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Injectable local drug delivery systems for glioblastoma: a systematic review and *meta*-analysis of progress to date†

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Glioblastoma (GBM) is an aggressive malignant cancer associated with bleak prognosis and high mortality. The current standard of care for GBM is maximum surgical resection plus radiotherapy and temozolomide (TMZ) chemotherapy. The blood brain barrier (BBB) remains the main obstacle for chemotherapy and severely limits the choice of therapeutic agents. Local treatment allows drugs to circumvent the BBB and reduces systemic side effects. Despite much research effort, to date, no drug delivery system (DDS) designed to be directly injected into brain tumors has been clinically approved, and a systematic overview of the progress in this field, or lack thereof, is missing. In this review, a systematic search of pre-clinical literature was conducted which resulted in 36 original articles on injectable DDS for local treatment of GBM which met the inclusion criteria. A wide range of injectable DDS have been developed and tested preclinically which include nanoparticles, liposomes, microspheres, hydrogels and others. *meta*-Analyses of the included studies showed that, overall, local administration of injectable DDS was beneficial to increase the animal's survival time. Finally, this review summarized the therapeutic effect after local treatment and discussed the shortcomings of the experimental setting in *in vivo* studies.

1. Introduction

Glioblastoma (GBM) is a primary malignant brain tumor of the central nervous system, associated with poor clinical outcome and high mortality.[1] Surgery is the mainstay of GBM management as it allows to reduce the tumor mass, relieve symptoms and obtain samples to confirm the diagnosis. Moreover, maximum safe resection also impacts the therapeutic outcome, leading to an increase of the patients overall survival.[2] However, because of the strong infiltrating nature of GBM cells into the healthy surrounding tissue, debulking of the tumor mass is insufficient to prevent GBM recurrence.[3] Moreover, 20–30% of GBM patients are not eligible for resection, either due to the extent of tumor spread, tumor location, impaired clinical condition of the patient or other reasons.[4,5] When surgery is not recommended, a stereotactic biopsy should be performed for tissue diagnosis and for clinical decision making.[6]

In 2005, a series of clinical trials were published showing the improvement of median survival (MS) after oral adminis-

tration of Temozolomide (TMZ) combined with radiotherapy (Stupp protocol), which shifted the paradigm of GBM treatment to a combined-modality regimen.[7–9] Since then, the standard of care therapy for newly diagnosed GBM patients is maximum surgical resection followed by the Stupp protocol. At present, GBM is still incurable. The overall MS is only 14.6 to 16.6 months after diagnosis, and only around 5% to 10% of GBM patients survive over five years.[9–11] Meanwhile, patients suffer from a decline in their quality of life and loss of cognitive function.[12]

The efficacy of radiotherapy is hampered by GBM cells showing radioresistance, even at high radiation doses,[13] which in part is driven by tumor hypoxia.[14] On the other hand, several innate or acquired chemoresistance mechanisms limit the efficacy of chemotherapy. For example, GBM cells can over-express O^6-methylguanine-DNA methyltransferase (MGMT), leading to resistance to alkylating agents such as TMZ.[15] MGMT, a DNA repair enzyme, can remove alkyl groups from the O^6 position of guanine, which is a crucial target site for alkylating agents. Hence, the gene mutation is reverted, leading to the avoidance of cell death. Due to the limitations of current treatment options, most patients still suffer from recurrence, which often appear within two years.[16] Even though no drugs have received regulatory approval for the treatment of newly diagnosed GBM patients since 2005, a non-pharmacological therapeutic approach based on tumor treating fields (TTF) was approved by the Food and Drug

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Administration in 2015.[17] The TTF device is applied to the scalp and delivers low-intensity, alternating electric fields delivered *via* transducer arrays. When added to TMZ chemotherapy, this approach can slightly improve progression-free survival.[18] However, its use in clinical practice is still limited.

Standard of care treatments for recurrent GBM patients are not well defined and may vary depending on the country and the hospital rules. They are established based on previous response to treatment, clinical status of the patient, MGMT promoter methylation and disease progression. Around a quarter of patients are eligible for the second surgery who are diagnosed with symptomatic and circumscribed recurrence more than a half year after the initial surgery.[6] Re-irradiation could be performed with an altered fractionated dose depending on the tumor size, while the efficacy remains controversial.[19] Systemic chemotherapy (TMZ rechallenge, nitrosoureas, bevacizumab) is also an option for recurrent GBM and patients could be included into clinical trials if they meet the inclusion criteria.[6] In addition, although bevacizumab received accelerated approval in the USA, it is not approved for the treatment of recurrent GBM in European countries, due to no sufficient data showing the improvement of overall survival time after the combined treatment between bevacizumab and lomustine compared with lomustine alone.[20]

GBM is an unmet medical need, and finding effective, safe and long-lasting therapeutic approaches is urgently required. Local drug delivery directly to the tumor or in the resection cavity has been proposed as a means of bypassing the blood–brain barrier (BBB). This strategy allows a higher drug concentration at the tumor site and reduces systemic side effects that often-limited systemic chemotherapy. Up to now, only one drug delivery system (DDS) – Gliadel®, delivering the chemotherapeutic drug carmustine from biodegradable wafers – has been approved for the local treatment of GBM. Gliadel® wafers contain 7.7 mg of carmustine and 192.3 mg of 1,3-bis-(*p*-carboxyphenoxy)propane (pCPP) and sebacic acid (SA) copolymer (in a ratio of 20 : 80) and can be directly positioned into the resection cavity during surgery.[21] A systematic review of clinical trials utilizing Gliadel® in combination with radiotherapy and TMZ showed that the MS was extended to 18.2 months compared to radiotherapy plus TMZ (14.6 months) from previous clinical trials.[9,22] Whilst Gliadel® wafers show a modest improvement in therapeutic outcomes, its clinical use is still limited. Its rigid structure can lead to mechanical mismatch between the stiff wafer and soft brain tissue causing side effects.[23] Moreover, the amount of drug that can be administered depends on the size of the cavity (8 wafers need to be placed to ensure therapeutic dose of carmustine) and the drug release profile is sub-optimal.[24–26] Whilst an array of local DDS is being developed to overcome these drawbacks,[27] there is still no clinically approved DDS designed for stereotactic injection directly into non-resectionable tumors.

Bypassing the BBB *via* direct intratumor administration would not only facilitate a high local concentration of a drug to be achieved at the target site, but also allow a wider range of therapeutics to be investigated. These factors allow a reduction

in dose-limiting systemic toxicity whilst opening possibilities for re-purposing existing anti-cancer therapeutics, previously unsuitable due to BBB impermeability. Several methods such as convection enhanced delivery (CED) or local injection of hydrogels or colloid systems are widely investigated in pre-clinical studies. However, to date, there is no systematic overview of the current state of these delivery systems nor a *meta*-analysis of their efficacy at improving the MS in GBM animal models.

The aim of this review was to undertake a systematic review of the pre-clinical literature to tabulate injectable DDS that have been developed for GBM therapies. Furthermore, we aimed to analyze whether injectable DDS directly administered intratumorally in animals bearing GBM tumors showed therapeutic efficacy compared to negative controls (no treatment or vehicle only controls). We also analyzed the role that the delivery system plays in comparison to direct injection of a free drug. Finally, we sought to compare local drug administration with systemic administration.

2. Methods

2.1 Search strategy

Three databases (PubMed, Web of Science and Scopus) were used in this review to systemically search articles which were relevant to the topic. The search for this review was completed on 9th June 2022.

The three search strategies were as follows:

Pubmed: ((glioblastoma[Title/Abstract] OR "brain tumo*" [Title/Abstract] OR glioma [Title/Abstract] OR gliosarcoma [Title/Abstract]) AND ("local delivery" [Title/Abstract] OR "drug delivery system" [Title/Abstract] OR "sustained release" [Title/Abstract] OR microsphere*[Title/Abstract] OR microparticle* [Title/Abstract] OR "convection enhanced delivery" [Title/Abstract] OR CED [Title/Abstract] OR hydrogel* [Title/Abstract])) AND ("in vivo" [Title/Abstract] OR "pre-clinical" [Title/Abstract] OR intracranial [Title/Abstract] OR resection [Title/Abstract]).

