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Photodynamic therapy in the treatment of intracranial gliomas: A review of current practice and considerations for future clinical directions

Carl J. Fisher* and Lothar Lilge*,†,‡
*Department of Medical Biophysics, University of Toronto
Princess Margaret Cancer Centre
101 College St, Rm. 15-710
Toronto, ON M5G 1L7
†University Health Network
Princess Margaret Cancer Centre
101 College St, Rm. 15-310, Toronto, ON M5G 1L7
‡llilge@uhnresearch.ca

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Invasive grade III and IV malignant gliomas remain difficult to treat with a typical survival time post-diagnosis hovering around 16 months with only minor extension thereof seen in the past decade, whereas some improvements have been obtained towards five-year survival rates for which completeness of resection is a prerequisite. Optical techniques such as fluorescence guided resection (FGR) and photodynamic therapy (PDT) are promising adjuvant techniques to increase the tumor volume reduction fraction. PDT has been used in combination with surgical resection or alternatively as standalone treatment strategy with some success in extending the median survival time of patients compared to surgery alone and the current standard of care. This document reviews the outcome of past clinical trials and highlights the general shift in PDT therapeutic approaches. It also looks at the current approaches for interstitial PDT and research options into increasing PDT's glioma treatment efficacy through exploiting both physical and biological-based approaches to maximize PDT selectivity and therapeutic index, particularly in brain adjacent to tumor (BAT). Potential reasons for failing to demonstrate a significant survival advantage in prior PDT clinical trials will become evident in light of the improved understanding of glioma biology and PDT dosimetry.

Keywords: Oncology; brain tumour; photodynamic dose; therapeutic index; photofin; aminoluvelinic acid.

‡Corresponding author.

List of Abbreviations

AAPM : The American Association of Physicists in Medicine
AIF : Apoptosis Inducing Factor
ALA : Aminolevulinic Acid
ALAS : ALA Synthase
BAT : Brain Adjacent to Tumor
BBB : Blood–Brain Barrier
BTB : Brain Tumor Barrier
CBTRUS : Central Brain Tumor Registry of the United States
CSC : Cancer Stem Cell
CTV : Clinical Tumor Volume
EGFR : Epidermal Growth Factor Receptor
EPO : Erythropoietin
FGR : Fluorescence-Guided Resection
GBM : Glioblastoma Multiforme
HpD : Hematoporphyrin derivative
IL-4 : Interleukin 4
MCF-7 : Michigan Cancer Foundation 7, Breast Cancer Cell Line
mRNA : Messenger RNA
MRI : Magnetic Resonance Imaging
mTHPC : meta-tetrahydroxyphenylchlorin
NIR : Near-Infrared Region
OAR : Organs at Risk
PDT : Photodynamic Therapy
PDGFR : Platelet-Derived Growth Factor
pO_2 : Oxygen Pressure
PpIX : Protoporphyrin IX, precursor of Heme
PS : Photosensitizer
ROS : Radical Oxygen Species
siRNA : Short Interfering RNA
SGZ : Subgranular Zone
SUR : Specific Uptake Ratio
SVZ : Subventricular Zone
TKIs : Tyrosine Kinase Inhibitors
TNFα : Tumor-Necrosis Factor alpha
WHO : World Health Organization

1. Introduction

Malignant gliomas, which account for 2% of all cancer deaths worldwide, despite only accounting for 1% of all tumors, are highly invasive and hence difficult to treat.[1] Of these tumors, glioblastoma multiforme (GBM) comprises the most common in adults and the most aggressive intracranial tumor group. Classification of these tumors is based on their cell of origin with the majority of tumors coming from glial precursors: astrocytes and oligodendrocytes. Morphological grouping is further done by major cell presence leading to the three main types of glioma: astrocytomas, oligodendrogliomas, and oligoastrocytomas according to World Health Organization (WHO) classification. Of these groups of tumors, malignant astrocytomas (also known as glioblastoma multiforme when classified as Grade IV) comprise the most common tumor type with about 50% incidence.[2] Further to this, according to WHO classification, tumors are graded (I, II, III, and IV) according to their anticipated biological behavior, with the higher-grade tumors (III and IV) being more aggressive in the absence of treatment.[3]

The prevalence of gliomas is approximately 18.7 per 100,000 in North America[4] and higher in females than males.[5] In high-income countries, the incidence is higher than in middle and low-income countries.[5] Once GBM diagnosis is rendered, the mean survival time is currently 16 months with aggressive standard therapy comprising surgery, radiation and chemotherapy[6] in their various forms including gamma knife and adjuvant chemotherapy.[7] Survival benefit for the most aggressive treatment combinations over standard therapies is only a few months.[1]

The difficulty in developing new treatment modalities for GBM is the need to retain eloquent and sensitive areas of the brain even when micro metastasis are present and the generally high sensitivity of normal brain tissue to most physical or chemical treatment agents. Treatment of micro metastasis in the brain adjacent to tumor (BAT) is additionally complicated by the blood–brain barrier (BBB) partially isolating normal brain tissue from the blood supply, opposed to the compromised vasculature of the tumor core. Vo and his colleagues have estimated the upper pore size in the Brain Tumor Barrier (BTB) to be 12 nm in an orthotopic murine glioma model[8] permitting relatively large molecules to enter the tumor proper. Most biomolecules cross the BBB by transmembrane diffusion whereby a low molecular weight and high lipid solubility are favored,[9] so the photosensitizer must then partition into the aqueous interstitial fluid for further transport. Photosensitizers which are too lipid soluble are sequestered by the capillary bed, whereby in particular the expression of P-glycoprotein can limit the uptake and needs to be considered specifically in the quarter of the population which under express P-glycoprotein and hence will be more sensitive to photodynamic therapy (PDT). Other drugs or photosensitizers can cross the BBB by the use of saturable transport systems.[10] Novel therapeutics, such as monoclonal antibodies that

inhibit extracellular receptors, are successful in some other cancers, but have found limited use in GBM,[11] and a search for other treatment modalities for GBM is ongoing. Any treatment modality for consideration needs to lead to a greater than 98% tumor volume reduction,[12–14] suggested as a barometer for a new treatment's efficacy, as survival time remains severely limited if this minimum resection rate is not met.

