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Rationally designed drug delivery systems for the local treatment of resected glioblastoma

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ABSTRACT

Glioblastoma (GBM) is a particularly aggressive brain cancer associated with high recurrence and poor prognosis. The standard of care, surgical resection followed by concomitant radio- and chemotherapy, leads to low survival rates. The local delivery of active agents within the tumor resection cavity has emerged as an attractive means to initiate oncological treatment immediately post-surgery. This complementary approach bypasses the blood–brain barrier, increases the local concentration at the tumor site while reducing or avoiding systemic side effects. This review will provide a global overview on the local treatment for GBM with an emphasis on the lessons learned from past clinical trials. The main parameters to be considered to rationally design fit-of-purpose biomaterials and develop drug delivery systems for local administration in the GBM resection cavity to prevent the tumor recurrence will be described. The intracavitary local treatment of GBM should i) use materials that facilitate translation to the clinic; ii) be characterized by easy GMP effective scaling up and easy-handling application by the neurosurgeons; iii) be adaptable to fill the tumor-resected niche, mold to the resection cavity or adhere to the exposed brain parenchyma; iv) be biocompatible and possess mechanical properties compatible with the brain; v) deliver a therapeutic dose of rationally-designed or repurposed drug compound(s) into the GBM infiltrative margin. Proof of concept with high translational potential will be provided. Finally, future perspectives to facilitate the clinical translation of the local perisurgical treatment of GBM will be discussed.

Contents

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1. Introduction

Glioblastoma (GBM) is the most common, aggressive, and neurological destructive primary brain tumor in adults. The standard care therapy includes safe maximal surgical resection of the accessible tumor followed by radiotherapy (RT) and chemotherapy with Temozolomide (TMZ) after an interval of 3–4 weeks [1]. Despite this, GBM remains incurable and more than two-thirds of GBM patients die within two years of diagnosis [2]. Long-lasting management of GBM patients is very challenging for several reasons including *i)* the tumor anatomical location (which restricts both neurosurgeons and drugs to effectively eradicate cancer cells), *ii)* ability to invade the healthy brain tissue [3], *iii)* direct intercellular communication via dynamic membrane protrusions [4], *iv)* a unique microenvironmental landscape (composed of immune, vascular and resident brain cells) [5] and *v)* developmental, genomic and epigenetic features that renders GBM tumors highly heterogeneous and chemoresistant [6]. The number of compounds approved for GBM is very limited, and a combination of advances in drug discovery and drug delivery will be necessary to properly address these challenges. Among the strategies investigated to find long-lasting therapies for the treatment of GBM, the local delivery of active agents within the tumor resection cavity have emerged. This approach bypasses the blood–brain barrier (BBB), increasing the local concentration at the tumor site while reducing or avoiding systemic side effects, opening the doors for many more molecules to be used to fight this devastating disease.

This opinion review will be divided into three sections: the first one will provide a global and concise overview on the local treatment for GBM and the different local delivery strategies that can be exploited for this purpose. Emphasis will be given to neurosurgical implants and the lessons learned from past clinical trials. In the next section, we will discuss the main parameters to be considered to rationally design fit-of-purpose biomaterials and develop drug delivery systems (DDS) for local administration in the GBM resection cavity. In the last section, recently published outstanding papers with high translational potential will be described as a proof of concept. They were selected because the DDS were designed to act not only as support/scaffold but also to increase their long-term therapeutic efficacy, or because the experimental models used to characterize the DDS were appropriately chosen to accelerate their clinical transition. Finally, future perspectives to facilitate the clinical translation of the local perisurgical treatment of GBM will be discussed.

2. Local treatment for glioblastoma

2.1. Influence of brain structure and tumor resection on local treatment

The limitations of systemic drug administration for cancer treatment (including *e.g.* short blood half-time, poor availability and drug distribution at the tumor site, off-site dose-limiting toxicities) as well as recent advances in materials science and technologies, have boosted research on loco-regional drug delivery as a promising strategy to circumvent biological barriers and increase therapeutic efficacy [7]. In particular, the local delivery of therapeutic agents at the tumor site or in the tumor resection cavity is appealing for brain tumors, which are surrounded by a unique and protective microenvironment strongly limiting the access to most chemotherapeutic agents. The brain has a complex neuroarchitecture with variable cellular and tissue composition, pH, texture and mechanical properties depending on the regions [8]. These parameters are further modified in presence of mechanical lesions (*e.g.* traumatic brain injury, tumor resection [9]) or diseases (*e.g.* cancers [10]) and play a major role in regulating drug diffusivity towards GBM cells. Drug diffusion in the brain can differ over the space since the composition, the stiffness, and the pressure of the diseased area of the brain are different. The drug is exchanged among several components including the blood plasma, the extracellular fluids (ECF), the cerebrospinal fluid (CSF) and the cells. Since the brain is a highly vascularized organ and needs to be supplied by oxygens and nutrients, an extensive network of arteries and veins penetrate the brain cortex generating the brain microcirculation. The density of vessels can be affected by physiological and pathological factors; for example, GBM can generate new blood vessels (neo-angiogenesis) or change the blood flow thus increasing intracranial pressure [11]. The high intracranial pressure might be an obstacle for drugs to accumulate in the targeted site. The capillary of the endothelial wall produces the brain ECF, made by the passive release of water though the ionic gradients. The secreted liquid moves through the brain cells and through a bulk flow sustained by hydrostatic pressure. The drug diffusion through the ECF is normally negligible due to the low volume and the presence of proteins and enzymes but distribution related to the ECF bulk flow is important for drug spreading in the brain, especially for high molecular weight (MW) drugs that have minimal diffusion due to the steric hindrance. The CSF is generated by the epithelial cells of the choroid plexus and circulates between the brain ventricles, the sub-arachnoid space and is connected also

with the lymphatic system. The CSF can lead to the clearance of the drug from the brain, reducing the effective drug concentration. The brain is composed of several types of cells such as neurons, astrocytes and microglia, with different characteristics and physiological properties, and the cellular composition and cellular density in the GBM microenvironment is heavily modified, thus affecting drug distribution. Another parameter to be considered is the metabolic activity in the brain and its modification in cases of disease, as enzymes (*e.g.* cytochrome P450, esterases) can reduce the therapeutic activity of the drugs by switching the active agents towards inactive or toxic metabolites [12]. Finally, the anatomical location of the tumor not only has an impact on the drug diffusivity or the possibility to perform a complete tumor resection (*e.g.* when the primary lesion is in close proximity to eloquent brain structures) but might also impact recurrence location and pattern [13].

Maximal safe resection is the mainstay of GBM management and is performed in all eligible patients (65–75% of GBM patients [14]) to remove as much tumor as possible without compromising neurological function [15]. Tumor debulking is essential for cytoreduction, to alleviate symptoms and increase the life span of patients and obtain tissue for histological and molecular diagnosis [16]. However, GBM cells can infiltrate healthy brain tissue several centimeters away from the tumor mass and outside the imaging contrast-enhancement area, escaping surgery [17]. Indeed, even with sophisticated imaging techniques, complete surgery is virtually impossible in GBM patients and there are always residual, infiltrating tumor cells capable of triggering the onset of recurrences. Ninety percent of these recurrences arise at the resection margins, in the macroscopically normal peritumoral zone [18]. This region is composed of highly proliferative residual tumor cells and other cells populations such as glioma stem cells (GSCs), reactive astrocytes, inflammatory cells (tumor-associated macrophages and microglia) and GBM-associated stromal cells able to interact intercellularly and to drive GBM cellular proliferation and migration [19].

Between surgical resection and initiation of RT (with concomitant and adjuvant TMZ), there is a scheduled delay of a minimum of 3 weeks which is the recommended time to avoid incomplete wound healing, postoperative deconditioning, suboptimal tumor reoxygenation and/or inflammatory changes within the tumor microenvironment [15,20]. The perisurgical administration of active agents directly in the brain at the time of surgery is promising because it allows drug(s) bypass of the BBB and to potentially achieve therapeutic drug concentrations in proximity of residual tumor cells, minimizing the risk of systemic side effects [21]. This approach accelerates the beginning of the pharmacological treatment, filling the oncological treatment time-gap between surgery and standard of care chemoradiation. Depending on the drug and DDS used, both the sustained release of the agent at therapeutic concentrations over a prolonged period, or its burst release leading to high concentrations in the first few days after surgery, could provide effective killing of residual tumor cells. An attractive strategy could be a combined DDS system in which a non-targeted cytotoxic compound is delivered at high concentrations for a burst release, then followed by the sustained release of a second molecularly targeted compound designed to penetrate the brain parenchyma and target the residual disease further (see Fig. 1).

Various strategies of localized drug delivery to improve survival rates and avoid local recurrence have been developed (Fig. 2). As described in Section 2.2, an Ommaya reservoir and convention enhanced delivery (CED) for intratumoral administration were used. Wafers and rigid implants for localized treatment in the resection cavity were developed, leading to the approval of Gliadel® implants (1996) (Section 2.3). More recently, hydrogels and nanofibers were studied in preclinical models. Finally, innovative DDS (*e.g.* spray devices) are currently under investigation (Sections 3 and 4).

2.2. Administration of therapeutic agents via Convection Enhanced Delivery

To circumvent the BBB and increase the concentration of therapeutics into the brain, active agents can be directly injected into the central nervous system (CNS) (*e.g.* into the tumor, the tumor resection cavity, the infiltrative brain parenchyma or into the ventricle) *via* repeated needle-based injection or catheter implants connected to a reservoir (*e.g.* Ommaya reservoir). This method avoids systemic toxicities, can be easily repeated, and allows the injection of large volumes of drugs. However, these systems are limited by catheter obstructions, local side effects (*e.g.* infections,

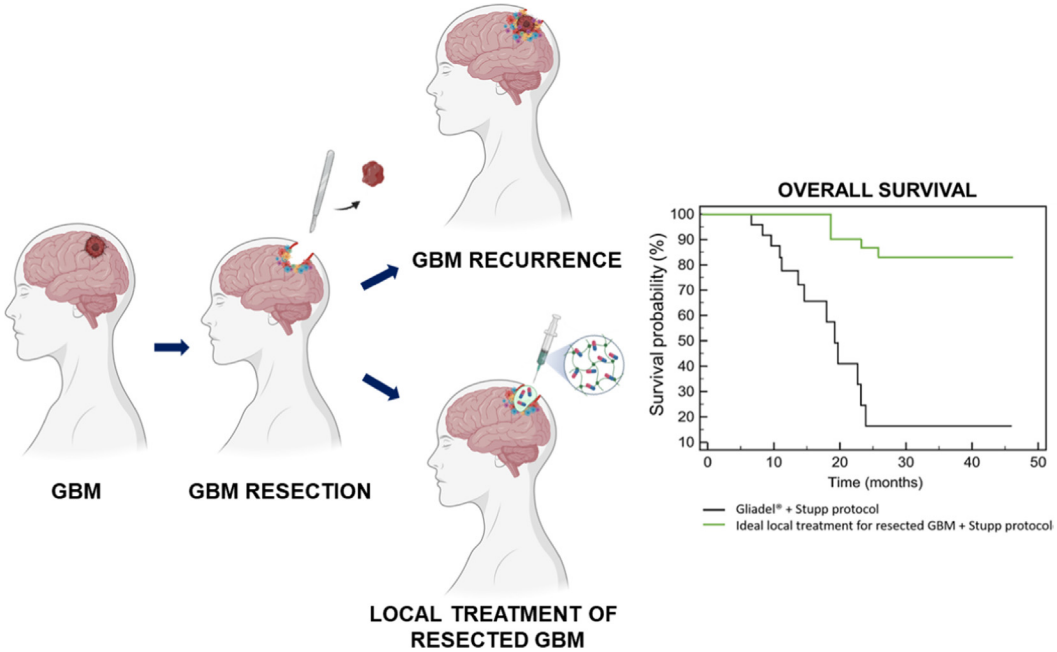

Fig. 1. Schematic representation of local treatment of resected GBM. The Kaplan–Meier curve is reproduced with permission from [22] and adapted for illustration purpose.

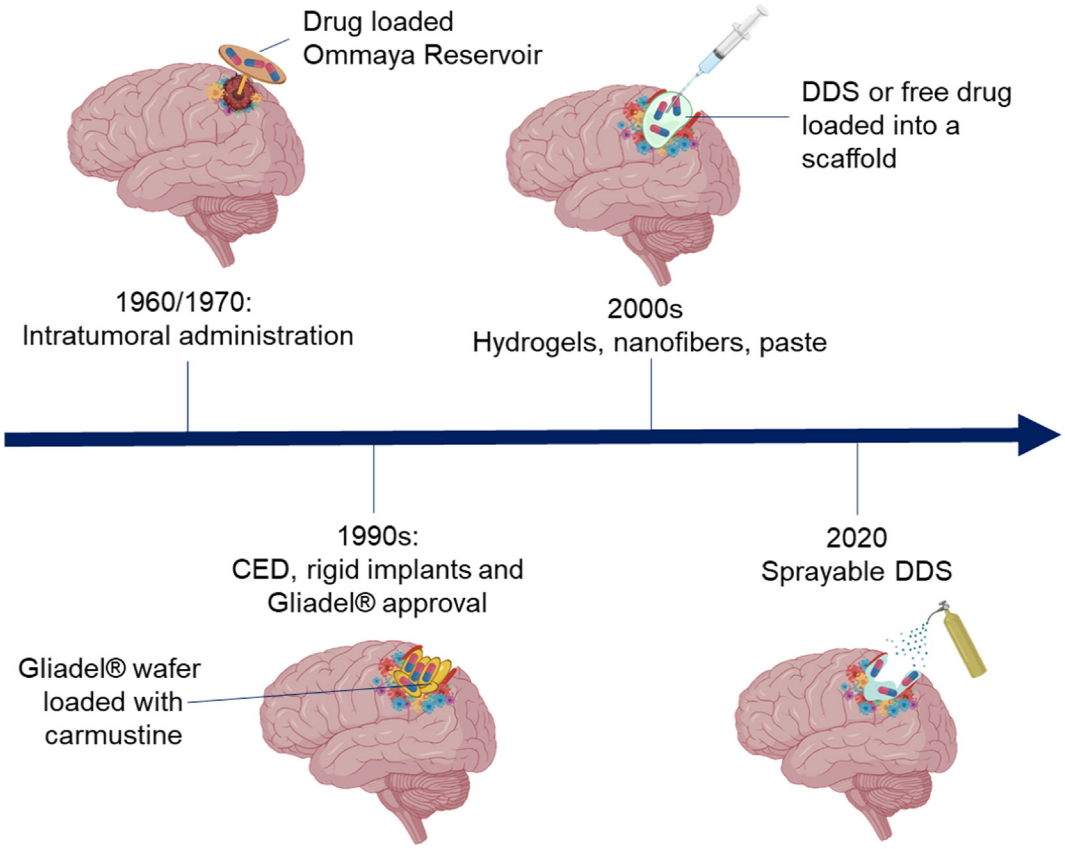

Fig. 2. Schematic illustrations of diverse strategies of localized drug delivery to tackle GBM historically. In the 1960/70s the treatment of GBM was based on an Ommaya reservoir and intratumoral administration of therapeutics; in the 1990s, convention enhanced delivery (CED) for intratumoral administration, wafers and rigid implants for localized treatment in the resection cavity were developed, leading to the approval of Gliadel® implants (1996); in the 2000s hydrogels and nanofibers were studied to increase the efficacy and the biocompatibility of the treatment. Finally, innovative DDS (*e.g.* spray devices) are currently under investigation.

intracranial hemorrhage) and the fact that drug distribution relies on passive diffusion. As diffusion depends on a free concentration gradient and the diffusivity of the compound or the drug-loaded nanocarrier in the tissue, the drug penetration depth is often limited either by the physicochemical properties of the drugs or their metabolism [23]. Alternatively, active agents can be directly infused in the brain parenchyma *via* convection-enhanced delivery (CED). This method relies on the use of micro-catheters that are stereotactically implanted into the brain and are connected to an infusion pump, which is able to create a pressure gradient which allows uniform drug distribution up to 2–3 cm ([21,24]. As CED drug distribution is based on the bulk flow of ECF, the concentration profile is constant during infusion and reduces the risk of neurotoxicity. There are no limitations in size and physicochemical properties of the drug that can be delivered by CED (even though drug diffusivity in the brain will vary), but infusion parameters such as drug concentration, volume, flow rate and duration need to be carefully adjusted. Key technical factors to consider to optimize the treatment efficacy and avoid side effects (induced by *e.g.* infusate backflow in the catheter, drug leakage into the CSF [25,26]) are the region of the brain to be treated (peritumoral region *vs* tumor core; tumor location in regions containing grey *vs* white matter), the catheter design, size, location and placement, and the infusate rate and volume. CED can be applicable to non-operable patients or recurrent tumors, enabling distribution of large volumes of high drug concentrations with minimum systemic toxicity. CED has been the most studied local delivery strategy for GBM and a wide range of active agents (*e.g.* chemotherapeutics, monoclonal antibodies, targeted toxins, proteins, viruses, nanomedicines) has been tested both in preclinical and early phase clinical trials. Refer to reviews focused on CED technique to learn more about advances in this field [23,27,28].

