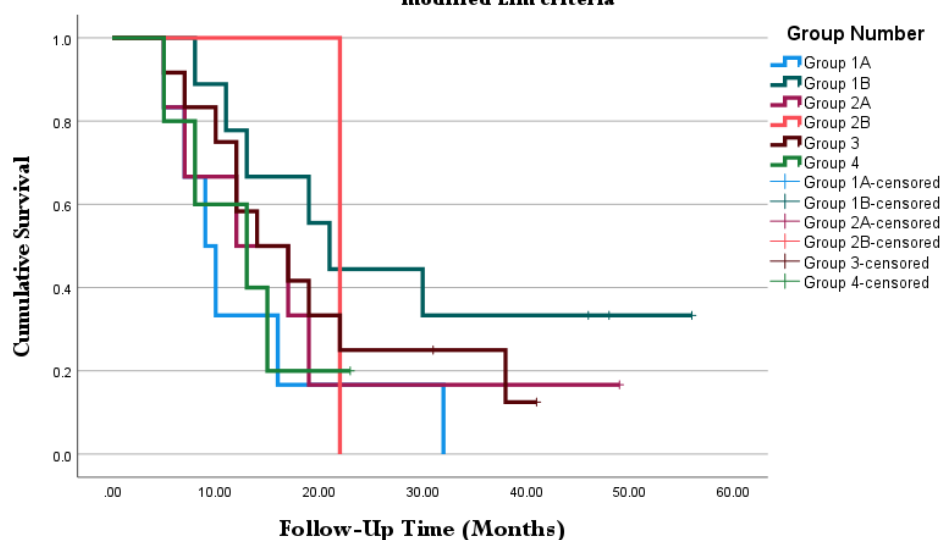
Figure 6.3. Comparison of median OS between patients categorised by the novel 'Modified-Lim' criteria into one of six groups.

Group Number	Tumour Contact Points (SVZ/Cerebral Cortex)	Invading SVZ?	Number of Patients	Median OS (Months)
Modified Lim Groups p=0.424				
1A	SVZ + Cerebral Cortex	Yes	6	9
1B	SVZ+ Cerebral Cortex	No	9	21
2A	SVZ Only	Yes	6	12
2B	SVZ Only	No	1	22
3	Cerebral Cortex Only	N/A	12	14
4	None	N/A	5	13

Table 6.5. Kaplan-Meier estimates of median OS between patients categorised by the novel 'Modified-Lim' criteria into one of six groups.

Cox Regression univariate analysis was performed (table 6.6) in order to compare the Hazard Ratios between the different group classifications. Compared to group 1A tumours, those in group 1B had a significant survival advantage (HR 0.312 (0.099-0.985), p=0.047) showing that those patients whose tumours that contacted both SVZ and Cortex, but did not invade

the SVZ, survived longer. On univariate analysis at least, the potential significance of SVZ-invasion on prognosis is therefore highlighted as an area of interest.

Covariate	HR (95% Confidence)	p-value
Modified Lim Group		0.490
1A	Reference Category	
1B	*0.312 (0.099-0.985)*	*0.047*
2A	0.595 (0.18-1.968)	0.395
2B	0.401 (0.048-3.388)	0.402
3	0.501 (0.180-1.391)	0.185
4	0.729 (0.204-2.605)	0.627

Table 6.6. Cox Regression univariate analysis for the 'Modified-Lim' criteria group classification proposed by this study.

Multivariate analysis was performed with once again dose, age and MGMT-methylation included as the other covariates (table 6.7). The significance of group 1B tumours unfortunately was lost on multivariate analysis and only MGMT-methylation and age showed significant impacts on survival with mean SVZ dose remaining non-significant.

Covariate	HR (95% Confidence)	p-value
Modified Lim Groups		0.921
1A	Reference Category	
1B	0.976 (0.234-4.069)	0.974
2A	0.773 (0.205-2.921)	0.705
2B	0.929 (0.093-9.284)	0.950
3	1.031 (0.219-4.852)	0.969
4	1.769 (0.388-8.074)	0.461
Mean SVZ Dose (Continuous)	0.980 (0.932-1.031)	0.436
Age	*1.091 (1.026-1.159)*	*0.005*
MGMT Methylation		0.033
No	Reference Category	
Yes	*0.370 (0.148-0.924)*	*0.033*

Table 6.7. Cox Regression multivariate analysis for the 'Modified Lim' criteria group classification proposed by this study.

6.2.4. SVZ-Contact Tumour Classification

Further groupings were performed to assess the effect on survival of SVZ-contact versus non-contact to permit literature comparisons. No significant difference in median OS was seen between contact and non-contact groups (table 6.8) and tumour contact with SVZ was not a significant hazard on Cox Regression univariate analysis (table 6.9). Age and MGMT status continued to be the only significant variables on multivariate Cox Regression (table 6.10).

Covariate	Number of Patients	Median OS (months)
Contact v non-Contact p=0.794		
Non-Contacting	17	14
Contacting	22	16

Table 6.8. Kaplan-Meier estimates of median OS between SVZ-contact and non-contact groups.

Covariate	HR (95% Confidence)	p-value
Contact v non-Contact		0.156
Non-Contacting	Reference Category	
Contacting	1.701 (0.816-3.545)	0.156

Table 6.9. Cox Regression univariate analysis for the SVZ-contact classification methodology.

Covariate	HR (95% Confidence)	p-value
Contact v Non- Contact		0.470
Non-Contacting	Reference Category	
Contacting	0.714 (0.287-1.779)	
Mean SVZ Dose (Continuous)	0.987 (0.942-1.034)	0.573
Age	*1.087 (1.029-1.148)*	*0.003*
MGMT Methylation		
No	Reference Category	
Yes	*0.356 (0.159-0.794)*	*0.012*

Table 6.10. Cox Regression multivariate analysis incorporating the SVZ-contact group classification methodology.

6.2.5. Invasive Tumour Classification

Cox Regression univariate analysis of the Modified Lim groups in section 6.2.3. showed the potential prognostic significance of SVZ invasion as group 1B tumours showed a statistically significant improved prognosis versus group 1A tumours, albeit a relationship that was lost on multivariate analysis. In order to investigate this further, patients were further grouped by the criteria of tumour invasion of the SVZ: combining groups 1A and 2A to form an SVZ-Invasive group (termed SVZ++) which was compared to the non-invasive groups (terms SVZ--). Though there was a visual difference in survival curves (figure 6.4), this was found to be not significant (table 6.11) and SVZ-invasion was not prognostically significant on univariate analysis (table 6.12).

Covariate	Number of Patients	Median OS (months)
Invasive v Non Invasive p= 0.141		
Non-Invasive	27	17
Invasive	12	10

Table 6.11. Kaplan-Meier estimates of median OS between Non-Invasive and Invasive tumour groups.

Covariate	HR (95% Confidence)	p-value
Invasive v Non-Invasive		0.156
Non-Invasive (SVZ--)	Reference Category	
SVZ-Invasive (SVZ++)	1.701 (0.816-3.545)	0.156

Table 6.12. Cox Regression univariate analysis for the invasive tumour group classification proposed by this study.
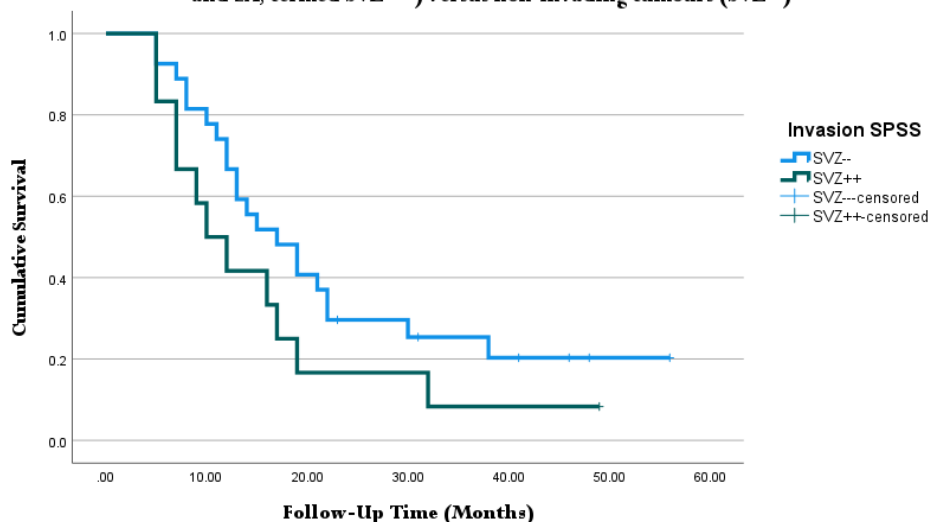
Figure 6.4. Comparison of median OS between patients by status of tumour SVZ invasion. Tumours invading the SVZ termed SVZ++, those with no SVZ invasion are SVZ--. Difference in median OS 10 months (SVZ++) versus 17 months (SVZ--), p=0.141.

The multivariate analysis that followed (table 6.13) showed that the trend towards significance of invasion was lost when adjusting for the other covariates and highlighting that age and MGMT-methylation continued to be the only statistically significant prognostic variables as dose once again proved to be non-significant.

Covariate	HR (95% Confidence)	p-value
Invasive v Non-Invasive		0.561
Non-Invasive (SVZ--)	Reference Category	
SVZ-Invasive (SVZ++)	0.744 (0.274-2.019)	0.561
Mean SVZ Dose (Continuous)	0.983 (0.941-1.028)	0.464
Age	*1.094 (1.031-1.160)*	*0.003*
MGMT Methylation		0.015
No	Reference Category	
Yes	*0.344 (0.145-0.814)*	*0.015*

Table 6.13. Cox Regression multivariate analysis for the invasive tumour criteria group classification proposed by this study.

The results presented in the previous sections highlight that whilst SVZ-invasion may have some potential prognostic relevance for OS (seen by the prognostic significance of group 1B versus group 1A in table 6.7), this was not shown to be significant in the current cohort when adjusted for the other covariates. The low patient numbers in this cohort could well be a factor in not revealing a statistically significant result and future studies would require a much larger cohort to verify any trends potentially revealed. In the current data, only age and MGMT-methylation status proved to be of prognostic significance for OS.

6.3. Commentary on Results

Hypothesis 2: Tumour location with respect to the SVZ has a significant impact on OS in GBM patients.

The results in this chapter highlight that at present there is no evidence to suggest that tumour location has a significant prognostic impact for the data in this cohort. Despite there being an apparent prognostic significance for OS of group 1B tumours versus group 1A tumours on univariate analysis, this was lost when adjusted for other covariates. Nonetheless, the findings described above in relation to tumour invasion of the SVZ were only discovered following the introduction of the two additional groups that formed the novel 'Modified-Lim' criteria. The introduction of this novel classification methodology has therefore provided a potential area of renewed research focus in the future.

6.3.1. Tumour Classification by Lim Criteria

Categorising the patients in this study firstly by only the original Lim criteria showed similar median OS estimates across all four groups (table 6.3), ranging from 13 months to 17 months with a median OS for the cohort being 15 months. Hazard ratios computed for each group showed no statistically significant findings as only age and MGMT-methylation status proved to be of prognostic significance on multivariate analysis. These findings agree well with the recent study from Comas *et al.* (2021) who also reported no significant differences in OS between the Lim groups (p=0.276) and likewise finding that age and MGMT-methylation were significant covariates on the multivariate analysis together with concomitant TMZ which was included in their study (p=0.007). The consistency of findings in this investigation compared to the much larger Comas *et al.* (2021) study of 133 patients is reassuring.

6.3.2. Justification for the Modified Lim Criteria

With the original Lim criteria showing no survival differences, it is only through the application of the full novel 'Modified-Lim' criteria to the analysis that some survival differences started to appear between the groups. Of particular note is a difference in median OS between groups 1A and 1B (9 months versus 21 months) with Group 1B tumours being shown to have statistically better prognosis than group 1A on Cox Regression univariate analysis (HR 0.312 (0-099-0.985), p=0.047). The distinction between patients in group 1A and 1B is tumour invasion of the ipsilateral SVZ and the observed statistically significant improved prognosis of 1B versus 1A, suggests that patient Overall Survival is potentially compromised by an invasion of the SVZ. However, when adjusting for other covariates in the multivariate analysis, this significance was lost and only age and MGMT-methylation status were seen to be of prognostic significance. No other similar works could be found in the literature that classified tumours based on macroscopic invasion and this methodology appears to be unique to this study. More work is needed to verify these suggested findings.

