Mol Neurobiol (2007) 36:36–44
DOI 10.1007/s12035-007-0016-z

Targeting Astrocytomas and Invading Immune Cells with Cannabinoids: A Promising Therapeutic Avenue

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Received: 5 February 2007 / Accepted: 11 May 2007 / Published online: 3 July 2007

Abstract The last quarter century has borne witness to great advances in both the detection and treatment of numerous cancers. Even so, malignancies of the central nervous system, especially high-grade astrocytomas, continue to thwart our best efforts toward effective chemotherapeutic strategies. With prognosis remaining bleak, the time for serious consideration of alternative therapies has arrived. Various preparations of the marijuana plant, *Cannabis sativa*, and related synthetic and endogenous compounds, may constitute just such an alternative. Cannabinoids, although much maligned historically for their psychotropic effects and clear abuse potential, have long been used medicinally and are now staging an impressive comeback, as recent studies have begun to explore their powerful anti-tumoral properties. In this study, we review in vitro and in vivo evidence supporting the use of cannabinoids for treatment of brain tumors. We further propose the continued intense investigation of cannabinoid efficacies as novel anti-cancer agents, especially in models recapitulating such properties within the unique environment of the brain.

Keywords Cannabinoids · Chemotherapy · Cancer · *Cannabis sativa*

According to a report released in 2003 by the Substance Abuse and Mental Health Services Administration [1], nearly 100 million Americans (40%) have used *Cannabis sativa* at least once, making this plant the most widely used illicit drug in the USA [2]. While abuse of *C. sativa* constitutes an obvious health problem, its controlled medicinal use is known to provide therapeutic and palliative benefits, as emphasized by a recent surge of case studies and anecdotes reported by patients [3]. Specifically, case studies report the spasmolytic and mild analgesic properties of controlled *C. sativa* intake [3, 4], and cancer patients that smoke or eat *C. sativa* before receiving chemotherapy report its powerful antiemetic and appetite-enhancing effects [2, 5]. Furthermore, GW Pharmaceuticals of the UK has reported that phase III clinical trials confirm cannabis-based medicines are generally well tolerated and have potential efficacies for neuropathic pain and spasticity associated with multiple sclerosis [6–8]. Considering this wide array of therapeutic potential carried by *C. sativa*, significant effort has been directed toward increasing our understanding of the molecular basis underlying cannabinoid action, with the aim of optimizing therapeutic properties while avoiding adverse effects.

In the 1970s, classic experiments tested the validity of the therapeutic properties associated with plant-derived cannabinoids (also known as phytocannabinoids), generating a body of cellular and whole-animal evidence for their anti-inflammatory, anti-proliferative, and cytotoxic effects [9, 10]. The 1980s and 1990s saw rapid advances toward understanding the molecular mechanism of their actions. Landmark discoveries include the molecular cloning of cannabinoid receptors CB1 and CB2 [11, 12] and the identification of two endogenous ligands—the endocannabinoids (eCBs) anandamide and 2-arachidonoylglycerol (2-AG)—as well as their respective biosynthetic and degrading enzymes [13–19]. These discoveries allowed the development of synthetic compounds targeting specific

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components of the eCB signaling system, opening the door to preclinical trials that could test putative therapeutic properties. An exciting avenue of research lies in the use of cannabinoid-based compounds for cancer treatment, particularly glioblastoma (GBM). Relevant evidence is reviewed here including both in vitro and in vivo results, as well as two possible mechanisms of action: cannabinoids acting either directly on tumors or indirectly through immune effectors (most likely microglia, the macrophages of the brain). Current understanding of eCB signaling and brain tumor biology will be considered first.

