Journal of Drug Targeting

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/idrt20

Vascular priming with RRx-001 to increase the uptake and accumulation of temozolomide and irinotecan in orthotopically implanted gliomas

Bryan Oronsky, Tony Reid & Pedro Cabrales

To cite this article: Bryan Oronsky, Tony Reid & Pedro Cabrales (2021): Vascular priming with RRx-001 to increase the uptake and accumulation of temozolomide and irinotecan in orthotopically implanted gliomas, Journal of Drug Targeting, DOI: 10.1080/1061186X.2021.1904248

To link to this article: https://doi.org/10.1080/1061186X.2021.1904248

Published online: 20 May 2021.

JOURNAL OF DRUG TARGETING
https://doi.org/10.1080/1061186X.2021.1904248

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

OPEN ACCESS Check for updates

Vascular priming with RRx-001 to increase the uptake and accumulation of temozolomide and irinotecan in orthotopically implanted gliomas

Bryan Oronsky[a], Tony Reid[a] and Pedro Cabrales[b]

[a]EpicentRx Inc, La Jolla, CA, USA; [b]Department of Bioengineering, University of California San Diego, La Jolla, CA, USA

ABSTRACT

Vascular normalisation refers to a 'remodeling' of the dysfunctional tumour capillary network, which regresses under the influence of anti-VEGF treatment, resulting in improved blood flow and oxygenation. RRx-001 is an anti-CD47-SIRPα small molecule with vascular normalising properties under investigation in clinical trials for the treatment of glioblastoma, brain metastases, lung cancer and colorectal cancer, with FDA Orphan Drug Designation in glioblastoma and other tumour types. This study investigated whether the improved oxygenation and perfusion that has been previously observed with RRx-001 both preclinically and clinically in the context of a brain metastasis trial was correlated with increased penetration and accumulation of the cytotoxic chemotherapies, irinotecan and temozolomide, in orthotopically implanted gliomas, priming tumours for improved response. The experiments demonstrate that administration of RRx-001 prior to temozolomide or irinotecan results in significantly increased uptake of irinotecan and temozolomide in orthotopic glioma tumours. Since the success of chemotherapy in the brain (and outside of it) is limited by subtherapeutic tumoral drug concentrations, vascular normalisation-enhanced delivery of standard cytotoxics as demonstrated with RRx-001 may mitigate or reverse clinical drug resistance and thereby improve the outcome of cancer therapy, particularly in the brain.

ARTICLE HISTORY
Received 24 December 2020
Revised 4 February 2021
Accepted 3 March 2021

KEYWORDS
Chemotherapy; RRx-001; temozolomide; irinotecan; vascular normalisation; vascular priming; tumor associated macrophage polarisation; CD-47 downregulation; BBB

Introduction

A hallmark of tumours, in general, and glioblastomas, in particular, is structurally and functionally abnormal blood vessels, which, compared to normal vasculature, are irregular in terms of size, shape and density [1] as well as leakiness, resulting in interstitial hypertension [2] as well as hypoxia. These abnormalities, which lead to blood vessel compression and a reduction in tumour perfusion, hinder the delivery, distribution and accumulation of anti-cancer therapeutics (Figure 1). Vascular priming, a variant of vascular normalisation, a term coined by Jain [3], in reference to the treatment-induced 'pruning' of the aberrant tumour vasculature with a concomitant increase in blood flow has emerged as a strategy to improve the uptake and penetration of anti-cancer therapies [4].

RRx-001 is a systemically non-toxic small molecule in a Phase 3 clinical trial [5] derived from 1,3,3-trinitroazetidine (TNAZ), a source of chemical energy for rockets and missiles, that skews tumour associated macrophage (TAM) polarisation from the protumor M2- to a tumour-inhibiting M1-phenotype [6]. RRx-001 is under investigation in clinical trials for the treatment of lung, colorectal, brain and other tumours. RRx-001 has received Orphan Drug Designation by the FDA in glioblastoma and other tumour types.