Wed of science: TI = (glioblastoma OR "brain tumo*" OR glioma OR gliosarcoma) AND AB = ("local delivery" OR "drug delivery system" OR "sustained release" OR microsphere* OR microparticle* OR "convection enhanced delivery" OR CED OR hydrogel*) AND AB = ("in vivo" OR "pre-clinical" OR intracranial OR resection).

Scopus: TITLE-ABS (glioblastoma OR "brain tumo*" OR glioma OR gliosarcoma) AND TITLE-ABS ("local delivery" OR "drug delivery system" OR "sustained release" OR microsphere* OR microparticle* OR "convection enhanced delivery" OR CED OR hydrogel*) AND TITLE-ABS ("in vivo" OR "pre-clinical" OR intracranial OR resection).

2.2 Inclusion and exclusion criteria

After the search, the results of all databases were downloaded to a Microsoft Excel file (Home and Student 2019). Duplicate results from the three databases were removed before screen-

ing. The following inclusion and exclusion criteria were used to create a selection framework that was used whilst analyzing titles/abstracts and full text manuscripts.

Inclusion criteria were as follows:

• Original research articles written in the English language.

• Articles had at least one *in vivo* antitumor efficacy study using an orthotopic GBM tumor model without resection of the tumor prior to treatment administration.

• The study had to analyze the local delivery of anticancer agents in comparison to a negative control of either untreated animals, or a vehicle solution.

• Drug preparations used were injectable. The DDS could be drug loaded nano/macro-carriers or scaffolds loaded with either free drugs or nanomedicines.

• Studies must have had quantitative results in terms of the MS time.

Exclusion criteria were as follows:

• Articles that were not available in English.

• Review articles, book chapters and conference proceedings where methodology was insufficient for correct evaluation of the results.

• Articles that had only *in vitro* studies, clinical studies or did not have a preclinical *in vivo* antitumor efficacy study.

• Articles that used either gene therapy, monoclonal antibodies, or genetically engineered cells as an antitumor treatment.

• Articles that did not use the orthotopic GBM tumor model in *in vivo* antitumor efficacy study (*e.g.*, they used a subcutaneous tumor).

• Studies that used a GBM tumor model with resection of the tumor prior to administration of treatment.

• Studies that delivered uninjectable preparations (*e.g.*, wafers), or only delivered liquid formulations of drugs that were not incorporated into a DDS.

• Articles that did not have an appropriate negative control group of either no treatment or empty vehicle control group in *in vivo* antitumor efficacy study.

• Articles that only researched radiotherapy or only had studies combined with radiotherapy.

• Articles that did not use MS to evaluate *in vivo* antitumor efficacy.

2.3 Data extraction

Data from included articles was extracted and entered into tables with different categories. The extracted data included the year of publication, first author, drug used, delivery vehicle, cancer model and MS time for each relevant treatment group. The results were listed in the table by *in vivo* antitumor efficacy studies rather than articles. Many articles had more than one *in vivo* efficacy study, and all data from different studies which met the inclusion criteria was extracted. Different studies in the same article meant that studies had at least one difference in drug used, delivery vehicle or cancer model. Different drug dosage, times of treatment or injection site were regarded as too similar to represent separately. If two or more of the same studies were in one article, the most

appropriate study was chosen and listed in the table according to the design of the *in vivo* antitumor efficacy study or the optimized study which was focused upon by the authors. Meanwhile, if two different drugs or single drug *vs.* double drug combination were in one study, the study was listed separately by drug used.

For MS time, some data was explicitly written in the article text or tables. However, in some cases the MS data had to be calculated from the Kaplan–Meier survival curves. To achieve this, figures were extracted into Microsoft PowerPoint (Home and Student 2019) and scaled appropriately to calculate the medium survival of the relevant experimental groups.

Percentage change in MS time between the no treatment group (negative control) and the treatment group was also calculated. If the study did not have a no treatment group, the empty vehicle group was used instead as negative control.

$$\% \text{ Change MS} = \frac{\text{MS}_{\text{Treatment}} - \text{MS}_{\text{Control}}}{\text{MS}_{\text{Control}}} \times 100\% \quad (1)$$

Eqn (1). % Change MS means percentage change in median survival time, $\text{MS}_{\text{Treatment}}$ means median survival time in treatment group, $\text{MS}_{\text{Control}}$ means median survival time in the negative control group.

2.4 Statistical analysis

Review Manager software (version: 5.4.1) was used to conduct the *meta*-analysis to compare the antitumor efficacy between locally administrated DDS and the most appropriate negative control group, locally administrated DDS and locally injected free drug, locally administrated DDS, and systemic administration of either the free drug or the DDS. The MS ratio was used to summarize the MS time data.[28] It was used as a method being consistent with the hazard ratio method.[29] The survival time ratio was calculated by the following equation. It was log-transformed to give the normal distribution and make the scale symmetric.[30,31] The log-transformed MS ratio, the standard deviation (or standard error) and the group size were used as inputs for the *meta*-analysis. Due to the high heterogeneity ($I^2 > 50\%$), the random model was used in the *meta*-analysis.

$$\text{Survival time ratio} = \frac{\text{survival time of an animal}}{\text{MS}_{\text{Control}}} \quad (2)$$

Eqn (2). $\text{MS}_{\text{Control}}$ means median survival time in the negative control group.

2.5 Quality assessment

The quality of each included article was assessed by a 12-point checklist described previously by Hirst *et al.*[28] One point was allocated to each item and the final score was the sum of all points. The 12-point checklist included: (1) peer-reviewed publication, (2) sample size calculation, (3) random allocation to groups, (4) blinded assessment of outcome, (5) compliance with animal welfare regulations, (6) statement of potential conflict of interests, (7) uniform volume or number of cells inoculated, (8) consistent site of tumor implantation, (9) reported number of animals in which the tumor did not grow, (10)

stated number of excluded animals, and given reasons for exclusion, including anomalies, (11) explanation of tumor model used, or multiple glioma models used and (12) presentation of evidence that the chemotherapeutic agent acts directly against tumor.

3. Results and discussion

3.1 Results of the systematic search as a bibliographic screening of literature data

Since TMZ was adopted as the gold standard therapy of GBM in 2005, the number of papers related to the development of DDS for GBM has increased considerably. To select the papers that could answer the scientific questions addressed in this review, we performed a systematic search using three search engines and defined inclusions and exclusion criteria.

The PRISMA diagram (Fig. 1(A)) shows the screening process conducted in this review. A total of 1854 articles were

Fig. 1 (A) PRISMA diagram showing the screening process of this systematic review. (B) The quantity distribution by publication year of included articles.

searched through three databases. 1038 duplicate articles were removed, and 816 articles remained. 520 articles were excluded during the title and abstract screen due to lacking primary data (*e.g.*, review articles), being irrelevant or clearly failing to meet the inclusion criteria. The remaining 296 articles were subject to analysis of the full text. Among these articles, the full text of two articles could not be accessed and no reply was received when the authors were contacted. Finally, 36 articles met all inclusion criteria for a total of 44 individual *in vivo* antitumor efficacy studies were eligible. These studies were classified into three categories according to what comparison groups were used as follows: 1 – local DDS *vs.* negative controls (no treatment or vehicle only); 2 – local DDS *vs.* locally administered free drug; 3 – local DDS *vs.* systemic administration of either the free drug or DDS. For all studies, a negative control group receiving either no intervention, or vehicle solution only, was required from which the percentage change in MS could be calculated. Data from these studies was extracted and entered into Tables 1, 2 and 3. Fig. 1(B) shows the quantity distribution by the publication year of all included articles in this review. Only two eligible articles were published before the establishment of Stupp protocol in 2005.[32,33] However, most of included articles were published after 2008, which indicates an increasing trend of interest for more effective and safer DDS for anti-GBM use.

