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Review

Malignant glioma: Should chemotherapy be overthrown by experimental treatments?

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Summary

Despite more than two decades of clinical research with chemotherapy, the outcome of malignant gliomas remains poor. Recent years have seen major advances in elucidation of the biology of these tumors, which in turn have led to the current development of innovative therapeutic strategies. The question confronting us at the end of the 1990s is whether we should continue to use and investigate chemotherapy or whether the time has come for experimental treatments.

As a contribution to this debate, we reviewed the abundant literature on chemotherapy of malignant glioma, paying special attention to methodological features. The new treatment approaches based on current knowledge about glioma biology are then briefly summarized.

Assessment of more than 20 years of chemotherapy trials is discouraging despite a few areas of modest success. Only patients with specific histology (oligodendroglioma, anaplastic astrocytoma) and good prognostic factors (young age, good performance status) may benefit from chemotherapy, with a possible reversal of neurological dysfunction. However, the real impact on survival is small (anaplastic astrocytoma) or undefined (oligodendroglioma). Furthermore, it is unfortunately obvious that the outcome of glioblastoma patients is not significantly modified by chemotherapy. We believe the time has come to explore the potential of novel biological therapies in glioblastoma patients. This could also be proposed for anaplastic astrocytoma and oligodendroglioma patients after failure of chemotherapy.

Key words: brain neoplasms, chemotherapy, gene therapy, glioma, immunotherapy, review literature

Introduction

Current therapies for malignant gliomas include tumor removal and irradiation. The neurosurgical procedure is a mandatory step for the establishment of a precise diagnosis, and its impact on prognosis is acknowledged [1, 2]. However, the infiltrative behavior of malignant gliomas precludes their complete resection, and 90% of glioblastomas recur within 2 cm of the primary site [3]. Postoperative irradiation is therefore commonly administered, with a significant and reproducible improvement in survival [4, 5]. Nevertheless, despite surgery and irradiation, the prognosis of high-grade glioma patients remains poor, with only a few patients alive two years after diagnosis [6].

A huge number of clinical studies were performed in recent decades to determine whether chemotherapy could influence the outcome of malignant glioma patients. Disappointing results of chemotherapy have raised the suggestion that chemotherapy should perhaps be abandoned. This is a provocative working hypothesis that may be valid in certain circumstances, but is probably inadequate in others. A critical appraisal of the potential of chemotherapy in malignant glioma treatment is complicated by numerous methodological problems encountered in neurooncology and discussed during this review.

The most obvious obstacles to effective chemotherapy for glioma remain, however, the intrinsic chemoresistance of these tumors [7], as well as their location within the central nervous system (CNS), limiting access of the drugs to their targets. A first theoretical difficulty which must be circumvented is the blood brain barrier (BBB) [8]. Diffusion of drugs through the BBB is dependent upon the size of the molecules (low molecular weight), liposolubility (high) and fixation rate to plasmatic proteins (low). However, the role of BBB in limiting drug penetration was probably overestimated. Contrast enhancement in neuro-imaging clearly shows that the BBB permeability is modified in brain tumors: neovessels often display loose tight junctions and defects in the capillary wall. But alterations of the BBB are generally less important at the border of the tumor, which also means in its most actively-growing zone. A second major obstacle once the drug has passed the BBB is its diffusion through a network of interstitial channels in the brain tissue. On their tortuous way to the target tumor, molecules can still be eliminated or deactivated by enzyme metabolization, non-specific binding to proteins, internalization to non-tumoral cells or reentry in the systemic circulation by recrossing the BBB [9].

Chemotherapy thus has to face a very potent adversary in an unfavorable microenvironment. In the first part of this paper, hopes for and limits of chemotherapy

will be critically reviewed. We will then discuss how the dramatic advances achieved in the understanding of glioma biology could open new avenues for the treatment of these tumors.

Monochemotherapy

Nitrosoureas are the leading drugs in glioma chemotherapy. Response rates to *BCNU/CCNU* as single agents can be estimated to vary from 10% to 40% [10–13], and even reach 64% in a subgroup of patients with anaplastic astrocytomas [2]. These results must be viewed with great caution. They are approximate, if not speculative, as they are from trials carried out in the 1970s before the CT era. A response rate in the absence of any radiologic evaluation is of questionable validity and certainly includes patients with stable disease. In these trials, the median time to tumor progression (MTTP) was 19 to 23 weeks [10–13].

As for nitrosoureas, *vincristine* was studied as a single agent 30 years ago and the authors observed several instances of clinical improvement [14]. In the absence of radiological evaluation, it is difficult to extrapolate the real efficacy of vincristine from these data, even if response rates of 20%–50% are generally reported in the literature [15, 16]. However, on the basis of these observations, vincristine was introduced in polychemotherapy regimens currently used for glioma.

Response rates reached 6%–26% in trials investigating *procarbazine,* with a MTTP of seven to 12 months in pretreated patients [17, 18]. More recently, procarbazine was reconsidered with interest because of its depleting activity towards methylguanine-DNA methyltransferase (MGMT) [19], an enzyme involved in malignant glioma resistance to nitrosoureas [7]. In a pilot study with 21 patients, procarbazine 200 mg/m^2 was delivered on days 1–5 to deplete MGMT before administration of BCNU 80 mg/m^2 on days 3–5. The response rate was 41%, and the MTTP and median survival 30 weeks [19]. However, these interesting results were accompanied by severe toxicity, and there was no evident correlation between MGMT depletion measured in monocytes and clinical responses. These preliminary data require confirmation.

Several other drugs have been evaluated as single agents, including platin compounds, epidophyllotoxins, thiotepa, piritrexim, 13-cis retinoid acid, all with very limited success [15, 16]. The use of *paclitaxel* in brain tumors is theoretically attractive because of its high lipophility but tempered by a high molecular weight and binding level to plasmatic proteins. Paclitaxel concentration was measured in the plasma, cerebro-spinal fluid, brain and tumor tissue of three previously irradiated patients after intravenous infusion (175 mg/m^2): tumor tissue drug concentrations were in the therapeutic range in all three patients [20], whereas the concentration was low in normal brain tissue. This suggested that paclitaxel could not cross the intact BBB. Paclitaxel was investigated on the basis of these experimental data, but only 10% to 20% of pretreated patients achieved a partial response (PR) [21, 22].

