Development and in vivo evaluation of Irinotecan-loaded Drug Eluting Seeds (iDES) for the localised treatment of recurrent glioblastoma multiforme

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Development and in vivo evaluation of Irinotecan-loaded Drug Eluting Seeds (iDES) for the Localised Treatment of Recurrent Glioblastoma Multiforme

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Key words: Glioblastoma; Irinotecan; PLGA; local delivery; sustained release; Drug Eluting Seeds.

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Abstract

Glioblastoma multiforme (GBM) is impossible to fully remove surgically and almost always recurs at the borders of the resection cavity, while systemic delivery of therapeutic drug levels to the brain tumour is limited by the blood-brain barrier. This research describes the development of a novel formulation of Irinotecan-loaded Drug Eluting Seeds (iDES) for insertion into the margin of the GBM resection cavity to provide a sustained high local dose with reduced systemic toxicities. We used primary GBM cells from both the tumour core and Brain Around the Tumour tissue from recurrent GBM patients to demonstrate that irinotecan is more effective than temozolomide. Irinotecan had a 75% response rate, while only 50% responded to temozolomide. With temozolomide the cell viability was never below 80% whereas irinotecan achieved cell viabilities of less than 44%. The iDES were manufactured using a hot melt extrusion process with accurate irinotecan drug loadings and the same cytotoxicity as unformulated irinotecan. The iDES released irinotecan in a sustained fashion for up to 7 days. However, only the 30, 40 and 50% w/w loaded iDES formulations released the 300 to 1000 μ g of irinotecan needed to be effective in vivo. The 30 and 40% w/w iDES formulations containing 10% plasticizer and either 60 or 50% PLGA prolonged survival from 27 to 70 days in a GBM xenograft mouse resection model with no sign of tumour recurrence. The 30% w/w iDES formulations showed equivalent toxicity to a placebo in non-tumour bearing mice. This innovative drug delivery approach could transform the treatment of recurrent GBM patients by improving survival and reducing toxicity.

Introduction

The most common malignant primary brain tumour in adults is glioblastoma multiforme (GBM) [1]. It is invasive and one of the most aggressive tumours [2] of the central nervous system, having the histological features of high cellularity, nuclear atypia, microvascular proliferation, brisk mitotic activity and necrosis [3]. Because GBM is extremely infiltrative it is impossible to fully remove surgically, which means that it almost always recurs at the borders of the resection cavity. Furthermore, there is no established chemotherapy regimen available to patients who recur. GBM accounts for 45.2% of primary malignant brain and CNS tumours gliomas [4], with an annual incidence rate of 3.9 per 100,000 worldwide [5]. Despite the development of new drugs for GBM the overall survival of patients remains at just 12–15 months with a 5-year survival of 5% [6]. Increasing numbers of patients with recurrent GBM are undergoing re-operation to control their disease where conventional second line therapy has failed [7-8]. Given the extremely poor survival statistics, it follows that current treatments clearly have much room for improvement and there is a great need for the development of novel and innovative methods of treating the disease in order to prolong survival and improve the quality of life of patients.

The reason most GBM treatments fail is because they are administered either via the intravenous or oral route. Systemic delivery of chemotherapeutic drugs to the brain is difficult due to the presence of the blood-brain barrier (BBB) [9] with only low molecular weight, electrically neutral, hydrophobic cross the BBB [10-12]. Most chemotherapeutic drugs are large, ionically charged, hydrophilic molecules and thus cannot easily cross the BBB at the levels required for therapeutic effect, which means a large systemic dose is required [11, 13-15]. Even if the drug crosses the BBB it can very quickly diffuse back making it difficult to obtain constant drug levels in the brain after systemic administration. Local administration would deliver the chemotherapeutic drug directly to the tumour offering

a number of advantages such as increased bioavailability, direct delivery to the site of action, lower dose of drug required and reduced side effects due to the avoidance of systemic circulation [16]. Furthermore, local drug delivery may be suitable for the treatment of GBM as approximately 80 to 90% recur within 2cm of the resection site [13]. As a result a number of local delivery strategies such as polymer millirods [17-19], gels [20-23], micro and nanoparticle formulations [24-30] have been investigated for direct administration into the brain parenchyma of the resection cavity.

The Gliadel[®] wafer is a local delivery device, which was approved by the Food and Drug Administration in 1996 for the treatment of recurring GBM [31-32]. It is a disc-shaped, 200mg biodegradable wafer containing 3.85% w/w of the chemotherapeutic agent Carmustine. Gliadel[®] has demonstrated a small but significant benefit in combination with surgery for patients with recurrent gliomas [31-35]. However, Gliadel[®] and other similar approaches are limited by the reliance on drug diffusion from the device into the brain parenchyma restricting penetration distances to a few millimetres. Brachytherapy is a localised treatment where a sealed radioactive source or seed is placed either inside or next to the cancerous tissue. The seeds are the size of a grain of rice (3mm X 7mm) and deliver a high dose of radioactivity to the cancerous tissue, with only a small amount delivered to normal tissue thus minimising side-effects. It is mainly used in the treatment of prostate, cervical, breast and liver cancer; however, recently it has been used in the treatment of GBM and has been shown to be a feasible option for extending the life of GBM patients [36]. Between 30 and 60 seeds are inserted into the brain parenchyma of the tumour resection cavity and deliver high dose radiation directly to the tumour margin [36]. The insertion of the radioactive seeds has been shown to be safe; however, adverse side-effects have been reported due to the high dose radiation [36]. Due to the safety of inserting the seeds into the brain parenchyma our group has developed irinotecan-loaded Drug Eluting Seeds (iDES),

similar in size to the radioactive seeds and made from the biocompatible and biodegradable polymer Poly(lactic-co-glycolic acid) (PLGA). Based on the diameter (2mm) of the iDES and drug diffusion of 3 mm either side of the iDES and in order to ensure homogenous drug distribution throughout the tumour margin one iDES will be implanted for every 8 mm of tumour margin, with each iDES being implanted approximately 6 mm apart, using Mick® TP/TPV applicator. Therefore, like the radioactive seeds, we anticipate that between 30 and 60 iDES would be inserted into the brain parenchyma, depending on the size of the resection cavity and tumour margin, increasing drug diffusion into the residual tumour tissue while reducing drug diffusion out of the cavity, alleviating the wound healing complications associated with Gliadel® and other local delivery strategies.

Irinotecan (IRN) is a semi-synthetic pro-drug [37], whose active metabolite 7-ethyl-10-hydroxycamptothecin, also referred to as SN-38, acts as an inhibitor of the Topoisomerase I group of enzymes [38]. Wang et al demonstrated that IRN is converted to SN-38 after intratumoural delivery to gliomas [39]. Topoisomerase I enzymes act within the cell to induce temporary cuts within one or both strands of DNA, allowing the DNA to uncoil for transcription and replication [40]. During this process, Topoisomerase I forms a covalent linkage with DNA, allowing it to form a cleavable complex [40]. SN-38 binds to Topoisomerase I in this conformation, inhibiting the enzymes from re-joining the strands of DNA, causing S-phase specific cell killing [38, 40-41]. Currently, IRN is part of the standard treatment regimen for advanced colorectal cancer, when used in combination with 5-fluorouracil (5-FU) and folinic acid [42]. However, it has been shown to be effective in the clinic against GBM as both a monotherapy [43-53] and in combination with other drugs such as temozolomide [54-55], carmustine [56-58] and bevacizumab [59-67]. As a monotherapy IRN had a response rate between 0 and 44% with progression free survival between 2 to 11 months [68]. In combination with other drugs IRN had a response rate between 13 and

100%, with progression free survival of 3 to 12 months [68]. The 100% response rate, which is very rare in GBM, was achieved with the IRN and bevacizumab combination [63, 67]. However, these clinical studies only involved 6 and 2 patients respectively.

Even though IRN crosses the BBB, high intravenous doses between 125 and 500 mg/m² are required to achieve therapeutic levels in the brain resulting in serious systemic side effects including gastrointestinal toxicity, leading to early and late onset diarrhoea, and severe neutropenia [69]. The issue of severe diarrhoea and neutropenia and the need to increase the levels of irinotecan in the brain has spurred on recent developments in improving IRN's ability to cross the BBB.

Local delivery of Irinotecan directly to the tumour resection site could improve therapeutic outcomes by allowing for the delivery of larger dose directly to the tumour site, while reducing systemic concentrations and thus alleviating the aforementioned side-effects. Drug Eluting Beads (DEB) made from a modified, biocompatible polyvinyl alcohol (PVA) hydrogel and containing either doxorubicin or irinotecan were implanted into the brains of healthy and tumour bearing B16 IX rats and histological and survival analysis performed [70]. They showed a significant difference in the survival of rats treated with either irinotecan or doxorubicin DEB, when compared to the placebo group. There was no significant difference between the survival curves of doxorubicin or irinotecan DEB, however, there was a degree of local toxicity associated with the use of doxorubicin DEB, resulting in pronounced haemorrhage around the area of implantation of the beads, while no such effect was observed with irinotecan [70]. Our research group was the first to clinically evaluate the local delivery of Irinotecan to the brain using DEBs in a Phase I clinical study (identifier: NCT02433392) [25, 71]. This study demonstrated promising results with an absence of swelling, inflammation or any suggestion of pseudo-abscess formation compared to the pattern

normally seen with Gliadel[®] wafers indicating reduced local toxicity and reduced risk of infection. This is due to IRN being less toxic than carmustine and thus more suited for local delivery to the brain as well as it being administered directly into the brain parenchyma, rather than being placed in the resection cavity. Moreover, there was an increase in survival from 6 to 8 months, which was comparable, if not slightly improved when compared to Gliadel[®] as a historic control. None of the patients demonstrated the normal systemic drug-related toxicities associated with IRN [25]. This study demonstrates that local delivery into the brain parenchyma offers a safe therapeutic advantage over systemic delivery in the treatment of GBM, provided the choice of drug and method of administration are appropriate. However, the DEBs were only capable of delivering IRN for up to 72 hours, while most of the DEB gel formulation was pushed out of the brain parenchyma and into the bed of the resection cavity. As the iDES are solid implants they have the capability to deliver drug for a much longer duration and will remain within the brain parenchyma.