The anti-cancer mechanisms of RRx-001 are multiple fold, including vascular normalisation [7] and CD47 downregulation with activation of tumour-associated macrophages [6]. Multiple studies have demonstrated the vascular normalising properties of RRx-001, resulting in more efficient vessel perfusion and improved access to hypoxic tumour regions[7] .RRx-001 anti-cancer effects are tumour-targeted. After intravenous infusion, RRx-001 binds to red blood cells, circulates systemically, adheres to aberrant tumour vessels whereby RRx-001 bound red blood cells are activated within hypoxic tumour regions to release RRx-001 and its metabolites into the tumour microenvironment. *Via* red-blood cell delivery, RRx-001 has been shown to cross the blood-brain barrier (BBB), and reach hypoxic tumours inside the brain [8,10]. Both preclinical and clinical studies have shown that RRx-001 penetrates into hypoxic tumours, resulting in central tumour necrosis [9,10].

In the Phase I/II clinical trial for brain metastases acronymed BRAINSTORM (NCT02215512), RRx-001 was dosed five (5) times—once alone four days prior to whole brain radiotherapy (WBRT) and then 2x/week for two weeks in combination with WBRT (30 Gy in 10 fractions). The effect of the single dose of RRx-001 alone prior to WBRT on vascular permeability measured with dynamic contrast enhanced magnetic resonance imaging (DCE-MRI)-K^{trans} (a volume transfer constant of contrast agent between the blood vessels and extra-cellular tissue compartment) demonstrated a significant reduction in vessel leakiness indicative of a more functional, normalised tumour vasculature [10] in twelve out of twelve patients on whom DCE-MRI was performed . In addition, previous experiments with microbubble contrast enhanced ultrasound (CEUS) [11] and multiplexed immunohistochemical [12] staining for hypoxia, blood flow and vasculature demonstrated changes in tumour blood flow that were characterised by an initial induction of regional hypoxia with a redistribution of blood flow followed by normalisation resulting in increased blood flow [13].

The purpose of these experiments was to determine whether the reversal of leakiness during single-agent therapy with RRx-001

CONTACT Bryan Oronsky boronsky@epicentrx.com EpicentRx Inc, 11099 North Torrey Pines Road, Suite 160, La Jolla, CA 92037, USA

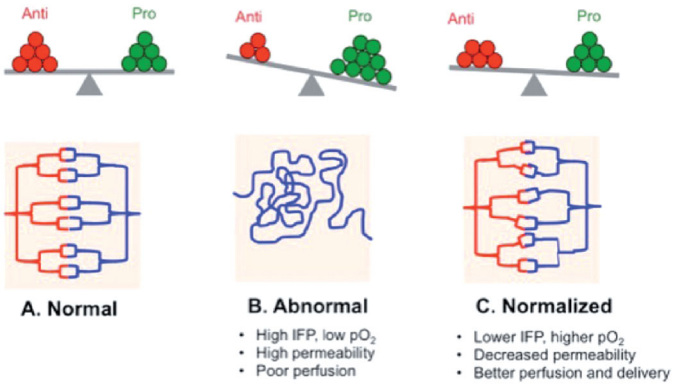

Figure 1. Schematic diagram to illustrate vascular normalisation. A. Normal tissues are characterised by an organised and efficient vascular supply. B. A hallmark of tumours is an abnormal inefficient vascular network due to an imbalance of pro-angiogenic and anti-angiogenic factors. C. Vascular normalisers like RRx-001 can restore balance to the inefficient vascular network which may promote the delivery of chemotherapy. Adapted from Jain RK et al. Angiogenesis and brain tumours. Nat Rev Neurosci 2007;8(8):610–622.

that has been observed both clinically in brain metastases and preclinically in heterotopically implanted syngeneic tumors [8,9], presumably as a result of lowered interstitial fluid pressure, would increase the delivery and accumulation of systemically administered irinotecan (CPT-11) and temozolomide (TMZ) in orthotopically implanted gliomas.

Materials and methods

All animal experiments were carried out using protocols reviewed and approved by the UCSD Institutional Animal Care and Use Committee (US National Research Council, 2010)

Irinotecan and temozolomide

Irinotecan hydrochloride (CPT-11) and temozolomide (Sigma-Aldrich, St Louis, MO, USA) were dissolved in saline. Oral administration of temozolomide (25 mg/kg) was by oral gavage in a 2.5 mg/mL solution containing 2.5 mg/ml Povidone K30, 0.015% citric acid, 50% Ora-Plus, 50% Ora-Sweet SF (Paddock Labs Minneapolis, MN) [14].

