Xiao Xue Zeng, Jianwen Zeng and Baoyi Zhu*

Future generation of combined multimodal approach to treat brain glioblastoma multiforme and potential impact on micturition control

https://doi.org/10.1515/revneuro-2021-0068
Received May 16, 2021; accepted August 26, 2021; published online September 16, 2021

Abstract: Glioblastoma remains lethal even when treated with standard therapy. This review aims to outline the recent development of various advanced therapeutics for glioblastoma and briefly discuss the potential impact of glioblastoma and some of its therapeutic approaches on the neurological function micturition control. Although immunotherapy led to success in treating hematological malignancies, but no similar success occurred in treatment for brain glioblastoma. Neither regenerative medicine nor stem cell therapy led to astounding success in glioblastoma. However, CRISPR Cas system holds potential in multiple applications due to its capacity to knock-in and knock-out genes, modify immune cells and cell receptors, which will enable it to address clinical challenges in immunotherapy such as CAR-T and regenerative therapy for brain glioblastoma, improving the precision and safety of these approaches. The studies mentioned in this review could indicate that glioblastoma is a malignant disease with multiple sophisticated barriers to be overcome and more challenges might arise in the attempt of researchers to yield a successful cure. A multimodal approach of future generation of refined and safe therapeutics derived from CRISPR Cas therapeutics, immunotherapy, and regenerative therapeutics mentioned in this review might prolong survival or even contribute towards a potential cure for glioblastoma.

Keywords: CAR-T; CRISPR Cas system; glioblastoma; immunotherapy; micturition; regenerative medicine

*Corresponding author: Baoyi Zhu, The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People’s Hospital, Yinquan Road B24, Qingyuan City, Postcode: 511500, Guangdong Province, P. R. China, E-mail: baoyizhu2019@163.com
Xiao Xue Zeng, Guangzhou United Family Hospital, Fangyuan Road 28, Haizhu District, Guangzhou, Postcode: 510000, Guangdong Province, P. R. China, E-mail: 2402312575@qq.com. https://orcid.org/0000-0002-9639-4010
Jianwen Zeng, The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People’s Hospital, Yinquan Road B24, Qingyuan City, Postcode: 511500, Guangdong Province, P. R. China, E-mail: zengjwen@gzhmu.edu.cn

Introduction

Glioblastomas is malignant brain tumor defined as grade IV glioma by the World Health Organization (WHO) (Louis et al. 2016), remaining fatal despite the utilization of various therapies combined (Weller et al. 2017). The central tendency of median overall survival in a recent systematic review was 13.5 months and in clinical trials, median overall survival for bevacizumab utilization was 18.2 months, median overall survival for the application of tumor treating fields was 20.7 months, and the median overall survival for using the vaccines was 19.2 months (Marenco-Hillembrand et al. 2020). Even by the standard regimen of surgery, with radiation therapy and chemotherapy followed by alkylating agent temozolomide, glioblastoma is incurable (Stupp et al. 2005). Although Maximal safety resection is usually performed to remove the glioblastoma, recurrence is inevitable thus repeated resection is required to prolong survival time (Sanai and Berger 2009). Some studies assessed the effect of repeated surgeries to treat glioblastoma (GBM) recurrence and suggest that it can improve survival in the selected patients in their studies (Bloch et al. 2012; Chaichana et al. 2013; Hervey-Jumper and Berger 2014). Also in some cases the patient might not respond to temozolomide therapy and but instead the GBM cells build up resistance to it (Lee 2016). The inevitable recurrence due to the fact that even post tumor resection the remaining Glioblastoma cells tend to spread and form a new glioblastoma within 2–3 cm of the original tumor surroundings. However, a main challenge lies in removing tumor cells remaining within the tumor margin without causing adverse impact to healthy brain tissues in the margin, which could result in damage to neurologic functions such as language and motor functions, or micturition disturbances. Intraoperative magnetic resonance imaging is also applied to improve the extent of tumor removal (Masuda et al. 2018). Awake craniotomy possesses the potential to avoid these adverse effects as the tumor resection is performed with the patient awake during the surgical procedure with a neuronavigation system, for instance the Stealth Station developed by Medtronic (Alphandéry 2018). However, 3–5% of patients can survive longer, for five years or more (Armocida et al. 2019; Scott...
et al. 1999; Steinbach et al. 2006). In extreme rare cases, patient survives glioblastoma for 20 years or more (Caruso et al. 2017; Sperduto et al. 2009). In this review we aim to outline the potential candidate therapeutics utilized to tackle glioblastoma and briefly discuss the potential effects of glioblastoma and glioblastoma therapeutics on micturition control. Moreover, CRISPR Cas system possess capacities applied in a variety of strategies to treat glioblastoma, including gene editing, immunotherapy, and regenerative therapy, and also enable personalized treatment for glioblastoma. The findings from the studies mentioned in this review could guide and inspire future researches (Figure 1).