There has recently been considerable interest in *temozolomide*, an imidotetrazine analog of dacarbazine (DTIC). Unlike DTIC, temozolomide does not require hepatic activation and displays an excellent oral bioavailability. Based on encouraging responses observed during a phase I study [23], temozolomide was administered to 48 patients with recurrent glioma after radiotherapy. Strict criteria of response were not applied. Objective clinical and radiologic responses were reported in 25% of the patients [24]. Because of these results, the low toxicity of the drug and the convenience of its use by mostly unfit patients, temozolomide was further developed. Two multicentric phase II studies including glioblastomas and anaplastic astrocytomas, respectively, have recently been completed. The design of these trials was excellent, with rigorous response criteria, centralized pathological and radiological reviews as well as monthly quality-of-life assessment. Preliminary data regarding the first 100 patients included are available for anaplastic astrocytomas only. The response rate is 42% with strict response criteria, but 41% of included patients were non-evaluable, mainly (29%) because of incorrect histological diagnoses [25]. This very high rate of non-evaluable patients in a well-designed study with pathological review raises doubts about the validity of data coming from most other studies which lack a centralized diagnosis review. Another item of information which is expected to emerge from these two studies including a large number of patients is the impact of temozolomide therapy on the quality of life in glioblastoma and anaplastic astrocytoma patients.

Polychemotherapy

The most commonly used multiagent regimen is PCV. Originally described in 1975, PCV was revised in 1978 and 1980, and comprises in its final form CCNU 110 mg/m^2 D1, procarbazine 60 mg/m^2 D8–21 and vincristine 1.4 mg/m^2 D8 + 29 [26]. Forty-six patients were treated by this final regimen, which for 40% of them was the first-line chemotherapy. The response rate for all patients was 26%, reached 42% in the subgroup of unpretreated patients and was only 17% in the subgroup of glioblastoma patients (pretreated or not). The overall MTTP was 26 weeks. In this study strict response criteria were not applied, and subsequent improvement of at least two of three parameters (neurologic examination, radionuclide and CT Scan) was considered as a response. Moreover, five 'low-grade astrocytomas' were included and their outcome was not detailed. However, these results formed the rationale for the further use of PCV in either relapsing or newly diagnosed patients.

Procarbazine and vincristine were also associated with mechlorethamine in a MOP combination. Interest in this regimen in brain tumors was triggered by the pediatric experience [27, 28]. Thirty-one adult patients

with recurrent malignant glioma entered a phase II study with separate results for measurable and evaluable diseases. The response rate (complete remission + PR) was 58% in measurable tumors, 50% in evaluable tumors, 37% in glioblastomas (n = 16) and 100% in anaplastic astrocytomas (n = 6). The median survival was 30 weeks for all assessable patients and 60 weeks for responders [29]. Interestingly, cyclophosphamide and the vincristine combination without procarbazine seems to have some activity as well [30]. Taken together, these data suggest that alkylating agents exert a potent antiglioma effect despite their limited diffusion through the BBB, and that a combination of procarbazine, vincristine and an alkylating agent deserves a larger-scale study.

More recently, interesting results were obtained with the ICE regimen (ifosfamide, carboplatin and etoposide) in a series of 36 well-defined patients who were all pretreated with surgery, irradiation and nitrosoureas [31]. Five complete responses (CR) and five PR were reported, with an overall response rate of 28% (95% CI: 14%–45%). Strict response criteria were used, and glioblastoma appeared to be as sensitive as anaplastic glioma to this regimen. MTTP (13 weeks) and median survival (29 weeks) for all patients remained low, but responders seemed to benefit from this effective regimen (MTTP 22 weeks, median survival 44 weeks). As clearly described by the authors, this was achieved at the price of a severe hematological toxicity.

A complex association of six drugs (thioguanine, procarbazine, dibromodulcitol, CCNU, 5-FU, hydroxyurea) was tested with the objective of overcoming a presumed resistance to nitrosoureas, with most of the drugs being administered at an infratherapeutic level [32]. Seventy-five evaluable patients with malignant glioma were included in this study. The response rate of 32% must be viewed with some caution, as the criteria were based on a subjective scale in the CT/MRI evaluation by the neuroradiologist.

A common feature observed in the majority of studies (mono- or poly-chemotherapy) is that chemotherapy is more likely to benefit young patients with a good performance status and an anaplastic astrocytoma subtype. This was confirmed in a retrospective study specially designed to address this question [33] and in adjuvant chemotherapy studies [5, 13].

Adjuvant chemotherapy

In the literature, 'adjuvant' chemotherapy in malignant gliomas is generally understood as chemotherapy following initial surgical excision of the tumor and subsequent irradiation. Unlike adjuvant treatment in other oncological fields, it does not imply the absence of residual disease at the time of treatment nor a tendency to its systemic control, but points to a better local control. Reading about response rates and not only survival in adjuvant studies is highly unusual for the medical oncologist. We believe that the term 'adjuvant therapies' should be restricted to the cases in which a macroscopically total or at least subtotal excision has been possible. Chemotherapy in unresectable malignant gliomas or after surgical biopsy alone should be refered to as first-line chemotherapy.

The first randomized study testing adjuvant chemotherapy was performed between 1972 and 1975 by the Brain Tumor Cooperative Group [5]. Patients treated with BCNU + radiotherapy had a median survival of 51 weeks compared to 36 weeks for patients treated with radiotherapy alone. Despite a trend in favor of adjuvant BCNU, this difference was not statistically significant. Whether or not there was a difference in the outcomes of anaplastic astrocytoma and glioblastoma patients was unfortunately not addressed.