6.3.3. Tumour Classification by SVZ-Contact

Further analysis of the data involved combining the groups to assess the effect of SVZ-contact on Overall Survival. This is an approach employed by many other studies in the literature and there appears to be widespread agreement that contact with the SVZ at presentation is detrimental to patient Overall Survival. The largest study of this type (Adeberg *et al.,* 2014) classified 607 GBM patients according to the Lim criteria and found a significant difference in Overall Survival in the SVZ-contacting groups 1 and 2 (12.3 months) versus the non-contact groups 3 and 4 (16.3 months) with p<0.001. These findings in a large retrospective study are well supported by more recent studies from Yamaki *et al.* (2020), Mistry *et al.* (2020), Hallaert *et al.* (2020) and Comas *et al.* (2021). In this present study, combining groups 1A, 1B, 2A and 2B formed a 'SVZ-Contact' cohort whose median OS was compared to the combination of groups 3 and 4 ('SVZ Non-Contact') in the same way as the previous studies. Despite a consistent methodology, no significant difference in median OS was found between the SVZ-contact and non SVZ-contact group combinations for the current study (16 months versus 14 months, p=0.794). It should be recognised that the Adeberg study had a large cohort of 607 patients with patient numbers in the other studies ranging from 133 (Comas study) to 502 (Mistry study). In this present work, with the application of several exclusion criteria, the patient cohort was limited to 39 patients and therefore much lower than the

cohorts in the literature. The absence of a significant difference in median OS in this present study could be attributed to these lower patient numbers. Given the resource limitations of this work, attaining such high patient numbers was beyond the capabilities of this present project but is a consideration for future work.

Many of the existing papers do not perform a multivariate analysis to include the effect of prognostic covariates on their results, as verified by the meta-analysis from Mistry *et al.* (2017a). Examining a selection of the papers that did and including the present study for comparison (table 6.14) shows that this study appears to stand out from the majority of the literature by including SVZ dose in the multivariate analysis of tumour location. The findings in the present study are similar to the most recent of these from Comas *et al.* (2021) which revealed that only the covariates of MGMT-methylation, concomitant chemotherapy and age were significant as SVZ-contact lost significance on multivariate analysis.

Study	Patients	SVZ-Contact Significant	Covariates Analysed	Significant Covariates
[Present Study]	36	No	Age, MGMT, Dose	Age, MGMT
Comas *et al.* (2021)	133	No	Age, MGMT, KPS, Chemo., Surgery	Age, MGMT, Concomitant TMZ, STR v Bx.
Hallaert *et al.* (2020)	214	Yes p=0.017	Age, MGMT, KPS, Surgery	All, including SVZ-contact p=0.035
Mistry *et al.* (2017b)	207	Yes p<0.001	Age, KPS, Chemo., Surgery	All, including SVZ-contact p=0.006

Table 6.14. Studies in the literature that included multivariate analysis of prognostic variables together with the effect of SVZ-contact on OS. Abbreviation: Chemo. – Chemotherapy, TMZ – Temozolomide, MGMT – MGMT-methylation status. Inclusion of dose in present study is highlighted.

6.3.4. Tumour Classification by SVZ-Invasion: A Novel Concept

Despite the abundance of evidence on the significance of SVZ-contact on patient survival, the application of the 'Modified-Lim' criteria to distinguish for SVZ-invasion and the subsequent survival analysis performed appears to be a novel concept with no similar studies found in the literature. Following the apparent significance of SVZ-invasion seen when comparing groups 1A and 1B, patients in groups 1A and 2A combined to form an 'SVZ Invasion' group which were compared to the combination of the remaining groups. This comparison showed a marked, albeit not statistically significant reduction in median OS (10 months versus 17 months, p=0.141) in the SVZ-invasion group. Again, the power of this study may be limited by the low patient numbers in the cohort, however these results together with the earlier

discussion on the difference between Group 1A and Group 1B patients do suggest a potential prognostic significance of SVZ invasion. This trend was lost on multivariate analysis as only age and MGMT-methylation showed prognostic significance. No other studies could be found in the literature which considered invasion (as opposed to contact) on patient prognosis and certainly none that included it as a multivariate covariate. Furthermore, the inclusion of SVZ dose together with SVZ-invasion plus age and MGMT-methylation as covariates appears to be unique to this present study.

6.3.5. Effect of Surgery

Those tumours invading the SVZ are by definition the least accessible for surgical resection. With extent of surgical resection being a significant prognostic factor to patient Overall Survival (Li *et al.*, 2016), the poorer survival outcomes for patients in Group 1A could be attributed to sub-total resection at surgery, though as identified in Chapter 2: surgical resection of the ventricles remains a controversial topic. Examining the mean post-operative tumour size, defined by the volume of the GTV structure measured in the Eclipse TPS, Group 1A patients did indeed have the highest mean post-operative GTV size of the cohort (53.6cc), implying that these tumours were most likely to be sub-totally resected in this cohort. Though the difference to the mean tumour size of the other patients was not statistically significant (53.6±30.8cc versus 34.8±31.0cc, p=0.212), this does imply that extent of surgical resection needs to be considered further for this analysis.

Unfortunately, surgical extent was excluded from the multivariate analyses in this chapter as the number of covariables had to be restricted to 4 due to the low numbers of events in the cohort. However, in Chapter 5 it was seen that for this cohort of patients, extent of surgery was not a significant prognostic indicator for OS and its inclusion in the multivariate analysis in this chapter (which analyses a sample of the same patient database as Chapter 5) is therefore unlikely to have revealed significance in this Chapter. Future research on a larger patient cohort should permit inclusion of surgical resection categorisation into the multivariate analyses.

6.3.6. More Data Needed? Future Areas of Research Focus

With SVZ-invasion potentially having some prognostic significance, not least due to the inaccessibility for surgical resection, the effect of radiotherapy for improving patient outcomes in this subset of patient gains renewed importance. In the analysis presented in this work, there are currently no findings to support such a strategy as SVZ dose and SVZ-

invasion proved to be non-statistically significant on multivariate analysis. At present, the only significant prognostic indicators in this cohort were age and MGMT-methylation status. Including the ipsilateral SVZ as a target in those patients with invasive tumour in the SVZ is therefore not suggested by this work and would require more retrospective data analysis and an increase in patient numbers.

The results in this current work do at least present a potential new focus for future research, as rather than simply repeating the same analysis for greater numbers of patients, a potentially interesting importance of SVZ-invasion has been revealed. An illustration of this potential trend is shown in figure 6.5, where those patients with SVZ-invasive tumours and who had a higher mean SVZ dose, appeared to survive longer than those with a dose less than 50Gy. This trend is not statistically significant and clearly this figure should be interpreted with caution given the low patient numbers, the use of a dosimetric threshold rather than as a continuous variable and the absence of prognostic covariates, but it does suggest a potentially promising area of future research: multivariate analysis of a larger patient cohort with respect to dose to the SVZ and SVZ-invasive properties of the tumour.
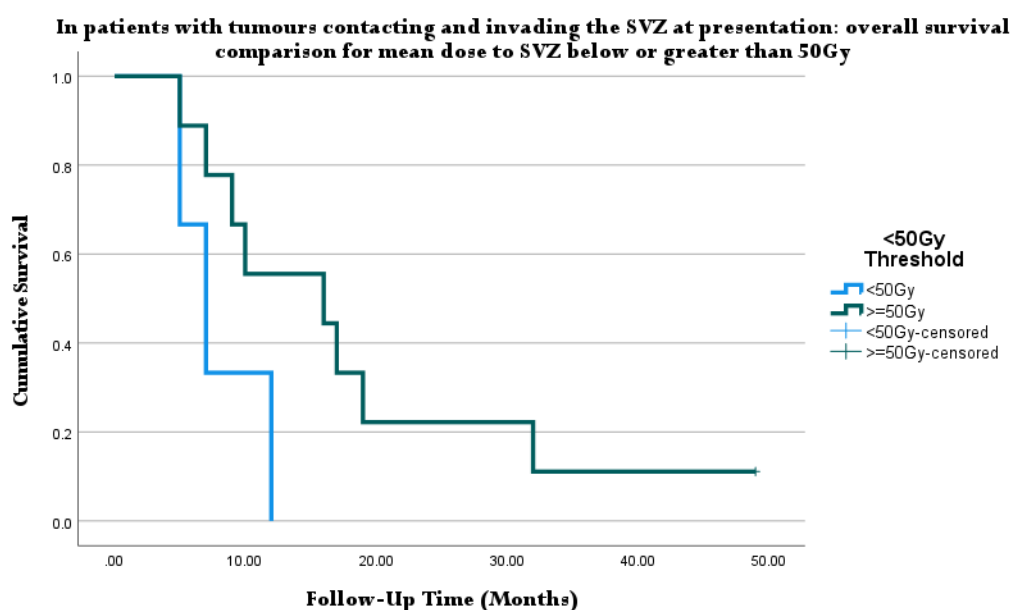
Figure 6.5. Comparison of median OS between patients by with SVZ-invasive tumours (Groups 1A and 2A) who's ipsilateral SVZ dose was below or above a threshold of 50Gy. For SVZ++ patients: median OS 7 months for mean ipsilateral SVZ dose <50Gy versus 16 months for >=50Gy (p=0.095). Note low patient numbers and data not adjusted for covariates.

6.4. Chapter Summary

The novel 'Modified-Lim' criteria has been introduced and employed to classify the patients in the study by their radiological tumour properties. Through this classification, it has been shown that whilst patients with SVZ-invasive tumours were suggestive of poorer survival outcomes, this significance was lost on multivariate analysis in the present study where age and MGMT-methylation status are seen as the only variables of prognostic significance. This study appears to be unique in incorporating SVZ dose into survival analysis that includes SVZ-contact and SVZ-invasion as covariables alongside age and MGMT-methylation status.

7. Investigating SVZ Contouring Accuracy

The dosimetric investigation of Chapter 5 found little evidence to support the targeting of the SVZ during radiotherapy for GBM patients, showing that mean SVZ dose was not a prognostic indicator on both univariate and multivariate Cox Regression analysis. Age and MGMT-methylation consistently proved to be of prognostic significance, agreeing with many other studies in the literature. A consideration of tumour location was provided in the analysis in Chapter 6, as a new classification methodology was introduced. Applying these criteria revealed the potential prognostic significance of SVZ invasion, as those patients who had SVZ invasion at presentation trended towards poorer survival outcomes, though this significance was lost on multivariate analysis. The multivariate analysis revealed that in the patients in this study, it was age and MGMT-methylation status that proved to be prognostically significant with SVZ dose, SVZ contact and SVZ invasive all proving to be non-significant.

The dosimetric findings described so far are all based on the contouring of the SVZ by a single observer (the author of this thesis) following the definition used by many studies in the literature where the SVZ is defined as a 5mm expansion lateral to the lateral ventricle. Though a contouring protocol was established to minimise subjectivity in the SVZ definition (described fully in Chapter 5) by using the higher contrast of the lateral ventricles on MRI and automatic contouring tools to define the SVZ, some manual adjustments were still required which inevitably introduces a potential source for inter-observer contour variability.

The accuracy and consistency of SVZ contouring is not only crucial to enable meaningful comparisons to be made between the findings of similar studies that are likewise investigating the potential significance of the SVZ, but if the SVZ were to be included as part of the radiotherapy target as part of future practice, the accuracy of the contouring of this structure becomes even more critical. Attention therefore now turns to the significance of SVZ contouring to the dosimetric metrics reported so far in this project. This chapter describes a study of contouring concordance between two observers for the first five patients in the study cohort that aims to address the final hypothesis of this thesis:

Hypothesis 3: Delineation precision has a significant effect on reported dosimetric metrics for the SVZ.