At this point, the data was split into three subcategories as outlined *via* the research questions below. While all the studies that met the inclusion criteria are described in the tables, a general discussion of selected papers was carried out to answer the subcategory questions. These are then followed by a *meta*-analysis of the data and a general discussion on the state of current research in this field.

3.2 Can the MS be improved by administering a DDS directly to the tumor tissue?

All the included studies had a no treatment or vehicle alone negative control group, but 19 of 44 studies had no comparison with local injection of the free drug or systemic administrations. The antitumor effect in these 19 studies, where the DDS was directly injected into the tumor, was variable (Table 1). Five studies prolonged the MS by more than 100% compared to the no treatment or vehicle control group,[34–38] whilst others barely showed an increase. In addition, two studies did not reach a significant difference between local DDS treatment group and control group.[39]

Local drug delivery widens the drug choice for the treatment of brain tumors, allowing drug repurposing of drugs that have never been exploited for this therapeutic purpose, but needs to be rationally conceived to effectively result in improved therapeutic outcome and clinical translation. For example, delivering drugs *via* nano-sized DDSs can increase the therapeutic efficacy and safety of active molecules for GBM.

Nanomedicines, which are commonly used for cancer diagnosis, therapeutic and monitoring, can target and reach tumor tissues by passive targeting (enhanced permeability and reten-
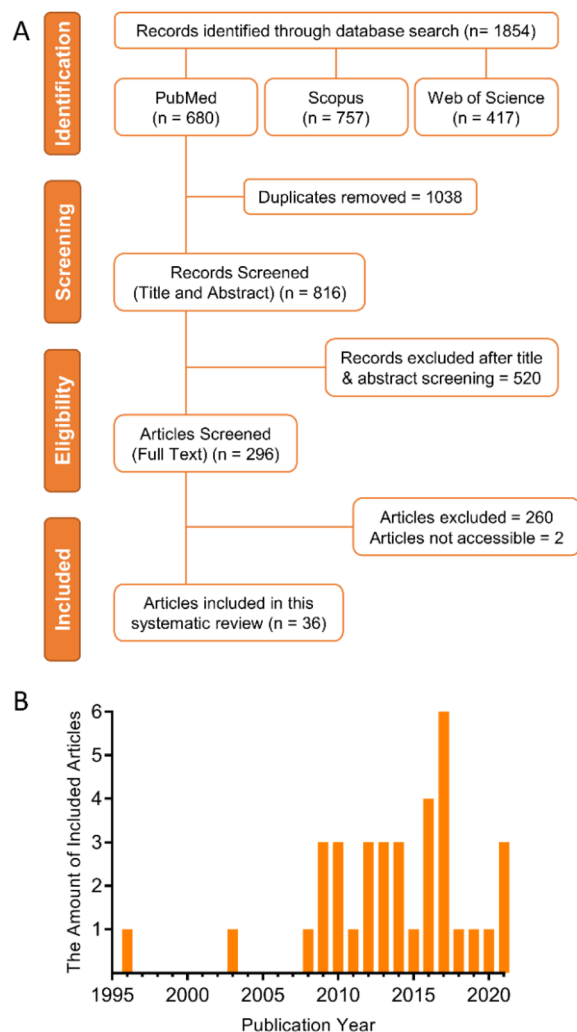
Table 1 Summary of direct injection studies with no drug comparison controls

Year	Drug used	Delivery vehicle	Cancer model	Treatment time (days) post-grafting	Number of implanted cells	Drug release pattern[a]	MS no treatment (days)	MS vehicle alone (days)	MS Local delivery via DDS (days)	% Change MS (local DDS vs. control)[b]	Ref.
2021	DOX and GEM	Hydrogel	A25 M cells – female NOD SCID mice	14	100 000	Rapid	N/A	61.5	79.5	54%	41
2021	DOX and GEM	Hydrogel	A25C cells – female NOD SCID mice	14	100 000	Rapid	N/A	54.5	82.5	51%	41
2020	CPT	Polymeric drug	GL261/fluc-DsRed glioma cells – unspecified gender NCr nu/nu mice	7	N/A	Prolonged	21.5[c]	N/A	28.5[c]	33%[d]	42
2019	EPI	Nanoparticles	U87MG Lu cells – male nu/nu mice	2 doses: 5, 12	100 000	Prolonged	N/A	27	>60	122%	34
2017	Cu(DDC)$_2$	Liposomes	F98 cells – male Fischer rats	10	10 000	N/A	N/A	20.5	25	22%	43
2017	Panobinostat	Micelles	F98 cells – unspecified gender Wistar rats	10	100 000	N/A	24.5	N/A	>60	145%	35
2016	SN-38	Micelles	U87MG cells – male F344/NJcl-rnu/rnu (nude) rats	5	200 000	N/A	N/A	21	28	33%	44
2014	BG	Nanoparticles	GBM6-luc cells – unspecified gender nude athymic mice	4 doses: 26, 29, 34, 36	20 000	Rapid	3	N/A	9	200%	36
2014	TMZ	Nanoparticles	U87 cells – male athymic nude mice	2 doses: 4, 7	500 000	Rapid	19.0[c]	N/A	28.0[c]	47%[d]	40
2013	PTX	Hydrogel	9L cells – female Fischer 344 rats	0	RP	N/A	15	N/A	33	120%	37
2013	Digoxin	Nanoparticles	BCSCs cells – unspecified gender nude rats	N/A	N/A	Prolonged	114.5[c]	N/A	124.0[c]	8%[d]	45
2012	DOX	Microspheres	BT4Ca cells – male and female BD IX rats	3	8000	N/A	19	N/A	20	5%	39
2012	Irinotecan	Microspheres	BT4Ca cells – male and female BD IX rats	3	8000	N/A	19	N/A	21	11%	39
2012	CPT	Hydrogel	C6 rat glioma cells – male Sprague-Dawley rats	7	1 000 000	RP	18	N/A	26	44%	46
2010	Fc-diOH	Lipid nanocapsules	9L cells – female Fischer 344 rats	6	1000	N/A	N/A	25	27	8%	47
2010	PTX	Hydrogel	9L cells – female Fischer 344 rats	0	100 000	RP	13	N/A	31	138%	38
2009	TPT and GD	Liposomes	U87MG cells – male nude rats	2 doses: 5, 8	500 000	N/A	20	N/A	33	65%	48
2008	DOX	Liposomes	U251MG cells – male nude (rnu/rnu, homozygous) rats	7	500 000	N/A	N/A	42.7	64.5[c]	51%[d]	49
2008	DOX	Liposomes	U87MG cells – male Fischer 344/NJc1-rnu/rnu (nude) rats	7	500 000	N/A	N/A	15.4	17.0[c]	10%[d]	49

Abbreviations: MS: median survival time; DDS: drug delivery system; Ref.: reference; GEM: gemcitabine; CPT: camptothecin; EPI: epirubicin; Cu(DDC)$_2$: copper diethyldithiocarbamate; BG: O6-benzylguanine; TMZ: temozolomide; PTX: paclitaxel; DOX: doxorubicin; Fc-diOH: ferrociphenol; TPT: topotecan; GD: gadodiamide; RP: referred to a previous protocol. [a] The drug release pattern was defined from the results of *in vitro* drug release studies. The prolonged release was defined as when the DDS released more than 10% of the loaded drugs after the first two days. Studies that did not have drug release data after day two, or the cumulative drug release from day two to the end of the experiment did not exceed 10%, were regarded as rapid release. [b] No treatment group was chosen as the negative control group to calculate the percentage change of MS. If the study did not have a no treatment group, the empty vehicle group was used instead. [c] The MS data was measured and calculated from the Kaplan–Meier survival curves (not directly stated in the article). [d] The calculation of the percentage change in the MS data used at least one MS data not directly stated in the article.