A further randomized comparison between postoperative radiotherapy alone or an adjuvant treatment of BCNU, dibromodulcitol and radiotherapy was conducted by the EORTC: chemotherapy induced a slight but significant improvement of median survival (13 *versus* 10.4 months) and MTTP (8.1 *versus* 6.7 months) in 255 eligible patients. This benefit was more pronounced for anaplastic astrocytoma than for glioblastoma but without reaching a significant level in any subgroup [34]. The real effect of dibromodulcitol in these results is questionable in view of the limited role of this drug in glioma treatment. Despite their statistical significance the previously cited results clearly indicate that the survival benefit is very limited, with minimal impact for the individual.

PCV was also tested as an adjuvant treatment and compared to BCNU. PCV induced a significant amelioration in MTTP (126 *versus* 63 weeks) and median survival (157 *versus* 82 weeks) compared to BCNU for anaplastic astrocytoma patients [35]. These patients had good prognostic factors, with a mean age of less than 46 years. In contrast, no difference appeared among glioblastoma patients.

All of the published studies on this topic included only a limited number of patients. A meta-analysis of 16 randomized trials was therefore performed in 1993 [13]. All of the 16 compared radiotherapy alone versus radiotherapy plus chemotherapy and were published between 1975 and 1989. The increase in survival with radiotherapy and chemotherapy was 10.1% at one year (95% CI: 6.8%–13.3%) and 8.6% at two years (95% CI: 5.2%–12.0%). The maximal survival advantage with chemotherapy was seen later (18–24 months) for glioblastoma patients than for anaplastic astrocytoma patients (12–18 months). This suggests that mainly long-survivor glioblastoma patients might benefit by adjuvant chemotherapy, which probably means patients with initial favorable prognostic factors (young age, minimal residual tumor, good performance status). Trials included in this meta-analysis were very heterogeneous, especially regarding histologic and age characteristics, statistical power was often insufficient and 'intent-to-treat' analysis was seldom used [36].

Finally, it is noteworthy that there has been no

randomized study comparing adjuvant chemotherapy with the same regimen at first relapse.

The special case of anaplastic oligodendrogliomas

Oligodendrogliomas are rare neoplasms arising from the oligodendrocytes and representing only 5%–10% of gliomas. They are generally slowly-progressing tumors, with a better prognosis than those of anaplastic astrocytomas and glioblastomas [37] but which may sometimes become more aggressive with 'contrast-enhancement' and acquire anaplastic characteristics. In addition to their histological and biological specificities [38], aggressive oligodendrogliomas (progressive symptoms or anaplastic subtype) were shown to be highly sensitive to chemotherapy. Thirty-three patients were treated after initial diagnosis or with recurrent disease in a multicentric phase II study [39]; most of them were preirradiated but all were chemotherapy-naïve. Of 24 evaluable patients, nine (38%) achieved a CR with an overall response rate (CR + PR) of 75%. Patients with preexisting low-grade oligodendroglioma had a response rate of 90% and patients with necrotic tumors 67%. It is noteworthy that four CR and three PR were achieved among nine patients treated by chemotherapy before irradiation. The MTTP was 25.2 months for patients with CR and 14.2 months for patients with PR. There was no significant difference in response rate between irradiated and non-irradiated patients. Even though the patients included had good prognostic factors, this trial, performed according to rigorous methodologic criteria, demonstrated the chemosensitivity of anaplastic oligodendrogliomas.

Despite this unusual efficacy, several questions remain unanswered; for instance, it is still not known whether PCV administered as an adjuvant therapy prolongs tumor control and/or survival. This question is currently being addressed in an RTOG randomized study comparing radiotherapy alone with PCV followed by radiotherapy. In addition, the questions of whether PCV must be given initially or later at the time of relapse, and whether PCV has the potential to delay radiotherapy remain unanswered. High-dose thiotepa with stem-cell rescue is also currently under investigation with the objective of deferring radiotherapy and its secondary neurological effects [40]. This regimen can also be considered a promising salvage treatment.

Whether the oligodendroglial component of mixed anaplastic oligoastrocytoma do confer to these tumors a sensitivity similar to that of oligodendroglioma is unknown. The major problem is the lack of well-defined histological criteria. However, some reports suggest that these tumors can respond to PCV as well [41].

Polymers and interstitial chemotherapy

As stated in the introduction 90% of malignant glioma relapses occur within 2 cm of the original resection site [3]; an improvement in local control might prevent or delay recurrence and have an impact on clinical symptoms or survival. Administration of chemotherapy in the tumor bed or in the tumor itself bestows the potential advantages of bypassing the BBB, reducing chemotherapy-induced systemic toxicity, increasing drug concentration at the target site and lengthening the duration of tumor exposure to the drug. Three main techniques for delivering drugs in the tumor or in the tumor bed have been employed: Ommaya reservoir, implantable pumps or surgically implantable polymer matrices loaded with the drugs.

Polymer matrices have theoretical advantages over the other possibilities. They are not subject to blockage by clogging or necrotic material and they do not require subcutaneous injections with their inherent infectious risk. They are not dependent on patient compliance and do not need maintenance. But they are not refillable and surgery is necessary for implantation [42].

In a phase I–II study assessing BCNU disks there was neither local nor systemic toxicity [43]. A multicentric randomized trial with 222 patients compared implantation of BCNU-wafers versus placebo-containing wafers. Indication for open surgical reintervention was determined by an independent surgeon not involved in the study. No unexpected toxicity was observed. Median survival was longer (31 *versus* 23 weeks) with drug-containing polymers. In patients with glioblastoma six-month survival was 50% greater in those treated with BCNU discs than in the placebo group but published survival curves are disappointingly superimposable afterwards [44]. Even if current benefits are limited, with minimal impact for the individual, polymer delivery systems provide novel ways to reach malignant gliomas with various agents. The minimal toxicity of this technique opens the door to the local administration of drugs that do not pass the BBB, or cytokines or genetic material that might be tested in an adjuvant setting. However, the probability that such approaches will allow deep penetration into cerebral tissues should not be over-estimated, since the penetration of antineoplastic drugs remains generally under 2 mm except for dextran, a high-molecular-weight water-soluble molecule [9].