PDT has been investigated as a potential treatment modality over the past decades albeit with variable success, only slightly increasing the median survival[15] although various reports of improved five-year survival can be found.[16] PDT is based on using light activated drugs or their pro-drugs, called photosensitizers, and red or NIR light in the 630 to $>800\,\text{nm}$ range.[17] Light is delivered intraoperatively either as cavity surface irradiation, at an irradiance of up to $400\,\text{mW}\,\text{cm}^{-2}$ or via interstitial diffusers at a power of less than $200\,\text{mW}\,\text{cm}^{-1}$ per diffuser length, with no significant thermal effects reported. The total delivered energy density at the tissue surface, termed radiant exposure, H, ranges clinically from 40 to $230\,\text{J}\,\text{cm}^{-2}$ so it is often not accessible from publication, see Table 1. Photons absorbed by the photosensitizer initiate the production of cytotoxic moieties, such as singlet oxygen or other radical oxygen species (ROS), predominately OH^- radicals, making spatial temporal O_2 availability for the photochemical reactions a prerequisite. This so-called type II photosensitization has the photosensitizer acting as a catalyst.[18,19] Cytotoxic damage to tumor tissue after light treatment (Fig. 1) results through these cytotoxins, which react indiscriminately with all cellular and tissue molecules, particularly lipids and proteins. The lifetime of $^1O_2$ is estimated at 30–200 ns depending on the actual microenvironment, resulting in an effective diffusion radius of 1–3 μm[20,21] emphasizing the need for efficient subcellular localization of the PS within the target cell. The result of PDT delivered in a single treatment session, sometimes referred to as acute PDT, and almost exclusively used clinically, is the previously observed sharp transition between coagulative necrosis and microscopically normal appearing tissue. As the reactive oxygen species do not target a particular signaling pathway or target tumors cannot develop resistance via upregulation of a different survival pathway. This enables the ability of repeat PDT based therapies as proposed[22,23] for metronomic PDT (mPDT) and already employed in a modified version *in vivo* by Eljamel and colleagues.[24] Photosensitizers can be administered in their photoactivable form, such as Hematoporphyrin derivative (HpD),[25] Photofrin,[26] chlorine in the form of meta-tetrahydroxyphenylchlorin (mTHPC),[27] Talaporfin sodium[28] or conversely as a pro-drug as in aminolevulinic acid induced protoporphyrin IX (PpIX) as photochemical active drug.[29] Various groups have used PDT treatment as a primary and adjuvant therapy following surgery with some initial success.[25,26,28,30]

Selectivity of PDT is provided by physical confinement of the minimum required light fluence Φ $[\text{J}\,\text{cm}^{-2}]$ to the boundary of the clinical target volume (CTV). This can be achieved by an appropriate photon source distribution within the limits governed by the optical properties of the tissue.[31–33] Additional selectivity is provided by the pharmacokinetics of the photosensitizers, leading to a local differential accumulation and retention in GBM tumor tissue versus normal intracranial tissues to provide selectivity particularly in BAT seeded with micro infiltrates.[34,35] For ALA, glioblastoma shows a larger synthesis and retention of PpIX compared to the normal surrounding brain tissue.[36–39] PpIX is synthesized via the heme biosynthesis pathway and preferentially retained in tumor cells which are deficient in Fe^{2+} and ferrochelatase, the enzyme responsible for the conversion of PpIX into heme (Fig. 2).[40] Kemmner and colleagues[41] showed that down regulation of the ferrochelatase gene expression is further augmented by siRNA in glioblastoma culture leading to a higher accumulation of PpIX. However, the inherent sensitivity and responsivity of the involved cells and tissues, such as GBM cells versus normal astrocytes, neurons and glial cells, dictate the attainable selectively of the PDT effect, as will be explained below. Additionally, the ability to synthesize PpIX depends on the metabolism of the tumor as demonstrated *in vitro* by Fisher *et al.*[42] and clinically by Johansson *et al.*,[30] however, the exact mechanism responsible for the observed variations is unknown. Recent transcription studies highlighting the variability within individual tumors[43] and the associated variability in photosensitizer PDT resilience may require a more proactive selection of patients for PDT to become the preferred therapeutic option. This suggests that

Table 1. Historical review of glioma PDT clinical trials and outcomes.

Year	Study	Patients [N]	Photosensitizer	Light delivery method	Optical energy [J], [J cm^{-1}], [J cm^{-2}]	Survival time [Months]	Ref.
1980	Perria *et al.*	9	HpD	NA	633 nm, 0.9–9 J cm^{-2}	NR	66
1981	Laws *et al.*	5	HpD	Optical fiber with an emission angle of 40° inserted into tumor center through a needle	630 nm 300 mW 15 mins–1 h = 270–1080 J	1 to >6	113
1983	Wharen *et al.*	3	HpD	6 mm diameter optical fiber coupled to a white light arc lamp, irradiating surgical bed post tumor resection	100 mW cm^{-2} over 385–700 nm, 30 min = 180 J cm^{-2}	NR	114
1984	McCulloch *et al.*	16	HpD	NA	620–720 nm, 1650–2520 J	Up to >24	115
1987	Kaye *et al.*	122	HpD	Flat cut fiber irradiating post resection surgical bed	630 nm, 70–230 J cm^{-2}	Recurrent GBM $\sim$ 6	70
1988	Perria *et al.*	8	HpD	Fiber bundle (4–6 fibers) irradiating interstitially (when complete resection was impossible) or the surgical bed (after resection)	630 nm (laser, interstitial) or 600–680 nm (lamp, surgical bed irradiation), 720–2400 J	1–13	116
1988	Kostron *et al.*	20	HpD	Surgical bed irradiation by laser or a broadband lamp, coupled to an isotropic fiber	630 nm (dye laser) or 590–750 nm (lamp) 25–120 J cm^{-2}	Multiple Recurrence 5 Single Recurrence 1 Primary 27	117
1988	Laws *et al.*	23	HpD	NA	630 nm, 500–1000 J		118
1990	Muller and Wilson	50	Photofrin	Surgical bed irradiation using an isotropic diffuser; eight patients received additional interstitial irradiation by cylindrical diffuser	630 nm, 440–3888 J; interstitial light delivery: 114–675 J	GBM 6.5 CT Complete 17.1	67
1991	Powers *et al.*	7	HpD	Intratumoral cylindrical fiber	630 nm, 658–2028 J	NR	119
1993	Origatamo and Reichman	18	Photofrin	Interstitial cylindrical diffuser and surgical bed isotropic diffuser	630 nm Surgical bed: 50 J cm^{-2} Interstitial 100 J cm^{-1}	5–22	120
1995	Kostron *et al.*	58	HpD	A combination of interstitial (fiber with cylindrical diffuser) and surgical bed irradiation (fiber with isotropic diffuser) using a laser	630 nm, $\leq$ 320 mW cm^{-2} (interstitial), and $\leq$1200 mW cm^{-2} (surgical bed), $\leq$ 8640 J in total	Primary 19 Recurrent 7	121
1995	Kaneko *et al.*	22	HpE	Intratumor irradiation using a fiber-coupled laser, at multiple target points	630 nm, 180 J per target point, 1–13 target points	NR	122