2.3. Administration of therapeutic agents in the glioblastoma resection cavity

2.3.1. Approved implant for the intracavitary treatment of glioblastoma (Gliadel®)

The DDS that opened the doors to local implant-based treatments for brain tumors, reliant on passive diffusion, and the only system currently on the market for newly diagnosed and recurrent GBM patients, is the carmustine-lodead wafer Gliadel®. This is a biodegradable random copolymer (polifeprosan 20) formed of 1,3-bis-(p-carboxyphenoxy)propane (CPP) and sebacic acid (SA) monomers in a 20:80 M ratio connected by anhydride bonds and loaded with 3.8% of carmustine (BCNU) [29,30]. Each wafer weighs 200 mg (192.3 mg of polifeprosan 20 and 7.7 mg of BCNU) has a cylindrical shape (diameter 14.5 mm, thickness 1 mm) and the recommended dose of drug for GBM patients is 61.6 mg. Therefore, a maximum of 8 wafers can be placed into the resection cavity during surgery to circumvent the BBB and achieve high local drug concentrations within the brain [31]. The copolymers erode in the brain releasing BCNU into the adjacent tissue over one week at a constant rate, even though diffusion is augmented in the days immediately following surgery by convective transport with interstitial flow that result from vasogenic oedema. Seventy percent of the wafer is biodegraded within 3 weeks, but in clinical trials polymer traces have been found in a few patients 13 to 23 weeks after

initial implantation [32]. As BCNU is highly lipophilic, the penetration of drug into the brain parenchyma surrounding the cavity implant is limited (in animal models: 3–6 mm from the polymer/tissue interface during the first 7 days, 2–3 mm for the next two weeks) because of rapid elimination through capillary walls or ependymal barriers. However, the drug might re-enter the interstitium providing a 'low dose' exposure in the peritumoral regions a few cm away from the implant [32–34]. Gliadel® was approved following extensive characterization, preclinical studies and several clinical trials in recurrent and newly diagnosed high-grade glioma patients [35–37] showing low systemic toxicities and prolonged overall survival compared to patients treated with placebo-wafers. In these trials, Gliadel® was compared to RT alone which was the standard treatment protocol for high-grade glioma patients at that time. Recently, several studies and *meta*-analysis and systematic reviews suggest an increased benefit of sequential Gliadel® treatment and RT/TMZ [38], even though a larger prospective study is now ongoing to collect information on the safety and effectiveness of Gliadel® in usual medical practice (NCT number: NCT02684838) [31]. Despite this, the use of Gliadel® is not included in the European Association of Neuro-Oncology guidelines for the treatment of GBM [15] and its use remains limited in the clinical practice. Price et al. reported that only 32% of suitable patients received BCNU wafers in the United Kingdom; [39]. Many neurosurgeons are reluctant to administer Gliadel® because of the expected postoperative complications and the possibility that it may preclude patient enrollment in subsequent clinical trials [40–42]. Complications associated with Gliadel® are cerebral edema, impaired neurosurgical wound healing, meningitis, CSF leakage, intracranial hypertension and seizures. Moreover, wafer dislodgement can occur increasing effective diffusion distance, and migration into the ventricular system can lead to obstructive hydrocephalus [43].

2.3.2. Local administration in the glioblastoma resection cavity: An overview on past clinical trials

The local drug delivery of active agents into the resection cavity using drug-impregnated gels, nanoparticles or polymeric-based DDS (*e.g.* wafers, films, disks, rods) that can be implanted or injected during surgery has been investigated for the treatment of GBM. These systems could guarantee a sustained release of the drug in the surrounding brain tissue by degradation or diffusion (depending on their biodegradability), providing therapeutic drug concentrations at the resection borders (where residual tumor cells are present) with limited systemic exposure [44]. Since Gliadel®'s approval, the interest in the development of DDS for the post-surgical local delivery of active agents as a therapeutic and long-lasting strategy against GBM recurrences has exploded. Even though encouraging preclinical results were obtained with several DDS in the last two decades, the translation to the clinic has been limited. Many recent reviews have detailed the different DDS and the main results obtained [45–47]. In this section, we would like to briefly discuss those systems that have been tested in clinical trials. It should however be stated that these trials have been performed over 25 years, a lapse of time in which there have been huge improvements in surgical and imaging techniques, molecular biology advances that led to a drift in brain tumor classification and diagnostics, and the introduction of concomitant and adjuvant TMZ chemotherapy as standard treatment (Stupp protocol). Therefore, modern studies should not be directly compared with more historical data.

2.3.2.1. OncoGel™. This technology consists in an injectable gel (OncoGel™) based on the DDS ReGel® and loaded with the anticancer drug paclitaxel (PTX). ReGel® is a thermosensitive, biodegradable triblock copolymer composed of poly(lactide-co-glycolide) (PLGA) and poly ethylene glycol (PEG). It is water soluble at temperatures below the gel transition temperature and forms a water-insoluble gel once injected into the body. OncoGel™ provides controlled PTX release during approximately 50 days and biodegrades within 4 to 6 weeks [48]. OncoGel™ was tested in preclinical models as potential local treatment for GBM in the Brem laboratory [49]. Its biocompatibility and safety following intracranial administration were demonstrated, as well as the drug distribution at lethal dose concentrations in the brain up to 6–9 mm away from the site of injection at 3 h and 3 days post-treatment, respectively. *In vivo* efficacy studies on the 9L gliosarcoma rat model showed increased survival time compared to controls when the treatment was administered at the same time as tumor cell allografting, alone or in combination with RT. Moreover, the combination of OncoGel™ with TMZ (either administered orally or loaded in CPP:SA polymer for local treatment) and RT [49], improved median survival and a significant increase in the number of long-term survivors of the combination treatment compared to oral TMZ and RT alone. OncoGel™ was produced by the pharmaceutical group BTG International and was granted the orphan drug status by the Food and Drug Administration (FDA) for the treatment of brain cancer in 2009, and by the European Medicines Agency (EMA) for the treatment of carcinoma of the oesophagus in 2010. This system was tested in the same period in two clinical trials: on recurrent GBM patients directly after tumor resection (Phase I/II dose escalation study, NCT number: NCT00479765) and on esophageal cancer patients in combination with standard of care chemotherapy (cisplatin and 5-Fluorouracil; 5-FU) and RT before surgery (Phase II study, NCT number: NCT00573131). In the first trial, four patients were included to evaluate the maximum tolerated dose of Oncogel™ following intracavitary administration after surgical resection of recurrent glioma; however, the study was then terminated due to a sponsor business decision (not based on safety or efficacy data). In the second study, Oncogel™ proved to be safe in combination with standard of care therapy in esophageal cancer patients, but there was no improvement in overall survival and therefore the study follow-up was discontinued [50].

2.3.2.2. Drug-eluting beads. BTG international also sponsored a phase I clinical trial aimed at determining the safety and feasibility of local injection of irinotecan hydrochloride drug-eluting beads (DEBs) directly into the resection cavity of recurrent GBM patients (NCT number: NCT02433392). Up to 60 DEBs were injected up to 5–8 mm depth into the cavity wall using a 24gauge flexible catheter (maximum volume 3 mL, drug dose 100 mg) in nine patients. The results showed a good safety profile, with less local side effects (swelling and wound healing issues) compared to BCNU wafers and no systemic toxicity and suggested a modest clinical benefit. However, the beads showed early offloading and only delivered irinotecan during 72 h. The trial was terminated based on the slow rate of patient recruitment [51,52].

2.3.2.3. PLGA micropsheres. Implantable, biodegradable PLGA microspheres loaded with the drug 5-FU were developed for localized and sustained release of the drug in GBM. This DDS was developed in Benoit's group [53]: formulations with different drug release rate were tested on rats showing no sign of neurological toxicity and increased animal survival following intratumoral administration, with a synergistic effect with RT in a C6 rat model [54,55]. Slow releasing microspheres had better therapeutic potential and Lemaire *et al.* showed by magnetic resonance imaging (MRI) that tumor proliferation was significantly reduced in the vicinity of the stereotactic injection site before regrowth, indicating that multi-injection protocols could be more promising. 5-FU microspheres could diffuse ~1.5 mm distance from the injection site and release 5-FU at a maximum of 3 mm [56,57]. For clinical

use, microspheres were freeze-dried and radiosterilized at 19 kGy in single-dose, vacuum-sealed vials to be reconstituted in the operating room with a sterile aqueous solution [58]. Three clinical trials were performed using this system. In a pilot study on eight newly diagnosed GBM patients undergoing surgical resection, perisurgical administration of 5-FU microspheres was performed around the walls of the surgical resection cavity (every 1 cm^2, to a depth of 2 cm; total volume: 1.5–2.5 mL; drug dose: 70 or 135 mg) followed by external beam radiation (total dose: 59.4 Gy) within 7 days [59]. The higher 5-FU dose caused recurrent brain swelling 3 weeks after RT and required steroid treatment before completing the radiation. Significant levels of 5-FU were present in the CSF one month after implantation, enabling optimal radiosensitization. The median survival time of patients treated with the 5-FU microspheres was 98 weeks at the last evaluation with two patients in disease remission at 139 and 153 weeks, respectively. A phase 1 study was performed on ten newly diagnosed inoperable grade 3 or 4 malignant glioma patients, who underwent stereotaxic implantation of 5-FU microspheres into the tumor in one or several trajectories (1–7 deposits per trajectory, depending on the size, shape, and necrotic/cystic components of the tumor; drug dose: 135 mg) followed by external beam radiation (total dose: 59.4 Gy) within 9 days [58]. The overall median survival was 40 weeks with 2 long-term survivors. Finally, a randomized multi-centre Phase 2 trial was performed including supratentorial high-grade glioma patients undergoing surgery, multiple injections of 5-FU microsphere suspension (drug dose: 130 mg) followed by early conventional fractionated RT (total dose: 59.4 Gy, within 7 days after surgery)[60]. In this study, which enrolled ninety-five randomised patients, only seventy-seven patients were included in the protocol as the others showed absence of perioperative confirmation of high-grade glioma. The treatment arm was compared to the early RT control arm only (TMZ was not the first line treatment at the time of this trial). The study showed acceptable safety of this treatment modality and a positive trend toward improved overall survival of the 5-FU microspheres arm compared to the control (15.2 *vs* 13.5 months, respectively), but no statistically significant benefit. The authors state that the study was not designed and sufficiently powered to demonstrate the potential of this DDS for GBM local treatment. The methodological issues and challenges related to this treatment strategy included: *i*) the decision regarding the most favorable target for administration (100 μL doses, at 2 mm depth of cavity borders spaced 1 cm apart: was the necessary treatment volume injected in the right places?); *ii*) lack of distribution analysis by dosimetry once the drug was delivered; *iii*) potential biases in patient selection as randomization was based on diagnostic assumption before histological confirmation [61].

2.3.2.4. CuboSphere™. A gel-like biodegradable matrix made of liquid crystalline cubic phases loaded with PTX and carboplatin (CuboSphere™) was developed and examined in a pilot study on GBM recurrent patients by Von Eckardstein *et al.* [62,63]. This DDS is adapted for application in the walls of the surgical resection cavity as it can adapt and adhere to its irregular shape promoting drug diffusion into the brain parenchyma. The system was firstly characterized *in vitro* (release studies) and *in vivo* for the intended application in the F98 rat model following partial resection and local treatment (tumor size and survival, drug diffusion, quantification in CSF and serum, histological analysis). Carboplatin and PTX were detectable for 6 h and 48 h at 3 mm from the site of implantation. While differences in tumor size showed a significant decrease in tumor growth following combination therapy, this result was not confirmed by the survival study and the authors could not explain if death of animals was attributable to local or systemic toxicity of the tested drugs [62]. Despite this, twelve patients with recurrent GBM were recruited for a pilot study and underwent re-resection followed by intracavitary application of PTX and carboplatin cubic phases (at PTX doses between 50 and 15 mg). Carboplatin was released from the matrix within 24 h, while PTX reached its peak after three to four days. Toxic brain swelling was observed in six of the patients receiving more than 15 mg of PTX, leading to necessary surgical removal of the matrix in the days following treatment. At 15 mg of PTX, only one patient showed extended brain edema. Three others experienced mild to moderate brain swelling which was treated medically and the remaining three showed no complications. No systemic side effects were observed in any patient. The authors concluded that intracavitary carboplatin/PTX chemotherapy in recurrent GBM using cubic phases is feasible and safe at a dose of 15 mg PTX [63].

2.3.2.5. 6-carboxylcellulose polymer. Another pilot study has been realized on GBM patients using cisplatin incorporated into biodegradable 6-carboxylcellulose polymer [64]. Twenty 1.5 × 1.5 cm wafers were implanted in the tumor resection cavity of seventeen patients (study group), to deliver a cisplatin dose of 45 mg. Two-three weeks after the surgery, patients started RT (total dose: 60 Gy). No local or systemic side effects were reported, and a significant increase in median overall survival was observed for patients administered local treatment compared to those receiving only surgery plus RT (427.5 *vs* 211.0 days, respectively), demonstrating that this treatment regimen is well tolerated and promising. However, to our knowledge, no further clinical trials have since been initiated for this therapeutic approach.

2.3.2.6. Hemostatic powders. Ferroli *et al.* mixed the anticancer drug mitoxantrone with the FDA approved hemostatic Surgifoam powder. Mitoxantrone is a type II topoisomerase inhibitor which has shown to be highly effective in animal GBM models [65] and safe when administered locally in GBM recurrent patients using intraventricular DDS [66]. The Surgifoam/mitoxantrone mix led to the obtention of a foam characterized by: *i)* ease of application; *ii)* ability to increase the exposure of tumor cells to the cytostatic drug by direct contact with the resection cavity borders and capability to conform to its surfaces, avoiding systemic drug diffusion; *iii)* ability to reduce the risk of postoperative hemorrhage due to the intrinsic properties of the hemostatic scaffold [67]. This DDS was tested in twenty-two recurrent GBM patients (with tumor size ranging between 3 and 6 cm), following gross total resection. To ensure lack of communication between the cavity and CSF spaces, attention was paid to avoid the lysis of postoperative cortical dural adhesion during surgery, obtaining closed surgical cavities. The dose of drug that could be administered depended on the dimension of the surgical resection cavity, and varied between 4 and 12 mg. An intracavity catheter was also inserted at the end of the surgery, connected with a Rickham subcutaneous reservoir of Mitoxantrone for further drug administration. No local or systemic side effects were observed in the patients included in the study, showing that this approach is safe and could be further exploited in the future. A similar approach was used by Abrahams et al. who started a dose-escalating phase I trial to evaluate the safety and tolerability of the local delivery in the tumor resection cavity of bevacizumab incorporated in a collagen sponge in GBM patients at first recurrence (NCT number: NCT01526837). No results have been published for this trial, which enrolled one patient and was terminated due to investigators' decision [68].