Intra-arterial chemotherapy and high-dose chemotherapy

Augmentation of the dose of chemotherapy delivered to glioma has been intensively attempted in the past decade using intra-arterial (i.a.) chemotherapy or intensive chemotherapy followed by bone marrow autograft or peripheric stem cell rescue. Most studies focused on nitrosoureas. Results for relapsing glioma or in the adjuvant setting are disappointing, with severe toxicities and high rates of therapy-related death [45–53]. A detailed description of this complex field is beyond the scope of this review. We believe, as the majority of the authors of the above-cited publications, that i.a. and high-dose chemo-

therapies do not improve the outcome of malignant gliomas and that these unacceptably toxic approaches should be avoided. Oligodendroglioma may be the exception, as discussed above [40].

Future studies on chemotherapy: A higher methodological standard is needed

The methodological difficulties pointed out in the field of malignant gliomas in the course of this review remain a major problem that contributes to the 'opacity' surrounding brain tumors in the field of medical oncology. Even if some specificities of malignant gliomas partly 'explain' these pitfalls, more rigorous rules must be applied in the conduct of future studies.

A first crucial prerequisite is a precise diagnosis. As discussed, glioblastoma, anaplastic astrocytoma and oligodendroglioma are tumors with widely differing behaviors and responses to chemotherapy. Phase II studies should focus on single tumor types and patients in phase III studies should be stratified according to the histology of their respective tumors, just as they should be for age, performance status and previous treatment. Whenever there is any doubt about diagnosis (change of tumor grade, radiation-induced necrosis), a new biopsy must be the rule. Furthermore, a central pathology review should be mandatory in any multicentric study.

Response criteria are to be strictly defined and endpoints should not be limited to symptomatic criteria or MTTP only [54, 55]. We believe that the interest in a new drug that helps to reduce symptoms should not be mistaken for scientific proof of an antitumoral effect. Both items of information are of interest for the clinical oncologist but evidence of tumor reduction remains the cornerstone of oncological evaluation. The classical definition of CR and PR ($\geqslant 50\%$ reduction in the product of the two largest perpendicular cross-sectional diameters of the tumor) was adopted to select in phase II studies the drugs with the best chances of yielding meaningful disease control or survival benefit and of preventing patients from being included in pointless larger phase III studies. Common adherence to these rules also remains necessary for a valid comparison of results, as outlined in the detailed recommendations of Macdonald [56]. Therefore, calling stable patients responders appears confusing. Unless expressly specified in the text, response rates in the present review adhere to the commonly accepted oncologic criteria.

Since objective measures of gliomas may be quite difficult, especially for infiltrative or multifocal tumors, MTTP can provide appropriate complementary information [54, 55]. In our opinion, lesions not amenable to the placement of perpendicular cross-sectional diameters because of their geometry should not be excluded from clinical studies for purely methodological reasons. Indeed, it seems appropriate to define objective qualitative response on CT or MRI for these evaluable but non-measurable tumors. The 'MOP study' [29] is a good example of this practice with two subgroups of patients (measurable vs. evaluable disease) analyzed and presented separately. Exclusion of evaluable non-measurable gliomas might constitute a significant bias as these tumors might have a worse prognosis than easily measurable lesions.

Furthermore, a central imaging review by one or several independent neuroradiologists is mandatory in multicentric studies. An additional confounding factor is the common use of cortisone. Any clinical or radiological stabilisation or improvement can be considered only under unchanged or reduced doses of steroids.

Finally, we would like to emphasize the absolute necessity of including quality-of-life and cost-effectiveness analyses in current clinical studies. As survival benefit induced by chemotherapy is minimal to date, we feel more and more strongly the absence of reliable information in these two fields. It is, of course, difficult and dangerous to evaluate the 'price' of a few weeks of life, but it does not appear to justify worsening of the patient's quality of life at high cost for society. Should therapeutic advances appear in the future, accurate data would be essential in any attempt to persuade the medical community and public health authorities to modify the current negative approach to malignant glioma treatment.

Experimental treatments

The genesis and progression of a tumor result from a series of complex biological mechanisms, including loss of cell cycle control, neo-angiogenesis and evasion of immune control. The remarkable advances achieved in these research fields open new avenues for treatment of malignant glioma patients by targeting cell proliferation, cell death, neo-angiogenesis or restoring an appropriate immune response. A detailed description of these fascinating topics is not the present objective, but these new therapeutical concepts are briefly summarized to explain why they should be integrated in the current management of glioma patients.

Oncogene inactivation and tumor suppressor gene replacement

As for most cancers, multiple genetic events contribute to the malignant transformation of astrocytes (Figure 1). Oncogenes have dominant effects and code for proteins stimulating cell growth or inhibiting apopotosis. Mostly by gene amplification, several members of the protein-tyrosine kinase receptor family are overexpressed in malignant glioma, including the epidermal growth factor receptor (EGFR), the platelet-derived growth factor receptor-α (PDGFRα) and the c-met genes [38]. Interestingly, the EGFR is also frequently mutated, leading to the constitutive activation of its tyrosine kinase activity and, consequently, to enhanced cell proliferation and