**Recent landscape of immunotherapy on glioblastoma**

**CAR-T therapy**

Since the surgical resections of brain glioblastoma require great skills and accuracy which can be difficult to perform and it is hard to target all the infiltrating tumor cells which allows remaining tumor cells to proliferate thus making recurrence inevitable, a more precise method of targeting the solid tumor or remaining glioblastoma cells post surgery would be ideal. As the CAR-T technique has brought evolution to the field of cancer, it is also researched for a development of cure for brain glioblastoma. T cells modified to express CAR are capable of targeting a specific antigen via the scFv binding domain (Eshhar et al. 1993). The CAR therapy has undergone various clinical trials and has revolutionized the field of cancer therapy (Yu et al. 2017). CAR therapy has gone through evolution of four generations (Muhammad et al. 2017). By using a retroviral vector it is possible to modify T4(+)-T-cells to have two chimeric receptors at the same time (Ghosh et al. 2017). CD19-specific CAR-T cells brought optimistic clinical results against leukemias or lymphomas (Maude et al. 2018). The FDA and EMA have approved Kymriah for the treatment of B-cell acute lymphoblastic leukemia (ALL), and Yescarta for the treatment of B-cell non-Hodgkin lymphoma (June and Sadelain 2018). The major challenge lies in the application of CAR therapy to treat solid tumors. T cells can target solid tumors by checkpoint therapy and infusions of lymphocytes were used against melanoma (Rosenberg et al. 1988). Until now, CAR techniques seemed to cause less regression on solid tumors than hematological tumors partly due to obstacle in finding suitable tumor-specific antigens that can be targeted by CAR-T cells, without possible on-target/off-tumor toxicity leading to the unintentional damage of healthy cells that express the same target antigen (Lamers et al. 2013). T cells have been genetically modified to target brain tumor associated antigens which presented optimistic preclinical effects. As for GBM, interleukin-13 receptor alpha 2 (IL13Rα2) was the first CAR-T cell target tested in trials due to its overexpression in tumors (Brown et al. 2012; Kahlon et al. 2004). The majority of clinical trials on GBM are carried out either in the USA or China. The clinical trials are performed with CAR-T cells in GBM targeting EGFRvIII, IL13Ra2, or HER2 which can be checked on www.clinicaltrials.gov. However, the sole tumor-specific antigen in human cancer often expressed in GBM for CAR is reported to be EGFRvIII (Li and Wong 2008). EGFRvIII is not expressed in other types of cancer and nor is it expressed on normal tissue. Although it is reported in a study that it was successful in slowing down glioblastoma progression in a mouse model, however in a study of phase I clinical trial involving some 10 patients with unmethylated GBM promoter both heterogeneity of EGFRvIII on the surface in various brain tissue and antigen escape happened which would pose the question whether it will be effective in treating GBM when CAR-T is engineered with just a single receptor of EGFRvIII antigen (Miao et al. 2014). A phase I study reported that just one infusion of EGFRvIII targeted CAR-T cells was carried out without inducing off-target toxicities and cytokine release syndrome. In the study, four glioblastoma patients who had CAR-T cells detected intratumorally during resection procedure to remove the tumor after intravenous CAR-T therapy (O’Rourke et al. 2017). In another study, IL13Ra2 CAR-T cells were injected into the brain where GBM resides in three patients. Followed by another study, the tumors of the same patients decreased after intracranial CAR-T cell injection (Brown et al. 2016). Although HER2 is overexpressed in some tumors but healthy cells carry them too, therefore it poses an off-target toxicity risk when used as target antigen in CAR-T therapy. Nevertheless, HER2-specific CARs in phase I trial was relative secure (Morgan et al. 2010). In a recent study it is discovered that p32, gC1qR, HABP, and C1qBP receptors are expressed on the surface of glioma cells and p32 CAR-T cells were engineered to specifically target p32 the binding receptor of CGKRK and LinTT1 on the surface of glioma cells in order to eliminate cancer cells in mouse models (Rousso-Noori et al. 2021). In a research, dual antigen targeting CAR-T cell for prostate cancers designed which coexpress prostate specific membrane antigen and prostate stem cell antigen (Zhang et al. 2018). Similar success was achieved in breast cancer (Wilkie et al. 2012). Such methods are used to avoid on-target off-tumor toxicity (Lamers et al. 2013). These
studies should inspire multiple antigen targeting approach in glioblastoma researches. Another method to enhance CAR-T therapy effect is to provide CAR-T cells with the ability to produce cytokines like IL-12, IL-15, IL-21, IL-7, or a mixture of them (Schaft 2020).

**Immune checkpoint strategy**

Since solid tumors can induce inhibitory receptors such as PD1 and CTLA-4 to decrease the ability of the CAR-T cells which are also attacked by the antibodies produced by the patient’s own immune cells (Haas et al. 2019). Efforts are taken to block PD1 or CTLA-4 in some CAR-T clinical trials, by combining it with anti-PD1, anti-CTLA-4 inhibitors (Heczey et al. 2017). Combined PD-1 and CTLA-4 inhibitors were effective when used in melanoma, although not without toxicities (Larkin et al. 2015). The immune checkpoint inhibitor therapy has also demonstrated effectiveness in other cancers including the breast cancer (Schmid et al. 2018). However, until now, immune checkpoint inhibitor strategy has shown no significant effect on Glioblastoma (Zhang et al. 2020).

