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Extended Abstract

Nanomedicine for treatment of glioblastoma : Local and targeted delivery of nanomedicines for the treatment of glioblastoma- Veronique Preat - University of Louvain, Belgium

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Abstract

Glioblastoma (GBM) treatment includes, when possible, surgical resection of the tumor followed by chemoradiotherapy, but the survival remains low mainly due to local recurrences. The local and targeted systemic delivery of anticancer drugloaded nanomedicines to treat GBM after surgical resection of the tumor is a promising strategy. Among the strategies that have been adopted in the last two decades to find new and efficacious therapies for the treatment of GBM, the local delivery of chemotherapeutic drugs in the tumor resection cavity emerged. We developed two formulations of anticancer nanomedicines that can be injected peri-surgically in the resection cavity of orthotopic GBM. Both PEG-DMA photopolymerizable hydrogel containing Paclitaxel loaded nanoparticles and lauryl-Gemcitabine lipid nanocapsules that spontaneously form a gel significantly improved the survival of the GBM-bearing mice. Another nanomedicine-based strategy could also improve GBM outcome. Targeted nano-theranostics are promising multifunctional system characterized by nano-size, possibility of surface functionalization, diagnostic and therapeutic capabilities. Due to the loss of BBB integrity in the GBM area, we showed that active targeting or magnetic targeting of SPIO/paclitaxel loaded nanoparticles enhanced the biodistribution of the nanoparticles in the brain and enhanced the survival time of GBM bearing mice after IV administration. The potential of other nanomedicine-based treatments of GBM will be discussed. Glioblastoma or glioblastoma multiforme (GBM) is a highly malignant form of glioma, which is the tumor associated with neoplastic glial cells in the brain, including oligo dendrocytes, astrocytes, and ependymal cells. According to the World Health Organization (WHO), GBM is classified as a grade IV brain tumor, which is the most aggressive variation of the malignancies of the central nervous system (CNS) . GBM is also one of the most prevalent malignant brain tumors, with an incidence rate of about 3.19 per 100,000 people per annum . The etiology of GBM remains unknown, although one of the identified risk factors is the abnormal exposure to ionizing radiation . This disease has a complex genetic expression, including gains of chromosomes 7 and 19, losses of chromosomes 10 and 13, amplification of epidermal growth factor receptor (EGFR) and MDM2, mutation of PTEN, NF1, PDGFRA1, IDH1/2, and deletion of CDKN2A/B. Moreover, the histological characteristics of GBM are quite as diverse as its genetic expression, including increasing mitotic and cellular activity, significant angiogenesis, and necrosis. The shape and size of tumor cells are also highly variable, thus the term multiforme]. GBM invades within the CNS and rarely metastasizes to distant regions . The common symptoms associated with GBM are headaches, cognitive impairment and personality changes, gait imbalances, incontinence, sensory loss, visual disturbances, seizures, confusion, and delirium. Most of the symptoms are nonspecific, therefore, the disease has the risk to be misdiagnosed as other neurological or psychological disorders, such as dementia, epilepsy, or stroke .

GBM possesses a number of unique properties that are associated with its generally poor prognosis, including: a large number of malignant cells that are dormant and may develop rapid resistance to anticancer drugs; glioma has a “crab claw-like” invasion pattern, creating unclear borders between malignant and healthy tissue, thus it is extremely difficult to resect completely the tumor tissue during surgery; the surgical procedure may stimulate the growth of malignant cells; the blood–brain tumor barrier prevents most chemotherapies or other anticancer treatments to reach the brain tumor tissue, resulting in a poor cytotoxic activity and the development of drug resistance . For all these reasons, the survival period for most of the patients with GBM is only approximately 1 year, and only 5% of patients survive longer than 5 years . The initial diagnostic approach for patients with suspected GBM is magnetic resonance imaging (MRI), which can determine the size, shape, and location of the tumor . Some advanced MRI techniques—such as diffusion-weighted MRI or dynamic susceptibility contrast MRI—may provide some additional information, including the differentiation between GBM and malignant lymphoma , or the prediction of EGFR gene amplification [10]. In addition, computed tomography might also be employed to determine the presence of the tumor, although its use in clinical practice for the diagnosis of GBM is not so frequent due to its relative lower resolution in comparison to MRI Positron emission tomography (PET) imaging—which utilizes 18-fludeoxyglucose and is considered as a standard diagnosis approach for many other cancers—offers little value in the diagnosis of GBM due to the significantly higher glucose uptake of the brain when