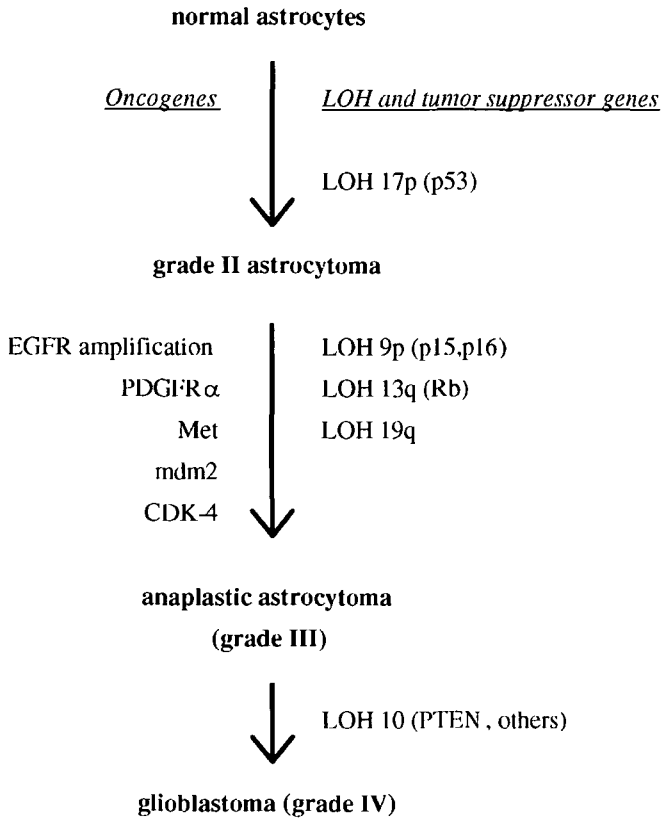

Figure 1. Simplified schema of oncogenes and tumor suppressor genes involved in the process of malignant transformation of astrocytes. Abbreviations: LOH – loss of heterozygoty; EGFR – epidermal growth factor-receptor; PDGFRα – platelet-derived growth factor receptor-α; Rb – retinoblastoma protein, mdm2 – minute double minute; CDK-4 – cyclin dependent kinase-4.

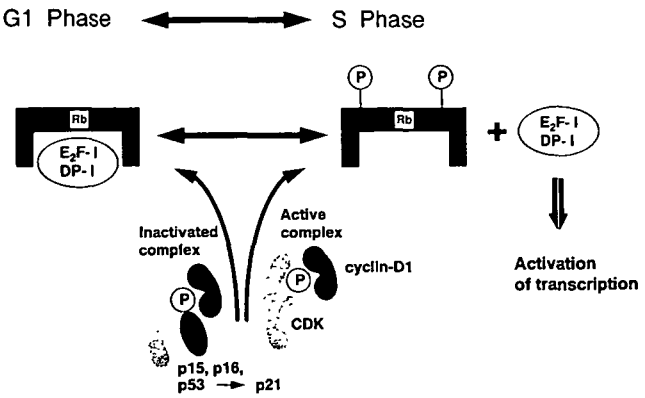

Figure 2. Simplified and schematic view of the main proteins involved in the control of cell cycle. The balance between cell proliferation (S phase) and cell quiescence (G1 phase) is tightly regulated. During the S phase, cyclin D1 proteins associate with cyclin-dependent kinases (CDK) to phosphorylate (P) the Rb protein, leading to the liberation of the E_2F-1 and DP-1 transcription factors and the subsequent synthesis of several molecules involved in cell proliferation. This proliferative activity is counter-balanced by CDK inhibitory factors (*p15*, *p16*, *p21*). The expression of *p21* is regulated by *p53*. The binding complex between cyclin D1, CDK and CDK inhibitory factors prevents the phosphorylation of the Rb protein. Rb is therefore able to retain E_2F-1 and DP-1 transcription factors and thus to prevent their effects (cells remain in G1 phase). The mechanisms identified that push the glioma cell into the S phase are described in the text.

tumorigenicity of glioma cell lines in nude mice [57, 58]. Numerous strategies are currently being investigated to specifically inhibit EGFR, using antibodies, immunoconjugates or antisense technology.

Several tumor suppressor genes have been shown to be altered in glioma. This was initially suspected because of the numerous genetic losses detected by caryotype and loss of heterozygoty (LOH) studies. LOH has been described in chromosomes 1p, 9p, 10p, 10q, 11p, 13q, 17p, 19q, and 22q. In some cases, the tumor suppressor genes present in the deleted chromosomic regions have been identified. For instance, the region deleted in chromosome 17p includes the *p53* gene [59]. *P53* plays crucial roles in cell homeostasis and can prevent malignant transformation, by inducing apoptosis or by blocking cells in G1 phase (Figure 2) to permit DNA reparation. Additional mechanisms lead to non-functional *p53* in glioma, including various types of mutation and the amplification of the mdm2 gene, whose product binds to *p53* and catalyses its proteolytic degradation [60]. Alterations of *p53* seem to be an early event in the genetic progression of glioma since they are observed in all grades of glioma.

Other suppressor genes, encoding *p16* and *p15*, are frequently deleted or not expressed in malignant glioma [61, 62]. By inhibiting cylin-dependent kinases (CDK), they also play a key role in the control of the cell cycle (Figure 2). Several mechanisms that can help propel the glioma cell into the S-phase have been identified: (i) absence of CDK inhibitors (*p15, p16, p21*); (ii) amplification of CDK-4 [63]; (iii) deletion or mutation of the Rb gene itself [64] which ensures that the E_2F-1 and DP-1 transcription factors continuously exert their proliferative effects. Interestingly, both of the latter events take place when the *p16* gene is intact and correctly expressed [65]. Finally, intense efforts are currently underway to identify the tumor suppressor genes assumed to be present in the chromosome 10 deletion, a genetic hallmark of glioblastoma. A first tumor suppressor gene candidate, called PTEN (Phosphatase and Tensin homolog deleted on chromosome *Ten*) was recently characterized [66].

Both alleles of a tumor suppressor gene usually have to be inactivated to alter the anti-proliferative functions of their products. It thus appears attractive to restore normal growth control by transfecting one functional copy of a given tumor suppressor gene. To date, major attempts have been made with the *p53* gene. Restoring a *p53* function should theoretically lead to growth arrest or induce apoptosis, and this was actually observed *in vitro* [67, 68]. Since the presence of functional *p53* has been shown to modulate chemoresistance [69], another possible advantage of the gene transfer of wild type (wt) *p53* may be sensitisation to chemotherapy. This was recently suggested in animal models where the intratumoral administration of an adenovirus containing wt *p53* drastically augmented the pro-apopototic properties of cisplatin [70]. Thus, combining biological therapies with current treatments appears to provide some ration-

ale for future studies. Several gene therapy clinical trials designed to restore the *p53* function are ongoing in different cancers [71], but to our knowledge not in malignant glioma. Some local responses have recently been reported following the administration of a retroviral-wt *p53* gene construct in lung cancer lesions [72]. Intense efforts are ongoing in an attempt to resolve some critical difficulties, such as the potential toxicities on normal cells, the low ratio of *in vivo* tumor cell-targeting with the current procedures, and immune responses against the vector limiting the efficacy of repetitive administrations.