**NK cell based strategy**

Although some relatively effective immunotherapeutic methods were developed against other solid tumors, scientists are still on their way to develop successful immunotherapeutic measures to deal with glioblastoma. However, NK cells might show potential prospects to treat glioblastoma in a way that they can kill the cancer cells as they don’t need to prime with the target antigens on tumor cells beforehand (Chu et al. 2019). The recent development in regards to glioblastoma includes the application of cord blood NK cells in treatment (Yvon et al. 2017). Moreover, NK cells-derived exosomes as a potential glioblastoma treatment is being researched in (Zhu et al. 2018) and a CAR-NK cell therapy targeting EGFR variant III has been generated to induce anti tumor effect, which was effective in vitro (Murakami et al. 2018). In addition, engineered ErbB2-specific NK-92/5.28.z cells (HER2. taNK) are under phase I clinical trial (Zhang et al. 2016).

**Challenges to CAR-T and other immunotherapies in glioblastoma**

T cell-based therapies to tumors in brains has to be effective in the immunosuppressive microenvironment, overcome on-target off-tumor toxicity problem such as antigen heterogeneity, and be able to cross the blood brain barrier (Li et al. 2020). In a case study a patient with a metastatic colon cancer died after being infused with ERBB2-targeted CAR T-cells infusion. This was due to the small amount of antigen ERBB2 on the healthy epithelial cells of the lung (Upreti et al. 2020). Cancer heterogeneity, antigen escape, and antigen loss are all problems to solve for CAR treatment in solid tumors (Morgan et al. 2010). Moreover, researchers have classified GBM into four main subtypes according to their genomic features, classical, proneural, mesenchymal, and neural, but researchers suggest that the neural subtype is merely a mixed with healthy normal cells (Martinez and Moon 2019). There can be a mixture of glioblastoma subtypes in the tumor, and the subtypes can alter themselves into any other subtype during the stages of tumor development (Verhaak et al. 2010). Though the shift from proneural and to mesenchymal subtype occurs with the greatest probability which brings more challenge to concocting an ideal strategy of cure in the form of CAR technique (Wang et al. 2018). Neurotoxicity induced by CAR-T therapy is another side effect with for unknown reasons which could cause manifestations such as transient cognitive impairments, hallucinations, and delirium, but also encephalopathy and seizures (Perales et al. 2017).

**Molecular based strategy**

Tumors are supplied with oxygen and nutrients by capillaries, enabling them to grow at a rapid pace. Angiogenic growth factors, such as vascular endothelial growth factor (VEGF) (Karamysheva 2008) are vital in promoting tumor angiogenesis and growth. Among them, VEGF is the key endothelial cell-specific mediator of angiogenesis hence various tactics to target VEGF/VEGFR-2 signaling have been developed. Strategies include inhibition of VEGF signaling by ligand sequestering agents (bevacizumab) (Hurwitz et al. 2004) or VEGFR-2 blocking antibodies such as ramucirumab (Fuchs et al. 2014; Wilke et al. 2014) are utilized, which also caused adverse impact. Tumors develop resistance toward such antiVEGF therapy and continue the process of angiogenesis, by some alternative pathway (Ebos et al. 2009; Pàez-Ribes et al. 2009). It is suggested that the reason behind tumor resistance toward anti VEGF therapy is that it merely targets VEGF without inhibiting other molecules in the angiogenesis pathway that promotes tumor cell proliferation, migration and survival (Piao et al. 2012).
Gene mutations in glioblastoma

In a case study a 29 year old man underwent glioblastoma resection therapy twice and BRAF V600E was found exclusively in the cancerous areas from the two specimens, suggesting that BRAF V600E mutation could be the malignant cause by influencing the transformation of diffuse astrocytoma along with CDKN2A and CDKN2B loci deletion into glioblastoma (Kanamori et al. 2016). In another case report, a nine-year-old male patient, after 10 years of initial diagnosis of an anaplastic astrocytoma in the left temporoparietal region, it underwent transformation into a secondary glioblastoma. After another seven years, it turned into epithelioid glioblastoma. Following the detection of a BRAF V600E mutation, dabrafenib treatment was administered, then the patient died 16 months after dabrafenib treatment initiation which suggests that BRAF inhibitors seems to be a life prolonging treatment option (Cecccon et al. 2018). CDKN2A is a tumor suppressive gene that encodes p16INK4a protein and inhibits the process of cell cycle. Sixty seven glioblastoma cases were included in a study and CDKN2A deletion was noted in 40.3% cases, and the most of it was homozygous deletion (74%) (Purkait et al. 2013). These findings will provide guidance to future CRISPR Cas based glioblastoma researches.