Cannabinoids and Their Receptors Collectively, cannabinoids include approximately sixty phytocannabinoids, two well-established eCBs, and a steadily increasing number of synthetic agonists and antagonists. Cannabinoids affect many biological functions—from impairing short-term memory and motor coordination to inducing analgesia and immunosuppression—by interacting with cannabinoid receptors [20], two of which, CB1 and CB2, have been cloned. These receptors share 44% protein identity and couple to Gi/o proteins, inhibiting adenylyl cyclase activity and activating the MAP kinase pathway. Interestingly, only CB1 regulates ion channel conductance [21]. CB1 and CB2 display unique pharmacological and expression profiles, a dichotomy that holds tremendous promise for targeted therapy. Specifically, CB1 is abundantly expressed by neurons and CB2 by peripheral immune cells, suggesting that selective CB2 ligands may regulate immune responses without producing psychotropic effects. Note that while recent studies demonstrated limited CB2 receptor expression in small subpopulations of neurons [22], these discrete brain regions are not likely involved in the psychotropic effects of *C. sativa*, as genetic studies have ascribed such adverse side effects of THC to specific activation of CB1 receptors [23, 24]. Thus, advancements in the chemical synthesis of cannabinoid ligands and increased understanding of the molecular targets they activate continue to strengthen the therapeutic potential of the eCB signaling system.

While the majority of classic studies have focused on THC, more recent reports suggest that the medical benefits of *C. sativa* likely result from the combined action of multiple phytocannabinoids activating distinct receptors. For example, cannabinol (CBN) likely acts through CB2 receptors and cannabidiol (CBD) through a distinct, as yet unidentified target [25, 26]. Supporting the existence of additional cannabinoid receptors are pharmacological studies carried out in $CB1^{-/-}/CB2^{-/-}$ mice showing the presence of atypical cannabinoid responses when both receptors are genetically deleted [27, 28]. Several investigators have hypothesized that the orphan receptor GPR55 may constitute such a novel cannabinoid receptor, although unambiguous data are still lacking [29, 30]. As many of the adverse effects of THC are mediated through neuronal CB_1 receptors, pharmacological agents specifically targeting CB2 receptors, or any of the orphan cannabinoid receptors, constitute an attractive approach for the development of cannabinoid-based therapeutics lacking psychoactive and neuropsychiatric side effects. Adding support to this view, induction of CB2 receptor expression occurs under specific pathological conditions (in microglia and tumor cells, see below) [31, 32], and thus, provides an opportunity for disease-specific targeting.

Astrocytoma Biology Cell constituents of healthy brain include neurons and glia, with glia further classified as astrocytes, oligodendrocytes, or microglia. While accounting for only 1.7% of all new cancers worldwide, the International Agency for Research on Cancer estimated more than 189,000 new cases of brain and central nervous system (CNS) cancers in 2002 [33]. That same year, deaths due to such cancers were estimated at nearly 142,000, accounting for 2.1% of global cancer deaths [33]. The term *glioma* broadly classifies primary neoplastic tissue of glial origin, with astrocytomas representing the most common group. In the USA alone, some 43,800 new cases of CNS tumors were projected for 2005, with gliomas making up nearly half of these [34].

Astrocytomas are highly aggressive tumors that express GFAP and have fibrillary cytoplasm and angular nuclei [35]. The tumor mass often contains a high degree of cellular heterogeneity and displays significant microvascular proliferation. The World Health Organization classifies astrocytomas into low (I and II) or high grade (III and IV), depending on their particular location and growth rate. Anaplastic astrocytomas (grade III) and GBM (grade IV) represent the most aggressive primary tumors of the CNS and account for nearly one third of all diagnosed brain tumors [36]. Their precise cellular origin remains debated principally because of their heterogeneity and broad etiology. Possible origins include terminally differentiated astrocytes, glial precursors, or cancer stem cells [35, 37]. An insight into their origin will likely come from correlations between histology and gene profiling. A great deal of attention is currently focused on abnormal cell cycle regulation [38], for example, dysfunction of the p53 pathway ($p14^{ARF}$, HDM2, and p53) and the RB1 pathway ($p15^{INK4B}$, $p16^{INK4A}$, CDK4, Cyclin D1, and RB1). Mitogenic signaling pathways may also be dysfunctional, for example, RAS, MAPK, and PI3K coupled to growth-factor receptors (IGF1R, EGFR, and PDGFR) [35]. In summary, while the origin of astrocytomas remains debated, the molecular mechanisms involved in their pathogenesis and insights into how these components relate to different grades of astrocytomas are being gained.