Neo-angiogenesis inhibition

It is now acknowledged that tumors may remain in a state of dormancy until they establish a blood supply for receiving oxygen and nutrients. This process of neo-angiogenesis implies successive ordered events in which a large number of players participate. Endothelial cells migrate, proliferate, should re-organize into a three-dimensional vascular structure and maintain the vessel continuity. Numerous factors with angiogenic properties have been identified, and their effects are counter-balanced by potent endogeneous inhibitors of angiogenesis (Table 1). Vascular endothelial growth factor (VEGF) was shown to play a key role in angiogenesis of glioma, recapitulating embryogenetic processes. VEGF binds to two specific tyrosine kinase receptors, called flk-1 and flt-1. VEGF and its receptors are down-regulated in the normal adult brain. In contrast, glioblastoma cells produce high levels of VEGF, whilst both flt-1 and flk-1 are expressed by proliferating endothelial cells of glioma, leading to the establishment of a paracrine loop. Interestingly, the expression of VEGF is strongly enhanced around areas of necrosis, in pseudopalisading tumor cells (Figure 3). This suggests that hypoxia can be a major inducer of VEGF expression, and hypoxia-sensing regions are actually present in the untranslated regions of the VEGF gene [73].

Several approaches have been developed to inhibit the effects mediated by VEGF, including antisense technology and monoclonal antibodies [74]. Perhaps the most impressive results were obtained by disrupting the VEGF-receptors signal transduction [75, 76]. The authors assumed that blocking the VEGF-receptor activities could inhibit the proliferation of endothelial cells and decrease the tumor growth. They tested their hypothesis using C6 rat glioma cells implanted either subcutaneously in nude mice [75] or intracerebrally [76]. Cells producing retrovirus encoding a dominant-negative mutant of the Flk-1 receptor were co-injected or administered five days after implantation of the C6 tumor cells. This recombinant retrovirus can infect endothelial cells *in vivo*, and a major inhibition of angiogenesis was observed, with delaying of tumor growth and prolonged survival in treated animals. More recently, the potential of anti-angiogenic factors was also pointed out, since an adenoviral vector expressing a modified platelet factor 4 (PF4) (see Table 1) was reported to inhibit the growth of glioma implanted intracerebrally [77]. These selected examples show that the anti-angiogenesis approach should now be investigated in the setting of human glioma.

Table 1. The process of angiogenesis is regulated by factors with a) pro- and b) anti-angiogenic effects (non exhaustive list).

a) Angiogenic factors	b) Anti-angiogenic factors
Vascular endothelial growth factor (VEGF)	Thrombospondin-1
Platelet derived growth factor α (PDGFα)	Platelet Factor 4
Basic fibroblast growth factor (bFGF)	Angiostatin
Transforming growth factor α (TGFα)	Interferon-α
Transforming growth factor β (TGFβ)	Tissue inhibitors of metallo-proteinases
Epidermal growth factor (EGF)	Endostatin
Fibroblast growth factors	
Interleukin-8	
Hepatocyte growth factor	

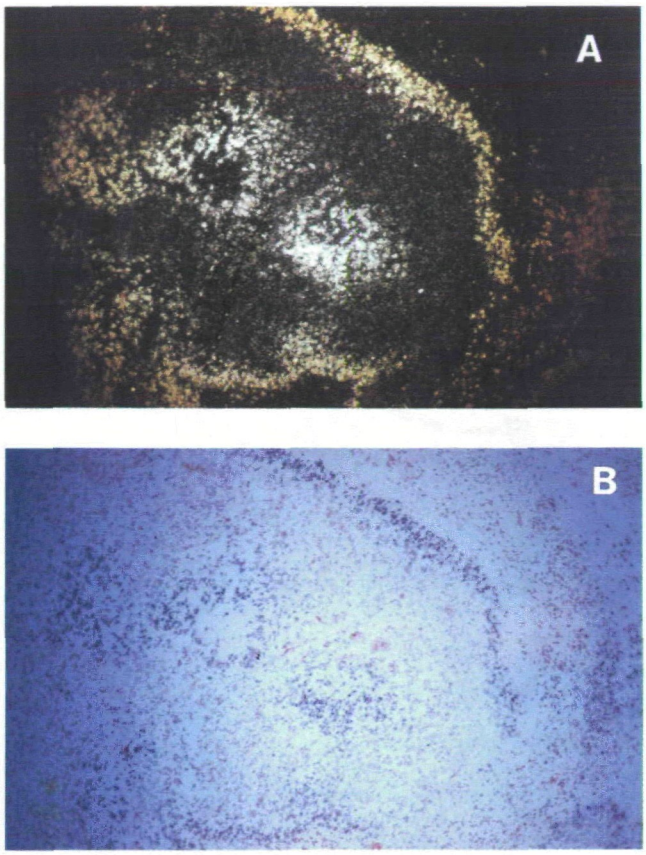

Figure 3. Expression of VEGF is more intense in regions surrounding necrosis. VEGF mRNA expression (*in situ* hybridization) is predominantly seen on palisading cells in a glioblastoma [a) dark-field and b) corresponding light-field micrographs] (courtesy I. Desbaillets and E. G. Van Meir).