In this study we report the development of iDES formulations for the treatment of recurrent glioma. The iDES were designed to be similar in size and shape to the radioactive seeds used in brachytherapy so that they can be safely administered either directly into the tumour using stereotactic guidance and the same catheters used in brachytherapy or 'free hand' into the margin of the resection cavity after tumour debulking. In this study we will first investigate the cytotoxicity of IRN against patient derived primary recurrent GBM cells taken from both the tumour core and Brain Adjacent to Tumour (BAT) tissue to determine the level and duration of IRN release needed for iDES to be effective. We will then investigate the influence of the implant length and IRN loading on the content, stability, drug release and cytotoxicity of the iDES. Finally, the most promising formulations will be tested for their toxicity in sham resection cavities of non-tumour bearing mice and for their efficacy in a GBM mouse resection model.

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Materials and Methods

Materials

The poly (lactic-co-glycolic acid) (PLGA) DLG 4A 50:50 lactide:glycolide ratio polymer with an inherent viscosity of 0.4 dl/g (Lakeshore biomaterials) was purchased from Evonik Industries (Birmingham, Alabama). Irinotecan was purchased from LGM Pharma (Nashville, TN). Kolliphor® plasticisers RH40 and P237 were purchased from BASF (Ludwigshafen, Germany). Kolliphor® P188, acetonitrile, dichloromethane and sodium phosphate were purchased from Sigma-Aldrich (Dorset, England). GBM cell lines were retrieved from patients who received resection surgery at the Queen Elizabeth Hospital, Birmingham, UK.

Determination of the cytotoxicity of irinotecan and temozolomide against patient derived GBM cells

Unfixed Tumour core and Brain Adjacent to Tumour (BAT) tissue was collected directly from recurrent GBM patients undergoing craniotomies at the Queen Elizabeth Hospital in accordance with ethical approval (application number: 11-029) from the Human Biomaterials Resource Centre (HBRC). The samples were immediately placed in collection fluid and transported to the laboratory. Extraction of the tumour cells from the tissue and culturing of the subsequent cells was performed using standard methods. The cells were plated onto 96-well flat-bottomed microtitre plates and cultured in the presence of 200µL of cell culture media containing varying concentrations (3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000, 10,000, 100,000nM) of irinotecan or temozolomide for either 3, 5, 7, 9, 11 13 day exposure time. Cytotoxicity testing was performed using the standard 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay [72]. All experiments were performed in triplicate.

Manufacture of blank PLGA iDES using micro-hot melt extrusion

The appropriate amount PLGA polymer and plasticizer (10, 20, 30, 40 and 50% w/w) were weighed into a sealed plastic container and roll mixed for 10 minutes. The active mix was subsequently fed into a 10mm 40:1 microlab extruder (Rondol Technology, Stoke-on-Trent, UK) at a feed rate of 90 grams per hour. The feeding, mixing and metering zones of the extruder were set at 45°C, 110°C and 70°C respectively. The melt was extruded through a 2mm die and subsequently cut into blank iDES of 6mm in length.

Determination of the diameters and weights of the blank PLGA iDES

Samples (n = 10) of each blank iDES were assessed for their average weight and diameter. Each iDES was measured in the middle and at both ends, using a digital Vernier calliper. The three measurements were averaged to give the diameter of each individual blank iDES.

Investigation of the influence of plasticizer type and loading on swelling of the blank PLGA iDES

Each blank iDES (n = 4) was placed in a glass vial with 3ml of distilled water and the vials placed into an orbital shaking incubator (Infors HT) at 37°C and 60 RPM. Their length and width were measured using a digital Vernier calliper at 0, 0.5, 1, 2, 4, 6, 24 and 48 hours.

Manufacture of irinotecan-loaded PLGA iDES using micro-hot melt extrusion

The appropriate amount PLGA polymer, irinotecan (10, 20, 30, 40 and 50% w/w) and plasticizer (10% w/w) were weighed into a sealed plastic container and roll mixed for 10 minutes. The active mix was subsequently fed into a 10mm 40:1 microlab extruder (Rondol Technology, Stoke-on-Trent, UK) at a feed rate of 90 grams per hour. The feeding, mixing and metering zones of the extruder were set at 45°C, 110°C and 70°C respectively. The melt

was extruded through a 2mm die and subsequently cut into iDES with varying lengths of 2, 3 or 6mm.

Content uniformity and drug stability of the PLGA iDES

A random sample ($n = 10$) of each iDES were selected, weighed and placed into a glass vial. Dichloromethane (3mL) was added to each vial and left for 1 hour to dissolve the iDES. Once dissolved, the vials were placed in a water bath set at 60°C to completely evaporate the dichloromethane. Once completely dry, 3mL of PBS was added to the Irinotecan/PLGA residue and the vial placed into an ultrasonic bath for one minute. The vial was then placed into an orbital shaking incubator (Unitron HT infors) at 37°C and 60 rpm overnight to ensure all of the IRN went into solution. The PBS was analysed using the irinotecan HPLC method.

In vitro release of the PLGA iDES into both sink conditioned and bio-relevant release media.

Each 2 X 3 mm iDES with varying (1, 2, 20, 30, 40 and 50% w/w) loadings and 30% w/w iDES with varying (2, 3 and 6 mm) lengths ($n = 4$) were placed into a sealed flask containing either 3mL of water (bio-relevant media) or 5mL of phosphate buffered (pH 7.4) solution (sink conditioned media) and placed into an orbital shaking incubator (Unitron HT infors) at 37°C and 60 rpm. Complete media replacement was performed at 1, 2, 3, 4, and 7 days. The samples were filtered using a 0.45µm filter and analysed using the irinotecan HPLC method.

Irinotecan HPLC Methodology

HPLC analysis was performed on a Dionex Ultimate 3000 HPLC with a Phenomenex Luna C18 4.6 x 150 mm column with a 5µM particle size. The mobile phase was comprised of 75% phosphate buffer with a pH of 2.7 and 25% acetonitrile. The flow rate was 1.00mL/min,

while UV detection was performed at a wavelength of 225nm with an injection volume of 20 μ L. Linearity was found to be in the range of 0.01 to 10mg/ml with an R^2 of 1.00.

Determination of the cytotoxicity of irinotecan within the PLGA iDES on patient derived GBM cells

400 μ L of the final content solutions from the PLGA iDES were diluted with 3600 μ L of sterile cell culture media and filtered. Control solutions were produced by dissolving the required amount (based on the actual content of irinotecan in each iDES) of irinotecan in 3mL of DCM and subsequently evaporating it off. The residual irinotecan was dissolved in 3mL of PBS and 400 μ L of this solution was diluted with 3600 μ L of cell culture media and filtered. The cytotoxicity of the solutions was determined against GBM cell lines harvested from patient 1 as mentioned previously and using a 5 day exposure time.

Determination of the cytotoxicity of the Irinotecan released under bio-relevant condition from the PLGA iDES on patient derived GBM cells

A 1mL sample of the bio-relevant release media from day 1 and day 7 was frozen and stored for later cytotoxicity testing against GBM cell lines harvested from patient 1. 400 μ L of each bio-relevant release sample was added to 3600 μ L of cell culture media and filtered using a syringe filter to ensure sterility. 200 μ L was subsequently added to the wells of a 96-well plate containing the cultured patient derived primary GBM cells and 200 μ L of cell culture media. Following 5 days of exposure an MTT assay was performed to assess % cell viability.

Cytotoxicity testing of the 2 X 3 mm PLGA iDES with varying IRN loadings by placing them directly onto GBM cells derived from the BAT sample from patient 1.

BAT cells from patient 1 were plated onto 6-well flat-bottomed plates and cultured in the presence of 3mL of cell culture media. The iDES were steam sterilised and placed into the

centre of the 6-well plate. The media was changed every day to represent the turnover of CSF as well as the removal of IRN via diffusion, metabolic elimination and permeation into nearby vasculature, which will reduce the concentration of IRN at the resection margin. The study was designed so that MTT assays could be performed on the cells at days 1, 2, 3, 4, 5 and 7. The patient 1 sample was used for this study as these were the fastest growing cells and more were available due to the tissue sample being bigger.

In vivo evaluation of the toxicity of the 30, 40 and 50% w/w iDES in sham resection cavities of non-tumour bearing mice.

To assess their toxicity, a single iDES implant with 0, 30, 40 and 50% w/w IRN loadings were implanted into sham resection cavities of non-tumour bearing immunocompetent C57/BL6 mice. There was no re-administration of implants. Mice were sacrificed via transcardial perfusion at 1, 2, 4, and 8-weeks post-implant. Brains were stored in 10% formalin upon collection, then moved to 2.5% formalin after 24 hr. Brains were cut in coronal orientation at the rostral and caudal edges of the resection cavity, then embedded in paraffin blocks. Blocks were sectioned into 4 µm thick slices and stained with H&E. The resulting histological slides were examined by a blinded clinical pathologist. Degree of acute inflammation, chronic inflammation, macrophage infiltration, and necrosis were individually scored on a scale of 0-2 (low, medium, high) and summed to achieve a 'toxicity score' for each iDES formulation at each time point. The in vivo toxicity study adhered to the NIH Guide for the Care and Use of Laboratory Animals.