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compared to other organs . However, alternative PET imaging using 11C-methionine could be useful as a diagnostic test to predict the prognosis of GBM patients .In terms of biomarkers for diagnosis and monitoring of GBM, isocitrate dehydrogenase (IDH) gene mutation and O6-methylguanine DNA methyltransferase (MGMT) hypermethylation have been confirmed to have some prognostic and predictive value. IDH mutation was integrated by the WHO in 2016 for the stratification of GBM into primary (IDH-wildtype) and secondary (IDH-mutant), with differences in prognosis . MGMT status was also included in the clinical guidelines for stratification and prediction of efficacy of chemotherapeutics . Other markers that may play a role in GBM diagnosis include EGFR gene mutation and amplification, p53 mutations, PTEN mutations, telomerase reverse transcriptase promoter mutation, or alpha thalassemia/mental retardation syndrome x-linked gene mutation. Despite all of the above, none of the current diagnosis standards provide real-time dynamic information on tumor progression and therapeutic efficacy, which would be extremely important to monitor the rapid progression of malignant tumors such as GBM [8].With regards to therapies against GBM, the standard for newly diagnosed patients consists of surgical resection, followed by radiotherapy of the surgical cavity and concurrent chemotherapy. Thus, the initial approach to manage a GBM patient includes surgical debulking, which may alleviate symptoms, and establish the diagnosis by biopsy. During the surgical procedure, Gliadel wafers—a registered product containing cytotoxic drug carmustine (bis-chloroethylnitrosourea, BCNU) incorporated into the biodegradable polymer polifeprosan 20—may be placed in the tumor cavity to slowly deliver the drug to the remaining tumor cells over a period of 3 weeks. In a large phase III clinical trial examining the efficacy of Gliadel wafers, patients treated with placebo wafers had a median survival of 11.6 months, while patients treated with Gliadel had a median survival of 13.9 months. A similar survival gain has been reported with the addition of adjuvant chemotherapy to the post-surgery therapy for GBM, which consists of radiotherapy (60 Gy in 30 fractions). The addition of systemic temozolomide (TMZ, 150–200 mg/m2/day continuously for 5 days every 4 weeks) has demonstrated only a limited benefit, with an increase in the median survival of the patients up to 2.5 months. The combined use of Gliadel wafers and TMZ has been associated with frequent adverse effects . An additional therapeutic approach, initially approved for recurrent GBM and more recently approved for newly diagnosed patients, consists of tumor-treating fields (TTF) produced by several transducer arrays attached to the shaved scalp of patients. These arrays are connected to an electrical device and generate low-intensity, intermediate-frequency alternating electrical fields that provoke antimitotic effects on malignant cells . A phase III clinical trial comparing TTF therapy plus TMZ with TMZ monotherapy as maintenance treatment in newly diagnosed GBM patients following conventional radiochemotherapy demonstrated a superior outcome with the combination of TTF and TMZ in both progression-free survival (7.1 months vs. 4.2 months in the TMZ-only group) and overall survival (19.4 vs. 16.6 months for the standard arm). Some concerns regarding the high cost/benefit ratio of this approach have been explained in the literature.The vast majority of GBM patients treated with the standard therapy experience recurrence of the disease, and only about 10% of the total number of these patients currently survive for more than 5 years . However, there is no standard consensus for the treatment of relapsed disease. Re-resection, re-radiation, and alternative dosing schemes of systemic TMZ, other chemotherapy agents such as cisplatin or irinotecan, and anti-angiogenic antibodies have been proposed as strategies for the treatment of recurrent disease, although only modest benefits to the patients have been shown by these treatments [5]. The anti-angiogenic drug bevacizumab has very recently received full approval from the U.S. Food and Drug Administration for the treatment of recurrent GBM . The results of a multicenter phase III trial evaluating the addition of bevacizumab to lomustine chemotherapy indicated an increase in the progression-free survival (4.2 months vs. 1.5 months for the chemotherapy alone). Despite this positive outcome, no significant differences were found in overall survival. Therefore, there is an urgent need to develop novel therapy approaches for GBM, improve the clinical outcomes, and reduce the rate of recurrence and adverse effects associated to current options. The blood–brain barrier (BBB) constitutes the main obstacle for the systemic treatment of brain tumors and other CNS disorders. The BBB consists of endothelial cells that enclose the brain and spinal cord capillaries and different types of perivascular cells, such as pericytes, astrocytes, microglial cells, and smooth muscle cells . The main anatomical difference of the BBB endothelial cells is the presence of tight junctions that form a continuous and almost impermeable barrier, resulting in limited paracellular transport of small and lipid-soluble molecules, a lack of fenestrations, and higher mitochondrial content required for the transport of solutes in and out of the brain The complex interactions between these components produce barrier functions that prevent most of the therapeutic compounds to reach the brain. In addition, the substantial presence of P-glycoprotein 1, that can recognize and pump out more than 60% of the marketed drugs, makes the penetration of the compounds into the brain much more difficult. In fact, the BBB only allows the free passage of water, ions, and a small number of lipophilic molecules, while the penetration of large molecules or hydrophilic drugs is often very limited. It has been reported that 98% of small molecules and 100% of large molecules