RRx-001

RRx-001 was provided by EpicentRx, Inc (La Jolla, CA). The synthesis and characterisation of RRx-001 is reported in detail elsewhere [15,16]. RRx-001 was dissolved in DMSO and then diluted with growth medium with a final concentration of DMSO at $<0.05\%$. RRx-001 solution was prepared immediately before use.

GBM xenografts

Human GBM primary tissues, GBM43, are maintained as serially passaged subcutaneous xenografts in athymic mice [17].

To prepare tumour cells from subcutaneous xenografts for transfer to the intracranial compartment, excised subcutaneous tumours were placed in culture dishes and minced with a scalpel then mechanically dispersed by repetitive pipetting to create small cellular aggregates that were passed repeatedly through 40-micron nylon mesh filters to produce single-cell suspensions. Cell suspensions were centrifuged at a rate of 1000 rpm for 10 min at 4 °C and supernatants aspirated before resuspending pellets in 1 ml of sterile DMEM media. Cells were suspended in DMEM media at 1×10^5 cells/μL for intracranial injection. 3 µL of tumour cell suspension (3×10^5 cells total) were injected into the right caudate putamen of 6w-old-female athymic mice (nu/nu). Prior injection, mice were anaesthetised IP [ketamine (100 mg/kg)/xylazine (10 mg/kg)].

Intracranial tumor establishment in athymic mice

Six-week-old female athymic mice (nu/nu, homozygous), housed under aseptic conditions, received intracranial tumour cell injection as previously described [13] and as approved by the UCSD Institutional Animal Care and Use Committee, and conducted accordingly to the Guide for the Care and Use of Laboratory Animals (US National Research Council, 2010).

Vascular administration of RRx-001, irinotecan and temozolomide

For vascular administration of therapy, mice were warmed for 5–10min with either a heating pad or a heat lamp to dilate tail vasculature. Injection sites were then cleaned with an alcohol swab, after which a 28 g insulin syringe was inserted that was loaded with RRx-001, irinotecan or temozolomide.

Oral administration of temozolomide

25 mg/kg temozolomide (TMZ) was administrated oral suspension vehicle containing 2.5 mg/ml Povidone K30, 0.015% citric acid, 50% Ora-Plus, 50% Ora-Sweet SF (Paddock Labs Minneapolis, MN) on Days 12, 16, 20, 24, 28 and 32.

Analysis of irinotecan content in intracranial tumors

For the experiment involving analysis of tumour irinotecan content, athymic mice with intracranial GBM43 were administered RRx-001 (10 mg/kg) followed 4 h later by 0.4 mg of irinotecan by tail vein on days 12, 18, 24 and 30 after implantation of tumour cells. Mice were euthanised 24 h after last irinotecan administration, with brains immediately resected and tumour tissue dissected prior to snap-freezing by immersion in liquid nitrogen. Analysis of irinotecan levels in tumour tissues was in brief as follows: water was added to tissues at a 20% (w/v) ratio, and tissues were then homogenised with a mechanical homogeniser in an ice bath. Homogenates were extracted for the lactone form of irinotecan with an acidic methanol solution by vortexing and centrifugation at 13 000 rpm for 10 min, with the supernatants then transferred to autosampler vials for high-performance liquid chromatography (HPLC) analysis (eluted peaks, 380/540 nm).

Analysis of temozolomide content in intracranial tumors

For the experiment involving analysis of tumour temozolomide (TMZ) content, athymic mice with intracranial GBM43 were administered RRx-001 (10 mg/kg) followed 4 h later by 20 mg/kg of TMZ by tail vein on days 12, 18, 24 and 30 after implantation of tumour cells. Mice were euthanised 24 h after last TMZ administration, with brains immediately resected and tumour tissue dissected prior to snap-freezing by immersion in liquid nitrogen. Analysis of TMZ levels in tumour tissues was in brief as follows: water was added to tissues at a 20% (w/v) ratio, and tissues were then homogenised with a mechanical homogeniser in an ice bath.

Homogenates were extracted with an acidic methanol solution by vortexing and centrifugation at 13,000 rpm for 10 min, and analysed with mass spectrometry (m/z 195).