7.1. Literature Review

Accuracy of target volume delineation in radiotherapy treatment planning has been widely investigated in the literature for many tumour sites as inter-observer variability (IOV) of target definition is considered by some to be the largest uncertainty in the radiotherapy process (Segedin and Petric, 2016). Indeed, recent guidance published by the Royal College of Radiologists acknowledges that incorrect outlining ultimately reduces the chances of cancer cure and recommends that "*radiotherapy target volume contours should be subject to systematic review by appropriately trained and experienced peer-professionals*" (Royal College of Radiologists, 2017, p.4.). In GBM specifically, a study by Cattaneo *et al.* (2005) found that IOV was improved when using co-registered MRI for volume definition and consensus radiotherapy guidelines have recently been devised by ESTRO-ACROP (Niyazi *et al.,* 2016) and the Radiation Therapy Oncology Group (Kruser *et al.,* 2019) to promote future consistency in CTV delineation.

Systematic reviews of studies that have examined IOV in target delineation include Jameson *et al.* (2010) and Vinod *et al.* (2016) who both found that such investigations lack consistency in study methodologies and the reporting of metrics for contour comparisons. When assessing contouring IOV, simple volume measurement comparisons give limited information on the spatial location of the respective volumes from two observers. Indeed, two structures with identical volumes but containing no mutual voxels of overlap could show as being identical on such simple volumetric comparisons. Analysis of contouring IOV is aided by two concordance analysis metrics, the Concordance Index (CI) and Dice Similarity Coefficient (DSC), which both provide spatial information to aid volumetric comparisons and are defined in figure 7.1.

$$\text{Concordance Index (CI)} = \frac{A \cap B}{A \cup B}$$

$$\text{Dice's Similarity Coefficient (DSC)} = \frac{2(A \cap B)}{A + B}$$
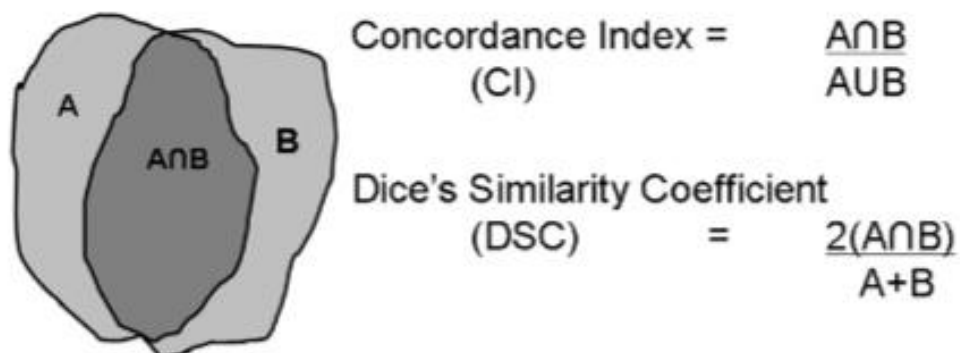
Figure 7.1. Definitions of Concordance Index (CI) and Dice's Similarity Coefficient (DSC) used for concordance analysis of two volumes A and B. From Hanna, Hounsell and O'Sullivan (2010) with copyright permission.

A systematic review by Hanna, Hounsell and O'Sullivan (2010) looked specifically at which metrics were used to report IOV. Surprisingly, they found that though the majority (84%) of 63 identified studies reported differences in volume measurements between observers, only 30% of studies included a concordance analysis metric such as the CI or DSC. Furthermore, fewer than half studied the dosimetric impact of such variations and only 3 studies compared patient outcomes based on the different contouring. The review recommends that IOV studies should report both a simple volumetric comparison and the concordance index. This recommendation will be applied in the work described in this chapter which furthermore will consider the impact on the survival analysis of the contour variations.

7.2. Methodology

The first five patients (study numbers 1-5) were included in this part of the project. The two independent observers were the author of this thesis (Observer A) and an experienced consultant oncologist specialising in GBM (Observer B) who was the local supervisor for this research project. Due to time restrictions, only five patients were studied. Observer A had completed contouring as part of the earlier parts of this project. Observer B completed their contouring blinded to the contours from Observer A. Observer A had followed the contouring protocol described in Chapter 5 whilst Observer B manually contoured the SVZ according to clinical experience. Both observers employed post-operative MRI sequences and used T2-weighted sequences where available due to the greater contrast between the lateral ventricle and adjacent white matter. Structures were contoured on the planning CT with co-registered MRI sequences (as described in section 5.1.6.) with structure resolution set to the Eclipse default and using the Eclipse paint brush tool which has a smallest available diameter of 0.4cm. Pixel resolution was recorded for all patients. Once contouring was completed, all subsequent data analysis was performed by Observer A.

All results were recorded within a database in Microsoft Excel (Microsoft Office, 2016). Volumetric information was obtained by using the 'measure volume' tool in the Eclipse TPS which reported the structure volume in cc to two decimal places of precision. The Boolean contouring tools were used in Eclipse to construct the Boolean union and intersection of the contours from Observers A and B and used to compute the Concordance Index as defined in figure 7.1.

7.3. Results

7.3.1. Pixel, Voxel and Contouring Tools Calculations

The transverse pixel resolution and CT slice thickness was recorded for all patients and used to calculate an approximate voxel size, the number of voxels per 1cc structure and the voxels per brush stroke for the contouring tool (table 7.1).

Parameter	Value
Transverse Pixel Resolution *[x,y]*	0.1cm
Slice Thickness *[z]*	0.3cm
Approximate Voxel Size *[xyz]*	0.003cc
Number of Voxels in 1cc *[1cc/Voxel Size]*	333
Volume of 0.4cm diameter paint brush $\left[\frac{4}{3}\pi r^3\right]$ + Voxels per brush stroke	0.03cc, 10
Volume of 0.5cm diameter paint brush $\left[\frac{4}{3}\pi r^3\right]$ + Voxels per brush stroke	0.07cc, 23

Table 7.1. Pixel and voxel resolution information for 5 patients in contouring study and calculation of Eclipse paint brush volumes.

The mean SVZ volume across all 10 contours (5 patients with contours from 2 observers) was approximately 12cc (more details in section 7.3.2). The average SVZ contour therefore comprised only approximately 4000 voxels. To put the small size of the SVZ into context in terms of practical contouring: the smallest paint brush tool in Eclipse (0.4cm diameter) would take only 400 individual brush strokes to contour the SVZ whilst using a 0.5cm diameter brush would take much fewer strokes at only 174. Increasing the brush diameter by only 1mm therefore more than halves the number of brush strokes required to contour the SVZ, giving an indication of the small size of the SVZ contour. As practical contouring does not consist of individual brush strokes/mouse clicks, rather it is a continuous 'painting' motion using the mouse formed of several individual brush strokes at a time, it is clear that contouring with too large a diameter brush can have a significant impact on the contouring accuracy of the SVZ. A single rogue brush stroke with the smallest brush (10 voxels) accounts for 0.3% of the SVZ volume whilst for a 0.5cm diameter brush it is 0.6%. Subsequent differences in volume will be contextualised using these practical contouring calculations.

7.3.2. Volumetric and Concordance Analysis

Table 7.2 lists the volumetric results for Observers A and B. The mean SVZ size across the 10 contours was 11.9±5.5cc. The error on the reported volumes in Eclipse for a 3mm CT slice thickness is estimated to be 20% for volumes <10cc and 1% for those ≥10cc (Srivastava, Cheng and Das, 2016). The range of possible volumes accounting for these errors are

excluded from the raw tabulated results for Observers A and B to aid clarity, but are included in the absolute difference calculations to show the maximum possible difference between observers. Subsequent data tables omit these volume ranges for simplicity, however the estimated 20% error on the reported volume measurements for structures <10cc should be considered when interpreting results.

Given the relatively small size of the SVZ contour, the small absolute volumetric differences actually account for a significant proportion of the SVZ contours. Across the cohort of 5 patients, the difference in volumes between Observers A and B was found to be almost statistically significant using a paired t-test (p=0.055).

Study Number	Observer A Volume (cc)	Observer B Volume (cc)	Difference + [*Max Difference*] (cc)	Percentage Difference [B versus A]
1	6.43	11.29	4.86 [*6.27*]	75.6%
2	7.44	15.35	7.91 [*9.55*]	106.3%
3	9.32	7.72	-1.60 [*-5.00*]	-17.2%
4	18.38	22.92	4.54 [*4.95*]	24.7%
5	7.22	12.6	5.38 [*6.95*]	74.5%

Table 7.2. Volumes of delineated ipsilateral SVZ performed by two independent Observers A and B. Error calculations omitted from raw results for clarity, however the absolute difference in contour volumes are stated together with maximum difference based on estimated error on reported volumes of 20% for volume <10cc and 1% for volume ≥10cc (Srivastava, Cheng and Das, 2016).

A considerable number of brush strokes accounted for the volumetric differences seen between the two observers (table 7.3). The differences therefore are not thought to be attributed to rogue brush strokes and appear to reflect a more systematic difference in contouring approach.

	A Volume (cc)	B Volume (cc)	Difference (cc)	Number of Voxels Difference (2s.f.)	Difference (Brush Strokes, 0.4cm brush, 10 voxels)	Difference (Brush Strokes, 0.5cm brush, 23 voxels)
1	6.43	11.29	4.86	1600	160	70
2	7.44	15.35	7.91	2600	260	113
3	9.32	7.72	-1.60	530	53	23
4	18.38	22.92	4.54	1500	150	65
5	7.22	12.6	5.38	1800	180	78

Table 7.3. Volumes of delineated ipsilateral SVZ performed by two independent Observers A and B. Absolute difference in contour volume expressed also as number of voxels and expressed as number of brush strokes for two brush sizes in Eclipse.

The largest difference between contours was seen for patient number 2 where Observer B contoured greater than double the volume of Observer A. Examining the contours on patient 2 visually (figure 7.2), the anatomical location and the superior and inferior extent from both observers appears consistent. However, Observer B is considerably more generous in their contours anteriorly, posteriorly and laterally, hence the large difference in absolute volume seen.
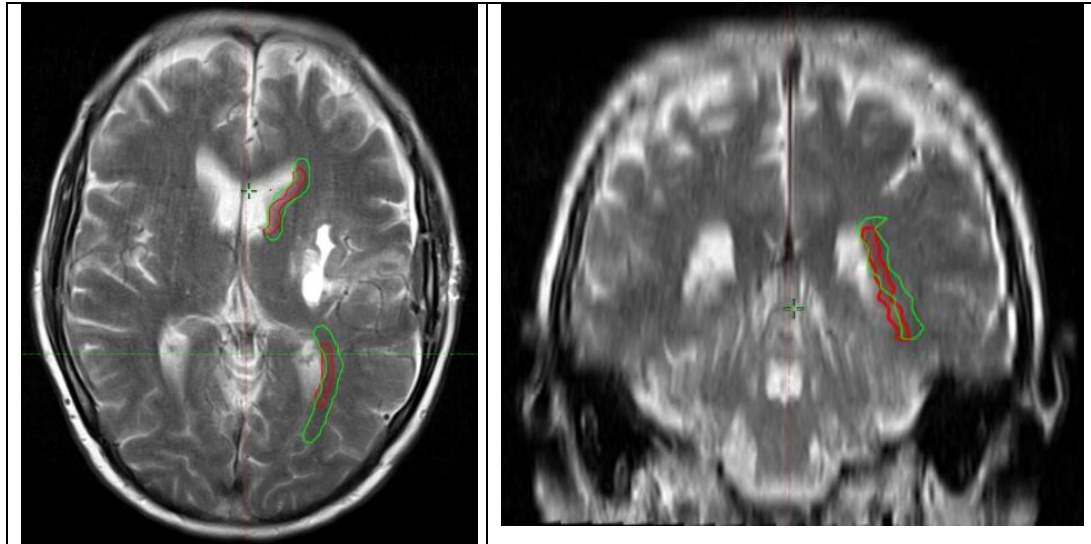
Figure 7.2. Contour comparison for patient 2. Observer A contour in red, Observer B in green. Spatial location of both contours very similar and accurate to SVZ anatomy. Observer B is considerably more generous, hence the greater measured volume.