cannot penetrate across the BBB. In addition to the paracellular pathway, there are several routes of transport for chemotherapeutics across the BBB. The transcellular route is associated with low molecular weight and high log D drugs; hence, molecules of less than 500 Da and with high lipophilicity are more favorable to transport across the BBB. However, highly lipophilic drugs can be extensively bound to plasma proteins, resulting in less free available drug, which can compromise brain uptake . The polar surface area is also a key descriptor for BBB permeability. An inverse correlation between polar surface area and brain permeability has been described. When chemotherapeutics possess a polar surface area above 80 Å2 and strong capacity to form H-bonds (>6), a higher free energy is necessary to move the molecule from the aqueous environment to the lipid cell membrane of the endothelial cells . Apart from paracellular and transcellular pathways, other alternative routes for drugs to cross the BBB are receptor-mediated transcytosis (by means of a receptor binding) or adsorptive-mediated transcytosis, which is induced nonspecifically by positively charged molecules.A few disorders and diseases—such as multiple sclerosis, dementia, stroke, autoimmune deficiency syndrome, and brain tumors—may affect the integrity of the BBB. It has been shown that the vascular network of the BBB is disrupted in brain tumors, although the extent of alteration is not likely to result in a massive increase of the amount of drug entering the CNS. Moreover, the invasive nature of high-grade gliomas may produce a widespread presence of malignant cells outside the disrupted region of the BBB. Furthermore, in brain tumor tissues, there is a dense network of tumor vessels—termed as blood–brain tumor barrier (BBTB)—which presents an additional impediment for anticancer drugs to reach malignant tissues. In addition, a large number of efflux transporters expressed on the surface of endothelial cells of tumor tissues may pump the drug out of the cells, thus augmenting the chemotherapy resistance ability of GBM.Due to all the factors described, there is an insufficient exposure to drugs at the site of action within the brain, and, therefore, the treatment for GBM and other types of intracranial tumors remains a big challenge. Only a small number of cytotoxic drugs, such as TMZ, which possesses an acceptable level of BBB penetration (about 20% of the systemic dose), are currently used to manage high-grade glioma . Despite their promising in vitro cytotoxic activity in GBM cell lines, other drugs, such as doxorubicin (DOX) , paclitaxel (PTX) , or cisplatin, have not been used in standard GBM care due to their poor CNS penetration ability. In these cases, high doses of systemic treatment is required to achieve an optimal concentration at the site of action, resulting in a higher frequency of adverse effects due to the high exposure of healthy tissues to these drugs. Local therapy involves the direct administration of therapeutic drugs that can include chemotherapeutics, immunotherapy, or gene therapy to the tumor location, as opposed to the systemic administration by intravenous injection or oral route. This drug delivery approach has gained significant attention in recent years, as it is thought to circumvent some disadvantages of the systemic administration. Thereby, the clinical efficacy of anticancer drugs can be greatly improved, while the incidence of adverse effects is substantially reduced. Localized delivery vehicles can also be designed in the form of depots, which is the pharmaceutical dosage form that can release the active drug over a long period. Localized drug depots present a number of advantages in the delivery of anticancer drugs: they increase the stability of the chemotherapeutic drugs, generate extended and controlled drug release patterns, thus offering better control on drug levels and reducing the number of invasive drug administrations, can incorporate poorly soluble compounds within the depot, decrease the total amount of drug in the formulation, and reduce side effects of chemotherapeutics. In terms of treatment of GBM and other brain disorders, the local delivery approach seems to offer additional benefits, as it is able to bypass the BBB and BBTB, concentrating higher amounts of drug in the malignant tissues. Furthermore, a recent study from Mathios et al. demonstrated that local chemotherapy can potentiate the efficacy of concurrent immunotherapy as well as reinforce the memory response of the host immune system in GBM mice models. This evidence was only obtained for the two standard chemotherapy drugs against GBM, BCNU, and TMZ. However, the result of this study can incentivize more researches and clinical trials focused on local therapy to treat GBM in the near future. Direct injection of therapies in the tumor resection cavity, in the surrounding brain parenchyma, or in the ventricle appears as the most straightforward approach to locally deliver therapeutic drugs against a brain tumor. Local delivery of chemotherapeutic drugs is considered a feasible approach to effectively manage GBM and other highly malignant brain tumors, since it can bypass the BBB and increase drug availability to tumor tissues. Although a number of reports in the literature and a few clinical trials have investigated the possibility of local delivery with a range of therapeutics, only a very limited number has been approved for clinical practice. One of the main limitations of local delivery modes—such as local injection, drug wafers, and implants—is the short distance of drug diffusion from the site of administration, severely hindering proper contact between chemotherapeutics and tumor tissues. Although CED has been proposed to overcome this limitation by enhancing diffusion into the tumor, it has failed to demonstrate clear benefits over standard therapy. The PRECISE study, the largest clinical trial investigating CED to date, indicated that more than half of the CED intratumoral catheters were improperly placed, which could be one of the reasons for the