Animal experiment design

All animal experiments were approved by the Animal Committee of UCSD and adhered to the experimental animal care guidelines. A total of 72 mice were used, including normal ($n = 18$) and tumour-bearing mice ($n = 54$).

In experimental group 1, the aim was to quantitate the IV TMZ concentration in tumour-bearing animals to determine a) survival and b) whether RRx-001 pre-treatment affects TMZ deposition in orthotopically implanted gliomas. Animals were divided into four groups: (1) control (saline) $n = 6$ (2) IV RRx-001 10 mg/kg only IV (3) IV TMZ administration (20 mg/kg) only ($n = 6$) and (4) TMZ administration (20 mg/kg) 4 h post RRx-001 ($n = 6$). Treatment was started 12 days after intracranial tumour cell injection and administered on Days on Days 12, 18, 24, and 30. Animals were sacrificed euthanised as the symptoms from the increasing tumour burden prevented access to food or water, and brain samples (including tumour and contralateral brain tissues) as well as plasma (total of 500 µL per each animal) were obtained for TMZ quantification (only 1 sample per each mouse tissue or blood).

In experimental group 2, the aim was to quantitate oral TMZ concentration in tumour-bearing animals to determine a) survival and b) whether RRx-001 pre-treatment affects TMZ deposition in orthotopically implanted gliomas. Animals were divided into four groups: (1) control (saline) $n = 6$ (2) IV RRx-001 10 mg/kg only IV (3) oral TMZ administration (25 mg/kg) only ($n = 6$) and (4) oral TMZ administration (25 mg/kg) 4 h post RRx-001 ($n = 6$). Treatment was started 12 days after intracranial tumour cell injection and administered on Days 12, 16, 20, 24, 28 and 32. Animals were sacrificed euthanised as the symptoms from the increasing tumour burden prevented access to food or water, and brain samples (including tumour and contralateral brain tissues) as well as plasma (total of 500 µL per each animal) were obtained for TMZ quantification (only 1 sample per each mouse tissue or blood).

In experimental group 3, the aim was to quantitate irinotecan concentration in tumour-bearing animals to determine a) survival and b) whether RRx-001 pre-treatment affects irinotecan deposition in orthotopically-implanted gliomas. Animals were divided into four groups: (1) control (saline) $n = 6$ (2) IV RRx-001 10 mg/kg only IV (3) IV irinotecan administration (0.4 mg) only ($n = 6$) and (4) IV irinotecan administration (0.4 mg) 4 h post RRx-001 ($n = 6$). Treatment was started 12 days after intracranial tumour cell injection and administered on Days 12, 18, 24, and 30. Animals were sacrificed euthanised as the symptoms from the increasing tumour burden prevented access to food or water, and brain samples (including tumour and contralateral brain tissues) as well as plasma (total of 500 µL per each animal) were obtained for irinotecan quantification (only 1 sample per each mouse tissue or blood).

Results

Effect of RRx-001 on survival when combined with intravascular irinotecan therapy

The design for these experiments is consistent with a clinical study in which investigational therapies are divided into separate treatment arms. To evaluate the activity of irinotecan in this context, we compared the anti-tumour activity of RRx-001 alone, irinotecan alone and RRx-001 + irinotecan administered intravenously every 6 days x4. While RRx-001 and TMZ alone provided minimal to moderate, respectively, survival benefit to mice with intracranial GBM43, when compared to control, the combination of RRx-001 and irinotecan provided the most extensive survival benefit of any treatment with half of the treatment group (3 of 6) experiencing apparent cure of tumour (Figure 2). The therapeutic regimens were well tolerated, with no animal subject experiencing >10% loss of pre-treatment body weight at completion of therapy (data not shown).

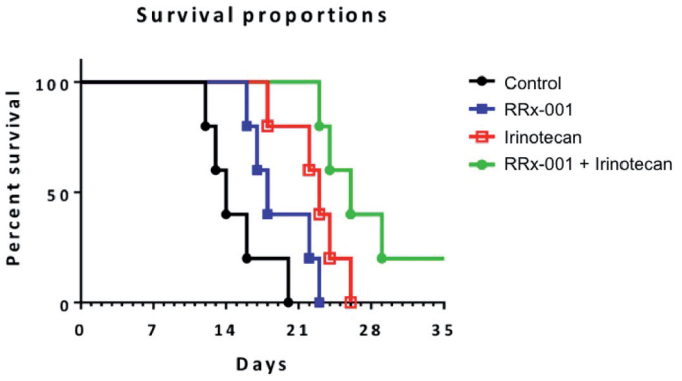

Figure 2. Corresponding survival plots for all treatment groups. The experiment was terminated at 35 days. 6 mice were included in the survival analysis for all treatment groups.