Table 2 Summary of direct injection studies with local free drug control

Year	Drug used	Delivery vehicle	Cancer model	Treatment time (days) post-grafting	Number of implanted cells	Drug release pattern[a]	MS no treatment (days)	MS vehicle alone (days)	MS Local free drug delivery (days)	MS Local delivery via DDS (days)	% Change MS (local free drug vs. control)[b]	% Change MS (local DDS vs. control)[b]	Ref.
2021	Chlorogenic acid	Gel	C6 cells – male Kunming mice	2	600 000	Prolonged	N/A	14.5	15	18.5	3%	28%	62
2021	MIT	Nanoparticles	Luci+ GL261^R132H cells – unspecified gender C57BL/6j mice	12	150 000	Rapid	23.0^c	N/A	36.0^c	97.0^c	57%^d	322%^d	53
2017	TMZ	Liposomes	CNS-1 cells – male Lewis rats	5	500 000	N/A	N/A	11.0^c	15.8	19.2	44%^d	75%^d	55
2017	TMZ	Hydrogel	GBM001 cells – unspecified gender nu/nu mice	7	20 000	N/A	N/A	20	28	38	40%	90%	57
2017	PTX	Gel	C6 cells – male Balb/c mice	3	500 000	Prolonged	N/A	15.5	18	26.5	16%	71%	56
2016	DTX	Cloudy suspension	C6 cells – male Sprague–Dawley rats	7	1 000 000	Rapid	N/A	23.8	33.4	70.2	40%	195%	58
2016	SN-38	Micelles	9L cells – male Fischer 344 rats	5	10 000	N/A	N/A	29	28	42	-3%	45%	44
2016	SQ-Gem	Nanoparticles	RG2 cells – male Fischer 344 rats	4	250 000	N/A	N/A	12	13.0^c	19.0^c	8%^d	58%^d	59
2016	Cisplatin	Polymeric drug	F98EGFR cells – unspecified gender Fischer rats	14	1000	N/A	21	N/A	76	19	262%	-10%	51
2015	PTX	Lipid nanocapsules	GL261 cells – female C57BL/6j mice	12	10 000	N/A	N/A	28	28	34	0%	21%	50
2014	DOX	Nanodiamonds	U251MG-Luc cells – unspecified gender NIH-RNU nude rats	14	RP	N/A	N/A	28.4	46.6	64.6	64%	127%	52
2014	DOX	Nanodiamonds	C6-Luc cells – male Fischer 344 rats	7	RP	N/A	N/A	31.0^c	43.8	78.2	41%^d	152%^d	52
2013	DI	Nanoparticles	GS5 cells – unspecified gender nude rats	10	500 000	Prolonged	147	N/A	177	>280	20%	90%	45
2013	PTX	Nanoparticles	U87MG cells – unspecified gender nude rats	7	500 000	Prolonged	27	N/A	30	46	11%	70%	45
2010	Am80	Micelles	U87MG cells – male Fischer 344 nude rats	7	500 000	RP	N/A	14.0^c	15.0^c	15.0^c	7%^d	7%^d	54
2009	BCNU	Nanoparticles	C6 cells – male Sprague–Dawley rats	5	1 000 000	Prolonged	12.9	N/A	14.7	22.0^c	14%	71%^d	60
2009	DOX	Micelles	9L cells – male Fischer 344 rats	7	500 000	N/A	N/A	16.9	19.6	36	16%	113%	61
2003	5-FU	Microspheres	F98 cells – female Fischer F344 rats	7	1,000	Prolonged	22.7	23.8	26.5	34.1	17%	50%	33
1996	5-FU	Microspheres	C6 cells – female Sprague–Dawley rats	7	200 000	RP	24.0^c	N/A	30.5^c	39.5^c	27%^d	65%^d	32

Abbreviations: MS: median survival time; DDS: drug delivery system; Ref.: reference; MIT: mitoxantrone; TMZ: temozolomide; PTX: paclitaxel; DTX: docetaxel; SQ-Gem: squalenoyl-gemcitabine; DOX: doxorubicin; DI: dithiazanine iodide; BCNU: carmustine; 5-FU: 5-fluorouracil; RP: referred to a previous protocol. [a] The drug release pattern was defined from the results of in vitro drug release studies. The prolonged release was defined as when the DDS released more than 10% of the loaded drugs after the first two days. Studies that did not have drug release data after day two, or the cumulative drug release from day two to the end of the experiment did not exceed 10%, were regarded as rapid release. [b] No treatment group was chosen as the negative control group to calculate the percentage change of MS. If the study did not have a no treatment group, the empty vehicle group was used instead. [c] The MS data was measured and calculated from the Kaplan–Meier survival curves (not directly stated in the article). [d] The calculation of the percentage change in the MS data used at least one MS data not directly stated in the article.

Table 3 Summary of direct injection studies with systemic direct control

Year	Drug used	Delivery vehicle	Cancer model	Treatment time (days) post-grafting	Number of implanted cells	Drug release pattern[a]	MS no treatment (days)	MS vehicle alone (days)	MS Systemic delivery (days)	MS Local delivery via DDS (days)	% Change MS (systemic vs. control)[b]	% Change MS (local DDS vs. control)[b]	Ref.
2018	TMZ	Liposomes	U87MG cells – male nu/nu mice	2 doses: 10, 17	100 000	Prolonged	25	N/A	28.5	50	14%	100%	64
2017	GemC$_{12}$	Hydrogel	U87MG cells – female NMRI nude mice	15	30 000	RP	24	28	36	49	50%	104%	65
2013	Irinotecan	Liposomes	GBM43 cells – female athymic mice	2 doses: 5, 8	300 000	N/A	19.0[c]	N/A	27.0[c]	55.0[c]	42%[d]	189%[d]	66
2013	Irinotecan	Liposomes	SF7796 cells – female athymic mice	20	300 000	N/A	41.0[c]	N/A	48.0[c]	58.5[c]	17%[d]	43%[d]	66
2012	Fc-diOH	Lipid nanocapsules	9L cells – female Fischer F344 rats	6	100 000	N/A	25	N/A	26.5[c]	11	6%[d]	-56%	68
2011	TMZ	Microspheres	C6 cells – male Sprague–Dawley rats	6	2 000 000	RP	19.5	N/A	27	46.5	38%	138%	67

Abbreviations: MS: median survival time; DDS: drug delivery system; Ref.: reference; TMZ: temozolomide; GemC$_{12}$: lauroyl-gemcitabine; Fc-diOH: ferrociphenol; RP: referred to a previous protocol. [a] The drug release pattern was defined from the results of in vitro drug release studies. The prolonged release was defined as when the DDS released more than 10% of loaded drugs after the first two days. Studies that did not have drug release data after day two, or the cumulative drug release from day two to the end of the experiment did not exceed 10%, were regarded as rapid release. [b] No treatment group was chosen as the negative control group to calculate the percentage change of MS. If the study did not have a no treatment group, the empty vehicle group was used instead. [c] The MS data was measured and calculated from the Kaplan–Meier survival curves (not directly stated in the article). [d] The calculation of the percentage change in the MS data used at least one MS data not directly stated in the article.

tion effect) or active targeting (via ligand receptor or antigen–antibody mechanisms). Singleton et al. designed a water-soluble poloxamer 407 micelle formulation loaded with the pan-histone deacetylase inhibitor Panobinostat.[35] The results showed that F98 bearing Fischer344 rats treated with the micelles by CED significantly prolonged the MS compared to untreated rats, and all treated animals survived until the end-point of the experiment (60 days). This study demonstrated a novel method of delivering a poorly water-soluble drug to brain tumor in a syngeneic infiltrative rat GBM model. In addition, the results of the in vivo toxicity study showed no adverse response after acute striatal infusion of unloaded micelles in healthy rats, showing the safety of this DDS.

Cancer theranostics represents a combined therapeutic and diagnosis approach that can be achieved using nanomedicines, to reduce delays in treatment and ease the subsequent treatment after diagnosis. Bernal et al. prepared a versatile nanoparticle formulation loaded with TMZ.[40] This multifunctional platform contained superparamagnetic iron oxide in the shell, which allowed the nanoparticles to be imaged in vivo by magnetic resonance imaging (MRI), and the surface of the nanoparticles was tagged with a fluorescent agent. Even though theranostic agents are often tested following systemic administration, the authors specifically conceived their system for a local administration (<100 nm diameter NPs with negative surface charge, to maximize convection through the extracellular space of brain parenchyma). Then, they exploited the theranostic properties of their nanomedicine to visualize by MRI their distribution in the brain following CED administration distant to the injection site. Moreover, the results of their antitumor efficacy study in the U87 MG xenograft model showed that nanoparticles significantly prolonged the MS of mice by 47% compared to untreated group.