Increased knowledge of the molecular mechanisms involved in astrocytoma pathogenesis has led to the develop-

ment of genetic mouse models that mirror important features of the clinical disease. Rodent models used during the mid-1970s mimicked de novo human brain tumors by direct mutagen administration or via allograft/xenograft procedures [35]. The first foray into genetic modeling of astrocytomas occurred in the late 1990s, using the GFAP promoter to drive expression of various forms of constitutively active mitogenic factors including Ras and Src [35, 39]. Simultaneously, Eric Holland (while in the laboratory of Harold Varmus) developed a novel mouse model for GBM which employed RCAS/*tv-a* transgenic mice and avian viral infection for cell-type-specific transfer of various oncogenic constructs [35, 40]. Interestingly, while independent or combined expression of constitutively active Ras or Akt failed to produce tumors in GFAP-selective viral infection studies, nestin-expressing cells infected with both constructs initiated tumor growth in more than a quarter of mice, reinforcing the notion that the origin of astrocytomas may lie in neural progenitor cells [35, 37, 40, 41].

Astrocytomas often arise from intricate CNS structures and are highly invasive in nature, and thus, constitute a major challenge for surgical resection and aggressive treatment protocols. Even after decades of research aimed at developing effective therapies, the current prognosis remains dismal upon high-grade astrocytoma diagnosis [42]. Treatments are a Pyrrhic victory at best, as they are nearly as invasive as the pathology itself, frequently offering a merely palliative therapy that typically includes initial surgical resection of the neoplastic region followed by a combination of intense radio- and chemotherapies. Targeted radiotherapy moderately improves patient outcome [42], increasing mean survival time of patients with grade IV astrocytomas by 22 weeks. Chemotherapeutic regimens often fall short of effectively opposing tumor progression, owing dose thresholds and abbreviated treatment cycles to this characteristically limited life expectancy after patient diagnosis. Clearly, the need is urgent for the development of novel therapies to treat what appears to be a quite resistant class of tumor. Could effective therapy be found in the new pharmacological platform that cannabinoid compounds represent?

Directly Targeting Astrocytomas with Cannabinoids Among the plethora of preclinical studies investigating the use of cannabinoids as chemotherapy for cancer cells was work performed in the 1970s testing the anti-neoplastic activity of cannabinoids in the available rodent models. Munson et al. [9] showed that oral administration of both THC and CBN, at concentrations as low as 25 mg/kg, inhibited tumor growth and increased mean life span in mice carrying Lewis lung carcinoma. Further work identified inhibition of nucleic acid synthesis and cellular respiration as possible mechanisms by which cannabinoids slowed the growth of both Lewis lung carcinoma and murine leukemia L1210 in vitro and in vivo [10, 43–45]. Critics of this work, however, cite Friedman's failure to find thymidine DNA incorporation, cytosine RNA incorporation, and leucine uptake into tumor proteins beyond several hours of acute selective inhibition in Lewis lung carcinomas in vivo [46], leaving the exact molecular mechanism by which cannabinoids inhibit tumor growth unresolved.

In some cases, CBD actually enhanced primary tumor growth, as indicated by increased thymidine incorporation [9, 26]. Such results suggest an indirect anti-inflammatory effect of CBD, as it could improve the ability of tumors to escape immune surveillance (see below). Accordingly, in 1980, the National Institute of Drug Abuse cautioned that while cannabinoid administration displayed an "attractive differential" in selective tumor growth inhibition over bone marrow toxicity, the use of "clearly cytotoxic agents may ultimately lead to the death of normal, non-transformed cells and tissues" [47]. Recent experiments have revisited this issue and show that cannabinoids have tumor-specific effects, leaving healthy tissue intact (see below).

For the next decade, cannabinoid-related research emphasized the effects of cannabinoids on immune responses and emesis control for patients undergoing chemotherapy rather than investigating their anti-tumoral properties. Much of this work was carried out with THC in the context of the possible immunosuppressive effects linked to marijuana consumption [3]. Schwarz et al. [48] subsequently showed that activation of cannabinoid receptors by non-THC ligands had anti-proliferative effects on peripheral immune cells, sometimes inducing apoptosis, thus reemphasizing the potential of cannabinoids as immune-based therapies.

In the mid-1990s, the French pharmaceutical group Sanofi Research found that human glioma cell lines, as well as human tumor tissue explants with various degrees of malignancy, expressed CB1 receptors [49]. Accordingly, cannabinoid agonists induced activation of the MAPK signal transduction pathway in human glioma cell lines and led to increased expression of the immediate-early gene *krox-24* [49, 50], suggesting that this signaling system likely influences cell viability and fate. These studies not only reintroduced the cannabinoid signaling system to the scientific community as an invaluable therapeutic target for cancer but also laid the groundwork for future studies investigating possible mechanisms of action.