2.3.2.7. Lessons learned from clinical trials. Overall, the limited clinical success of local DDS for GBM can be explained by lack of insufficient interdisciplinary interactions between experts in different fields (*e.g.* material and biomedical scientists, clinicians) and technical difficulties to translate preclinical results due to physical dis-

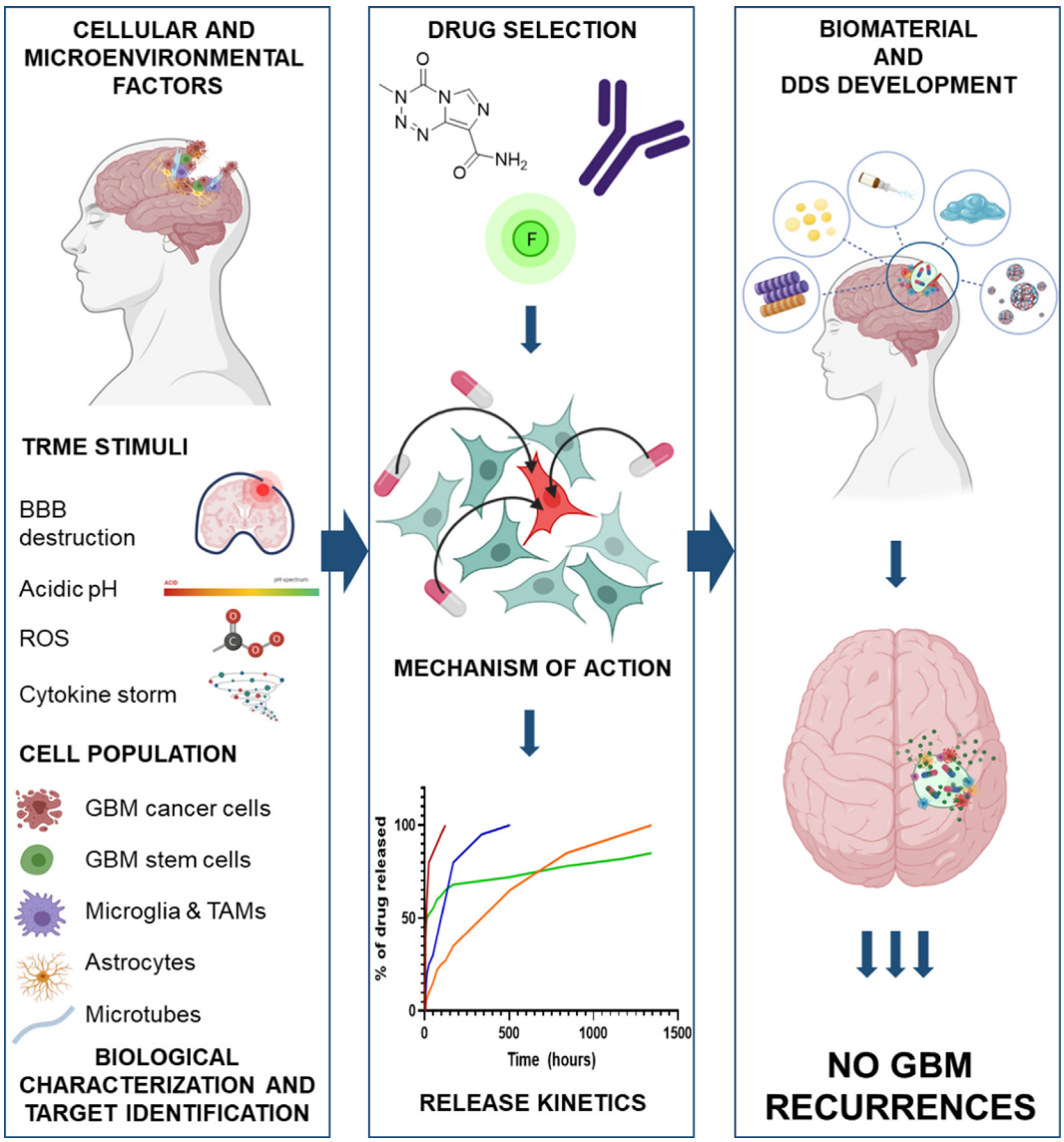

Fig. 3. Rationally-designed approach to treat GBM using biomaterial-based localised DDS. Starting from the analysis of the cell populations and the target identification within and adjacent to the GBM resection cavity, drugs need to be developed in order to possess high tumor cell toxicity and low off target effects; moreover, high penetration into the brain parenchyma and prolonged sustained concentration over time are desired. Finally, a tailored biomaterial needs to be developed in order to potentiate the therapeutic efficacy of the drug. Legend: GBM: glioblastoma; TMRE: tumor resection microenvironment; BBB: blood–brain-barrier; ROS: reactive oxygen species; TAM: tumor associated macrophages.

tances between research laboratories and hospitals. Moreover, incomplete understanding of the disease pathophysiology and DDS-brain interactions combined with the lack of adequate preclinical models able to predict the efficacy in humans, has hampered the success of clinical translation. Finally, technical issues (*e.g.* challenges regarding chemistry, good manufacturing practice, scalability, sterilisation and controls required for clinical translation and commercialization) and poor interest of pharmaceutical companies into the development of DDS for relatively rare diseases, may also have played a role [69,70]. Indeed, despite the positive and encouraging results of some of the clinical trials discussed here, globally showing the safety and feasibility of intracavitary application of DDS in GBM patients, these remain limited to small cohorts of patients reducing the potential impact of their outcomes. Often the results are not sufficiently promising as to convince sponsors to continue the clinical development of the products. Moreover, most of these trials are performed in recurrent GBM patients. Recurrent GBM tumors are very different from their primary tumors, as treatments (surgery, radiation and chemotherapy) induce overall changes in the tumor microenvironment favoring tumor aggressiveness, heterogeneity, chemoresistance and immune suppression [71–73]. The degree of tumour infiltration is likely far greater in the recurrent setting and therefore presents a much more challenging test-bed for clinical trials, relative to primary tumors. Therefore, the results of these trials might be biased by patient selection [74]. Testing new local DDS in these patients might not be representative of the therapeutic response that could be obtained in newly diagnosed (thus previously untreated) patients immediately after first surgery. This consideration will be particularly true in the future, if the drug selection and biomaterials for local GBM treatments will be adapted to the new findings involving the Tumor Resection MicroEnvironment (TRME) to specifically target cellular subpopulations present at the resection cavity borders and potentially leading to the onset of tumor recurrences. As the brain microenvironment is highly dynamic over time and space, DDS should be conceived by carefully selecting the

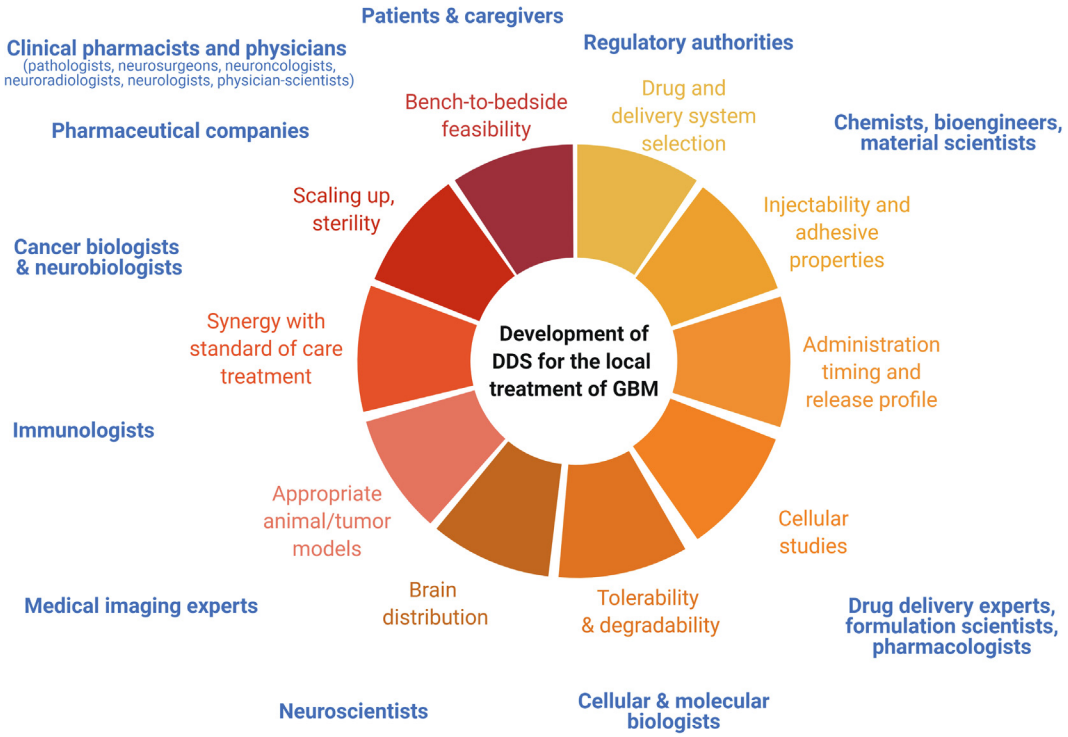

Fig. 4. List of main parameters to consider for the rational and optimal development of DDS for the local treatment of GBM and different expertise required to increase the chances of clinical translation. Modified from [45].

materials for their intended application (intratumoral administration *vs* post-surgical application) and considering the limitations of the formulation at each step. To maximize the clinical potential of local DDS for GBM, researchers should rationally develop innovative systems capable of satisfying the medical needs identified by the academic and clinical communities.

3. Strategies to develop rationally-designed biomaterial and drug delivery systems for local administration within the glioblastoma resection cavity

The increasing knowledge on brain cancer anatomy and the critical analysis of previous clinical study failures (see Section 2), has provided important feedbacks to tackle GBM. Combining these achievements with the development of cutting-edge technologies will lead to the development of the next generation of DDS for this therapeutic indication. In the following paragraphs, we will summarize those parameters that we consider central for the implementation of fit-for-purpose biomaterials and DDS for the local treatment of GBM. This section will first focus on the biomaterials scaffold that will be applied the resection cavity (*e.g.* hydrogel, nanofibers). We will then discuss the drugs that can be used for the local treatment of GBM. Finally, we will discuss the optimal properties and experimental approaches that need to be considered to characterize rationally designed DDS towards resected GBM (Figs. 3 and 4).

3.1. Biomaterials and drug delivery systems as therapeutic platforms for the tumor resection cavity

The residual tumor cells left in the resection margins or infiltrating the brain parenchyma represent the main contribution to the risk of recurrence [17–19,75]. Therefore, the selection of appropriate biomaterials has a pivotal role on the modulation of the GBM responses after surgical resection by enhancing the therapeutic benefits for GBM, while minimizing the invasiveness of the treatment. Globally, biomaterials developed for an application in the brain resection cavity should have the following desirable properties: *i*) adaptability; *ii*) lack of toxicity, biocompatibility and low immunogenicity; *iii*) biodegradability; *iv*) chemical and mechanical stability; *v*) provide controlled and sustained release of bioactive compounds [76]. Biomaterials represent a means for site- and time-controlled therapeutic delivery in the brain, they can act both as scaffold and DDS, and can interact with both GBM cells, healthy brain cells and the TRME.

By modulating their chemical composition (*e.g.* natural, synthetic or hybrid materials of the scaffold), mechanical properties, linking chemistry (inducing selective stimuli-responsive release of payloads, adhesion molecules decoration and controlled degradation) and texture (porosity, viscosity), biomaterials possess a versatility and tuneability that can provide suitable applications for the treatment of post-resection GBM. Engineered biomaterials including micro- and nanoparticles, lipidic nanocapsules (LNC), hydrogels and implantable scaffolds, and have been studied to prepare depots for sustained local drug release and/or scaffolds to fill the tumor-resected niche, mold to the resection cavity or adhere to the exposed brain parenchyma to prevent the tumor recurrence [77,78]. A scaffold (*e.g.* hydrogel, spray or nanofiber) able to provide persistent close contact with the brain parenchyma is often but not always associated with a DDS enabling a sustained and controlled drug release.

However, the delicate nature of the brain tissue imposes strict criteria for the biomaterial design. In particular, the materials must be compatible with the brain tissue, which is extremely sensitive to both mechanical and environmental stresses [79]. Optimal biomaterials should display properties that simultaneously promote the tumor eradication and avoid wound healing impairment. Also,

future biomaterials will need to display extremely high neuroprotection towards mechano-chemical injuries that might be induced by the dislodgement of implants. Thus, various biomaterials features are being investigated for safer and improved local delivery into the resection cavity including material nature, stiffness, drug release and diffusion, tissue adhesion and healing properties of the surrounding damaged parenchyma, interaction with the TRME, biodegradation and linking chemistry.

While most of the local systems developed for GBM aim at being implanted and are reliant on drug diffusion into the brain to kill residual cancer cells, some systems have achieved the opposite by exploiting the concept of cancer cell traps for GBM treatment [80]. Their scope is to chemo-attract cancer cells away from the tumor or brain parenchyma, and then kill them once they have migrated into the DDS (*e.g.* [81,82]).

3.1.1. Biomaterial: Structure determines function

The selection of the biomaterial is the first step for the generation of a successful local delivery treatment for GBM. Indeed, by taking advantage of the intrinsic properties of the biomaterial it is possible not only to obtain scaffolds with appropriate properties for drug delivery but also to ameliorate the outcome of the treatment. Materials used to make these systems can be broadly divided into three categories: natural, synthetic and hybrid materials. Natural materials are cost-effective, elicit excellent tolerability *in vivo* and may show bioactivity in the resection cavity ameliorating the treatment. Examples of investigated materials include polysaccharides (*e.g.* hyaluronic acid (HA) [83], alginates [84], dextran [85] and chitosan [86]), polypeptides (*e.g.* gelatin, elastin and collagen [87]) and lipids (*e.g.* lecithin, phospholipids [88]). These biomaterials can form hydrogels by self-assembly or following chemical modification, and can be locally injected as gels or liquids that undergo sol–gel transition depending on the linking chemistries, the physical binding or upon exposure to environmental stimuli (*e.g.* pH, light, temperature or ionic strength) [89]. The drawbacks of these materials reside on processability problems, reduced opportunities to tune drug release kinetics and degradation by modifications of polymer composition [44]. Moreover, if the biomaterial derives from other organisms (*e.g.* by extraction), a cross-species reaction may manifest, limiting its biocompatibility. Polymers such as chitosan and fibrins possess an intrinsic advantage to be retained in the resection cavity due to their bioadhesive properties.

Synthetic biomaterials display several advantages related to the tunable design allowing desired mechanical properties, drug release kinetics and provide highly controlled biodegradation rates. Examples of these materials are N-(2-Hydroxypropyl)metha crylamide (HPMA), PLGA, linear or branched PEG, dendrimers, polyamides and synthetic lipids. Such biomaterials are more customizable, offering the possibility to be grafted with or to encapsulate drugs and to alter their features to have adapted properties. In addition, they show prolonged stability in the resection cavity due to the possibility to be decorated with adhesion moieties and to avoid degradation by the insertion of uncleavable sites or highly hindrance molecules that reduce enzymatic degradation. However, this last point is also the most significant drawback; indeed, side degradation products can accumulate in healthy parts of the brain or cause inflammation in the resected cavity borders. Similar to natural materials, synthetic materials can also be injected to fill the resected cavity as gels directly, or liquids that form gels following internal/external stimuli. Their constituents require regulatory agency (FDA, EMA) approval before clinical application [90].

When regarding the characteristics of the brain, the TRME and the desired pharmacological outcome, the selection of the material should be based on its intrinsic properties and its ability to revert the malignancy-trend of GBM and avoid the onset of recurrences.

3.1.2. Stiffness and fibrousness of the biomaterials scaffold

The mechanical properties of biomaterials can modulate GBM progression, acting on several key parameters of tumor growth such as proliferation, invasion and GSCs fate [91,92].

Biomaterials stiffer than brain tissue have been demonstrated to promote durotaxis – an event in which cells are guided by rigidity gradients - of GBM cells and GSCs but reduce the migration of neural cells, which prefer softer substrates. This phenomenon can be ascribed to the mechano-similarity of the biomaterials with the extracellular matrix (ECM), which is stiffer than the healthy cellular brain; GBM cells are encouraged to move by mechanosensation through the microenvironmental stiffness (10 kPa GBM *vs* 1.7 kPa normal brain) [93,94]. For example, higher spreading of GBM tumor cells and increased migration speed was observed in stiffer fibronectin-based scaffolds while proliferation rate decreased compared to softer substrates [95,96]. However, from the evidence provided by the Gliadel® wafers, the stiffness of the biomaterial can be a "double edged sward": tools stiffer than the host tissue can lead to increased gliosis, inflammation, and worse outcomes. Indeed, *in vitro* studies have identified stiffness as a strong modulator of GBM proliferation and invasion directly out of the implant [69,97]. In addition, materials that are too rigid can reduce GBM invasion due to the decreased nutrient diffusion. Wang *et al.* reported decreased U-87 MG cell proliferation correlated with the higher cross-linking density in stiffer PEG hydrogels [98]. Conversely, materials softer than brain tissue led to poor material stability and fixation at the implant site and resulted in being less effective [99–101]. A combined solution might be the development of a gradually softer matrix (*e.g.* stimuli-responsive cross-linker or degradable matrix) to firstly encourage the durotaxis and to then enhance the healing of the damaged tissue [102,103]. Unsolved challenges related to the stiffness of the biomaterials reside on the low characterization of the mechanical properties of the brain (and their modifications following resection) which have not been fully characterized to date [79].

