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CLINICAL STUDY

A Phase I clinical trial of dose-escalated metabolic therapy combined with concomitant radiation therapy in high-grade glioma

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Abstract

Background Animal brain-tumor models have demonstrated a synergistic interaction between radiation therapy and a ketogenic diet (KD). Metformin has in-vitro anti-cancer activity, through AMPK activation and mTOR inhibition. We hypothesized that the metabolic stress induced by a KD combined with metformin would enhance radiation's efficacy. We sought to assess the tolerability and feasibility of this approach.

Methods A single-institution phase I clinical trial. Radiotherapy was either 60 or 35 Gy over 6 or 2 weeks, for newly diagnosed and recurrent gliomas, respectively. The dietary intervention consisted of a Modified Atkins Diet (ModAD) supplemented with medium chain triglycerides (MCT). There were three cohorts: Dietary intervention alone, and dietary intervention combined with low-dose or high-dose metformin; all patients received radiotherapy. Factors associated with blood ketone levels were investigated using a mixed-model analysis.

Results A total of 13 patients were accrued, median age 61 years, of whom six had newly diagnosed and seven with recurrent disease. All completed radiation therapy; five patients stopped the metabolic intervention early. Metformin 850 mg three-times daily was poorly tolerated. There were no serious adverse events. Ketone levels were associated with dietary factors (ketogenic ratio, $p < 0.001$), use of metformin ($p = 0.02$) and low insulin levels ($p = 0.002$). Median progression free survival was ten and four months for newly diagnosed and recurrent disease, respectively.

Conclusions The intervention was well tolerated. Higher serum ketone levels were associated with both dietary intake and metformin use. The recommended phase II dose is eight weeks of a ModAD combined with 850 mg metformin twice daily.

Keywords Ketogenic diet · Glioblastoma · Radiation · Metformin · Phase I

Abbreviations

KD	Ketogenic diet
ModAD	Modified Atkins diet
HGG	High-grade gliomas
AMPK	AMP-activated protein kinase
LDL	Low-density lipoprotein
MCT	Medium chain triglyceride
β-OHB	β-hydroxybutyrate
VMAT	Volumetric modulated arc therapy
HbA1C	hemoglobin A1C
IGF1	Insulin like growth factor1
TRAM	Treatment response assessment maps
BMI	Body mass index

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Introduction

High-grade gliomas (HGG) are the most common primary adult brain tumor. Despite adjuvant chemoradiation, relapses are universal and median survival short [1]. Biochemically, the tumors exhibit metabolic reprogramming [2], with increased glucose uptake being driven by the PI3K-AKT pathway [3]. There is both laboratory and clinical evidence that carbohydrate metabolism influences outcomes: glioma cells express growth-promoting insulin receptor [4], exogenous glucose induces an aggressive in-vitro phenotype [5], and multiple observational studies have shown hyperglycemia to be a poor prognostic factor in glioblastoma patients [6–8].

Although increased glucose metabolism is thought to contribute to the tumor's aggressive phenotype [5], it may also be the cancer's Achilles' heel [9] since tumors may become glucose-dependent. When blood glucose levels drop, ketone bodies provide an alternative source of energy for the normal brain. Preclinical evidence suggests that glioma cells are less able to metabolize ketones [10], hence hypoglycemia may selectively starve tumors cells. Furthermore, there is some evidence that ketones themselves, especially β-hydroxybutyrate(β-OHB), optimize the response to radiation therapy through its action as a HDAC inhibitor [11] and enhancement of DNA damage G2/M checkpoint cell cycle arrest [12, 13]. Ketogenic diets, such as the Modified Atkins Diet (ModAD) [14], or a classical strict KD, rely on fats as the primary source of energy. The combination of a KD with radiation therapy has been shown effective in preclinical models [15, 16]. Retrospective studies have suggested a KD to be beneficial to glioblastoma patients [17, 18], however it is unclear how applicable these studies are to the wider population.