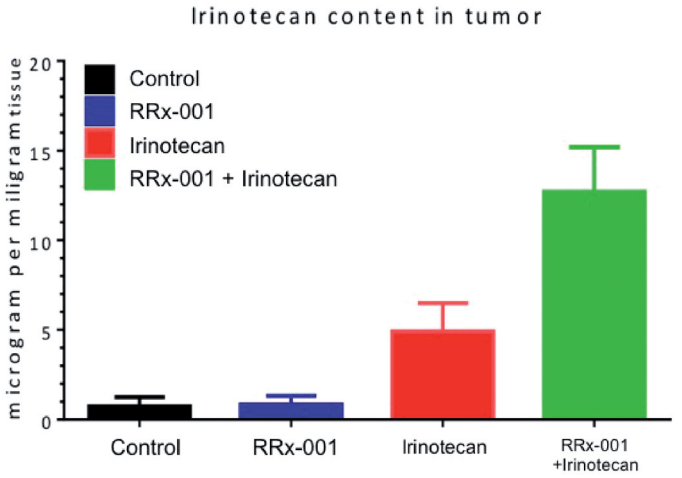

Figure 3. Tumour Irinotecan Content. 24 h after irinotecan administration, mice bearing the tumour (one per group) were euthanised. Their brains and tumour tissue were dissected. Irinotecan levels, measured in tumour tissues with HPLC, demonstrated significantly higher levels in combination with RRx-001 pre-treatment.

Analysis of irinotecan content in intracranial xenografts

To obtain information addressing whether RRx-001 alters access of peripherally administered irinotecan to intracranial tumour, twenty-four hours after last irinotecan administration, one mouse from each group was euthanised and tumour was dissected from surrounding normal brain of the euthanised mice, with dissected tumours subsequently examined for irinotecan content. The results of this analysis showed that irinotecan accumulation was substantially and significantly higher in the tumours of mice receiving pre-treatment with RRx-001 (Figure 3).

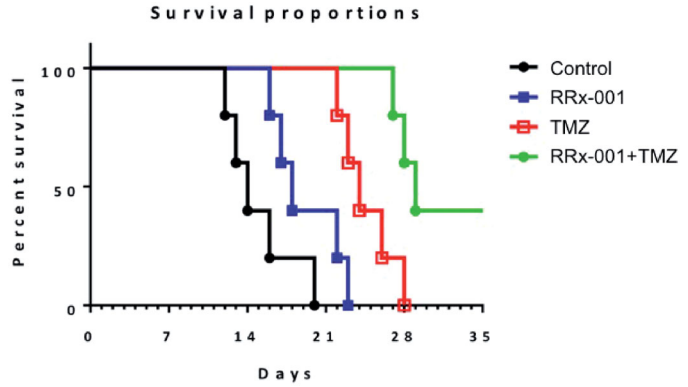

Figure 4. Corresponding survival plots for all treatment groups. The experiment was terminated at 35 days. 6 mice were included in the survival analysis for all treatment groups.

Effect of RRx-001 on survival when combined with intravascular temozolomide (TMZ) therapy

The design for these experiments is consistent with a clinical study in which investigational therapies are divided into separate treatment arms. To evaluate the activity of irinotecan in this context, we compared the anti-tumour activity of RRx-001 alone, TMZ alone (20 mg/kg) and RRx-001 + TMZ (20 mg/kg) administered intravenously every 6 days x4. While RRx-001 and TMZ alone provided minimal to moderate, respectively, survival benefit to mice with intracranial GBM43, when compared to control, the combination of RRx-001 and TMZ provided the most extensive survival benefit of any treatment with half of the treatment group (3 of 6) experiencing apparent cure of tumour (Figure 4). The therapeutic regimens were well tolerated, with no animal subject experiencing >10% loss of pre-treatment body weight at completion of therapy (data not shown).