Fibrous biomaterials are commonly made using electrospinning techniques and are constituted by small fibers (also called nanofibers). Advantages of fibrous materials are represented by the low-generated intracranial pressure due to the structure of the fibers that confers reduced swelling. Conversely, it has been reported that fibrous biomaterials would not be a good choice for implants into the resection cavity since they appear to promote GBM recurrence [96]. Segura *et al.* switched this drawback into a strength by using nanofibers as a means for a tumoricidal stem cell implant [87]. PLGA nanofibers containing salinomycin were fabricated by electrospinning, showing a sustained release of the drug for at least a 2-week period and stability for approximately 30 days. The efficacy of the fibers was tested on human GBM U-251 cells showing an increment of reactive oxygen species (ROS) leading to cell apoptosis compared to the free drug [104]. However, they have yet to assess the potential of this biomaterial *in vivo*. Jain *et al.* developed engineered aligned poly-caprolactone (PCL)-based nanofibres to attract and drive GBM cells from the primary tumor site to a more accessible, extracortical location. This nanofiber consists of two compartments: a primary empty reservoir based on PCL/polyurethane and a second compartment made of cyclopamine-conjugated collagen hydrogel that serves as an apoptotic 'tumor sink' located above the skull surface whose role is to receive the tumor cells that 'invade' the cortical surface. This new scaffold resulted in a significant reduction of GBM volume [81]. This strategy received the FDA Breakthrough Status in 2019 and is currently under investigation [105].

3.1.3. Injectability and adhesive properties

Most of the post-operative complications induced by Gliadel® can be attributed to the rigid structure which does not conform

to the irregular shape of the tumor resection cavity, limiting the area of contact with the cavity walls and leading to uneven drug delivery which might reduce the therapeutic effect due to an increase of the effective diffusion distance to residual disease. As their size and shape are not adapted to the anatomy of the resection cavity and do not bio-adhere to its walls, these wafers can migrate and collapse on the cavity floor. This mechanical and physical mismatch creates micro-shearing of the surrounding tissue, causing scar formation and neuroinflammatory response which might lead to brain edema and impaired would healing [43]. Obstructive hydrocephalus can also appear due to wafer migration into the ventricular system [106]. Moreover, the drug content is low, and the adjustment of several wafers is needed to obtain therapeutic doses of BNCU; therefore, the size of the cavity determines the amount of drug that can be administered.

The biomaterial tissue adhesion to the brain parenchyma is crucial to avoid a dislocation of the matrix and reduce off-target drug release. Scaffolds with a thin and flexible structure (*e.g.* electrospun scaffolds) can easily conform to the cavity borders maximizing the contact surface area and avoiding mass effect. Similarly, *in situ* assembling hydrogels and polymeric pastes have also emerged as good solutions to bypass the limitations of pre-formed solid implants, as they are softer materials capable of molding to the contours of the resection cavity lining and adhering to it, thus avoiding implant collapse and decreasing the diffusion distance of the released drugs between the DDS and the residual tumor cells [45]. As CNS cells are mechanosensitive [107], characterization of the DDS mechanical properties needs to be assessed to ensure that its viscoelastic properties are suitable for brain implantation to avoid inflammatory and pathological changes (*e.g.* gliosis, foreign body reactions (FBR), stem cell differentiation [108]) and reduce the risk of excessive intracranial pressure following intracerebral administration. If the DDS is injectable, it should be confirmed that the rheological properties are maintained after extrusion from syringes. Moreover, the bioadhesive properties of the biomaterial should be considered in the selection of the appropriate DDS for application in the tumor resection cavity. If the system adheres to the resection cavity walls, expulsion from the cavity induced by interstitial fluid or bleeding can be avoided.

3.1.4. Interaction of the biomaterials with the tumor resection microenvironment

The relationship between the nanocarriers and the immune system in GBM are also under investigation. Biomaterials developed at the beginning of the 2000 s were aimed at inhibiting the immune response. Indeed, the mechanical injury induced by tumor resection surgery induces BBB disruption, as well as recruitment of immune cells and the release of inflammation factors (*e.g.* cytokines) that can be associated with tumorigenesis and angiogenesis, enhancing the development of recurrences. Therefore, biomaterials able to tune down inflammation and promote wound healing were developed [109]. Conversely, biomaterials have also been developed as tools to boost the immune-system against GBM, despite GBM being referred to as a "cold tumor" and therefore with low immunogenic cell infiltration [110]. More recently, reports showed that a fair balance between pro- and anti-inflammatory input is required to re-shape the pro-tumor polarization of M2-macrophages and microglial cells in anti-GBM strategies [111]. Therefore, the selection of the biomaterials should also keep in consideration the immunomodulatory and immunotherapeutic properties associated with a temporally and selective controlled release of different immune-factors, and the correct ratio for a combination with antitumor therapeutics. As an example, polysaccharides are commonly used as scaffolds for GBM 3D cultures; HA, for example, plays a role in the diffusion and migration of GBM cells. However, the use of HA with a 100–500 kDa molecular weight range, promotes local anti-tumor inflammation by a dual interaction with GBM-associated macrophages and inhibits leukocyte migration interfering with growth factor signaling through CD44 binding [112]. Similarly, chondroitin sulfate proteoglycans (CSPGs) might be used to induce inflammation, inhibiting tumor invasiveness. A concerted mechanism is required that involves the controlled release and diffusion of anticancer drugs in combination with the immunomodulation properties of the infiltrating immune-cells, and finally healing the damaged tissue to restore homeostasis in the TRME.

It is well established that the GBM core microenvironment is highly hypoxic and that tumor growth may physically destroy the BBB, whilst secreting high levels of angiogenic factors (*e.g.* vascular endothelial growth factor A or fibroblast growth factor) which promote the tumor-blood vessel network. Therefore, introducing rationally-designed biomaterials bearing immobilized angiogenic factors into the resection cavity, can potentially restore the BBB network and provide adequate oxygen levels. Moreover, since hypoxia and cytokines are implicated in different events such as tumor angiogenesis, immunosuppression and GSCs maintenance, biomaterials able to provide oxygen, reduce cytokine levels and enhance the regeneration of the disrupted BBB, are preferred as this may limit GBM recurrence [113].

3.1.5. Biodegradability and biocompatibility of biomaterials

Gliadel® wafers showed substantial drug release 1-week post implantation, but thereafter, the empty scaffolds remained in the cavity for a prolonged period, increasing the risk of adverse effects. Achieving sustained long-term release kinetics (from days to months) with a safe biomaterial, remains a major drug delivery challenge for GBM local treatment. Therefore, all biomaterials developed to be applied to the tumor resection cavity must be biocompatible, biodegradable by enzymatic/nonenzymatic means and/or resorbable, to circumvent the requirement of a second surgery for device removal. Indeed, it has been demonstrated that long-term or non-biodegradable implants, such as those made from silicone, induce chronic inflammation, scarring and neuronal death [114].

Unfortunately, degradation studies of implants in the brain under physiological/pathological conditions are very rare. CSF in the brain contains molecules (*e.g.* proteins, sugars, peptides, ions) that can impact and degrade the material used. Furthermore, neuroinflammation in the resection cavity can produce ROS and recall immune cells that can contribute to implant degradation [115].

Another issue for safe biomaterial development resides on the FBR, a self-defense mechanism of the body which can lead to an over-reacted immune response, fibrosis and collagen encapsulation within the implanted materials. Many materials and implants do not achieve the expected performance because the host tissue severely resists these "foreign objects" as potential threats [116]. Several biomaterials are currently under investigation in order to overcome the FBR reactions [116].

3.1.6. Examples of biomaterials suitable for resected glioblastoma

The field of biomaterials represents an ever-growing body of research with the potential to bypass the clinical limitations that currently restrict efficacious GBM treatment. This research area is advancing at impressive rates providing new exciting scaffolds and DDS (combined or alone). Tailored biomaterials whether injectable, implantable or sprayable, will ameliorate the therapeutic profile of small molecules, proteins, cell-based treatment and recently emerging immunotherapy with a potential overall survival benefit for patients. In this section, we will summarize the features of two classes of biomaterials – hydrogels and electrospun nanofibers – as a paradigm of scaffolds applied to the GBM resection cavity. New exciting approaches are emerging in recent years

(*e.g.* sprays, nanofibers, paste; see Section 4) with enormous future potential, but still too early to define their impact on GBM treatment.

3.1.6.1. Hydrogels. Injectable hydrogels are excellent candidates for the local treatment of GBM and represents the first biomaterial designed for the application in the human body [117,118]. These composites consist of water swollen 3D polymeric network biomaterials, which reach a defined volume when administered into the brain cavity. The development of controlled polymerization has provided the potential to produce macromolecules with a narrow molecular weight distribution and tailored features to produce custom-sized biomaterials for hydrogels. Traditional methods of production involve structurally modified biomaterials that induce physical or chemical gelation. The derivatization of hydrogels with chemically reactive moieties induces the formation of covalent bonds that provide higher mechanical stability and strength. Reactive moieties for chemically-based hydrogels include azides, amines, maleimides, thiols, alkynes. In contrast, physically-based hydrogels are made by polymers designed to self-assemble in aqueous solvents and the sol–gel transition can be driven by hydrophobic interactions or by coulombic interactions. Examples of derivatized moieties for physical hydrogels are cyclodextrins/adamantane. Physical based hydrogels possess a high degree of swelling in aqueous buffers and a high stretching ratio. Recently, lipid-based biomaterials have also been successfully utilised to prepare hydrogels for the treatment of GBM [45,119]. Examples of biomaterials used for hydrogel development include PEG, PCL, PLGA and poly(lactic acid) (PLA) [120]. Advantages in the use of hydrogels resides in persistent retention within the resection cavity, with a tunable drug release; indeed, the crosslinked structure allows for variable drug release, protecting drugs from enzymatic/-chemical degradation. Moreover, the superior biocompatibility, the customizable synthesis and properties and the easy scalability, make them an attractive tool for GBM treatment. The major issues required to be overcome, relate to the 3D structures of hydrogels, which can often support the growth of GBM cells. The delivery issue that must be addressed in the use of hydrogels resides in the homogeneity of the network and therefore the crosslinking moieties, the gelation time and the rheological properties of the final composite. Examples of hydrogel application for GBM can be found here[77,121,122].

3.1.6.2. Electrospun nanofibers. Electrospun nanofibers are biomaterials with highly versatile and tunable physical and chemical properties made by electrospinning techniques that allow the synthesis of continues filaments with variable diameters (from 10 nm to microns) and length (meters) [123]. This procedure can be implemented with two fluid coaxial nozzle electrospinning, yielding core-sheath fibers. Rational-design of controlled fibers with desired surface area, morphologies and compositions can be obtained by the appropriate selection of the biomaterial (polymers, inorganics and hybrid organic–inorganic compounds), the matrix concentration, the selection of the extrusion solvent and the additives used. The nanofibers can be kept together on the same "macro filament" by hydrophobic interactions, hydrogen bonds or chemical ligands. These characteristics enable *i*) combination of different properties from two different polymers into one fiber (to obtain the desired stiffness and porosity grade, for example); *ii*) encapsulation of drugs or active molecules in different spatial positions (in the inner fiber core or in the outer part of the fiber), therefore controlling drug release (from burst to sustained); *iii*) different shapes (*e.g.* discs or pills-like), surface area and highly controlled compositions; *iv*) bio-adhesivity to the brain parenchyma; *v*) biomaterial degradation [124]. Examples of nanofibers developed are PLGA, poly(L-lactic acid-co-ε-caprolactone) (P(LLA-CL)) and polyethylene glycol – poly(L-lactic acid)(PEG-PLLA). Over the years, nanofibers have been loaded with several anticancer drugs (*e.g.* BCNU, 5-FU, PTX) and recently nanofibers have been used for the local delivery if tumoricidal stem cells showing promising results [87]. A -TMZ nanofibers library was developed combining different parameters such as polymer composition, release kinetics and matrix degradation to obtain a custom-designed 3D wafer. The outcome of this study showed that tuning the drug release for specific periods, ranging from hours to one month, is a key parameter in reducing the recurrence of GBM [125]. However, the site of drug release is also crucial. PLGA nanofibers were studied as a biomaterial for delivery of several anticancer drugs such as BCNU and vancomycin, with results showing that even upon prolonged drug release (up to 8 weeks), poor drug tissue penetration (only 5 mm) was evident and in the wrong location (subarachnoid space instead of the brain cortex), reducing the efficacy of the treatment [126].

3.2. Step-by-step local treatments progression towards clinical translation

In our opinion, an ideal DDS for the intracavitary local treatment of GBM should *i*) use materials that facilitate and expedite translation to the clinic and adoption by health services (*e.g.* Generally Recognized as Safe (GRAS), Good Manufacturing Practice (GMP) and FDA approved materials; *ii*) be characterized by simple formulation and ease of sterile manufacturing for effective scaling up; *iii*) easy-handling application by the neurosurgeons *iv*) be adaptable to the resection cavity shape and stick/adhere to its inner border to guarantee its full coverage, or be applied in those regions that have been detected as possible recurrence foci, such as the infiltrative margins; *v*) be soft and possess mechanical properties compatible with the brain, thus avoiding swelling and increased intracranial pressure; *vi*) include a drug content sufficient to reach a therapeutic dose without necessarily filling the entire cavity and inducing local toxicity.

Even if the translation to clinic is taken into account from the start of the rational design, these ideal features might seem simple to achieve and to test in preclinical models, but they are surely not readily applied to a human brain. To ensure the clinical success of DDS for the local treatment of GBM, inter-disciplinary collaboration between diverse experts in different fields is essential. The need for preclinical and clinical expertise emphasizes the importance of collaborative efforts to achieve the final goal of increasing the quality of life of patients [127,128]. A scheme of positive and collaborative interactions among chemists, bioengineers, material scientists, biomedical scientists, biologists, neuroscientists, physicists and bioimaging experts, immunologists, tumor microenvironment experts, clinicians and physician-scientists in academia and pharmaceutical industries, must be adopted from the early stages of preclinical development and strategic planning. The competences and responsibilities of each expert need to be defined and integrated to build an effective team with a scaling up and bench-to-bedside vision to develop safe and long-term GBM treatments. These distinct expertise and interactions are essential to properly define the drug to be used, select an adapted tailored scaffold and appropriately characterize the DDS on appropriate experimental models (Fig. 4). In the next sections we will summarize the parameters that we consider essential to anticipate and ensure the clinical translation of newly developed DDS for the local treatment of GBM.

3.2.1. Selection of the drug

So far, the most successful chemotherapeutic drug for GBM treatment is the alkylating agent TMZ, which is the gold standard for newly diagnosed patients since 2005. TMZ is a prodrug able to cross the BBB following oral administration and converts into

its active metabolite 5-(3-methyl-triazen-1-yl) imidazole-4-carboxamide (MTIC) in physiological conditions [129]. TMZ is generally well tolerated with myelosuppression (thrombocytopenia) as the main dose-limiting toxicity [130,131]. As TMZ is unstable at physiological pH, does not require metabolic activation, and conversion to MTIC can occur after uptake by GBM cells, increasing the doses of TMZ at the tumor site by local administration is a promising strategy and has shown good therapeutic benefit in preclinical models [49,132–134]. However, high intrinsic and/or acquired chemoresistance limit the response rate of alkylating agents in one third of GBM patients with O^6-methylguanine–DNA methyltransferase (MGMT) promoter unmethylated tumors [135,136], highlighting the necessity to find alternative, curative, and long-lasting treatments.