Shortly after these publications, the group of Manuel Guzman undertook a series of elegant studies testing the hypothesis that cannabinoid agonists may serve as powerful anti-tumoral agents in the treatment of astrocytomas. First, Sanchez et al. [51] showed that THC reduced the proliferation of C6 rat glioma cells in culture while having no effects on primary neurons and astrocytes in culture. Interestingly, although THC administration induced apo-

ptosis in C6 cells (as inferred from DNA fragmentation analysis and membrane morphology observations), coadministration of the CB1-selective antagonist, SR141716A, failed to block the cannabinoid-induced cytotoxicity, suggesting that activation of CB1 receptors was not sufficient to induce cell death [51]. Even more intriguing was the subsequent finding that a specific C6 sub-clone, C6.9, was sensitive to THC administration and that this toxic effect was not antagonized by either SR141716A or SR144528 (CB2-selective antagonist) alone, but rather by their combination, suggesting that activation of both receptor subtypes was required to kill astrocytomas [52]. While this study did not compare the relative levels of CB1 and CB2 receptor expression between C6 sub-clones, one wonders if the observed sensitivity to THC could be due to increased expression of one subtype of cannabinoid receptor by C6.9 cells. Their very recent study shows that p8, a stress-regulated mediator of cell fate, constitutes a key player in THC-induced apoptosis of C6 cells [53].

In addition to clonal variation within this glioma line, it should be noted that existing data regarding cannabinoid-induced cytotoxicity in C6 cells are potentially complicated by the fact that specific treatment regimens (i.e., single versus repetitive administration) vary from study to study. Indeed, in vitro culture conditions, especially serum concentrations, greatly influence the sensitivity of cell lines to many compounds, including cannabinoids [54]. Furthermore, other groups have shown that THC-induced death of C6 cells [55], as well as of various human glioma lines [56], can actually be antagonized by SR141716A or SR144528. While at first glance contradictory, these studies provide valuable information regarding the type of agonists and treatment schedule required to efficiently eradicate astrocytomas.

Building upon their results using C6 cells in culture, the Guzman group tested the anti-tumoral properties of cannabinoids on C6 cells stereotactically injected into rat brain. Highly invasive tumors formed within 2 weeks of injection and tumor-bearing animals did not survive beyond 3 weeks [52]. Intratumoral administration of either THC or the potent synthetic cannabinoid agonist, WIN55212-2, slowed the progression of these malignant tumors, increasing mean survival times post-inoculation [52]. These results were quite spectacular: in 25% of the cases, gliomas were eradicated, and rats survived beyond study observations [52]. As mentioned above, cannabinoid treatment did not damage neighboring healthy tissue. This potent therapeutic effect of THC on astrocytomas growing in vivo constitutes a stepping-stone for further testing the efficacy of such compounds at killing high-grade astrocytomas in other animal models as well as in patients.

A handful of studies addressed the cytotoxic action of other cannabinoids toward C6 gliomas in culture. For example, ajulemic acid, a non-psychotropic synthetic THC analog, is more effective than THC at inhibiting C6 and U87 (a human glioma line) proliferation in culture [57]. The group of Christopher Fowler made significant progress in addressing the complex role that cannabinoids may play in glioma proliferation. After showing that C6 sensitivity to the chemotherapeutic agent tamoxifen is increased by coadministration of THC [54], this group found that several other cannabinoids, including AEA, 2-AG, CP 55,940, and the CB2 agonist, JWH-015, also inhibited C6 proliferation [58, 59]. While the anti-proliferative effect of the eCBs is blocked by cannabinoid receptor antagonists, the anti-proliferative effect of synthetic agonists was not [58]. Interestingly, AEA also induces apoptosis in a variety of human glioma cell lines through TRPV1 [58, 60–62], and chemical disruption of lipid rafts blocks this effect [63]. Several other cannabinoids have also been shown to induce apoptosis in C6 cells, including the eCB analogue stearoylethanolamide [64, 65].