Table 1. (*Continued*)

Year	Study	Patients [N]	Photosensitizer	Light delivery method	Optical energy [J], [J cm^{-1}], [J cm^{-2}]	Survival time [Months]	Ref.
1996	Muller and Wilson	56	Photofrin	Surgical bed irradiation using an isotropic diffuser; 15 patients received additional interstitial irradiation using a cylindrical emitting fiber	630 nm 80-J cm^{-2}, to a total of 440–4500 J (surgical bed PDT); 65–450 J cm^{-1}, to a total of 120–150 J per fiber (interstitial)	Total (Diagnosis) 29.5 Post-PDT 7.8	68
2001	Rosenthal *et al.*	29	Boronated Porphyrin	Surgical bed irradiation using a fiber-coupled laser	630 nm, 25–100 J cm^{-2}	NR	123
2004	Stylli *et al.*	358	HpD	Surgical bed irradiation using an isotropic fiber	630 nm, 240 J cm^{-2}	AA 60 GBM 12	124
2005	Stylli *et al.*	136	HpD	Surgical bed irradiation using an isotropic fiber	630 nm, 70–240 J cm^{-2}	Primary GBM 14.3 Recurrent GBM 13.5	125
2006	Kostron *et al.*	112	Photofrin	NA	630 nm, 40–150 J cm^{-2}	10.5	126
2006	Muller and Wilson	26	mTHPC	Combination of surgical bed and interstitial PDT	652 nm, 20 J cm^{-2}	9	26
2007	Beck *et al.*	10	ALA-PpIX	Intratumor PDT using up to 6 fibers with cylindrical diffuser	635 nm, 4320–11520 J	15	29
2008	Eljamel *et al.*	27	Photofrin & ALA	Post-tumor resection surgical bed irradiation, 5 PDT sessions per patient	630 nm, 100 J cm^{-2} per session	13.2	24
2013	Muragaki *et al.*	27	Talaporfin Sodium	Surgical bed irradiation at 1 or 2 sites using a laser coupled into the surgical microscope	664 nm 27 J cm^{-2} per site (1 cm^2 treatment spot)	26	28
2013	Johannson *et al.*	5	ALA-PPIX	Interstitial PDT with stereotactically implanted cylindrical light diffusers.	635 nm, 720 J cm^{-1} per treatment fibre	>29 months in responders, <9 months in nonresponsders	30

personalized cancer medicine concepts are equally applying to PDT for GBM.

The pharmacokinetic-based selectivity of the various photosensitizers translate at least partially into a clinical impact and prolong the survival times in early clinical trials, but not to the extent anticipated based on the large differences in photosensitizer uptake initially observed in preclinical studies for ALA induced PpIX[36,37] and also in some clinical studies for Photofrin,[26] HpD[25] and ALA induced PpIX[30] reporting specific uptake ratio (SUR) of less than 10. In part, this is due to the intrinsic high sensitivity or low resilience of the normal tissue structures, particular astrocytes and neurons within the PDT treatment field as shown by Lilge and Wilson.[44,42] Neurological deficits due to high radiant exposure [J cm^{-2}] and hence high PDT dose, see below, are a limiting factor within the current PDT delivery concepts. Thus, most trials selected a more moderate light dose at the expense of tumor resection, to provide for a high quality of life for these patients post therapy, particularly when photosensitizer photobleaching is not a PDT-dose limiting effect.[12,30]

The fact that prolonging of the survival times is clinically evident, particularly in recent studies, encourages various groups to maintain interest in evaluating and improving PDT for primary and recurrent glioma although clinical studies activity is currently low with only one phase I trial (2011) related to childhood tumors at the Medical College of Wisconsin registered on ClinicalTrials.gov (June 2012) and one trial for recurrent and *de novo* PDT with up to five therapy sessions underway in Dundee Scotland[24] as well as a continuation of the Munich study.[30]

Prior to designing new GBM PDT clinical trials, the biological or clinical outcome needs to be related to an appropriate definition of the PDT dose. Additionally, the PDT therapeutic ratio between particular invading malignant glioblastoma cells and normal brain structures at the border of the CTV needs to be maximized by exploiting established and novel efficacy-determining parameters. Microinvasions, if left untreated, are the probable causes of GBM recurrence and need to be eradicated or minimized post-tumor resection[45] despite the fact that these microinvasions contribute only a minor fraction of the original tumor mass, essentially $\ll 2\%$, thus affecting the overall resection rate only minimally.

2. PDT Dose Definition and Its Peculiarities in Glioma

Any definition of the PDT dose needs to be based on an "effective" pharmaceutical equivalent "cytotoxic" dose equivalent and hence, it should refer to the concentration of cytotoxic radicals, such as, $^1O_2$, OH^-, H_2O_2, produced and other reactive oxygen species (ROS) according to Fig. 1. Niedre *et al.*[46] determined the number of $^1O_2$ molecules required to destroy a MCF7 cell at 10^7 or approximately 100 μM, a concentration anticipated for an indiscriminate oxidation of biomolecules. While optical quantification of the $^1O_2$ as surrogate for the "cytotoxic" dose is possible *in vitro* based on its very weak phosphorescence emission at 1270 nm, *in vivo* it is detectable only at the tissue surface,[47] so novel developments using fiber coupled with nanostructured detectors will enable interstitial monitoring of $^1O_2$.[48]

The challenge to the medical physicist is to predict and possibly monitor the local $^1O_2$ concentration and hence the PDT-dose with high spatial resolution throughout the CTV, whereas the oncologist needs to consider also the biological response and relate it to the clinical outcome. As shown in Fig. 1, the $^1O_2$ production requires spatial temporal co-localization of the photosensitizer, molecular oxygen $^3O_2$, and the activating photon.

To date, no PDT tissue response model, considering all the three established efficacy determining parameters, has been validated. For example, the optical fluence model[49] states that necrosis will occur if a particular fluence [J cm^{-2}] is exceeded in the tissue for a fixed administered photosensitizer dose and assuming equal pharmacokinetics across the patient population resulting in a known absorption coefficient [cm^{-1}] of the photosensitizer and additionally assuming ubiquitous availability of oxygen to determine the resulting PDT dose gradient essentially solely by the light attenuation gradient. For a normal brain tissue, the effective attenuation coefficient μ_{eff} varies from 4.1 to 2.0 cm^{-1} for wavelength in the 630–690 nm range.[36,50–52]

The photodynamic threshold model,[44,53] see Eq. (1), states that once the photosensitizer absorbed a minimum quantity of photons [hv] per unit tissue volume [cm^{-3}] necrosis results. The model assumes ubiquitous availability of molecular oxygen [$^3O_2$] and the number of absorbed photons is given by the photosensitizer concentrations in the tumor and normal tissue $[PS]_t$ and $[PS]_n$, respectively and