Physicochemical and pharmacological properties of the drug are important predictors of drug diffusion in the brain, which is an important parameter for local treatment. These properties are intrinsically correlated to each other and need to be analyzed holistically. Molecules with high cytotoxic activity against GBM cells and not requiring hepatic drug activation, showing no adverse neurological effects, dose-limiting systemic side effects and poor BBB permeability, are ideal candidates for direct local delivery to the brain. The drugs should also present the following features once administered locally: *i*) low local toxicity; *ii*) high diffusion into the brain parenchyma; *iii*) constant threshold concentration over time [62]. However, free drugs often lack appropriate stability, physicochemical properties and toxicity profiles and therefore can be chemically modified or incorporated into carriers (*e.g.* micro- or nano-sized vehicles) to increase their sustained release, selectivity and cellular uptake and reverse drug-resistance mechanisms, thus reducing local side effects and prolonging the therapeutic effect [137]. Aiming at combinatory therapies with standard of care chemoradiation, molecules acting in synergy with RT or other chemotherapeutic drugs and with a different mechanism of action compared to TMZ, should be privileged to avoid crossed-linked resistance [138]. Moreover, molecules with immunomodulatory properties or acting on specific cell-subtypes present in the tumor microenvironment (*e.g.* TAMs, GSCs) can also increase the therapeutic efficacy, by specifically reversing tumorigenesis at the resection cavity borders and addressing the complex heterogeneity of GBM and TRME [139].

Physicochemical properties such as the nature of the molecule (small drug, proteins, antibodies), the lipophilicity and the molecule size, correlates with diffusion in the brain and therefore anticipates if the drug can effectively reach the desired target [140]. For example, small molecules with low MW show better brain distribution than larger molecules. This pattern can be ascribed to the steric hindrance of the drug (the space that the drug occupies in the medium); therefore, the diffusion coefficient is inversely related to the molecule size and to the interaction of the drug in the brain environment [141]. Lipophilicity can estimate drug diffusion in the brain; drugs with higher lipophilicity (log P > 1) possess better penetration in brain tissue and greater cell membrane permeability than hydrophilic drugs. In contrast, these drugs show higher binding with the proteins and lipidic membranes reducing the effective dose. pKa of drugs is related to the presence of ionizable moieties in the chemical structure, allowing the molecule to shift from an uncharged to charge state depending on the pH. pKa is important for drugs to discriminate the TME from healthy brain, since the former possess lower pH than the latter and increases the number of hydrogen bond donors and acceptors modifying the interaction with enzymes and proteins in the brain. Also, charged moieties ameliorate the solubility of the molecule in the aqueous phase resulting in a better distribution though ECF and CSF, whilst uncharged moieties confer higher solubility in the lipophilic area of the brain and increase the cell membrane permeability. Collectively, the drug properties influence specific or non-specific binding of brain components. For example, drugs like BCNU which possess a log P of 1.375, show very poor penetration in the brain (diffusion of 3 mm) since they are drained out before their diffusion. Conversely, smaller hydrophilic drugs like 5-FU and Lauroyl-gemcitabine ($GemC_{12}$), show a smaller transvascular permeability than BCNU and therefore a longer retention time in the brain with better local efficacy [57,142]. To date, there are a few mathematical models able to describe and predict drug distribution in the brain [12].

3.2.2. Administration timing and drug-release profile

Considering that local delivery for GBM would be useful as an adjunct to standard of care chemoradiation, which starts 3 to 6 weeks after surgery, the optimal release kinetics from a DDS should in theory be at least 1 month, thus commencing treatment during an otherwise oncological treatment gap. However, this time range depends on the mechanism of action of the cytotoxic drug selected for the local treatment (is the drug directed against GBM infiltrating cells? is it used to potentiate an immunotherapy approach? does it target a non-cancer cell population within the TRME?) which will thus define the DDS to be used. Cell cycle non-phase specific drugs (*e.g.* TMZ, BCNU, lomustine) are concentration-dependent meaning that their maximal efficacy depends on the dose that can be administered. For these drugs, repeated "bolus" release profiles leading to high peaks of exposure, must be privileged. On the contrary, cell cycle phase specific drugs (*e.g.* 5-FU, gemcitabine) are time-dependent meaning that their efficacy relies on the duration of exposure, requiring continuous and sustained release [69]. The guidelines on the diagnosis and treatment of GBM of the European Association of Neuro-Oncology state that "benefit of alkylating agents has to be weighed against the potential long-term toxicities and the risk of inducing a hypermutator phenotype that is associated with a more malignant phenotype, in particular in patients with IDH-mutant gliomas, who have a longer life expectancy" [15]. This should also be valid for local treatment approaches: indeed - considering that the brain will be exposed to low drug concentrations for prolonged periods following local implantation of DDS - the evolution of tumor cells under therapy (*e.g.* TMZ-induced hypermutation [143]) should be considered when selecting the drugs and release profiles for local treatment as they might be more risky than beneficial and limit the efficacy of the drug in the longer term (*e.g.* potentially depriving patients from the treatment with that drug at a later stage, such as recurrence). Finally, in case of dual drug delivery, the optimal release profile might differ between the two loaded drugs depending, for example, on the cellular population targeted by the drug/DDS (*e.g.* GBM cells with slow or fast proliferation rates, reactive astrocytes, immune cells, GSCs), the time window of the microenvironmental change targeted by each drug (*e.g.* glutamate release, free radical formation, ischemia) or the possible synergy when acting together or sequentially.

In vitro release studies from the DDS should be performed in buffers able to mimic the brain microenvironment such as artificial CSF, even though many authors perform studies in water or Phosphate-Buffered Saline. If the drug release is triggered by an external or endogenous stimulus or if an activation of the active compound is required for the therapeutic activity, this should also be considered in the setup of the release experiments. Finally, authors might want to evaluate if the released agents from the DDS retain their cytotoxic capability *in vitro*. For example, Rahman *et al.* have placed their PLGA/PEG matrices in culture medium for 24 h or 14 h and then have seeded GBM cells on top of the matrices to evaluate if they had retained the cytotoxic function [144]. Gawley *et al.* have determined the cytotoxicity of irinotecan released after 1 day or 7 days from drug loaded eluting beds under biorele-

Table 1
Comparison of methodologies described, used to assess drug diffusion in the brain. Legend: ● High definition; ■ Mid definition; ▲ Low definition.

	MRI	Optical Imaging	ToF-SIMS	Microdialysis
Drug distribution	■	■	●	●
Biodegradation	●	■	●	■
Spatial resolution	●	●	●	▲
Resolution time	■	●	▲	●
Neuroinflammation	●	■	●	●
Applied in clinical settings	●	▲	▲	▲
Strength	- Low invasivity; - High spatial resolution [~1 mm (clinical); ~0.1 mm (preclinical)]; - Physiological and anatomical feedbacks.	- Fast method; - Real-time analysis; - Combination of tracers/ probes.	- Label-free; - Simultaneous measurements of both endogenous and exogenous compounds; - Quantitative measurement.	- Unbound drug concentration
Weakness	- Contrast-agents can induce toxicity; - Indirect quantification.	- Fluorescent dyes is required; - Limited tissue depth penetration; - Often used only in the preclinical settings.	- Invasive; - Drug specific methodology.	- Invasive - Hydrophilic molecules

vant conditions (water) and exposed patient derived GBM cells to this biomaterial for five days [145]. It is important to note that caution should be applied when attempting to extrapolate *in vitro* release profiles to the preclinical modelling setting, due to the inability to mimic the turbulent and dynamic tumor microenvironment *in vitro*. Dose-escalation studies should be utilized to determine maximum tolerated doses *in vivo*, prior to a sufficiently powered therapy study.

3.2.3. Drug retention and diffusion in the brain

3.2.3.1. Drug diffusion in the brain. In the development and optimization of DDS to prevent GBM recurrences, a cornerstone is the drug diffusion depth into the brain following local administration. As the majority of GBM recurrences arise within 2 cm from the resected margins, appropriate drug doses should reach this penetration depth following local administration in the resection cavity [125]. Therefore, tools able to promote the diffusion of the drug with a prolonged 0th or 1st order release kinetics over time with concerted mechanism between the drug-release and biomaterial degradation, is desired [69].

When drugs are incorporated into a nanocarrier and are administered in the brain, the drug needs to diffuse at a sufficient concentration to act on with GBM cells. The fate of the drug molecule within the brain is related to a complex combination of factors including diffusion, the cerebral fluids (both ECF and CSF), extra/intracellular exchange, target and off-target bindings and drug metabolism [146]. Therefore, drug distribution can be related to myriad factors, namely brain anatomy, the characteristic of the selected drug and drug-release kinetics related to the nanocarrier properties. The size, shape and charge of the nanocarriers also impact drug diffusion in the brain, affecting distribution in the brain parenchyma. [147]. Rigid nanoparticles will diffuse slower than "fluffy" biomaterials due to the higher deformability and possibility to pass through extracellular matrix pores (50 nm). Haynes and colleagues developed PLGA-PEG nanoparticles that can rapidly penetrate the brain tumor microenvironment leading to improved tumor growth suppression when compared to drugs delivered by otherwise similar, but nonpenetrating, NPs [148]. The physicochemical properties of the drug incorporated into the nanocarrier need to be considered while selecting the nanocarrier, as their drug loading and release kinetics vary accordingly [69]. Depending on the drug release kinetics pursued, the main mechanisms which can be varied are based on diffusion, erosion, swelling and osmosis [149]. Linking chemistry can also be used to tune the drug release from nanocarriers. The resected cavity possesses an acidic environment with a high ROS content. Therefore, direct conjugation of drug with a given matrix using hydrazones, self-immolative or disulphide bonds, can induce the release of the molecule after a reducing environment-dependent trigger [150]. Another strategy is based on chemotherapeutic drug-impregnated microchip delivery; these complex systems are made of pumps, valves and channels at the micrometer scale and are remotely controlled for single or combined release of chemotherapeutic agents. Compared to Gliadel®, these strategies reduce the bolus mechanism of release, potentially producing a more efficacious effect against tumor recurrences [21]. However, the lack of methodology to quantify drug diffusion in the brain *in vivo*, make it difficult to predict treatment efficacy and to ameliorate the nanocarriers for this purpose and avoiding the collateral damage of the healthy tissue.

3.2.3.2. Methods to assess drug diffusion in the brain. Several methods summarized in Table 1 have been developed over the years to visualize at different scales, the nanoparticle and drug diffusion and distribution in the brain, as well as confirming the presence and degradation of DDS following intracerebral administration. These platforms help to understand the strong or weak points of local delivery systems for GBM and the mechanism(s) that lead to the success/failure of the treatment for future development in a preclinical setting. Moreover, due to the huge intra- and inter-heterogenicity of the GBM, the implementation of these methods can help to develop more personalized and patient-tailored therapies. Some of these methods are limited to preclinical use but provide useful information for clinical translation.

Magnetic Resonance Imaging (MRI). MRI has revolutionized GBM patient care and can be used for tumor detection, diagnosis and as a tool to grade GBM by estimating the spreading, necrosis, angiogenesis, metabolite expression, tumor growth and recurrence and therefore providing an insight on the GBM physical processes. Factors that affect the efficacy of MRI are the relaxation and signal contrast, the magnetic susceptibility of the tissue (the feedback of the materials after a magnetic field application), the water diffusion and displacement (that is a prediction of GBM spreading), the chemical shift and the electron screening of C^{13} metabolites (*e.g.* lactate) [151]. Moreover, MRI can be used to visualize nanoparticle and drug diffusion in the brain. For this purpose, PLGA-based microspheres were loaded with tritiated 5-FU, stereotactically implanted and administered by CED. MRI showed a limited drug diffusion area with a maximum radius of 3 mm from the implantation site over time [57]. Similar findings were observed using other radiolabeled drugs [152]. In recent years, emerging strategies

involving ^{18}F compounds has been validated to better understand drug/nanocarrier diffusion in the brain [153]. However, due to the limited surface of analysis and the low concentration of the fluorine, this technique needs further implementation. MRI can also be harnessed for real-time imaging of paramagnetic nanocarriers with high imaging contrast capability (*e.g.* iron oxide nanoparticles) [154].

Optical imaging. In vivo bioluminescence and fluorescence have gained attention in the last few years to trace nanocarriers in the animal body in a non-invasive manner. The derived-images of these techniques give an insight regarding the fate of the DDS in the body and therefore can be applied to understand the behavior of drug-loaded biomaterials used for localized drug delivery to GBM. The advantage of these techniques resides on their safety through avoiding the use of radiation and low-time consuming analysis. However, optical imaging shows drawbacks such as background fluorescence from cellular components, chemical compounds (*e.g.* drugs) and tissue depth, photo-bleaching of the dyes, photo-toxicity related to the excitation light and incompatibility with optogenetic tools. The most important application of optical imaging is the Förster resonance energy transfer (FRET) [155]. The concept of this technique is based on the energy transfer of a "donor" to an "acceptor" fluorophore, resulting in the excitation and light emission of the latter. Example of acceptor/donor dyes are Cyanine 3 (λex 554 nm–λem 568 nm) and Cy5 (λex 649 nm–λem 666 nm). Compared to other resonance energy transfer techniques like chemiluminescence resonance energy transfer (CRET) and bioluminescence resonance energy transfer (BRET), which utilize donor molecules excited through a chemical stimuli (CRET) or a bioluminescent molecule (BRET) and can cause toxicity or immunogenicity, FRET only requires external photoexcitation. Factors that affect the efficiency of FRET include the distance between the fluorophores (the acceptor should be about 1–10 nm from the donor), the spatial orientation and the excitation/emission spectrum overlap of the selected dyes [156]. FRET measurements can be elegantly used to reveal information about the fate of nanocarriers in the resected cavity and how its content is released over the time, mimicking drug diffusion towards the GBM cells. In our group we developed a $GemC_{12}$-LNC hydrogel to be injected in the GBM resection cavity containing DiI (λex 549 nm–λem 575 nm) and DiD (λex 644 nm–λem 665 nm) as donor and acceptor fluorophore respectively. The hydrogels were injected in healthy rat brains following surgery and the animals were sacrificed at different time points. The brains were analyzed by observing that the progressive degradation of the formulation corresponds to a release of the fluorophores in proximity of the cavity borders over time [78]. However, even if fluorescent labelled nanocarriers can provide some insight on diffusion in the brain, dyes possess different properties compared to the drugs. The use of some fluorescent drugs (such as doxorubicin) can be convenient, but it is still elusive for *in vivo* application due to the low fluorescence quantum yields of these molecules which can preclude the detection at low concentration [157]. Strategies to make drugs trackable consist of the conjugation with dyes. Drawbacks of this strategy are the increment of the MW of the molecule and possibility to alter their biodistribution profile or decreasing the pharmacological effect. Finally, this strategy can reside on the dual-loading of the dye and the drug onto the same nanocarriers and assessing if the dye can alter the physicochemical properties [157].

Time-of-flight secondary ion mass spectrometry (ToF-SIMS). Mass spectrometry imaging (MSI) is the analytical gold technique to both identify and quantify molecular components with high sensitivity in different biological samples without the use of fluorescent labels or radioactive tracers. In recent years, further development of the technique made it possible to couple MSI with time-of-flight analyzers, generating time-of-flight secondary ion mass spectrometry in imaging mode (ToF-SIMS) and allowing the analysis of biological samples at the cellular and subcellular level. TOF-SIMS analysis employs a pulsed primary ion beam that can generate large fragment ions (up to 1000 Da) permitting the quantification of lipids, metabolites and drugs at a spatial resolution of 100 nm [158]. Recently, ToF-SIMS has been used for mapping GBM samples to produce clinically relevant data on tumor behavior and heterogeneity [159]. *In vitro*, ToF-SIMS was used to quantify the cell uptake and intracellular localization in T98G GBM cells, of p-boronphenylalanine and sodium borocaptate, a clinically used boron neutron capture therapy agent for cancer therapy [160]. By labelling each drug with a different boron isotope it was possible to image the subcellular distribution of both drugs in the same cells. In another study, the B-cell lymphoma 2 (BCL-2) inhibitor ABT-737 was visualized in the A-172 human GBM cell line with high spatial and high mass resolution [161].

Microdialysis. This tool is currently the only method allowing direct information on unbounded drug concentration in the desired tissue to be obtained, and was also applied to monitor drug diffusion in the brain [162]. Microdialysis relies on sampling a localized area of the desired tissue permitting direct quantification of the drug concentration in the targeted area and estimation of the relationship between the concentration and the pharmacological effect. The methodology is based on the insertion into the brain area of interest, a probe made of a tube and a semi-permeable membrane with a cut-off at variable range (from 6 to 100 kDa). Upon tissue insertion, the probe pumps a perfusion liquid with a constant flow; the drugs dispersed in the area move into the probe by passive diffusion and are sampled outside the tissue for collection and analyses. The advantages of this technique are related to the quantification of the unbonded drug only, which is the moiety potentially active against the molecular target. Compared to other techniques, microdialysis has shown interesting advantages such as the capability to sample and measure at scheduled times on the same animals (i.e. longitudinally) with a dual benefit: reducing the number of animals and acquiring richer information, allowing the distribution of the drug over time to be traced. Over the years, this technique become more versatile due to the coupling with advanced analytical techniques that reduced the quantification limits of the analysis, and the use of new materials as membranes with larger cut-offs which are able to quantify molecules with different properties. The major drawbacks of the technique are its invasiveness and its dependence on the nature of the drug. Indeed, lipophilic drugs cannot be sampled since they are stacked to the membrane [163].