Metformin, an oral biguanide anti-diabetic drug, has multiple actions including preventing hepatic gluconeogenesis [19] and increasing insulin sensitivity. In non-diabetic patients metformin decreases post-prandial hyperglycemia [20]. Biochemically, metformin activates AMP-activated protein kinase (AMPK) [21] and downregulates mTOR signaling [22]. There is current interest in the drug's potential (though clinically unproven) anti-neoplastic activity [23]. Recently published work has shown that the combination of fasting-induced hypoglycemia with metformin impairs tumor growth through modulation of the PP2A-GSK3β-MCL-1 Axis [24], a tumorigenic pathway in glioblastoma [25].

In this trial we examined two metabolic interventions in non-diabetic glioma patients being treated with radiation therapy: (1) ModAD and (2) concomitant administration of metformin. Both metabolic treatments are being expected to downregulate the mTOR pathway [22, 26]. We hypothesized that metabolic stress (relative hypoglycemia) would enhance the anti-tumor efficacy of radiation-induced DNA damage. We further hypothesized that metformin would reduce insulin resistance and consequently elevate ketone blood levels. We performed a formal prospective phase I dose-escalation trial to assess the tolerability and feasibility of this approach.

Methods

Trial design

A prospective single-institution phase I dose-escalation clinical trial of combined metabolic and radiotherapy and amongst adults with brain gliomas, both newly diagnosed and recurrent, was conducted at the Chaim Sheba Medical Center, Fig. 1. The trial was approved by the local Institutional Review Board (IRB) - SMC 0712 – 13, and registered on Clinicaltrials.gov NCT02149459. All patients were required to sign the study informed consent. Adverse events were graded using Common Terminology Criteria for Adverse Event (CTCAE) version 4.03.

All patients received radiation therapy. There were three cohorts of metabolic therapy; (1) dietary intervention alone, (2) low-dose metformin combined with dietary intervention and (3) high-dose metformin combined with dietary intervention (Suppl Table 1). A cohort "−1" was defined as normal diet with very low dose metformin in case cohort 1 was not tolerated. Dose escalation proceeded according to a "3 + 3 design", there was no intra-patient dose escalation. The recommended phase II dose was defined as the maximal tolerated dose.

Eligibility criteria

Inclusion criteria were adult patients with histologically proven high-grade glioma, (WHO grade III and IV) whether newly diagnosed or recurrent, who required radiation therapy. Astrocytic and oligodendroglia supratentorial tumors, grades 3 or 4 according to the WHO 2007 classification were eligible. Patients were required to have a performance status ECOG of two or less, and life expectancy of at least two months. For patients with recurrent disease, there was no limit regarding the number/type of previous systemic treatments or surgeries, however at least a 2-week break between prior treatment and enrollment was required, and patients were required to have recovered from the toxic effects of prior therapies. Regarding previous irradiation in patients with recurrent glioma - all were required to have received a course of fractionated radiation therapy, and subjects were permitted to have received up to one prior radiosurgical procedure within the treatment field. Exclusion criteria included patients with diabetes mellitus, known inborn errors of metabolism, hyperlipidemia (defined as total cholesterol over 400 mg/dL, low-density lipoprotein (LDL) above 300 mg/dL, and/or triglycerides over 500 mg/dl.), and contraindications to metformin use (drug allergy, renal failure: creatinine levels over 150 μmol/l (1.7 mg/dL), liver disease, ongoing alcohol abuse).

Concurrent anti-tumor medications

Concurrent anti-tumor medications were allowed, including temozolomide, bevacizumab and medical cannabis. The use of steroids was allowed, but discouraged.