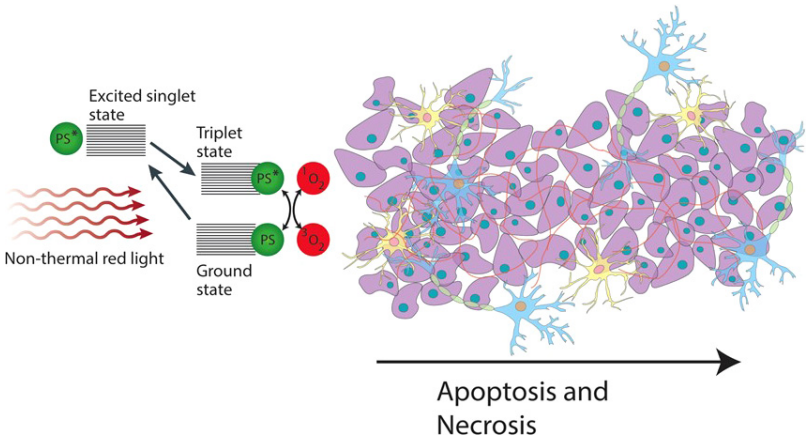

Fig. 1. Overview of PDT mechanism in the brain. Here we are demonstrating a mixed tissue volume similar to that seen beyond the resection boundary where there is a mix of tumor cells (purple cells in illustration) and neuronal cells (blue and yellow cells in illustration).

its molar extinction coefficient.

$$T_x = 2.3\varepsilon[PS]_x\phi(d), \tag{1}$$

where x represents either normal, n, or tumor tissue, t. The available photo density ϕ as function of fluence, H is given by Eq. (2)

$$\phi(d) = H(d)\frac{c}{hv}. \tag{2}$$

Whereby c is the speed of light in vacuum h is Planck's constant $(6.63 \times 10^{-34}\text{m}^2\text{kg s}^{-1})$ and v is

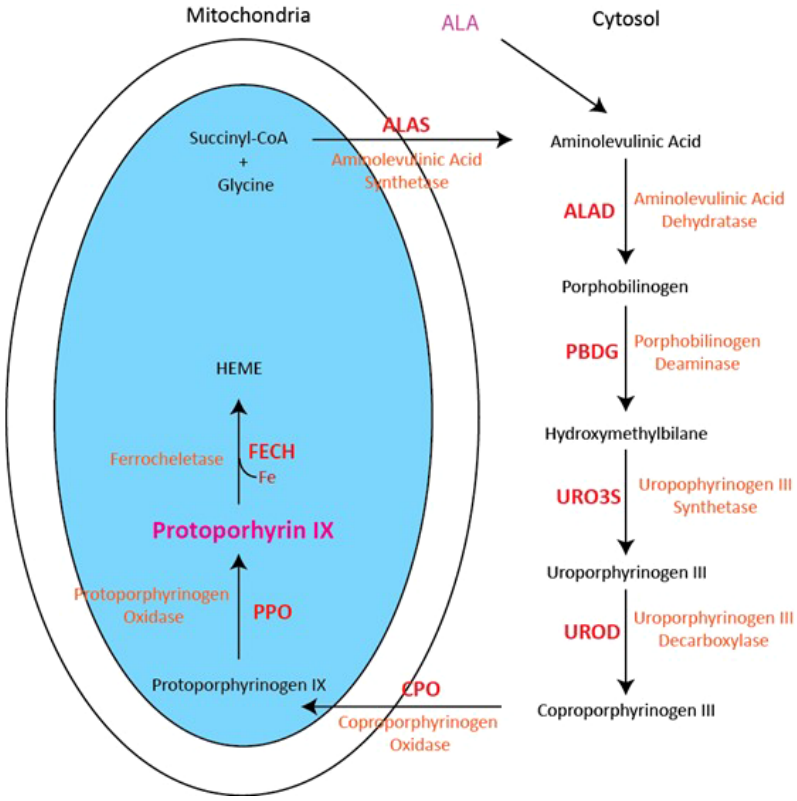

Fig. 2. Overview of the Heme Biosynthesis Pathway. The Heme Biosynthesis pathway is present in most mammalian cells with the rate-determining enzyme being ALAS (or the synthesis of aminolevulinic acid), thus ALA induced PpIX bypasses the rate-limiting step through use of exogenous ALA. Also note that in tumor cells there is a deficiency of Ferrochelatase or a reduction in the amount of free iron in the cells leading to a build-up of protoporphyrin IX (PpIX).

the inverse of the wavelength. In order to achieve PDT selectivity at a particular depth or distance d from the surface or light source, on needs $[PS]_t > [PS]_n$ and preferably $T_t < T_n$ also to be fulfilled. Thus, the PDT cytotoxic dose can be calculated by the product of the fluence rate [mW cm^{-2}], converted into Photon density hv cm^{-2} based on Eq. (2), exposure time [s] and the molar extinction coefficient of the photosensitizer at the activation wavelength [cm^{-1}M^{-1}] times the photosensitizer's tissue concentration [ug g^{-1}]. This definition of the photodynamic dose, proposed by Farrell *et al.*,[53] also required ubiquitous availability of molecular oxygen, which is correct for well-perfused tissues and moderate irradiance or fluence rates (< 100 mW cm^{-2}), when PDT will not cause insufficient oxygen availability. For most tissues the threshold values are in the 10^{18}hv cm^{-3} range when the tissue pO_2 exceeds 2% and represents the inherent sensitivity of the tissue to PDT mediated by a particular photosensitizer.[44,53]

The pharmacokinetics governing the uptake and retention of photosensitizers on an individual patient basis are beyond the physicians control and hence the delivered PDT cytotoxic dose can currently only be modulated via the fluence rate [J cm^{-1}] and possibly the irradiance [W cm^{-1}]. Thus, research efforts have focused on confining the fluence delivered to the bulk tissue within the limitations given by the optical properties of the tissue.[54–57] Quantification of the photon density can be achieved using either analytical or numerical methods based on the source power and the tissue's optical properties[58,59] or by direct interstitial measurements by optical fibers.[60,61] Knowledge of the spatially resolved effective attenuation coefficient μ_{eff} [cm^{-1}] enables determination of the fluence at all distances from a source. Knowledge of the pO_2 and local photosensitizer concentration [μM] allows the use of a tissue response model, such as the threshold model, to adjust the fluence rate so that the threshold value or critical fluence is exceeded throughout the CTV while avoiding undue damage to adjacent organs at risk (OAR).