In vivo testing of the irinotecan-loaded PLGA millirods in a GBM mouse resection model.

Orthotopic GBM tumours were established in the brains of immunodeficient athymic nude mice (n = 5 in each group) via stereotaxic injection as previously described [73-74]. Briefly,

1×10^5 U87 mCherry-Fluc (U87 mChFl) cells in 3 μ l serum-free DMEM were loaded into a 10 μ l capacity Hamilton syringe. The needle was positioned at stereotaxic coordinates [3.0, -0.5, -1.0] from the bregma point. Tumour cells were then injected at 1 μ l/min, allowed to settle for 5 min, then the needle was retracted at 0.5 mm/min. The tumours were given one week to engraft and grow. Established tumours were then resected under fluorescent guidance, and a single 2 mm X 2 mm iDES was implanted into the resulting resection cavity. There was no re-administration of implants. Changes in tumour volume were tracked by bioluminescence. Mice were injected with 150 mg/kg luciferin H_2O , and then imaged 10 min later in an IVIS Kinetic imager under isoflurane anaesthesia. Identically-sized regions of interest were drawn over the heads of each mouse, and average radiance was recorded. The mice were monitored for survival and Kaplan-Meier survival curves produced. The in vivo efficacy study adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Statistical analysis

Statistical analysis was performed using a one way analysis of variance (ANOVA) (GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego, CA). Post-hoc comparisons of the means were performed using Tukey's Honestly Significance Difference test. A significance level of $p < 0.05$ was accepted to denote significance in all cases. Significance between groups in the Kaplan-Meier survival analysis was determined by Chi-square test.

Results

Cytotoxicity of IRN and TMZ against patient derived recurrent GBM cells taken from the tumour core

When tested against recurrent GBM cells derived from the tumour core of eight individual patients IRN and TMZ had response rates of 75.0 and 50.0% respectively (Figure 1). However, with TMZ the cell viability never went below 80.0%, even at the higher doses of 5.0 Log nM (100.0 μ M), while high dose IRN achieved cell viabilities of less than 43.8%. This data demonstrates that if IRN can be delivered at sufficient concentrations to the tumour margin after resection it has the potential to be more effective than TMZ in the treatment of recurrent GBM. The TMZ results were surprising as only one of the GBM samples (patient 6) had an unmethylated MGMT promoter. However, as these were recurrent GBMs the patients would have been treated with systemic TMZ after their initial resection surgery, resulting in the recurring GBM being chemo resistant to TMZ. The two GBMs (patient 2 and patient 6) that did not respond to IRN both had a wild type isocitrate dehydrogenase (IDH) gene making them more aggressive as mutations in the IDH gene are associated with improved survival and response to treatment in GBM patients [75-78]. This coupled with their wild type α -thalassaemia/mental retardation syndrome X-linked (ATRX) gene, which makes them less sensitive to DNA damaging agents such as IRN and TMZ [79], would make them very aggressive GBMs. However, the GBMs from patients 1, 3, 7 and 8 all responded to treatment with IRN (Figure 1) even though they also had wild type IDH and ATRX genes and thus are also considered to be very aggressive.

Fig. 1. The cell viability of primary GBM cells taken from the tumour core of 8 patients with recurrent GBM after five days exposure to increasing concentrations of either irinotecan or temozolomide.

There was no relationship between the response to IRN and the mutation status of IDH genes. The lowest IC_{50} value of 2.36 Log nM (229.1 nM) was for the GBM from patient 8 which had wild type IDH and ATRX genes (Figure 2A). However, the IC_{50} values for the GBMs from patients 1, 3 and 7 were significantly ($P = 0.01$) higher than that for patient 8, even though they also had wild type IDH and ATRX genes (Figure 2A). The sample taken from patient 4 had the same (P value = 0.84) IC_{50} value as the sample taken from patient 7. As we have seen the GBM from patient 7 was considered aggressive with wild type IDH and ATRX genes, while the GBM from patient 4 was considered less aggressive as it had a mutated IDH with a wild type ATRX gene and thus should have been more responsive to treatment [75-78].

For the 6 patient samples that did respond to IPN treatment an IRN concentration of greater than 6.2 $\mu\text{g/mL}$ (Log 4 nM) was required to achieve a reduction in cell viability of greater than 50% in most cases, while an IRN concentration of approximately 62.3 $\mu\text{g/mL}$ (Log 5 nM) reduced cell viability by between 37.8 and 52.6% (Figure 2B). We also investigated the influence of exposure time on the response of the primary tissue samples from patients 1, 3, 4 and 8 to IRN (Figure 2C). We found that there was a significant difference ($P = 0.015$) between 3 and 5 days exposure on the IC_{50} value of the patient samples we investigated. There was no significance ($P = 0.125$) on the IC_{50} value going from 5 to 10 days exposure (Figure 2C). This result is not surprising as the mechanism by which IRN mediates its cytotoxicity requires a minimum of 3 days exposure as the cancer cell must enter into the S-phase of its cell cycle where SN-38 binds to Topoisomerase I inhibiting the enzyme from re-joining the DNA strands, causing S-phase specific cell killing [38, 40-41].

Based on the above data we believe that for the iDES to be most effective in vivo, each one, of which there will be between 30 and 60 implanted in the brain parenchyma of the resection

cavity, needs to deliver at least 300 to 1000 μ g of IRN per day for a period of at least 5 to 7 days ensuring that all GBM cells are exposed to IRN during the S-phase of their cell cycle.

Fig. 2. The cytotoxicity of IRN in relation to patient, concentration and exposure time. **(A)** IC₅₀ values for primary recurrent GBM cells taken from the tumour core and after five day exposure to irinotecan. **(B)** Cell viability values for the same recurrent GBM cells after five days exposure to 3, 4 and 5 Log nM concentrations of irinotecan. **(C)** Effect of IRN exposure time on the IC₅₀ value of recurrent GBM cells taken from the tumour core.

During surgery the tumour core is resected leaving behind the BAT margin that is resistant to therapy and the source of recurrence [80-84]. As the iDES are designed to be placed into this

margin we evaluated the cytotoxicity of IRN, in comparison to TMZ, against the BAT margin for patients 1, 3, 5 and 8. Figure 3 demonstrates that the BAT samples are more responsive to both IRN and TMZ when compared to the corresponding tumour core (Figure 1). However, the response to IRN was significantly ($P = 0.01$) higher when compared to TMZ (Figure 3A to D). Even at the highest dose TMZ was only able to reduce cell viability to below 50% in one BAT sample (Figure 3A), the remaining three samples were all above 70%. IRN was able to reduce the cell viability to below 50% in all BAT samples, with the higher doses reducing cell viability to below 10% (Figure 3A to D). The IC_{50} data in figure 3E demonstrates that the BAT samples have a greater response to IRN treatment than the corresponding tumour core from the same patient. Therefore, IRN treatment of the resection margin with the iDES will be more effective than previously thought.

Optimisation of the manufacture of the doxorubicin-loaded PLGA iDES

Choice of plasticizer type and loading

To reduce the risk of adverse reactions at the site of implantation in the brain parenchyma the neurosurgeons advised that the iDES should be between 1.8 and 2.2mm in diameter, no more than 6mm in length, have a smooth surface (i.e. no shark skinning) and non-swelling. IRN has a melt temperature of approximately 220°C and because the processing temperature is 110°C it will have no plasticising effect on the polymer. Therefore, to ensure ease of extrusion, a consistent diameter and no shark skinning a plasticiser will need to be added to the formulation.

Fig. 3. The cytotoxicity of IRN on brain around the tumour (BAT) cells in comparison to TMZ and the tumour core. **(A-D)** The cell viability data for BAT GBM cells taken from the resection margin of patients with a recurrent GBM and after five days exposure to increasing concentrations of either IRN or TMZ. **(E)** The IC_{50} values for both BAT and Core samples taken from the same patients and after treatment with IRN.

Figure 4 demonstrates the influence of plasticizer type and loading on the diameter consistency and swelling of the iDES. The higher the loading the more consistent the diameter, with Kolliphor P188 and RH 40 requiring a minimum loading of 10% w/w to produce iDES with a consistent diameter, while Kolliphor P237 required a minimum loading of 15% w/w (Figure 4A). The RH 40 plasticizer resulted in significant ($P = 0.025$) swelling at all loadings (Figure 4B). The P 188 and P 237 plasticizers had significant ($P = 0.019$) swelling at loadings of 15 and 20% w/w (Figures 3 C and D). Therefore, based on this data we decided to use the P 188 plasticizer at a loading of 10% w/w as it produced iDES with a consistent diameter of approximately 2mm and did not induce swelling.

Content and cytotoxicity of the IRN in the PLGA iDES

To ensure that our HME process produced iDES with the correct IRN content and that the IRN retained its cytotoxicity during the manufacturing process we tested the iDES for their IRN content and used this solution to determine their cytotoxicity against the sample from patient 1 in comparison to an unprocessed control solution of the same concentration. A series of iDES containing various loadings (10 to 50% w/w) of IRN and of various (2, 3 and 6mm) lengths were investigated. The 2mm long iDES will be used for testing in the GBM mouse resection model (section 3.3), while the 3mm and 6mm iDES will be used in any future clinical trial depending on how deep seated the residual tumour is within the brain parenchyma. Figures 5A and B demonstrate that the iDES have contents between 98 and 104% of their theoretical value. Figures 5C and D show that the IRN extracted from the iDES retained its cytotoxicity. When compared to an unprocessed control solution of the same concentration there was no significant ($P = 0.562$) difference in cell viability. This data demonstrates that our HME process is capable of producing iDES that have the correct drug content and in which the IRN retains its cytotoxicity.