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disappointing results . The optimization of catheter devices and their placement protocols, together with the use of computer modeling for real-time monitoring of placement and infusion processes, are expected to considerably improve the efficacy of CED approaches to treat GBM. Nanosystems carrying chemotherapeutic drugs or as mediators of alternative therapies offer several advantages over the standard dosing forms in the treatment of GBM. Firstly, nanosystems can protect labile therapeutics from environmental degradation before reaching the target tissues. In addition to raising the concentration of therapeutic or diagnostic agents at the site of action and limiting their systemic clearance, the local administration of nanoparticles or injectable hydrogels can extend and control the release profiles, which open new opportunities for personalized treatments. Secondly, they are able to combine different drugs and diagnostic agents in the same system, with potential synergistic antitumor effects and the possibility to integrate treatment with disease monitoring. As explained by Chiarelli et al. , nanostructures are flexible platforms that can incorporate the required building blocks, from metal cores for imaging contrast enhancement and radiation/magnetic therapy to polymeric shells in order to provide biocompatibility and functional sites for the attachment of homing molecules, chemotherapeutics, nucleic acids, or optically active moieties. Thirdly, nanosystem characteristics, such as size, morphology, and surface functionalization, can be tuned with the aim to increase the extracellular matrix penetration and uptake by the tumor tissues.Despite the promising results in preclinical research using nanoparticles, a limited number of nanomedicines have been approved for clinical practice. Currently, there is only one nanotherapeutic, Nanotherm SPIONs, approved for use in the clinical treatment of GBM, and very few are undergoing phase I and phase II clinical trials. The complexity of some nanoparticle designs, the high production costs, and a significant failure rate in clinical trials have been proposed as possible factors for the low clinical uptake. Research efforts aimed to develop nanotherapeutics for the diagnosis and treatment of GBM should be directed to bridging the gap between preclinical studies and the clinical phase. As a starting point, ongoing research on elucidating the mechanisms of brain tumor growth should be able to reveal novel potential molecular targets for local therapy that could enhance targeting efficiency. Moreover, there is a need to select and optimize the animal models that reflect the heterogeneity of brain tumors in order to efficiently predict therapeutic outcomes and adverse effects of nanotherapeutics. Additionally, reproducibility and scalability of nanoparticle synthetic methods should be improved, and cost should be minimized, to facilitate the translation of novel developments, which promises significant advances in the treatment of GBM.

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