While light delivery can principally be conformed to the general shape of the bulk tumor by appropriate placement of sources within the confines of the effective attenuation coefficient, it does not provide a basis for selectivity in BAT or the leading edge of the tumor, as the size of the remaining micro invasions, found up to 2 cm beyond the surgical resection cavity, are small ($< 200\,\mu$m) compared to the inverse of the penetration length (> 0.8 mm) of 630–760 nm treatment light,[62] exposing all structures in the BAT to the same photon density gradient.

Similarly, the oxygen diffusion distance, $\sim 100\,\mu$m, and thus the pO_2 distribution is similar between the microinvasion and surrounding normal cells. Hence, the selective uptake/retention of the photosensitizers provides the primary source for PDT treatment selectivity for GBM microinvasion within normal tissue.[36,63–65]

At a fixed depth, d, beyond the bulk tumor resection, or distance from an interstitial light source with T_t, T_n, $[PS]_t$, and $[PS]_n$ denoting the threshold values and photosensitizer concentrations for tumor and normal tissue respectively, selectivity requires the ratio of $T_t/[PS]_t$ to be smaller than the respective ratio of normal neuronal tissues.

$$\frac{T_t}{[PS]_t}\,\frac{T_n}{[PS]_n} \tag{3}$$

However, the intrinsic threshold values, as determined by Lilge and Wilson[36] are unfavorable for PDT with $T_n < T_t$, resulting in an almost complete loss of tumor specific PDT selectivity in the region of the microinvasion according to Eq. (3), despite the intrinsic photosensitizer selectivity being favorable as noted by Refs. 63–65. Thus, as noted in the clinical trials, an increase in light dose leads to higher cell death across all tissue types (including normal brain) or conversely normal brain conserving light doses result in a PDT dose insufficient to eradicate the clinically required fraction of the tumor. The problem is further aggravated by the need to satisfy Eq. (3) for all depth or distance, d, beyond the resection cavity or photon source, at which invasive GBM cells can be found. This depth is commonly set at 20 mm.[34] In this extreme case, the conditions:

$$T_t \le 2.3\varepsilon[PS]_t\phi(20) \tag{4a}$$

$$T_n > 2.3\varepsilon[PS]_n\phi(0) \tag{4b}$$

need to be satisfied. Equation (4) can also be written as

$$\frac{T_t}{[PS]_t} = 2.3\varepsilon\phi(20) < \frac{T_n}{[PS]_n} = 2.3\varepsilon\phi(0) \tag{5}$$

for $d = 20$ mm or directly as

$$\frac{T_t\phi(0)}{[PS]_t\phi(20)}\,\frac{T_n}{[PS]_n}. \tag{6}$$

Using the one-dimensional diffusion equation for homogenous media as solution of the transport equation.[51] We get

$$\phi(d) = \frac{\phi(0)}{D \cdot d} \mathrm{e}^{-\mu_{\mathrm{eff}} d}. \tag{7}$$

With D being the diffusion coefficient given by,

$$D = \frac{1}{3\mu_a + \mu_s'} [\mathrm{cm}^{-1}], \tag{8}$$

leads to,

$$\frac{T_t}{[PS]_t} \frac{D \cdot d}{e^{-\mu_{\mathrm{eff}} d}} < \frac{T_n}{[PS]_n}. \tag{9}$$

Assuming an average effective attenuation coefficient $\mu_{\mathrm{eff}} = 2.4\,\mathrm{cm}^{-1}$ in normal brain it follows that for equal T between tumor and normal tissue $[PS]_t > 3000[PS]_n$ to achieve a selectivity up to $d = 20\,\mathrm{mm}$. This drug selectivity is not attainable by pharmacokinetics nontargeted delivery. Assuming equal T values and currently demonstrated photosensitizer selectivity, PDT selectivity can only be achieved over a narrow depth of $d \approx 1 - 2\frac{1}{\mu_{\mathrm{eff}}}$. Other delivery mechanisms utilizing antibody or nanoparticles, developed with the intention to improve photosensitizer selectively and which are able to reach the tumor mass via the compromised blood tumor barrier (BTB) may not be applicable in the brain as these constructs cannot cross the intact BBB to access in particular the invading front of the tumor.

3. Clinical Evaluations of PDT's Added Value in Treating Gliomas

In the past three decades following the initial work by Perria *et al.*,[66] numerous PDT clinical trials involving primary and recurrent gliomas have been performed using predominantly porphyrin-based photosensitizer such as Photofrin and HpD, so expertise with other photosensitizers exist (see Table 1). Most of the previous brain PDT clinical trials had small numbers and results were mixed, with single center trials tending to show significant improvements due to the use of PDT compared to multicenter trials, where for example, surgical skill can mask the PDT effect. For example, Mueller and Wilson,[67] show a 60-week median survival, whereas the resulting 1995 multicenter study failed to demonstrate a survival advantage of at least three months as stipulated at the clinical trial onset.[68] Some studies demonstrated a significant increase in median survival time compared to surgery alone or standard therapy when using adjuvant PDT.

One common problem in evaluating clinical trials is the inconsistent reporting of the light dose delivered particularly if neither irradiance nor radiant exposure is quoted but only total delivered energy is quoted. As recently noted in the American Association of Physicists in Medicine (AAPM) task force 140 report,[69] the appropriate quantities of irradiance or fluence rate [$\mathrm{W\,cm^{-2}}$] and radiance exposure or fluence [$\mathrm{J\,cm^{-2}}$] should be quoted.

The survival advantage extends for the majority of the studies by only a few months (max $\sim$ 12), so a retrospective analysis by Stylli and Kaye showed a cohort of long-term survivors ($>$120 months past diagnosis) following HPD-mediated PDT at high fluence ($240\,\mathrm{J\,cm^{-2}}$),[25] pointing to the need to deliver a large total dose to achieve a very high tumor volume reduction rate. Similarly, the Munich group showed recently that in a small cohort[30] 3 out of 5 long-term survivors had good intra tumor PPIX synthesis which also translated into an above 30 month survival.

For intracavity photoactivation of the PS, a liquid filled balloon creating a spherical cavity with central light source for simplified light dosimetry calculation is often selected. Alternatively, direct filling of the cavity with a low light scattering medium, commonly based on intralipid or its various analogues can be used. While most studies listed in Table 1 used moderate radiant exposure doses of 40–$120\,\mathrm{J\,cm^{-2}}$, higher doses show more promise and have been reported in literature,[70] so often it is not accessible due to the unknown size of the malignancy.