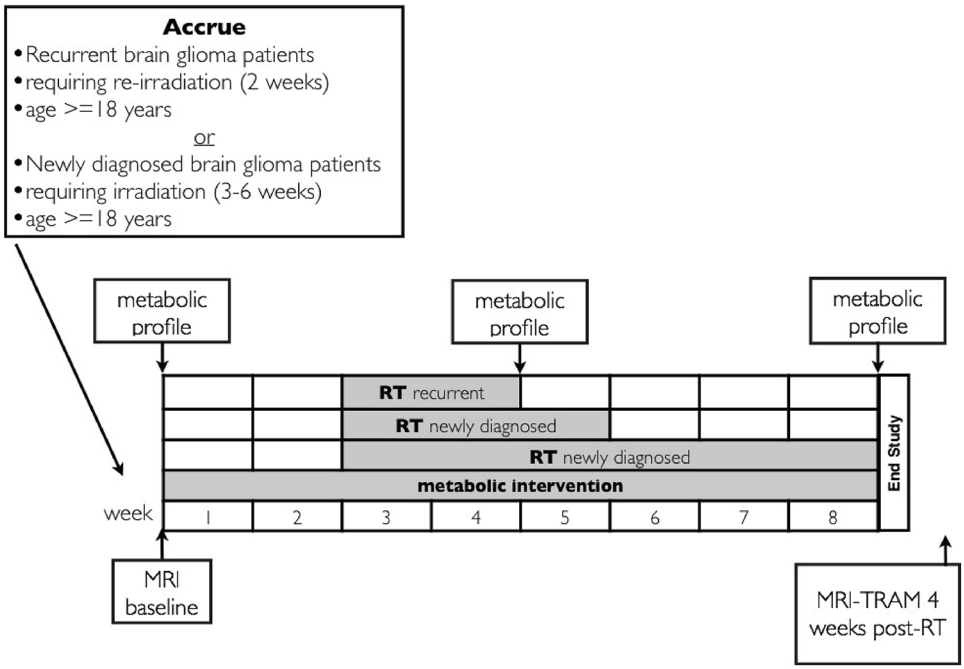

Fig. 1 Trial Schema

Dietary intervention

Subjects were instructed to conform to the ModAD based upon a low carbohydrate, high-fat intake [14], (details in supplementary material) for eight weeks. The target 'ketogenic ratio', calculated as total fat intake (gram) divided by the sum of total protein intake (gram) and total carbohydrate intake (gram), was between 1:1 and 3:1. The diet was supplemented with coconut-extracted MCT oil, known to stimulate hepatic ketogenesis [27], at the dietician's discretion. No calorie restriction was applied, patients were instructed to eat to satiety. Dieticians kept close contact with the patients through weekly meetings and daily telephone calls. Dietary nutritional intake was calculated using the Tzameret program (Maymone Software, Jerusalem) [28] based upon detailed food diaries. At the conclusion of the eight-week period, subjects were gradually returned to a normal diet.

Metformin

Participants in cohort #2 and #3 received metformin therapy in addition to the diet. Cohort #2 received #850 mg twice daily, cohort #3 received #850 mg three times daily. Cohort #-1 were to receive #850 mg once daily. In order increase tolerability a reduced dose metformin was administered during the first week (Suppl. Table 1).

Radiation therapy

Radiation therapy for newly diagnosed HGGs was 60 Gy over six weeks in 30 fractions planned using 3-dimensional or Volumetric modulated arc therapy (VMAT) techniques. Radiation for recurrence disease was 30-35 Gy in ten fractions delivered over two weeks [29]. Image guidance was performed daily.

Correlative studies

Patients underwent a comprehensive metabolic assessment including weight, height and blood tests (electrolytes, liver and kidney function, lipid profile, hemoglobin A1C(HbA1C), insulin, C peptide, insulin like growth factor1 (IGF1), leptin, adiponectin, β-OHB) prior to and during the metabolic therapy. MRI scans, including treatment response assessment maps (TRAM) [30], were performed prior to, and 8–12 weeks following treatment.