A controversial question central to this line of research is whether or not CB2 is expressed in brain tumor tissue [31, 49, 50]. Although pharmacological data suggest that brain tumor tissue expresses CB2 receptors, the equivalent expression data obtained using commercially available antibodies have long been more difficult to interpret. Indeed, these antibodies have shown great variation in the predicted molecular weight of the receptor, and most of the published data lack the required genetic controls. To corroborate their findings, Guzman and colleagues reported that both C6 cells and human glioma tissues express CB1 and CB2 receptor mRNA and protein [31], contradicting an earlier study that found no CB2 mRNA in a panel of human astrocytoma cell lines and tissues [as assessed by real-time polymerase chain reaction (PCR)] [49]. A recent study reported the quantitative-PCR and immunohistochemical analysis of biopsies from 37 human astrocytomas of varying malignancy and suggested a positive correlation between CB2 receptor expression and tumor malignancy [66], confirming earlier immunohistochemical observations from a similar panel of human tumor samples [31]. Accordingly, our group also found CB1 and CB2 mRNA expression in a panel of human tumor tissue biopsies (Fig. 1). Thus, these tumors express CB2 receptors; but whether receptor expression derives from tumor cells specifically or from invading immune or endothelial cells which also express these receptors remains unclear (see below).

In summary, while a large body of evidence supports the toxic effect of cannabinoids on astrocytomas in culture, significant differences in the types of compounds, concentrations, and treatment schedules render it difficult to compare the results. Furthermore, to our knowledge, only one study has addressed the therapeutic potential of cannabinoids on astrocytomas growing in the brain (using the stereotatic model). Recently developed genetic mouse

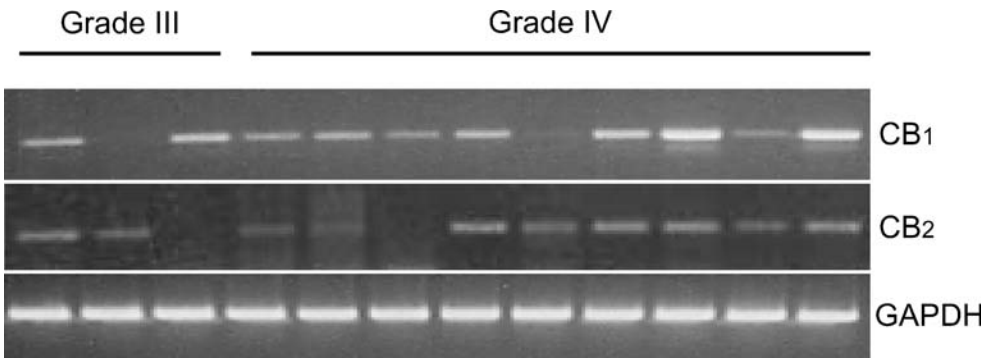

Fig. 1 Human malignant brain tumors express cannabinoid receptor mRNAs. RNA was isolated from 12 brain tumor biopsies (kind gift of Dr. Robert Rostomily, Department of Neurological Surgery, University of Washington) of WHO grade IIIor IV. RT-PCR analysis (35 cycles) conducted using primers CB1-(5′CATC-CAGTGTGGGGAGAACT-3′ and 5′-TACCTGTCGATGGCTGTGAG-3′) and CB2-specific (5′-GATTGG-CAGCGTGACTATGA-3′and 5′-GATTCCGGAAAAGAGGAAGG-3′) primers. Analysis of HEK 293 cells stably expressing either full-length CB1or CB2confirmed amplicon specificity; no-RT controls confirmed the absence of genomic DNA contamination, as primer pairs are not intron-spanning; CB1but not CB2was amplified from normal brain (data not shown)

models of astrocytoma more accurately mimic actual pathology and should be used to further test the efficacy of cannabinoids at eradicating astrocytomas.

The Interplay Between Astrocytomas and Microglia The heterogeneous character of astrocytomas extends beyond the varied morphology of cell shape and transformed nuclei, increased proliferation of local microvasculature, and regions of pseudo-palisading necrosis. A diverse panel of cell types accumulates in brain tumors. By far, the most abundant immune cells that accumulate are macrophages (as visualized by Iba-1 immunostaining) [67, 68]. Indeed, while lymphocytes infrequently survey healthy CNS, peripheral monocytes frequently pass across the blood–brain barrier (BBB), especially when disrupted, contributing to the pool of resident microglia. Accordingly, using bone marrow labeling studies, Flugel et al. [68] found that the majority of tumor-associated macrophages originate in the periphery, a finding that suggests the use of these invading macrophages as invaluable vehicles for the delivery of specific therapies [69].