Specific immunotherapy

It is now well recognized that immune cells, in a complex network of intercellular interactions and cytokine-

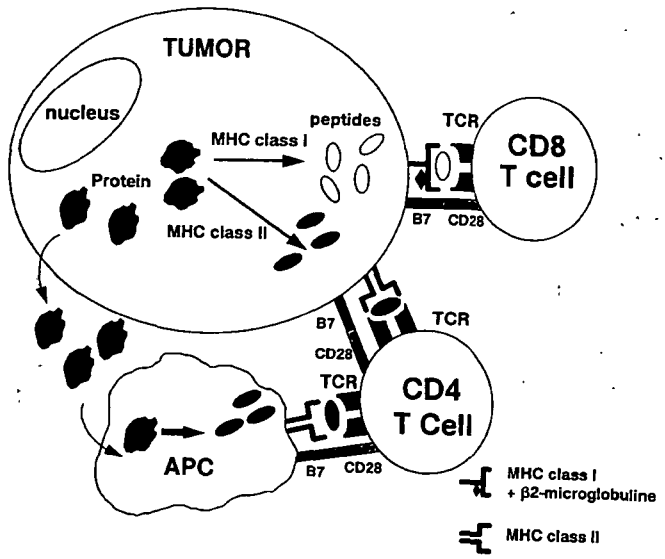

Figure 4 Schematic view of the antigen-specific interactions between tumor cells and T lymphocytes. Tumor antigens are processed by cancer cells and presented by major histocompatibility antigens (MHC) class I molecules to CD8+ T cells and in some circumstances by MHC class II molecules to CD4+ T cells. Tumor cells are probably helped in this function by specialized cells (APC – antigen presenting cells; eg., dendritic cells, Langerhans cells). APC can capture some tumor antigens, process and present them mainly (but not exclusively) in a MHC class II restricted way. In addition to this antigen-specific interaction between MHC-peptide complexes and the T cell receptor (TCR), T cell clonal activation requires a second signal provided by costimulatory molecules. The absence of a second signal results in the specific unresponsiveness of T cells, or anergy. Interactions between B7 and CD28 molecules play a pivotal role in determining immune reactivity *versus* anergy [78]. However, recent results suggest that costimulation pathways are more complex than initially thought. It appears that CD28 mediates stimulatory effects, whereas CTLA-4, a molecule with high homology to CD28, may be a negative regulator of T cell responses [79].

mediated signals, can in some circumstances help protect the host against cancer. There is also cumulative evidence that T lymphocytes play a critical role in the anti-tumor response. The initiation or priming of a specific T cell response requires an antigen-specific interaction between tumor cells and T lymphocytes, probably with the help of professional antigen presenting cells (APC) (Figure 4).

In the case of glioma, several aspects of the dialogue between tumor cells and T cells remain poorly characterized (see ref. [80] for a more detailed discussion of this topics). Moreover, several mechanisms concur to create a state of immunosuppression in the glioma microenvironment, including the secretion of various cytokines such as TGF-β2 or interleukin-10 [81, 82], or cell-cell interactions with Fas ligand expression by glioma cells as a first candidate [83, 84]. Consequently, the growth of aggressive tumors such as malignant glioma may be due to an imbalance in tumor-host relationships that could result from many possible alterations in the different components of the immune response (Table 2). It has been proposed that immunizations using either tumor antigens themselves (but to date no glioma antigen able to elicit an immune response has been characterized *in vivo*) or genetically modified tumor cells as

Table 2. Possible mechanisms of immune-escape by glioma (most are hypothetical).

Tumor antigens are not presented to immune cells
Lack of tumor antigen
Lack of MHC class I or II molecules
Lack of TAP (transporter associated with antigen processing) molecules
Lack of professional APC in the local environment of glioma
Tumor antigens are presented, but do not elicit an immune response
Tumor peptide density below the threshold level required for T-cell activation
Onco-fetal antigen for which immune tolerance has been established
Costimulatory signal absent or with inhibitory effect
Appearance of new epitopes that are tolerogenic rather than immunogenic
Inefficacy of immune response
Limited accessibility of immune cells to the CNS
Inappropriate immune stimulation (e.g., solely T helper and antibody response)
Immunosuppressive microenvironment
Soluble factors (TGF-β2, IL-10, PGE2, IL-1R antagonist)
Cell–cell contact (FasL)

immunogens could tilt the balance in favor of an efficient antitumoral response. It has been shown in *in vivo* models that such immunogens could induce not only a local immune response at the site of injection, but also systemic effects [85]. In the particular case of glioma, these vaccination approaches must take into account the accessibility of effector cells to their targets in the CNS. It is now clear that there is a significant trafficking of activated T cells through the CNS [86, 87] and that T cells primed by glioma cells in the periphery can recirculate and reach the brain to mediate their antitumor effects [88–91].

Several approaches using genetically modified tumor cells as vaccines have been investigated in animal models in an attempt to enhance the immunogenicity of glioma. The most interesting results reported to date were obtained by enhancement of lymphocyte responsiveness with tumor cells transfected with the interleukin-7 gene [88], and by inhibition of the local immunosuppression using TGF-β2 antisense constructs [92]. The enhancement of antigen presentation and T cell costimulation is another promising approach and results achieved with GM-CSF, B7, or CIITA (a transcription factor playing a critical role in the regulation of MHC class II molecules) genes should be available in the near future. In addition, promising results have recently been reported in a model of melanoma (B16 murine melanoma) located in the CNS. Efficient antitumor response was obtained using GM-CSF gene modified melanoma cells [93] or bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA [94].

The strict localization of glioma in the CNS, with only occasional metastases, may appear to be an advantage for approaches employing intratumoral delivery. Genetically modified cells can be implanted directly in the tumor (or the tumor bed after surgical removal) in order to sustain a local secretion of cytokines in the tumor microenvironment. This has the advantage of

more closely mimicking the natural biology of cytokine action (paracrine effect) than does the intravenous administration of recombinant cytokines. Cell lines producing a viral vector containing the gene of interest can also be placed intratumorally, leading to the *in vivo* transfection of tumor cells. However, locoregional gene therapy for glioma has major limitations. Firstly, genetic material is injected in a site where glioma-associated immunosuppression is probably the strongest, and secondly, only a small percentage of cells are actually transfected *in vivo* with current gene delivery techniques. This latter problem is a major impediment for a tumor that has infiltrated normal brain structures at an early stage of development. Despite these theoretical disadvantages, these approaches are being experimentally investigated in glioma, particularly with interleukin-4 (IL-4), and interesting results have been observed in nude mice [95] and in immunocompetent animals [96].