Concordance analysis performed by calculating the CI between observers also showed poor agreement between observers (table 7.4). All patients showed lower than 30% concordance between observers (range 18.9% to 28.7%). Despite seeing the largest absolute difference in volume, patient 2 contours showed the highest concordance of 28.7% and examining figure 7.2. it can be seen visually that there is a relatively good degree of agreement in terms of overlap between Observers A and B. The poorest concordance was seen for patient 3 where a low degree of overlap can be verified visually (figure 7.3), note that the GTV is in close proximity to the SVZ for this patient.

Study Number	Observer A Volume (cc)	Observer B Volume (cc)	CI (%)
1	6.43	11.29	27.1
2	7.44	15.35	28.7
3	9.32	7.72	18.9
4	18.38	22.92	20.5
5	7.22	12.6	23.0

Table 7.4. Volumes of delineated ipsilateral SVZ performed by two independent Observers A and B using T2 MRI sequences and compared using Conformity Index expressed as a percentage.
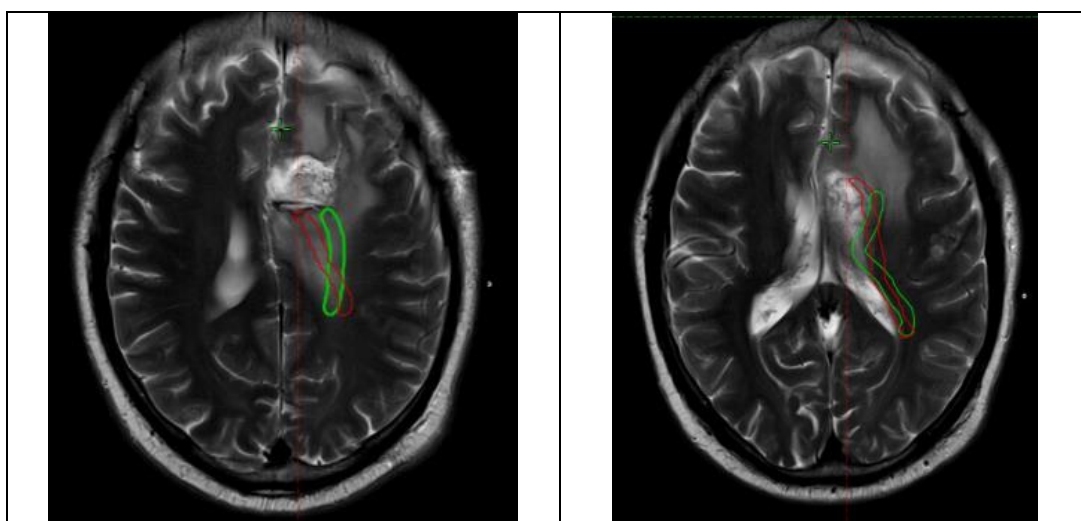
Figure 7.3. Ipsilateral SVZ contours for patient 3 from Observer A (red) and Observer B (green). Tumour is in contact with the SVZ causing difficulty in interpreting the surrounding anatomy.

7.3.3. Effect on Dosimetric Results

Given the variability between the contours of the SVZ, the sensitivity to variable SVZ contouring was examined for both percentage overlap of SVZ with PTV and mean dose to ipsilateral SVZ. These were calculated for the contours of both Observer A and B (table 7.5). Despite the lack of concordance in contouring, strong correlation was noted between observers for both percentage of SVZ overlap (Pearson correlation = 0.874) and for mean dose to SVZ (Pearson correlation = 0.932). Furthermore, paired t-tests showed no significant difference between observers for percentage of SVZ overlap (p=0.904) and mean dose to SVZ (p=0.858).

Study Number	% Ipsi SVZ in PTV (A)	% Ipsi SVZ in PTV (B)	Difference (B-A, %)	Mean Dose Ipsi SVZ (Gy) A	Mean Dose Ipsi SVZ (Gy) B	% Difference
1	99.22%	96.63%	-2.59	60.6	60.5	-0.2
2	97.18%	96.40%	-0.78	61.3	61.3	0.0
3	78.43%	96.50%	18.07	55.3	59.9	8.3
4	80.09%	73.34%	-6.75	58.3	57.4	-1.5
5	55.40%	50.32%	-5.08	46.8	44.3	-5.3

Table 7.5. Using SVZ contours from two Observers A and B, comparison of percentage SVZ overlap and mean dose to SVZ between the two observers.

The poor concordance for patient 3 is also reflected in the consequential dosimetric analysis, where the largest differences in percentage overlap (18%) and in the mean dose to the ipsilateral SVZ (8.3%) were seen due to discordance between observers. Examining the

contours on this patient, the reason for this appears to be Observer B failing to contour the SVZ to its full inferior extent (figure 7.4) which has an associated impact on both the percentage of PTV overlap and the mean dose to the SVZ.
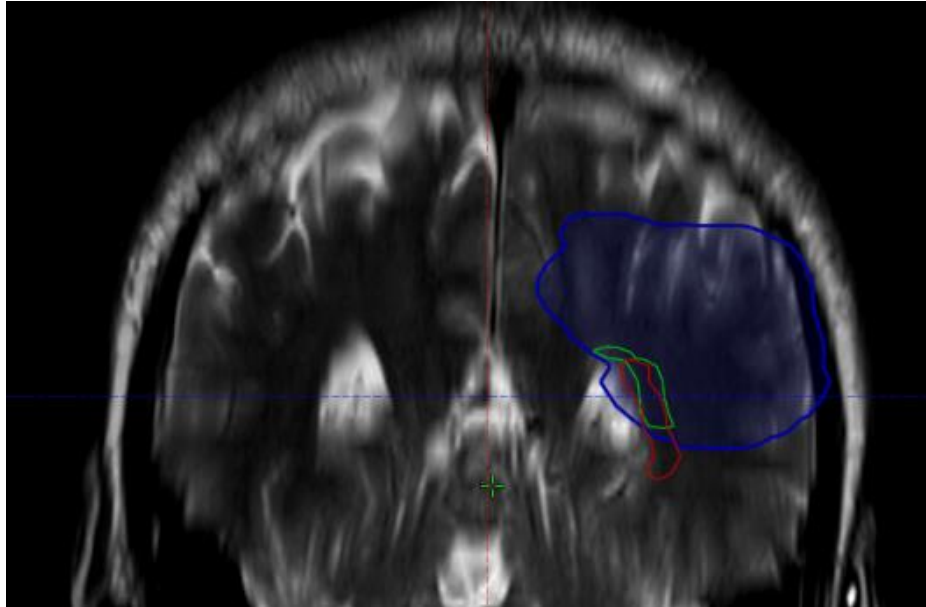
Figure 7.4. Contours for Observer A (red) and Observer B (green) together with PTV (blue) on patient 3. Observer B does not contour the full inferior extent of the SVZ.

7.4. Commentary on Results

Hypothesis 3: Delineation precision has a significant effect on reported dosimetric metrics for the SVZ.

7.4.1. Study Numbers

Due to time restrictions, this small concordance analysis was limited to a study of the first 5 patients with contouring performed by only two observers. An obvious extension to the study would be to extend the analysis to more patients in the investigation cohort and to include contours from further observers. An obvious candidate for Observer C would be the consultant radiologist who categorised tumours in Chapter 6 for this study, though Joskowicz *et al.* (2019) warn that two or three observers is still insufficient to establish the full range of IOV for a particular site.

7.4.2. Volumetric Analysis

There appears to be significant IOV for SVZ delineation for the five patients in this study with large volumetric differences seen between observers. As the overall volume of these structures is relatively small (~10cc), small absolute volumetric differences account for a high

proportion of the total SVZ volume. As a result, the size of the contouring brush becomes significantly important, something that this study has examined. Even using the smallest brush sizes in Eclipse, a single rogue brush stroke can account for 0.3% of the SVZ volume. However, the considerable volumetric differences observed in this study account for a high number of brush strokes and are not thought to be down to random observer contouring error. Differences observed appear to be more systematic and reflect the need for a clear SVZ contouring protocol.

The transverse pixel resolution of approximately 1mm contrasts with a relatively large 3mm slice thickness in the cranio-caudal direction. As argued by Bellon *et al.* (2014), the slice thickness and FOV for CT planning scans can affect the accuracy of target delineation. It should be noted that the 3mm slice thickness for GBM patients has recently been reduced to 2mm in the author's centre. This analysis should be repeated on patients scanned on the finer slice resolution to reassess contouring concordance in view of the improved resolution.

High resolution segmentation in Eclipse is possible and can be employed for contouring critical structures close to regions of steep dose gradient such as in stereotactic radiosurgery (Karen *et al.,* 2017). If the SVZ were to be included as a target volume in radiotherapy, it would be in close proximity to both the PTV and other organs at risk such as the brainstem and therefore would be subjected to significant local dose gradient variations. There is therefore an argument that contouring of a structure of this small size (and potential therapeutic significance) should be performed using the high-resolution segmentation option. Unfortunately, owing to the resource limitations of this HSST-based project, there was no time to investigate this option further in the current study.

Operator training plays an important role in delineation for radiotherapy, being not only a legal requirement under the Ionising Radiation (Medical Exposure) Regulations (2017) but also conceptually vital to ensuring accurate contour definition. Arculeo *et al.* (2020) found that 100 hours of training was needed for accurate OAR contouring. With this in mind it should be noted that Observer A (the author of this thesis) is a clinical scientist with no prior training in contouring structures within the brain, whereas Observer B has over 5 years of experience as a consultant clinical oncologist. Variations in contour accuracy must be considered in the context of the previous experience described here.

7.4.3. Concordance Analysis

There was relatively poor concordance between the observers in this study for all 5 patients. The poorest concordance was seen for patient 3 whose tumour was categorised as a Group 1B tumour in Chapter 6. These tumours by definition are in contact with the SVZ at presentation, though do not invade it. Consequently, given the bulk of the tumour mass, they are highly likely to distort and deform the anatomy in relation to the SVZ which makes the delineation of the SVZ particularly challenging. This is noticeable in figure 7.3 where the close proximity of the GTV to the SVZ causes blurring of the usual anatomical boundaries for SVZ delineation due to the presence of anatomical compression and tumour oedema. Defining the SVZ for these tumours therefore needs careful consideration of disease processes that may be distorting the usual definitions of SVZ.

There were no comparable studies found in the literature that specifically examined SVZ contouring concordance between observers. However, a study of IOV in brain metastasis delineation by Stanley *et al.* (2013) offers perhaps the best opportunity for comparable findings, as this work examined the variation when contouring small targets within the brain. The contours from 8 physicians were compared for concordance, however the contouring analysis performed was limited to a ratio of volumes between observers rather than a spatial location-based metric such as the CI or DSC. Notwithstanding the limitations of the reported metrics, the median ratio of largest to smallest volume of 1.68 shows that even for a high contrast target such as a solitary brain metastasis, there is still considerable variation in contour delineation.

7.4.4. Dosimetric Analysis

Despite the significant volumetric differences observed, the impact on the mean dose to the SVZ is <5% for most of the patients. The larger difference seen for patient 3 can be likely attributed to the incomplete contouring of the SVZ for Observer B, again this reflects the importance of clear contouring protocols to ensure consistency.

This concordance study sampled approximately 10% of the total patient cohort from Chapters 5 and 6 where mean dose to the SVZ was consistently found to be a non-significant prognostic indicator for OS. The relatively low numbers of patients sampled from the study cohort for this concordance analysis, coupled with the small differences seen in the reported mean dose between the two observers are therefore considered highly unlikely to turn mean SVZ dose from being non-significant to significant in this work.

In order to illustrate this, it should be noted that three of the five patients had absolute differences in mean dose between the two observers of <1Gy. Even for the largest difference in mean dose seen for patient 3 (8.3%), replacing the mean dose from Observer A with that from Observer B would be a difference in absolute dose terms of only 4.6Gy or 8% of the patient's prescription dose. With only five data points changing out of a cohort of 50 (used in the multivariate analysis in Chapter 5) and three out of the five changes being <1Gy, the reported survival results from Chapter 5 are unlikely to be affected. This is perhaps best demonstrated by considering that none of the differences seen would have changed the patient's position either side of the dosimetric thresholds analysed in table 5.11 and in which all thresholds showed non-significant results. As such, the impact on the survival analysis presented so far is thought to be insignificant at present, however with the caveat that the concordance analysis presented above is only for a sample of the patient cohort.