Control of micturition in various brain location

The pelvic nerve transmit signals of the bladder filling sensation to the sacral cord, which then relays it to the caudal periaqueductal gray that transmits signals to excite the premotor interneurons in the M-region when the volume of urine in the bladder exceeds a certain amount which, transmit signals through descending fibers to the motor neurons in the sacral cord, to cause the start of voiding, whereas the emotional motor system plays a role in assessing whether the time and environment is appropriate (Blok and Holstege 1996). A study involving resting-state functional MRI conclude that brain regions including the frontal lobe, cingulate cortex, caudate nucleus, hypothalamus, and temporal lobes hold power in controlling the sensation to void (Gao et al. 2015). Moreover, functional neuroimaging studies show that locations including frontal lobe, anterior cingulate cortex, and insula are involved in controlling the filling, storage, or withholding of the bladder (Blok et al. 1997a,b; Fowler and Griffiths 2010; Griffiths and Tadic 2008). Posterior portion of the right anterior cingulate gyrus processes a person’s bladder sensation information and controls the inhibition of the pontine micturition center with or without the trigger of the L-region to control continence. Anterior portion of the right anterior cingulate gyrus controls the sensation of the bladder and the urge to void due to external stimuli and or due to behavioral reason (Gehring and Knight 2000). Right anterior insula controls the suppression of mechanoreceptor discharge in the bladder wall. Right inferior frontal gyrus controls the willingness to void (Blok et al. 1997a,b). The cerebellum controls the motor of micturition and processes the sensory information from the bladder as the animal studies manifest that stimulation or tumor within the cerebellum would influence urine storage and micturition (Bradley and Teague 1969; Nishizawa et al. 1989).

Neurogenic dysfunction of micturition

Neurogenic bladder is the dysfunction of urinary bladder and urethra caused by diseases of the central nervous system or peripheral nerves (Stohrer et al. 2009). In a systematic study, it concluded that micturition disorders in brain tumors due to increased intracranial pressure could include dysuria, incontinence, and pollakiuria depending on the location of the tumor (Ueki 1960) and maybe its size too. Lesions in the cingulate gyrus are also associated with bladder disturbances of various types, especially urge incontinence (Andrew and Nathan 1964). Glioblastoma in brain could lead to the overactive bladder when it affects the frontal cortex, basal ganglia, hypothalamus, and cerebellum. If the tumor affects the brainstem it could cause either overactive bladder or urinary retention. Brain tumor derived urinary incontinence can be classified into either neurogenic urinary incontinence or functional urinary incontinence, although they can coexist. Therapeutic for neurogenic urinary incontinence utilizing anticholinergic drugs which are rather hard to penetrate the blood–brain barrier, whereas treatment for functional urinary incontinence are behavioral therapy (regular voiding and pelvic floor exercise) and medication to boost bladder cognition which all contribute to better life quality (Sakakibara 2015).

Potential effects of glioblastoma therapeutics on micturition control

In a case study regarding a woman who underwent the process of low grade glioma resection which involved the posterior part of the anterior cingulate gyrus and the supplementary motor area. There was no urinary disorder
before the surgery but the patient suffered from urinary incontinence without bladder sensation. She was then with given oxybutynin chloride, 15 mg daily for two years which did not cause any side effects. In another case a man underwent low grade glioma resection which involved the inferior frontal gyrus and the anterior insula and also suffered from no micturition disorder prior surgery but suffered from the condition post surgery without incognition of the bladder and was treated under the same regimen of drug for four years or more (Duaffu and Capelle 2005). In another case report, a 52 year old woman suddenly began to suffer from incontinence and underwent subtotal frontal pole glioblastoma multiforme resection; the micturition control was reestablished after discharge but with nocturnal frequency. Six months later she had complete control over micturition again and suffered from no urinary disorder. The same study suggested that the frontal lobes controls the micturition and in another systematic review it mentioned approximately 500 patients who underwent, frontal lobectomy or bilateral anterior cingulectomy and it suggested that the frontal lobes exercise an inhibitory effect on micturition, the left frontal lobe more apparent (Maurice-Williams 1974; Ueki 1960). A detailed understanding of the central nervous system on micturition control and continence could guide the brain glioblastoma surgery towards function maintenance and treatments for post surgery urinary dysfunctions (Duffau and Capelle 2005). In another case study a 75-year-old man suffered progressively from lumbosacral spinal subdural hematoma after surgical removal of a right temporal glioblastoma multiforme two weeks post hospital discharge as he recovered, the symptoms included urinary retention which deteriorated (Paisan et al. 2017). The preservation of micturition control post the application of tumor elimination therapeutics also depends on the glioblastoma size and its position. Theoretically, all glioblastoma cells ought to be eliminated to prevent recurrence which is currently inevitable. The currently advanced therapeutics utilized in the eradication of glioblastoma cells cannot guarantee absolute intactness of brain tissue or neurons around the area where glioblastoma is located. The current findings suggest that CAR-T therapy is not yet entirely clinically safe to use against Glioblastoma in its current development stage, as it still needs to overcome the on-target off-tumor toxicity problem such as antigen heterogeneity (Li et al. 2020), therefore it is possible that CAR-T could cause unintentional damage to the healthy cells of brain areas that controls micturition. Hypothetically, if a successful immunotherapy is developed, it could relieve the intracranial pressure by effective elimination of tumor cells without harming the healthy cells, thereby relieving micturition disturbances. Due to a lack of sufficient studies reporting on the effect of various immunotherapies against glioblastoma on micturition control, the impact remains to be observed in future researches as more studies are needed to conduct an evidence based assessment. Stem cell therapeutics such as autologous muscle-derived stem cells were utilized to deal with urinary incontinence by direct intrasphincter injection (Carr et al. 2012). A vast number of studies of stem cell therapeutics in kind were done on directly dealing with urological disturbance by bladder tissue engineering (Lai et al. 2002). Studies of grafts depending on the generation of urothelial cells or smooth muscle cells (Feil et al. 2008), but more detail are beyond the scope of this review. More researches should be carried out on the application of regenerative therapy to repair brain tissue or nerve damage which will repair the control function brain has over micturition. Current regenerative therapy is not particularly effective which needs the application of CRISPR Cas technology to enhance it.