Determination of Ketone body levels

β-OHB ketone bodies were measured in venous blood using the Precision Xtra Ketone Monitoring System (Abbott Laboratories, Lake Bluff, Illinois USA), and in urine using a dipstick (Combur10 Test strip, Roche Diagnostics, Basel). Patients were instructed to self-monitor urine ketones daily for the first two weeks, and subsequently on alternative days for six weeks using urine dipsticks.

Amendment

The original protocol recruited only patients with recurrent disease scheduled to receive re-irradiation. In September 2017 the protocol was amended to include accrual of patients with newly diagnosed disease together with concomitant temozolomide.

Statistics

Levels of metabolites, weight and body mass index (BMI) before and during the intervention were compared using Student's paired t-test. The relationship between blood ketone and HbA1C levels with various covariates was examined using a linear mixed model. Survival was estimated by Kaplan-Meier analysis. Median follow up time was calculated using the 'Reverse Kaplan-Meier' approach. Statistical analysis was performed using Stata version IC 16.1 (Stata, College Station, TX).

Results

Thirteen patients were enrolled between November 2014 and February 2020. Median age 61 years, 62 % male, 77 % glioblastoma (Table 1). Six had newly diagnosed disease and seven patients had recurrent disease. For those with recurrent disease, all had previously received at least two previous treatments including radiation therapy. Median follow up was 18 months.

Treatment delivery

All patients completed radiation as planned. Five out of 13 (38 %) discontinued the metabolic intervention after a mean of 4 weeks, all from cohort two: one patient with pre-existing gout experienced asymptomatic hyperuricemia (grade 3), one patient with recurrent glioma and known seizures was hospitalized for seizures and general deterioration, one patient due to rapid tumor progression within two weeks of commencing the trial, two patients requested to come off study due to complaints of anorexia, nausea and difficulty in meeting dietary goals. Regarding metformin, amongst the patients in cohort two 850 mg twice daily was well tolerated. Of the three patients in cohort three, two decreased their daily dose to 850 mg twice daily due

Table 1 Baseline demographics of trial participants

Patient #	Age	Sex	Original histology	Time since first diag (months)	Clinical setting	Previous treatments	RT dose (Gy)	Concomitant therapy	Metabolic intervention therapy
1	62	m	GBM	28	Rec	RT + Tem, Bev	30	Bev + steroids	Cohort 1: Diet alone
2	61	m	GBM	12	Rec	RT + Tem, Bev + CCNU	30	Bev	
3	66	f	GBM	44	Rec	RT + Tem, re-Tmz	35	-	
4	61	f	AA	13	Rec	RT + Tem, Bev + CCNU	30	-	Cohort 2: Diet + Metformin 850 mg twice-daily
5	58	m	GBM	43	Rec	RT + Tem, re-Tmz, re-op, Bev	30	Rindopepimut	
6	57	f	AA	24	Rec	RT, re-op, Tem, bev	30	Steroids	
7	58	m	GBM	36	Rec	RT + Tem, Bev + CCNU	30	Bev	
8	62	m	GBM	2	New diag		60	Tmz	
9	58	m	GBM	2	New diag		60	Tmz	
10	74	f	GBM	0	New diag		60	Tmz + steroids	
11	52	f	AA	5	New diag		60	Tmz	Cohort 3: Diet + Metformin 850 mg three-times daily
12	66	m	GBM	1	New diag		60	Tmz	
13	63	m	GBM	1	New diag		60	Tmz + steroids	

AA anaplastic astrocytoma grade 3, *Bev* bevacizumab, *CCNU* lomustine, *diag* diagnosis, *GBM* glioblastoma, *Rec* recurrence, *re-op* re-operation, *RT* radiotherapy, *re-TMZ* reintroduction Temozolomide, Tmz Temozolomide

to nausea. One patient stopped concomitant temozolomide due to thrombocytopenia.

Toxicity and tolerability

There were two grade 3 adverse events: nausea and asymptomatic hyperuricemia; there were no grade 4/5 events (Table 2). Amongst some patients, ketone levels dropped slightly towards the end of the eight weeks, suggesting decreased compliance. Conversely, two patients requested to maintain the diet beyond the prescribed eight weeks.