Resident microglia and invading macrophages accumulate in both human and experimental astrocytomas, with intratumoral densities being higher than peri-tumoral and normal brain tissue densities and total numbers increasing with grade of malignancy [67, 70, 71]. Infiltrating microglia/macrophages constitute as much as one third of the tumor mass of high-grade astrocytomas [71]. Within tumor masses, these cells change their morphology from a ramified appearance in lower grade tumors to amoeboid in high-grade astrocytomas [71]. While distinctions in microglia/macrophage morphology have been implicated in the extent of their activation [72, 73], it still remains unclear whether a fully activated and immunologically competent microglia/macrophage promotes or hinders tumor eradication.

A positive correlation exists between astrocytoma malignancy and the number of invading microglia/macrophages [71], which can closely interact with tumor cells, engulf them, and promote cell death [74, 75]. Engulfed debris may then be presented by MHC class II and I (presumably due to cross-presentation), and prime $CD4^+$ and $CD8^+$ T cells, respectively. While the likelihood of interaction between activated microglia/macrophages and T cells increases in regions of malignancy, especially where the BBB is disrupted, T cell priming rarely occurs because of the silencing of microglia/macrophages by astrocytomas [76, 77]. Most likely, the immunosuppressive nature of the astrocytoma environment tempers the immune cytotoxic function [67], thus, contributing to the lack of primed T cells within and around tumors. Furthermore, microglia/macrophages within high-grade tumors (grades III and IV) have decreased MHCII expression, suggesting a compromise in their innate ability to inhibit tumor growth [78]. It has been suggested that gliomas in vivo actively dampen immune responses via increases in local concentrations of immunosuppressive cytokines such as IL-10 and TGF-β [71]. Several groups have suggested that pharmacologically blocking this silencing could boost a directed immune attack against the tumors, and thus, constitute a promising immunotherapeutic approach for astrocytomas.

Astrocytomas, Microglia, and Cannabinoids: A Powerful Ménage à Trois The precise cellular mechanism mediating the therapeutic effect of cannabinoids on astrocytomas remains an open question. While the brain is uniquely isolated from the body's peripheral immune system by the BBB [79], in the C6 model described above, the BBB is compromised as a result of the intracranial injection of tumorigenic cells and subsequent tumor growth. Astrocytoma growth in humans also disrupts the BBB and allows for bidirectional passage of immune cells [70, 80]. Two

important questions arise from these facts. Do cannabinoids act on the tumor cells directly or indirectly through a modulation of invading immune cells? Is it possible that microglia/macrophage function may be targeted by cannabinoids? As an initial attempt at addressing these questions, the Guzman group inoculated C6 cells into the flanks of immuno-compromised Rag-$2^{-/-}$ mice [52]. Intratumoral administration of THC or WIN55212-2 significantly reduced tumor mass, but never completely eradicated them (as had been reported with the stereotactic cranial model). They next showed that JWH-133, a synthetic CB2-selective agonist, induced regression of both C6 and human astrocytoma growth in the flanks of Rag-$2^{-/-}$ mice, an effect blocked by the CB2 antagonist SR144528 [31]. The identification of non-psychotropic anti-proliferative cannabinoid agonists in this model system constituted a very exciting result for the field.

Microglial (in addition to other peripheral immune effectors) expression of cannabinoid receptors, as well as their functional response to cannabinoid administration, is well documented [81] and is worth considering within the context of this review. Primary mouse microglia and the murine microglial cell line BV-2 express both CB1 and CB2 [32, 82], although levels appear dependent on the particular state of activation. In addition, cannabinoids increase MAPK activity in BV-2 cells [82], confirming functional coupling and target value. Of particular importance to cannabinoid impact on tumor regression, cannabinoids significantly decrease cytokine production by microglia [83]. In addition, while THC administration and CB1 receptor activation failed to elicit changes in the migration of microglia in culture [84], AEA, 2-AG, and CBN regulate chemokinesis and chemotaxis in a CB2-dependent fashion [82]. How cannabinoids regulate the migration and function of microglia/macrophages invading astrocytomas constitutes a fascinating question that requires further investigation.