Regenerative medicine for reparation of damaged brain tissues and nerves

Glioblastomas are highly recurrent even after surgical resection due to the fact that the boundary between tumor cells and bodily healthy cells isn’t entirely clear (Perry and Wesseling 2016) thus it’s difficult to eradicate all the tumor cells which possess the ability to infiltrate further and the immunotherapy and gene therapy for brain glioblastoma have not yet achieved astounding success in this endeavor. Even if researchers once manage to achieve breakthrough in developing successful methods in removing all the tumor cells of glioblastoma in brain entirely, a greater challenge needs to be overcome such as repairing the loss of neurologic functions, thus reparation or replacement of healthy brain tissues or neurons becomes an essential step for the glioblastoma patients to regain functions. Therefore, regenerative medicine is crucial to the function revival of the central nervous system and restoration of micturition control.

Nerve repair

There are various medications or therapeutics which might be utilized to contribute towards nerve repair, the detail of which is beyond the scope of this paper. However, nerve repair techniques, essential to restoration of the functions of the central nervous system post cancer cell elimination,
include epineurial repair, perineurial repair, and group fascicular repair (Ramachandran and Midha 2019). Autologous nerve grafting brings the benefit of fast recovery for long nerve gaps (>3 cm), more proximal injuries and critical nerve injuries (Grinsell and Keating, 2014). Nerve allograft is another option, where nerves can be obtained from a cadaver or donor for nerve graft (Trehan et al. 2016). Moreover, the cadaver nerve allografts are highly accessible and both endoneurial microstructure and Schwann cells (SC) of the donor, accelerating the regeneration (Moore et al. 2011). By surgical procedure the nerve transfer therapeutic is a method utilized for complete loss of sensory and muscle functions (Moore 2014). The technique functions by transferring distal motor nerve by utilizing extended nerve grafts for the middle and high-level injuries, reducing the regeneration distance and period, which could also be an potential option for brain nerve reconstruction (Tung and Mackinnon 2010). Nerve injury with a relative long gap requires clinical treatment such as nerve autograft therapy which requires a long time for recovery (Deumens et al. 2010). Successes of these therapies are insufficient despite they are performed at the expense of healthy nerves and along with its functions. Other various technique are utilized, such as linking the nerve gaps utilizing some kind of scaffold, or a combination of intervention methods of scaffold bridging and stem cell transplantation, however it seems that scaffold bridging along with stem cell transplantation are more effective than scaffold bridging alone (Pfister et al. 2011).