Metabolic outcomes

Ketones were detected in all patients' blood at least once, with the mean blood ketone level during the intervention (weeks 1–8 inclusive) ranging from 0.17 to 1.3 mmol/l, and maximal recorded values ranging from 0.2 to 2.5 mmol/l; likewise, ketones were consistently found in urine specimens ranging from −/+ to +++ (median ++). The only other consistent finding was an asymptomatic increase in blood uric acid levels. Numerical, but non-significant, decreases were noted in blood glucose and HbA1c and increases in blood lipid values (Suppl Table 2).

Associations with blood ketone levels

On mixed-model univariate analysis, high dietary fat intake, low dietary carbohydrate intake, low dietary protein intake (borderline significance), MCT intake, ketogenic ratio, metformin dose, and lower serum insulin levels were associated with higher blood ketone levels (Fig. 2, Suppl. Table 3). In the final mixed-model multivariant analysis, the only significant covariates were ketogenic ratio and metformin use (Suppl. Table 4).

Table 2 Adverse events during the trial, as classified by CTCAE 5.0

Adverse event (CTCAE)	Grade 1–2 (n)	Grade 3 (n)
Anorexia	6	
Nausea	5	1
Weight loss	1	
Vomiting	3	
Constipation	3	
Diarrhea	1	
Hiccups	1	
Hypercholesterolemia	8	
Hyperuricemia		1
Seizures	1	

Associations with HbA1C level

On mixed-model univariate analysis, total daily caloric intake, weight and BMI were associated with higher HbA1C levels (Suppl. Table 5). On mixed-model multivariate analysis the only significant covariate was total daily caloric intake (Suppl. Table 6).

Clinical outcomes

Representative examples of radiological responses are presented (Fig. 3). Median progression free survival was 10 months for newly diagnosed disease and 4 months for recurrent disease; median overall survival was 21 months and 8 months respectively (Suppl. Figure 1a,1b).

Discussion

We report a prospective phase I dose-escalation trial of metabolic therapy combined with radiotherapy in patients with high-grade glioma. The recommended phase II dose is eight weeks ModAD combined with metformin 850 mg twice daily. The purpose of the study was not to assess efficacy, nonetheless, the patients performed favorably compared to historical controls [1, 31, 32].

The metabolic impact of the intervention was modest: only low levels of ketones in peripheral blood were obtained, and the decreases in blood glucose, insulin and HbA1c levels were non-significant. Possible reasons for this small metabolic impact include: (1) The serum concentration of metabolites, such as insulin, are highly labile making measurement challenging. (2) We originally planned to administer a strict ModAD to patients in which carbohydrate intake is tightly controlled, at the request of the local IRB this was replaced with a more relaxed ModAD. Even very small quantities of dietary carbohydrates severely impair ketone production. (3) The short duration of the metabolic intervention (eight weeks), this is especially relevant for HbA1c that has a half-life of approximately five weeks. (4) Possible poor patient dietary compliance, nutritional intake was calculated based upon patients' food diaries. (5) The small size of the trial. (6) Clinical trials of low-carbohydrate diets in adult cancer patients have shown difficulty in generating even moderate levels of ketones [33–35]. (7) Lack of caloric restriction. Interestingly we noted that HbA1c levels correlated with caloric intake (Suppl. Tables 4, 5). Hence, we speculate that caloric restriction would be usefully combined with a KD. This has indeed been demonstrated in rodents [16, 36], however the ability to tolerate such a diet clinically is uncertain. (8) Several patients received steroid treatment during the trial (Table 1), known to induce hyperglycemia.