Much more data is therefore needed by extending the concordance analysis to include all patients from the study and repeating the Cox Regression analyses from Chapters 5 and 6. This will provide a more accurate and complete picture on the effect of contouring variation on reported dosimetric and consequently survival parameters.

7.4.5. Implications for Clinical Practice

The findings of this small concordance study highlight that despite the SVZ being a defined anatomical structure within the brain, there is still considerable variation between two observers. Should the SVZ be included as a target for radiotherapy in future clinical trials or indeed in future clinical practice, robust delineation protocols are clearly vital. In the author's centre, peer review of radiotherapy targets in the brain has recently been implemented as recommended by the RCR (Royal College of Radiologists, 2017). Furthermore, Patrick, Souhami and Kildea (2020) outline how the introduction of an IOV meeting in their centre improved concordance of target volume delineation between oncologists. A peer review process and potentially a reflective IOV meeting is likely to play an important role in ensuring consistent and accurate SVZ delineation if it were ever to be included as a radiotherapy target in routine practice.

There is perhaps a role to be played by automated segmentation to reduce human variation in contour definition. One promising paper on this subject by Biswas, Bhattacharya and Maity (2018) proposes an algorithm for the volumetric segmentation of the lateral ventricles on MR images which when tested gave highly encouraging DSC scores of 0.96. The accuracy of such

algorithms is likely to be tested however by the presence of complex underlying anatomy such as that seen in patient 3 in this study, where the tumour proximity to the SVZ caused deformation of the lateral ventricle which had an assumed impact on the contouring concordance. Such similar cases could cause a problem for automatic segmentation software. It should be noted though that anatomical changes in the ventricle areas of the brain are not limited to disease processes from a nearby GBM. Ventricular changes can be seen in patients with hydrocephalus (Kang *et al.,* 2018) and ischemic stroke (Hijdra and Verbeeten, 1991), the latter potentially causing significant ventricular deformation. By considering the lateral ventricles in a wider medical context, rather than through the lens of radiotherapy volume delineation, potential automated segmentation solutions can perhaps be found. One such promising algorithm proposed by Ferdian *et al.* (2017) showed DSC scores of 0.93 even when delineating the deformed lateral ventricles in patients with ischemic stroke. This is perhaps a clear indication that the solutions for automated segmentation of structures in the brain may already lie in other medical disciplines. Future literature reviews and research should be careful to avoid missing potentially vital sources of information that could aid future radiotherapy practice, even if their current application is not based in oncology.

7.5. Chapter Summary

A study of inter-observer variability when contouring the ipsilateral SVZ has been presented. Poor concordance was seen between the SVZ contours of two independent observers which was not thought to be down to random brush stroke contouring errors and more likely due to systematic differences which can be attributed to the complexity of the clinical cases and the absence of a coherent SVZ definition in the literature. The findings advocate the importance of robust and clear delineation protocols for the SVZ, particularly if it were to be included as a radiotherapy target volume in the future. Disease processes that affect the underlying anatomy are seen to cause particular issues with accurate delineation and as such automatic contouring algorithms could offer a solution. Automatic segmentation software that already exists in other neurological applications could be employed in radiotherapy, particularly in the difficult cases caused by anatomical deformation.

8. Summary, Discussion and Critical Appraisal of Project Findings

This investigation was undertaken to establish the significance of the SVZ in defining survival outcomes for patients with GBM. Given the conflicting findings seen in the literature to date, this worked aimed to provided clarity through a coherent investigation that examined the potential role that radiotherapy to the SVZ may have in improving patient Overall Survival in GBM. It was hypothesised that patient Overall Survival could be improved by irradiating a potential sanctuary for glioma stem cells that otherwise would escape therapeutic intervention and many authors have proposed clinical trials to this effect. This project was motivated by the continued poor survival data for patients diagnosed with GBM and the relative absence of truly successful treatment outcomes. A GBM diagnosis therefore remains a devastating outcome for patients and their families. The opportunity and resources presented by the HSST programme and the relative lack of investment in GBM research compared to other tumour sites provided additional incentive to perform this research. The project was underpinned by the author's own personal interest in neuro-oncology and expertise as a clinical scientist working in radiotherapy physics and further supported by academic and clinical supervisors with specialist interest and expertise in the field of neuro-oncology.

The project was sub-divided into three distinct areas and the preceding chapters of this work have individually described the methodology, results and key findings of each part of the investigation. This chapter now seeks to unify the key findings and theories identified in this work, provide a critical appraisal of the entire project and identify the areas of focus for future work on the subject. This chapter begins by summarising the key research findings from this project, ensuring clarity on the fundamental message that this work conveys by limiting the accompanying prose in this section. Attention then turns to an in-depth discussion of the findings, placing the conclusions drawn into context in relation to the existing scientific literature and the implications for current and future clinical practice. A critical appraisal of the project then follows, identifying areas of relative strength in the research performed, including a discussion of the features that sets this project aside from the current scientific works on the subject. Areas of relative weakness are also identified and used as a basis for suggesting the future direction of follow-up research work.

8.1. Overview of Key Findings

The primary research question proposed in the introduction to this thesis and the three hypotheses generated following the review of the literature in Chapter 2 are now addressed. In order to ensure clarity on the reported findings, they are listed below in individual text boxes under their respective chapter headings and hypotheses, with the principal points for each conclusion being underlined.

8.1.1. Chapter 5: Investigating the effect of Ipsilateral SVZ Dose on Overall Survival in GBM

This chapter began by examining the significance of mean dose to the ipsilateral SVZ on GBM patient survival outcomes. The survival analysis performed included data on prognostic covariables including age, sex, Performance Status, surgical extent, chemotherapy treatment and MGMT-methylation status. On Cox Regression multivariate analysis, it was found that age, chemotherapy treatment and MGMT-methylation status were shown to be of prognostic significance, however no such findings were recorded for mean SVZ dose. In relation to hypothesis 1 therefore:

> **Hypothesis 1: A higher mean incidental dose delivered to the SVZ during radiotherapy leads to longer OS in GBM patients.**
>
> Mean dose to the ipsilateral SVZ was found to be of no prognostic significance on Cox Regression multivariate analysis. Hypothesis 1 therefore does not universally hold true for all GBM patients in this study.

8.1.2. Chapter 6: Investigating the effect of Tumour Location and Invasive Properties on Overall Survival in GBM

The novel Modified-Lim tumour classification criteria was introduced in this chapter as the effect on Overall Survival of tumour proximity to and invasive of the SVZ was investigated. In respect of the second hypothesis of this work, the following can be concluded:

> **Hypothesis 2: Tumour location with respect to the SVZ has a significant impact on OS in GBM patients.**
>
> Applying the novel 'Modified-Lim' tumour classification criteria suggested in this study, Group 1B tumours had a significantly improved prognosis than Group 1A tumours on univariate analysis (HR 0.312 (0.099-0.985, p=0.047). However, this significance was lost when including the covariate data obtained from Chapter 5 in a multivariate analysis. Hypothesis 2 therefore remains inconclusive and more research is required.

8.1.3. Chapter 7: Investigating SVZ Contouring Accuracy

The final principal chapter of this thesis investigated the concordance between two observers when contouring the SVZ in order to address the third and final hypothesis:

> **Hypothesis 3: Delineation precision has a significant effect on reported dosimetric metrics for the SVZ.**
>
> In the small study of 5 patients, no significant differences in the reported dosimetric metrics were seen, however this finding is for a small sample of the study cohort analysed in Chapters 5 and 6 and a complete dataset is needed to draw more firm conclusions. Notwithstanding the non-significant effect on the dose metrics, concordance between the two observers was poor. Clear SVZ delineation protocols are therefore required to ensure consistent definition of the SVZ if it is to be included as a radiotherapy target volume.

8.1.4. Primary Research Question

> **"Is there evidence that the inclusion of the ipsilateral SVZ as a target in a radiotherapy treatment plan could lead to improved survival for patients with GBM?"**
>
> At present, the results of this study **do not** provide clear evidence for the inclusion of the ipsilateral SVZ as a target in a patient's radiotherapy treatment plan. However, an area of potential future research interest has been identified through the novel Modified Lim criteria introduced in this work, and the potential significance of SVZ invasion requires further investigation. With age and MGMT-methylation status proving prognostically significant, it may be that future work can identify a subset of patients for which SVZ irradiation may be of benefit.

The evidence reported in this investigation does not provide clear basis for a clinical trial at present as no significant impact on patient OS has been found for either mean SVZ dose or tumour proximity to the SVZ. A potential new line of research interest has however been

identified in the potential significance of SVZ-invasion. This sets this research apart from the existing literature in identifying a specific subset of patients for which SVZ-irradiation may be of potential benefit, though more evidence is needed at this time.

8.2. Discussion on Key Findings

The preceding principal thesis chapters each provided an interim discussion of their respective findings in the context of the existing literature on SVZ irradiation. Having now summarised the key findings from this project, these prior commentaries are now unified through a narrative that seeks to draw together the work performed in this investigation.

8.2.1. SVZ Irradiation does not improve Overall Survival for all patients

The literature on the subject of SVZ irradiation analysed in Chapter 2 revealed largely contradictory findings between individual, mainly retrospective studies that examined patient survival with respect to the incidental dose delivered to the ipsilateral SVZ in radiotherapy for GBM. On this basis, Nourallah *et al.* (2017) presented the case for a prospective clinical trial based on the hypothesis that irradiating the SVZ may extend survival in GBM. Despite the conflicting evidence between individual studies, the rationale for such a trial received support from the systematic review and meta-analysis of Susman *et al.* (2019) whose results indicated that the dose delivered to the ipsilateral SVZ may indeed be of prognostic significance. Despite the findings of the meta-analysis, a continued lack of prospective trial data is reflected in the recently updated radiotherapy treatment guidelines issued by the American Association of Neurological Surgeons (AANS) in 2020 (Ziu *et al.,* 2020) which fail to recommend SVZ irradiation, citing continued contradictory evidence in the literature. Such conflicting literature continues to be published, where either no improvement in patient survival is seen with SVZ irradiation (Bender *et al.,* 2021) or promising neurocognitive preservation is seen by instead actively sparing the SVZ during radiotherapy (Gui *et al,* 2020).

Examining the meta-analysis from the Susman group in detail, the statistically significant improvement found in survival for high dose versus low dose SVZ irradiation was for Progression-Free Survival only with no such improvement seen for Overall Survival. The findings in the present study showed no significant impact on Overall Survival, reassuringly agreeing with these findings from the meta-analysis.

8.2.2. Macroscopic Tumour Invasion of SVZ may be Significant

The application of the novel 'Modified Lim' classification criteria proposed in this study has revealed that the macroscopic tumour invasion of the SVZ may be of prognostic significance to patient Overall Survival and may have a role in determining which patients could benefit from potential SVZ radiotherapy. The meta-analysis from Mistry *et al.* (2017a) identified that GBM contacting the lateral ventricles was significant for lower patient survival, finding hazard ratios of 1.58 (1.35-1.85) for OS and 1.41 (1.22-1.64) for PFS. This present research project did not reach this conclusion for SVZ-contact however has gone further than the previous studies and identified that macroscopic invasion of the lateral ventricle, rather than purely SVZ contact may be of significance in determining patient outcomes. The effect in this study was however lost on multivariate analysis, therefore more research is required in this area to draw firmer conclusions.

A significant factor in this survival detriment may be the surgical challenge of resecting a tumour that invades the lateral ventricle given that the extent of surgical resection has been identified by many studies as a prognostic indicator. Lee *et al.* (2013) found that OS was significantly improved in patients with gross total resection versus sub-total resection and biopsy (24.9 months versus 19.1 months versus 14.8 months, p=0.0016). Achieving gross total resection of a tumour invading the lateral ventricle would require surgical entry into this part of the brain. Ventricular entry during GBM surgery is itself a controversial topic with studies showing that such a procedure is well-tolerated with few postoperative complications (Young *et al.,* 2021) but with conflicting evidence on survival benefits (Sonoda *et al.* (2017) and Saito *et al.* (2020)). Given the surgical controversies in these patients, radiotherapy perhaps may have a significant role to play in a patient's treatment if irradiating an invaded lateral ventricle proves to be a safer alternative than attempting a risky invasive surgical resection.