The combination of data collected using different techniques can provide more information to better understand nanocarrier distribution and drug diffusion in the brain. For example, to have more complete information, MRI and optical imaging have been used in a synergistic manner by the use of combined fluorescent and MRI probes, thus taking advantage of the high sensitivity of the fluorescent imaging and the higher tissue penetration and higher special resolution of MRI. For example, neural stem cells were loaded with ^{111}In-MSN exhibiting a strong fluorescent profile making them a suitable tool for real-time tracing after intracranial administration in GBM xenografts [164]. Focused ultrasound (FUS) technique is a recently developed approach to facilitate the permeation of drugs in the brain though a reversible opening of junctions increasing vascular permeability of drugs. FUS was combined with MRI to better localize drugs to the tumor recurrence and to monitor drug diffusion and penetration [165].

3.2.4. In vitro cellular studies to test drug delivery system for local glioblastoma treatment

The first step to evaluate the anti-tumor efficacy of a treatment is to test the cytotoxic activity of the free drug and, if pertinent, the

loaded drug using *in vitro* cellular models. First, the drugs are tested in 2D (monolayers) or 3D (spheroids) culture models [166], often using established GBM cell lines and standard cell viability/cytotoxicity assays (*e.g.* MTT, Alamar Blue, PrestoBlue, Titer-Glo). Recently, many authors are also using GBM/macrophage co-cultures [167] or human 3D GBM models (*e.g.* tumor spheres, organotypic slices, explants, tumoroids, GBM-derived from cerebral organoids) [168] composed of primary glioma cells and GSCs. These models are able to mimic GBM composition and microenvironment, organization, physical constraints, drug resistance and penetration. Each cellular model has advantages and drawbacks: the choice of the appropriate model should be carefully considered according to the scientific question at stake and the relevance of the conclusions that can be drawn should be put into context knowing the limitations of the model used. When developing DDS for the local treatment of GBM, most authors perform drug cytotoxicity studies on monolayers of GBM cell lines (*e.g.* U87-MG, GL261, T-98G, U-251, 9L, C6 cells) or, more recently and when collaborations with hospitals are established, on primary cells derived from patients.

In the future, *in vitro* injury models able to mimic the complexity of the TRME should also be used to evaluate the efficacy of innovative DDS for local administration in the resection cavity, for example using co-cultures of GBM and non-tumor cells (*e.g.* astrocytes and microglia, the first cells responding to a brain injury). *In vitro* co-culture models simulating mechanical injuries represent major tools to study the role of different brain cell populations under both physiological and pathological conditions [169,170]. In the context of the TRME, two studies have established models capable of demonstrating the beneficial role of brain-resident cells (astrocytes and microglia) on GBM tumor cell growth and could potentially serve as a basis for future developments. Okolie *et al.* developed an injury model that showed that reactive astrocytes play a major role in tumor progression, potentiating tumor aggressiveness at resection and recurrence in mice. Astrocytes and tumor cells were seeded into two separate chambers until confluence before removing the insert and applying a scratch on the astrocytes to observe tumor cell migration. The astrocytic response strongly influenced tumor growth and migration, suggesting that reactive astrocytic gliosis could potentiate the aggressiveness of residual tumor cells after resection [171]. In contrast, as GBM cells strongly interact with the surrounding tissues attracting astrocytes and stimulating their proliferation [172], Schmitt *et al.* developed two *in vitro* models to mimic complete or incomplete resection of the tumor mass [172]. The authors seeded different proportions of GBM cells, astrocytes and microglia estimating the cell populations amounts and ratios following resection (incomplete resection: 70%, 29% and 1%; complete resection: 10%, 80% and 10%, respectively) providing a model that could be exploited to screen molecules aimed at reducing the onset of tumor recurrence and reversing tumor cell growth and infiltration.

Finally, *in vitro* studies evaluating DDS-induced and the chemotherapy-induced neurological damage are very rare in articles describing DDS for local GBM. However, normal tissue controls should be included from the early-stage of drug screening to establish tumor selectivity and lack of normal tissue toxicity [173]. Indeed, comparing the neurotoxic doses and exposure times in healthy brain cells (*e.g.* reactive astrocytes [174]) with cytotoxic necessary to eliminate tumor cells, could define appropriate therapeutic windows and reduce the number of *in vivo* experiments needed to test the safety of DDS for local GBM treatment.

3.2.5. Bio-tolerability

Brain biocompatibility and neurotoxicity is a major concern in the development of DDS for brain use; therefore accurate and methodic assessment should be performed to evaluate if the DDS is suitable for brain implantation and to guarantee its safety [175]. Moreover, as microglia and reactive astrocytes can contribute to tumor development and the instauration of an immunosuppressive environment in contact with the tumor [176–178], it is important to avoid the possibility that DDS implantation and degradation could contribute and support chronic inflammation that might support the onset of tumor recurrences. Therefore, the inflammatory events produced both by the mechanical trauma (*e.g.* GBM resection, DDS intracerebral administration, intracranial pressure increase or brain swelling) and the brain tissue contact with the DDS, should be analyzed in the short and long-term (acute and chronic tissue response). Therefore, physicochemical parameters of the DDS such as pH, surface charge and isotonicity of the formulations should be tested from the early phases of DDS development to ensure its suitability for application in the brain. Thereafter, bio-compatibility studies on the non-drug loaded DDS should be performed *in vitro* on healthy brain cells (*e.g.* immortalized or primary astrocytes and microglia) and GBM cells. Finally, *in vivo* studies with appropriate imaging follow-up, biochemical and histological analysis, should confirm that the DDS is chemically inert.

3.2.6. Appropriate animal/tumor models and drug delivery system impact on the tumor resection microenvironment

Testing treatment candidates in appropriate and clinically relevant preclinical models is essential to accurately demonstrate successful drug delivery to brain tumors. Indeed, the limited transfer to the clinics of effective treatments for GBM can partially be attributed to the inability of current preclinical models to properly mimic human GBM heterogeneity and tumor microenvironment, leading to lack of predictability of therapeutic effect in patients [6]. An ideal model should faithfully recapitulate the key histopathological, genetic and imaging features encountered in GBM – including intratumoral heterogeneity and invasiveness - as well as being reproducible, reliable and stable over time [179,180]. A wide variety of GBM preclinical rodent models exist with different levels of accuracy, complexity and cost, where the choice of the model should be selected based on the scientific question addressed. To choose the most suitable model, researchers should consider several factors.

Firstly, the animal size/species is a crucial parameter both for technical reasons (feasibility of performing the experiments, cost, ethical reasons) and for how experimental results and conclusions should be drawn. For example, the size of the tumor resection cavity is around 9 mm^3 in mice and 28 mm^3 in rats [78,181] (the body weight difference between these species is 1:10 but their brain weight difference is 1:3). The resection cavity in humans is highly variable, irregular and depends on the size of the tumor at surgery and the extent of resection (*e.g.* 14–55 cm^3 and 92% respectively, in a study by Chaichana *et al.* on 292 patients [182]). A recent study by Ermis *et al.* evaluating the volumes of resection cavities in 30 patients provided a median volume of approximately 22–27 cm^3 [183]. This huge difference between rodents and humans should be considered and discussed for the specific purpose of the experiment (*e.g.* adhesivity and tolerability studies), to evaluate if this parameter can impact the interpretation of the results and the eventual scaling up of the system. For example, the DDS will likely fill the entire cavity in the rodent models due to practicality and lack of induced raised intracranial pressure, but in the clinical setting, will need to properly adhere to the cavity walls to provide sufficient drug dose and even drug distribution, while avoiding injecting high volumes which fill the entire cavity with subsequent potential for raised intracranial pressure. Therefore, the size and the surface of the cavity and the adhesion properties should be considered in the development of the system; films, sprays and nanofibers can be optimal scaffolds as they have intrinsic properties that take these features into account. The drug dose will also

have to be adapted, and for many DDS the drug loading correlates to the amount of hydrogel/implant that should be administered.

Secondly, the invading capacity of GBM cells from the tumor mass to the brain parenchyma differs depending on the GBM cell line and preclinical model used. This is a very important parameter when testing DDS for local treatment of GBM, as higher concentrations of drug will be released in the proximity of the tumor resection cavity thus showing good therapeutic effects in non-infiltrative tumors even if the drug diffusion depth is low.

Thirdly, human orthotopic preclinical xenograft models obtained by transplantation of human cell lines or patient-derived cells into mice or rats are closer to the clinical scenario, but they require the use of immunodeficient animals [184]. This is a limitation as both the tolerability and anticancer efficacy studies will be unable to provide information on the host immune responses to the DDS and the treatment. In contrast, the use of syngeneic models obtained by grafting murine cells into their host, permits the study of the entire tumoral microenvironment (including innate and adaptative immune cells and mediators), and can be performed on transgenic mice and transfected cells allowing advanced cellular imaging techniques for spatio-temporal characterization of tumor growth and response to treatment (*e.g.* two-photon imaging [185]). However, they lack genomic and microenvironmental heterogeneity (in part due to the lack of cancer stem cells and other progenitor populations) and tumor growth does not allow for the natural development of the tumor microenvironment (TME) [186], which may manifest in *de novo* GBM models using transgenic animals. Moreover, tumor cell grafting can produce inflammatory immune responses which can confound the interpretation of efficacy data [186]. Some authors are trying to develop immunocompetent murine GBM models able to recapitulate molecular and morphological characteristics of human tumors fully and faithfully [187–189], but their use is still rare in studies concerning the development and characterization of DDS for local GBM treatment.

Finally, in most cases the development of DDS for local treatment of GBM is aimed at post-surgical application. Surgical debulking of brain tumors creates an environment (characterized by excessive and chronic inflammation and persisting wound, astrogliosis, activated microglia and GSCs) able to stimulate the proliferation of infiltrating tumor cells causing tumor recurrences [9]. Considering the resection border microenvironment in the development of DDS for GBM is therefore essential to develop specific, effective, and long-lasting treatments. Indeed, reporting therapeutic efficacy of local DDS on preclinical models designed to treat established GBM does not necessarily guarantee that the therapeutic effect will be maintained following administration into the resection cavity. Considering that there is no optimal model to evaluate the efficacy of DDS for local GBM treatment, experiments on multiple models with different characteristics can alternatively be used to evaluate distinct scientific questions in a stepwise manner.

3.2.7. Synergy with standard of care treatment and combinatory treatments

GBM is a very aggressive tumor, and combined strategies are required to target tumor heterogeneity and obtain long-lasting therapeutic effects [138]. This means that local DDS are developed to be used as adjunct therapy to the Stupp protocol or standard of care treatment [15]. Therefore, if a given DDS demonstrates adaptability for local application into the brain using carefully designed studies and models *in vitro* and *in vivo*, its combination with standard treatment (TMZ and RT) should be evaluated to assess eventual toxicities and efficacy. The effect in combination with any other treatment commonly administered before/after tumor resection (*e.g.* corticosteroids, commonly administered to manage brain edema) could also be assessed, if relevant. Moreover, combination with other treatment strategies, in particular immunotherapy, can also be envisaged and should properly be addressed including appropriate control groups. It is important to note that localised DDS for GBM offers two potential principle outcome measures: i) significantly longer survival relative to standard-of-care treatment arms; ii) comparable survival relative to standard-of-care, but with significantly lower side-effects.

4. Proof of concept and future perspectives on local drug delivery in the glioblastoma resection cavity

Examples of drugs that have been used in preclinical models alone or in combination for local GBM treatment are: *i*) chemotherapeutic (pro)drugs: cisplatin [31], BCNU [190], doxorubicin [85,191], 5-FU) [54,58,60], epirubicin [134], PTX [192], $GemC_{12}$ [142], curcumin [193], mitoxantrone [65]; *ii*) anti-glutamatergic agents: riluzole, memantine [194]; *iii*) glycolytic inhibitors: 3-bromopyruvate, dichloroacetate [195]; *iv*) salinomycin [104]; *v*) steroids: dexamethasone [196,197]; *vi*) angiogenesis inhibitors: cediranib [197], rapamycin [192], minocycline [198]; *vii*) immunotherapies: IL-2 [199].

However, even though the choice of the appropriate drug is very important, the technical drug delivery approach is also critical. Following Gliadel® approval, pCPP:SA polymers at different ratios were loaded with several drugs and safely and effectively delivered intracranially in GBM-bearing animals (*e.g.* [65,191,194,200–202]). For example, Mangraviti *et al.* have recently used CPP:SA polymers to deliver the hydrophilic drug acriflavine, an FDA-approved small molecule able to inhibit hypoxia-inducible factor (HIF-1), at different doses (10%, 25%, 50% w/w) [203]. This therapeutic approach is promising, as transcriptional activity of HIF-1α has shown to play a crucial role in determining the extension of tumor invasion and recurrence [204]. The authors demonstrated *in vitro*, a burst release during the first 24 h followed by a sustained release during the following 120 days in PBS. *Ex vivo*, they demonstrated that the drug is actively released and homogeneously dispersed around the tumor site up to 60 days post-implantation. *In vivo* studies using the 9L gliosarcoma models showed excellent antitumor efficacy response, with 50%, 90–100% and 83% long-term survivors following local treatment with acriflavine wafers at 10%, 25% and 50% w/w, respectively. However, clinical experience with Gliadel® wafers showed some limitations: it was a monotherapy system with a rigid structure; poor drug loading and fast drug release; limited penetration depth into the brain; dependence on the resection cavity size to administer appropriate drug doses. For these reasons, several groups tried to improve the efficacy of polymer-mediated implants by developing fit-for-purpose DDS (using biomaterials forming foams, hydrogels, paste, sprays) more adapted for brain implantation for the controlled release of other chemotherapeutic drugs in the GBM resection cavity. Some excellent examples are reported below (summarized in Table 2).

McCrorie *et al.* developed an unconventional sprayable bioadhesive hydrogel made of low methoxyl pectin containing drug (etoposide or olaparib) nanocrystals coated with polylactic acid-polyethylene glycol (NCPPs) [205]. They delivered the hydrogel via a spray device, to further increase the adaptability to the GBM resection cavity. They carefully characterized the hydrogel (*in vitro* biocompatibility on GBM cells and astrocytes, degradation in CSF, gelling capability in the brain, *ex vivo* bioadhesion studies), the NCPPs (stability, drug loading and release, eventual variations following formulation spraying) and the whole DDS (*in vivo* biocompatibility at 1, 7 and 14 days in mouse brain and *ex vivo* in a pseudo-resection cavity on fresh porcine cadaver brain to assess the depth of penetration). Even though no efficacy studies have

Table 2
Selection of outstanding research achievements describing biomaterials and DDS for the local treatment of GBM. The main properties addressed by the system, its innovation and/or strengths in terms of experimental models used, have been highlighted. Legend: PLGA: poly(lactic-co-glycolic acid); PLA: polylactic acid; PEG: Polyethylene glycol; CXCL10: chemokine ligand 10; siIDO1: small interfering RNA targeting indoleamine 2.3-dioxygenase-1.