Stephen et al. designed a multifunctional magnetic nanoparticle to deliver O^6-benzylguanine by CED thus improving its biodistribution and efficacy.[36] This DDS consisted of an iron oxide core which could be used to image the nanoparticles by MRI and evaluate the brain diffusion via CED. The chitosan shell of the nanoparticle contained sulfhydryl groups which were sensitive to a redox environment for localized drug delivery within the cells. The chlorotoxin peptide was linked to the PEG surface for active targeting of GBM cells via MMP2 and Annexin A2 binding. Following CED administration, this DDS produced an excellent volume of distribution within the brain. The results of the antitumor efficacy study in GBM6-Luc xenograft model showed that the mice treated with this DDS combined with oral TMZ significantly prolonged the MS compared to the untreated mice ($p < 0.001$). Of note, in this study, the author defined the first treatment day as day 0, which was 26 days after tumor inoculation, therefore effecting the percentage change in MS. If the tumor inoculation day was defined as day 0 just as the other studies, the MS would have been 29 days (control group) and 35 days (treatment group), meaning that the percentage change in MS would have been 21%.

In another study, the BT4Ca glioma model was used to evaluate the antitumor efficacy of doxorubicin (DOX) micro-

spheres and irinotecan microspheres administered intratumorally.[39] The results showed that the MS was only prolonged by 5% and 11% respectively compared to the no treatment group, and both treatment groups did not reach a statistically significant difference ($p = 0.85, 0.33$, respectively). The results of another study in this article showed that rats treated with unloaded microspheres or saline boosted the tumor growth.[39] The authors speculated that this might have been due to microsphere injection occurring too soon (three days) after tumor inoculation, though clearly more research is needed for better understanding of this. This negative result shows how the time of treatment impacts therapeutic benefit in GBM preclinical orthotopic models. Indeed, following grafting of cells in the brain, it is important to wait for them to form a tumor mass before treating it intratumorally. This will avoid further changes in the tissue architecture and the spreading of tumor cells from the injection site when administering liquids by CED, which would result in more infiltrated tumors and faster tumor growth.

Kikuchi et al. used two different xenograft tumor models to evaluate the antitumor efficacy of DOX liposomes.[49] For the U251 MG tumor model, nude rats treated with DOX liposomes had a significantly prolonged MS by 51%. However, for the U87 MG tumor model, nude rats that received DOX liposomes showed an improvement of MS by only 10%, which still had a significant difference ($p = 0.016$) but minimal therapeutic benefit. This difference in efficacies displayed using these two tumor models might be due – among other factors – to the difference in tumor size at the time of treatment, life span of animals within each model or the different cellular sensitivity to DOX in these two cell lines. The MS of untreated rats inoculated with U251 MG cells was 42.7 days while that of rats inoculated with U87 MG cells was just 15.4 days. In this experimental set-up the life span of the U87 MG model was rather short, perhaps limiting the evaluation of therapeutic efficacy in this experimental setting.

3.3 Does using a DDS improve the efficacy of a locally administered drug?

Similarly to the studies outlined in Table 1, a further nineteen studies analyzed direct injection of a DDS into a tumor model, but this time with the addition of a locally injected free drug comparison group. Separation of these studies (shown in Table 2) allows us to analyze whether the DDS confers any therapeutic benefit compared to the same drug administered without a delivery system. However, first it should be noted that the percentage change in MS between local DDS treatment group and the control group was highly variable, confirming the findings from Table 1.

For 89% of the studies, the animals treated locally with a DDS showed an improvement in MS compared to animals treated locally with free drug by the same administration approach. Injecting free drugs intratumorally did not prolong the MS in two studies compared to control group.[44,50] By contrast, in a few studies, local free drug administration prolonged the MS greatly.[51–53] However, except for two studies,[51,54] local

DDS treatment was more effective than local free drug treatment.[32,33,44,45,50,52,53,55–62]

Zhou et al. designed a brain-penetrating PLGA nanoparticle DDS to overcome the infiltrative and heterogeneous nature of GBM and chemotherapy resistance.[45] The surface of the nanoparticles could be modified by [^{18}F]NPB4, which allowed the DDS to be tracked by PET imaging. The antitumor efficacy of paclitaxel (PTX)-loaded brain-penetrating nanoparticles was evaluated in the U87 MG model. The results showed that the MS of rats treated with PTX-loaded nanoparticles was significantly longer than the one of animals treated with free PTX or without treatment. However, the authors were aware of the limitations of the model, including the fact that the U87 MG cells are not infiltrative and therefore do not properly mimic human GBMs. Hence, the GS5 cell line – a well-characterized brain cancer stem cell (BCSC) line – which could recapitulate the histopathology of human GBM, was used in subsequent studies. Around 2000 FDA approved compounds were screened to identify drugs which could inhibit the growth of GS5 cells by the thiazolyl blue tetrazolium bromide (MTT) assay. In vitro analysis showed that dithiazanine iodide, an anti-helminthic cyanine dye, effectively inhibited the proliferation of GS5 cells. The authors next evaluated the antitumor efficacy of dithiazanine iodide loaded brain-penetrating nanoparticles in vivo. Rats treated with dithiazanine iodide loaded nanoparticles by CED significantly prolonged the MS compared to untreated rats and rats treated with the free drug by CED.

Chen et al. developed an injectable phospholipid-based gel to deliver PTX locally.[56] The viscosity of drug-loaded gel was less than 100cp, which was suitable for injection (for injection, the viscosity should be less than 300cp).[63] The gel was added into the dialysis bag and incubated in the artificial cerebrospinal fluid to evaluate the drug release profile in vitro. The results showed that PTX release could be sustained over 30 days. The in vivo efficacy study showed that C6 bearing mice treated with PTX gel significantly prolonged the MS compared to mice treated with saline ($p < 0.01$) and mice treated with local free PTX ($p < 0.05$). On the other hand, a different study showed a totally opposite result. Barth et al. used the fifth-generation polyamidoamine dendrimer to conjugate cisplatin and cetuximab.[51] Compared with the control group, local free cisplatin prolonged the MS of rats by 262%, while the DDS slightly reduced the MS by 10% in the F98EGFR model. The in vitro results also showed that the DDS were devoid of cytotoxicity perhaps indicating that the cisplatin failed to be released from the bioconjugates.

In summary, a wide range of delivery systems have been analyzed in a variety of rodent models of GBM. It is therefore unsurprising that a broad range of outcomes has been observed, leading us to undertake a meta-analysis of the data as described later on.

3.4 Can local drug administration give higher efficacy than systemic delivery?

Of all the eligible studies included in this review, only six of them compared systemic vs. intratumoral administration of

the same drug. In all these studies, outlined in Table 3, the systemic treatment group improved the MS by less than 50% compared to untreated controls, while animals treated with local DDS showed a prolonged MS of more than 100% in four of six studies.[64–67] Except for one study,[68] local DDSs showed a better antitumor effect than the systemic administration of the same drug.

Chen *et al.* compared the antitumor efficacy of irinotecan liposomes by different routes of delivery.[66] Two different types of GBM xenograft models which use cells derived from primary human GBM tissues were used to evaluate the *in vivo* antitumor efficacy of this DDS. GBM43 cells were shown to be radioresistant and SF7796 cells were derived from a recurrent tumor. The results showed that local delivery of irinotecan liposomes by CED significantly enhanced the antitumor efficacy in both models compared to systemic administration

by tail vein ($p < 0.001$ in GBM43 model and $p = 0.048$ in SF7796 model). Laine *et al.* developed an active targeting strategy to deliver ferrociphenol.[68] NFL-TBS.40–63 peptide (cell-penetrating peptide) was inserted onto the surface of lipid nanocapsules. The results of the *in vivo* study in the 9L model showed that the intratumoral delivery by CED significantly reduced the MS, while animals treated with intracarotid administration had increased MS. While this study showed the beneficial effect of active targeting in intracarotid treatment, it also showed that CED administration of ferrociphenol lipid nanocapsules was not safe in this experimental setting leading to severe side effects.