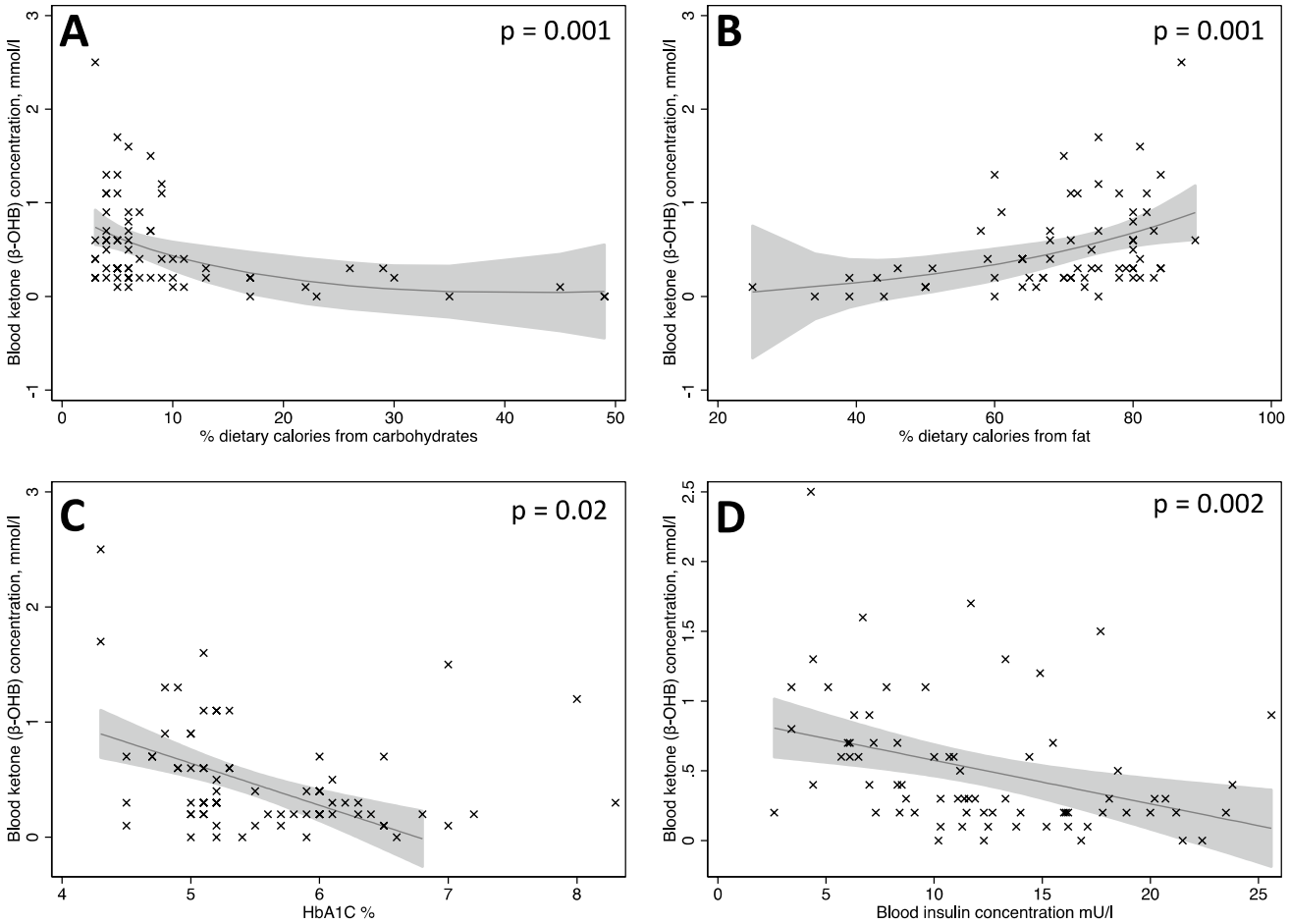

Fig. 2 Impact of dietary intervention on ketone levels. Grey zone represents 95% confidence interval on line of best fit. **a** Relationship between dietary carbohydrate (as % of total caloric input) and blood ketone levels, **b** Relationship between dietary fat intake (as % of total caloric input) and blood ketone levels, and **c** Relationship between Glycated hemoglobin (percentage, HbA1c) and blood ketone levels. **d** Relationship between blood insulin levels and blood ketone levels