New approaches to induce apoptosis and death of glioma cells

Other strategies designed to specifically kill tumor cells are currently being investigated. Exciting results were obtained in a rat glioma model using 'suicide genes' [97]. This approach is the prototype of prodrug activation systems, and consists in transferring into tumor cells a drug-susceptibility gene which encodes an enzyme that can activate a prodrug intratumorally. Transduced tumor cells are thus rendered sensitive to drugs that are otherwise non-cytotoxic. In the particular case of the 'suicide gene', the gene coding for the thymidine kinase enzyme of the herpes simplex virus (HSV-tk) is transferred into tumor cells using a viral vector. This gene is not present in normal cells which are insensitive to gancyclovir. In contrast, like HSV-infected cells, tumor cells transduced by HSV-tk gene are killed by gancyclovir since the tk enzyme is able to phosphorylate the antiviral drug. In the 9L glioma rat model, major tumor regressions were obtained, although only a small fraction of tumor cells were shown to be transfected *in vivo* by retroviral HSV-tk [97]. The mechanisms responsible for this so-called 'bystander effect' are not fully understood, even though the passage of phosphorylated gancyclovir through gap junctions is thought to play an important role. This 'suicide' strategy is being intensively investigated in humans, but it is too early to estimate its significance with the current procedures. Some necroses around stereotactical injection sites are observed, but the rate of cell transfection *in vivo* and the bystander effect are more limited in humans than in rats. Other prodrug activation systems are of interest in CNS tumors. Tumor cells may be transfected with a cDNA encoding the cytochrome P450 2B1, the liver enzyme catalyzing cyclophosphamide and ifosfamide activation. When stably transfected with the P450 2B1 gene, the C6 and 9L rat glioma cells are highly sensitive to cyclophosphamide in *in vitro* and *in vivo* experiments [98].

Another new and exciting way to treat malig ,ant glioma emerged from the discovery that these tumors often express functional Fas (CD95) [99]. Fas is a transmembrane glycoprotein belonging to the nerve growth factor/TNF receptor superfamily: when activated, it can, like TNF-receptor 1, transduce an apoptotic signal through its cytoplasmic domain. Apoptosis is triggered by the binding of Fas to its natural ligand (FasL) or by cross-linking with anti-Fas antibodies. A high proportion of human glioma cell lines are sensitive to apoptosis mediated by anti-Fas antibody *in vitro*. Others are resistant, but may be rendered sensitive after stable transfection of a human Fas cDNA (100). These results offer new possibilities for treating glioma with anti-Fas antibodies or soluble FasL. One possible drawback to such an approach is that other Fas-positive cells may be affected. Thus, infiltrating leukocyte activity may be reduced, restricting strategies relying upon simultaneous immunoenhancement [84].

Finally, the use of virus as killers was recently suggested to be an additional way to destroy cancer cells. Adenovirus lacking the E1B protein do not replicate in normal cells but only in *p53*-deficient cells. Such a 'defective' virus can thus destroy tumor cells and spread to other tumor cells devoid of functional *p53*. Dramatic effects have been obtained *in vitro* and in models using human xenografts implanted in immunocompromised mice [101, 102]. No data are available in immunocompetent animals. Thus, a possible limitation of this approach could be an anti-adenovirus immune response, precluding repeated administrations of these killers. In addition, the absence of toxicity of this human adenovirus on normal cells was demonstrated in rodents, but remains to be established in man.

Concluding remarks

The place of chemotherapy in the management of malignant glioma patients is limited. However, it is critical that the different types of gliomas should not be considered as a unique entity, and that chemotherapy should be precisely tailored to the histological diagnosis. The discovery that oligodendroglioma are chemosensitive tumors is one of the most important advances in these last years [39]. PCV is clearly indicated at relapse and its place as an adjuvant therapy will be defined in the near future. In marked contrast, glioblastoma patients do not benefit from chemotherapy. Their survival is not improved, and their quality of life has rarely been evaluated. Development of new drugs with strict methodology is required, but with little validity if not accompanied by a rigorous assessment of quality of life and cost effectiveness. Anaplastic astrocytomas are more chemosensitive than glioblastomas, and there is sometimes obvious reversal of neurological defects with palliative chemotherapy. In addition, adjuvant PCV provided young anaplastic astrocytoma patients with some benefit in survival and time to progression [35].

The limited efficacy of chemotherapy (with the exception of oligodendroglioma) and the absence of survival improvement for CNS tumors during recent decades [103] indicate that innovative strategies to treat glioma patients must be intensively explored. In our opinion, it is time to propose such experimental strategies instead of chemotherapy to glioblastoma patients. Anaplastic astrocytoma patients could also be considered after failure of a first-line chemotherapy. The dramatic advances achieved in molecular biology, vector technology and the current understanding of tumor pathogenesis provide novel opportunities to attack cancer in a variety of different ways. It is clear that future biological therapies will face great difficulty, since malignant gliomas are diseases with multiple genetic events, high heterogeneity and constant modifications. However, clinical observation and biological data suggest that the tumor growth results from an imbalance between factors promoting cell proliferation *versus* cell death, angiogenesis stimulators *versus* inhibitors and immune evasion *versus* immune response. Therefore, there is hope that the correction of one key biological factor could tilt the balance towards tumor regression. Indeed, the repair of a single biological defect can sometimes induce unexpected effects. Wild-type *p53* may modify the expression of some angiogenic and anti-angiogenic factors [104], inhibition of VEGF could contribute to the maturation of dendritic cells [105], and interleukin-12 exhibits antiangiogenic activities [106]. Nevertheless, the current experience in cancer treatment suggests that several components contributing to carcinogenesis should be targeted to provide maximal chances of tumor control. Therefore, therapies based on immuno-enhancement should be joined with other approaches such as those designed to inhibit neoangiogenesis, to restore the control of cell cycle, or to induce apoptosis. Such an integrated treatment strategy combining non-antagonistic low toxicity procedures that attack different components of tumor growth present an opportunity to improve the disastrous prognosis of patients suffering from malignant glioma.

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