**Stem cell therapy**

Neural stem cells which are selfregenerative are present in the subependymal ventricular zone, the subgranular zone of the dentate gyrus of the hippocampus, and the subcortical white matter of the human brain (Eriksson et al. 1998; Nunes et al. 2003; Sanai et al. 2004). Stem cells that derived from embryonic and adult tissues have the ability to regenerate and have differentiation potential, which can be used to restore the functions of central nervous system including micturition control. Stem cells are divided into pluripotent stem cells, multipotent stem cells, and multipotent stem cells depending on their developmental potential (De los Angeles et al. 2015). For nerve regeneration, Schwann cells and/or Schwann-like cells are derived from bone marrow stromal cells are utilized (Dezawa et al. 2005). Schwann cells produce nerve growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, platelet-derived growth factor and neuropeptide Y which promote nerve regeneration (Terenghi 1999). Moreover, Schwann cells are able to self proliferate, modulate the immune system, remyelinate, and migrate which all contribute to regeneration (Ciu et al. 2012). Schwann cell therapeutic utilized in an experimental model of traumatic peripheral nerve injury was effective in causing regeneration and remyelination (Borlongan 2010; Guénard et al. 1992). Skin-derived precursor stem cells are found in the dermis and can differentiate into neurons and glial cells whereas hair follicle stem cells can differentiate into Schwann cells directly (Herberts et al. 2011). For the cell-based therapy to succeed in nerve regeneration, it depends on transplanted cells differentiating into Schwann-like cells, to produce neurotrophic growth factors and to contribute to myelination of axons. There are other stem cells that carry regenerative abilities. Embryonic stem cells possess differentiation feature and the ability to proliferate although it causes ethical concerns if acquired for transplantation purposes. Neural stem cells have the capacity to differentiate into neurons and glial cells. However these cells are hard to cultivate and it is possible for them to develop into neuroblastoma (Maris and Matthy 1999). Fetal stem cells can be obtained from amniotic fluid, amniotic membrane, umbilical cord and Wharton’s jelly. However amniotic tissue-derived stem cells and umbilical cord-derived mesenchymal stem cells also possess the capacity for differentiation and proliferation. Main benefits of fetal-derived stem cells are they are easy to acquire although they also cause ethical concerns (Widgerow et al. 2014). Multipotent somatic stem cells obtained from bone marrow, mesenchymal stem cells, demonstrated some potential promoting axon myelination when utilized in artificial conduits along with acellular grafts (Chen et al. 2007; Dezawa et al. 2001; Walsh and Midha 2009). Moreover, one study concluded that astrocyte lineage cells are required for neural differentiation and synapses to mature (Klapper et al. 2019). Pluripotent stem cells are able to differentiate into all tissues/cells with various functions depending upon the culture conditions (Yagi et al. 2017). Pluripotent stem cells or typical embryonic stem cells can differentiate into specialized cells to regenerate specific tissues for instance lungs or brain tissues (Yang and Huang 2019). However, human induced pluripotent cells have the potential to differentiate into a variety of cells (Sampaziotis et al. 2015; Zhang et al. 2009). Autologous patient-derived induced pluripotent stem cells transformed into dopaminergic progenitor cells are implemented as a therapeutic to treat Parkinson’s disease, inspiring the application of iPSCs to various treat human diseases (Schweitzer et al. 2020). Moreover, in a study, stem cells have been injected directly into the lesion of the spinal cord indicating that transplanting neural progenitor cells or other stem cells
can restore bladder control (Neuhuber et al. 2008). Studies to repair impaired cardiac tissue by the direct injection of human induced pluripotent cells into the area in need of tissue regeneration (Liu et al. 2018; Nelson et al. 2009; Shiba et al. 2016). However, implementation of human induced pluripotent technology has met challenges including the purity of the desired cell and the teratoma formation risk due to the undifferentiated cells after transplantation (Ma et al. 2011). To overcome this challenge, the lactose replaced glucose supplemented medium in order to eliminate undifferentiated cells (Ma et al. 2011; Zhang et al. 2012). A study involves deriving glial enriched progenitors from human induced pluripotent stem cells by the transient treatment of human induced pluripotent stem cell derived neural progenitors with the small molecule deferoxamine for three days (Llorente et al. 2021). These studies might spark inspiration towards future researches of stem cell therapy on brain tissue or brain nerve regeneration. However, obstruction of induced pluripotent stem cell application in routine clinical treatments includes immune rejections, carcinogenicity, genomic instability and shortage in standard quality controls (Deinsberger et al. 2020). Other disadvantages of stem cell therapeutics include from stem cell cultivation to transplantation safety and cell preparations, require a rather long period and are quite expensive, however nerve repair or restoration cannot always wait (Hussain et al. 2020).

Stem cell therapeutics can be found on ClinicalTrials.gov, see Table 1.

The potential of CRISPR/Cas system in multiple applications

The CRISPR Cas system carries potential in developing a cure for brain glioblastoma see (Figure 1). The CRISPR sequences and their associated Cas proteins prevents cell invasion of foreign genetic materials from similarly to the eukaryotic RNAi system and it can then create a memory file of the foreign genetic material that is stored to induce immediate reactions in case of repeated invasions from the same foreign genetic material, acting the role of a defensive system (Zhang et al. 2018). There are two classes CRISPR/Cas systems due to the variation in Cas proteins. The first class system requires multiple Cas proteins to form the CRISPR-associated complex relied upon for antiviral defense, whereas the second class system depends merely on a single Cas protein consisting of more than one domains. The class 1 system is divided into type I, III, and IV groups and the class 2 system is divided into type II and type V groups (Makarova et al. 2015). The CRISPR gene editing method is able to make specific and precise modifications to genes, even to tiny sequences which bears a greater advantage in relative to other methods for the same purpose, with strong complementarity between the guide sequences (crRNAs) and their targets, as well as the precision of Cas cleavage at the target site (Brown et al. 2017; Shmakov et al. 2015).