Fig. 4. The influence of plasticizer type and plasticizer loading on the consistency of diameter during manufacture (A) and swelling of the iDES (B, C and D).

Fig. 5. IRN content depending on loading percentage and size of the iDES and their respective cell viability of the extracted IRN. (A) IRN content of the 10, 20, 30, 40 and 50% iDES (A) and (B) 2 x 2mm, 2 x 3mm and 2 x 6mm iDES. (C) The cytotoxicity of the IRN extracted from the 2 X 3 mm 10, 20, 30, 40 and 50% iDES and (D) 30% w/w 2 x 2 mm, 2 x 3 mm and 2 x 6 mm iDES in comparison to an unprocessed control of the same concentration.

***In vitro* release of IRN from the PLGA iDES**

In vitro drug release was performed in both sink conditioned and bio-relevant release media for the various iDES formulations. Sink conditions is a volume of dissolution medium that is at least three to ten times the saturation volume of the total drug in the formulation and were used to investigate how the formulation variables of drug loading and iDES dimensions would influence drug release. Sink conditions are rarely achieved *in vivo*, particularly with

implants, were they are surrounded by a small volume of aqueous fluid. Therefore, bio-relevant (or non-sink) release conditions were used to determine how these same parameters would influence drug release *in vivo*. Under sink conditions (Figure 6) the iDES demonstrated a standard matrix release profile with an ‘initial burst’ on day one followed by decreasing drug release until day 7 (Figure 6A and B). The drug release was influenced by both drug loading and iDES dimensions (Figure 6A and B). An increase in drug loading from 10 to 50% significantly ($P = 0.0103$) increased release on day one from 1.2mg to 6.8mg, while the total release over the 7 days increased ($P = 0.0111$) from 1.9mg to 10.9mg (Figure 6A). Increasing the dimensions of the iDES from 2 X 2 mm to 2 X 3 mm had no significant ($P = 0.543$) influence on drug release (Figure 6B). This is not surprising as both sets of iDES had very similar weights and IRN content values. However, increasing the dimensions to 2 X 6 mm significantly ($P = 0.0107$) increased release, with an increase on day one from 2.6mg to 5.2mg and an increase in the total release from 2.9mg to 8.4mg. We have demonstrated that IRN release can be controlled by both drug loading and iDES dimensions, which provides us with a platform technology that can be used to accurately control IRN levels *in vivo*. Under sink conditions all of the iDES formulations released greater than 92% of their drug-loading over 7 days of release. This is due to their small size and the porous nature of the PLGA allowing the release media to completely diffuse into the iDES solubilizing the majority of the IRN enabling it to be released.

Under bio-relevant conditions the iDES also demonstrated a standard matrix release profile with an ‘initial burst’ on day one followed by decreasing drug release until day 7 (Figure 6C and D). The IRN levels released were lower than those released under sink conditions, however, not as low as expected, which is due to the hydrochloride salt form of IRN being used making it water soluble. IRN-HCL has a water solubility of 25mg/mL and thus our 3mL of bio-relevant media, was able to provide sink conditions for most of the iDES

formulations. Therefore, we believe that the increased drug release under sink conditions was due to the increased volume (5mL compared to 3mL) of the media rather than its composition. The increased volume decreases the thickness and concentration of the IRN diffusion layer at the iDES/release media interface, which increases drug release. This has the potential to influence IRN release in vivo, with a high local concentration of IRN around the iDES, controlling release rather than the iDES itself [16].

Fig. 6. In vitro cumulative release of the iDES under sink (A and B) and bio-relevant conditions (C and D).

Despite this, the bio-relevant drug release was also influenced by both drug loading and iDES dimensions (Figure 6C and D). However, there was no significant ($P = 0.582$) difference in release between the 40 and 50% w/w iDES. This is due to the large amount of IRN on the surface and within the iDES being released and saturating the diffusion layer at the iDES/release media interface. As has been mentioned previously this may have implications *in vivo*, with an increase in IRN loading from 40 to 50% w/w not necessarily resulting in an increase in the amount of IRN delivered *in vivo*. An increase in drug loading from 10 to 40/50% significantly ($P = 0.0213$) increased release on day one from 1.0mg to approximately 4.0mg, while the total release over the 7 days increased ($P = 0.0237$) from 1.8mg to approximately 8mg (Figure 6C).

As with the sink conditioned media increasing the dimensions of the iDES from 2 X 2 mm to 2 X 3 mm had no significant ($P = 0.487$) influence on drug release (Figure 6D). However, increasing the dimensions to 2 X 6 mm significantly ($P = 0.0107$) increased release, with an increase on day one from 2.3mg to 4.9mg and an increase in the total release from 3.2mg to 7.3mg. Based on the bio-relevant release data we believe that the *in vivo* release of IRN will be controlled by both drug loading and iDES dimensions. However, the lack of sink conditions *in vivo* and saturation of the diffusion layer at the iDES/cerebral spinal fluid (CSF) interface, will limit the amount of IRN released. Alternatively, the daily production rate of CSF (which is three to four times the total volume) and the removal of IRN via diffusion, metabolic elimination and permeation into nearby vasculature may reduce the IRN concentration of the diffusion layer providing some level of sink conditions. Under bio-relevant release conditions the iDES formulations released between 71 and 102% of their drug-loading over 7 days of release. Those iDES formulations that released greater than 90% of their content were the 10% w/w 2 X 6 mm and the 40% w/w 2 X 2 mm and 2 X 3 mm. This is not surprising as due to either their low IRN loading or smaller mass the 3mL of bio-

relevant release media was able to provide sink conditions. The remaining iDES formulations released between 71 and 87% of their IRN loading, which means their duration of release *in vivo* may be extended beyond 7 days.

The *in vitro* daily release profiles for the iDES released under bio-relevant conditions are presented in figure 7A and B. There was a large day one burst, which increased significantly ($P = 0.0213$) with an increase in drug loading. The day one burst will provide a large dose of IRN that will kill the majority of tumour cells already in the S-phase while building up a large concentration of IRN in the brain tissue increasing the diffusion of the IRN into the brain parenchyma enabling it reach those deep seated tumour cells. The iDES then demonstrated a typical matrix release profile, with a decrease in drug release over time. The 2 X 3 mm 30, 40 and 50% w/w and the 2 X 6 mm 30% w/w iDES formulations were capable of releasing the required 300 to 1000 μ g of IRN per day for the full 7 days (Figure 7A and B).

Cytotoxicity of the IRN released from the PLGA iDES into the bio-relevant release media

The IRN released from iDES formulations on day 1 and day 7 into the bio-relevant release media was tested for its cytotoxicity against the tumour core sample from patient 1 (Figure 7C and D). An increase in IRN loading from 10 to 50% w/w resulted in an a decrease in the cell viability from 21.3 to 11.2% (Figure 7C), while an increase in the length of the iDES from 2 to 6 mm resulted in a decrease in cell viability from 34.0 to 25.4% (Figure 7D). This is due to the increased IRN released on day 1 as a result of an increase in drug loading and length (Figure 7A and B). The cell viability on day 7 is higher than that observed for day 1, which is to be expected due to the lower amount of IRN released on day 7 (Figure 7A and B). The cell viability ranges from 34.0 to 21.5% depending on the loading or length of the iDES. Based on the IC_{50} data for the tumour core sample from patient 1 (Figure 1), the cell viability

values are what would be expected for the concentration of IRN in the media. Therefore, the IRN released retained its cytotoxicity through formulation, storage and release.

Fig. 7. The influence of IRN loading on the in vitro daily release and the cytotoxicity of the iDES. (A) In vitro daily release of the 2 X 3mm iDES with varying IRN loadings and (B) the 30% w/w iDES with varying lengths. (C and D) Cytotoxicity of the day 1 and 2 bio-relevant release samples on

the BAT sample from patient 1. (E) The cytotoxicity of the 2 X 3 mm iDES with varying irinotecan-loadings when placed directly onto the BAT sample from patient 1.

Cytotoxicity of the PLGA iDES when placed directly onto the BAT cells

To mimic the *in vivo* scenario as close as possible BAT cells from patient 1 were cultured using 6-well plates. 6-well plates were used to provide sufficient media (3 mL) to cover the iDES. The media was changed every day to represent the turnover of CSF as well as the removal of IRN via diffusion, metabolic elimination and permeation into nearby vasculature, which will reduce the concentration of IRN at the resection margin. Figure 7E demonstrates that the placebo (unloaded) iDES reduced cell viability to 92.1 by day 3. Although there was no significant difference (P value = 0.73) between day 1, 2 and 3 we felt that it was important to highlight as the slight decrease is probably due to the lowering of the pH of the culture media as a result of the release of lactic acid from the PLGA. Both the 10 and 20% w/w iDES reduced cell viability to 58.2 and 38.1% respectively by day 4 (Figure 7E). However, from day 5 to day 7 the cell viability began increase reaching 71.2 and 62.3% by day 7. These observations are not surprising and are similar to what is observed when patients are treated with chemotherapy. The IRN released on days 1 to 4 kills of the less aggressive clones leaving behind the more aggressive clones. The IRN released between day 5 and day 7 is insufficient, due to a combination of IRN exhaustion in the iDES and higher concentrations being required to kill of the more aggressive clones, to reduce cell viability any further and thus the more aggressive clones start to grow back (Figure 7E). However, when compared to the 0% w/w iDES control the rate of re-growth for the 10 and 20% w/w iDES is much slower, which suggests that they would reduce the rate of recurrence *in vivo*.

The 30% w/w iDES reduced cell viability to 0% by day 5, while the 40 and 50% w/w iDES achieved this by day 4 (Figure 7E). There was no sign of cell re-growth, which suggests that all of the GBM cells were killed off. Based on the results above we believe that the 30, 40 and 50% w/w iDES deliver sufficient IRN over the 7 days to completely kill off all of the residual tumour cells remaining after resection surgery. Therefore, these iDES formulations will be evaluated in mice for both toxicity and efficacy.