8.2.3. More Research Needed: Consideration for Trial Designs

Nourallah *et al.* (2017) argued that a carefully designed prospective clinical trial is needed that takes the whole ipsilateral SVZ dose to 60Gy. Based on the findings from the present study, there is no evidence to support this proposal at present and more work is needed to determine the exact design of such a trial. It is suggested that a future trial design may need to include strict inclusion criteria for patients based on the anatomical features of the tumour with respect to macroscopic SVZ invasion, depending on the outcome of future work. The

combination of the well-documented importance of surgical resection on patient survival and the controversies surrounding surgical resection in the lateral ventricle leaves a potential role for radiotherapy in achieving improved clinical outcomes in this well-defined subset of patients. It is hypothesised that delivering higher doses of radiotherapy to the ipsilateral SVZ when infiltrated by invasive tumour may provide a therapeutic benefit for a certain group of patients who otherwise may have a poor prognosis, but more retrospective research is needed before considering such a trial.

Nourallah *et al.* (2017) advocate a radiotherapy dose of 60Gy to the entire ipsilateral SVZ. A different dose level was used in the prospective study of Valiyaveetil *et al.* (2017) where all patients were planned such that the ipsilateral SVZ and periventricular zone (PVZ, defined as a 5mm isotropic expansion of the lateral ventricles) received a mean dose ≥50Gy. Disappointingly, no such survival improvements were seen in this study and the authors actually argue that future studies should instead spare these areas to preserve neurocognitive function. The Valiyaveetil study, though prospective, is not a randomised trial and omitted several elements in the analysis including MGMT-methylation status and neurocognitive function. Furthermore, no consideration was given to tumour location in the patient selection, instead the planning approach was applied to all patients regardless of tumour location. The argument put forward by this present study is different, suggesting that there may be a subset of tumours with respect to SVZ-invasion for which radiotherapy may be of prognostic benefit.

In order to achieve a given prescription dose for the SVZ in any future trial, it is proposed that the mathematical relationship derived to equate degree of overlap with mean dose could be employed. The advantage of this is avoiding over-irradiation of stem cell regions that could be contributing to neuro-cognitive repair and that have been implicated as having detrimental outcomes on patient neurocognitive function (Gui *et al.*, 2020). With GTV, CTV and SVZ delineated and the PTV expanded, a calculation of expected mean dose to SVZ for a given overlap could be performed and used to determine whether the remaining SVZ should be included in the plan.

8.2.4. Clarity on SVZ Delineation is Critical

The early study on SVZ radiotherapy from Barani *et al.* (2007) is cited by many including Evers *et al.* (2010) for its definition of the SVZ, describing it as "*the 5mm of tissue immediately adjacent to the lateral walls of the lateral ventricle*". However, the accompanying figure to this

definition (figure 8.1) highlights where there is room for ambiguity in contouring, as the SVZ does not follow the entire contour of the lateral wall of the lateral ventricle as per the definition and instead has a well-defined inferior aspect.
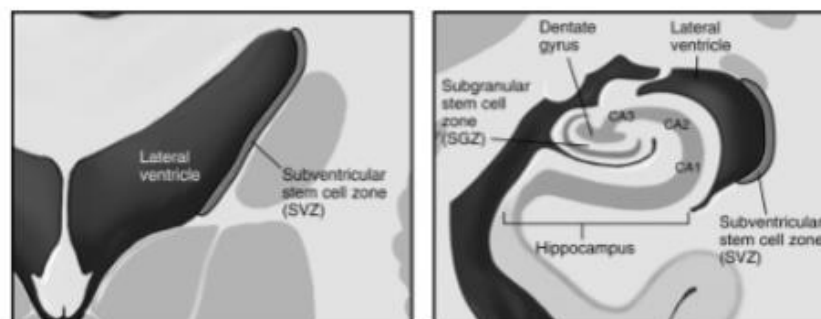
Figure 8.1. Definition of the SVZ from Barani et al. (2007). Note the absence of anatomical orientation in this figure and the well-defined inferior limit of the SVZ with respect to the lateral ventricle. Reproduced with copyright permission.

This subtle discrepancy in the inferior extent of the defined SVZ may seem trivial but in a paper cited by many as being the 'gold-standard' definition, actually propagates to create the lack of consensus seen in the wider literature. The absence of consensus is acknowledged by Nourallah *et al.* (2017) who cite the respective studies of Gupta *et al.* (2012) and Lee *et al.* (2013) where the former omits the inferior aspects of the SVZ included by the latter (figure 2.4a). The inconsistent definition for the inferior limit of the SVZ was also seen in a patient in this present study (patient 3, described in section 7.3.2.) and is an area where future protocols should be much more specific, defining anatomical landmarks for the limits of the SVZ contour rather than potentially ambiguous statements that lack fixed anatomical boundaries.

8.3. Critical Appraisal of Research Project

8.3.1. Strengths of Study

Compared to other works that have been outlined and appraised in the literature review of Chapter 2, this study has provided greater clarity and full details of the radiotherapy treatments delivered to patients, including confirmation of treatment completion, details of treatment planning protocols and technical machine hardware and software specifications. A reproducible SVZ contouring protocol has been devised which relies on the higher-contrast delineation of the ventricle rather than the subjective delineation of the SVZ itself. Overall Survival has been used as a well-defined endpoint which avoids the uncertainties associated with the use of Progression-Free Survival. The inclusion of dose and age as continuous

variables in the multivariate analyses throughout this work are rarely seen elsewhere in the existing published literature with previous studies also excluding key prognostic variables such as age and MGMT-methylation status from their analyses, variables which this study have found to be prognostically significant.

This study has also not been restricted to a single retrospective analysis of either delivered dose to the SVZ (as in studies including Evers *et al.* (2010) and Chen *et al.* (2013)) or of tumour proximity to the SVZ (in works such as Adeberg *et al.* (2014) or Yamaki *et al.* (2020)). Based on the proposed significance of the SVZ in GBM in relation to migrating glioma stem cells, this study has looked at both tumour proximity and dosimetric data with respect to the SVZ as it seeks to establish the significance of the SVZ in patient survival in GBM.

During the initial dosimetric investigation, a relationship has been derived between SVZ dose and degree of SVZ overlap with the PTV. The two concepts are related conceptually, as a close-proximity tumour will ensure a high degree of SVZ-overlap with the PTV and lead to a higher mean dose. Both Nourallah *et al.* (2017) and Hallaert *et al.* (2021) argue that to this degree it is almost impossible to derive a prognostic effect from tumour contact with the SVZ. Notwithstanding this collinearity, this present study has nonetheless derived a potentially useful relationship between the degree of SVZ overlap with the PTV and the mean dose delivered, which could be significant when determining any future prescription doses for the SVZ to ensure a certain mean dose is achieved.

When examining the significance of tumour location, this study went further than the existing literature by proposing new classification groups based on the macroscopic invasive properties of the tumour with respect to the SVZ, not solely based on observed contact. Through this novel classification methodology and a combined investigation of both tumour proximity and SVZ dosimetry, the potential importance of tumours that invade the SVZ has potentially been revealed although as described above much more research is needed to fully investigate this field.

This study furthermore has included a contouring concordance analysis in which the importance of consistent SVZ delineation has been identified. No other such concordance analyses of SVZ contouring have been found in the current literature.

8.3.2. Weaknesses of Study

The present study is a retrospective analysis of patients previously treated in the author's centre, as such it carries the usual limitations of retrospective studies including the potential for selection and sampling bias. Bias in patient selection was minimised by using an automated search of the radiotherapy database to generate a list of all potential patients within a range of years and stipulating strict inclusion criteria for the patients in this study (Chapter 4). The strict inclusion criteria limited the study cohort to 54 patients for the first part of the investigation and availability of imaging sequences reduced this to only 39 for the radiological classification. As such this study also has a weakness of a relatively small sample size, though it is comparable to other similar studies in the literature such as Slotman *et al.* (2011, n=40). With greater resources including more time for data processing, more patients could have been included in this investigation.

Many of the comparative studies in the literature report the impact of SVZ dose on patients' Progression-Free Survival. PFS was not analysed in this current study for two principal reasons. Firstly, analysing PFS is more resource intensive, as it requires careful examination of follow-up clinical data to establish dates of disease progression. In the author's centre, patients are referred for radiotherapy from a number of other centres spanning a large catchment area in South-East England, hence obtaining patient follow-up data would have many practical challenges associated with its procurement. Secondly, the determination of the date of disease progression and hence PFS calculations can be significantly biased by the frequency of follow-up imaging or follow-up clinical reviews. Given the resource limitations of the current HSST-based study, a well-defined end point such as Overall Survival was chosen for the data analysis.

Resource limitations prevented the acquisition of more patients to the current investigation. Low patient numbers reduce the number of events in an Overall Survival analysis and hence placed a statistical restriction on the number of covariables that can be included in the multivariate analyses presented here. With an expanded study, the number of included covariates could be increased with a higher number of events and provide a clearer picture on, for example, the effect of surgery on the data in Chapter 6.

Proximity to the SVZ has been implicated as a high-risk factor for multifocal and distant disease recurrence by Adeberg *et al.* (2014). Such patterns of disease recurrence have been reported by many studies that classified tumour according to the original groups proposed by

Lim *et al.* (2007), including the Lim study itself, Jafri *et al.* (2013), Yamaki *et al.* (2020) and Hallaert *et al.* (2021). The absence from this current study of recurrent disease analysis provides an unfortunate area of weakness, however the lack of recurrence analysis was not unique to this study and several other studies including Chaichana *et al.* (2008) and Mistry *et al.* (2020) also do not report on disease recurrence.

The importance of the contralateral SVZ (CL SVZ) in determining patient survival outcomes was also not considered in this study. Again, this is down to resource limitations particularly in regard to the amount of time taken to contour each patient. Though not all studies in the literature consider this in their analysis, those that do report mixed results that only add to the controversy on the subject. Gupta *et al.* (2012) and Ellicin *et al.* (2014) both report worse outcomes for high doses to the CL SVZ; Adeberg *et al.* (2016) and Arnalot *et al.* (2017) find improved outcomes and Ravind, Prameela and Dinesh (2015) find no significant impact. Rather than the omission of the CL SVZ from this investigation being an outright weakness, its absence may actually provide greater clarity on the findings that are reported for the ipsilateral SVZ and have given the investigation performed here a more specific focus.

8.4. Future Work and Innovation Proposal

8.4.1. Study Extension and Expansion

One obvious area of future work is the extension and expansion of the retrospective study described in this thesis. As a general improvement, a greater number of patients could be included in the analysis to increase the statistical power of the study. This includes a greater number of patients in the concordance analysis element which was currently limited to 5 patients and two observers. Greater numbers of both patients and observers will likely strengthen the statistical findings reported.

As described in the previous sections, there are some areas where this current study lacked the resources to fully investigate. Future study extension could be performed in the areas that are listed below with reference to the sections in which they are commented on further:

1. Increased patient numbers in retrospective survival analysis presented in Chapter 5 to permit more covariates in the multivariate analysis and increase statistical power (section 8.3.2).
2. Inclusion of toxicity data when comparing patient outcomes based on SVZ dose (section 5.4.10).

3. Increased patient numbers in retrospective study of tumour location and survival analysis thereof (section 6.3.6).

4. As a result of point (3), further patient numbers would permit inclusion of surgical data in the multivariate analyses in Chapter 6 (section 6.3.5).

5. Concordance analysis performed for more patients in the study and with additional observers (section 7.4.1).

6. Repetition of concordance analysis on more recent patients scanned with a 2mm CT slice thickness (section 7.4.2).

7. Investigation on the significance of high-resolution segmentation in Eclipse on contouring accuracy and concordance (section 7.4.2).

8. Analysis of tumour recurrence patterns with respect to SVZ proximity (section 8.3.2)

9. Study of the effects of dose to the contralateral SVZ (section 8.3.2).

10. Obtain follow-up clinical and imaging data for the patients to determine the potential prognostic impact on Progression-Free Survival (section 8.3.2).