Most groups reported improved clinical outcomes as measured by reduced MRI or contrast enhanced CT around the resection cavity for surrogate to validated tumor volume reduction rates and hence indicators for a potential longer term recurrence-free survival. Infrequent transient reduction in the cognitive brain function has been observed following adjuvant PDT; however, some reports indicate that at higher radiant exposures the patient's subsequent Karnofsky score decreases in an inverse relationship with PDT dose, indicating potential cognitive deficits following PDT. Most clinical reports provide an insufficient discussion of neurological deficits post-PDT or the appropriate progression-free survival time points. The Muller group,[26] showed for Photofrin-mediated PDT a

reduction in the Karnofsky score for a radiant exposure dose of 80 J cm^{-2} compared to the 40 J cm^{-2} group with a statistical significant reduction evident up to three months post-PDT. This is of concern if prolonged longer survival is to be achieved by means of higher radiant exposure alone. It should be noted that not all clinical trials can report on short and long term neural deficits as the disease inevitably will lead to diminishing mental capacity as recently reviewed by Eljamel *et al.*[24]

The meta analysis by Eljamel[71] on adjuvant PDT in high grade gliomas comprising more than 1000 patients, utilizing a variety of photosensitizers, Photofrin ($\lambda = 630$ nm), ALA induced PpIX ($\lambda = 635$ nm), mTHPC ($\lambda = 652$ nm), Chlorine e6 ($\lambda = 660$ nm) showed median survival of > 16 and > 10 months for de novo and recurrent GBM, respectively. Independent of the clinical trial a high number of early failures is notable where PDT demonstrated no benefit. There are two possible reasons as outlined in the past sections. Physically achieving an adequate PDT dose at the location of the recurrence, for example, if these are > 0.5 cm beyond the resection cavity; or; biologically as this particular tumor did not respond to PDT or had a very high threshold value T_t possibly due to constitutively active survival signals as anticipated for mesenchymal versus proneural GBM.[72,73] To date, no studies are available quantifying the PDT response as a function of genetic expression and mutations in GBM, as well as the tumor's growth sustaining signaling pathways. This should become a requirement particularly within the concept of personalized cancer medicine. This suggests an important departure from the previously held doctrine in PDT-research that PDT generated ROS dominated cytotoxic effects are independent of any particular signaling pathway. In particular, the tumor's ability to accumulate or synthesize the photosensitizer selectively as well as inherent defenses against ROS need to be considered.

In general, the clinical trials so far appear to demonstrate that success or failure of adjuvant PDT treatment in prolonging survival is not determined by removal of the bulk tumor even if it constitutes above 98% of the total tumor burden but rather the extent of destroying the infiltrative tumor cell beyond the surgically visible tumor boundary, as demonstrated by fluorescence or MRI image guided resection. Over 80% of all recurrences are observed within 2 cm of the resection boundary,[45] emphasizing the need for control beyond the resection boundary. Lastly, an overall biomedical field effect needs to be considered as there is additionally recent evidence that Hif2a overexpression in brain tumors can reprogram nonstem-like glial cells to a glioma stem cell-like behavior leading to a faster reestablishment of a novel glioma.[74]

In summary, a review of all prior clinical studies demonstrates a lack of molecular information about the tumor driving mutations, expression of hypoxia inducing factors which not only leads to potential differences in the intrinsic tumor sensitivity and responsivity to PDT, but also towards a tumor's inherent resistance and repopulation potential.

4. Cellular Responses to PDT and Options for Selectivity Modulation

Historically, the most commonly used photosensitizers for adjuvant or standalone glioblastoma PDT treatment have been Photofrin (Pinnacle Biologics Inc., Muller and Others), or its alternative form HpD and ALA induced PpIX. Additional expertise exists for mTHPC (BioleticPharma Ltd., Kostron), Talaporfin Sodium (Light Sciences Oncology, Akimoto), and Benzoporhyrin derivative (BPD) by Schmidt and others.[75] All these photosensitizers can cross the compromised BTB within the tumor bulk but none or to a significantly lesser extent the intact BBB within the BAT, where again the expression of the p-glycoprotein is known to regulate the photosensitizer uptake.[9] Evidence suggests that ALA can cross the BBB also in normal brain tissue structures.[76]

In vitro cells sensitized with ALA induced PpIX will undergo cell death through a primarily apoptotic mechanism. Clinically, primary cell death will follow a similar path, with a first wave of cell death occurring within the first 8 h. However, the majority of the apoptotic bodies lyse due to the lack of local phagocytosing microglia, as they have been equally affected by the PDT treatment in the CTV. During this lysis process, cytokines such as tumor necrosis factor alpha (TNFα)[77] are released, which are thought to be primarily responsible for the second, delayed, additional cell death.[78] This could also possibly be due to the disruption of the BBB following ALA induced PpIX-mediated PDT as shown by Ref. 79. These two distinct mechanisms following PDT have led to attempts to modulate both delayed lysis induced inflammation and delayed cell death, via the proposed metronomic PDT delivery described by Bisland *et al.*[22] Here the

overall goal is to reduce the cytotoxic dose rate allowing normal tissue repair for microglia survival and completion of the immunological quiescent apoptotic cell death. Other already mentioned approaches include improving ALA-induced PpIX-mediated PDT selectively by using the already mentioned siRNA to down-regulate ferrochelatase.[41]

Photofrin and other photosensitizers (mTHPC), including Talaporfin sodium, and BPD have a somewhat different subcellular distribution and can be found in the plasma membrane of cells when the BBB is compromised or in the vascular endothelial cells within the intact blood vessels and as such the cellular damage dynamics and the dose response are different. Dereski *et al.*[80] determined that Photofrin and similar photosensitizers do not require subcellular uptake to elicit a PDT effect on normal brain tissues or tumor. While the lipophilic photosensitizers can penetrate into the membrane layers of the BBB they cannot exit into the hydrophilic environment of the brain parenchyma. Hence, light exposure treatment produces a disruption of the blood vessels in the light exposed tissue volume, resulting in wide-spread platelet activation and adhesion, vascular occlusion and subsequent starvation of cells from oxygen and nutrients leading to necrosis.[81] Photofrin-mediated PDT produces a large lesion volume albeit selectivity between normal cells and GBM microinvasions is lessened or eliminated. The primary mechanism of cell death is necrotic, followed by an immediate inflammatory response. To enable selective therapy, therapeutic approaches must focus on exploiting different mechanisms of cell death through modulation of treatment delivery such as modulating the immune response or options to modulate the immediate intrinsic cellular sensitivity and secondary effects following the delayed PDT response, while maintaining equal tumor and tumor stroma response to PDT. This may allow increasing the overall tumor response. In this respect, Seshadri *et al.*[82] showed greater sustained reduction of pO_2 in the target area using HPPH (A Hexyloxyethyl derivative of pyrophenphoxide), a vascular acting photosensitizer, and low-rate light delivery up to 4 h. This resulted in a large necrotic tumor volume in a rat model.