In vivo evaluation of the toxicity of the 30, 40 and 50% w/w iDES in sham resection cavities of non-tumour bearing mice.

The toxicity results presented in Figure 8A shed light on the toxicity profile of the iDES relative to a placebo control. Regardless of IRN loading, moderate levels of acute inflammation are present one week after implantation, however, this inflammation subsides over time. This behaviour is attributable to the wound healing response to resection-induced surgical brain injury. Chronic inflammation due to persistent drug release from the iDES is likewise highest at the onset of implantation as a result of the initial burst of IRN from the iDES and is generally resolved over time, with the exception of 40% which still has detectable levels on day 56. While only mild chronic inflammation is observed in the 0% and 30% groups, moderate inflammation is seen on day 14 in both the 40% and 50% groups, meaning these higher concentrations of IRN elicit a more intense inflammatory response. This observation is further supported by the presence of necrosis in both the 40% and 50% groups. In summary, the 30% iDES does not appear any more toxic than the placebo control in any substantive way, whereas the 40% and 50% iDES cause elevated immune activity and temporary damage to parenchymal tissue.

Fig. 8. In vivo toxicity (A) and efficacy (B) of the placebo, 30, 40 and 50% w/w iDES.

In vivo evaluation of the efficacy of the 30, 40 and 50% w/w iDES in a GBM mouse resection model.

The efficacy of the iDES was evaluated over 70 days using a GBM mouse resection model (Figure 8B). The data demonstrates that 100% of the mice in the sham (untreated control)

and placebo groups had died by day 27 and 31 respectively (Figure 8B). However, 100% of the mice in the 50% w/w iDES group died by day 22, which is not surprising given the level of toxicity observed in the toxicity study (Figure 8A). The mice in the 30 and 40% iDES groups showed long-term survival with 40% of the mice still alive at day 70. Furthermore, the surviving mice show no expected symptoms associated with a recurring brain tumour and when imaged using Fluc bioluminescence imaging none of them showed any sign of tumour recurrence (Figure 9).

Fig 9. Fluc bioluminescence imaging of a positive control mouse at day 29 with a recurrent tumour clearly visible (A) and the surviving mice at day 70 post implantation with no visible recurrent tumour (B).

Discussion

There currently is no standard treatment option for recurrent GBM patients. Due to there being so few options the National Institute of Health and Care Excellence in the UK approved temozolomide as a ‘second line’ therapy for recurrent brain tumours in 2001, which has shown moderate activity in the treatment of recurrent GBM [85]. However, this activity is significantly reduced in patients with unmethylated MGMT and these patients should be considered for an alternative treatment approach [86]. This demonstrates a clear need for the development of novel and innovative treatments in order to prolong survival and improve the quality of life of recurrent GBM patients. Here, we provide evidence that the local delivery of irinotecan to the margin of the resection cavity using drug eluting seeds is a viable treatment option for recurrent GBM and may be more effective than treatment with temozolomide. Using patient derived recurrent GBM cells we demonstrated that IRN is significantly more effective than TMZ for treating recurrent GBM. Furthermore, the doses required to produce a significant reduction in cell viability were much lower for IRN compared to TMZ. Using a clinically relevant GBM mouse resection model we show that the 30% w/w iDES completely suppressed recurrence of postsurgical GBM with no additional toxicity when compared to the placebo. These results position the iDES as a feasible ‘first line’ treatment for recurrent glioblastoma.

We sought to compare the efficacy of IRN and TMZ against a panel of patient derived recurrent GBM cells taken from the tumour core. Our response rate for TMZ was 50%, which is similar to response rates of recurrent GBM patients in the clinic when treated with TMZ alone [87, 88]. However, in those patients who responded the cell viability never went below 80%. IRN had a response rate of 75% and reduced cell viabilities to below 45% and in some to as low as 10%. Based on this data IRN is clearly a more effective drug for treating recurrent GBM when administered directly to the cancer cells. Because TMZ crosses the

BBB to a much greater extent that IRN and IRN's dose is limited as a result of diarrhea and severe neutropenia [69] patients receiving TMZ tend to perform significantly better in the clinic when systemic administration is used. However, in the case of the iDES, local administration will be used at re-operation with the IRN administered directly to the cancerous cells.

We found that the efficacy of IRN was 1.8 to 3.1 fold greater when tested against a panel of patient derived recurrent GBM BAT (margin) cells compared to the tumour core from the same patient. This is due to intra-tumour heterogeneity, where different fragments of the same tumour respond differently to different drugs. Furthermore the BAT tissue consists of fast dividing cells that will be more responsive to those drugs such as IRN that target DNA replication. This data is significant as during surgery the tumour core is resected leaving behind the BAT margin, which has been shown to be resistant to therapy and the source of recurrence [80-84]. Therefore, the fact that IRN is significantly more effective against the BAT than the core would suggest that the iDES would be an effective treatment strategy for targeting the margin left behind after surgery.

One of the major issues with Gliadel[®], besides the choice of drug, is its disc-shaped design. The disc-shape means that it has to be placed onto the surface of the resection cavity rather than being inserted into the brain parenchyma. Thus, to reach the deep seated tumour cells the carmustine must rely on diffusion through brain tissue which restricts penetration distances to a few millimetres. Furthermore, because the wafers are sitting in the resection cavity, the carmustine is in close proximity to the wound and can cause impaired neurosurgical wound healing. We designed our iDES based on the size and shape of the radioactive seeds used in brachytherapy. These seeds are the size of a grain of rice (3mm X 7mm) and cylindrical in shape. Between 30 and 60 seeds are inserted into the brain

parenchyma of the tumour resection cavity, with the insertion of the seeds shown to be safe. Inserting the between 30 and 60 iDES into the brain parenchyma will increase drug penetration into the residual tumour tissue, while reducing drug diffusion out of the cavity alleviating the wound healing complications associated with Gliadel® and other local delivery strategies.

It was decided to use Hot Melt Extrusion to manufacture the iDES as it was easy to control the diameter of the extrudate and thus the diameter of the iDES. However, the biodegradable polymer PLGA is not designed for use in HME processes and thus we needed to add a plasticizer as a processing aid. Without the use of a plasticizer PLGA extrudates can develop what is called shark skin, where the surface of the extrudate forms cracks and becomes rough and jagged, which would not be conducive for safe insertion into brain tissue. The use of certain types and amounts of plasticizers can cause the extrudate to absorb water and swell. For safety reasons we do not want the iDES to swell upon implantation into the brain. We selected three of the most common types of plasticizer currently used in HME, Kolliphor P237 Kolliphor P188 and RH 40, and evaluated them for consistency of diameter, the presence of shark skinning and swelling when placed into water. The addition of a plasticizer produced iDES implants with a smooth surface and a consistent diameter. The plasticizer Kolliphor P237 required loadings greater than 15% w/w to have any effect while the plasticizers Kolliphor P188 and RH 40 only required loadings of 10% w/w to have an effect. However, the RH 40 plasticizer resulted in significant swelling at all loadings, while the Kolliphor P237 and P188 plasticizers resulted in swelling at loadings greater than 15%. It was decided to use the Kolliphor P188 plasticizer at a loading of 10% w/w to manufacture the iDES. The above results are due to P188 having a lower melting temperature than P237, therefore, less P188 is needed to reduce the viscosity of the formulation. Furthermore, RH 40 is semi-solid, whereas P188 and P237 are both solid, which means that RH 40 has a greater

influence on decreasing the viscosity of the formulation. RH 40 is hygroscopic, which means that it absorbs water and it is the absorption of water into the implants that has caused them to swell.

Our HME process was capable of producing iDES with an acceptable content uniformity of IRN, which was shown to be stable and have the same cytotoxicity as an unformulated control. This demonstrates that our manufacturing process is suitable for the manufacture of stable iDES containing the correct amount of IRN. On the advice of clinicians we investigated the release of IRN from iDES that were both 3 and 6mm long. We also investigated 2mm long iDES as these would be used in the subsequent animal study. The in vitro release was performed under standard sink conditions as well as bio-relevant conditions to try and get an understanding of how the iDES would release in vivo. In order to achieve the 300 µg to 1000 µg release per day it is important to understand how the dimensions of the implant influence release. Under both conditions the iDES demonstrated the standard matrix release profile of an 'initial burst' on day one followed by decreasing drug release until day seven. The sink conditioned release was higher than the bio-relevant release which is to be expected. The in vitro release was influenced by both drug loading and the length of the iDES, with the higher the drug loading and the longer the iDES the greater the release. All of the iDES formulations were capable of providing IRN release for up to 7 days, however, only the 2 X 3 mm 30, 40 and 50% w/w and the 2 X 6 mm 30% w/w iDES formulations were capable of releasing the required 300 to 1000µg of IRN over the 7 days.

The IRN released into bio-relevant media on days 1 and 7 had similar cytotoxicity to a control solution of the same concentration demonstrating that the IRN released retained its cytotoxicity through formulation, storage and release. Furthermore, when the 2 X 3mm iDES were placed directly onto the BAT cells the 30% w/w iDES reduced cell viability to 0% by

day 5, while the 40 and 50% w/w iDES achieved this by day 4. There was no sign of cell re-growth for either the 30, 40 or 50% w/w iDES which suggests that all of the GBM cells were killed off.

The in vivo toxicity study showed that the 30% w/w iDES do not appear any more toxic than the placebo control, whereas the 40% and 50% w/w iDES cause elevated immune activity and temporary damage to parenchymal tissue. The in vivo efficacy study demonstrated that the 30 and 40% w/w iDES induced long-term survival with 40% of the mice still alive at day 70 and with no sign of tumour recurrence.