A limitation of our study is the inclusion of two distinct patient populations: those with recurrent disease and those with newly diagnosed disease. For the purposes of determining the phase II dose we did not differentiate between the populations, however this may be an over simplification. Inadvertently, these populations were distributed unevenly across the trial: all subjects in cohort #1 had recurrent disease, cohort #2 was mixed, whereas all patients in cohort #3 were newly diagnosed (Table 1). The influence upon treatment tolerability is hard to ascertain – whereas subjects with newly diagnosed disease had a tougher concurrent treatment regimen (long course radiation therapy combined with temozolomide) they were treatment naïve and consequently had better overall wellbeing, with the converse being true for subjects with recurrent disease. Potentially, subjects with recurrent disease may tolerate the treatment regimen of cohort #3, but this was not tested in our trial.

The theoretical basis for this trial is the work of Seyfried and others, suggesting addiction to glucose is cancer cells' Achilles' heel. They have proposed that the combination of low blood glucose levels and high blood ketone levels will be therapeutic in brain tumors, and perhaps cancer in general. Seyfried has proposed a therapeutic window of glucose 55–65 mg/dl and ketones 2.5–7.0 mM [37]. Despite intensive counselling delivered by a dedicated multi-disciplinary team (dieticians, nurses and physicians) and the combination with an antidiabetic agent, we were far from achieving these goals, suggesting that although malignant cell growth may be driven by metabolic reprogramming [38], a dietary approach combined with metformin is inadequate. Moreover, some recent work suggests that the dietary-induced hypoglycemia as a treatment for brain tumors may be simplistic - for instance cancer cells attempt to compensate for low glucose levels by upregulating glucose transporter GLUT1 [39], and glioma cells may even adapt to utilize ketones as an energy source [40, 41]. Furthermore the role of AMPK (activated by metformin) appears to be complex and context-dependent: whereas in healthy cells AMPK switches