Application in gene editing

The CRISPR systems could make alterations to genes and a strategy was created to produce guide RNAs, tracrRNA, and the Cas9 cleavage protein in the body cells (Cong et al. 2013). CRISPR/Cas9 system simply consists of a crRNA guide sequence, the Cas9 nuclease protein and a tracrRNA (Liu et al. 2015). CRISPR/Cas9 is one of the most valued technologies for gene editing as the Cas9 endonuclease, guided by RNA (gRNA), can target and cleave specific DNA sequences, enabling it to insert or delete sequences (Jinek et al. 2012), possessing enormous potential for developing a variety of cancer treatments as it is quite efficient (Sanchez-River and Jacks 2015). Researches conducted utilizing CRISPR/Cas9 for *in vivo* in animal models in correcting various human genetic diseases (Birling et al. 2017). CRISPR therapeutic is used as a strategy for Leber congenital amaurosis 10, a rare disease by removing the CEP 290 gene mutation responsible from patient retinal cells *ex vivo*, by gene editing and re-implanting it back into the eye (Ledford 2020). In the pursuit of developing treatments for glioblastoma, CRISPR plasmids are utilized in a study to knock out regulators of G-protein signaling 4 in the endeavor to deter the growth, proliferation, and infiltration of glioblastoma cells (Guda et al. 2020). In Wu et al. CRISPR-Cas13a is applied to edit FGFR3-TACC3 fusion genes (Wu et al. 2021). Another research came to the conclusion that SOX2 regulatory region 2 deletion by CRISPR/Cas9 technology reduces SOX2 expression in glioma cells, as a result cell growth and proliferation are reduced in tumor cells, impairing self regeneration in glioblastoma stem cells (Saenz-Antoñanzas et al. 2021).

Application in immunotherapy

Scientists utilized CRISPR/Cas9 to engineer CAR-T cells resistant to PD-1 inhibition, which prevents the T-cells from recognizing cancer cells (Ren et al. 2017). Moreover, CRISPR therapies on phase 1 trials in humans, mainly culture the donor cells, having them modified, and then reintroduced
into the human body (Xu et al. 2019). For instance a phase 1 trial involving a patient diagnosed with lipo sarcoma and two patients suffering from myeloma whose T cells were modified by CRISPR therapy to express a T cell receptor specific to the NY-ESO-1 tumor antigen, which were detectable in all three patients for three months at least. Then in a period of nine months following the treatment two patients had survived and one patient had deceased (Stadtmauer et al. 2020). In Jung et al., CRISPR/Cas9 improved anti tumor function of T cells by knockout of diacylglycerol kinase to provide CAR T cells with resistance to immunosuppressive factors such as TGFβ and prostaglandin E2 as treatment for glioblastoma (Jung et al. 2018).

In a study, CRISPR Cas9 system was applied to generate universal EGFRvIII-targeted CAR-T cells resistant to PD-1 checkpoint inhibition by gene editing of endogenous T-cell receptor, beta-2 microglobulin and PD-1 against glioblastoma. Prolonged survival in mice models was achieved via intracerebral administration of those engineered CAR-T cells rather than by intravenous infusion (Choi et al. 2019). The benefits of creating universal immune cell banks such as engineered allogeneic CAR T cells is that they are readily available even in cases when it’s urgent or when it is not convenient to acquire autologous T cells from health compromised patient for ex vivo expansion or modification into target CAR-T cells (Thommen and Schumacher 2018).