Formulation	Delivery System	Key points of the research	Ref.
Hydrogels	Lauroyl-Gemcitabine loaded lipid nanocapsules	• Simple, all-in-one system • Complete adaptation to resection cavity • *In vivo* hydrogel degradation studies • *In vivo* efficacy on different models following intratumoral administration or in tumor resection cavity; • Short-, mid-, long-term tolerability studies	[78,142,181]
	Nanocrystals coated with PLA-PEG	• Complete adaptation to resection cavity, adhesivity • Combination therapy • Characterization of DDS, nanocarrier and mix • Spray device to deliver the gel • *Ex vivo* bioadhesion studies in porcine brain	[205]
	Triglycerol monostearate	• Fit-for-purpose material for tumor resection microenvironment • Matrix metalloproteinase (MMP) enzyme-responsive hydrogel • *In vivo* studies in C6 rat resection model	[213]
	Alginate	• Combination with immunotherapy • *In vivo* studies compared with TMZ • Orthotopic isogenic glioma mouse model • Clinical translation planned in 3–5 years	[216]
	Oligopeptide hydrogel as a drug reservoir	• *In situ* gelation in the resection cavity • Combination of CXCL10 and Zinc 2-methylimidazole nanoparticles loaded with mitoxantrone and siIDO1 and camouflaged with macrophages membrane • Stimuli-mediated drug release (acid-dependent) • Strong apoptosis induction and higher levels of CD3+ CD4+ helper T cells and cytotoxic T cells compared with the drugs alone • Prolonged survival after both orthotopic intratumoral injection and administration in the resection cavity	[217]
	Dextran phosphate	• Approved in Belarus since 2015 • Clinical trials on coming in Europe in 2021	[221]
Wafers	Acetylated dextran	• *In vivo* drug release studies on animals with and without tumor • Drug diffusion in the brain • Biocompatibility up to 3 months	[212]
	Polymers impregnated with chemotherapeutic agent	• Stimuli-mediated drug release (acid-dependent) • *In vivo* drug release studies in resection cavity of animals with and without tumor	[215]
Paste	Blend of PLGA and PEG	• Complete adaptation to resection cavity • TMZ stability • Cytotoxicity on patient-derived GBM cell lines, isolated from the invasive margins of the tumor during resection • *In vitro* efficacy drug-loaded DDS on inserts suspended over 9L cells • Efficacy and safety studies on 9L resection model, compared to standard-of-care treatment	[207]
Patches	Oxidized starch-based patch	• Flexible, sticky and bioresorbable • Integrated with wireless electronics to control intracranial drug release *via* mild-thermic actuation • Stimuli-mediated drug release and brain diffusion (temperature-dependent) • Adhesion force studies on bovine muscle tissue • Efficacy studies on tumor resection models in mice and mongrel dogs • Clinical translation expected soon	[218]
Injectable drug eluting seeds	PLGA polymer (50:50 lactide:glycolide ratio), Kolliphor® plasticisers RH40, P237 and Kolliphor® P188	• Primary GBM cells from both the tumor core and brain around the tumor tissue of recurrent GBM patients • Cytotoxicity studies following drug release (1, 7 days) under biorelevant conditions (water)	[145]

been reported yet, this innovative sprayable DDS seems promising for further development.

Ramachandran *et al.* developed a flexible, polymeric theranostic 3D nano brain implant delivering TMZ for localized GBM treatment (Fig. 5A) [125]. They rationally selected the composition and ratios of the polymers (PLGA, PLA and Polycaprolactone) to obtain nanofiber implants (wafers) with different release profiles and selected an optimal formulation with better control on burst release for *in vivo* studies. They performed *in vivo* drug release studies on tumor-bearing mice and biocompatibility studies on healthy mice, showing very different degradation profiles in the two models (7 days *vs* 3 months, respectively). This sharp difference can be attributed to the tumor microenvironment (acidic pH, presence of enzymes, necrotic fluid, chemokines, cytokines and tumor associated immune cells) that can accelerate the degradation of the nanofibers. The authors adapted the wafer composition and mixed nanofibers with different release kinetics to obtain wafers able to provide a sufficient dose of the drug at a constant rate for prolonged times (either 1 week or 1 month). They showed that TMZ diffusion from the fast-releasing wafer could be detected up to 8 mm toward each side of the implant by 48 h from the site of implantation without systemic leakage. The biocompatibility of empty and drug-loaded wafers was demonstrated in healthy rat brain for up to three months by assessing behavior or body weight changes, brain edema (MRI) and inflammation by blood analysis (hematological parameters, pro- and anti-inflammatory cytokine levels) and histological analysis of the brain (leucocyte, immune cell infiltration, tissue thickness) and other organs. Finally, the

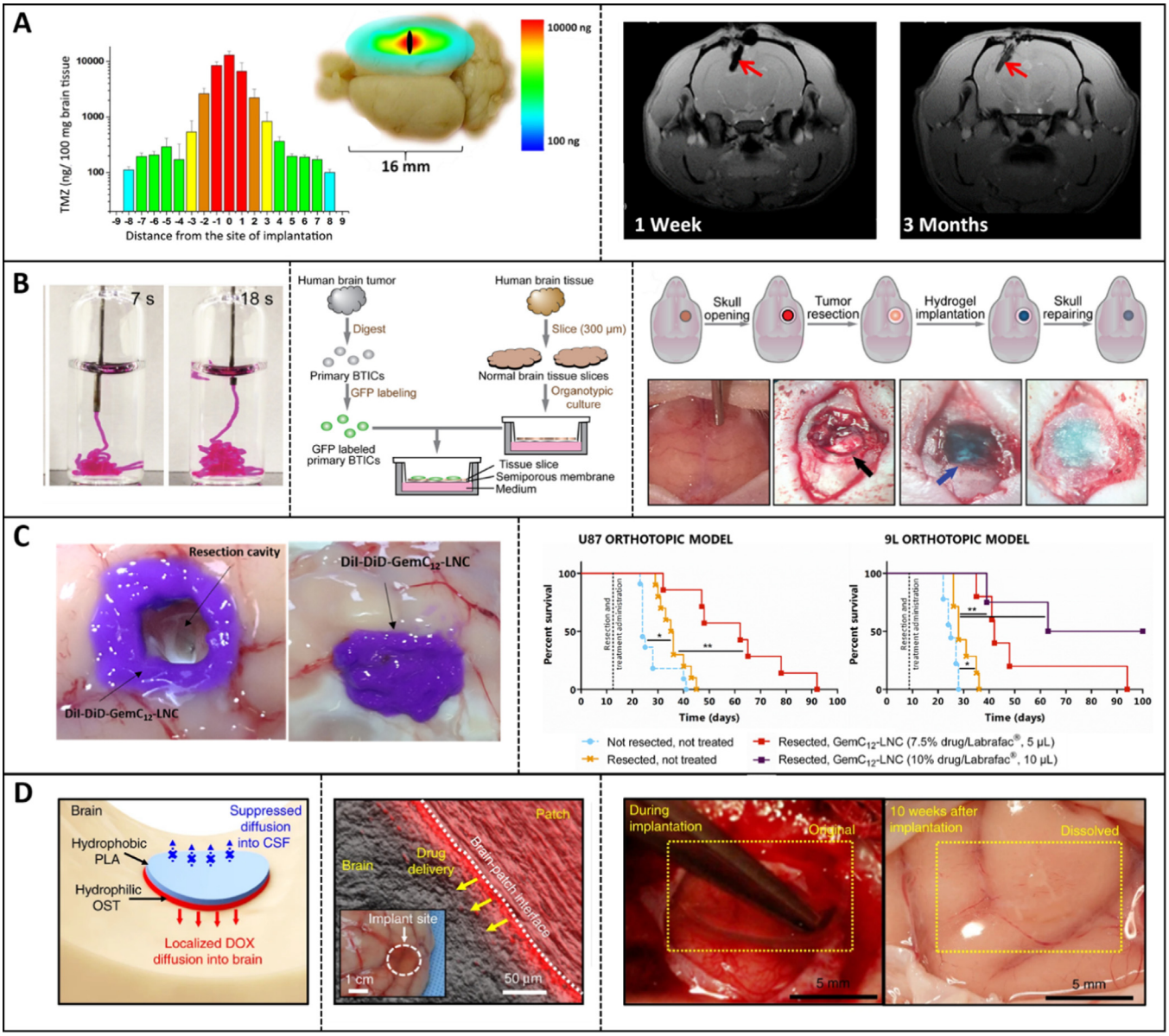

Fig. 5. Illustration of DDS for the local treatment of GBM. A) *In vivo* studies showing the TMZ distribution in the brain following intracerebral administration of nanofiber wafers (left panel) and MRI images showing the wafer in the brain at different time points (right panel) [125]; B) Injectability of the camptothecin-based self-assembling prodrug hydrogel (left panel), a schematic illustration of the brain-tissue organotypic model (central panel) and *in vivo* orthotopic tumor resection model (right panel) used to evaluate the efficacy of the DDS against brain tumor initiating cells [208]; C) Adhesion of the $GemC_{12}$-LNC hydrogel to the resection cavity borders in a pig brain (left panel) and survival curves obtained in rodent orthotopic models following local administration in the tumor resection cavity showing a delay in the onset of tumor recurrences (right panel); D) Illustration of the bioresorbable electronic patch (BEP) developed by Lee et al. (left panel), image showing the adhesivity of the BEP to the brain surface (central panel) and its *in vivo* biodegradation in canine brain (right panel). Adapted and reproduced with permission from Schiapparelli *et al.* [208], Ramachandran *et al.* [125], Bastiancich *et al.* [209] and Lee *et al.* [218]. For panels B and C, reuse is permitted under the terms of the Creative Commons CC BY license (http://creativecommons.org/licenses/by/4.0/).

therapeutic potential of these systems was evaluated using an orthotopic C6 rat model. A surgical cut was performed 3 days following cell grafting and wafers were implanted. At equal TMZ doses (3.5 mg/animal), animals treated with fast releasing wafers had a delay in tumor recurrences onset increasing their median survival compared to controls, but eventually died, whilst animals treated with slow releasing wafers showed excellent anti-tumor response (87.5% long-term survivors with no sign of recurrence at 90 days).

Gawley *et al.* tested irinotecan-loaded drug eluting seeds on primary GBM cells from both the tumor core and brain around the tumor tissue of recurrent GBM patients, to show that irinotecan is more effective than TMZ [145]. As a first step in the development of a dual polymer pro-drug/depot delivery system for GBM, Vasey *et al.* evaluated the cytotoxicity of DOX and DOX-nanoparticles on primary cancer cell lines derived from patients following GBM resection, isolated from the invasive margins of the tumor (GIN lines) [206]. These models provide responses on the potential therapeutic efficacy of the drug (or, better, on the sensitivity of that specific cell line to the compound), but they do not mimic the physiological conditions in which the DDS will release the drug. Therefore, they do not provide information on their impact on the TME, TRME or the response to standard of care treatment (*e.g.* increase of expression of genes correlated to TMZ resistance). To determine how the DDS and sustained drug-release play a role on the cytotoxic effect and how they might interact with the TME,

two recent studies have used more complex models for evaluating DDS *in vitro*. Smith *et al.* developed a PLGA/PEG microparticle matrix tailored to incorporate etoposide and active TMZ within a low pH environment, obtaining a dual DDS able to target high intratumor molecular heterogeneity of GBM [207]. The system is a paste at room temperature when drug and polymer is mixed with saline, and which solidifies (sinters) at body temperature. The drug-loaded paste can be placed in close contact to the resection cavity borders, minimizing the diffusion distance to the invasive tumor margins. This formulation shows a high burst release of the two drugs (70% of TMZ and 60% of etoposide are released at day 1). The *in vitro* cytotoxicity of the free drugs was tested on two established GBM cell lines and four primary patient-derived GIN lines. To address TMZ instability, the authors used an organic acid-based carrier to ensure that TMZ would not be converted into its active form until diffusion-mediated release from the polymer. To confirm this, they used inserts suspended over 9L glioma cells where they applied their drug loaded PLGA/PEG matrices to evaluate the cytotoxic effect on cells directly exposed to drugs released from the DDS. Combination indexes confirmed the synergy between the two drugs on this cell line. The 9L orthotopic tumor resection model was selected both for the safety and efficacy *in vivo* experiments. The efficacy of the system was compared to standard of care treatment and showed the high potential of intracavitary TMZ/etoposide PLGA/PEG paste treatment as adjuvant of RT, with a significant overall survival benefit and long-term survivorship with post-sacrificial brain histological sections revealing disease-free brains in animals treated with PLGA/PEG/TMZ/etoposide.

Schiapparelli *et al.* developed a camptothecin-based self-assembling prodrug able to spontaneously form a supramolecular filament hydrogel upon contact with brain tissue (Fig. 5B) [208]. The drug could be steadily released from the gel (17% of the prodrug was released over 30 days in DPBS), but this rate could be easily tuned and optimized to the required profile by varying the molecular design and the concentration of the prodrug. The prodrug was efficiently converted to free camptothecin (glutathione-triggered activation) and shown to be cytotoxic on human-derived brain tumor initiating cells (BTICs). To study the viability of BTICs in response to the gel, the authors established human organotypic explants grafted with BTICs and directly applied the DDS on top of the infiltrated BTICs-slices. Cell growth was monitored by fluorescent microscopy over one week, to analyze the impact of the DDS on the proliferation and infiltrative behavior of the tumor cells. This was a very elegant way to appropriately address the release of the drug and its cytotoxic efficacy at the same time, on a pertinent and well-conceived cellular model. Finally, the authors showed the antitumor efficacy of their system using a highly aggressive orthotopic primary GBM resection mouse model, showing a significant delay to eventual recurrence relative to controls. In the future, it would be interesting to evaluate how this promising DDS - which is injectable, tunable and has a very simple composition - acts in combination with TMZ and RT.

In our group, we evaluated the feasibility, safety and efficacy of an injectable gel-like nanodelivery system consisting of lipid nanocapsules (LNC) loaded with the prodrug $GemC_{12}$ for the local treatment of GBM (Fig. 5C) [45,78,142,209]. This injectable and biodegradable hydrogel is easy to formulate and scale-up, possesses mechanical properties adapted for brain implantation and shows sustained release of the drug for one month *in vitro*. To mimic the clinical setting, we developed and validated a 'biopsy punch' surgical technique to resect orthotopic U-87 MG tumors providing a reliable and clinically relevant tool to test the efficacy of a wide range of DDS [78,181]. After perisurgical administration in the tumor resection cavity, $GemC_{12}$-LNC hydrogel delayed the formation of tumor recurrences. In syngeneic immunocompetent rat bearing 9L gliosarcoma, we showed that both $GemC_{12}$-LNC and $GemC_{12}$ can delay or even inhibit the formation tumor recurrences depending on the dose administered. We had to increase the drug loading and tune mechanical properties to reduce the volume administered. As the volumes of CSF and blood, and the intracranial pressure in humans, are much higher compared to rodents we will eventually have to find solutions to increase the bio-adhesivity of our system for effective clinical translation. Moreover, while this system led to promising results delaying the onset of tumor recurrences, our results confirmed that monotherapeutic DDS (as Gliadel®) aimed at only killing tumor cells might not be enough to avoid GBM relapse in the long-term. This is true for several reasons: firstly, the high degree of heterogeneity of GBM tumors requires combination strategies to act on different tumor cellular populations and to overcome suboptimal efficacy due to acquired chemoresistance or technical constraints related to the therapeutic strategy used; secondly, the non-cancerous cells in the post-resection cavity borders can significantly differ in type, number and activation state compared to the TME, where they can vary over time depending on the inflammatory response phase and can modify the TRME response to treatments. The nanomedicine $GemC_{12}$-LNC has a targeting capacity for myeloid derived suppressor cells (MDSCs), and it was shown that surgical resection induces a reduction of MDSCs compared to untreated animals both immediately after resection [210] and in the recurrent tumors [211]. Lastly, in syngeneic tumor models, recurrences appear very fast after surgery and they are more aggressive than the primary tumors [78]. These results confirm how combining information from different preclinical models, and including models able to mimic the TRME, can provide a better overview of the clinical potential of the DDS.

Graham-Gurysh *et al.* developed biodegradable, acid responsive PTX-loaded acetylated dextran nanofibrous scaffolds with different degradability rates and combined them to obtain the best performing drug release rate *in vivo* [212]. Based on analysis of the translational impediment of previous scaffolds, the authors developed their DDS with a nanostructure able to maintain a high surface area to volume ratio when scaled up, while still ensuring consistent drug release kinetics. They evaluated drug release from the scaffolds *in vivo* after implantation into a resection cavity in mice with or without tumors. Interestingly, not only did they observe a difference in PTX release between healthy and tumor-bearing animals, but also differential release depending on the size of the tumors (smaller tumor, with smaller acidic surface area released PTX at a slower rate). Finally, the authors evaluated the efficacy of their DDS on a mCherry-U87-MG resection model showing complete inhibition of tumor recurrences in 78% of the animals treated with the fast/slow release mixed scaffold (78% long-term survivors).