Because IRN has already been administered directly into the brain parenchyma in a Phase I study (NCT02433392) with no local toxicities such as swelling, inflammation or any suggestion of pseudo-abscess formation and the fact that in brachytherapy solid metal implants are safely implanted into the brain parenchyma makes the regulatory pathway for the iDES to enter the clinic much easier. Based on the evidence presented in this paper our iDES are as safe as the DEBs and brachytherapy and could potentially be more effective against recurrent GBM than treatment with TMZ alone. The HME process first needs to be transferred to a Good Manufacturing Practices facility so that a clinical batch of iDES can be manufactured. One of the advantages of HME is its ease of technology transfer and scale-up and the extruder in our lab is currently used by a number of GMP manufacturers. Furthermore, we have a wealth of experience in the technology transfer and scale-up of HME processes for implantable devices. Once the iDES clinical batch is produced they need to be evaluated for their safety in Good Laboratory Practices toxicity study. This study should evaluate the effect of the number of iDES implanted on local toxicity. Once the iDES are proven to be safe then a Phase I clinical trial involving 50 recurrent GBM patient should begin.

The Phase I clinical trial of the IRN DEB gel formulation demonstrated an increase in survival from 6 to 8 months, which was comparable, if not slightly improved when compared to Gliadel[®] as a historic control. However, the pharmacokinetic data demonstrated that the majority of IRN was removed from the brain within 48 hours, while all of it was removed by 72 hours. IRN works during the S-Phase of the cell cycle, which occurs around day 3. Thus, the efficacy of IRN could be improved if therapeutic levels can be maintained at the tumour site for at least 5 to 7 days. The iDES are capable of delivering therapeutic levels of IRN for up to 7 days and offer the potential of a non-toxic and effective treatment for recurrent GBM. After the successful Phase I clinical testing of the IRN DEB gel formulation, the next logical step is to evaluate the iDES for safety and efficacy in a Phase I clinical trial.

In conclusion these studies provide evidence that IRN is effective against recurrent GBM and that the iDES inhibit GBM recurrence and result in long-term survival in mouse models, which reflect the clinical scenario of GBM therapy. These findings as well as the demonstrated safety of local delivery of IRN to the brain parenchyma should encourage the Phase I clinical assessment of the iDES and ultimately the translation of this novel reformulation therapy into clinical practice for the treatment of recurrent GBM.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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wrote the manuscript with all authors contributing. **Competing interests:** All authors declare that they have no competing interests. Data and materials availability: Access to the primary tumour tissue used in this study is available from the Human Biomaterials Resource Centre at the University of Birmingham. Access to the Drug Eluting Seed technology is available by contacting C.M.C directly.

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References

1. T.A. Dolecek, J.M. Propp, N.E. Stroup, C. Kruchko, CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro. Oncol.* **14**, Suppl 5:v1-49 (2012).
2. A. Munshi A, Chloroquine in glioblastoma--new horizons for an old drug. *Cancer.* **115**, 2380-23833 (2009).
3. D.N. Louis, A. Perry, G. Reifenberger, A. von Deimling, D. Figarella-Branger, W.K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* **131**, 803-820 (2016).
4. Q.T. Ostrom, H. Gittleman, P. Farah, A. Gordtcek, Y. Chen, Y. Wolinsky, N.E. Stroup, C. Kruchko, J.S. Barnholtz-Sloan, CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro. Oncol.* **15**, 1-56 (2013).
5. J.P. Thakkar, T.A. Dolecek, C. Horbinski, Q.T. Ostrom, D.D. Lightner, J.S Barnholtz-Sloan, J.L. Villanoet Epidemiologic and Molecular Prognostic Review of Glioblastoma. *Cancer Epidemiol. Biomarkers Prev.* **23**, 1985-1996 (2014).
6. Q.T. Ostrom, T. Bauchet, F. Davis, I. Deltour, J. Fisher, C. Langer, M. Pekmezci, J.A. Schwartzbaum, M.C. Turner, K.M. Walsh, M.R. Wrensch, J.S. Barnholtz-Sloan JS, The epidemiology of glioma in adults: a “state of the science” review. *Neuro. Oncol.* **16**, 896-913 (2014).
7. J. Dejaegher J, S. De Vleeschouwer, in *Glioblastoma*, S. De Vleeschouwer S, Ed. (Codon Publications, Brisbane, 2017), vol 27, chap. 14.
8. A.M. Robin, I. Lee, S.N. Kalkanis, Reoperation for Recurrent Glioblastoma Multiforme. *Neurosurg. Clin. N. Am.* **28**, 407-428 (2017).

9. T.S. Reese, M.J. Karnovsky, Fine structural localization of a blood–brain barrier to exogenous peroxidase. *J. Cell. Biol.* **34**, 207-217 (1967).
10. A. Seelig, R. Gottschlich, R.M. Devant, A method to determine the ability of drugs to diffuse through the blood-brain barrier. *PNAS.* **91**, 68-72 (1994).
11. N.J. Abbott, I.A. Romero, Transporting therapeutics across the blood-brain barrier. *Mol. Med. Today.* **2**, 106-113 (1996).
12. E.M. Kemper, A.E van Zandbergen, C. Cleypool, H.A. Mos, W. Boogerd, J.H. Beijnen, O. van Tellingen, Increased Penetration of Paclitaxel into the Brain by Inhibition of P-Glycoprotein. *Clin. Cancer Res.* **9**, 2842-2855 (2003).
13. P.P. Wang, J. Frazier, H. Brem, Local drug delivery to the brain. *Adv. Drug Deliv. Rev.* **54**, 987-1013 (2002).
14. N.H. Grieg, Optimizing drug delivery to brain tumors. *Cancer Treat. Rev.* **14**, 1–28 (1987).
15. J. Siepmann, F. Siepmann, A.T. Florence, Local controlled drug delivery to the brain: Mathematical modeling of the underlying mass transport mechanisms. *Int. J. Pharm.* **314**, 101-119 (2006).
16. J.B. Wolinsky, Y.I. Carlson, M.W. Grinstaff, Local drug delivery strategies for cancer treatment: Gels, nanoparticles, polymeric films, rods, and wafers. *J. Con. Rel.* **159**, 14-26 (2012).
17. F. Qian, A. Szymanski, J. Gao, Fabrication and characterization of controlled release poly(D,L-lactide-co-glycolide) millirods. *J. Biomed. Mater. Res.* **55**, 512-522 (2001).
18. B.D. Weinberg, E. Blanco, J. Gao, Polymer Implants for Intratumoral Drug Delivery and Cancer Therapy. *J. Pharm. Sci.* **97**, 1681-1702 (2008).

19. C. McConville, P. Tawari, W. Wang, Hot melt extruded and injection moulded disulfiram-loaded PLGA millirods for the treatment of glioblastoma multiforme via stereotactic injection. *Int. J. pharm.* **494**, 73-82 (2015).
20. T.M. Krupka, B.D. Weinberg, N.P. Ziats, J.R. Haaga, A.A. Exner, Injectable polymer depot combined with radiofrequency ablation for treatment of experimental carcinoma in rat. *Invest. Radiol.* **41**, 890–897 (2006).
21. J.K. Jackson, M.E. Gleave, V. Yago, E. Beraldi, W.L. Hunter, H.M. Burt, The suppression of human prostate tumor growth in mice by the intratumoral injection of a slow-release polymeric paste formulation of paclitaxel. *Cancer Res.* **60**, 4146–4151 (2000).
22. T.J. Vogl, K. Engelmann, M.G. Mack, F. Straub, S. Zangos, K. Eichler, K. Hochmuth, E. Orenberg, CT-guided intratumoural administration of cisplatin/epinephrine gel for treatment of malignant liver tumours. *Br. J. Cancer* **86**, 524–529 (2002).
23. S.J. Vukelja, S.P. Anthony, T.C. Arseneau, B.S. Berman, C.C. Cunningham, J.J. Nemunaitis, W.E. Sanjowski, K.D. Fowers, Phase 1 study of escalating-dose OncoGel (ReGel/paclitaxel) depot injection, a controlled-release formulation of paclitaxel, for local management of superficial solid tumor lesions. *Anticancer Drugs.* **18**, 283–289 (2007).
24. E. Reguera-Núñez, C. Roca, E. Hardy, M. de la Fuente, N. Csaba, M. Garcia-Fuentes, Implantable controlled release devices for BMP-7 delivery and suppression of glioblastoma initiating cells. *Biomaterials.* **35**, 2859-2867 (2014).
25. G. Cruickshank, O. Fayeye, D. Ngoga, J. Connor, A. Detta, Intraoperative intraparenchymal injection of irinotecan drug loaded beads in patients with recurrent

- glioblastoma (GBM): A safe new and depot approach for loco-regional therapy (NCT02433392). *Neuro. Oncol.* 2015. **17**, 11 (2015).
26. P. Menei, L. Capelle, J. Guyotat, S. Fuentes, R. Assaker, B. Bataille, P. François, D. Dorwling-Carter, P. Paquis, L. Bauchet, F. Parker, J. Sabatier, N. Faisant, J.P. Benoit, Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of malignant glioma: a randomized phase II trial. *Neurosurgery.* **56**, 242-248 (2005).
 27. A. Beduneau, P. Saulnier, J.P. Benoit, Active targeting of brain tumors using nanocarriers. *Biomaterials.* **28**, 4947–4967 (2007).
 28. H. Koo, M.S. Huh, I.C. Sun, S.H. Yuk, K. Choi, Y. Kim, I.C. Kwon, In vivo targeted delivery of nanoparticles for theranosis. *Acc. Chem. Res.* **44**, 1018–1028 (2011).
 29. J.D. Meyers, T. Doane, C. Burda, J.P. Basilion, Nanoparticles for imaging and treating brain cancer. *Nanomedicine.* **8**, 123–143 (2013).
 30. A.Z. Wang, Nanoparticle drug delivery: focusing on the therapeutic cargo. *Nanomedicine.* **7**, 1463–1465 (2012).
 31. A.B. Fleming, W.M. Saltzman, Pharmacokinetics of the carmustine implant. *Clin. Pharm.* **41**, 403-419 (2002).
 32. S. Valtonen, U. Törmänen, P. Toivanen, H. Kalimo, L. Kivipelto, O. Heiskanen, G. Unsgaard, T. Kuurne, Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study. *Neurosurgery.* **41**, 44-48 (1997).
 33. M. Westphal, D.C. Hilt, E. Bortey, P. Delavault, R. Olivares, P.C. Warnke, I.R. Whittle, J. Jaaskelainen, Z. Ram, A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro. Oncol.* **5**, 79-88 (2003).