8.4.2. Additional Areas of Research

The Potential for Spectroscopic MRI

This investigation quantified macroscopic SVZ invasion via observer interpretation of pre-operative imaging. Establishing the degree of SVZ invasion at a microscopic level could be achieved by way of spectroscopic MRI (sMRI). The benefits of sMRI in GBM were recognised by Cordova *et al.* (2016) who found that sMRI was able to diagnose tumour infiltration and recurrence before contrast enhancement on conventional MRI. Such is the potential of sMRI that an ongoing dose-escalation trial (NCT03137888) in the USA reported by Mellon *et al.* (2019) is using sMRI to find actively proliferating tumour beyond the areas of T1-enhancement that will serve as targets for radiotherapy dose escalation. If appropriate funding and resources are secured, the use of sMRI could confirm microscopic tumour invasion of the SVZ and guide both surgery and also potentially radiotherapy targets.

Delivered versus Planned Dose and the Role of Adaptive Radiotherapy

The study reported in this thesis was able to only record the planned dose to the SVZ when reporting on correlation with patient survival outcomes. As the patient undergoes 6 weeks of fractionated radiotherapy treatment, it is not unreasonable to expect some degree of anatomical changes within the brain as a result of the radiation as well as potentially progressing disease processes. Furthermore, the majority of patients undergo radiotherapy in

a post-operative setting and the dynamics of changes within the cavity volume have been observed and reported by Atalar *et al.* (2013). As a result, the actual delivered dose to the SVZ may differ from the planned dose reported by the TPS. In the same way that the 'VoxTox' study from Cambridge saw that delivered dose was a better predictor of rectal toxicity than planned dose in prostate radiotherapy (Shelley *et al.*, 2017), it is suggested that delivered dose to the SVZ may provide a better correlation with outcomes.

A study by Darazs *et al.* (2019) confirms that the suggested anatomical changes are indeed observed in GBM. The patients in this study all underwent an adaptive radiotherapy protocol, where additional CT/MRI planning imaging was performed four weeks into the standard 30 fraction radiotherapy schedule and an additional radiotherapy plan created for the final two weeks of treatment, that accounted for anatomical changes in the brain. The retrospective study examined changes in the volumes of the SVZ on the adaptive planning CT and found significant volumetric changes of up to 17% with an associated statistically significant impact on reported dosimetric parameters for the SVZ. As noted towards the end of the literature review in Chapter 2, adaptive radiotherapy for GBM is an area of growing interest. If irradiation of the SVZ became routine clinical practice following suitable clinical trials, anatomical changes within the brain can affect the accuracy of the delivered dose and there is potentially justification to pursue an adaptive radiotherapy strategy for GBM patients, though such an approach would require consideration of the additional resources required.

8.4.3. Innovation Proposal – HSST-specific

IMPORTANT DISCLAIMER:

The proposal described here is added in order to satisfy the requirements for HSST assessment in providing a theoretical proposal for a new technique innovation. The author recognises that at present, the results in this current study do not justify the initiation of the trial in this proposal. Much more research is required including the study of additional patients to increase the numbers in this retrospective study and findings at present are not statistically significant. Only once the retrospective study is expanded, published and peer-reviewed would consideration to such a clinical trial be given and any trial proposal would not be initiated without the required peer-reviewed scrutiny and Regulatory approvals.

As per the requirements of HSST, this thesis includes an innovation proposal which is detailed in full in appendix A. The innovation proposed is a purely theoretical small '3+3' Phase I

prospective clinical trial in which participants with macroscopic invasion of the SVZ by the enhancing tumour seen on pre-operative imaging, undergo radiotherapy to the ipsilateral SVZ to give a mean dose of at least 50Gy to that structure. As described in the disclaimer above, this trial is most definitely not for initiation at present as much more retrospective data is needed to provide the theoretical justification for the trial schema proposed. The proposal is presented only due to the stipulated requirements of the HSST programme and is on the assumption that statistically significant findings are obtained at a later date once the current retrospective study is significant expanded in terms of patient numbers.

8.5. Summary of Novel Methodologies and New Knowledge Gained

- A new SVZ contouring protocol has been devised which uses the higher-contrast lateral ventricles for reproducible delineation of the SVZ rather than subjective contouring of the SVZ itself.

- A new categorisation of chemotherapy treatment has been used in the analysis in this work.

- Univariate and multivariate analysis has been performed using mean SVZ dose and age as continuous variables in contrast to the threshold methods employed by much of the existing literature.

- The Cox Regression analyses in Chapter 5 include all three treatment modalities as covariates. The multivariate analyses include these plus the other prognostic covariates of age, chemotherapy treatment and MGMT-methylation status. No other studies in the literature could be found that included this combination of covariates in multivariate analysis, hence it is felt that this study contributes new knowledge to the scientific literature in this respect.

- Age has been shown to be of prognostic significance in GBM when analysed as a continuous variable. No other papers studying the effect of SVZ radiotherapy were found in which age was prognostically significant when analysed as a continuous variable.

- Many of the existing works on the subject employ age thresholds in their dosimetric analysis and this work has tested a previously unused threshold of 55 years of age in which the difference in Overall Survival between the groups was seen to be significant. Increasing age is confirmed as a poor prognostic indicator in GBM.

- A novel classification methodology termed the 'Modified Lim Criteria' has been proposed and used by this work to categorise tumour types in Chapter 6. Through this novel methodology, it has been seen that tumour invasion of the SVZ may be of potential prognostic significance and a new area of future research focus has been identified.

- The multivariate analyses in Chapter 6 included both tumour location group and SVZ dose together with age and MGMT-methylation status. No other similar studies were found in the literature.

- SVZ contouring analysis has not previously been reported in the literature and this study has presented a concordance analysis for two observers delineating the SVZ on 5 patients. It has revealed the importance of a clear, unambiguous delineation protocol for the SVZ.

9. Conclusion

A GBM is a highly aggressive form of malignant brain tumour whose poor prognosis means an ominous diagnosis for a patient. Though treatment options and technologies have developed over recent decades, there remains a funding shortfall for brain tumour research in comparison to other cancers. Despite combination therapies of surgery, chemotherapy and radiotherapy, tumour recurrence is a major factor behind the poor patient outcomes in GBM and many authors have proposed that the survival of glioma stem cells in the SVZ is a likely source of recurrent disease and relative treatment failure. On this basis, inclusion of the SVZ in radiotherapy treatments for GBM has been proposed, however existing research on the subject has yielded mixed and often contradictory results when examining retrospective data. Given the conflicting evidence in the literature, a prospective randomised trial for SVZ radiotherapy seemingly lacks the current conclusive supporting evidence and such a proposal remains controversial.

This thesis has described an investigation into the effect of radiotherapy to the SVZ in GBM patients that sought to explore the existing gaps in the literature to provide further evidence and clarity on the potential survival benefits from SVZ irradiation. Three principal chapters in this thesis have described the work performed to address the primary research question and investigate each of three hypotheses generated following a review of the existing literature.

In GBM patients as a whole, increasing dose to the ipsilateral SVZ was found to have no significant prognostic impact on patient Overall Survival, a finding that is in line with a recent meta-analysis on the subject. However, a novel tumour classification methodology proposed by this current study has revealed a potentially interesting finding that could be the focus of future research interest. Invasion of the SVZ by the enhancing tumour was shown to be potentially detrimental to patient survival compared to non-invasive tumours. Though the prognostic significance of this was not seen on the current multivariate analyses for relatively low patient numbers, it does provide an area of renewed research focus. Given the surgical challenges discussed in this work when attempting to resect a tumour within the lateral ventricle, it may be that radiotherapy has an important future role in the management of these patients.

By performing this work and presenting this thesis as part of the HSST programme, the author hopes that a small contribution has been made to the ongoing research into this

dreadful disease. The author intends to continue researching the topic of SVZ radiotherapy with several areas of future research being identified in this thesis.

This thesis ends as it began with some poignant words from the late Dame Tessa Jowell. By reflecting on these words, and continuing with a drive and motivation to research improved treatments, patients with stories similar to hers may have improved hope in their lives.

"It's all about sharing knowledge…if we achieve this, we will go a long way to crack GBM and other cancers too"

Dame Tessa Jowell, House of Lords, 2018 (ITV News, 2018)

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Appendix A – Innovation Proposal

A1: Lay Summary

The innovation proposal described here is for a clinical trial that will test whether including an extra part of the brain in a radiotherapy treatment will help patients with brain tumours to live longer. Scientists think that this part of the brain, called the 'SVZ', is responsible for tumours regrowing after treatment. The results from several pilot research projects[1] suggest that the SVZ should be included in radiotherapy treatments for some patients to stop tumours growing back and help the patients to live longer.

As the new treatment would involve treating more of the brain with radiation than normal, we need to test that the idea is safe first and that patients don't have any side effects. Clinical trials are the best way to test if new treatments are safe and effective for patients. They involve patients volunteering to take part and the trial conditions are carefully controlled to ensure the results that are seen are accurate and fair. The clinical trial proposed here is the simplest form, termed a 'Phase One' trial, which will test the new treatment on a very small number of volunteer brain tumour patients to check it is safe before using it on other patients. If a patient volunteers, they will have the SVZ included in their radiotherapy treatment. We will then monitor their treatment carefully and record any side effects that may arise to test that no harm is being done. If the trial is successful, we will undergo a 'Phase Two' trial where we will test the new treatment on a larger number of brain tumour patient volunteers to see if they live longer and don't have worse side effects than those who don't have the treatment. This will show whether the new treatment is successful, and if it is, could be used for all eligible brain tumour patients in the future.

A2: Introduction

The results of this thesis recognised the potentially significant role of the SVZ in determining survival outcomes for a subset of GBM patients, in particular the significance of SVZ-invasive tumours. There now follows a purely theoretical proposal as required for HSST assessment in which the ipsilateral SVZ is irradiated to a dose greater than 50Gy with the aim of improving patient Overall Survival, but with the specific caveat that this is only in those patients for which there is macroscopic invasion of the SVZ at presentation. This chapter now sets out the proposition for such a trial which forms the innovation proposal required as part of the HSST assessment.

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[1] This proposal is written assuming that the further research suggested by the main thesis has been performed and found results that support the initiation of the trial proposed here.

A3: Stakeholder Engagement

A3.1. Healthcare Professionals

A meeting was held with the Consultant Clinical Oncologist who supervised the project that is the subject of this thesis, and an MVCC research and development (R&D) manager. The idea for the potential future trial was discussed and agreed as being potentially feasible in principle, subject to completion of the further work discussed in this thesis, verification of the findings thereof, application for formal sponsorship through the Trust's R&D governance procedures, approval from the Health Research Authority (HRA) and ethical approval from a Research Ethics Committee (REC). A separate discussion was also held with the Clinical Director at MVCC who offered their encouragement and support towards conducting such a trial in the future if such encouraging results from an extended retrospective study were obtained.

A3.2. Patients

In conducting healthcare research, Ehlers *et al.* (2017) warn of 'the ivory tower': a disconnect between what academic researchers perceive are important questions and those that patients most need answering. Engagement with patients has been identified as a critical element in the design of patient-centred research studies and patient engagement has been undertaken via research focus groups and even through social media channels (Kim *et al.,* 2018).

When in the early design stage, it is the intention of the trial investigators to perform such patient engagement events to assist in the trial design. Given the distressing circumstances which accompany a GBM diagnosis for a patient, such engagement sessions will have to be sensitively held and be performed through appropriate channels. As all eligible patients for the trial would undergo an informed consent process and be given appropriate time to reach an informed decision, one area of engagement would be on the design of patient information for the trial: ensuring that the appropriate language and tone was used to explain the key concepts. Another potentially useful area of patient engagement would be to determine neuro-cognitive endpoints and toxicity scoring, as whilst the researchers may perceive what is most important to measure and be based on traditional measures such as verbal memory and fluency (Lee and Winton Hall, 2019), this may differ significantly from what a patient may see as the worst side effects, for example, the feelings of exhaustion, losing personal memories or developing hallucinations (The Brain Tumour Charity, 2015, p.6).