Conclusion and Perspective High-grade astrocytomas are rarely diagnosed in their early stages of pathogenesis and are therefore already intricately embedded in healthy brain tissue, making thorough surgical resection difficult. Thus, astrocytomas constitute a seemingly insurmountable therapeutic quandary for most neurosurgeons, as even a small number of cancer cells left behind can remit into deadly tumors, emphasizing the premise that *all* tumor cells must be completely removed or eradicated for effective treatment. While this fact certainly rationalizes the use of chemotherapy on paper, in practice, the therapeutic window of classic cytotoxic agents (such as the DNA methylating agent telozolamide) is quite narrow and continues to limit clinical effectiveness [85]. The acquired resistance to such approaches by tumor cells further complicates the use of these chemotherapeutic agents [85]. Patients often live less than 12 months after the diagnosis of a high-grade astrocytoma, and shorter treatment schedules combined with conservative dosing greatly reduce the chances of continued remission. It seems clear that the time for purely palliative considerations has come and gone, and other therapeutic and pharmacological avenues for treatment of these aggressive tumors must thoroughly be investigated.

Cannabinoids likely represent such a venue and have indeed received renewed attention from both researchers and clinicians alike [86–88]. The vast majority of data support their potent and selective anti-proliferative and pro-apoptotic properties, especially in transformed glial cells. Both synthetic and plant-derived cannabinoids offer a unique prospect for cancer chemotherapy in that escalated doses can be safely administered. Indeed, cannabinoid receptors are absent from the respiratory and blood pressure centers of the brain stem, and thus, these compounds have no clinically relevant lethal dose [2]. While cannabinoids are hydrophobic and can easily pass the BBB, their intratumoral administration is also practical. In a recent European clinical study of individuals with recurrent GBM, intratumoral administration of THC led to shrinkage of the tumor mass [87]. Finally, the ability to target CB2 receptors for astrocytoma eradication constitutes an equally viable avenue for non-psychotropic therapy and should be further investigated in vivo.

Conversely, there are still many unanswered questions, and the molecular and cellular mechanisms underlying this anti-tumoral effect remain unclear. In fact, whether the only mechanism by which cannabinoids act is through binding and activation of cannabinoid receptors, thereby directly killing astrocytomas, is not certain. Indeed, systematic testing of selective cannabinoid receptor antagonists is often lacking, and in many cases, the effective dose exceeds that which is classically considered a receptor-dependent concentration. While pharmacology will certainly guide this course of research, genetic approaches are required to unambiguously identify targets mediating the anti-tumoral properties of cannabinoids. Given the availability of genetic tools, including CB1, CB2, and CB1/CB2 receptor knockout mice, efforts should be made to investigate the requirement of cannabinoid receptor expression by astrocytomas and/or immune effectors for mediating the tumor cytotoxicity of cannabinoids. While in vitro studies certainly provide evidence for proof-of-concept and target validation, it is likely that directing scientific resources toward the investigation of in vivo cannabinoid-induced astrocytoma eradication will offer the greatest likelihood of success and bring this novel therapy from the bench to the clinic. With new evidence challenging the canonical theory of an immuno-privileged CNS environment [79], it is likely

that the brain immune system will influence how cannabinoids lead to the elimination of astrocytomas. For example, clinical data now support the use of autologous tumor lysate-pulsed dendritic cell therapy for the treatment of malignant astrocytomas [89, 90]. Could the intersection of cannabinoids, astrocytomas, and microglia favor such a mechanism? In addition, it has been suggested that cannabinoids may reduce tumor proliferation by inhibiting angiogenesis, a mechanism that could also participate in the anti-tumoral effect of these compounds [91, 92].

Significant improvements in existing analytical tools have led to a greater understanding of how cannabinoids may act. Much of the medicinal chemistry is completed, and numerous receptor-selective derivatives are available. Mice lacking CB1, CB2, or both receptors have been generated. The signal transduction pathways coupled to cannabinoid receptors and how they traffic in response to specific cannabinoids is becoming clear. And finally, the intricacies of cancer biology and neuro-immunology are better understood. It is thus time to revisit the seminal work of Manuel Guzman and colleagues in which non-selective cannabinoids were shown to completely eliminate tumors in the brains of rats and optimize the very promising anti-tumoral properties of cannabinoids [52].

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