As stated earlier, the current standard of care therapy for GBM is surgical resection followed by the Stupp protocol. TMZ is an alkylating agent able to pass the BBB following oral administration, so it is a good drug candidate to assess
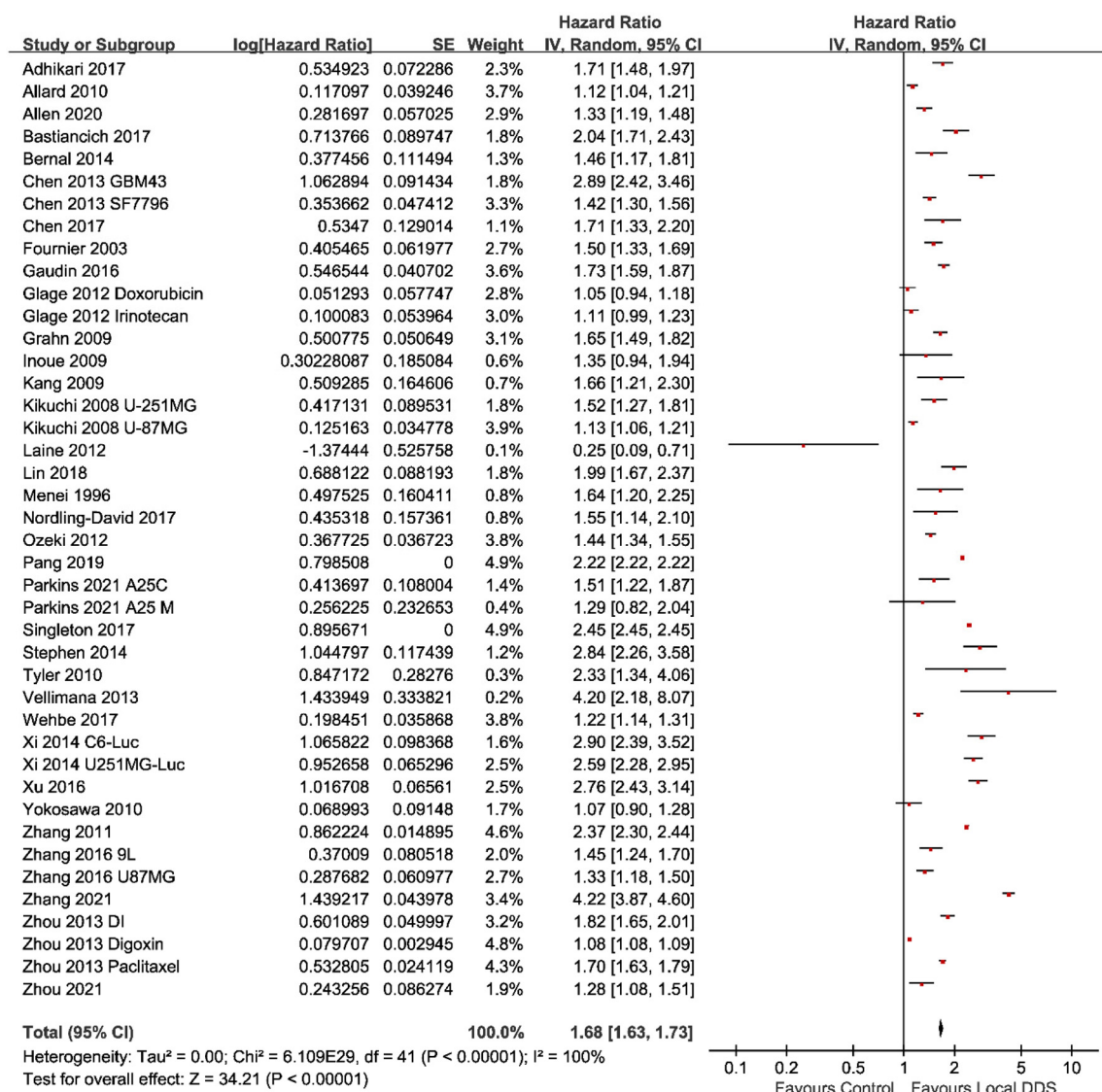
Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Adhikari 2017	0.534923	0.072286	2.3%	1.71 [1.48, 1.97]
Allard 2010	0.117097	0.039246	3.7%	1.12 [1.04, 1.21]
Allen 2020	0.281697	0.057025	2.9%	1.33 [1.19, 1.48]
Bastiancich 2017	0.713766	0.089747	1.8%	2.04 [1.71, 2.43]
Bernal 2014	0.377456	0.111494	1.3%	1.46 [1.17, 1.81]
Chen 2013 GBM43	1.062894	0.091434	1.8%	2.89 [2.42, 3.46]
Chen 2013 SF7796	0.353662	0.047412	3.3%	1.42 [1.30, 1.56]
Chen 2017	0.5347	0.129014	1.1%	1.71 [1.33, 2.20]
Fournier 2003	0.405465	0.061977	2.7%	1.50 [1.33, 1.69]
Gaudin 2016	0.546544	0.040702	3.6%	1.73 [1.59, 1.87]
Glage 2012 Doxorubicin	0.051293	0.057747	2.8%	1.05 [0.94, 1.18]
Glage 2012 Irinotecan	0.100083	0.053964	3.0%	1.11 [0.99, 1.23]
Grahn 2009	0.500775	0.050649	3.1%	1.65 [1.49, 1.82]
Inoue 2009	0.30228087	0.185084	0.6%	1.35 [0.94, 1.94]
Kang 2009	0.509285	0.164606	0.7%	1.66 [1.21, 2.30]
Kikuchi 2008 U-251MG	0.417131	0.089531	1.8%	1.52 [1.27, 1.81]
Kikuchi 2008 U-87MG	0.125163	0.034778	3.9%	1.13 [1.06, 1.21]
Laine 2012	-1.37444	0.525758	0.1%	0.25 [0.09, 0.71]
Lin 2018	0.688122	0.088193	1.8%	1.99 [1.67, 2.37]
Menei 1996	0.497525	0.160411	0.8%	1.64 [1.20, 2.25]
Nordling-David 2017	0.435318	0.157361	0.8%	1.55 [1.14, 2.10]
Ozeki 2012	0.367725	0.036723	3.8%	1.44 [1.34, 1.55]
Pang 2019	0.798508	0	4.9%	2.22 [2.22, 2.22]
Parkins 2021 A25C	0.413697	0.108004	1.4%	1.51 [1.22, 1.87]
Parkins 2021 A25 M	0.256225	0.232653	0.4%	1.29 [0.82, 2.04]
Singleton 2017	0.895671	0	4.9%	2.45 [2.45, 2.45]
Stephen 2014	1.044797	0.117439	1.2%	2.84 [2.26, 3.58]
Tyler 2010	0.847172	0.28276	0.3%	2.33 [1.34, 4.06]
Vellimana 2013	1.433949	0.333821	0.2%	4.20 [2.18, 8.07]
Wehbe 2017	0.198451	0.035868	3.8%	1.22 [1.14, 1.31]
Xi 2014 C6-Luc	1.065822	0.098368	1.6%	2.90 [2.39, 3.52]
Xi 2014 U251MG-Luc	0.952658	0.065296	2.5%	2.59 [2.28, 2.95]
Xu 2016	1.016708	0.06561	2.5%	2.76 [2.43, 3.14]
Yokosawa 2010	0.068993	0.09148	1.7%	1.07 [0.90, 1.28]
Zhang 2011	0.862224	0.014895	4.6%	2.37 [2.30, 2.44]
Zhang 2016 9L	0.37009	0.080518	2.0%	1.45 [1.24, 1.70]
Zhang 2016 U87MG	0.287682	0.060977	2.7%	1.33 [1.18, 1.50]
Zhang 2021	1.439217	0.043978	3.4%	4.22 [3.87, 4.60]
Zhou 2013 DI	0.601089	0.049997	3.2%	1.82 [1.65, 2.01]
Zhou 2013 Digoxin	0.079707	0.002945	4.8%	1.08 [1.08, 1.09]
Zhou 2013 Paclitaxel	0.532805	0.024119	4.3%	1.70 [1.63, 1.79]
Zhou 2021	0.243256	0.086274	1.9%	1.28 [1.08, 1.51]
Total (95% CI)			**100.0%**	**1.68 [1.63, 1.73]**