34. H. Brem, S. Piantadosi, P.C. Burger, Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrence. *The Lancet*. **345**, 1008–1012 (1995).
35. M.G. Hart, R. Grant, R. Garside, G. Rogers, M. Somerville, K. Stein, Chemotherapeutic wafers for High Grade Glioma. *Cochrane Database Syst. Rev.* **16**, CD007294 (2011).
36. E. Barbarite, J.T. Sick, E. Berchmans, A. Bregy, A.H. Shah, N. Elsayyad, R.J. Komotar, The role of brachytherapy in the treatment of glioblastoma multiforme. *Neurosurg. Rev.* **40**, 195-211 (2017).
37. M. Ramesh, P. Ahlawat, N. Srinivas, Irinotecan and its active metabolite, SN-38: review of bioanalytical methods and recent update from clinical pharmacology perspectives. *Biomed. Chromatogr.* **24**, 114-123 (2010).
38. Y. Xu, Irinotecan: mechanism of tumor resistance and novel strategies for modulating its activity. *Ann. Oncol.* **13**, 1841-1851 (2002).
39. W. Wang, A. Ghandi, L. Liebes, S.G. Louie, F.M. Hofman, A.H. Schönthal, T.C. Chen, Effective conversion of irinotecan to SN-38 after intratumoral drug delivery to an intracranial murine glioma model in vivo. *J Neurosurg.* **114**, 689-694 (2011).
40. B. Sinha, Topoisomerase Inhibitors. *Drugs.* **49**, 11-19 (1995).
41. J. Vrendenburgh, A. Desjardins, D.A. Reardon, H.S. Friedman, Experience with irinotecan for the treatment of malignant glioma. *Neuro. Oncol.* **11**, 80-91 (2009).
42. M. Saunders, T. Iveson, Management of advanced colorectal cancer: state of the art. *Br. J. Cancer.* **95**, 131-138 (2006).
43. H.S. Friedman, W.P. Petros, A.H. Friedman, L.J. Schaaf, T. Kerby, J. Lawyer, M. Parry, P.J. Houghton, S. Lovell, K. Rasheed, T. Cloughsey, E.S. Stewart, O.M. Colvin, J.M. Provenzale, R.E. McLendon, D.D. Bigner, I. Cokgor, M. Haglund, J.

- Rich, D. Ashley, J. Malczyn, G.L. Elfring, L.L. Miller, Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J. Clin. Oncol.* **17**, 1516-1525 (1999).
44. J.C. Buckner, J.M. Reid, K. Wright, S.H. Kaufmann, C. Erlichman, M. Ames, S. Cha, J.R. O'Fallon, L.J. Schaaf, L.L. Miller, Irinotecan in the treatment of glioma patients: current and future studies of the North Central Cancer Treatment Group. *Cancer.* **97**, 2352-2358 (2003).
 45. E. Raymond, M. Fabbro, V. Boige, O. Rixe, M. Frenay, G. Vassal, S. Faivre, E. Sicard, C. Germa, J.M. Rodier, L. Vernillet, J.P. Armand, Multicentre phase II study and pharmacokinetic analysis of irinotecan in chemotherapy-naïve patients with glioblastoma. *Ann. Oncol.* **14**, 603-614 (2003).
 46. M.C. Chamberlain, Salvage chemotherapy with CPT-11 for recurrent glioblastoma multiforme. *J. Neurooncol.* **56**, 183-183 (2002).
 47. C.D. Turner, S. Gururangan, J. Eastwood, K. Bottom, M. Watral, R. Beason, R.E. McLendon, A.H. Friedman, S. Tourt-Uhlig, L.L. Miller, H.S. Friedman, Phase II study of irinotecan (CPT-11) in children with high-risk malignant brain tumors: the Duke experience. *Neuro. Oncol.* **4**, 102-108 (2004).
 48. T.F. Cloughesy, E. Filka, G. Nelson, F. Kabbinavar, H. Friedman, L.L. Miller, G.L. Elfring, Irinotecan treatment for recurrent malignant glioma using an every-three-week regimen. *Am. J. Clin. Oncol.* **25**, 204-208 (2002).
 49. T.F. Cloughesy, E. Filka, J. Kuhn, G. Nelson, F. Kabbinavar, H. Friedman, L.L. Miller, G.L. Elfring, Two studies evaluating irinotecan treatment for recurrent malignant glioma using an every-three-week regimen. *Cancer.* **97**, 2381-2386 (2003).
 50. T.T. Batchelor, M.R. Gilbert, J.G. Supko, K.A. Carson, L.B. Nabors, S.A. Grossman, G.J. Lesser, T. Mikkelsen, S. Phuphanich, NABTT CNS Consortium, Phase 2 study

- of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97-11. *Neuro. Oncol.* **6**, 21-27 (2004).
51. M.R. Gilbert, J.G. Supko, T. Batchelor, G. Lesser, J.D. Fisher, S. Piantadosi, S. Grossman, Phase I clinical and pharmacokinetic study of irinotecan in adults with recurrent malignant glioma. *Clin. Cancer Res.* **9**, 2940-2949 (2003).
 52. M.D. Prados, W.K.A. Yung, K.A. Jaeckle, H.I. Robins, M.P. Mehta, H.A. Fine, P.Y. Wen, T.F. Cloughesy, S.M. Chang, M.K. Nicholas, D. Schiff, H.S. Greenberg, L. Junck, K.L. Fink, K.R. Hess, J. Kuhn, North American Brain Tumor Consortium study, Phase 1 trial of irinotecan (CPT-11) in patients with recurrent malignant glioma: a North American Brain Tumor Consortium study. *Neuro. Oncol.* **6**, 44-54 (2006).
 53. M.D. Prados, K. Lamborn, W.K.A. Yung, K.A. Jaeckle, H.I. Robins, M.P. Mehta, H.A. Fine, P.Y. Wen, T.F. Cloughesy, S.M. Chang, M.K. Nicholas, D. Schiff, H.S. Greenberg, L. Junck, K.L. Fink, K.R. Hess, J. Kuhn, North American Brain Tumor Consortium study, A phase 2 trial of irinotecan (CPT-11) in patients with recurrent malignant glioma: a North American Brain Tumor Consortium study. *Neuro. Oncol.* **8**, 189-193 (2006).
 54. W. K. A. Yung, F. S. Lieberman, P. Wen, I. Robin, M. Gilbert, S. Chang, L. Junck, T. Cloughesy, K. Lamborn, and M. Prados, Combination of temozolomide (TMZ) and irinotecan (CPT-11) showed enhanced activity for recurrent malignant gliomas: A North American Brain Tumor Consortium (NABTC) phase II study. *J. Clin. Oncol.* **23**, 1521-1521 (2005).
 55. M.L. Gruber, W.P. Buster, Temozolomide in combination with irinotecan for treatment of recurrent malignant glioma. *Am. J. Clin. Oncol.* **27**, 33-38 (2004).

56. A.A. Brandes, A. Tosoni, U. Basso, M. Reni, F. Valduga, S. Monfardini, P. Amistà, L. Nicolardi, G. Sotti, M. Ermani, Second-line chemotherapy with irinotecan plus carmustine in glioblastoma recurrent or progressive after first-line temozolomide chemotherapy: a phase II study of the Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *J. Clin. Oncol.* **22**, 4779-4786 (2004).
57. D.A. Reardon, J.A. Quinn, J.N. Rich, S. Gururangan, J. Vredenburgh, J.H. Sampson, J.M. Provenzale, A. Walker, M. Badruddoja, S. Tourt-Uhlig, J.E. Herndon, J.M. Dowell, M.L. Affronti, S. Jackson, D. Allen, K. Ziegler, S. Silverman, C. Bohlin, A.H. Friedman, D.D. Bigner, H.S. Friedman, Phase 2 trial of BCNU plus irinotecan in adults with malignant glioma. *Neuro. Oncol.* **6**, 134-144 (2004).
58. J.A. Quinn, D.A. Reardon, A.H. Friedman, J.N. Rich, J.H. Sampson, J. Vredenburgh, S. Gururangan, J.M. Provenzale, A. Walker, H. Schweitzer, D.D. Bigner, S. Tourt-Uhlig, J.E. Herndon, M.L. Affronti, S. Jackson, D. Allen, K. Ziegler, C. Bohlin, C. Lentz, H.S. Friedman, Phase 1 trial of irinotecan plus BCNU in patients with progressive or recurrent malignant glioma. *Neuro. Oncol.* **6**, 145-153 (2004).
59. B. Purow, H.A. Fine, Antiangiogenic therapy for primary and metastatic brain tumors. *Hematol. Oncol. Clin. North. Am.* **18**, 1161-1181 (2004).
60. J.J. Vredenburgh, A. Desjardins, J.E. Herndon, J.M. Dowell, D.A. Reardon, J.A. Quinn, J.N. Rich, S. Sathornsumetee, S. Gururangan, M. Wagner, D.D. Bigner, A.H. Friedman, H.S. Friedman, Phase II trial of bevacizumab and irinotecan in recurrent malignant gliomas. *Clin. Cancer Res.* **13**, 1253-1259 (2007).
61. K.J. Goli, A. Desjardins, J.E. Herndon, J.N. Rich, D.A. Reardon, J.A. Quinn, S. Sathornsumetee, D.A. Bota, H.S. Friedman, and J.J. Vredenburgh, Phase II trial of bevacizumab and irinotecan in the treatment of malignant gliomas. *J. Clin. Oncol.* **25**, 2003-2003 (2007).