A recent example of a DDS that has been developed carefully considering the TMRE, using fit-for-purpose biomaterials and characterized using appropriate *in vitro* and *in vivo* models, is the injectable matrix metalloproteinase (MMP) enzyme-responsive hydrogel described by Zhao *et al.* [213]. The authors evaluated the release of TMZ and O6-benzylamine (BG, MGMT inhibitor) from these MMP-responsive hydrogels in the presence or absence of MMP (first in PBS +/- MMP9; then in PBS +/- CSF from post-operative GBM patients, with and without MMP inhibitor), demonstrating that their presence was required for hydrogel disassembly and drug release. Then they proved that MMP9 enzyme is present in high concentrations in the postsurgical environment of glioma-bearing mice, demonstrating how their system can specifically exploit a microenvironmental change in the TMRE to release the drugs and kill infiltrating cancer cells. The therapeutic potential of this DDS was confirmed *in vivo* using a C6 rat resection model, showing that the local administration of the TMZ + BG gel had

superior anti-glioma efficacy than TMZ alone (administered either locally or systemically).

Combining immunotherapy and local delivery of anticancer drugs for brain tumors has increasingly attracted the attention of researchers [214], with three recent studies renewing this therapeutic approach. Mathios *et al.* have tried to evaluate in which conditions local or systemic chemotherapy can potentiate immunotherapy [215]. They showed that locally delivered chemotherapy (50% TMZ or 3.8% BCNU loaded PCPP-SA wafers) can maintain and potentiate glioma immunotherapy (anti-programmed cell death protein 1 (PD1) monoclonal antibody) to a much higher extent compared to systemic chemotherapy, which is immunosuppressive, does not work in synergy with anti-PD1 and causes severe lymphodepletion when combined with immunotherapy. Their study emphasizes the fact that evaluating the order, timing and delivery methods of combination strategies can have a positive impact on the obtained efficacy and opens the doors for future combinatory treatments. Chao *et al.* used a cocktail chemoimmunotherapeutic hydrogel formulation mixing immunogenic cell death-inducing chemotherapeutics (DOX), immune adjuvants (imiquimod) and alginate for brain tumors [216]. They combined it with local (mix in the gel) or systemic (intravenous administration) anti-PDL1 immunotherapy and compared it with systemic TMZ treatment, showing high therapeutic potential (powerful systemic antitumor immune response leading to complete remission of the tumors in 100% of animals) in an orthotopic isogenic glioma mouse model. This model was generated from engineered glioma cancer cells (P5 C57 neural stem cells transformed by transducing lentivirus containing P53 and NF1 tumor suppressor guide sequences) to evaluate the therapeutic effects of the combined treatment. Even though the authors did not provide further characterization on the glioma model generated and therefore we do not know if it recapitulates key characteristics of human GBM or lower-grade gliomas, this is an excellent and promising example of local DDS for future clinical application in GBM. The DDS is simple to prepare and easy to scale up, is formulated as a lyophilized powder with long stability, and can be dispersed in aqueous solution to form a gel. It is produced under GMP standards and the sterilization and endotoxin controls of alginate have been realized. The authors state that a startup company has been established, and we might therefore expect to see this formulation in clinical trials in the next few years. A self-assembly injectable oligopeptide hydrogel able to stimulate tumoricidal immunity towards GBM cells following surgical resection has also recently been developed. The hydrogel was loaded with *i*) the chemotactic CXC chemokine ligand 10 and *ii*) a DDS consisting of Zinc 2-methylimidazole-based nanoparticles loaded with mitoxantrone (immune cell death trigger) and small interfering RNA targeting indoleamine 2.3-dioxygenase (endogenous immunosuppressive mediator). The nanocarrier was coated with glioma-associated macrophage membrane to obtain a tumour-homing immune nanoregulator DDS. After local administration in the resected cavity, the hydrogel can switch the "cold" tumor immunity of GBM into "hot", significantly reducing the postoperative recurrences by inducing sustained T-cell infiltration [217].

Lee *et al.* recently published impressive data on a drug-loaded, flexible, sticky and bioresorbable electronic patch (BEP) integrated with wireless electronics to control intracranial drug release via mild-thermic actuation (Fig. 5D) [218]. This system shows very high potential for clinical application, and all experiments were performed to ensure its translation to the clinics within a short time-frame. The BEP DDS has a bifacial structure composed of a hydrophilic drug-loaded oxidized starch film with imine conjugation to guarantee adhesivity to the brain tissue and provides long-term sustained release, and a hydrophobic PLA encapsulation film which reduces undesirable drug leakage into the CSF. Magnesium-based ultrathin electronic devices are embedded between these films and work as wireless heater and thermic sensors, controlling drug release, accelerating intercellular drug diffusion, and enhancing drug penetration depth. The BEP are packaged and sterilized before use. The softness and strong adhesion of the system were adapted to allow for complete adaptability and bioadhesiveness to the tumor resection cavity tissue, as demonstrated by adhesion force studies between bovine muscle tissue and OST films. The conformal contact between the BEP and the brain tissue is also maintained during degradation, as demonstrated in a canine model. All the components of the BEP are biodegradable and dissolved in canine brain within 10-weeks without debris or side effects. BEP can be loaded with different anticancer drugs (*e.g.* DOX and TMZ) for combination therapy. The biocompatibility of DOX-loaded BEP was evaluated in nude mice, showing absence of local immune responses and of no neurological deficits or abnormal behaviors following implantation in the surgical cavity. DOX release can be triggered and controlled by wireless mild-thermic actuation at 42 °C, which also enhances drug diffusion due to increased cell membrane permeability. The anti-tumor efficacy of DOX-loaded BEP followed by mild-thermic treatment was tested on orthotopic tumor resection models in mice and mongrel dogs (mouse model: 3 mm diameter, 0.13 mg DOX; canine model: BEP 12 mm in diameter, 1 mg DOX), showing superior tumor growth suppression compared to BCNU-loaded CPP:SA wafers [218].

A treatment that might lead to promising results and an increase in patient survival in the future is Temodex. Temodex is a gel formed of a polymeric carrier (dextran phosphate sodium salt) encapsulating TMZ. It is stored as powder and once reconstituted, it rapidly forms a gel that can be administered following GBM surgery, allowing the delivery of high local concentrations of TMZ in the tumor resection cavity [219]. The TMZ release from the inert carrier is very fast, and initiates chemotherapy immediately following surgery using high doses [220]. This product was developed at Belorussian State University in Minsk thanks to state sponsorship and has been approved in Belarus for intracerebral administration as first line treatment in GBM patients since 2014 as adjuvant to standard therapy (surgery, RT and systemic TMZ). In clinical trials performed in Belarus, Temodex showed an increase in the overall survival of patients in the treatment arm compared to the controls (median overall survival 55.57 *vs* 41.36 weeks, respectively, 10% MGMT methylation threshold), and analysis on the tumor tissue samples showed that its efficacy is independent on the MGMT promoter methylation status of the patients [221]. The authors suggest that this effect can be due to the high local concentration of TMZ (which is fastly released from the gel) which leads to a more potent and rapid cytotoxic effect on tumor cells compared to systemic treatment as the cytotoxic effect of TMZ relies on the regulation of several signaling pathways and tumor cells apoptosis can be induced independently of MGMT [222]. Since 2015, the Swedish public company Double Bond acquired the marketing rights for Temodex worldwide (except Eurasian Economic Union and Ukraine), and was granted Orphan Drug Designation by the European Medicines Agency in August 2016 for the treatment of Glioma (EMA number: EMA/OD/085/16). The company is now pushing the development and validation of this DDS to obtain its registration in the EU under the name SI-053. They have identified ten preclinical and clinical milestones (*e.g.* key opinion leader meeting with Westphal and Dirven to fine-tune the Phase 1 clinical plan; *in vivo* efficacy in mice; stability studies; pharmacokinetic studies in rats; sterilization; long term toxicity in rats in combination with TMZ chemo-radiation) that

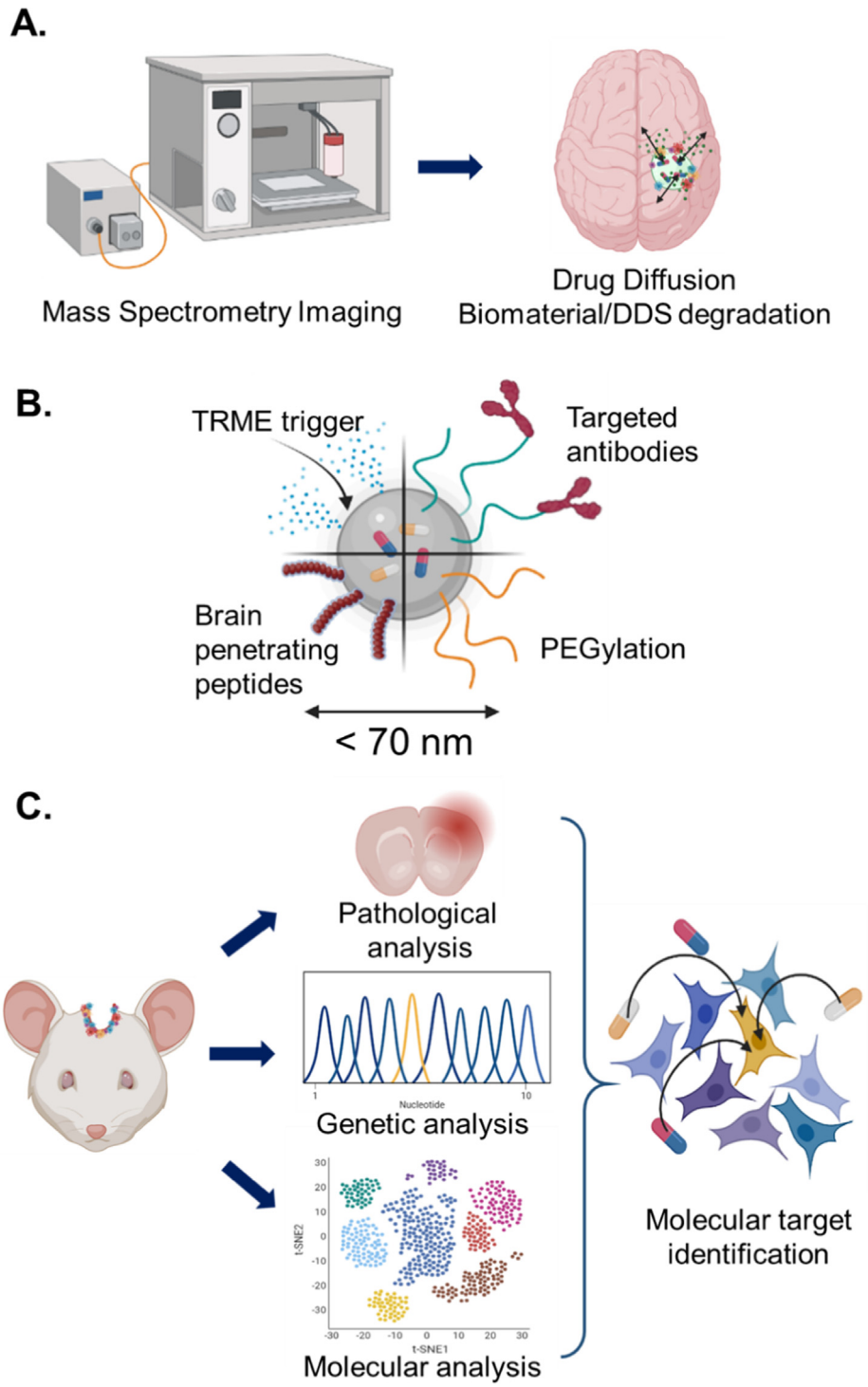

Fig. 6. Illustration of future perspectives for efficient DDS development applied to the local treatment of GBM. A) Mass Spectrometry Imaging as a tool to detect brain penetration of drug and degradation of biomaterials/DDS; B) Schematic representation of nanoparticle with distinct moieties to help brain penetration and diffusion; C) Illustration of combined pathological, molecular and genomic analysis for identification molecularly targeted drug compounds for next generation DDS.

will be reached between 2020 and 2021 to start Phase 1 clinical dose escalating trials in Europe on newly diagnosed GBM patients in addition to standard of care treatment in the second semester of 2021 [223].

5. From bench to bed side: Bridging the translational gap

A detailed, comprehensive, and accurate characterization of the DDS – combined with appropriate intellectual property, technology transfer and financial strategy - is necessary in order to bridge the translational gap and initiate clinical trials [224]. For example, experiments on more advanced animal models (*e.g.* spontaneous tumors, companion animals) or phase 0 trials might be developed to provide more information on the safety and potential benefit of the DDS [225].

Almost all pre-clinical models used to evaluate GBM drug delivery technologies to date, including those recently reported by us [45,207] rely on overall survival as an indirect proxy of brain pen-

etration of efficacious drug concentrations. Whilst overall survival is a desirable success outcome, reliance on this metric raises challenging questions when a significant survival benefit is not observed (*e.g.*, whether lack of efficacy is due to poor drug tissue penetration at therapeutic concentrations, or due to GBM-intrinsic cellular and molecular resistance mechanisms). Indeed, accurate quantitative or even semi-quantitative measurement of drug penetration within brain parenchyma is an unmet scientific bottleneck. We encourage the brain tumour drug delivery research community to strive to decouple drug brain penetration from the distinct successful application of a localized drug delivery system, and to build in relevant complementary tools when designing pre-clinical studies. For example, the emergence of next-generation label-free MSI modalities such as matrix-assisted laser desorption/ionization MS and 3D-orbitrap secondary ion MS, provides a potential means to visualise and quantitate delivered drug and tissue analytes in the brain, recently expounded by us and others [226,227]. (Fig. 6A)

Advances in therapeutic nanoparticle design have shown promise in enhancing penetration of brain parenchyma and include dense PEGylation with nanoparticle diameter $\leq$ 70 nm to promote movement of nanoparticles along white matter tracts [148], cell penetrating peptides [228] and brain tumor-specific targeting of nanoparticles [229] (Fig. 6B). However, an overarching caveat remains, whereby current rodent pre-clinical models may be inappropriate to recapitulate the true infiltrative extent of GBM which manifests clinically. At present, rodent xenograft and allograft orthotopic brain tumor models are particularly amenable for surgical resection and are widely utilized in the evaluation of GBM intra-cavity and direct interstitial delivery. Spontaneously occurring *de novo* GBM in canines [230,231] presents a potential viable alternative for assessing localized drug delivery against infiltrative disease; however, these studies typically provide anecdotal evidence from relatively few animals, permitted on compassionate grounds and where there is no means to compare a DDS against standard-of-care controls. Even where a veterinary research facility may prospectively attempt to overcome this, there are significant challenges for adequate treatment and control arms. Ultimately, more clinically-accurate *de novo* transgenic GBM syngeneic rodent models which better recapitulate infiltration, may emerge as the most reliable pre-clinical models which predict phase I safety and phase II response in GBM patient clinical trials.

Next-generation localized DDS for GBM must also consider rationally designed or repurposed molecular targeted drug compounds in combination, predicated on integrative omics of GBM infiltrative disease. (Fig. 6C) We have shown that GBM cells derived from the 5-aminolevulinic acid-based fluorescent invasive margin, harbour a sub-population(s) in closest proximity to residual disease spared by surgery, and which better reflects residual GBM genotype and phenotype [232]. Whilst personalised genomics may predicate personalised drug delivery, new challenges are presented for clinical trial design. Furthermore, as localized DDS will almost certainly be applied post-surgery and thereby the target tumor tissue will be infiltrative residual disease, DDS which are designed to deliver therapeutic cargos in hypoxic/reducing microenvironments, may not be optimal. Rather, a better understanding of the molecular and cellular basis of the brain microenvironment within the GBM infiltrative margin, will enable the design of more clinically-accurate DDS.

It is also imperative that next-generation localized delivery systems for GBM are designed to be fit-for-purpose for surgical theatre. Ease and rapidity of product application by the operating neurosurgeon should be given high priority. In addition, scalability of a DDS for clinical use, GMP-able characterisations and early engagement with regulatory agencies should be considered as early as possible during the pre-clinical research pipeline, particularly as most DDS are likely to be regulated as 'drug' due to a lack of medicinal value from a drug-free DDS.

As almost all GBM patients will undergo maximal tumor resection as a first-line intervention, localized drug delivery will endure as an attractive means to initiate oncological treatment immediately post-surgery. If many of the challenges outlined in this review are overcome in the coming years, there is much reason for optimism that localized delivery of high therapeutic concentrations of drug combinations may significantly prolong survival, whilst minimizing/avoiding dose-limiting systemic toxicities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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