A4: Innovation Justification

This trial represents a suitable innovation project as required by HSST as though prospective clinical trials have previously been performed which include the SVZ in the radiotherapy target volume (Valiyaveettil *et al.,* 2020), this trial would be innovative in its design. Previous trials have included all eligible GBM patients in their recruitment for SVZ irradiation, however as determined by the findings of the retrospective research described in this thesis, more selective criteria are instead required as it is hypothesised that inclusion of the SVZ in the radiotherapy treatment only offers survival benefits for those with macroscopic SVZ invasion at tumour presentation. Moreover, the discovery of the significance of SVZ-invasion over purely SVZ-contact did itself come from an innovation in the thesis, as the tumour classification first proposed by Lim *et al.* (2007) was modified by the author to form a novel classification that included two further groups based on the criteria of macroscopic tumour invasion.

A5: Proposed Trial Protocol

A draft clinical trial protocol is included at the end of this innovation proposal in figure A2 and table A1. The proposal is drafted using the Trust's approved template for sponsored studies and would be peer reviewed by the Trust's R&D management board prior to approval. Health Research Authority authorisation would be obtained via an IRAS application and ethics approval would also be required as the study would be trialling radiotherapeutic interventions.

A6: Radiotherapy Treatment Planning Protocol

Including the ipsilateral SVZ in the radiotherapy treatment plan would be a novel concept at MVCC. A feasibility assessment was performed to check that the current radiotherapy techniques in use at our centre would be able to support a proposed future clinical trial. Shown in figure A1 are the target volumes, dose distributions and beam arrangements from the planning study which prescribed a mean dose of 60Gy to the entire ipsilateral SVZ. This prescription is the higher of the two test prescriptions in the proposed trial and was chosen intentionally to determine if OAR tolerance doses could still be achieved from the maximum dose level for the ipsilateral SVZ. All OAR constraints were achieved for this test plan and the total planning and treatment time were of similar magnitudes to current treatments.
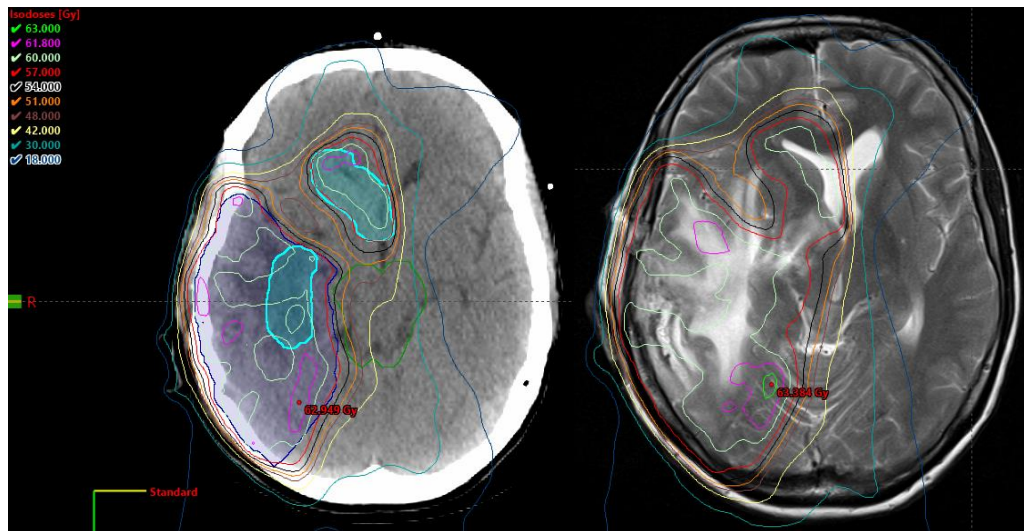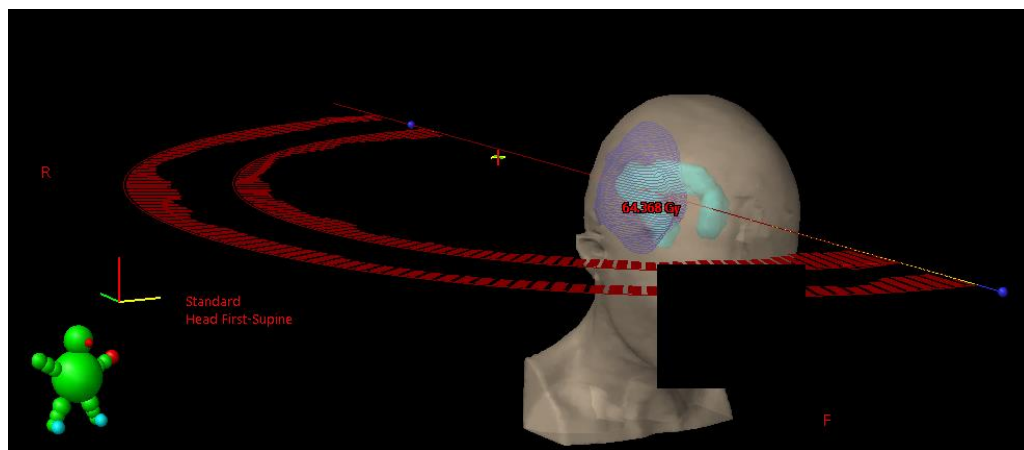
Figure A1: (a) Axial CT slice showing planned dose distribution for a prescribed mean dose to the ipsilateral SVZ (cyan blue) of 60Gy in 30# concurrent with PTV prescription of 60Gy in 30# (dark blue volume). (b) Dose distribution superimposed on co-registered T2TSE MRI showing underlying SVZ anatomy. (c) Two co-planar ipsilateral RapidArc treatment beams and 3D view of target volumes.

A7: Conclusion

This appendix has set out the details for the innovation proposal in the form of a theoretical Phase I trial. A lay summary has been provided together with details of stakeholder engagement, including proposed patient involvement. A feasibility planning study has shown that the planned treatment is achievable and the draft protocol that follows provides full details of the proposed trial.

East and North Hertfordshire NHS

NHS Trust

Prospective Trial of SubVentricular**Zo**ne **R**adiotherapy in patients with SVZ-**in**vasive Glioblastoma Multiforme

Short Title**:** ZORIN Study

Protocol Reference/RD no: RD2021-09

Version number: 1.0

Version date: 7th September 2021

Study Chief Investigator: Dr Anup Vinayan

Study Co-Chief Investigator: Mr Thomas Hague

Study Sponsor: East & North Hertfordshire NHS Trust (incorporating the Mount Vernon Cancer Centre), of Lister Hospital, Coreys Mill Lane, Stevenage, SG1 4AB

IRAS ID: tbc

This protocol has regard for the HRA guidance

Figure A2: Proposed protocol front cover.

Protocol Synopsis

Study Title	Prospective Trial of SubVentricular Zone Radiotherapy in patients with SVZ-invasive Glioblastoma Multiforme (GBM)
Short Title	ZORIN Study
Type of Trial	Prospective
Study Period	January 2023 – January 2025
Primary Objective	*Phase I*: To assess the toxicity and deliverability of concurrent ipsilateral SVZ radiation during radiotherapy for GBM in patients with macroscopic SVZ-invasive tumours.
	Phase II: To determine if concurrent irradiation of the ipsilateral SVZ during radiotherapy for GBM prolongs Overall Survival for patients with macroscopic SVZ-invasive tumours.
Outcome measures	*Phase I*: Toxicity Data
	Phase II: Overall Survival, Progression-Free Survival, Toxicity Data
Study Design and Methodology	Phase II will only commence once independent data monitoring has occurred of the Phase I toxicity data.
	Phase I Methodology:
	Patients will be consented for trial participation and complete a baseline neuro-cognitive questionnaire.
	Patients will undergo a planning CT scan as per standard of care for GBM radiotherapy.
	As well as the standard gross tumour volume (GTV) and clinical target volume (CTV), the ipsilateral SVZ will be delineated by the Consultant Clinical Oncologist prior to planning by using the post-operative co-registered planning MRI.
	A 5mm PTV margin will be added to the SVZ in line with the margin applied to the CTV to form the PTV.
	The ipsilateral SVZ will be prescribed a mean dose of 50Gy in 30# (test arm 1, n=3) concurrently with the PTV which is prescribed a median dose of 60Gy in 30# as per standard protocol. Test arm 2 (n=3) if proceeding, will prescribe a mean dose of 60Gy in 30# to the ipsilateral SVZ.
	Patients will be planned using inversely planned RapidArc using 2-3 coplanar partial arcs. Organs at risk (OAR) will have the same tolerance doses as standard protocol.
	Compromise of the ipsilateral SVZ PTV may be required if overlapping with a planning organ at risk volume (PRV) for an OAR, in order to achieve dose constraints.
	Patients will undergo follow-up MRI scans and further neuro-cognitive questionnaires to assess disease progression and toxicity. Details of questionnaire to be determined following patient stakeholder engagement events.

	Phase II Methodology: As above but a randomised controlled trial with at least two groups – those receiving SVZ radiotherapy (test arm 1±2) and those receiving standard of care (control arm). If both 50Gy and 60Gy mean SVZ dose are well tolerated, two test arms will be employed.
Planned Trial sites	Mount Vernon Cancer Centre
No of Participants	**Phase I:** Standard 3+3 trial design for dose escalation studies [Storer (1989) + Hansen *et al.* (2014)]. 3 patients with mean SVZ dose to 50Gy then potentially 3 patients with mean SVZ dose to 60Gy = 6 patients in total. **Phase II:** Randomised controlled trial with sample size determined via calculation using method from Schoenfeld (1983), assuming significance level α =0.05 and power γ=0.8.
Eligibility Criteria	<u>Inclusion criteria:</u> 1)Diagnosis of WHO Grade IV Glioblastoma Multiforme (GBM) 2) Macroscopic invasion of the ipsilateral SVZ as confirmed on pre-operative magnetic resonance imaging (MRI) 3) Adults aged ≥18 4) WHO Performance Status 0-1. 5) Eligible for 6 weeks of concurrent chemoradiation <u>Exclusion criteria:</u> 1)Previous radiotherapy treatment to the brain, head or neck, 2) Grade III high grade gliomas 3) Non-invasion of the SVZ 4) Contraindicated for MRI scanning 5) Unable to consent for trial
Statistical Methodology and analysis	**Phase I:** Neurocognitive assessments at baseline and during treatment using MoCA (Montreal Cognitive Assessment) and EQD5-BN20. **Phase II**: Kaplan-Meier survival analysis will be performed to determine Overall and Progression-Free Survival statistics. Toxicity assessment as per Phase I.

Table A1: Proposed trial protocol for the 'ZORIN' study.

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Appendix B: List of DClinSci Modules Completed by the Author

DClinSci Appendix – List of AMBS A units and Medical Physics B units together with assignments – Thomas Hague

AMBS – A Units		
Unit title	**Credits**	**Assignment wordcount**
A1: Professionalism and professional development in the healthcare environment	30	A1 – assignment 1 – 2500 words Group work/presentation – 10 minutes (10%) A1 – assignment 2 – 3000 words
A2: Theoretical foundations of leadership	20	A2 – assignment 1 – 3000 words A2 – assignment 2 – 3000 words
A3: Personal and professional development to enhance performance	30	A3 – assignment 1 – 1500 words A3 – assignment 2 – 4000 words
A4: Leadership and quality improvement in the clinical and scientific environment	20	A4 – assignment 1 – 3000 words A4 – assignment 2 – 3000 words
A5: Research and innovation in health and social care	20	A5 – Group work/presentation – 15 minutes (25%) A5 – assignment – 4000 words
Medical Physics – B Units		
B1: Medical Equipment Management	10	Group presentation 1500 word assignment
B2: Clinical and Scientific Computing	10	Group presentation 1500 word assignment
B3: Dosimetry	10	Group presentation 1500 word assignment
B4: Optimisation in Radiotherapy and Imaging	10	Group presentation 1500 word assignment
B6: Medical statistics in medical physics	10	3000 word assignment

B8: Health technology assessment	10	3000 word assignment
B9: Clinical applications of medical imaging technologies in radiotherapy physics	20	Group presentation 2000 word assignment
B10a: Advanced Radiobiology	10	Virtual experiment/1500 word report
B10c: Novel & External Beam Therapy	10	1500 word report
B10f: Radiation Protection Advice	10	1500 word report/piece of evidence for portfolio
Generic B Units		
B5: Contemporary issues in healthcare science	20	1500 word assignment + creative project
B7: Teaching Learning Assessment	20	20 minute group presentation