5. Modulating PDT Response: Focusing on Normal Brain Sensitivity

The well-documented secondary wave of neuronal death following PDT or stroke provides ample opportunity to modulate biological signaling cascades in normal brain with the aim to enhance the survival of the exposed neural tissue. The secondary delayed cell death is initiated via inflammatory processes.[81] The focus needs to be on examining agents or procedures which demonstrated protection of neural tissue in these situations. These treatments should focus on preventing damage similar to that seen in traumatic brain injury, or other injury types leading to apoptosis and necrosis such as stroke and hemorrhage.

Preferably, these adjuvant treatments need to address all PDT induced direct cell death mechanisms, those due to the strong inflammatory response as well as due to secondary hypoxia following vascular occlusion. For the inflammatory response, Mroz *et al.* noted that the release of interleukins, such as IL-4 and TNFα, promotes tumor cell destruction, which is beneficial when additional tumor cells are destroyed.[83] This is of particular interest when it is combined with specific tumor markers as suggested through research conducted by Korbelik *et al.*[84] However, as PDT is currently employed, nonspecific implementation of the opposite effect is observed, where the release of the interleukins impacts more normal neural tissue, leading to neuronal cell destruction visibly beyond the direct PDT associated necrotic boundary, as reported by Lilge and Wilson.[36]

The use of neuroprotectants was proposed by many,[85–87] for stroke-related therapy, but when administered prior or during light activation, could protect normal neurons tissue throughout and beyond the CTV by a similar mechanism as seen in stroke neuroprotection against the direct cytotoxic insult and the toxic aspects of the immune response. The disruption of blood vessels, as observed for vascular active photosensitizers, can cause hypoxic regions extending beyond the BAT,[88] and a situation similar to stroke is created, whereby a neuroprotectant improving the neuron survival during hypoxia *in vivo* can prove beneficial in PDT. Thus, in theory, the therapeutic index can be increased in mixed target tissues by effectively increasing T_n, according to Eq. (9), permitting an increase in the light dose throughout the region beyond the resection boundary, thus greater tumor resection rates become feasible.

EPO has been investigated in numerous stroke associated clinical trials and an increase in the Barthel Index, a clinical measure for cognitive

function following stroke was demonstrated.[89] This represents either preservation of neuronal connections or retaining plasticity in neuronal reconnections. EPO was investigated pre-clinically *in vivo* and shown to provide a protective benefit following excitotoxic neuronal injuries,[90] traumatic brain injury,[91] and arterial occlusion.[92] The use of EPO as neuroprotection is based on the presence of the EPO receptor (EPOR) on neuronal cells at birth and again with increasing age as shown in a murine model.[93] Using mRNA assays we confirmed this also in human brain tissue biopsies providing an opportunity for neuroprotection in conjunction with PDT to reduce the delayed or secondary PDT response in patients. Most GBMs do not express significant amounts of EPO receptor or IGF-1 receptors known to augment EPO effects.[94]

The function of EPO as neuroprotectant with PDT is similar to that of its normal role in the hematopoietic system, where EPOR activation initiates Hif1-alpha.[95] In the brain, astrocytes sensing an oxygen deficient environment release EPO, which acts in a paracrine and autocrine manner on neural tissue, lead to their protection.[96] Further studies are required to determine the magnitude of an EPO effect for intracranial PDT, particularly during the acute phase of the PDT induced damage. Furthermore, it is to be determined whether in a mixed tissue target, the benefit of a potential neuroprotectant carries across other cell families making up the tissue, particularly in tumors with various growth signals, which would not result in an improvement of the therapeutic index.

In conjunction with this, hypothermia has also been used as a neuroprotectant for stroke-related therapies and at least once for PDT. Dereski *et al.* demonstrated that hypothermia led to an upward of 50% reduction in normal brain lesion volume following Photofrin-mediated PDT.[97] The exact mechanism of hypothermia-mediated protection has not been fully elucidated. There has been some progress pointing to an anti-apoptotic mechanism, or a reduction in ROS generation. Specifically, hypothermia has been demonstrated to lead to a decrease in AIF release following cerebral artery occlusion.[98] The mechanism for hypothermia-mediated protection is important if it is to be used in any PDT-mediated scheme.

If the reduction in lesion volume following PDT is mediated through reduction in ROS generation, it can be inferred that one would manipulate the threshold values in T_t and T_n in a similar manner, depending on whether the reduction is enzyme dependent (increase in scavenger proteins with hypothermia) or physically mediated. Physical reduction in ROS generation would impact PDT efficacy negatively, and thus needs to be determined early on, while enzyme specific reductions, through the increase in Superoxide dismutase, Catalase, Glutathione peroxidase, will need to be determined in each tissue type if that is the mechanism.

Independent of the neuroprotection avenue followed, the increase in PDT threshold values needs to be significantly large for a normal brain than GBM and additionally, the impact of the particular approach on the cells ability to retain or synthesize the PS, as in the care of ALA induced PpIX, needs to be investigated. Fisher *et al.*,[42] have shown recently that hypothermia at 32°C resulted in an increased PpIX synthesis in various GBM cells *in vitro*, albeit without any changes in PpIX concentration in primary rat neurons *in vitro*, providing good support about the feasibility of this approach.

6. Modulating PDT Response: Focusing on the PS Accumulation and Intrinsic Sensitivity of Tumor Cells

A second complementary approach to increase the therapeutic index is by reducing the resilience of GBM cells to PDT generated cytotoxins. The potential synergies between PDT and Tyrosine Kinase Inhibitors (TKIs) are beginning to become evident.[99]

TKIs lead to inhibition of the cells survival signaling by blocking the binding pocket of the receptor preventing the phosphorylation signal cascade. These agents have been used clinically for various cancers, as a cytostatic agent. While all TKIs failed to provide a long-term tumor control in clinical trials due to the onset of resistance through switching to a different survival signal by the cells, PDT provides an acute setting, whereby the TKI effect of inhibition of survival signals potentiating cell death needs to be achieved only for a period of 1–3 days for a possible synergistic effect to take place. GBM provide an ideal situation to exploit this temporary inhibition mechanism in conjunction with PDT, as many GBM tumors are driven by a small number of survival signals, commonly one receptor tyrosine kinases in conjunction with the loss of a tumor suppressor gene, most commonly

PTEN. The two dominant kinases that drive GBMs in particular, are the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor receptor (PDGFR) families accordingly combined for over 60% of all GBMs. In most instances, only one receptor is present; however, some tumors express both.[100] For both of these receptors, clinically approved TKIs are available, which can cross the BBB, and thus provide an opportunity for *in vivo* evaluation. Clinical inhibitors such as Erlotinib (Roche, Basel, Switzerland), Lapatinib (GSK, London, England), and Nilotinib (Novartis, Basel, Switzerland) can provide a starting point, albeit knowledge of the tumor receptor status is required prior to choose a particular inhibitor to combine with PDT. For this scenario to provide improved PDT efficacy, one needs to determine whether these inhibitors have an effect on normal neural tissue and the magnitude of improvement by this combination on the therapeutic index, as well as their influence of PS retention or synthesis in the tumor and normal surrounding tissue.