### Table 1: Stem cell therapeutics for brain tumors listed on www.clinicaltrials.gov.

<table>
<thead>
<tr>
<th>Clinical trial identifier</th>
<th>Title</th>
<th>Status</th>
<th>Interventions</th>
<th>Phase of study</th>
<th>Location</th>
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<tr>
<td>NCT0002619</td>
<td>Chemotherapy followed by bone marrow or peripheral stem cell transplantation in treating patients with glioblastoma multiforme or brain stem tumors</td>
<td>Completed</td>
<td>Peripheral blood stem cell transplantation</td>
<td>Phase II</td>
<td>Kaplan Cancer Center, New York, New York, United States</td>
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<tr>
<td>NCT01759810</td>
<td>Proteome-based personalized immunotherapy of glioblastoma</td>
<td>Unknown</td>
<td>Allogeneic hematopoietic stem cells and autologous hematopoietic stem cells</td>
<td>Phase II/III</td>
<td>Moscow, Russian Federation</td>
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<td>NCT01269424</td>
<td>BG and TMZ therapy of glioblastoma multiforme</td>
<td>Terminated</td>
<td>Autologous hematopoietic stem cell transplantation</td>
<td>Phase I</td>
<td>University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States</td>
</tr>
<tr>
<td>NCT00179803</td>
<td>Stem cell transplant for high risk central nervous system (CNS) tumors</td>
<td>Completed</td>
<td>Stem cell transplant</td>
<td>Phase II</td>
<td>Children's Memorial Hospital, United States</td>
</tr>
<tr>
<td>NCT03072134</td>
<td>Neural stem cell based virotherapy of newly diagnosed malignant glioma</td>
<td>Active, not recruiting</td>
<td>Neural stem cells loaded with an oncolytic adenovirus</td>
<td>Phase I</td>
<td>United States</td>
</tr>
<tr>
<td>NCT00078988</td>
<td>High-dose chemotherapy plus autologous stem cell transplantation compared with intermediate-dose chemotherapy plus autologous stem cell transplantation with or without isotretinoin in treating young patients with recurrent high-grade gliomas</td>
<td>Completed</td>
<td>Peripheral blood stem cell transplantation</td>
<td>Phase III</td>
<td>United States</td>
</tr>
<tr>
<td>NCT00253487</td>
<td>Combination chemotherapy and radiation therapy in treating younger patients who are undergoing an autologous stem cell transplant for newly diagnosed gliomas</td>
<td>Completed</td>
<td>Peripheral blood stem cell transplantation</td>
<td>None applicable</td>
<td>Children's Hospital Medical Center, United States</td>
</tr>
<tr>
<td>NCT02820584</td>
<td>A phase I study of immunotherapy with GSC-loaded dendritic cells in patients with recurrent glioblastoma</td>
<td>Completed</td>
<td>GSC loaded autologous dendritic cells</td>
<td>Phase I</td>
<td>I.R.C.C.S.Istituto Neurologico Carlo Besta</td>
</tr>
<tr>
<td>NCT00576641</td>
<td>Immunotherapy for patients with brain stem glioma and glioblastoma</td>
<td>Completed</td>
<td>Autologous dendritic cells</td>
<td>Phase I</td>
<td>Cedars Sinai Medical Center, United States</td>
</tr>
<tr>
<td>NCT00392886</td>
<td>Combination chemotherapy with or without etoposide followed by an autologous stem cell transplant in treating young patients with previously untreated malignant brain tumors</td>
<td>Completed</td>
<td>Autologous hematopoietic stem cell transplantation, peripheral blood stem cell transplantation</td>
<td>Phase II</td>
<td>United States</td>
</tr>
</tbody>
</table>
In Sun et al., laminin-411 expression inhibited in glioblastoma cells in mice models by CRISPR/Cas9 technology slowed down tumor growth leading to reduced tumor size and increased mice survival (Sun et al. 2019).

Application in regenerative medicine

CRISPR systems are utilized by researchers in regenerative medicine. Despite the advances in regenerative therapeutics to replace damaged cells or tissues it faces barriers such as graft versus host disease (Blazar et al. 2012). The gene editing and hiPSCs technologies inspired studies to correct or introduce genetic mutations into patient-specific hiPSCs to overcome potential rejection (Hockemeyer and Jaenisch 2016). These engineered autologous cells can then be transplanted back into the specific patient, avoiding immune rejection (Kishino et al. 2020). CRISPR/Cas9 systems implemented along with iPSC technology are utilized to generate universal iPSC cells to match the various human leukocyte antigen phenotypes, differentiating into any desired cell type (Koga et al. 2020).

Conclusion

Despite various advances in recent decade, brain glioblastoma still remained fatal. Although immunotherapy led to success in treating hematological malignancies, it has not led to similar success in brain glioblastoma, and nor has astounding success been achieved in the application of regenerative medicine or stem cell therapy to find cure for glioblastoma. A cure for brain glioblastoma requires at least the following steps: 1. Early screening in brain cancer for early interventions is vital to preserve micturition control from impairment, prolong survival and to achievement a relative optimistic prognosis. 2. Complete tumor cell elimination in brain or effective measures undertaken to inhibit tumor growth to prevent recurrence is the next step after early screening or diagnosis. 3. Regenerative therapy is the next step required to repair or replace any damaged nerves or brain tissues in order to restore impaired neurological functions including micturition control. Moreover, CRISPR Cas technology demonstrate great promise in its capacity for gene modification, the knock-in and knock-out of enzymes, cell regulators, and
cell surface receptors which could manipulate future development of immunotherapy or molecular therapy against glioblastoma to reduce unintentional harm to the healthy tissues or nerves in the brain which could impair neurological functions including micturition control. The findings in the researches mentioned in this review indicate that glioblastoma is a malignant disease with multiple sophisticated barriers to be overcome before it can be cured. Many more mysteries might unravel in future researches and more challenges might arise. A combined multimodal treatment approach consisting of clinically safer and more effective therapeutics derived from CRISPR-Cas approach, immunotherapy, and regenerative therapies, might build towards a potential effective strategy in surviving or even contribute partly towards a successful cure.

Author contributions: Xiao Xue Zeng and Baoyi Zhu planned the paper, Xiao Xue Zeng wrote the manuscript and Baoyi Zhu revised the manuscript. Jianwen Zeng summarized the references.

Research funding: This work was supported by the grants from National Natural Science Foundation of China (grant numbers: 81900688 to BZ), Natural Science Foundation of Guangdong Province (2019A1515011107 to BZ), the Medical Research Foundation of Guangdong Province (2019 A2019473 to BZ), and the Science and Technology Foundation of Qingyuan City (2020KJJH009 to BZ).

Conflict of interest statement: The authors have no conflict of interest to disclose.

References