Heterogeneity: Tau² = 0.00; Chi² = 6.109E29, df = 41 (P < 0.00001); I² = 100%
Test for overall effect: Z = 34.21 (P < 0.00001)

Fig. 2 A forest plot of the hazard ratio showing the antitumor efficacy of locally administered injectable DDSs compared with control groups. The table showed the median survival ratio (hazard ratio), the standard error (SE) and 95% confidence intervals (CI) of each study.

whether local delivery can outperform systemic delivery. Lin *et al.* delivered TMZ-loaded liposomes directly into the tumor by CED.[64] The U87 MG-bearing mice treated twice with local TMZ liposomes had a significantly longer MS compared with the control group, while mice treated three times with oral TMZ had no significant improvement in survival. Zhang *et al.* implanted TMZ poly (D,L-lactide-*co*-glycolide) (PLGA) microparticles on the surface of the tumor tissue to evaluate the antitumor effect in the C6 glioma model.[67] Rats treated with TMZ microparticles locally had a significantly longer MS than rats treated with TMZ orally ($p = 0.002$). These studies demonstrate that local delivery can enhance antitumor effect and reduce systemic toxicities by decreasing the TMZ concentration in circulating blood.

3.5 *meta*-Analyses

meta-Analyses of the included studies were carried out to determine the overall effect size for the questions posed previously. Two eligible studies were excluded from the *meta*-analysis as the survival time of each animal was not available.[50,51] A *meta*-analysis which compared locally administrated injectable DDS with negative control groups across 42 studies was conducted firstly to analyze whether a DDS directly administrated into the tumor tissue could improve the MS *via* the hazard ratio method.[29] For the purpose of this study, the hazard ratio-based "time to event" methodology was used, which gives the MS ratio between the intervention of the local DDS *vs.* no DDS treatment controls. The diamond in the forest plot (Fig. 2) showed that the total MS ratio (indicated as hazard ratio) was 1.68 (95% confidence interval (CI), 1.63–1.73), which reached a significant difference ($p < 0.00001$). This result indicated that local administration of injectable DDS was beneficial for the survival time of animals. The heterogeneity in this *meta*-analysis was at a high degree ($I^2 = 100\%$). The reason for this is probably because studies used different model drugs, delivery vehicles, tumor cells, animal models and administration

methods. According to Tables 1–3, time of tumor incubation prior to the treatment varied from the same day of implantation to 26 days after implantation; the number of implanted cells varied from 1000 to 2 000 000. These experiment settings obviously affected the tumor size when starting the treatment, and the survival time of animals without treatment or animals in treatment groups, which would further affect the value of MS ratio. Only 16 of the 44 studies had *in vitro* drug release results included in the study. Some studies showed that a DDS with a rapid release pattern could prolong the MS of animals treated with the DDS by more than 100% compared with that in the control group,[36,53,58] while a sustained-release DDS only slightly increased the MS by less than 30% in some studies.[45,62] It might indicate that sustained-release pattern does not necessarily mean a better antitumor efficacy.

Another *meta*-analysis across seventeen studies was conducted to analyze whether using a DDS improved the efficacy of a locally administered drug. In this case, as a direct comparison was carried out between two therapeutic interventions, the mean difference method was used. Despite a large amount of heterogeneity ($I^2 = 75\%$), Fig. 3, shows that local administration of injectable DDS could enhance the antitumor efficacy significantly (mean difference = 0.17; 95% CI, 0.13–0.21; $p < 0.00001$) compared with locally administrated free drugs.

Six of all the eligible studies had a systemic treatment group using the same drug as in the local administration. A *meta*-analysis was conducted across these studies to compare the antitumor efficacy between systemic treatment and local treatment. As shown in Fig. 4, animals locally treated with DDS had a significantly prolonged MS compared with animals systemically treated with either the free drug or the DDS (mean difference = 0.17; 95% CI, 0.07–0.26; $p = 0.0007$).

3.6 Quality assessment of the included articles

Study quality was assessed by the 12-point checklist in all 36 included articles. The ESI Table S1† shows the full breakdown
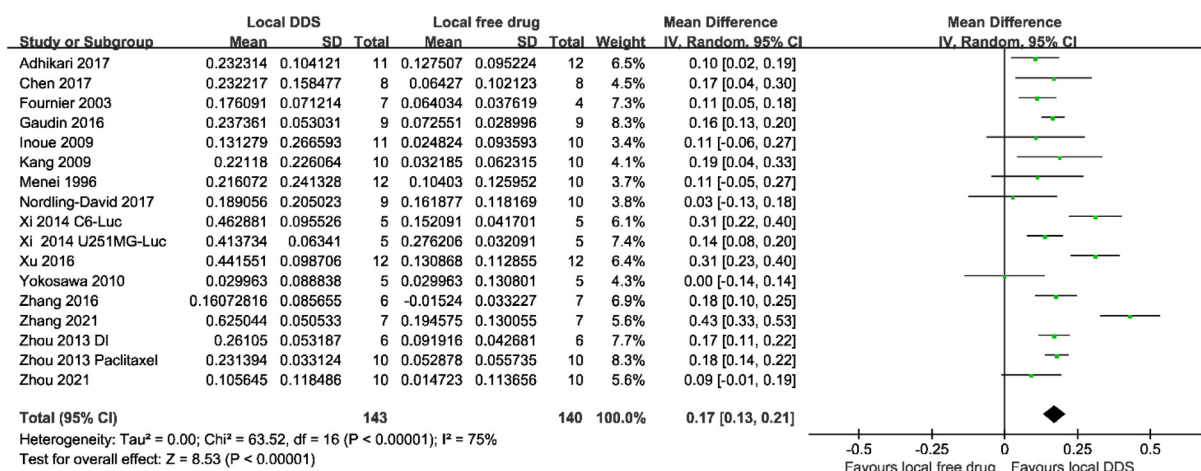

Study or Subgroup	Local DDS Mean	SD	Total	Local free drug Mean	SD	Total	Weight	Mean Difference IV. Random, 95% CI
Adhikari 2017	0.232314	0.104121	11	0.127507	0.095224	12	6.5%	0.10 [0.02, 0.19]
Chen 2017	0.232217	0.158477	8	0.06427	0.102123	8	4.5%	0.17 [0.04, 0.30]
Fournier 2003	0.176091	0.071214	7	0.064034	0.037619	4	7.3%	0.11 [0.05, 0.18]
Gaudin 2016	0.237361	0.053031	9	0.072551	0.028996	9	8.3%	0.16 [0.13, 0.20]
Inoue 2009	0.131279	0.266593	11	0.024824	0.093593	10	3.4%	0.11 [-0.06, 0.27]
Kang 2009	0.22118	0.226064	10	0.032185	0.062315	10	4.1%	0.19 [0.04, 0.33]
Menei 1996	0.216072	0.241328	12	0.10403	0.125952	10	3.7%	0.11 [-0.05, 0.27]
Nordling-David 2017	0.189056	0.205023	9	0.161877	0.118169	10	3.8%	0.03 [-0.13, 0.18]
Xi 2014 C6-Luc	0.462881	0.095526	5	0.152091	0.041701	5	6.1%	0.31 [0.22, 0.40]
Xi 2014 U251MG-Luc	0.413734	0.06341	5	0.276206	0.032091	5	7.4%	0.14 [0.08, 0.20]
Xu 2016	0.441551	0.098706	12	0.130868	0.112855	12	6.4%	0.31 [0.23, 0.40]
Yokosawa 2010	0.029963	0.088838	5	0.029963	0.130801	5	4.3%	0.00 [-0.14, 0.14]
Zhang 2016	0.16072816	0.085655	6	-0.01524	0.033227	7	6.9%	0.18 [0.10, 0.25]
Zhang 2021	0.625044	0.050533	7	0.194575	0.130055	7	5.6%	0.43 [0.33, 0.53]
Zhou 2013 DI	0.26105	0.053187	6	0.091916	0.042681	6	7.7%	0.17 [0.11, 0.22]
Zhou 2013 Paclitaxel	0.231394	0.033124	10	0.052878	0.055735	10	8.3%	0.18 [0.14, 0.22]
Zhou 2021	0.105645	0.118486	10	0.014723	0.113656	10	5.6%	0.09 [-0.01, 0.19]
Total (95% CI)			143			140	100.0%	0.17 [0.13, 0.21]