Analysis of TMZ content in intracranial xenografts

To obtain information addressing whether RRx-001 alters access of peripherally administered TMZ to intracranial tumour, twenty-four hours after last TMZ administration, one mouse from each group was euthanised and tumour was dissected from surrounding normal brain of the euthanised mice, with dissected tumours subsequently examined for TMZ content. The results of this analysis showed that TMZ accumulation was substantially and significantly higher in the tumours of mice receiving pre-treatment with RRx-001 (Figure 5).

Effect of RRx-001 on survival when combined with oral temozolomide (TMZ) therapy

To evaluate the activity of TMZ in this cohort, we compared the anti-tumour activity of RRx-001 alone, TMZ alone (25 mg/kg) and RRx-001 + TMZ (25 mg/kg) administered by oral gavage every 6 days x4. While RRx-001 and TMZ alone provided minimal to moderate, respectively, survival benefit to mice with intracranial GBM43, when compared to control, the combination of RRx-001 and TMZ provided the most extensive survival benefit of any treatment. The therapeutic regimens were well tolerated, with no animal subject experiencing >10% loss of pre-treatment body weight at completion of therapy (data not shown).

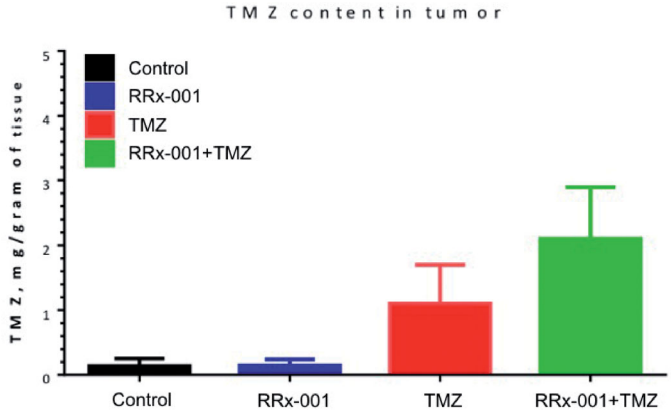

Figure 5. Tumour TMZ Content. 24 h after TMZ administration, mice bearing the tumour (one per group) were euthanised. Their brains and tumour tissue were dissected. TMZ levels, measured in tumour tissues with HPLC, demonstrated significantly higher levels in combination with RRx-001 pre-treatment.

Analysis of TMZ content in intracranial xenografts

To obtain information addressing whether RRx-001 alters access of orally administered TMZ to intracranial tumour, twenty-four hours after last TMZ administration, one mouse from each group was euthanised and tumour was dissected from surrounding normal brain of the euthanised mice, with dissected tumours subsequently examined for TMZ content. The results of this analysis showed that TMZ accumulation was substantially and significantly higher in the tumours of mice receiving pre-treatment with RRx-001.

Discussion

While heart disease is currently the leading cause of death in the United States (without taking into account the acute surge in mortality from COVID-19), cancer is expected to surpass it in the near term [18]. The development of resistance to radiation, chemotherapy and molecularly targeted therapies is the underlying basis for this increase in mortality, which, in part, results from tumour hypoperfusion with poor drug and oxygen penetration. The 'abnormalisation' of the tumour vasculature with increased interstitial fluid pressure (IFP) is directly responsible for the inefficiency and stasis of blood flow [19]. To optimise perfusion and subsequent drug delivery, vascular priming, which combines agents that normalise tumour blood vessels with traditional therapies [20], is broadly applicable as a generic strategy to improve the specificity of treatment independent of the specificity of the drug itself, through a modulation of blood flow and biodistribution.

The experiments in this paper, which demonstrate that administration of RRx-001 prior to temozolomide or irinotecan results in significantly increased uptake of irinotecan and temozolomide in orthotopic glioma tumours are consistent with an RRx-001-induced vascular 'priming' effect that has been observed both preclinically and clinically in the Phase I/II BRAINSTORM clinical trial (NCT02215512).

These results, while theoretically applicable to multiple tumour types, are particularly relevant to the treatment of CNS malignancies, a source of considerable morbidity and mortality, given the added layer of 'protection' afforded to the tumour cells by the blood-brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSF) [21], even if they are partially disrupted in cancer, which impedes drug delivery and bioavailability.