When considering pathway inhibitors, an overlooked important tumor fact is that gliomas are quite diverse spatially and temporally, in terms of tumor niches compared to the founding clone. As Sottoriva *et al.*[43] demonstrated that any glioma can contain five to six distinct tumor subpopulations that develop in different locations over time, often representing various classical GBM notations. However, EGFR mutations or amplifications are normally present in founding clone, its interruption should target most of the tumor subpopulations, although there is a possibility of a limited efficacy in some cases.

The SUR of the photosensitizer, and hence a desirable condition to increase d, the distance over which selectivity can be achieved according to Eq. (9), can possibly be achieved for nanoparticle-based delivery, albeit due to their size (>5 um) a temporary opening of the BBB may need to be realized. The latter can be achieved by injecting a salt solution, peptides as proposed by Sarkar *et al.*[101] or locally the use of micro or nanoparticle-mediated ultrasound BBB disruption.[102] PDT research in gliomas, and more broadly other cancers, is following two different approaches to increase the efficacy of treatment. For gliomas, some groups worked on physical approaches to treat a specific CTV, such as interstitial PDT with image guided fiber placement to deliver the minimum required photon density.[30,65] In small case studies, these groups have found some success in a limited number of patients. However, various reports showed that some tumors do not synthesize PpIX from exogenous ALA,[30] leading to insufficient PpIX in the CTV. This is a challenge to clinicians and researchers as it basically invalidates the applicability of the threshold model as $[PS]_t$ being zero does not allow any solution to Eq. (9), and in the extension utility of any adjuvant therapy.

Various PDT anti-tumour effect enhancements were suggested and tested on glioma *in vitro* and occasionally *in vivo*, whereby increased photosensitizer concentration in the cells or the tumor as well as the cell and tumor response. Takahashi and colleagues[103] demonstrated a combination effect of hyperthermia (43°C) and ALA induced PpIX-mediated PDT and showed a significant reduction in tumor growth 5 days post-therapy. Similarly Charkrabarti *et al.*[104] noted a strong augmentation of the anti-tumor effects also *in vivo* for Photofrin-mediated PDT when miR-99a was co-administered to inhibit the PI3K/AKT signaling pathways in p53 wild-type tumors. Similarly, recently Chen *et al.*[105] proposed the use of 1,25-dihydroxyvitamin D3 which resulted in an increase of coproporphyrinogen oxidase at the transcript level and translated into a higher PpIX synthesis and PDT effect. Use of the antibiotic ciprofloxacin to augment the lethal effect of ALA-mediated PDT was demonstrated by Cornelius *et al.*[106] However, in all of these studies the effect on normal brain structures is not reported and needs to be tested prior to considering these avenues for a future increase in the therapeutic index according to Eq. (9) as required to treat the infiltrating front of GBMs clinically.

7. Conclusions

While physical approaches to confirm PDT response to a particular treatment area have proven beneficial in well-circumscribed tumors or situations where the CTV is delineated by organ boundaries, such as the prostate and skin, they are often inadequate in treating GBM beyond the resection boundary. In the solid tumor cases, a steep PDT dose gradient at the tumor boundary is attainable by the change in tissue optical properties and photosensitizer concentration. Physical PDT dose confinement schemes using confined light delivery are

unable to provide a high therapeutic index in mixed tissues as exemplified in Eqs. (3) and (8). As in heterogeneous tissues with a mix of both tumor and normal cells, the task is to achieve a sufficient dose throughout the invasion zone for tumor cell eradication without causing clinically significant neuronal damage. Hence, if repeated treatments as proposed by Eljamel *et al.*[24] and PDT responsivity modifications are considered, PDT can provide an additional survival advantage to GBM patients. In these situations, selectivity and targeted PDT response can only be achieved by either improving selectivity in PS uptake and retention through their intrinsic pharmacokinetic properties and antibody co-treatment,[107–109] or alternatively by a biological approach which modulates the intrinsic threshold values of tumor and normal cells in opposite directions to widen the therapeutic index. The threshold value of tumor or healthy cells, which represents the cell's sensitivity to PDT, now needs to be considered as an independent modifiable response parameter which can be personalized in addition to the light delivery.

The preceding manuscript described how the local control of GBM could be improved by optimizing the PDT dose, employing neuroprotective strategies, as well as inhibiting tumor survival signaling. These strategies could certainly lead to a clinical increase in patient survival, with the potential to achieve high five-year survival rates. However, recent finding pertaining to the origin of GBM-forming stem cells needs to be carefully considered to determine the ability of a local therapy, such as a single PDT treatment, to achieve a cure on its own. This is due to the fact that the brain's ability to maintain itself is organized distinctively from other organs, particularly in the intra-organ distribution of adult stem cells. In most organs, stem cells are homogenously distributed throughout the organ and cancer stem cells (CSC) co-localize with the tumor and hence are also subject to PDT dose and associated elimination. In the brain, however, glial and neuronal stem cells are located in selective areas within the subventricular zone (SVZ) lining the lateral ventricles, and the subgranular zone (SGZ), part of the dentate gyrus of hippocampus, where both harboring neural stem cells and progenitor cells constantly generate new neuronal cells. On one side, there is a recent review by Heywood *et al.*[110] pointing to mounting evidence that GBM initiating cells are located at the same selective locations and contribute to the development and re-establishment of brain tumors, thus they are outside the PDT-CTV. Similar results were observed by Zong *et al.*[111] and expanded to glial progenitors and astrocytes, which could all serve as *de novo* origin for glioma. The difference between the interpretations by Heywood versus Zong is the speculation that CSCs can also be further transformed progeny cells from the original neuronal and glial progenitors. Therefore, it is still possible that GBM CSCs co-localize with the tumor and hence are accessible for a local therapy. This is also suggested by Piccirillo *et al.*[112] showing the presence of cells within the tumor margin permitting high engraftment rates *in vivo*, so these cells appear not to enable the self-renewal of a major identifier of cancer stem cells.

Identifying the actual location of brain CSC is a prerequisite step to determine if a single PDT application as adjuvant therapy could potentially provide a cure in the case of the CSC spatially co-localized with the tumor; or if multiple PDT applications are required in the case of brain CSC residing away from the actual cancer essentially considering GBM a treatable chronic disease. Alternatively, the SVZ and the SGZ can become treatment target volumes, if a differential sensitivity of CSC from that of normal neuronal stem cells can be established.

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