62. J.J. Vredenburgh, A. Desjardins, J.E. Herndon, J. Marcello, D.A. Reardon, J.A. Quinn, J.N. Rich, S. Sathornsumetee, S. Gururangan, J. Sampson, M. Wagner, L. Bailey, D.D. Bigner, A.H. Friedman, H.S. Friedman, Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J. Clin. Oncol.* **25**, 4722-4729 (2007).
63. S. Raval, S. Hwang, L. Dorsett, Bevacizumab and irinotecan in patients (pts) with recurrent glioblastoma multiforme (GBM). *J. Clin. Oncol.* **25**, 2078-2078 (2007)
64. F. Bokstein, S. Shpigel, D.T. Blumenthal, Treatment with bevacizumab and irinotecan for recurrent high-grade glial tumors. *Cancer.* **15**, 2267-2272 (2008).
65. H.S. Friedman, M.D. Prados, P.Y. Wen, T. Mikkelsen, D. Schiff, L.E. Abrey, W.K. Yung, N. Paleologos, M.K. Nicholas, R. Jensen, J. Vredenburgh, J. Huang, M. Zheng, T. Cloughesy, Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J. Clin. Oncol.* **1**, 4733-4740 (2009).
66. T. Mesti, M.E. Moltara, M. Boc, M. Rebersek, J. Ocvirk, Bevacizumab and irinotecan in recurrent malignant glioma, a single institution experience. *Radiol. Oncol.* **49**, 80–85 (2015).
67. O. Ozel, M. Kurt, O. Ozdemir, J. Bayram, H. Akdeniz, D. Koca, Complete response to bevacizumab plus irinotecan in patients with rapidly progressive GBM: Cases report and literature review. *J. Oncol. Sci.* **2**, 87-94 (2016).
68. J.J. Vredenburgh, A.D. David A. Reardon, H. S. Friedman, Experience with irinotecan for the treatment of malignant glioma. *Neuro. Oncol.* **11**, 80–91 (2009).
69. J.R. Hecht, Gastrointestinal Toxicity of Irinotecan. *Oncology.* **12**, 73-78 (1998).
70. S. Baltes, I. Freund, A. Lewis, I. Nolte, T. Brinker, Doxorubicin and irinotecan drug-eluting beads for treatment of glioma: a pilot study in a rat model. *J. Mat. Sci.* **21**, 1393-1402 (2010).

71. G. Cruickshank, D. Ngoga, A. Detta, A. Lewis, R. Holden, O. Fayaye, Local Delivery Of Irinotecan To Recurrent Glioblastoma At Reoperation Offers A Safe Therapeutic Advantage Over Systemic Delivery. Presentation at the annual British Neuro Oncology Society Conference, June 21-23, 2017.
72. J.A. Plumb, R. Milroy, S.B. Kaye, Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Res.* **49**, 4435–4440 (1989).
73. K. Shah, S. Hingtgen, R. Kasmieh, J.L. Figueiredo, E. Garcia-Garcia, A. Martinez-Serrano, X. Breakefield, R. Weissleder, Bimodal viral vectors and in vivo imaging reveal the fate of human neural stem cells in experimental glioma model. *J. Neurosci.* **28**, 4406-4413 (2008).
74. M. van Eekelen, L.S. Sasportas, R. Kasmieh, S. Yip, J.L. Figueiredo, D.N. Louis, R. Weissleder, K. Shah, Human stem cells expressing novel TSP-1 variant have anti-angiogenic effect on brain tumors. *Oncogene.* **29**, 3185-95 (2010).
75. J.R. Chen, Y. Yao, H.Z. Xu, Z.Y. Qin, Isocitrate Dehydrogenase (IDH)1/2 Mutations as Prognostic Markers in Patients With Glioblastomas. *Medicine.* **95**, e2583 (2016).
76. T.A. Juratli, M. Kirsch, K. Robel, S. Soucek, K. Geiger, R. von Kummer, G. Schackert, D. Krex, IDH mutations as an early and consistent marker in lowgrade astrocytomas WHO grade II and their consecutive secondary high-grade gliomas. *J. Neurooncol.* **108**, 403-410 (2012).
77. Q. SongTao, Y. Lei, G. Si G, D, YanQing, H, HuiXia, Z, XueLin, W. LanXiao, Y. Fei, IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci.* **103**, 269–273 (2012).

78. C. Houillier, X. Wang, G. Kaloshi, K. Mokhtari, R. Guillevin, J. Laffaire, S. Paris, B. Boisselier, A. Idbaih, F. Laigle-Donadey, K. Hoang-Xuan, M. Sanson, J.Y. Delattre, IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. *Neurology*. **75**, 1560–1566 (2010).
79. D. Conte, M. Huh, E. Goodall, M. Delorme, R.J. Parks, D.J. Picketts, Loss of Atrx sensitizes cells to DNA damaging agents through p53-mediated death pathways PLoS One. **7**, e52167 (2012).
80. M.E. Berens, A. Giest, "...those left behind." Biology and oncology of invasive glioma cells. *Neoplasia*. **1**, 208-219 (1999).
81. S.J. Price, R. Jena, N.G. Burnet, P.J. Hutchinson, A.F. Dean, A. Peña, J.D. Pickard, T.A. Carpenter, J.H. Gillard, Improved delineation of glioma margins and regions of infiltration with the use of diffusion tensor imaging: an image-guided biopsy study. *Am. J. Neuroradiol*. **27**, 1969-1974 (2006).
82. A.C. Maia, S.M. Malheiros, A.J. da Rocha, J.N. Stávale, I.F. Guimarães, L.R. Borges, A.J. Santos, C.J. da Silva, J.C. de Melo, O.P. Lanzoni, A.A. Gabbai, F.A. Ferraz, Stereotactic biopsy guidance in adults with supratentorial nonenhancing gliomas: role of perfusion-weighted magnetic resonance imaging. *J. Neurosurg*. **101**, 970-976 (2004).
83. R.F. Barajas, J.G. Hodgson, J.S Chang, S.R. Vandenberg, R.F. Yeh, A.T. Parsa, M.W. McDermott, M.S. Berger, W.P. Dillon, S. Cha, Glioblastoma multiforme regional genetic and cellular expressions patterns: influence on anatomic and physiologic MR imaging. *Radiology*. **254**, 564-76 (2010).
84. T. Van Meter, C. Dumur, N. Hafez, C. Garrett, H. Fillmore, W.C. Boraddus, Microarray analysis of MRI-defined tissue samples in glioblastoma reveals

- differences in regional expression of therapeutic targets. *Diagn. Mol. Pathol.* **15**, 195-205 (2006).
85. J.R. Perry, K. Bélanger, W.P. Mason, D. Fulton, P. Kavan, J. Easaw, C. Shields, S. Kirby, D.R. Macdonald, D.D. Eisenstat, B. Thiessen, P. Forsyth, J.F. Pouliot, Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. *J. Clin. Oncol.* **28**, 2051-2057 (2010).
 86. M. Weller, G. Tabatabai, B. Kästner, J. Felsberg, J.P. Steinbach, A. Wick, O. Schnell, P. Hau, U. Herrlinger, M.C. Sabel, H.G. Wirsching, P. Kötter, O. Bähr, M. Platten, J.C. Tonn, U. Schlegel, C. Marosi, R. Goldbrunner, R. Stupp, K. Homicsko, J. Pichler, G. Nikkhah, J. Meixensberger, P. Vajkoczy, S. Kollias, J. Hüsing, G. Reifenberger, W. Wick W, DIRECTOR Study Group, MGMT promoter methylation is a strong prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive glioblastoma: the DIRECTOR trial. *Clin. Cancer Res.* **21**, 2057-2064 (2015).
 87. M. Brada, K. Hoang-Xuan, P. Rampling, P.Y. Dietrich, L.Y. Dirix, D. Macdonald, J.J. Heimans, B.A. Zonnenberg, J.M. Bravo-Marques, R. Henriksson, R. Stupp, N. Yue, J. Bruner, M. Dugan, S. Rao, S. Zaknoen, Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. *Ann. Oncol.* **12**, 259-266 (2001).
 88. W. Wick, J.P. Steinbach, W.M. Küker, J. Dichgans, M. Bamberg, M. Weller. One week on/one week off: A novel active regimen of temozolomide for recurrent glioblastoma. *Neurology.* **62**, 2113-2115 (2004).

Matthew Gawley, Lorna Almound and Senam Daniel conducted the formulation, characterisation and in vitro release testing of the iDES. **Sarah Lastakchi, Sharnjit Kaur and Allah Detta** conducted the IC₅₀ studies and tested the release media and iDES for cytotoxicity. **Garth Cruickshank** assisted with experimental design and provided clinical advice. **Ryan Miller, Shawn Hingtgen and Kevin Sheets** deigned and conducted the in vivo toxicity and efficacy studies. **Christopher McConville** devised the iDES concept, designed and supervised all of the experiments. C.M.C wrote the manuscript with all authors contributing.

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