Heterogeneity: Tau² = 0.00; Chi² = 63.52, df = 16 (P < 0.00001); I² = 75%
Test for overall effect: Z = 8.53 (P < 0.00001)

Fig. 3 A forest plot showing the antitumor efficacy of locally administered injectable DDSs compared with locally injected free drug. The table showed the log-transformed data of the median survival ratio (Mean), the standard deviation (SD) and the number of animals in each group (Total). The mean difference showed the 95% confidence interval of each study.
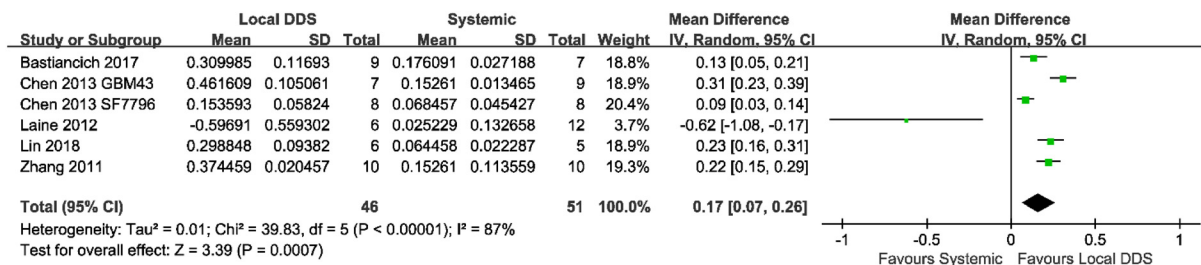
Study or Subgroup	Local DDS Mean	SD	Total	Systemic Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
Bastiancich 2017	0.309985	0.11693	9	0.176091	0.027188	7	18.8%	0.13 [0.05, 0.21]	
Chen 2013 GBM43	0.461609	0.105061	7	0.15261	0.013465	9	18.9%	0.31 [0.23, 0.39]	
Chen 2013 SF7796	0.153593	0.05824	8	0.068457	0.045427	8	20.4%	0.09 [0.03, 0.14]	
Laine 2012	-0.59691	0.559302	6	0.025229	0.132658	12	3.7%	-0.62 [-1.08, -0.17]	
Lin 2018	0.298848	0.09382	6	0.064458	0.022287	5	18.9%	0.23 [0.16, 0.31]	
Zhang 2011	0.374459	0.020457	10	0.15261	0.113559	10	19.3%	0.22 [0.15, 0.29]	
Total (95% CI)			**46**			**51**	**100.0%**	**0.17 [0.07, 0.26]**	

Heterogeneity: Tau² = 0.01; Chi² = 39.83, df = 5 (P < 0.00001); I² = 87%
Test for overall effect: Z = 3.39 (P = 0.0007)

Fig. 4 A forest plot showing the antitumor efficacy of locally administered injectable DDSs compared with systemic administration of either the free drug or the DDS. The table showed the log-transformed data of the median survival ratio (Mean), the standard deviation (SD) and the number of animals in each group (Total). Mean difference showed the 95% confidence interval of each study.

score of the quality assessment. Except one article, all articles were peer-reviewed publications. None of them mentioned how to calculate the sample size. Most of articles stated the accurate site and the number of inoculated tumor cells for the establishment of cancer model. Only three articles reported the number of animals in which the tumor did not grow and two articles used a blinded assessment for *in vivo* studies. For greater research transparency and reproducibility, we think that the points on this checklist are important for researchers of future studies to consider. In particular the blinded assessment of outcomes is simple way to remove biases in the study.

4. Conclusions

Overall, the results of this study show that local drug delivery within the tumor mass is a promising approach to overcome the intrinsic GBM therapeutic challenges. Bypassing the BBB allows researchers to increase the number of active molecules that can be explored to defeat this devastating tumor and achieve high local drug concentrations thus maximizing their therapeutic effect. While the number of articles describing the use of local treatments for GBM in preclinical models has increased exponentially since Gliadel®'s approval, the clinical trials have been limited leading to no new local treatments approved for GBM since 1997.[23] Indeed, the development of local treatments for GBM is challenging, especially for non-resectable tumors where no cavity exists for implantation. Several parameters need to be considered to properly achieve the therapeutic goal. These include a DDS design and formulation development which considers the GBM physiopathology and unique microenvironment, adapted properties for a local application in the brain and a careful characterization of its *in vivo* safety and efficacy using appropriate preclinical models.[69]

The goal of this systematic review and *meta*-analysis was to evaluate the therapeutic efficacy of DDS intratumorally delivered *via* injection in unresected GBM preclinical models. In addition, we asked whether the DDS itself plays a role in outcomes by comparison to locally injected free drug. The *meta*-analyses showed that whilst there was a high degree of variability across studies, local injection of delivery systems resulted

in an improvement of MS compared to control groups and to local injection of free drugs. This study also showed that local drug administration *via* a DDS could outperform systematic administration of free drugs. These results indicate that local delivery still holds promise for this scope, but efforts should be made to standardize the methods used to test such systems to compare their efficacy.

Indeed, the therapeutic effect observed following intratumoral administration can be subject to many variables and biases. For example, the GBM model chosen for the study can directly impact the outcome as less infiltrative cell lines are likely to respond better to local treatment compared to more aggressive models. Also, the volume injected, the injection rate, the drug dose, and the time of administration – which vary between studies – can make it very difficult to compare data between different groups (or between different investigators working in the same group). Moreover, the tumor size at time of treatment is not always measured and/or reported so it is difficult to replicate the studies and understand the real impact of the treatment in the long-term.

Most of the papers reported in this review used xenograft models using established GBM cell lines to test the efficacy of local treatments. While these models lead to reproducible and reliable experiments with high engraftment and growth rates – and some of them have shown to still mimic the true biological nature of GBM[70] – cells are injected in the brain of immunodeficient animals. In recent years, the cross talk between glial cells and immune cells (brain resident macrophages as well as immune cells recruited from the periphery) has been demonstrated to impact tumor cell behavior. Testing local treatments on several models could help our understanding of the real therapeutic efficacy and potential long-term impact, thus partially filling the preclinical-clinical gap.

Whilst the prognosis for GBM is universally poor, there is no local intervention for non-resectable tumors. Stiff wafers such as Gliadel® are not only unsuitable but suffer from rapid release of a monotherapy. The studies in this review show promise in this regard (soft hydrogels and injectable formulations) though it is likely that delivery of multiple drugs acting *via* differing mechanisms would be beneficial. Key considerations moving forward will be the evaluation of drug penetration to residual GBM cells proliferating deeper into the

brain parenchyma,[71] obtaining optimal drug release profiles *in vivo*,[23] and multifaceted modes of therapeutic action. However, to date the *meta*-analysis conducted herein shows clear rationale for the continued development of injectable local drug delivery devices for GBM therapeutics.

Author contributions

Yu Wang: methodology, formal analysis, data curation, writing – original draft; Chiara Bastiancich: writing – original draft, writing – review & editing; Ben Newland: conceptualization, methodology, writing – review & editing.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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