Novel therapeutic strategies are urgently needed for the treatment of CNS malignancies, many of which are characterised by aggressive tumour phenotypes and associated with dismal prognoses [22–24]. Despite wide-spread research efforts, few advances have been made in recent years, with few additions to the standard-of-care regimens for brain malignancies [25].

Over the last decades, advances in anticancer therapies have largely bypassed the treatment of patients with brain cancer [26]. As a case in point, all currently used anti-GBM agents including temozolomide and irinotecan as well as carmustine (BCNU) and cis-platinum (cisplatin) have no greater than a 30–40% response rate, and most fall into the range of 10–20% [27]. In fact, temozolomide is the only systemically delivered chemotherapy proven to significantly improve survival in patients with glioblastoma [28]. A possible explanation for these poor response rates is suboptimal CNS concentrations of chemotherapy to which the tumours are exposed, in turn at least partly attributable to the common feature of aberrant tumour neovascularity that, in combination with the BBB and BCSF, as well as intravascular coagulation/thrombosis [29], shelters CNS tumours from cytotoxicity. For example, temozolomide levels in the CNS only reach approximately 30–40% of plasma levels [30], while in one study with irinotecan-infused non-human primates the level of irinotecan in the CSF was 14% of the plasma level; no SN-38 (the primary metabolite of irinotecan) was detectable intrathecally [31].

Novel agents such as RRx-001 have the potential to improve outcomes for patients with brain malignancies, in combination with existing treatment modalities such as chemotherapy and radiation, or potentially as single agents. Preclinical and clinical studies have demonstrated RRx-001 capability to cross the BBB and penetrate brain tumours, with promising anti-cancer activity. In clinical trials to date, RRx-001 has been investigated for the treatment of multiple solid tumour types including brain metastases (NCT02215512) and glioblastoma (NCT02871843). Future advanced-stage trials are planned featuring RRx-001 for the treatment of CNS malignancies.

Conclusions

Since the success of chemotherapy in the brain (and outside of it) is limited by subtherapeutic tumoral drug concentrations, vascular normalisation-enhanced delivery of standard cytotoxics as demonstrated with RRx-001 may mitigate or reverse clinical drug resistance and thereby improve the outcome of cancer therapy, particularly in the brain.

Ethical approval

All animal experiments were carried out using protocols reviewed and approved by the UCSD Institutional Animal Care and Use Committee and conducted accordingly to the Guide for the Care and Use of Laboratory Animals (US National Research Council, 2010).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

Authors B.O, T.R and P.C. participated in the study conception, design, analysis and interpretation of the data; as well as the manuscript drafting, writing and editing. of the paper or revising it critically for intellectual content; All authors provided final approval of the manuscript.

Funding

EpicentRx Inc. funds research of RRx-001.

Data availability statement

No additional data available.

References

[1] McDonald DM, Choyke PL. Imaging of angiogenesis: from microscope to clinic. Nat Med. 2003;9:713–725.
[2] Boucher Y, Leunig M, Jain RK. Tumor angiogenesis and interstitial hypertension. Cancer Res. 1996;56:4264–4266.
[3] Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307:58–62.
[4] Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. J Clin Oncol. 2013;31:2205–2218.
[5] Oronsky B, Reid TR, Larson C, et al. REPLATINUM Phase III randomized study: RRx-001 + platinum doublet versus platinum doublet in third-line small cell lung cancer. Future Oncol. 2019;15:3427–3433. Oct
[6] Cabrales P. RRx-001 acts as a dual small molecule checkpoint inhibitor by downregulating CD47 on cancer cells and SIRP-α on monocytes/macrophages. Transl Oncol. 2019;12:626–632.
[7] Oronsky B, Scicinski J, Cabrales P, et al. RRx-001, an epigenetic-based radio- and chemosensitizer, has vascular normalizing effects on SCCVII and U87 tumors. Clin Epigenetics. 2016;8:53.
[8] Oronsky B, Oronsky N, Fanger G, et al. A review of two promising radiosensitizers in brain metastases: RRx-001 and 2-deoxyglucose. J Cancer Sci Ther. 2015;07:5.
[9] Brzezniak C, Schmitz BA, Peterson PG, et al. RRx-001-induced tumor necrosis and immune cell infiltration in an EGFR mutation-positive NSCLC with resistance to EGFR tyrosine kinase inhibitors: a case report. Case Rep Oncol. 2016;9:45–50.
[10] Kim M, Parmar H, Cao Y, et al. Whole brain radiotherapy and RRx-001: two partial responses in radioresistant melanoma brain metastases from a phase I/II clinical trial: a TITE-CRM phase I/II clinical trial. Transl Oncol. 2016;9:108–113.
[11] Ning S, Bednarski M, Oronsky B, et al. Dinitroazetidines are a novel class of anticancer agents and hypoxia-activated radiation sensitizers developed from highly energetic materials. Cancer Res. 2012;72:2600–2608.
[12] Scicinski J, Oronsky B, Ning S, et al. RRx-001 modulates intratumor blood flow in SCCVII and U87 tumors. Cancer Res. 2012;72:4371.
[13] Aiken R, Fine H, Butowski N, et al. Phase I, trial of radiation, temozolomide and RRx-001 followed by maintenance temozolomide with or without Rrx-001 in newly diagnosed glioblastoma patients. Neuro-Oncology. 2019;21:vi11–vi12.