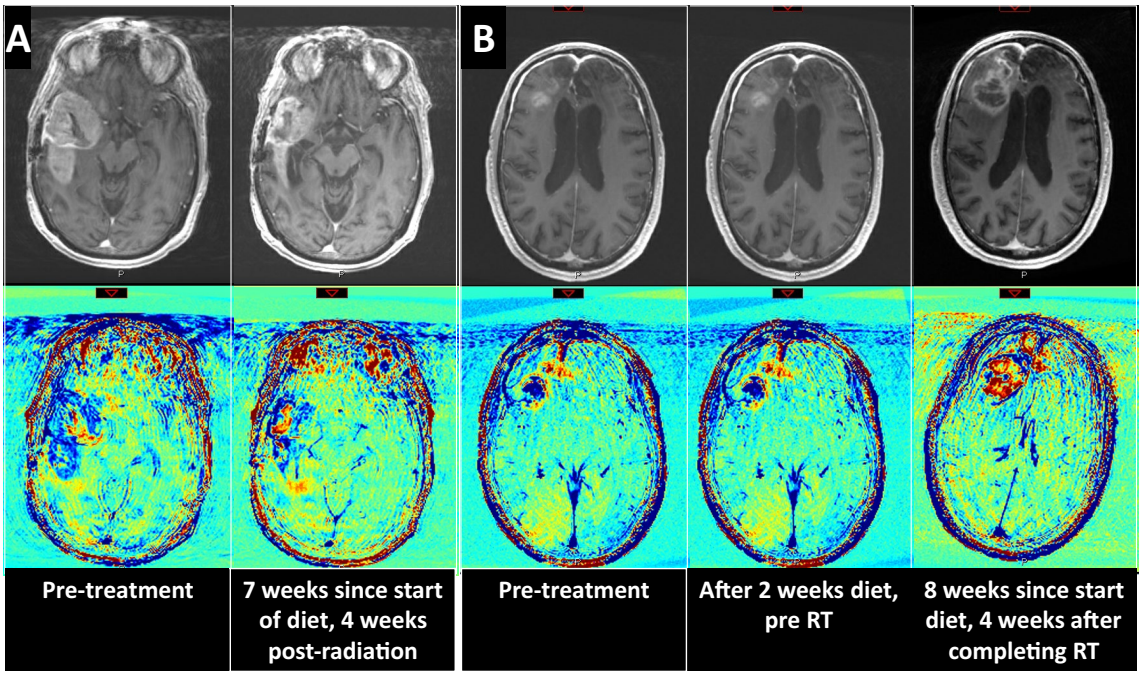

Fig. 3 Representative imaging of participants. Upper row - Fast spoiled gradient-echo (FSPGR) Gadolinium-enhanced Magnetic Resonance Imaging (MRI), lower row - Treatment response assessment maps (TRAMs) based upon the difference between early and late enhancement. **a** Patient #1 Recurrent glioblastoma, post treatment the lesion shrunk and became more necrotic (supported by the red TRAM image). **b** Patient #5 Recurrent glioblastoma, Over the course of the trial the lesion enlarged, and subsequently became cystic. The TRAM images suggest that the lesion became progressively necrotic

off cell growth and proliferation at times of stress, acting as a tumor-suppressor, in cancer cells AMPK may actually promote tumor growth [42] possibly through phosphorylation of Phosphoinositide 3-kinase enhancer-activating Akt (PIKE-A) [43]. Fortunately, a new generation of pharmaceutical agents targeting metabolic pathways are in development, that may target these weaknesses more effectively [44, 45].

Our trial is unique in combining metformin with a low-carbohydrate diet in non-diabetic patients, and intriguing in suggesting that metformin promotes ketogenesis (Suppl. Tables 2, 3). Previous investigators have proposed and investigated metformin combined with a KD as an anti-cancer treatment [46, 47], however there is little data regarding the influence of metformin on ketogenesis. In-vitro metformin stimulates the production of β-OHB in isolated hepatocytes [48], cultured neurons and astroglia [49]. Mechanistic explanations for metformin's pro-ketogenic activity include the drug's ability to overcome insulin resistance, and in particular, metformin's inhibition of liver mitochondrial complex I - favoring fat metabolism [50] and ketone body production. If validated, the addition of metformin would be beneficial in other disease settings in which ketone production is desired.

In conclusion, we have completed a Phase I trial of combined metabolic and radiation therapy in adult patients with high-grade gliomas. Clinical efficacy appears promising. The recommended phase II dose is 8-weeks ModAD with concomitant metformin (850 mg twice daily).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11060-021-03786-8.

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Author contributions Keren Porper, Leor Zach, Colin E. Champ, and Yaacov R Lawrence designed the study, wrote the manuscript, and analyzed the data. All authors implemented the research protocol, contributed to the interpretation of the data, read and approved the final manuscript.

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Availability of data and material The data that support the findings of this study are available on request from the corresponding author (Y.R.L).

Declarations

Conflict of interest Yaacov Lawrence: Research funding: Karyopharm Therapeutics, Checkmate Pharmaceuticals, Bristol-Myers Squibb and pending from Merck Serono. Honoria/consultancy fees: Bristol-Myers

Squibb, Clinigen Group and Roche Genetech. Stock ownership: Protean Biodiagnostics Inc. Colin E. Champ receives compensation for his dietary books, Leor Zach is an advisor of Trail-IN Pharma. Scientific advisory board for Atkins, and scientific advisory board for Biosense. The other authors declare that they have no competing interests.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. The trial was approved by the local Institutional Review Board (IRB) - SMC 0712 – 13, and registered on Clinicaltrials.gov NCT02149459.

Consent to participate All patients signed an IRB approved informed consent form, in accordance with the principals of Good Clinical Practice (GCP).

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