[14] Trissel L, Zhang YB, Koontz SEPB. Temozolomide stability in extemporaneously compounded oral suspensions. Int J Pharm Compd. 2006;10:396–399.
[15] Scicinski J, Oronsky B, Taylor M, et al. Preclinical evaluation of the metabolism and disposition of RRx-001, a novel investigative anticancer agent. Drug Metab Dispos. 2012;40: 1810–1816.
[16] Straessler NA, Lesley MW, Cannizzo LF. Development of a safe and efficient two-step synthesis for preparing 1-bromoacetyl-3,3-dinitroazetidine, a novel clinical anticancer candidate. Org Process Res Dev. 2012;16:512–517.
[17] Giannini C, Sarkaria JN, Saito A, et al. Patient tumor EGFR and PDGFRA gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. Neuro Oncol. 2005;7:164–176.
[18] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65:5–29.
[19] De Bock K, Cauwenberghs S, Carmeliet P. Vessel abnormalization: another hallmark of cancer? Molecular mechanisms and therapeutic implications. Curr Opin Genet Dev. 2011; 21:73–79.
[20] Oronsky BT, Scicinski JJ, Reid T, et al. Beyond antiangiogenesis: vascular modulation as an anticancer therapy – a review. Transl Oncol. 2012;5:133–140.
[21] Bradbury M, Begley D, Kreuter J. 2000. The blood-brain barrier and drug delivery to the CNS. Boca Raton (FL): CRC Press.
[22] Arvanitis CD, Ferraro GB, Jain RK. The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat Rev Cancer. 2020;20:26–41.
[23] Aldape K, Brindle KM, Chesler L, et al. Challenges to curing primary brain tumours. Nat Rev Clin Oncol. 2019;16: 509–520.
[24] Mellinghoff IK, Gilbertson RJ. Brain Tumors: Challenges and Opportunities to Cure. J Clin Oncol. 2017;35: 2343–2345.
[25] Lah T, Novak M, Breznik B. Brain malignancies: glioblastoma and brain metastases. Semin Cancer Biol. 2020;60: 262–273.
[26] Laquintana V, Trapani A, Denora N, et al. New strategies to deliver anticancer drugs to brain tumors. Expert Opin Drug Deliv. 2009;6:1017–1032.
[27] Tivnan A, Zakaria Z, O'Leary C, et al. Inhibition of multidrug resistance protein 1 (MRP1) improves chemotherapy drug response in primary and recurrent glioblastoma multiforme. Front Neurosci. 2015;9:218.
[28] Jackson S, Anders N, Mangraviti A, et al. The effect of regadenoson-induced transient disruption of the blood brain barrier on temozolomide delivery to rat brain parenchyma. J Clin Oncol. 2015;33:2066–2066.
[29] Falanga A, Marchetti M, Vignoli A. Coagulation and cancer: biological and clinical aspects. J Thromb Haemost. 2013;11: 223–233.
[30] Ostermann S, Csajka C, Buclin T, et al. Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. Clin Cancer Res. 2004;10: 3728–3736.
[31] Hamberg P, Donders RC, ten Bokkel Huinink D. Central nervous system toxicity induced by irinotecan. J Natl Cancer Inst. 2006;98:219.